Ecology and Evolution Affect Network Structure in an Intimate Marine Mutualism

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abstract: Elucidating patterns and causes of interaction among mutualistic species is a major focus of ecology, and recent meta-analyses of terrestrial networks show that network-level reciprocal specialization tends to be higher in intimate mutualisms than in nonintimate mutualisms. It is largely unknown, however, whether this pattern holds for and what factors affect specialization in marine mutualisms. Here we present the first analysis of network specialization (H_2') for marine mutualistic networks. Specialization among eight Indo-Pacific networks of obligate mutualistic gobies and shrimps was indistinguishable from that among comparably intimate terrestrial mutualisms (ants-myrmecophytes) and higher than that among nonintimate ones (seed dispersers). Specialization was affected by variability in habitat use for both gobies and shrimps and by phylogenetic history for shrimps. Habitat use was phylogenetically conserved among shrimp, and thus effects of shrimp phylogeny on partner choice were mediated in part by habitat. By contrast, habitat use and pairing patterns in gobies were not related to phylogenetic history. This asymmetry appears to result from evolutionary constraints on partner use in shrimps and convergence among distantly related gobies to utilize burrows provided by multiple shrimp species. Results indicate that the evolution of mutualism is affected by life-history characteristics that transcend environments and that different factors constrain interactions in disparate ecosystems.

Keywords: mutualism, network topology, specialization, shrimp goby, forbidden link.

Introduction

It is now widely appreciated that mutualistic interactions are ubiquitous in most environments and often have far-reaching effects on the dynamics of entire ecosystems (Stachowicz 2001; Thompson et al. 2006). Thus, there has been extensive effort to describe and compare the topology of

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mutualistic networks (Bascompte et al. 2003; Bascompte and Jordano 2007; Mello et al. 2011), as interaction characteristics provide insight into features such as network stability (Bascompte et al. 2006; Okuyama and Holland 2008) and coevolution dynamics (Guimaraes et al. 2007a, 2011). Initial analyses of multiple networks supported the generality that most mutualisms are characterized by nested interactions, where specialists from one group (e.g., pollinators that interact with only one or a few plants) interact with subsets of the partner species (e.g., plants) with which generalists interact (Bascompte et al. 2003, 2006; Bascompte and Jordano 2007). These early studies suggested that reciprocal specialization—for example, a mutualistic relationship between two specialists—is rare in mutualistic networks (Bascompte et al. 2003).

Recent research, however, has questioned the idea that reciprocal specialization is uncommon in mutualisms (Bluthgen et al. 2007). One key difference that could explain these conflicting results is that initial meta-analyses focused primarily on mutualisms with relatively loose (i.e., nonintimate) partner interactions (Bascompte et al. 2003; Guimaraes et al. 2006, 2007b). However, interaction characteristics of mutualisms run the gamut from highly intimate (i.e., symbiotic), where partners spend the majority of their lives in contact with one another, to nonintimate (i.e., nonsymbiotic), where interactions among partners are fleeting or infrequent (Ollerton 2006; Thompson et al. 2006; Bluthgen et al. 2007). Two studies that considered degree of intimacy among different types of mutualisms (e.g., seed dispersal, pollination, ant-myrmecophyte, ant-nectar) found that, consistent with predictions from coevolutionary theory (Thompson 2005b), reciprocal specialization was higher (Bluthgen et al. 2007) or nestedness lower (Guimaraes et al. 2007a) in intimate (e.g., ant-myrmecophyte) versus nonintimate (seed dispersal) mutualisms. In addition, most

early analyses of network topology used network statistics (e.g., connectivity, nestedness) that considered only binary connections between species, that is, a presence/absence interaction matrix (but see Vazquez et al. 2007). However, an analysis of network properties based on an index of reciprocal specialization (Bluthgen et al. 2006) explicitly incorporating data on the frequency of interactions showed that specialization is higher in many mutualistic networks than was previously believed, based on binary measures of network structure (Bluthgen et al. 2007). To evaluate how intimacy might affect network properties in general, it is necessary to expand the scope of analyses to include mutualisms from disparate environments with taxonomically diverse species, using quantitative statistical tools (Ings et al. 2009).

To date, most empirical research on the organization of mutualistic networks has been conducted on plant-animal interactions in terrestrial environments (but see Guimaraes et al. 2007b; Ollerton et al. 2007; Ricciardi et al. 2010). However, mutualisms are also common in marine systems (Hay et al. 2004; Thompson et al. 2006), and these interactions are vital to the functioning of many marine environments, such as coral reefs, where the very fabric of the ecosystem is built on a coral-zooxanthellae mutualism (Baums 2008). To determine whether nascent generalities that have emerged from comparative studies of terrestrial systems pertain to marine mutualisms, it is first necessary to characterize similarities and differences in the nature of the interactions between mutualists in each environment. For example, if species in a marine network are intimately associated, then we would expect their degree of specialization to be more similar to a comparably intimate (e.g., ant-myrmecophyte) terrestrial network than to networks composed of more loosely interacting species (e.g., seed dispersers; Bluthgen et al. 2007). Here, we characterize the degree of reciprocal specialization (H_2') in multiple networks using an intimate marine mutualism involving fishes (gobies) that form lifelong pairings with crustaceans (alpheid shrimps) and explicitly compare levels of specialization in this mutualism with intimate and nonintimate terrestrial mutualisms.

While comparisons of specialization patterns among disparate systems will provide insight into the generalizability of information gleaned from terrestrial plant-animal interactions, it is necessary to go beyond specialization analyses to obtain a deeper understanding of the processes that determine why particular mutualist species do or do not interact (Rezende et al. 2007a, 2007b; Vazquez et al. 2009; Olesen et al. 2011). Insurmountable barriers that prevent contact among mutualists are termed "forbidden links" (Jordano et al. 2006) and can originate from ecological factors such as differences in phenology (Jordano et al. 2003; Vazquez et al. 2009), phenotypic (i.e., morphological) complementarity (Stang et al. 2009; Hultgren and Duffy 2010), and/or habitat specialization (Snow and Snow 1972) between potential part-

ners. Further, these constraints can be phylogenetically mediated, such that closely related species share traits that allow them to interact with only a subset of potential mutualistic partners. Discerning the particular factors that induce forbidden links and whether they are phylogenetically linked is critical because each type of constraint can have a different impact on the stability (Memmott et al. 2007; Rezende et al. 2007b; Aizen et al. 2012) and evolution (Rezende et al. 2007a) of mutualistic networks. Here, in addition to comparing levels of specialization among disparate marine and terrestrial systems, we elucidate for the first time ecological and evolutionary constraints that jointly affect specialization patterns in a marine mutualism.

With more than 120 species of gobies, and at least 20 species of shrimps, the goby-shrimp mutualism is highly diverse relative to other well-studied marine mutualisms (e.g., anemone-anemonefish), ubiquitous on coral reefs circumtropically, and thus a model system to understand the causes and consequences of specialization (Karplus and Thompson 2011). In this mutualism, one or two gobies share a burrow that is constructed by one or two shrimps. The burrow provides effective shelter against predators of both species, and the goby warns the shrimp of the presence of danger through a complex, tactile communication system based on tail flicks that are sensed by the antennae of the shrimp (Karplus 1979; Karplus and Tuvia 1979; Karplus and Thompson 2011). In addition to mediating death rates, each partner potentially augments nutrition for the other, as gobies gain access to food items excavated by the shrimps during burrowing and the shrimp spends more time foraging when a goby is present (Karplus and Thompson 2011). The association is obligate for most participating species (but see Karplus 1992; Randall et al. 2005; Wirtz 2008; and Kramer et al. 2009 for examples of facultative goby-shrimp interactions), as gobies separated from shrimps are quickly consumed by piscivores and shrimp cease to feed and grow when separated from a goby (Karplus and Thompson 2011). Obligate shrimp-associated gobies fall into 12 genera and are known to have evolved independently at least twice in the Indo-Pacific (Thacker et al. 2011) and once in the Caribbean Sea (Thacker and Roje 2011). The shrimps are all in the genus *Alpheus* (primarily in the *brevirostris* species group; Banner and Banner 1981), but it is unknown how many times the obligate association with gobies has evolved.

Methods

Sampling

To characterize specialization patterns in obligate goby-shrimp communities worldwide, we obtained quantitative (count) data from several published scuba-based surveys

conducted in five Indo-Pacific locations (table S1; tables S1 and S2 available online): Seychelles Islands (Polunin and Lubbock 1977); Great Barrier Reef, Australia (Cummins 1979); Red Sea, Israel (Karplus et al. 1981); Uchiumi Bay, Shikoku Island, Japan (Yanagisawa 1984); and the Gulf of Thailand, Thailand (Nakasone and Manthachitra 1986). With the exception of Cummins (1979), the data in each study were collected through single-pass, systematic surveys of a section of reef, where divers determined the identity of each goby and shrimp species within individual burrows. Cummins (1979) also conducted systematic surveys but visited particular quadrats on multiple occasions. Hence, it is likely that the same individual gobies and shrimps were counted on more than one occasion during these surveys. To avoid pseudoreplication, we calculated the mean number of unique goby-shrimp associations that Cummins detected in each quadrat per survey and then summed these values across quadrats.

In addition to the previously published surveys, we quantified for this study goby and shrimp associations and environmental covariates at three Indo-Pacific locations between 1999 and 2011. Specifically, we surveyed reefs in Kenting, Taiwan (21.95°N, 120.76°E), from May to June 2005; in Kimbe Bay, New Britain, Papua New Guinea (PNG; 5.44°S, 150.12°E), in October 2005; and in Moorea, French Polynesia (17.49°S, 149.85°W), in July 1999 and August 2011 (survey data deposited on the public Moorea Coral Reef Long-Term Ecological Research website: http://dx.doi.org/10.6073/pasta /a7447f66c3570c879b88bf25c605a2c9; table S1). Within each location (with the exception of Moorea; see below), we randomly placed 22–25 1 × 30-m transects on the benthos at sites with potentially suitable shrimp-goby habitat. The starting location of each transect was haphazardly selected on arrival at a reef, and transects were extended in a random direction, with the constraint that depth remain approximately equal along their lengths. Once transects were established, a scuba diver hovering ∼1 m from the bottom recorded all mutualistic goby and shrimp species observed within 0.5 m on either side of the transect line. At 1-m intervals along the transect line, the diver recorded substrate type (silt, sand, rubble, live coral, dead coral) and whether the location was found below overhanging reef (see Thompson 2004 for definition of substrate types). We surveyed the fore-reef in Moorea in 2011 by randomly placing three 30 × 1-m transects on the benthos and using the techniques described above. Data from the lagoon in Moorea were taken from 1999 surveys detailed in Thompson (2004), in which gobies and shrimps were surveyed at 682 3 × 3-m quadrats that were distributed on grids covering the northern part of the island (Thompson 2004). Because this extensive survey effort showed that there was only one goby-shrimp combination (*Ctenogobiops feroculus–Alpheus djeddensis*) in the lagoon, we were able to use a subset of the quadrat data for this study without sacrificing

information on the number of unique goby-shrimp associations. To approximately match the amount of area that was surveyed in 1999 and 2011 in Moorea, we selected for this study nine contiguous quadrats from a region of the lagoon that contained optimal shrimp-goby habitat. At each Indo-Pacific site, we attempted to sample each location until the accumulation of unique associations of goby-shrimp species was saturated. Estimates of association accumulation based on bootstrap analysis implemented with PRIMER, version 6 (Clarke and Gorley 2006), indicated that we observed 100% of the associations in the habitats sampled in Moorea, 86% in PNG, and 95% in Taiwan (fig. S1; figs. S1–S4 available online). We also attempted to collect and preserve in 95% ethanol at least one individual from each goby and shrimp species for genetic analysis. In both PNG and Moorea, we collected every species that was surveyed. In Taiwan, we collected 9 of 10 goby species (we missed *Lotilia graciliosa*) and 3 of 5 shrimp species (we missed *Alpheus djiboutensis* and *Alpheus rubromaculatus*). Once at the lab, the ethanol was drained and the specimens were stored at −80°C.

Network Topology

To evaluate the degree of goby-shrimp specificity at each location, we used H_2', a quantitative measurement of reciprocal specialization among mutualistic partners (Bluthgen et al. 2006, 2008; app. A; apps. A–C available online). This index measures the degree of network specialization on a scale of 0–1, where 1 indicates that all species from one group interact exclusively with only one species from the other group and vice versa, and 0 means that reciprocal specialization is absent. Thus, H_2' is directly comparable to the commonly used nestedness statistic (reviews in Bascompte and Jordano 2007; Bluthgen 2010), and when H_2' is 0, it describes a perfectly nested network (i.e., generalists and specialists interact with generalists, and specialist-specialist interactions are absent). Although several statistics have been used to describe network structure (Dormann et al. 2009; Rivera-Hutinel et al. 2012), we chose H_2' because it accounts for the observation frequencies of individual species, is not biased by the total number of species in a network (Bluthgen et al. 2007; Bluthgen 2010), and was thus ideal for our study, which utilized data collected from disparate researchers in systems with variable numbers of species. To provide context for the goby-shrimp mutualism, we compared H_2' values from our study with four different types of terrestrial mutualisms (Bluthgen et al. 2007) using a nonparametric one-way Kruskal-Wallis test and a post hoc Nemenyi test (Zar 1996). We determined whether network size or sampling effort induced bias on specialization values in the goby-shrimp networks by correlating both the total number of species (m) and the total number of recorded individuals

in a network against H'_2. We excluded *Ctenogobiops* species in PNG from these analyses, because the genetic results revealed that we did not reliably identify *Ctenogobiops* species during field surveys in this location (see "Results"). We used the bipartite package (Dormann et al. 2008) in R, version 2.15.0 (R Development Core Team 2012), to compute H'_2 values and the coin package (Hothorn et al. 2008) for the Kruskal-Wallis and Nemenyi tests.

Influence of Ecology and Evolution on Shrimp and Goby Partner Use

We determined in two ways whether ecological and/or phylogenetic constraints affected goby-shrimp associations. First, we asked whether similarity in partner use for both gobies and shrimps correlated with similarity in habitat use, phylogeny, and biogeographic distribution. Second, we determined whether species that live with phylogenetically similar partners (i.e., "phylocommunities"; Webb et al. 2002) were also phylogenetically similar themselves, utilized similar habitats, or were found only on particular islands.

These analyses were based on comparison of dissimilarity matrices. We built matrices based on the systematic data we collected in Moorea, PNG, and Taiwan. From the perspective of first shrimps and then gobies, we constructed explanatory (i.e., predictor) dissimilarity matrices based on habitat use (X_H), phylogeny (X_P), and presence on an island (X_B). We then used simple and partial Mantel tests and path analyses to determine the effects of these predictor matrices on partner-use (Y_{PU}) and phylocommunity (Y_{PC}) response (i.e., criterion) matrices.

To construct habitat dissimilarity matrices (X_H), we first calculated weighted mean values for each species (means were weighted by the relative abundance of that species on each transect for each habitat parameter, e.g., depth, percent silt, etc.). Next, we normalized each habitat variable (mean zero and unit variance) and calculated Euclidian distances between pairs of shrimps and then pairs of gobies. Similarly, to characterize biogeographic dissimilarity (Y_{PU}), we coded the presence or absence of each species on an island as 1 or 0 and again calculated Euclidian distances between species.

Phylogenetic dissimilarity matrices (X_P) were calculated using the cophenetic function in the Picante package (Kembel et al. 2010) in R, which converts a phylogenetic tree into an interspecific phylogenetic distance matrix. For the gobies, we used a previously published Bayesian phylogenetic tree with branch lengths (expressed in substitutions/site) for 54 species of shrimp-associated and free-living gobies (Thacker et al. 2011). For the shrimps, we constructed a dissimilarity matrix specifically for this study that was based on a Bayesian phylogenetic tree from 564

bp of mitochondrial cytochrome oxidase subunit I (COI) for the 10 collected species plus 2 goby-associated shrimps from the Caribbean (Williams et al. 2001). Sequence for the Caribbean shrimps was taken from GenBank (table S2) and generated for the Indo-Pacific shrimps in this study (details on shrimp-sequencing and tree-construction methodology are provided in app. B).

The first set of response matrices (Y_{PU}) characterized dissimilarity between pairs of shrimps and pairs of gobies based on their use of partner species. These matrices were calculated by first determining the density of each unique association at each location (raw count divided by the total area surveyed at each location) and then summing densities across locations. Next, we used these shrimp × goby matrices to calculate Hellinger distances (app. C) between each pair of shrimp species and then each pair of goby species. We used Hellinger distance because this metric has been shown to be well suited for analysis of multivariate species data where zeros are prevalent and because it is not unduly influenced by extreme values (Legendre and Gallagher 2001). Habitat (X_H), biogeography (X_B), and partner-dissimilarity (Y_{PU}) matrices were calculated with PRIMER, version 6 (Clarke and Gorley 2006).

The second set of response matrices (Y_{PC}) were based on phylocommunity structure as calculated using the comdist function of the Picante package (Kembel 2010; Kembel et al. 2010) in R. The comdist function measures the mean phylogenetic distance between pairs of species drawn from different assemblages (Webb et al. 2002; Kembel 2010). For example, from the perspective of the shrimps, this function measures the mean phylogenetic distance between pairs of goby species used by a given shrimp versus every other shrimp. It then outputs a dissimilarity matrix of phylogenetic "beta diversity," that is, the phylogenetic distance between different goby species associated with different shrimps (for more details, see Webb et al. 2002). Thus, if two shrimp species are similar in a phylocommunity analysis, then this means that they both pair with phylogenetically similar gobies. To account for differences in frequency in partner pairings, we weighed the pairwise measurements based on Hellinger-transformed densities (app. C) of gobies used by each shrimp species. We then repeated the analysis from the perspective of the gobies, using the shrimp phylogeny generated for this study.

To help visualize the patterns characterized by the dissimilarity analyses, we constructed group-averaged dendrograms based on each matrix. In addition, to discern in greater detail how habitat heterogeneity affected distribution patterns of shrimps and gobies, we performed nonmetric multidimensional scaling (nmds) analyses on the habitat dissimilarity matrices. These analyses were per-
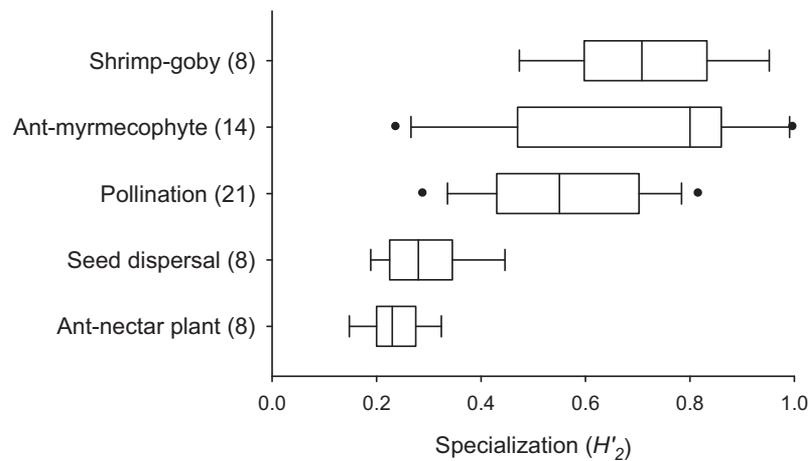
Figure 1: Box plot showing median specialization (H'_2) for the goby-shrimp and four terrestrial mutualisms with varying degrees of partner intimacy. Boxes encompass the twenty-fifth and seventy-fifth quartiles, bars show the tenth and ninetieth percentiles, and circles show the fifth and ninety-fifth percentiles. Percentiles were calculated using the Cleveland method in SigmaPlot, version 10.0 (Systat, San Jose, CA).

formed using PRIMER, version 6 (Clarke and Gorley 2006).

Comparison of matrices was accomplished using simple and partial Mantel tests and path analyses. Importantly, phylogenetic history can affect pairing patterns via a number of different mechanisms, including by influencing the ecological traits measured in this study. Accordingly, we used path analysis to estimate the independent and combined effects of the predictor matrices ($\mathbf{X}_H$, $\mathbf{X}_P$, $\mathbf{X}_B$) on the response matrices ($\mathbf{Y}_{PU}$, $\mathbf{Y}_{PC}$). Specifically, we hypothesized that phylogenetic history would affect habitat use and biogeography (i.e., there would be positive correlations between $\mathbf{X}_P$ and $\mathbf{X}_H$ and between $\mathbf{X}_P$ and $\mathbf{X}_B$) and that both of these ecological factors would affect the structure of partner use ($\mathbf{Y}_{PU}$) and/or phylocommunity ($\mathbf{Y}_{PC}$) matrices. In addition, we hypothesized that phylogeny would also influence pairing patterns directly, independent of habitat and biogeography (see fig. 4). Correlation coefficients for the path analysis were obtained from simple Mantel tests, with the significance of particular paths inferred from partial Mantel tests following Legendre and Legendre (1998). Simple and partial Mantel correlation coefficients and one-tailed significance tests based on 1,000 permutations were calculated in R using the ecodist package (Goslee and Urban 2007), and path coefficients were determined using the sem package (Fox 2006). Because a small number of shrimps and gobies were not available for genetic analysis, Mantel tests and path analyses included only species that were present in all matrices. Again we excluded *Cteno-gobiops* species in PNG from these analyses, because we were not confident that we accurately identified species in this genus in the field (see "Results"). Thus, the tests to explain partner use and phylocommunity structure based on shrimps included 10 species, and those based on gobies encompassed 20 species for partner similarity and 19 for phylocommunity analyses. (*Cryptocentrus nigrocellatus* was removed from the phylocommunity analyses because it paired only with a shrimp, *A. djiboutensis*, that was not sequenced.)

Results

Network Properties

Surveys from the eight Indo-Pacific locations identified 2–15 goby species and 2–10 shrimp species at a location (table S1). Comparison of H'_2 among the goby-shrimp and four terrestrial mutualisms (Bluthgen et al. 2007) showed that there was a highly significant difference among systems ($\chi^2 = 30.90$, df $= 4$, $P < .0001$) and that specialization in the goby-shrimp networks was most similar to the intimate terrestrial mutualism involving ants and myrmecophytes (fig. 1). Post hoc Nemenyi tests indicated that H'_2 was significantly higher in the goby-shrimp system than in the less intimate seed-dispersal ($P = .005$) and ant-nectar plant ($P = .0004$) systems. Although the shrimp-goby data were collected by disparate researchers at variable locations, sampling bias did not seem to affect the goby-shrimp results, as neither the total number of observed interactions (m) nor the network size (total number of species in a study) correlated with H'_2 ($R^2 < 0.04$ and $P > .68$ for both) among goby-shrimp networks.

*Influence of Ecology and Evolution on Goby
and Shrimp Partner Use*

Cluster analyses based on dissimilarity matrices from data collected in Moorea, PNG, and Taiwan identified groups of shrimps and gobies that utilized similar habitats, were phylogenetically similar, and were found on different islands (figs. 2, 3, S2). From a habitat perspective, dendrograms and nmds plots identified groups of 1–4 shrimp species (fig. 2A, S3) and 1–11 goby species (fig. 3A, S4) that were found in five distinct habitats characterized primarily as (1) sandy and shallow, (2) sandy and deep, (3) high coral, (4) all silt, or (5) embedded within a vertical wall with overhanging hard substrate above sand-silt substrate (i.e., overhang).

Although the shrimp phylogeny must be interpreted cautiously as it is based only on the mitochondrial COI gene, the five species clusters (fig. 2B) largely corresponded with alpheid morphology and revealed several undescribed species that could easily be distinguished by variation in color patterns, as noted for other *Alpheus* studies (e.g., Anker et al. 2008). For example, *Alpheus* sp. 5, *Alpheus ochrostriatus*, and *Alpheus bellulus* are relatively pale with fine white lines running from anterior to posterior along the surface of the carapace and abdomen. In addition, *Alpheus* sp. A (dark), *Alpheus* sp. 1 (striped), and *Alpheus djeddensis* share the presence of dark blotches on the lateral carapace, white saddles on the first and fourth abdominal somites, and an overall dark-blue to brownish-yellow mottled aspect. Importantly, although several of the *Alpheus* species in this study were not formally identified, they are not unknown: identifications of *A.* sp. 1 (striped), *A.* sp. 5 (pink), and *A.* sp. 7 (white) are based on published photo records (Hayashi and Shiratori 2003). We were unable to find a published record of only one morphotype, *A.* sp. A (dark). As shown by Thacker et al. (2011), gobies separated phylogenetically by genus and fell into two major clades, where clade 1 included species in the genera *Amblyeleotris*, *Ctenogobiops*, and *Vanderhorstia*, and clade 2 contained the genera *Cryptocentrus*, *Mahidolia*, *Tomiyamichthys*, and *Stonogobiops* (fig. 3B). The genetic analysis also revealed that there were actually three *Ctenogobiops* species (*Ctenogobiops aurocingulus*, *Ctenogobiops mitodes*, and *Ctenogobiops tongaensis*) in PNG, even though we discerned only two in the field.
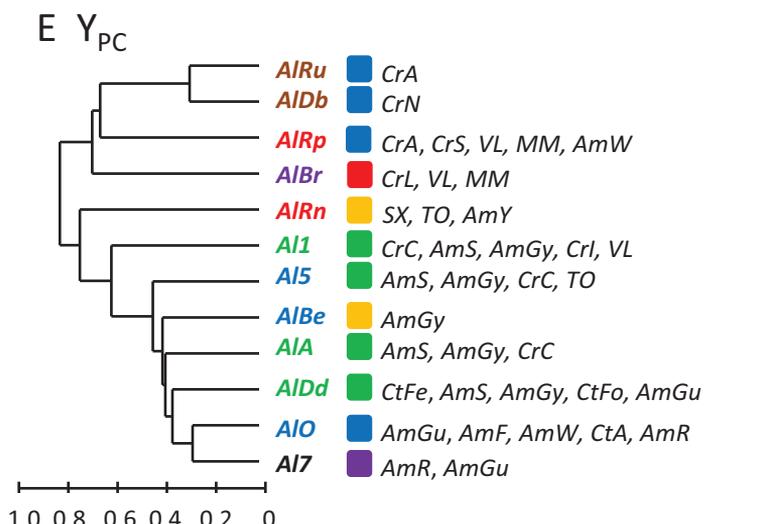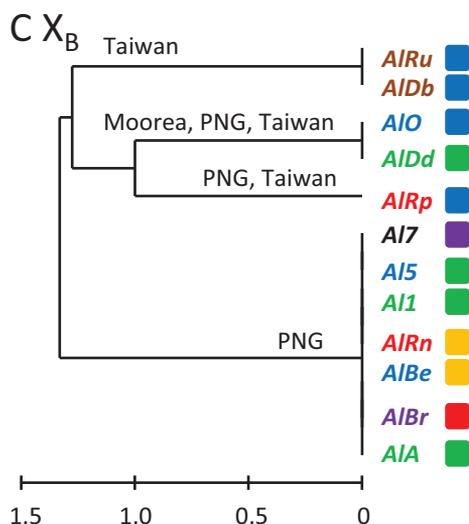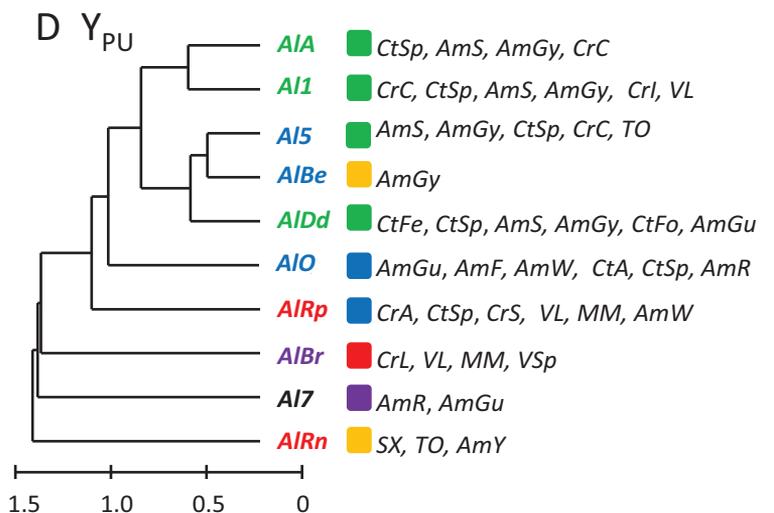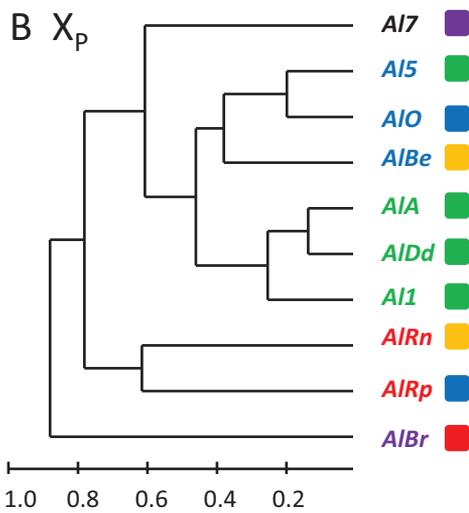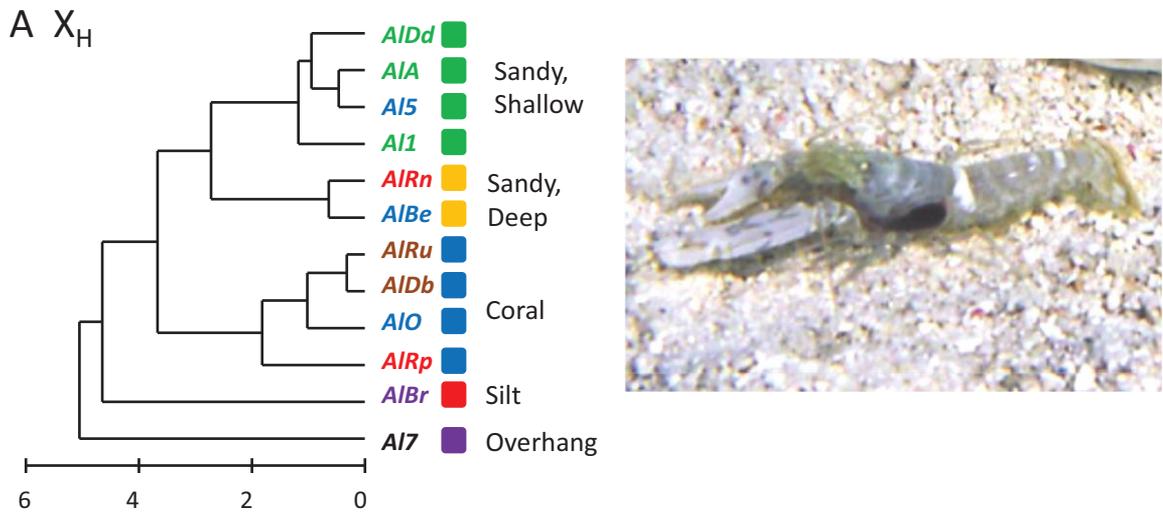
Finally, cluster analysis based on dissimilarity matrices of species presence in Moorea, PNG, and Taiwan grouped species found at one location to all three locations. Two shrimp species (*A. djeddensis* and *A. ochrostriatus*) were present in all three locations, while one shrimp (*Alpheus rapax*) was found in both PNG and Taiwan (fig. 2C). Seven shrimps were found only in PNG, and two only in Taiwan, but no species were unique to Moorea (fig. 2C). In contrast

to the shrimps, most gobies were found at only one of the islands, and only *Amblyeleotris guttata* and *C. aurocingulus* were identified at two islands (PNG and Taiwan), with *C. aurocingulus* being excluded from this analysis because we were unable to definitively identify it in field surveys in PNG (fig. 3C).

Analysis of the influence of shrimp predictor on response matrices elucidated a strong phylogenetic signal on partner use, as closely related species paired with similar gobies (table 1; figs. 2D, 4A). In addition, shrimps that used similar habitats paired with similar gobies and were phylogenetically similar (table 1; figs. 2D, 4A). The effect of phylogeny on shrimp partner use, however, was only partly mediated by habitat (fig. 4A). Indeed, the path analysis discerned a strong independent effect of phylogeny on partner pairing, with the effect of phylogeny on habitat use accounting for only ~25% of the correlation between phylogeny and partner pairing (fig. 4A). By contrast, no phylogenetic signal on habitat use or partner pairing was found for gobies (table 1; fig. 4B). There was, however, a strong correlation between habitat and partner use, with gobies in similar habitats pairing with similar shrimp (figs. 3D, 4B). The biogeography matrices did not correlate with partner use matrices for either shrimps or gobies (table 1; fig. 4A, 4B).

Phylocommunity analysis separated shrimps into two main groups (fig. 2E). The first group contained four shrimps that associated mainly with clade 2 gobies (Thacker et al. 2011) in the genera *Cryptocentrus*, *Mahidolia*, *Tomiyamichthys*, and *Stonogobiops* (fig. 2E). The second group included a gradient between shrimps that paired exclusively with clade 1 gobies (*Amblyeleotris*, *Ctenogobiops*, and *Vanderhorstia*; Thacker et al. 2011) and those that paired with both clade 1 and clade 2 gobies. There was a significant correlation between the shrimp phylocommunity and the shrimp phylogenetic matrices, indicating that shrimps that used phylogenetically similar gobies were also phylogenetically similar themselves (table 2; fig. 4C). There were no correlations between the shrimp phylocommunity and shrimp habitat or biogeography matrices (table 2), and path analysis showed that the correlation with phylogeny was not mediated by habitat or biogeography (fig. 4C).

The goby phylocommunity analysis elucidated five groups (fig. 3E). The first group interacted mainly with *A. ochrostriatus*, the second with the shrimp closely related to *A. djeddensis*, the third with *A. rapax*, the fourth with *Alpheus randalli*, and the fifth with *Alpheus brevicristatus*. There was a significant correlation between the goby habitat use and the phylocommunity matrices (table 2, fig. 4D), indicating that gobies in similar habitats resided with closely related shrimps (fig. 4D). Most goby phylocommunity groups included species from both goby phylo-

A X_H

AlDd	🟩	
AlA	🟩	Sandy, Shallow
Al5	🟩	
Al1	🟩	
AlRn	🟨	Sandy, Deep
AlBe	🟨	
AlRu	🟦	
AlDb	🟦	Coral
AlO	🟦	
AlRp	🟦	
AlBr	🟥	Silt
Al7	🟪	Overhang

6   4   2   0

B X_P

Al7	🟪
Al5	🟩
AlO	🟦
AlBe	🟨
AlA	🟩
AlDd	🟩
Al1	🟩
AlRn	🟨
AlRp	🟦
AlBr	🟥

1.0   0.8   0.6   0.4   0.2

D Y_{PU}

AlA	🟩	CtSp, AmS, AmGy, CrC
Al1	🟩	CrC, CtSp, AmS, AmGy, CrI, VL
Al5	🟩	AmS, AmGy, CtSp, CrC, TO
AlBe	🟨	AmGy
AlDd	🟩	CtFe, CtSp, AmS, AmGy, CtFo, AmGu
AlO	🟦	AmGu, AmF, AmW, CtA, CtSp, AmR
AlRp	🟦	CrA, CtSp, CrS, VL, MM, AmW
AlBr	🟥	CrL, VL, MM, VSp
Al7	🟪	AmR, AmGu
AlRn	🟨	SX, TO, AmY

1.5   1.0   0.5   0

C X_B

Taiwan

AlRu	🟦
AlDb	🟦
AlO	🟦
AlDd	🟩
AlRp	🟦
Al7	🟪
Al5	🟩
Al1	🟩
AlRn	🟨
AlBe	🟨
AlBr	🟥
AlA	🟩

Moorea, PNG, Taiwan

PNG, Taiwan

PNG

1.5   1.0   0.5   0

E Y_{PC}

AlRu	🟦	CrA
AlDb	🟦	CrN
AlRp	🟦	CrA, CrS, VL, MM, AmW
AlBr	🟥	CrL, VL, MM
AlRn	🟨	SX, TO, AmY
Al1	🟩	CrC, AmS, AmGy, CrI, VL
Al5	🟩	AmS, AmGy, CrC, TO
AlBe	🟨	AmGy
AlA	🟩	AmS, AmGy, CrC
AlDd	🟩	CtFe, AmS, AmGy, CtFo, AmGu
AlO	🟦	AmGu, AmF, AmW, CtA, AmR
Al7	🟪	AmR, AmGu

1.0   0.8   0.6   0.4   0.2   0

E64

genetic clades 1 and 2 (fig. 3*E*), and thus there was no correlation between the goby phylocommunity and phylogeny matrices (table 2). As with the shrimps, there was no correlation between phylocommunity and biogeography matrices for the gobies (table 2).

In sum, we found that shrimp-pairing patterns were influenced by habitat use, shrimp phylogeny, and goby phylogeny. From the perspective of the gobies, however, only habitat affinity significantly affected the use of shrimp partners.

Discussion

Our understanding of how mutualism evolves and persists has been expanded greatly by analyses of terrestrial mutualisms (Bascompte and Jordano 2007; Bluthgen et al. 2007; Guimaraes et al. 2007*a*). Here we evaluated for the first time the degree of specialization in multiple networks of marine mutualists and found that H_2' among goby and shrimp species was very similar to that among ants and myrmecophytes and much higher than that among seeds and their dispersers. Despite the superficial differences between gobies and shrimps versus ants and myrmecophytes, the demographic rates affected by mutualism in each system are very similar (Thompson et al. 2006). For example, one partner (myrmecophyte or shrimp) creates habitat that sets the carrying capacity of its associate (ant or goby). Further, the shelter user mitigates rates of death of its partner by signaling when predators are nearby (goby) or physically defending its host from herbivores (ant). These results suggest that evolutionary processes that shape patterns of specialization depend on fundamental life-history characteristics of participating species and transcend the particular environment in which they live.

In addition to our research, network analyses (but not quantitative measures of specialization) have been conducted on two additional marine mutualisms. Serendipitously, these two mutualisms are characterized by very different degrees of partner intimacy. On the one hand, three networks of cleaner fish from the Caribbean and western South Atlantic were found to have high degrees

of nestedness, indicating low specificity (Guimaraes et al. 2007*b*). To facilitate comparison with our results, we calculated H_2' for the two Caribbean networks and found that specialization was much lower in the cleaner fish than in the goby-shrimp networks (0.28 for the St. Croix [Johnson and Ruben 1988] and 0.20 for the Bonaire network [Wicksten 1998]) but very similar to reported values in seed-dispersal networks (Bluthgen et al. 2007; Mello et al. 2011). Such low values are expected in these systems, as both cleaners and seed dispersers engage in fleeting encounters with their mutualistic partners. On the other hand, a census of anemonefish-anemone interactions on Indonesian reefs showed that these mutualists were not nested and that all fish had a significantly preferred host anemone species (Ricciardi et al. 2010). Here H_2' was 0.70 (based on table 2 of Ricciardi et al. 2010), a value that is almost equivalent to the mean value from our goby-shrimp meta-analysis (fig. 1). Again, this result corroborates theoretical expectations (Thompson 2005*b*) because, similar to the goby-shrimp interaction, partner association between anemonefishes and anemones is much more intimate than that in the cleaner-fish system, as anemonefishes obligately depend on the shelter provided by their hosts and the anemones rely on the fish to ward off predators (Holbrook and Schmitt 2005; Thompson et al. 2006). These findings support the prediction that intimate and nonintimate mutualisms differ fundamentally in their network structure, regardless of whether the interaction takes place in a terrestrial or marine setting.

On top of comparing the topology of mutualistic networks among diverse systems, it is important to elucidate whether similar ecological and evolutionary factors constrain pairing patterns in disparate environments. We found that partner use by shrimps was strongly related to phylogeny and habitat, with closely related shrimps tending to partner with phylogenetically similar gobies. Importantly, while habitat use of shrimps was also influenced by phylogeny (i.e., closely related species tended to occupy similar habitats), shrimps exhibited a strong phylogenetic signal in partner pairing even after controlling for habitat, indicating that habitat segregation alone was not respon-

Figure 2: Group-averaged dendrograms based on dissimilarity matrices of shrimp: *A*, habitat use ($\mathbf{X}_{\mathrm{H}}$); *B*, phylogeny ($\mathbf{X}_{\mathrm{P}}$); *C*, biogeography ($\mathbf{X}_{\mathrm{B}}$); *D*, association with different species of gobies ($\mathbf{Y}_{\mathrm{PU}}$); *E*, phylocommunities ($\mathbf{Y}_{\mathrm{PC}}$). Colored fonts indicate phylogenetic relatedness. Colored squares represent habitat affinities: sandy, shallow (green); sandy, deep (orange); high coral (blue); all silt (red); overhang (purple). Gobies found to associate with a particular shrimp species are shown in *D* and *E*, with the most frequent association listed first. Shrimp species abbreviations, genus *Alpheus*: *bellulus* = AlBe, *brevicristatus* = AlBr, *djeddensis* = AlDd, *djiboutensis* = AlDb, *ochrostriatus* = AlO, *randalli* = AlRn, *rapax* = AlRp, *rubromaculatus* = AlRu, sp. 1 = Al1, sp. 5 = Al5, sp. 7 = Al7, and sp. A = AlA. Goby species abbreviations, genus *Amblyeleotris*: *fasciata* = AmF, *guttata* = AmGu, *gymnocephala* = AmGy, *randalli* = AmR, *steinitzi* = AmS, *yanoi* = AmY, *wheeleri* = AmW; genus *Ctenogobiops*: *aurocingulus* = CtA, *maculosus* = CtM, *feroculus* = CtFe, *formosa* = CtFo, spp. = CtSp; genus *Cryptocentrus*: *albidorsus* = CrAl, *cinctus* = CrC, *inexplicatus* = CrI, *leptocephalus* = CrL, *lutheri* = CrLu, *nigrocellatus* = CrN, *strigilliceps* = CrS, sp. A = CrA; *Lotilia graciliosa* = LG; *Mahidolia mystacina* = MM; *Stonogobiops xanthorhinica* = SX; *Tomiyamichthys oni* = TO; genus *Vanderhorstia*: *lanceolata* = VL, sp. = VSp. Photo of *A. djeddensis* by Daniel Geiger.
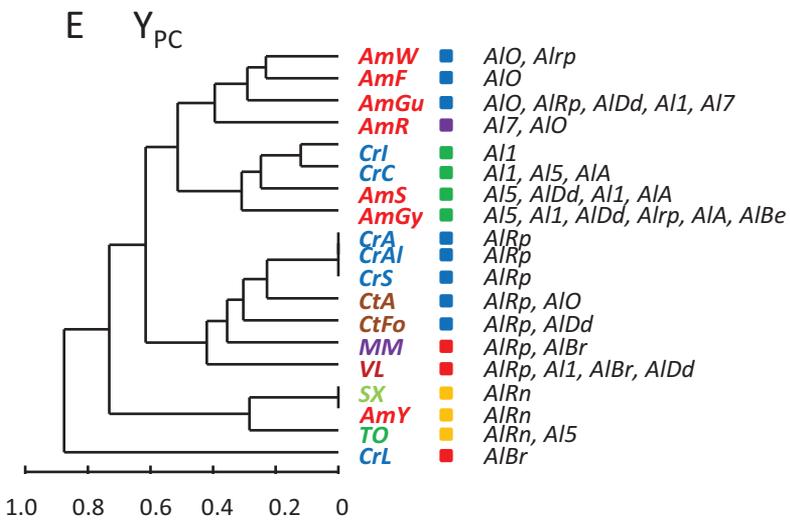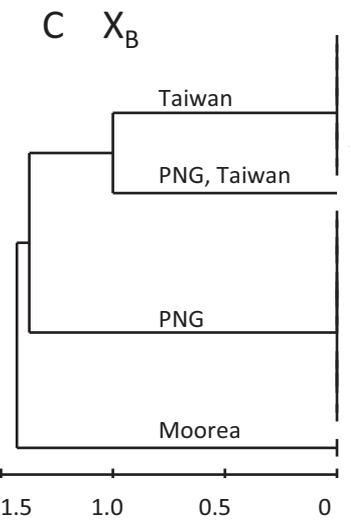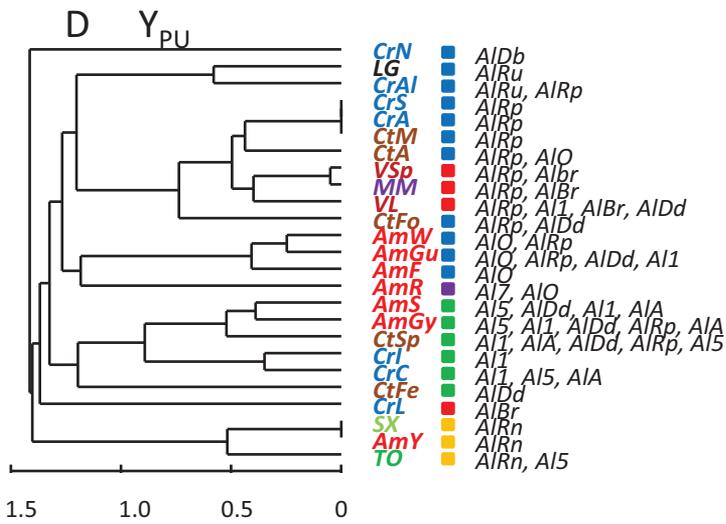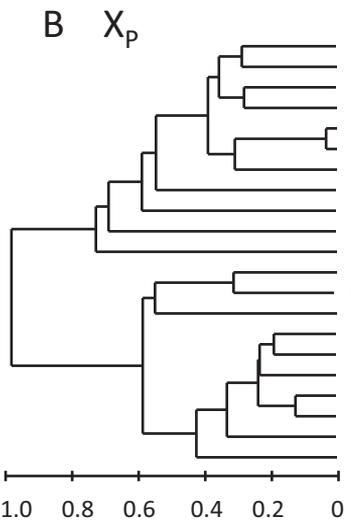
Figure with panels A–E showing dendrograms.

A X_H

Taxon	Habitat
VSp	Silt
VL	
MM	
CrL	
CtFo	Coral
LG	
CtM	
CrN	
CrAl	
CtA	
CrS	
AmW	
AmGu	
CrA	
AmF	
SX	Sandy, Deep
AmY	
TO	
AmS	Sandy, Shallow
AmGy	
CtSp	
CtFe	
CrI	
CrC	
AmR	Overhang

Scale: 8 6 4 2 0

B X_P

CrLu, CrL, CrA, CrI, CrS, CrN, CrAl, CrC, TO, MM, SX, CtFo, CtA, VL, AmY, AmF, AmW, AmS, AmGu, AmR, AmGy

Scale: 1.0 0.8 0.6 0.4 0.2 0

D Y_{PU}

Taxon	Alleles
CrN	AlDb
LG	AlRu
CrAl	AlRu, AlRp
CrS	AlRp
CrA	AlRp
CtM	AlRp
CtA	AlRp, AlO
VSp	AlRp, Albr
MM	AlRp, AlBr
VL	AlRp, Al1, AlBr, AlDd
CtFo	AlRp, AlDd
AmW	AlO, AlRp
AmGu	AlO, AlRp, AlDd, Al1
AmF	AlO
AmR	Al7, AlO
AmS	Al5, AlDd, Al1, AlA
AmGy	Al5, Al1, AlDd, AlRp, AlA
CtSp	Al1, AlA, AlDd, AlRp, Al5
CrI	Al1
CrC	Al1, Al5, AlA
CtFe	AlDd
CrL	AlBr
SX	AlRn
AmY	AlRn
TO	AlRn, Al5

Scale: 1.5 1.0 0.5 0

C X_B

Taiwan: CtA, AmW, VSp, LG
PNG, Taiwan: CrS, CrN, CrAl, CtFo, CtM, AmGu
MM, CrA, CrL, CrI, CrC, Ctsp, AmY, AmS, AmR, AmGy
PNG: VL, TO, SX, CtFe
Moorea: AmF

Scale: 1.5 1.0 0.5 0

E Y_{PC}

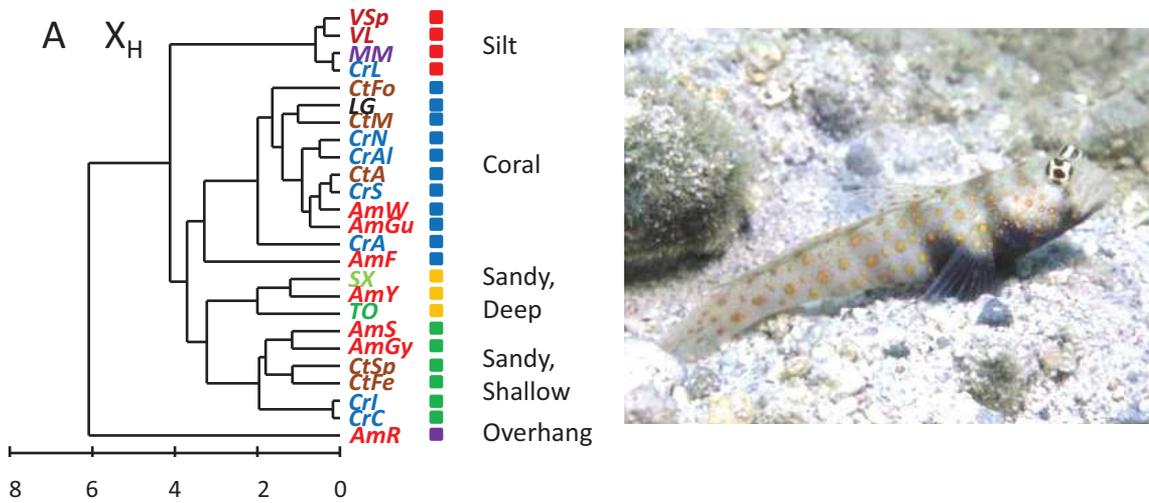| Taxon	Alleles
AmW	AlO, Alrp
AmF	AlO
AmGu	AlO, AlRp, AlDd, Al1, Al7
AmR	Al7, AlO
CrI	Al1
CrC	Al1, Al5, AlA
AmS	Al5, AlDd, Al1, AlA
AmGy	Al5, Al1, AlDd, Alrp, AlA, AlBe
CrA	AlRp
CrAl	AlRp
CrS	AlRp
CtA	AlRp, AlO
CtFo	AlRp, AlDd
MM	AlRp, AlBr
VL	AlRp, Al1, AlBr, AlDd
SX	AlRn
AmY	AlRn
TO	AlRn, Al5
CrL	AlBr

Scale: 1.0 0.8 0.6 0.4 0.2 0

E66

Table 1: Simple and partial Mantel tests evaluating relationships between predictor and partner similarity (response) matrices and between predictor matrices

	Y × X (simple)	Y × X/X (partial)				X × X (simple and partial)[a]		
	$Y_{PU} \times X$	$Y \times X/X_H$	$Y \times X/X_P$	$Y \times X/X_B$	$Y \times X/all$	X_H	X_P	X_B
$X_{SHRIMPS}$:								
Habitat (X_H)	**.69 (.001)**	...	**.49 (.01)**	**.68 (.002)**	**.49 (.008)**	...	**.56 (.012)**	.14 (.36)
Phylogeny (X_P)	**.76 (.001)**	**.63 (.008)**	...	**.76 (.001)**	**.63 (.005)**	**.54 (.011)**	...	.29 (.10)
Biogeography (X_B)	.16 (.269)	.08 (.41)	−.10 (.72)	...	−.10 (.73)	−.02 (.58)	.25 (.075)	...
X_{GOBIES}:								
Habitat (X_H)	**.49 (.001)**	...	**.49 (.001)**	**.48 (.001)**	**.48 (.001)**	...	.04 (.28)	.10 (.17)
Phylogeny (X_P)	.03 (.36)	.01 (.41)	...	.03 (.271)	.01 (.44)	.05 (.23)	...	−.09 (.95)
Biogeography (X_B)	.09 (.14)	.04 (.26)	.09 (.11)	...	.05 (.27)	.11 (.14)	−.10 (.95)	...

Note: Values shown are Mantel correlation coefficients (Mantel *r*) and *P* values (in parentheses). Correlations with *P* values <.05 are in bold.

[a] Results from simple tests are above the diagonals formed by empty cells, and those from partial tests are below them.

sible for the observed pairing patterns. Partner similarity in gobies, however, was not correlated with phylogeny. Asymmetry in phylogenetic constraints among interacting species have also been documented in host-parasite interactions (Krasnov et al. 2012), seed-dispersal networks (Rezende et al. 2007*a*, 2007*b*), and food webs (Rossberg et al. 2006; Bersier and Kehrli 2008). In seed-dispersal mutualisms, phylogenetic constraints were much more common in animals than in plants, and it was hypothesized that birds with morphologically similar beaks were phylogenetically closely related and thus constrained to utilize seeds that were the same size (Rezende et al. 2007*a*). Unlike in seed dispersal and most other studied mutualisms, partner selection in the goby-shrimp system is mediated directly by the active behavior of both gobies and shrimps. Further, field studies suggest that population sizes of shrimps are limited by the amount of habitat that is suitable for constructing burrows, whereas gobies are limited by the availability of shrimp burrows. For example, field studies in Japan demonstrated that on settlement, each *Alpheus bellulus* shrimp constructs its own burrow and pairs with the goby *Amblyeleotris japonica*. Once the shrimps become sexually mature, however, males and females combine burrows, thus cutting in half the number of burrows available to *A. japonica* (Yanagisawa 1984). The resulting competition for burrows reduces greatly the population size of *A. japonica*, and larger gobies were found to expel smaller individuals from burrows, typically resulting in the death of the vanquished goby. Similarly, a field experiment in Moorea demonstrated that increasing the density of the goby *Ctenogobiops feroculus* produced intraspecific competition for the burrows of *Alpheus djed-*

densis and that larger fish evicted from these burrows smaller competitors, who were then rapidly consumed by predators (Thompson 2005*a*). Hence, it is possible that there is selective pressure on the shrimps to pair with gobies that provide them with the best protection via tactile communication, while for the gobies it is most important to merely gain access to burrows. Indeed, seminal work on partner choice in the goby-shrimp system demonstrated that tactile communication between gobies and shrimps can be very complex and that communication patterns vary among different goby species (Karplus 1979; Karplus et al. 1979; Karplus and Thompson 2011). In addition, Karplus (1981) found that shrimps were highly selective in pairing with different goby species and refused to form antennal contact with certain gobies with which they do not naturally occur, even though such gobies had attempted to enter the burrow. It is thus feasible that closely related shrimps pair only with gobies whose "language" they understand and that the shrimps' ability to discern different goby signals is evolutionarily conserved. Because there is intense pressure on gobies to gain access to a burrow, however, it is possible that convergence in communication patterns occurred among distantly related gobies, thus allowing these species access to burrows not available to their congeners. More research is needed to evaluate the generality of asymmetry in evolutionary constraints on partner choice among networks of closely interacting species, but our results add to a growing list of empirical studies demonstrating this phenomenon.

In addition to phylogeny, we found that selection of abiotic habitat contributes to the segregation of gobies and shrimps in the Indo-Pacific, as different groups of species

Figure 3: Group-averaged dendrograms based on dissimilarity matrices of gobies. Descriptor and response matrices, colored fonts, colored squares, and shrimp and goby species abbreviations are as in figure 2. Shrimps found to associate with a particular goby species are shown in *D* and *E*, with the most frequent association listed first. Note that *Cryptocentrus lutheri* (*CrLu*; *B*) was not observed in our field studies but is included to provide the most complete goby phylogeny. Photo of *Amblyeleotris guttata* by D. Geiger.
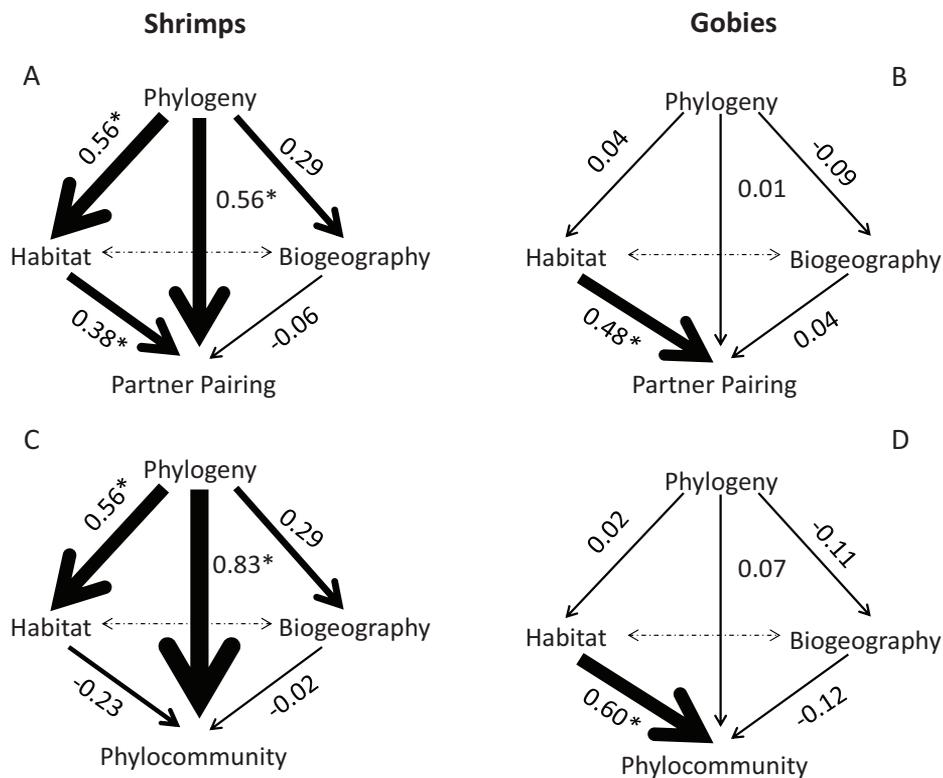
Figure 4: Path models illustrating relationships between predictor (phylogeny: X_P; habitat: X_H; biogeography: X_B) and response matrices (partner pairing: Y_{PU}, phylocommunity) for shrimps and gobies. Arrows point in the hypothesized direction of causality and are drawn with their width proportional to the path coefficients. The dashed line with a double-headed arrow represents a correlation between habitat and biogeography with no statement about causality (this path coefficient has been excluded from the figure for clarity). Asterisks denote significant paths inferred from simple and partial Mantel tests (tables 1, 2).

were found in habitats with fine versus coarse sediment as well as in deep versus shallow locations. These variables appear to influence goby-shrimp distributions worldwide; research in the Red Sea (Karplus et al. 1981; Freinschlag and Patzner 2012), Great Barrier Reef (Syms and Jones 2004), and Japan (Yanagisawa 1984) also show that sediment and depth affect assemblage structure. A likely mechanism dictating the importance of sediment composition is the capacity of different shrimp species to construct burrows under varying conditions. For example, aquarium experiments demonstrated that *A. djeddensis* was unable to build stable burrows in sediments that differed from preferred microhabitat in the field (Thompson 2004). Similarly, *Alpheus macellarius* burrows collapsed when the shrimp attempted to tunnel in suboptimal substrate (Palomar et al. 2005). For the gobies, the mechanism that drives habitat selection is still unclear. On the one hand, it is possible that gobies settle in particular types of habitat and then pair with preferred shrimps after arriving at that habitat. On the other hand, larvae of coral reef fish have been shown to use olfactory cues to discriminate among

settlement sites (Elliott et al. 1995; Munday et al. 2009), and thus settlement may be induced directly by chemical cues emanating from preferred shrimps. Regardless of the specific mechanisms, it is clear that habitat heterogeneity plays a large role in constraining interactions among mutualistic gobies and shrimps.

Although the role of phenological and morphological mismatch in constraining species interactions (i.e., inducing missing links) has been demonstrated repeatedly in the well-studied terrestrial mutualisms (Jordano et al. 2003; Stang et al. 2009; Vazquez et al. 2009; Olesen et al. 2011), the effect of habitat has received less attention. A notable exception was found in a network of 9 hummingbirds and 58 plants in a tropical rain forest (Snow and Snow 1972). Here, 185 out of a possible 522 interactions between birds and plants were observed. Of the 337 interactions that were not recorded, 29% were due to habitat uncoupling based on the preference of birds for different parts of the canopy (Jordano et al. 2006). The lack of emphasis on habitat in most terrestrial studies may be a product of the scale of sampling, as surveys are often made in relatively homoge-

Table 2: Simple and partial Mantel tests evaluating relationships between predictor and phylocommunity (response) matrices and between predictor matrices

	Y × X (simple)	Y × X/X (partial)				X × X (simple and partial)[a]		
	$Y_{PC} \times X$	Y × X /X_H	Y × X/X_P	Y × X/X_B	Y × X/all	X_H	X_P	X_B
$X_{SHRIMPS}$:								
Habitat (X_H)	.22 (.17)	...	−.27 (.90)	.20 (.192)	−.27 (.913)	...	**.56 (.012)**	.14 (.36)
Phylogeny (X_P)	**.70 (.003)**	**.70 (.004)**	...	**.68 (.008)**	**.70 (.003)**	**.54 (.011)**	...	.29 (.10)
Biogeography (X_B)	.19 (.22)	.16 (.27)	−.02 (.61)	...	−.02 (.62)	−.02 (.58)	.25 (.075)	...
X_{GOBIES}:								
Habitat (X_H)	**.59 (.001)**	...	**.59 (.001)**	**.60 (.001)**	**.60 (.001)**	...	.02 (.38)	.05 (.33)
Phylogeny (X_P)	.10 (.15)	.10 (.13)	...	.09 (.14)	.09 (.15)	.03 (.343)	...	−.11 (.95)
Biogeography (X_B)	−.09 (.78)	−.16 (.93)	−.08 (.73)	...	−.15 (.9)	.06 (.31)	−.11 (.96)	...

Note: Mantel correlation coefficients and *P* values are as presented in table 1.

[a] Results from simple tests are above the diagonals formed by empty cells, and those from partial tests are below them.

neous regions (e.g., fields) where animals are capable of visiting the entire sample frame. Similar to tropical rain forests, however, habitat on coral reefs is extremely heterogeneous from very small (10^0 m) to large (10^3 km) scales and varies vertically as well as horizontally. It is thus possible that habitat is more important in constraining interspecific interaction among mutualists in environments with structurally complex versus simple habitat.

Our research suggests that evolutionary history and habitat heterogeneity induce missing links in the marine goby-shrimp mutualism. The inability of species to overcome these constraints has implications for the potential of a mutualistic assemblage to recover following a perturbation. Rezende et al. (2007*b*) found that a cascade of extinctions is likely to result following the initial loss of one or a small number species in phylogenetically constrained—but not phylogenetically independent—mutualisms. Declines of particular species in the goby-shrimp mutualism could be induced, for example, by disease or targeted collection of particular species for the aquarium trade (Shuman et al. 2005). Large changes in the availability of abiotic habitat caused by global climatic changes could also destabilize mutualistic networks if at least one partner depends obligately on a specific type of habitat. For example, there is empirical evidence that climate-driven fluctuation in habitat availability influenced a goby-shrimp network in the eastern South Pacific in the recent past (Thompson et al. 2005). During the last glacial maximum, sea level in this region was up to 22 m lower than at present, thus drying out lagoon habitats on oceanic islands such as Moorea (Deschamps et al. 2012). This habitat loss induced local extinctions in *C. feroculus* and *A. djeddensis* that reside in the shallow, sandy lagoons, even though another shrimp-goby association (*Alpheus ochrostriatus* and *Amblyeleotris fasciata*) was found on the nearby fore-reef habitat (Thompson et al. 2005). Thus, habitat-mediated constraints on partner pairing apparently contributed to the local demise of a large part of the shrimp-goby network in this region. These results emphasize the importance of discerning the particular mechanisms that affect partner pairing and distribution patterns to provide insight toward how mutualistic networks respond to various types of perturbations.

In conclusion, we found that the degree of specialization in the relatively intimate marine goby-shrimp mutualism is (1) comparable to specialization values in networks with similar levels of intimacy and (2) much higher than those with less intimate interactions, regardless of whether species are in terrestrial or marine environments. In addition, we showed that phylogenetic history and habitat preferences help explain patterns of interaction and suggest that the role of habitat be considered more often in analyses of species interactions, particularly when mutualists live in heterogeneous environments. These results suggest that similar processes influence the ecology and evolution of mutualisms in both terrestrial and marine environments but that it is critical to understand the life history of species to contextualize network patterns.

Literature Cited

Aizen, M. A., M. Sabatino, and J. M. Tylianakis. 2012. Specialization and rarity predict nonrandom loss of interactions from mutualistic networks. Science 335:1486–1489.

Anker, A., C. Hurt, and N. Knowlton. 2008. Revision of the *Alpheus websteri* Kingsley, 1880 species complex (Crustacea: Decapoda: Alpheidae), with revalidation of *A. arenensis* (Chace, 1937). Zootaxa 1694:51–68.

Banner, D. M., and A. H. Banner. 1981. The alpheid shrimp of Australia part 3: the remaining alpheids, principally the genus *Alpheus*, and the family Ogyrididae. Records of the Australian Museum Supplement 34:1–357.

Bascompte, J., and P. Jordano. 2007. Plant-animal mutualistic networks: the architecture of biodiversity. Annual Review of Ecology Evolution and Systematics 38:567–593.

Bascompte, J., P. Jordano, C. J. Melian, and J. M. Olesen. 2003. The nested assembly of plant-animal mutualistic networks. Proceedings of the National Academy of Sciences of the USA 100:9383–9387.

Bascompte, J., P. Jordano, and J. M. Olesen. 2006. Asymmetric coevolutionary networks facilitate biodiversity maintenance. Science 312:431–433.

Baums, I. B. 2008. A restoration genetics guide for coral reef conservation. Molecular Ecology 17:2796–2811.

Bersier, L. F., and P. Kehrli. 2008. The signature of phylogenetic constraints on food-web structure. Ecological Complexity 5:132–139.

Bluthgen, N. 2010. Why network analysis is often disconnected from community ecology: a critique and an ecologist's guide. Basic and Applied Ecology 11:185–195.

Bluthgen, N., J. Frund, D. P. Vazquez, and F. Menzel. 2008. What do interaction network metrics tell us about specialization and biological traits? Ecology 89:3387–3399.

Bluthgen, N., F. Menzel, and N. Bluthgen. 2006. Measuring specialization in species interaction networks. BMC Ecology 6:9.

Bluthgen, N., F. Menzel, T. Hovestadt, B. Fiala, and N. Bluthgen. 2007. Specialization, constraints, and conflicting interests in mutualistic networks. Current Biology 17:341–346.

Clarke, K. R., and R. N. Gorley. 2006. PRIMER v6: user manual/tutorial. PRIMER-E, Plymouth.

Cummins, R. A. 1979. Ecology of gobiid fishes associated with alpheid shrimps at One Tree Reef. PhD diss. University of Sydney, Australia.

Deschamps, P., N. Durand, E. Bard, B. Hamelin, G. Camoin, A. L. Thomas, G. M. Henderson, J. Okuno, and Y. Yokoyama. 2012. Ice-sheet collapse and sea-level rise at the Bølling warming 14,600 years ago. Nature 483:559–564.

Dormann, C. F., J. Frund, N. Bluthgen, and B. Gruber. 2009. Indices, graphs and null models: analyzing bipartite ecological networks. Open Ecology Journal 2:7–24.

Dormann, C. F., B. Gruber, and J. Frund. 2008. Introducing the bipartite package: analysing ecological networks. R News 8:8–11.

Elliott, J. K., J. M. Elliott, and R. N. Mariscal. 1995. Host selection, location, and association behaviors of anemonefishes in field settlement experiments. Marine Biology 122:377–389.

Fox, J. 2006. Structural equation modeling with the sem package in

R. Structural Equation Modeling: A Multidisciplinary Journal 13: 465–486.

Freinschlag, M., and R. A. Patzner. 2012. Shrimp-gobies in the southern Gulf of Aqaba (Red Sea). Zoology in the Middle East 55:41–46.

Goslee, S. C., and D. L. Urban. 2007. The ecodist package for dissimilarity-based analysis of ecological data. Journal of Statistical Software 22:1–19.

Guimaraes, P. R., Jr., P. Jordano, and J. N. Thompson. 2011. Evolution and coevolution in mutualistic networks. Ecology Letters 14:877–885.

Guimaraes, P. R., V. Rico-Gray, S. F. dos Reis, and J. N. Thompson. 2006. Asymmetries in specialization in ant-plant mutualistic networks. Proceedings of the Royal Society B: Biological Sciences 273: 2041–2047.

Guimaraes, P. R., V. Rico-Gray, P. S. Oliveira, T. J. Izzo, S. F. dos Reis, and J. N. Thompson. 2007a. Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. Current Biology 17:1797–1803.

Guimaraes, P. R., C. Sazima, S. F. dos Reis, and I. Sazima. 2007b. The nested structure of marine cleaning symbiosis: is it like flowers and bees? Biology Letters 3:51–54.

Hay, M. E., J. D. Parker, D. E. Burkepile, C. C. Caudill, A. E. Wilson, Z. P. Hallinan, and A. D. Chequer. 2004. Mutualisms and aquatic community structure: the enemy of my enemy is my friend. Annual Review of Ecology Evolution and Systematics 35:175–197.

Hayashi, M., and T. Shiratori. 2003. Gobies of Japanese waters. TBS Buritanica, Tokyo.

Holbrook, S. J., and R. J. Schmitt. 2005. Growth, reproduction and survival of a tropical sea anemone (Actiniaria): benefits of hosting anemonefish. Coral Reefs 24:67–73.

Hothorn, T., K. Hornik, M. A. V. van de Wiel, and A. Zeileis. 2008. Implementing a class of permutation tests: the coin package. Journal of Statistical Software 28:1–23.

Hultgren, K. M., and J. E. Duffy. 2010. Sponge host characteristics shape the community structure of their shrimp associates. Marine Ecology Progress Series 407:1–12.

Ings, T. C., J. M. Montoya, J. Bascompte, N. Bluthgen, L. Brown, C. F. Dormann, F. Edwards, et al. 2009. Ecological networks—beyond food webs. Journal of Animal Ecology 78:253–269.

Johnson, W. S., and P. Ruben. 1988. Cleaning behavior of *Bodianus rufus*, *Thalassoma bifasciatum*, *Gobiosoma evelynae*, and *Periclimenes pedersoni* along a depth gradient at Salt River Submarine Canyon, St. Croix. Environmental Biology of Fishes 23:225–232.

Jordano, P., J. Bascompte, and J. M. Olesen. 2003. Invariant properties in coevolutionary networks of plant-animal interactions. Ecology Letters 6:69–81.

———. 2006. The ecological consequences of complex topology and nested structure in pollination webs. Pages 173–199 *in* N. M. Waser and J. Ollerton, eds. Specialization and generalization in plant-pollinator interactions. University of Chicago Press, Chicago.

Karplus, I. 1979. Tactile communication between *Cryptocentrus steinitzi* (Pisces, Gobiidae) and *Alpheus purpurilenticularis* (Crustacea, Alpheidae). Zeitschrift für Tierpsychologie 49:173–196.

———. 1981. Goby-shrimp partner specificity. 2. The behavioral mechanisms regulating partner specificity. Journal of Experimental Marine Biology and Ecology 51:21–35.

———. 1992. Obligatory and facultative goby-shrimp partnerships in the western tropical Atlantic. Symbiosis 12:275–291.

Karplus, I., R. Szlep, and M. Tsurnamal. 1981. Goby-shrimp partner specificity. 1. Distribution in the northern Red Sea and partner

specificity. Journal of Experimental Marine Biology and Ecology 51:1–19.

Karplus, I., and A. R. Thompson. 2011. The partnership between gobiid fishes and burrowing alpheid shrimps. Pages 559–608 *in* R. A. Patzner, J. L. Van Tassell, M. Kovacic, and B. G. Kapoor, eds. The biology of gobies. Science, Boca Raton, FL.

Karplus, I., M. Tsurnamal, R. Szlep, and D. Algom. 1979. Film analysis of the tactile communication between *Cryptocentrus steinitzi* (Pisces, Gobiidae) and *Alpheus purpurilenticularis* (Crustacea, Alpheidae). Zeitschrift für Tierpsychologie 49:337–351.

Karplus, I., and S. B. Tuvia. 1979. Warning signals of *Cryptocentrus steinitzi* (Pisces, Gobiidae) and predator models. Zeitschrift für Tierpsychologie 51:225–232.

Kembel, S. W. 2010. An introduction to the Picante package. picante.r-forge.r-project.org/picante-intro.pdf.

Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464.

Kramer, A., J. L. Van Tassell, and R. A. Patzner. 2009. A comparative study of two goby shrimp associations in the Caribbean Sea. Symbiosis 49:137–141.

Krasnov, B. R., M. A. Fortuna, D. Mouillot, I. S. Khokhlova, G. I. Shenbrot, and R. Poulin. 2012. Phylogenetic signal in module composition and species connectivity in compartmentalized host-parasite networks. American Naturalist 179:501–511.

Legendre, P., and E. D. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia (Berlin) 129:271–280.

Legendre, P., and L. Legendre. 1998. Numerical ecology. Elsevier, Amsterdam.

Mello, M. A. R., F. M. D. Marquitti, P. R. Guimaraes, E. K. V. Kalko, P. Jordano, and M. A. M. de Aguiar. 2011. The missing part of seed dispersal networks: structure and robustness of bat-fruit interactions. PloS ONE 6(2):e17395.

Memmott, J., P. G. Craze, N. M. Waser, and M. V. Price. 2007. Global warming and the disruption of plant-pollinator interactions. Ecology Letters 10:710–717.

Munday, P. L., D. L. Dixson, J. M. Donelson, G. P. Jones, M. S. Pratchett, G. V. Devitsina, and K. B. Doving. 2009. Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. Proceedings of the National Academy of Sciences of the USA 106:1848–1852.

Nakasone, Y., and V. Manthachitra. 1986. A preliminary report on the associations between gobies and alpheid shrimps in the Sichang Island, the Gulf of Thailand. Galaxea 5:157–162.

Okuyama, T., and J. N. Holland. 2008. Network structural properties mediate the stability of mutualistic communities. Ecology Letters 11:208–216.

Olesen, J. M., J. Bascompte, Y. L. Dupont, H. Elberling, C. Rasmussen, and P. Jordano. 2011. Missing and forbidden links in mutualistic networks. Proceedings of the Royal Society B: Biological Sciences 278:725–732.

Ollerton, J. 2006. "Biological barter": patterns of specialization compared across different mutualisms. Pages 411–435 *in* N. M. Waser and J. Ollerton, eds. Plant-pollinator interactions: from specialization to generalization. University of Chicago Press, Chicago.

Ollerton, J., D. McCollin, D. G. Fautin, and G. R. Allen. 2007. Finding NEMO: nestedness engendered by mutualistic organization in anemonefish and their hosts. Proceedings of the Royal Society B: Biological Sciences 274:591–598.

Palomar, N. E., M. A. Juinio-Menez, and I. Karplus. 2005. Behavior of the burrowing shrimp *Alpheus macellarius* in varying gravel substrate conditions. Journal of Ethology 23:173–180.

Polunin, N. V. C., and R. Lubbock. 1977. Prawn-associated gobies (Teleostei: Gobiidae) from the Seychelles, western Indian Ocean: systematics and ecology. Journal of Zoology 183:63–101.

R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Randall, J. E., P. S. Lobel, and C. W. Kennedy. 2005. Comparative ecology of the gobies *Nes longus* and *Ctenogobius saepepallens*, both symbiotic with the snapping shrimp *Alpheus floridanus*. Environmental Biology of Fishes 74:119–127.

Rezende, E. L., P. Jordano, and J. Bascompte. 2007*a*. Effects of phenotypic complementarity and phylogeny on the nested structure of mutualistic networks. Oikos 116:1919–1929.

Rezende, E. L., J. E. Lavabre, P. R. Guimaraes, P. Jordano, and J. Bascompte. 2007*b*. Non-random coextinctions in phylogenetically structured mutualistic networks. Nature 448:925–926.

Ricciardi, F., M. Boyer, and J. Ollerton. 2010. Assemblage and interaction structure of the anemonefish-anemone mutualism across the Manado region of Sulawesi, Indonesia. Environmental Biology of Fishes 87:333–347.

Rivera-Hutinel, A., R. O. Bustamante, V. H. Marin, and R. Medel. 2012. Effects of sampling completeness on the structure of plant-pollinator networks. Ecology 93:1593–1603.

Rossberg, A. G., H. Matsuda, T. Amemiya, and K. Itoh. 2006. Food webs: experts consuming families of experts. Journal of Theoretical Biology 241:552–563.

Shuman, C. S., G. Hodgson, and R. F. Ambrose. 2005. Population impacts of collecting sea anemones and anemonefish for the marine aquarium trade in the Philippines. Coral Reefs 24:564–573.

Snow, B. K., and D. W. Snow. 1972. Feeding niches of hummingbirds in a Trinidad valley. Journal of Animal Ecology 41:471–485.

Stachowicz, J. J. 2001. Mutualism, facilitation, and the structure of ecological communities. BioScience 51:235–246.

Stang, M., P. G. Klinkhamer, N. M. Waser, I. Stang, and E. van der Meijden. 2009. Size-specific interaction patterns and size matching in a plant-pollinator interaction web. Annals of Botany 103:1459–1469.

Syms, C., and G. P. Jones. 2004. Habitat structure, disturbance and the composition of sand-dwelling goby assemblages in a coral reef lagoon. Marine Ecology Progress Series 268:221–230.

Thacker, C. E., and D. M. Roje. 2011. Phylogeny of Gobiidae and identification of gobiid lineages. Systematics and Biodiversity 9: 329–347.

Thacker, C. E., A. R. Thompson, and D. M. Roje. 2011. Phylogeny and evolution of Indo-Pacific shrimp-associated gobies (Gobiiformes: Gobiidae). Molecular Phylogenetics and Evolution 59: 168–176.

Thompson, A. R. 2004. Habitat and mutualism affect the distribution and abundance of a shrimp-associated goby. Marine and Freshwater Research 55:105–113.

———. 2005*a*. Dynamics of demographically open mutualists: immigration, intraspecific competition, and predation impact goby populations. Oecologia (Berlin) 143:61–69.

Thompson, A. R., R. M. Nisbet, and R. J. Schmitt. 2006. Dynamics

of mutualist populations that are demographically open. Journal of Animal Ecology 75:1239–1251.

Thompson, A. R., C. E. Thacker, and E. Y. Shaw. 2005. Phylogeography of marine mutualists: parallel patterns of genetic structure between obligate goby and shrimp partners. Molecular Ecology 14:3557–3572.

Thompson, J. N. 2005*b*. The geographic mosaic of coevolution. University of Chicago Press, Chicago.

Vazquez, D. P., N. P. Chacoff, and L. Cagnolo. 2009. Evaluating multiple determinants of the structure of plant-animal mutualistic networks. Ecology 90:2039–2046.

Vazquez, D. P., C. J. Melian, N. M. Williams, N. Bluthgen, B. R. Krasnov, and R. Poulin. 2007. Species abundance and asymmetric interaction strength in ecological networks. Oikos 116:1120–1127.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics 33:475–505.

Wicksten, M. K. 1998. Behaviour of cleaners and their client fishes at Bonaire, Netherlands Antilles. Journal of Natural History 32: 13–30.

Williams, S. T., N. Knowlton, L. A. Weigt, and J. A. Jara. 2001. Evidence for three major clades within the snapping shrimp genus *Alpheus* inferred from nuclear and mitochondrial gene sequence data. Molecular Phylogenetics and Evolution 20:375–389.

Wirtz, P. 2008. The Gulf of Guinea goby-shrimp symbiosis and a review of goby-thalassinidean associations. Life and Marine Sciences 25:71–76.

Yanagisawa, Y. 1984. Studies on the interspecific relationship between gobiid fish and snapping shrimp. II. Life history and pair formation of snapping shrimp *Alpheus bellulus*. Publications of the Seto Marine Biological Laboratory 29:93–116.

Zar, J. H. 1996. Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ.

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The fierce shrimp goby, *Ctenogobiops feroculus*, and the snapping shrimp, *Alpheus djeddensis*, in a sandy lagoon off Moorea, French Polynesia (Photo © Daniel L. Geiger/Santa Barbara Museum of Natural History).