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PERIPATRIC SPECIATION DRIVES DIVERSIFICATION AND DISTRIBUTIONAL PATTERN OF REEF HERMIT CRABS (DECAPODA: DIOGENIDAE: *CALCINUS*)

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The diversity on coral reefs has long captivated observers. We examine the mechanisms of speciation, role of ecology in speciation, and patterns of species distribution in a typical reef-associated clade—the diverse and colorful Calcinus hermit crabs—to address the origin of tropical marine diversity. We sequenced COI, 16S, and H3 gene regions for ~90% of 56 putative species, including nine undescribed, "cryptic" taxa, and mapped their distributions. Speciation in Calcinus is largely peripatric at remote locations. Allopatric species pairs are younger than sympatric ones, and molecular clock analyses suggest that >2 million years are needed for secondary sympatry. Substantial niche conservatism is evident within clades, as well as a few major ecological shifts between sister species. Color patterns follow species boundaries and evolve rapidly, suggesting a role in species recognition. Most species prefer and several are restricted to oceanic areas, suggesting great dispersal abilities and giving rise to an ocean-centric diversity pattern. Calcinus diversity patterns are atypical in that the diversity peaks in the west-central oceanic Pacific rather than in the Indo-Malayan "diversity center." Calcinus speciation patterns do not match well-worn models put forth to explain the origin of Indo-West Pacific diversity, but underscore the complexity of marine diversification.

KEY WORDS: **Allopatric speciation, biodiversity, biogeography, color pattern evolution, circumtropical speciation, coral reef, coral triangle, cryptic species, crustacea, ESU, Indo-Malayan hot spot, molecular phylogenetics, sympatric speciation.**

The marine tropics can be divided into four broad regions defined by largely endemic biotas: the East Atlantic (EA; West African tropical coastline and offshore islands, Mediterranean), West Atlantic (WA; East American tropical coastline, Caribbean, and offshore islands including Bermuda), East Pacific (EP; West American tropical coastline to offshore islands including Galapagos and Clipperton), and Indo-west Pacific (IWP; from East Africa to Easter Island) regions (Ekman 1953; Briggs 1974). Diversity is lowest in the EA and highest, by about an order of magnitude, in the IWP (Paulay 1997). Further patterns are evident within the vast IWP, where marine biodiversity peaks in the Indo-Malayan triangle bounded by the Philippines, Indonesia, and New Guinea and decreases in a striking manner toward the central Pacific (Stehli and Wells 1971). Hermit crabs show a similar diversity pattern, although this has not been systematically documented (see below). Much early work focused on this striking IWP diversity pattern and attempted to find single or at least dominant processes to explain diversification in the IWP based on this pattern. Although numerous hypotheses of diversification have been proposed (Rosen 1988), three have been most emphasized: the center of origin (Ekman 1953; Briggs 1974), center of overlap (Woodland 1983), and center of accumulation hypotheses (Ladd 1960;

Jokiel and Martinelli 1992). These three hypotheses have attributed the origin of IWP diversity to geographically localized diversity pumps: within the Indo-Malayan area, at the boundary between the Indian and Pacific basins, and on remote, peripheral islands, respectively; with subsequent range expansion from these areas. Increasing documentation of variation in spatial diversity patterns as well as modes of speciation have, however, led to the realization that multiple processes must be involved in generating the diversity (Palumbi 1997; Paulay 1997; Williams 2007).

Molecular phylogenetics provides a powerful tool for understanding the origins of observed patterns of species richness. By analyzing diverse taxa, we can address questions of diversification from a quantitative, mechanistic perspective: what is the relative importance of different modes of speciation in generating species richness and spatial patterns of diversity? In the past, analyses of spatial patterns of diversity were largely inferential (i.e., top-down): by examining biota-level patterns, researchers inferred likely processes of diversification. In contrast a quantitative phylogenetic approach provides a mechanistic (i.e., bottom-up) perspective: by documenting numerous speciation events, we can investigate how regional-level diversity patterns arise. Such an approach necessitates thorough spatial and taxonomic sampling, so that most or all speciation events in a clade are identified and characterized. Thorough taxonomic coverage is also one of the most important factors in recovering an accurate tree topology, as shown by both empirical and simulation studies (e.g., Graybeal 1998; Zwickl and Hillis 2002; Soltis et al. 2004).

The objectives of this study are to pursue a comprehensive phylogenetic and biogeographic analysis of the reef-associated hermit crab genus *Calcinus*, to: (1) determine spatial and temporal patterns of diversification, (2) evaluate the relative importance of different modes of speciation and how they gave rise to observed patterns of diversity and distribution, and (3) assess the roles of color and ecology in diversification. *Calcinus* is a diverse group of diurnal, colorful, and abundant diogenid hermit crabs. The Diogenidae is the second largest of the seven families that comprise the Paguroidea, the hermit crabs, with 19 genera currently recognized (McLaughlin 2003). Diogenids are most diverse in shallow tropical waters and most medium- to large-sized hermit crabs on coral reefs are diogenids. All known *Calcinus* species are tropical or subtropical, most live on coral reefs, and several are facultative coral associates, frequently encountered within branching corals. The genus is circumtropical, with 41 described species (Table 1): 33 in the IWP and two to four in each of the other tropical regions (EA, WA, EP). There is substantial variation in ranges among IWP species, with some extending from East Africa to Hawaii, whereas others are known from single islands or archipelagos (Table 1).

Calcinus species are most readily identified from, and a few can only be reliably differentiated based on, their color pattern (e.g., Poupin and McLaughlin 1998; Poupin and Lemaitre 2003). Partly because colors fade in preserved specimens, coloration has been underutilized in crustacean taxonomy in the past. However more effective field methods, including SCUBA, field photography, increased sampling, and appreciation of color differences, have substantially improved our knowledge of *Calcinus* in recent decades. Alpha taxonomy and geographic distributions are now comparatively well documented (Poupin 2003), making *Calcinus* an excellent focus for evolutionary and biogeographic study.

We constructed mitochondrial and nuclear gene phylogenies of *Calcinus* based on most described species in the genus, including samples from multiple locations spanning the known ranges of most widespread species. Sequence data provide evidence for substantial cryptic diversity in the genus. In some species, color pattern appears to have evolved so rapidly that sister species with strikingly different color patterns are only slightly or not genetically differentiated. Most young sister species pairs have allopatric distributions, indicating that allopatric speciation is the main or only mechanism for diversification. Isolation on remote island groups appears to be the most common cause of speciation. The diversity pattern of *Calcinus* is atypical in that it peaks in the central Pacific, a pattern driven by an apparent ecological preference of many species to oceanic habitats, great dispersal ability, and predominance of peripatric speciation.

Materials and Methods

SPECIMENS

We sampled 37 of the 43 nominal species of *Calcinus* and nine additional, undescribed phylogenetic species recognizable on the basis of sequence data (Table 1). The species not sequenced are *Calcinus urabaensis*, known from a single specimen in Colombia, *Calcinus kurozumii*, known only from a single collection on Pagan Island (Marianas), *C. tropidomanus*, known from a single collection in Somalia, and *C. sirius* from Australia. We also did not sample *"Calcinus" paradoxus*, a species based on a single specimen collected in much deeper (500 m) water than any other *Calcinus*, whose generic assignment even its author questioned (Bouvier 1922); nor the dwarf species *C. revi*, suspected to be the juvenile of more common *Calcinus* species (J. Poupin, pers. comm.). Much of the material was collected by reef walking, snorkeling, or scuba-diving, fixed in 75–95% ethanol, and deposited in the Invertebrate Collections of the Florida Museum of Natural History, University of Florida (UF; Table 1). Additional specimens were borrowed from other institutions (Table 1). Whenever possible living animals were photographed to record color pattern. We identified specimens using Poupin's (2003) interactive taxonomic key, the primary taxonomic literature, and in consultation with J. Poupin and P. McLaughlin. Data on geographic ranges and ecology of species were compiled from the

Table 1. Known species of *Calcinus* and new ESUs, including their geographic ranges and accession information for all the sequenced specimens (including outgroup specimens).

ESU count	Species	Reported geographic range			Specimen info		Museum Catalog no.	COI seq	16S seq	H3 seq
		Region	W	E	Specimen provenance	Specimen no.				
1	*Calcinus albengai* Poupin and Lemaitre 2003—deep morph	IWP	Australs	Australs	Australs	H92	MNHN Pg.6378	+	+	+
2	*Calcinus albengai* aff. Poupin and Lemaitre 2003—shallow morph	IWP	Australs	Australs	Australs	H94	MNHN Pg.6385	+	+	+
3	*Calcinus anani* Poupin and McLaughlin 1998	IWP	Japan	Tuamotus; Marquesas	Bismarck Arch. (PNG)	H77	UF 4808	+	+	+
					Marquesas	H95	MNHN Pg.6357	+	+	+
					Bismarck Arch. (PNG)	H324	UF 11740	+		
4	*Calcinus argus* Wooster 1984	IWP	Mascarenes	Hawaii	Mascarenes	H62	UF 5437	+		
					Mascarenes	H114	UF 5446	+		
					N Marianas	H96b	UF 5714	+		
					Hawaii	H146	UF 7364	+	+	+
					Hawaii	H192	UF 8038	+		
					New Caledonia	H203	MNHN	+		
					Mascarenes	H312	UF 12814	+		
					Mascarenes	H313	UF 13022	+	+	+
5	*Calcinus dapsiles* Morgan 1989	IWP	W Australia	W Australia	W Australia	H101	UF 6297	+	+	+
					W Australia	H133	UF 6297	+	+	+
6	*Calcinus elegans* (H. Milne Edwards 1836)	IWP	S Africa	Tuamotus; Marquesas	Marianas	H5	UF 325	+	+	+
					Mascarenes	H67	UF 5504	+		
					Tuamotus	IP31	UF 1351	+	+	+
					Lines	H306	UF 11487	+		
					Lines	H317	UF 11204	+		
7	*Calcinus elegans* aff.—Hawaii	IWP	Hawaii	Hawaii	Hawaii	H15	UF 3216	+	+	+
					Hawaii	H113	UF 8350	+	+	+
					NW Hawaiian Ids.	H292	UF 12060	+		
					NW Hawaiian Ids.	H293	UF 12064	+		
					NW Hawaiian Ids.	H294	UF 12064	+		
					NW Hawaiian Ids.	H304	UF 12068	+		
					Hawaii	H307	UF 14838	+		
8	*Calcinus gaimardii* (H. Milne Edwards 1848)	IWP	Maldives	Fiji	Palau	H42	UF 3924	+	+	+
					Philippines	H136	UF 6744	+	+	+

Continued.

Table 1. Continued.

ESU count	Species	Reported geographic range			Specimen info		Museum Catalog no.	COI seq	16S seq	H3 seq
		Region	W	E	Specimen provenance	Specimen no.				
9	*Calcinus gouti* Poupin 1997	IWP	Lines	Tuamotus	Tuamotus	H25	UF 1349	+		
					Tuamotus	IP5	UF 1863	+	+	+
					Lines	H190	UF 8604	+	+	+
10	*Calcinus guamensis* Wooster 1984	IWP	Somalia	Hawaii; Marquesas	Samoa	H23	UF 3224	+		
					Hawaii	H60b	UF 3219	+		
					Marquesas	H49	UF 5171	+	+	+
					Societies	IP44	UF 1888	+		
					Mascarenes	H58	UF 5418	+	+	+
					Hawaii	H142	UF 3219	+		
11	*Calcinus haigae* Wooster 1984	IWP	Red Sea	Hawaii; Tuamotus	Samoa	H41	UF 3225	+		
					Marianas	H83	UF 5713	+	+	+
					Tuamotus	H82	UF 1744	+		
					Tuamotus	H120	UF 1332	+	+	+
					Tuamotus	H232	UF 9270	+	+	+
					Lines	H175	UF 8372	+		
					Hawaii	H139	UF 8035	+		
					Hawaii	H230	UF 8035	+	+	+
					Lines	H176	UF 8379	+		
					Tuamotus	H231	UF 9269	+		
12	*Calcinus hakahau* Poupin and Mclaughlin 1998	IWP	Marquesas	Marquesas	Marquesas	H51	UF 5175	+	+	+
					Marquesas	H117	UF 5166	+	+	+
13	*Calcinus hazletti* Haig and McLaughlin 1984	IWP	Hawaii	Hawaii	Hawaii	H119	UF 8349	+	+	+
					NW Hawaiian Ids.	H295	UF 12157	+	+	+
					NW Hawaiian Ids.	H302	UF 12158	+		
14	*Calcinus hazletti* aff.—Northern Marianas	IWP	Japan?/N Marianas	N Marianas	N Marianas	H79	UF 5732	+	+	+
					N Marianas	H90	UF 5728	+	+	+
					Wake Atoll	H191	UF 8438	+		
15	*Calcinus imperialis* Whitelegge 1901	IWP	E Australia	Easter Is.	Easter Is.	H38	UF 3646	+	+	+
16	*Calcinus inconspicuus* Morgan 1991	IWP	E Australia	New Caledonia	New Caledonia	H107	MNHN	+	+	+

Continued.

Table 1. Continued.

ESU count	Species	Reported geographic range			Specimen info		Museum Catalog no.	COI seq	16S seq	H3 seq
		Region	W	E	Specimen provenance	Specimen no.				
17	*Calcinus isabellae* Poupin 1997	IWP	Marianas	Hawaii; Pitcairn	Marianas	IP42	UF 732	+	+	+
					Tuamotus	IP20	UF 1758	+		
					Lines	H174	UF 8371	+		
					Wake Atoll	H199	UF 8449	+	+	+
					Cooks	H228	UF 10354	+		
18	*Calcinus kurozumii* Asakura and Tachikawa 2000	IWP	N Marianas	N Marianas						
19	*Calcinus laevimanus* Randall 1840	IWP	S Africa	Hawaii; Tuamotus	Hawaii	H75	UF 3221	+		
					Mascarenes	H66	UF 5426	+	+	+
					Marianas	IP28	UF 601	+		
					Tuamotus	H76	UF 1720	+		
					Hawaii	H13	UF 3221	+		
					Wake Atoll	H198	UF 8445	+	+	+
20	*Calcinus latens* Randall 1840	IWP	Mozambique; Yemen	Tuamotus	Marianas	IP39	UF 460	+		
					Mascarenes	H316	UF 12564			
					Mascarenes	H110	UF 5450	+	+	+
					Tuamotus	IP7	UF 1712	+		
					Tuamotus	IP9	UF 1712	+		
					Lines	H322	UF 10805	+		
					Cooks	H297	UF 10339	+		
					Wake Atoll	H320	UF 8440	+		
					Lines	H308	UF 10686	+		
					Cooks	H298	UF 10339	+	+	+
					Wake Atoll	H321	UF 8440	+		
21	*Calcinus latens* aff.—Hawaii	IWP	Hawaii	Hawaii	Hawaii	H16	UF 3217	+	+	+
					Hawaii	H109	UF 3217	+	+	+
					NW Hawaiian Ids.	H299	UF 12066	+		
					NW Hawaiian Ids.	H300	UF 12066	+		
22	*Calcinus latens* aff.—Oman	IWP	Oman	Oman	Oman	H81	UF 5428	+	+	+
					Oman	H314	UF 5416	+	+	+
23	*Calcinus laurentae* Haig and McLaughlin 1984	IWP	Hawaii	Hawaii	Hawaii	H39	UF 3625	+	+	+
					NW Hawaiian Ids.	H291	UF 12059	+	+	+
					NW Hawaiian Ids.	H303	UF 12278	+		

Continued.

Table 1. Continued.

ESU count	Species	Reported geographic range			Specimen info		Museum Catalog no.	COI seq	16S seq	H3 seq
		Region	W	E	Specimen provenance	Specimen no.				
24	*Calcinus lineapropodus* Morgan and Forest 1991	IWP	Cocos Keeling	Tuamotus	Samoa	H84	UF3255	+		
					Marianas	IP19	UF 1322	+	+	+
					Ryukyus	H137	UF 6990	+	+	+
					Lines	H177	UF 8600	+		
25	*Calcinus minutus* Buitendijk 1937	IWP	Cocos Keeling	Samoa	Samoa	H86	UF3263	+	+	+
					Marianas	IP32	UF 1321	+		
					Philippines	H140	UF 6511	+		
					Ryukyus	H149	UF 6982	+	+	+
26	*Calcinus morgani* Rahayu and Forest 1999	IWP	S Africa	Tuamotus	Samoa	H27	UF 3236	+	+	+
					Societies	IP33	UF 1350	+		
					Marianas	IP43	UF 652	+	+	+
					Palau	H130	UF 3992	+	+	+
					Ryukyus	H145	UF 6995	+	+	+
					Ryukyus	H147	UF 7237	+		
27	*Calcinus nitidus* Heller 1865	IWP	Society	Tuamotus	Societies	H26	UF 1334	+	+	+
					Societies	H121	UF 1334	+		
					Societies	H129	UF 6886	+		
					Tuamotus	IP2	UF 1347	+	+	+
28	*Calcinus orchidae* Poupin 1997	IWP	Marquesas	Marquesas	Marquesas	H50	UF 5177	+	+	+
29	*Calcinus pascuensis* Haig 1974	IWP	Easter Is.	Easter Is.	Easter Is.	H37	UF 3648	+	+	+
30	*Calcinus pulcher* Forest 1958	IWP	Seychelles	New Caledonia	Palau	H40	UF 3890	+	+	+
					Mascarenes	H59	UF 5430	+	+	+
					Philippines	H135	UF 8357	+	+	+
					Micronesia	H193	UF 5396	+		
					Philippines	H194	UF 6531	+	+	+
					Papua New Guinea (Milne Bay)	H148	UF 5553	+	+	+
31	*Calcinus pulcher* aff.—Mascarenes	IWP	Mascarenes	Mascarenes	Mascarenes	H309	UF 12741	+		
					Mascarenes	H144	UF 5430	+	+	+
32	*Calcinus revi* Poupin and McLaughlin 1998	IWP	Japan	Tuamotus	Tuamotus					
33	*Calcinus rosaceus* Heller 1861	IWP	Red Sea	Gulf of Oman; Mauritius	Oman	H63	UF 5427	+	+	+
					Oman	H118b	UF 5435	+	+	+
					Mascarenes	H310	UF 12781	+		
					Mascarenes	H305	UF 12635	+	+	+

Continued.

Table 1. Continued.

ESU count	Species	Reported geographic range			Specimen info		Museum Catalog no.	COI seq	16S seq	H3 seq
		Region	W	E	Specimen provenance	Specimen no.				
34	*Calcinus seurati* Forest 1951	IWP	Somalia	Hawaii; Tuamotus	Marianas	IP36	UF 562	+	+	+
					Hawaii	H14	UF3223	+	+	+
35	*Calcinus sirius* Morgan 1991	IWP	W Australia	E Australia						
36	*Calcinus sirius* aff. Poupin 1997	IWP	Australs	Australs	Australs	H93b	MNHN Pg.6395	+	+	+
37	*Calcinus spicatus* Forest 1951	IWP	E Australia	Pitcairn Is.	New Caledonia	H106	MNHN	+	+	+
					Cooks	H229	UF 10337	+	+	+
38	*Calcinus tropidomanus* Lewinsohn 1981	IWP	Somalia	Somalia						
39	*Calcinus vachoni* Forest 1958	IWP	Mascarenes	Easter Is.	N Marianas	H88	UF 5742	+	+	+
					Ryukyus	H131	UF 6992	+		
					Philippines	H132	UF 6748	+	+	+
40	*Calcinus vachoni* aff.—Cook Islands	IWP	Cooks	Cooks	Cooks	H47	UF 1377	+	+	+
					Cooks	H301	UF 11702	+		
41	*Calcinus vachoni* aff.—Réunion	IWP	Mascarenes	Mascarenes	Mascarenes	H311	UF 12634	+	+	+
					Mascarenes	H318	UF 13011	+		
					Mascarenes	H319	UF 13011	+	+	+
42	*Calcinus vanninii* Gherardi and McLaughlin 1994	IWP	Mascarenes	Mauritius	Mascarenes	H80	UF 5425	+	+	+
					Mascarenes	H87	UF 5412	+	+	+
43	*Calcinus californiensis* Bouvier 1898	EP	Baja California	El Salvador	Baja CA Sur	H98	UF 8367	+	+	+
					Baja CA Sur	H99	UF 15221	+	+	+
44	*Calcinus explorator* Boone 1930	EP	Gulf of CA	Galapagos	Clipperton Atoll	H179	MNHN Pg.7617	+		
					Clipperton Atoll	H204	MNHN	+	+	+
45	*Calcinus obscurus* Stimpson 1859	EP	El Salvador	Ecuador; Colombia	Panama	H105	UF 8359	+	+	+
					Panama	H111	UF 8359	+	+	+
46	*Calcinus mclaughlinae* Poupin 2006	EP	Clipperton Atoll	Clipperton Atoll	Clipperton Atoll	H178	MNHN Pg.7622	+	+	+
47	*Calcinus tibicen* Herbst 1791	WA	Belize	Ubatuba Brazil	Florida	H102	UF 8363	+	+	+
					Florida	H103	UF 8364	+		
					Tobago	H124	UF 8358	+	+	+
48	*Calcinus urabaensis* Campos and Lemaitre 1994	WA	Colombia	Colombia						
49	*Calcinus verrilli* Rathbun 1901	WA	Bermuda	Bermuda	Bermuda	H138	UF 8365	+	+	+
50	*Calcinus paradoxus* Bouvier 1922	EA	Azores	Azores						
51	*Calcinus talismani* A. Milne Edwards and Bouvier 1892	EA	Cape Verde	Guinea	Cape Verde	H206	MNHN		+	+

Continued.

Table 1. Continued.

ESU count	Species	Reported geographic range			Specimen info		Museum	COI	16S	H3
		Region	W	E	Specimen provenance	Specimen no.	Catalog no.	seq	seq	seq
52	*Calcinus tubularis* Linnaeus 1767	EA	Ascension Is.; Madeira	Lebanon	Madeira	H91	UF 8361	+	+	+
					Madeira	H97	UF 8361	+	+	+
Outgroups										
	Ciliopagurus strigatus Herbst 1804				Marianas	IP21	UF 1871	+	+	+
	Ciliopagurus tricolor Forest 1995				Mascarenes	H68	UF 5433	+	+	+
	Ciliopagurus galzini Poupin and Malay 2009				Tuamotus	H32	UF 1742	+	+	+
	Dardanus lagopodes Forskal 1775				Marianas	IP15	UF 326	+	+	+
					Tuamotus	IP18	UF 1760	+	+	+
	Dardanus sanguinocarpus Degener 1925				Hawaii	H45	UF 3507	+	+	+
	Dardanus longior Asakura 2006				Marquesas	H56	UF 3639	+	+	+

taxonomic literature, Poupin's (2003) website on the genus, the UF specimen database, and the authors' field observations (see Supporting Information). The diogenid hermit crabs *Ciliopagurus* (e.g., *C. strigatus*, *C. tricolor*, and *C. galzini*) and *Dardanus* (e.g., *D. lagopodes*, *D. sanguinocarpus*, and *D. longior*) were chosen as the closest outgroup taxa based on a phylogenetic analysis (not shown) of a larger set of hermit crab taxa (including nine diogenid, four pagurid, and two coenobitid genera).

Samples for sequencing were selected to span as much of the geographic range of each species as available material permitted (Table 1, Figs. 3–14). We collected DNA sequence data from 150 operational taxonomic units (OTUs). All but one (*C. talismani*) of the 150 OTUs were sequenced for the cytochrome oxidase I (COI) mitochondrial gene fragment. We generated phylogenetic trees for the COI-only dataset, and on the basis of these trees we selected a subset of 96 OTUs for further sequencing of 16S rDNA and Histone 3 (H3) genes. The 96-OTU subset was comprised of only the two genetically most divergent individuals in each species or genetically distinct putative new species. Thus, the full 150-OTU taxon set was used for constructing the COI-only tree whereas a "pruned" subset of 96 OTUs was used for constructing individual gene trees and a concatenated three-gene tree. The ILD test for data combinability (see below) was also performed on the 96-OTU subset. Lastly, molecular clock analyses (see below) were performed on a further reduced 50-OTU taxon subset, to keep computations manageable.

MOLECULAR METHODS

DNA was extracted from muscle tissue using DNAzol and proteinase K following the protocol given in Meyer 2003. Sequence data were collected for two mitochondrial DNA markers (COI and 16S) and one nuclear marker (H3). Average length of the amplified fragments and PCR primers used are as follows: COI: ~645 base pairs (bp), primers dgLCO (5′-GGT CAA CAA ATC ATA AAG AYA TYG G-3′) and dgHCO (5′-TAA ACT TCA GGG TGA CCA AAR AAY CA-3′; Meyer 2003). 16S: ~550 bp, primers 16SAR (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16SBR (5′-GCC GGT CTG AAC TCA GAT CAC GT-3′; Palumbi 1996). H3: ~350 bp, primers H3af (5′-ATG GCT CGT ACC AAG CAG ACV GC-3′) and H3ar (5′-ATA TCC TTR GGC ATR ATR GTG AC-3′; Colgan et al. 1998). PCR thermocycler profiles for COI and 16S were as in Meyer (2003), whereas the PCR profile for H3 followed Perez-Losada et al. (2004). PCR products were either (1) cleaned using Wizard PCR Preps (Promega, Madison, WI) and sequenced using the ABI Big Dye protocol and a Perkin-Elmer ABI Automated Sequencer (Perkin-Elmer Applied Biosystems Inc., Foster City, CA); or (2) cleaned using the exo-sap cleanup protocol and sequenced at the high-throughput sequencing facility of the University of Florida's Interdisciplinary Center for Biotechnology research (ICBR) in a 96-well format using

BigDyeTerminator (Applied Biosystems, Foster City, CA) cycle sequencing reactions, employing an ABI-3730-XL for electrophoresis. Initially, mitochondrial DNA sequencing was done along both directions of a DNA fragment, and as our confidence in base calls increased in later stages, only one strand was sequenced (unless base ambiguities were noted, in which case the second direction was sequenced). Histone 3 sequencing was always done on both directions.

SEQUENCE ANALYSIS

Chromatograms of the sequences were manually checked and edited using the software Sequencher version 4.2 (Gene Codes Corporation, Ann Arbor, MI). Sequence alignment was done by eye using Se-Al version 2.0a11 (Rambaut, http://tree.bio.ed.ac.uk/software/seal/). Sequences are available in GenBank (accession nos. FJ620149-FJ620493, EF683559-EF683561) and aligned sequences are available from the authors. We also included COI data from GenBank for *Calcinus obscurus* (AF436039). In all analyses, all sites were weighted equally, characters were unordered, and gaps were treated as missing data.

We used two approaches to decide whether to pool the three-gene fragments into a single analysis. First, we used the incongruence length difference (ILD) test (Farris et al. 1995), a parsimony-based statistical test of data combinability commonly employed in phylogenetic studies. We used PAUP* version 4.0b10 (Swofford 2002) to perform the ILD test simultaneously for the three data partitions. No significant incongruences were noted among the three gene trees. However, the usefulness of the ILD test for evaluating data combinability has been called into question (e.g., Yoder et al. 2001; Barker and Lutzoni 2002). To address these concerns and to explore our data further, we also visually compared Bayesian tree topologies resulting from independent searches for each of the three gene regions. The gene trees were not in conflict with each other or with the three-gene concatenated analysis (data not shown). Based on this evidence, we decided that a combined analysis was appropriate.

We determined the simplest model of evolution that best fit our COI-only dataset as well as our three-gene dataset using the Akaike Information Criterion (AIC) as implemented by the program Modeltest 3.6 (Posada and Crandall 1998). Phylogenetic relationships were estimated using maximum likelihood (ML), maximum parsimony (MP), and Bayesian statistics (BS). Parsimony analyses were done using PAUP, ML analyses were implemented using both PAUP and GARLI version 0.951–1 (Zwickl 2006, http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html), whereas Bayesian analyses were implemented using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). In the MP and ML analyses using PAUP, heuristic searches started with random addition of taxa replicated 10 times using the tree-bisection-reconnection (TBR) branch-swapping algorithm. Branch support in the MP analyses was estimated by bootstrap support values, calculated as above with 1000 (for the three-gene tree) or 200 (for the COI tree) replicates. ML branch support values were not calculated using PAUP due to computational constraints. In the ML analyses using GARLI, we used random starting trees and performed—five to seven independent runs to obtain the best tree. Branch support values were estimated in GARLI using 2200 and 1300 bootstrap replicates for the COI-only and three-gene datasets, respectively. In the Bayesian analyses, we ran two independent chains for 1 million generations each; each chain was sampled every 100 generations. The MCMC runs reached stationarity in 60k generations or less. We discarded the initial 25% of the trees as the burn-in phase. Bayesian posterior probabilities were calculated based on the remaining 75% of the trees.

We calculated pairwise COI genetic distances for each sister species pair identified in our phylogenetic trees using PAUP. We used Kimura's (1980) K2P distance metric to facilitate comparison with earlier studies.

ANALYSIS OF SPECIATION AND BIOGEOGRAPHY

Our analysis of speciation patterns focuses on described species as well as previously undescribed, but genetically distinct evolutionary significant units (ESUs; sensu Moritz 1994). ESUs are defined as reciprocally monophyletic populations for the locus investigated (here 16S and COI mtDNA; H3 was not considered due to low levels of interspecific divergence), that have at least one other independent, defining attribute such as distinct color pattern, structural morphology, distribution, or reciprocal monophyly in another, independent marker. ESUs satisfy the phylogenetic species concept, and are clades with an evolutionary history separate from other ESUs. Some ESUs are as morphologically and genetically distinctive as described species; conversely a few described species are not reciprocally monophyletic in mtDNA (see below). ESUs are thus species-level units which, unlike biological species, can be defined in allopatric as well as in sympatric settings without experimental tests of interbreeding.

We call the divergence of ESUs from each other evolutionary significant events (ESEs). ESEs are to speciation what ESUs are to species: they are objectively defined diversification events that give rise to ESUs. To quantify the relative importance of different modes of diversification, we enumerated all identifiable ESEs that have given rise to at least one individual ESU (or described species). That is, we considered ESEs that have given rise to either two separate ESUs, or led to the separation of one ESU from a clade that subsequently further diversified.

Species occurrence records were mapped in ArcGIS, and species ranges inferred by drawing a polygon around bordering record points. Species were considered allopatric when they had separate ranges; such ranges may end on adjacent islands, but are

Table 2. List of ESUs used in biogeographic analyses, and their geographic distributions relative to each other.

Clade	ESU pair	Distribution
I	*C. verrilli–C. tubularis*	allopatric
II	*C. latens–C.* aff. *latens* Hawaii	allopatric
II	*C. latens–C.* aff. *latens* Oman	allopatric
III	*C. hazletti–C.* aff. *hazletti* N Marianas	allopatric
III	*C. minutus–C. rosaceus*	allopatric
III	*C. minutus–C. nitidus*	allopatric
III	*C. haigae–C. minutus/C. rosaceus/ C. nitidus*	sympatric
III	*C. inconspicuus*–rest of clade III	parapatric
IV	*C. vachoni–C.* aff. *vachoni* Cooks	allopatric
IV	*C. vachoni–C.* aff. *vachoni* Mascarenes	allopatric
V	*C. spicatus–C. pascuensis*	allopatric
VI	*C. mclaughlinae–C. obscurus*	allopatric
VI	*C. californiensis–C. mclaughlinae/ C. obscurus*	parapatric
VI	*C. tibicen–C. talismani*	allopatric
VI	*C. explorator–C. tibicen/C. talismani*	allopatric
VII	*C. gaimardii–C. morgani*	sympatric
VII	*C. elegans–C.* aff. *elegans* Hawaii	allopatric
VII	*C. imperialis–C. vanninii*	allopatric
VII	*C. isabellae–C. imperialis/C. vanninii*	parapatric
VIII	*C. laevimanus–C. seurati*	sympatric (depth-separated)
IX	*C. pulcher–C.* aff. *pulcher* Mascarenes	allopatric
IX	*C. hakahau–C. gouti*	allopatric
IX	*C. laurentae–C. hakahau/C. gouti*	allopatric
IX	*C. lineapropodus*–rest of clade IX	sympatric
X	*C. albengai–C.* aff. *albengai deep*	sympatric (depth-separated)
X	*C. dapsiles–C. albengai* complex	allopatric
X	*C. argus–C.* aff. *sirius*	allopatric
X	*C. anani–C. argus/C. sirius*	sympatric

separated by an open ocean. Species ranges that truly abut, or overlap for $<10\%$ of the range of the sister taxon with the smaller distribution, were termed parapatric. Table 2 lists the ESUs used in the biogeographic analyses.

Diversity contour maps were generated from this data by superimposing the inferred distributional range of each species. For each species inferred species ranges were represented by a polygon as described above. These ranges were superimposed on each other to give the total number of species inferred to occur at any locality. Iso-diversity contour lines were then drawn around areas within which a given number of species are expected to occur based on the stacked species ranges. Such diversity contour maps can be biased in that (1) diversity in interior areas can be overestimated when species are actually absent from there but inferred to occur because of peripheral records, and (2) lack of sampling of marginal occurrence will lead to an underestimation of marginal range, but lack of sampling of central occurrence will not lead to an underestimation of central range. As a second method for estimating local diversity, we also assembled species lists for relatively well-studied areas and have indicated the number of species known from these on the contour maps. The latter method is prone to the biases of geographically varied sampling methods and efforts.

MOLECULAR CLOCK ANALYSIS

We used BEAST 1.4.8 (Drummond and Rambaut 2007) to estimate divergence times of *Calcinus* sister taxa. We did a partitioned analysis for all three genes (3-nucleotide codon partitions for COI and H3 and 1 partition for 16S) using an uncorrelated, log-normal, relaxed clock. For each partition, we specified a GTR + I + G model of sequence evolution. We estimated the time to most recent common ancestor (TMRCA) of each pair of sister species using a Yule tree prior, a UPGMA starting tree, and two independent runs of 1×10^7 generations each. Posterior distributions were sampled every 1000 generations after removing the first 10% of the MCMC chain as the burn-in. Convergence of the results was checked by loading the posterior distributions into the program Tracer. The fossil record in this group is too poor for fossil-based calibration. Instead the analysis was calibrated by specifying a prior on the divergence date of the transisthmian species pair *Calcinus tibicen* and *C. explorator*. The timing of vicariance of transisthmian sister species varies substantially among taxa, with many falling around 3.1 my (Coates and Obando 1996), but others are older (cf. Knowlton and Weigt 1998; Lessios 2008). As a preliminary approximation, we set a prior with a lognormal distribution with a mean of 1.21352716 and standard deviation of 0.28012786. This approximates a normal distribution with a mean of 3.5 my and a standard deviation of 1.0 (a normal distribution was not used because a transisthmian divergence time of zero would have had a positive probability, which is unrealistic; A. J. Drummond, pers. comm.).

Results

SEQUENCE ATTRIBUTES

The COI region sequenced was 609 base pairs (bp) long, with 368 invariable and 238 parsimony-informative sites. Mean base frequencies were: 0.25A, 0.17C, 0.23G, 0.35T, showing an A–T bias of 60%. The 16S gene fragment contained some regions that could not be confidently aligned across all taxa. We tested the importance of these hypervariable regions by running separate analyses with and without them. The inclusion or exclusion of hypervariable regions did not result in substantial topological differences, thus they were included in the final analyses. The

16S gene fragment was 459 bp long, with 276 bp invariable and 125 bp parsimony-informative sites, and mean base frequencies of 0.32A, 0.18C, 0.13G, 0.36T (A–T bias 68%). The H3 gene fragment was 336 bp long, with 279 bp invariable and 53 bp parsimony informative sites, and mean base frequencies of 0.19A, 0.34C, 0.28G, 0.19T (A–T bias 38%). The best-fit models were GTR + I + G for COI, 16S, and the combined three-gene set, and GTR-I for H3. We observed 27 insertions and deletions (indels) in the 16S gene fragment whereas COI and H3 had no indels.

PHYLOGENY RECONSTRUCTION AND SPECIES BOUNDARIES

The three methods of phylogenetic analyses used (MP, ML, and BS) gave congruent results, and the topologies generated from the three-gene and COI-only datasets were likewise congruent (Fig. 1A and B). Bootstrap values were higher in the three-gene trees (particularly at the deeper nodes), as expected. We thus used the three-gene trees to identify supra-specific clades within *Calcinus*. Ten strongly supported clades were identifiable within the genus. We defined strong phylogenetic support as >70% bootstrap values in the MP and ML trees and >95% posterior probabilities in the BS trees (see clades I–X in Fig. 1A; the sole exceptions to our criteria for defining clades were clade VII, which had a 62% bootstrap value for the ML analysis; and clade IV, which was supported by both ML and BS analysis, but had no bootstrap support under MP, nonetheless this grouping was recovered in all methods of analysis used). Relationships of ESUs within these clades were generally well resolved, but the relationships of the clades to each other was generally poorly resolved. Thus, these clades served as the basic units for our analyses of speciation patterns.

Because the COI analyses (Fig. 1B) covered more individuals from more geographic locations, these were used to delineate species and ESUs. Analyses revealed nine ESUs (22% of the sampled IWP fauna) that do not correspond to previously described species. Eight of these nine are allopatrically divergent populations of described species whereas one is codistributed with its sister-species but has a nonoverlapping depth range. Three described species were not reciprocally monophyletic: *Calcinus minutus, C. nitidus*, and *C. rosaceus* are interdigitated in a mostly unresolved species complex (Fig. 1A, B, Clade III). All other nominal species for which multiple individuals were sequenced were recovered as monophyletic units with high bootstrap/posterior probability support values. Thus most named species fulfilled the ESU criterion and phylogenetic species concept (Wheeler and Meier 2000).

Note that the EA species *C. talismani* is not represented in the COI-only phylogeny because we were unable to amplify this gene region from the available specimen. Nonetheless in 16S-only, two- and three-gene trees, *C. talismani* is recovered as sister to *C. tibicen*.

Excluding the *C. minutus* complex (see Discussion), intraspecific K2P distances ranged from 0% to 6% (1.3 ± 1.0%), with only one outlier with K2P > 4%. Pairwise, interspecific K2P distances within clades ranged from 4% to 25% (K2P) (Fig. 2A). Thus, there was no barcoding gap (Hebert et al. 2003; Meyer and Paulay 2005), but also little overlap between intraspecific and interspecific distances. Including the *C. minutus* complex creates a much larger overlap between intra- and interspecific differences (Fig. 2B).

Of 267 pairwise intraspecific K2P distance comparisons, 10% had values >2.7%. These were within *C. argus, C. pulcher s.s., C. haigae,* and *C. anani*. These species appear to exhibit substantial geographic structuring across their range : *C. argus* appears to have divergent populations in the Mascarenes and Hawaii (Fig. 1B, Clade X); *C. pulcher* has a distinct population in the Philippines (Fig. 1B, Clade IX); *C. haigae* shows divergence in the Tuamotus (except for 1 individual; Fig. 1B, Clade III); and *C. anani* from the Marquesas and Papua New Guinea appear genetically differentiated (Fig. 1B, Clade X). Interestingly, we observed distinct color morphs for a *C. anani* specimen from the Philippines (not sequenced) and for juvenile *C. haigae* from the Tuamotus (illustrated in Poupin 2003). However, the distinct groupings were not consistently supported across all methods of analysis and small sample sizes also limit our ability to further investigate differentiation within these species.

DISTRIBUTION OF *CALCINUS* SPECIES

Our surveys led to numerous new geographic records and substantial improvement in the documentation of the distribution of *Calcinus* species (see Supporting Information and http://www.flmnh.ufl.edu/scripts/dbs/malacol_pub.asp for source of records). Figures 3–14 show the presently known geographic range of each species.

DIVERSITY PATTERNS IN *CALCINUS*

The species richness of *Calcinus* is highest in the oceanic Pacific, and does not peak in the Indo-Malayan triangle (Fig. 15). Both projected and known diversity peak in the Mariana and Tuamotu Islands, in NW and SE Oceania. Sixteen species have been recorded from the Marianas and 15 from the Tuamotus. Diversity in the Indo-Malayan triangle is substantially lower, with eight species recorded from the Philippines, nine from Indonesia, and 10 from all of New Guinea. Only 12 species have been recorded from the entire Indo-Malayan archipelago, compared with 21 species from SE Polynesia.

EVOLUTIONARILY SIGNIFICANT EVENTS

Twenty-four ESEs were identified in the IWP and four in other regions. Six of the IWP ESEs separate sympatric sister taxa, others are geographically structured. Of the 22 geographically structured

ESEs, 20 separate allopatric sister taxa and two split parapatric sisters with narrow areas of distributional overlap. Sympatric sister taxa are generally separated by deeper genetic distances than allopatric or parapatric taxa (see the following paragraphs). All 21 pairs of allopatric or parapatric sister taxa appear to have adjacent ranges as far as current sampling can document.

Geographically structured ESEs span the globe, but cluster in some areas (Fig. 16); most fall in areas previously recognized as potentially important in speciation, as evidenced by a high proportion of endemics. Within the IWP, four ESEs separate ESUs in Hawaii. Three ESEs each separate ESUs between the tropical and subtropical S Pacific, and across the Indian Ocean

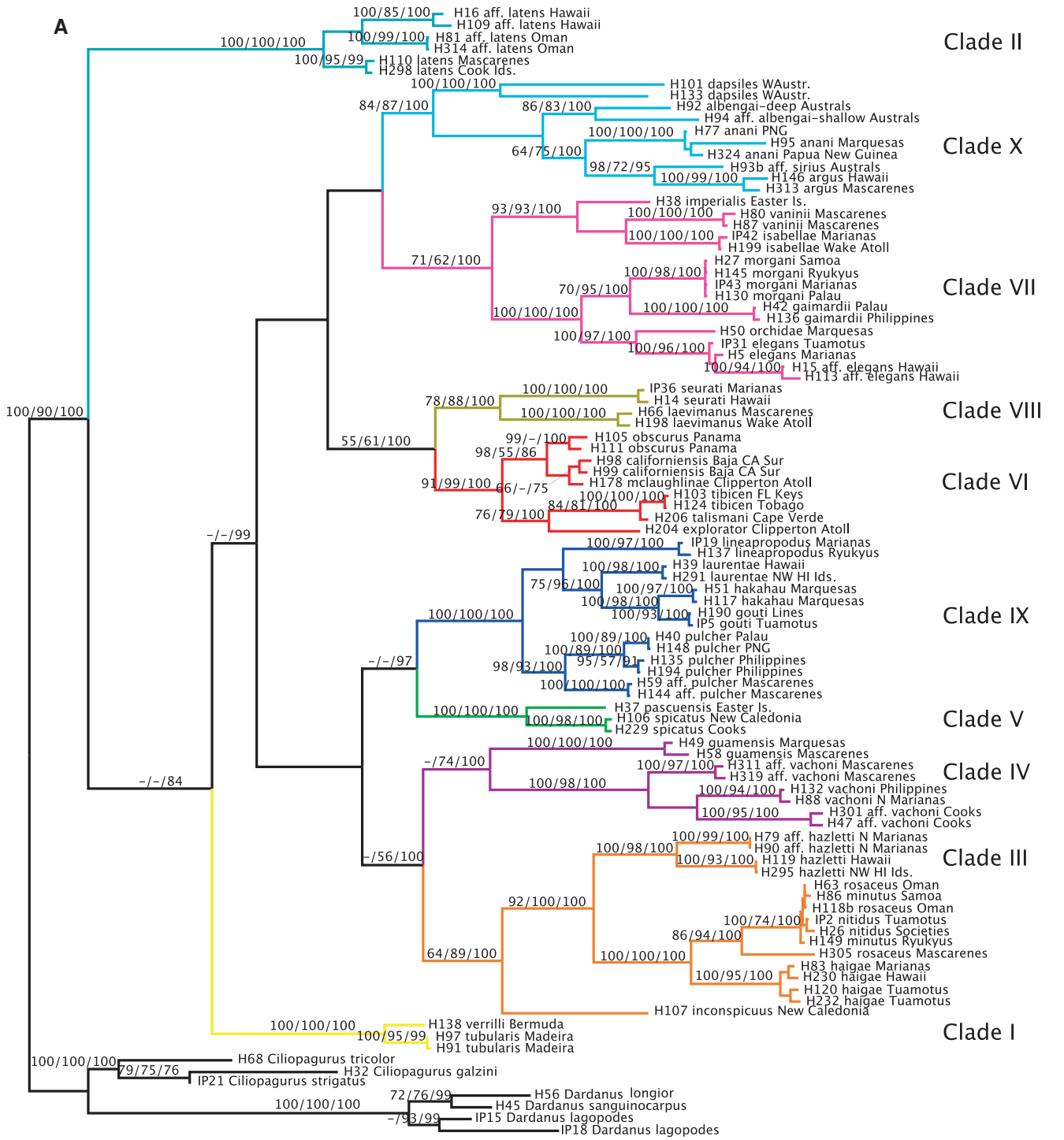


Figure 1. **Bayesian phylograms constructed using (A) three concatenated genes and (B) COI only. The values above the branches represent parsimony bootstraps, maximum likelihood bootstraps, Bayesian posterior probabilities, respectively.**

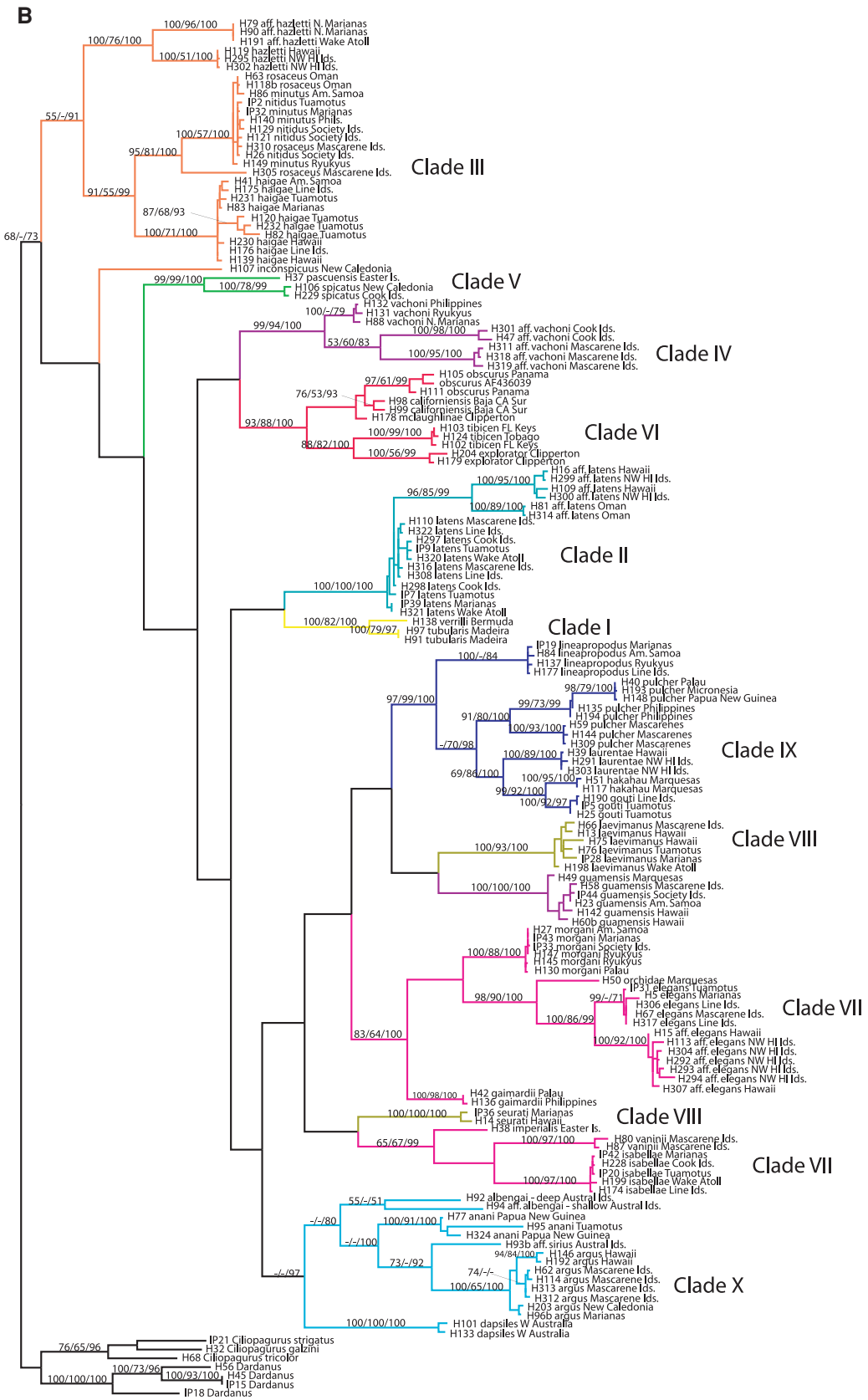


Figure 1. Continued.

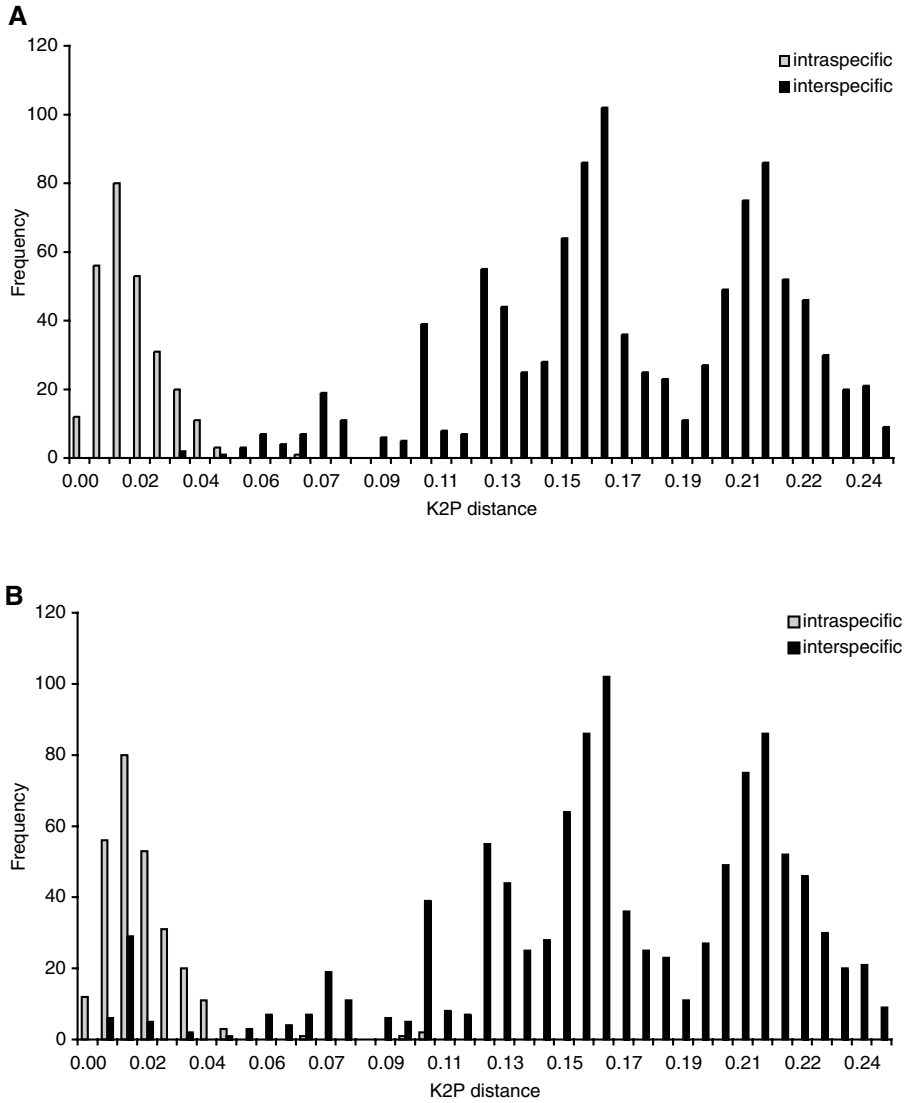


Figure 2. Frequency distribution of K2P distances for intraspecific variation and interspecific distances in *Calcinus*, without (A) and with (B) the *C. minutus* complex.

(although the exact locations of the latter separations are poorly constrained because of sparse sampling in the Indian Ocean). Two ESEs each separate ESUs in the Marquesas, SE Polynesia, and at subtropical latitudes across Australia. Single ESEs separate ESUs in Arabia and Easter Island (Fig. 16). Outside the IWP, two ESEs separate ESUs between adjacent regions (EP–WA, and Bermuda–EA), and two separate sister taxa within the EP: along the central American coast, and between Clipperton Island and the central American coast.

TIME TO ALLOPATRY

The molecular clock analyses showed that allopatrically distributed sister species pairs were significantly younger than sympatric sister species (allopatric sister species: mean = 2.0 my, range = 0.4–6.3 my; sympatric sister species: mean = 5.8 my, range = 2.2–10.2 my; Fig. 17; $P > 0.05$, *t*-test) and all young (<2.5 my) divergences were among allopatric sister taxa. There is considerable spread in TMRCAs, particularly for sympatric sister species pairs. There is no temporal gap dividing the ages of strictly allopatric species from sister species that have broadly overlapping geographic ranges (Fig. 17).

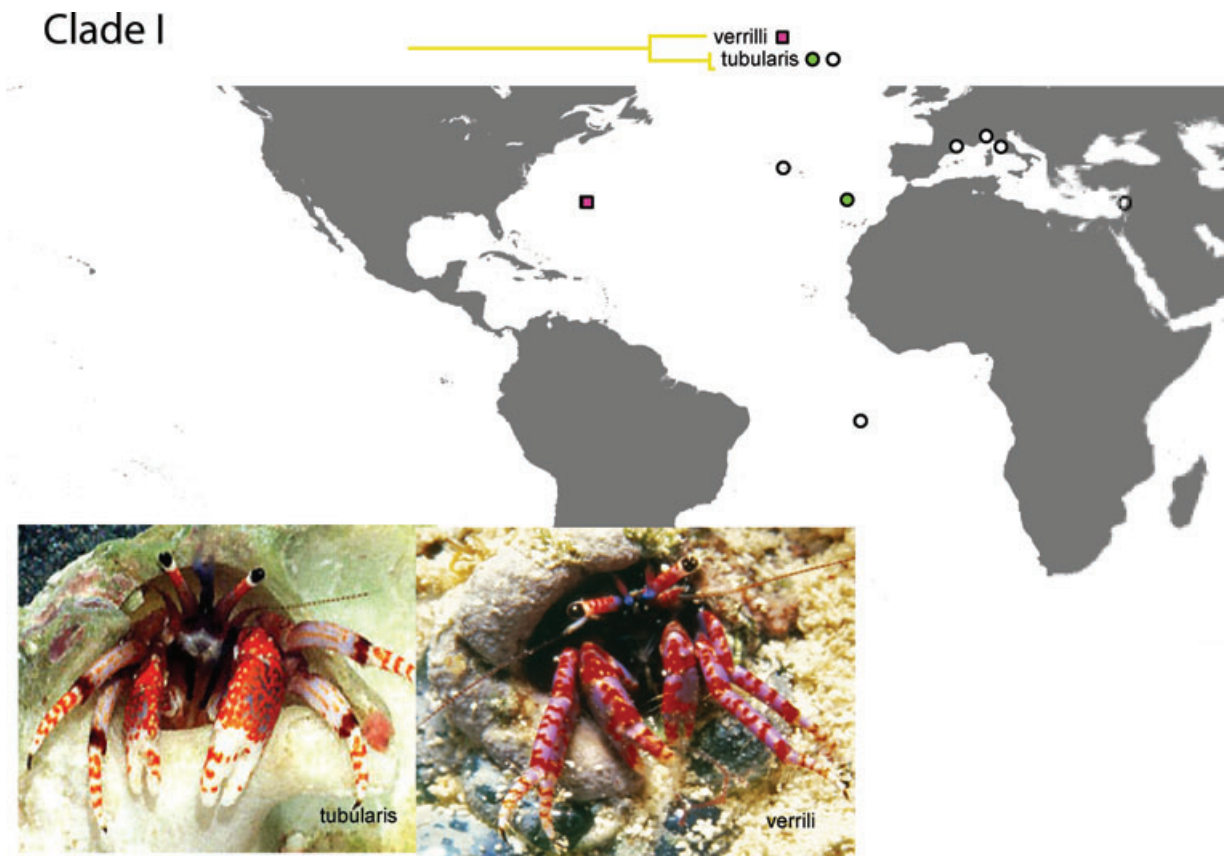
Discussion

SPECIES BOUNDARIES

Although there is a general correspondence between described species and genetically defined ESUs, 22% of the taxa examined were not concordant. Three described species were not reciprocally monophyletic whereas nine ESUs represent previously undescribed (and mostly unrecognized) forms.

Calcinus minutus, C. nitidus, and *C. rosaceus* failed to sort into monophyletic units (Fig. 1A, B, Clade III). Most specimens in this complex form a tight cluster (K2P $< 2\%$, with all species combinations represented at K2P $\leq 0.5\%$), except for one *C. rosaceus* from the Mascarene Islands (K2P $= 10\%$). These three species have allopatric, abutting ranges and are very similar morphologically; with *C. minutus* and *C. nitidus*, in particular, nearly impossible to distinguish except by color (Fig. 5; Morgan 1991; Poupin and McLaughlin 1998; Poupin 2003). Several factors can cause species-level nonmonophyly (Funk and Omland 2003). First, there may be insufficient differences in the marker used to differentiate species. We consider this unlikely because mitochondrial gene regions used cleanly resolve other *Calcinus* species. Second, ancestral polymorphisms may have been retained because of a slow rate of evolution or recent speciation. Although there is no evidence for a slow-down in the rate of evolution in this lineage, species divergence may have been so recent that ancestral haplotypes have not had sufficient time to sort into monophyletic clades. The virtual lack of morphological differentiation (other than color) between *C. nitidus* and *C. minutus* is suggestive of recent divergence. Third, mitochondrial haplotypes could have introgressed across species boundaries. The occurrence of a divergent sequence in one *C. rosaceus* specimen, sister to all others in the complex, suggests that introgression is a plausible explanation. Morphologically as well as in color pattern *C. rosaceus* is closest to *C. haigae*, the sister taxon to this complex (Fig. 5; Poupin 2003; Asakura and Tachikawa 2003). This suggests that the divergent sequence may represent the original *C. rosaceus* genotype, which has largely been replaced by a sweep of *C. minutus* haplotypes. Independent markers could provide a test of this hypothesis. The H3 nuclear sequences are not variable across this complex, and appear to lack the power to resolve this problem. Color patterns are likely under genetic, nuclear control, thus they represent separate "genetic" markers; however color may be under selection and could thus have evolved more rapidly than potentially neutral mitochondrial markers (see below). Future work with other markers is needed to resolve the status of these species.

In contrast, six previously described species show marked differentiation into two or three ESUs each. In two (*C. albengai* (Clade X, Fig. 14) and *C. elegans* (Clade VII, Fig. 9)), the differentiated ESUs are conspicuous and previously noted color



Figures 3–14. Distributions, color patterns, and COI phylogeny of Clade I *Calcinus* species. Colored symbols represent specimens available in the FLMNH collection; unfilled/black symbols represent records derived from the literature.

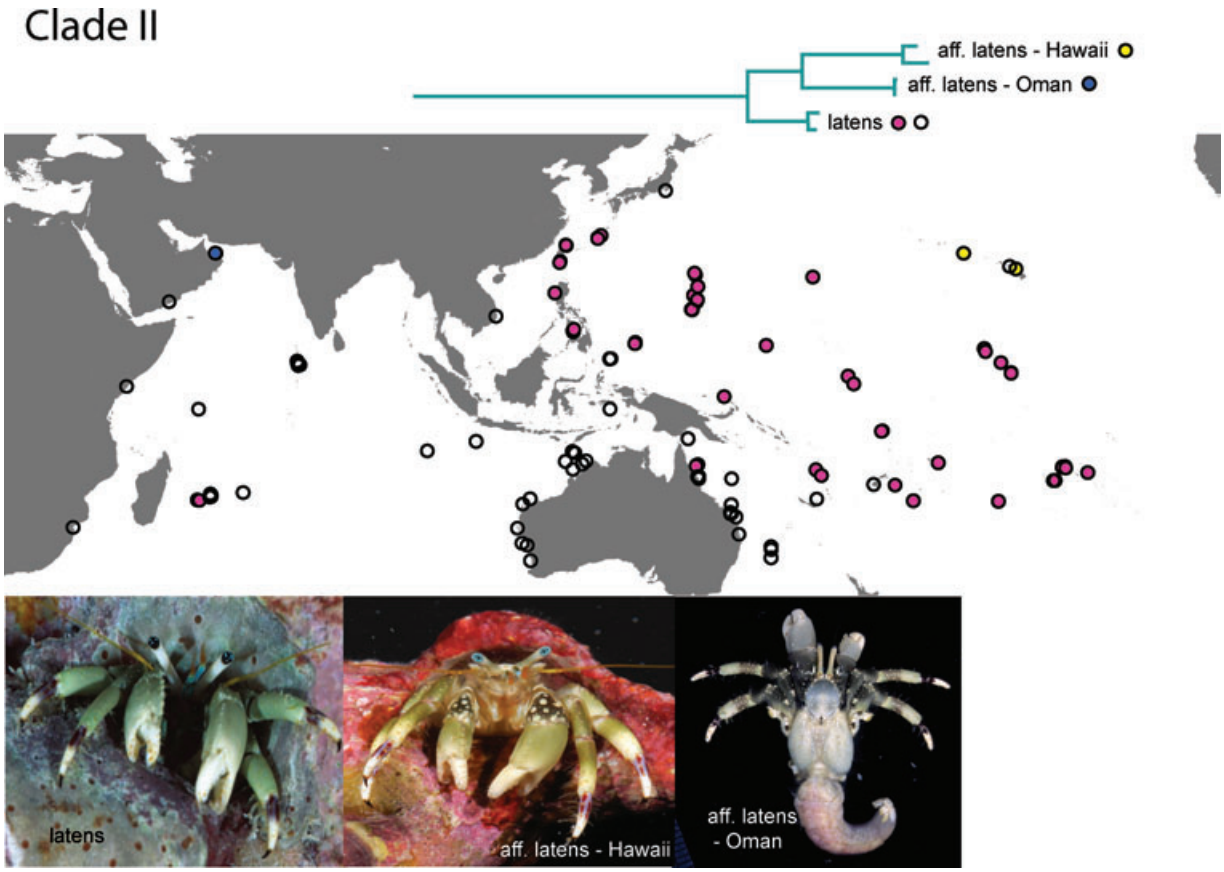


Figure 4. Distributions, color patterns, and COI phylogeny of Clade II *Calcinus* species. Symbols follow Fig. 3.

forms that have not been taxonomically recognized (Poupin and Lemaitre 2003; Haig and McLaughlin 1984). In the six others (*C. hazletti* (Clade III, Fig. 5), *C. vachoni* (Clade IV, Fig. 6), *C. latens* X2 (Clade II, Fig. 4), and *C. pulcher* (Clade IX, Fig. 13), no color differences were noted during collection, but are evident in four of the five for which live images were taken. Color differences could not be discerned only in photographs of *C. hazletti* ESUs in Micronesia and the Hawaiian Islands (although color polymorphism has been reported in this species in Japan; Asakura 2004). No images were available for the Cook Islands *C. vachoni* ESU.

Much of the incongruence between morphology-based species and genetic ESUs results from changing taxonomic traditions, and reflects a lack of systematic revision. Historically, carcinologists hesitated to describe species distinguished solely by a color pattern; thus, the strikingly distinctive Hawaiian color form of *C. elegans* (Fig. 9) has not been named (Haig and McLaughlin 1984). More recently, workers have tended to recognize such structurally similar color forms, such as the Marquesan endemic *C. hakahau* (Fig. 12), as distinct species (Poupin and McLaughlin 1998). A well-executed revision should rectify alternate species concepts currently in use.

Species boundaries can be defined based on a variety of criteria and characters (e.g., Wheeler and Meier 2000). When taxa are sympatric and co-occurring, species limits are usually straightforward, however species delimitations are more subjective for allopatric taxa not subjected to potential interbreeding. Genetics, color pattern, structural morphology, and/or geography can all inform taxonomic delineations. We defined ESUs as reciprocally monophyletic taxa in a genetic marker, which are also distinguishable by at least one additional independent character. Three described species (*C. minutus*, *C. nitidus*, and *C. rosaceus*) do not meet this definition, as they are not demonstrably reciprocally monophyletic with the genetic markers used. However these three forms do have other, independent characters that correlate: color pattern and geography, implying that they are on independent evolutionary trajectories.

EVOLUTION OF COLOR PATTERNS

The general correspondence between color forms and ESUs indicates that color patterns are almost always reliable and sufficient for differentiating *Calcinus* species. Color pattern-level differentiation between morphologically similar sister species is common among hermit crabs (e.g., Asakura and Paulay 2003; Lemaitre and Poupin 2003; Poupin and Malay 2009), other crustaceans (e.g., Knowlton 1993; Macpherson and Machordom 2001; Ravago and Juinio-Meñez 2003), as well as in other taxa, such as reef fish (e.g.,

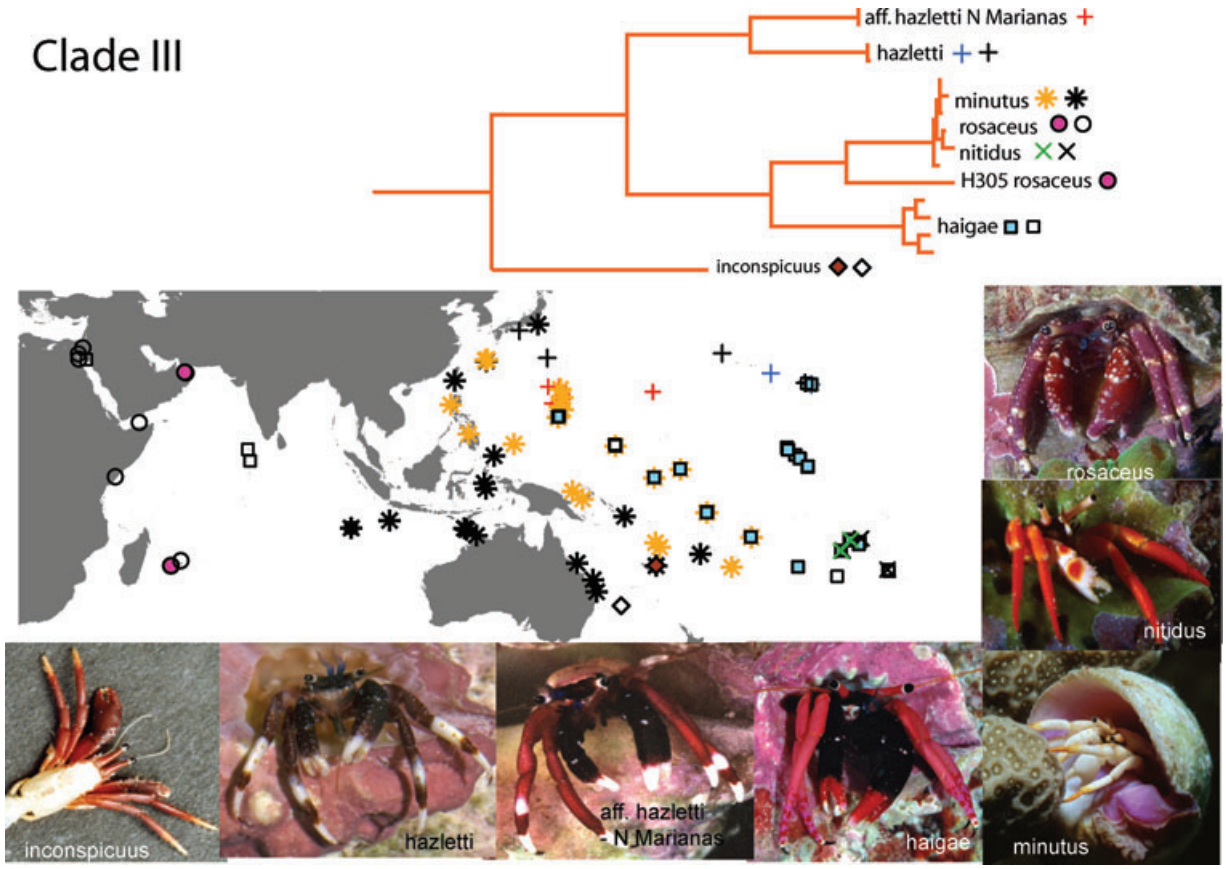


Figure 5. Distributions, color patterns, and COI phylogeny of Clade III *Calcinus* species. Symbols follow Fig. 3.

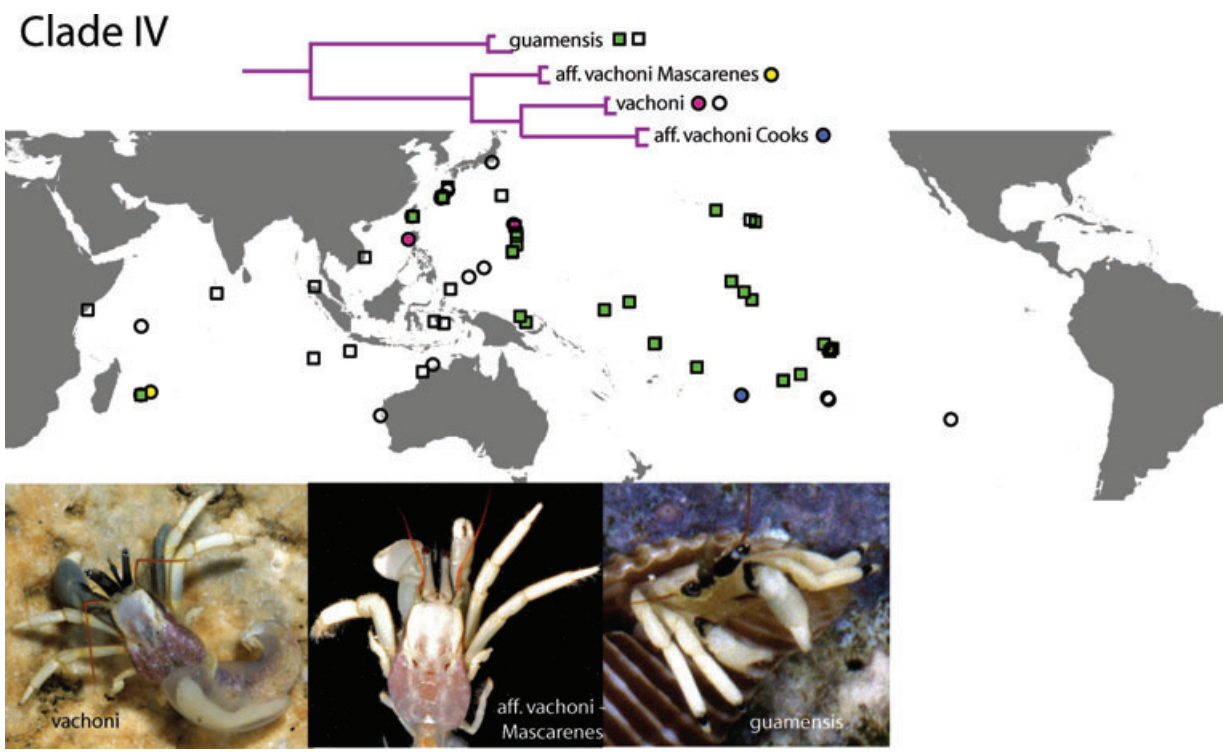


Figure 6. Distributions, color patterns, and COI phylogeny of Clade IV *Calcinus* species. Symbols follow Fig. 3.

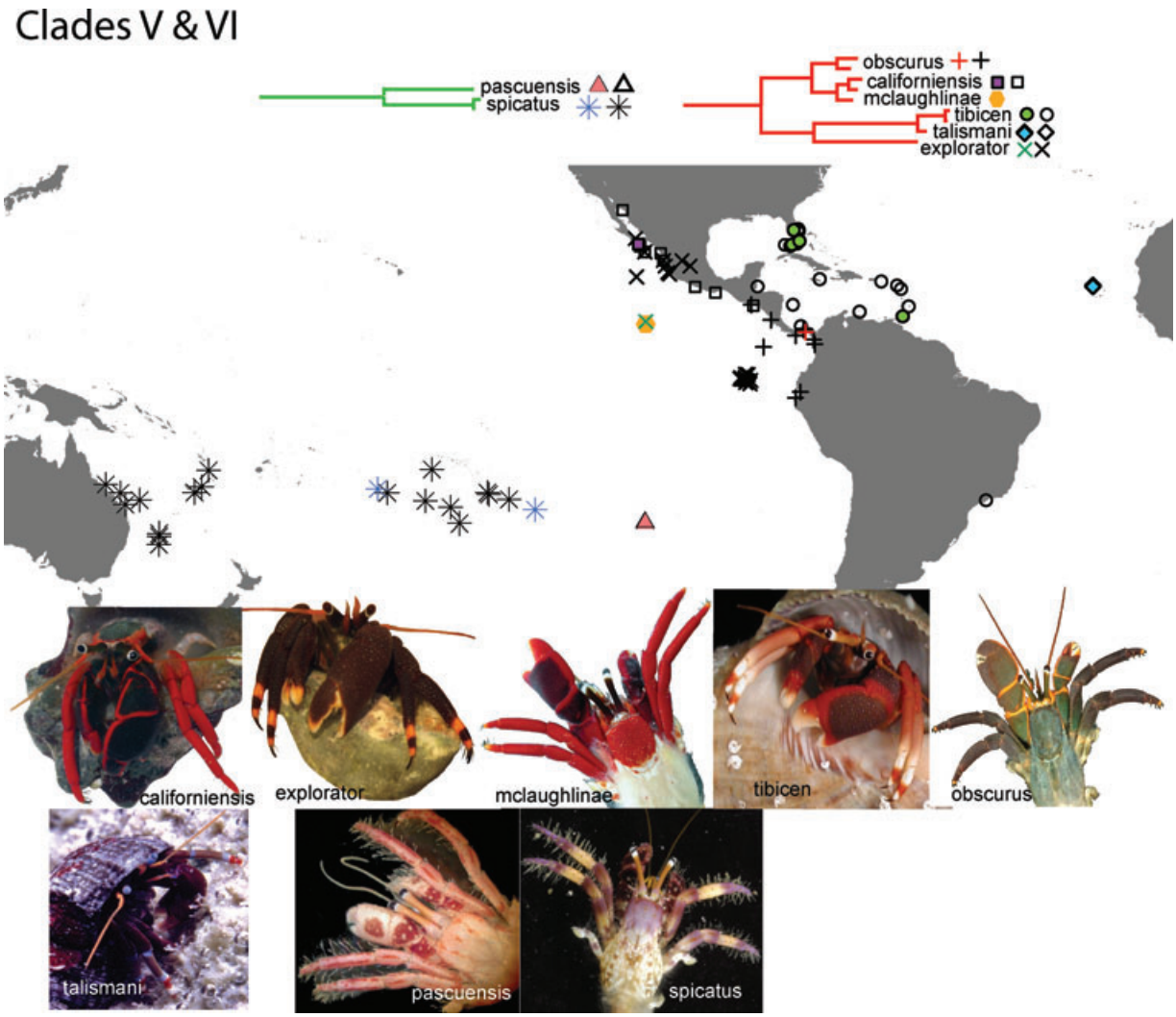


Figure 7. **Distributions, color patterns, and COI phylogeny of Clades V and VI *Calcinus* species. Symbols follow Fig. 3.**

McMillan et al. 1999; Bowen et al. 2006; reviewed in Knowlton 1993). Among reef fish, there have also been documented cases of closely related species that differ strikingly in color and yet show few (if any) structural differences and are not reciprocally monophyletic at the mitochondrial level (e.g., McMillan et al. 1999; Bowen et al. 2006). If the lack of monophyly is not due to introgression, these findings imply that the rate of color pattern evolution can equal or outpace mitochondrial sequence divergence, which suggests that differentiation in coloration may be driven by selection.

In *Calcinus*, coloration is so conspicuous and varied that it can be reasonably assumed to serve a purpose and thus be acted upon by natural selection. For example, it has been demonstrated that the size of the white chelar patch in *C. laevimanus* (Fig. 11) influences success in interspecific agonistic encounters (Dunham 1978). It is likely that other *Calcinus* species use color patterns in adaptive ways. If coloration is involved in conspecific interactions, then strong selection on these visual cues could result in the rapid color evolution, and genetically isolated populations may diverge in these cues over relatively short periods of time. Moreover, if color patterns are used for species recognition, then divergence in color may lead to the development of reproductive isolation barriers and thus speciation. Color patterns have been shown to serve in species recognition and mate choice in other marine groups, including fiddler crabs (Detto et al. 2006) and fish (McMillan et al. 1999; Puebla et al. 2007; Seehausen et al. 2008).

GEOGRAPHY OF SPECIATION

Speciation appears to be largely or exclusively allopatric in *Calcinus*, as in most animals (Coyne and Orr 2004), and allopatric separation of sister taxa is retained for more than 2 my (Fig. 17). The narrowly allopatric to parapatric ranges of all young sister taxa imply either that the geography of the original speciation event has been maintained in these taxa and there has been little postspeciational changes in distribution, or that such changes were

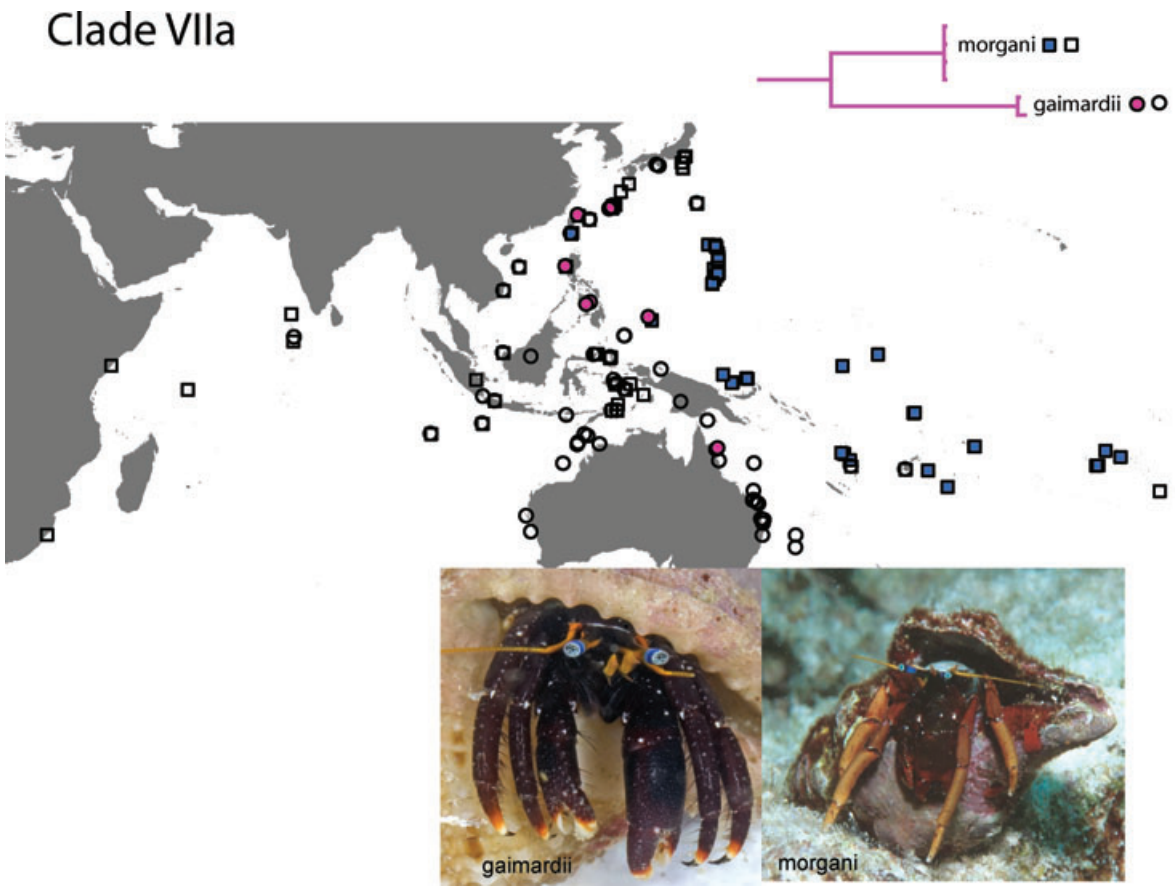


Figure 8. Distributions, color patterns, and COI phylogeny of Clade VIIa *Calcinus* species. Symbols follow Fig. 3.

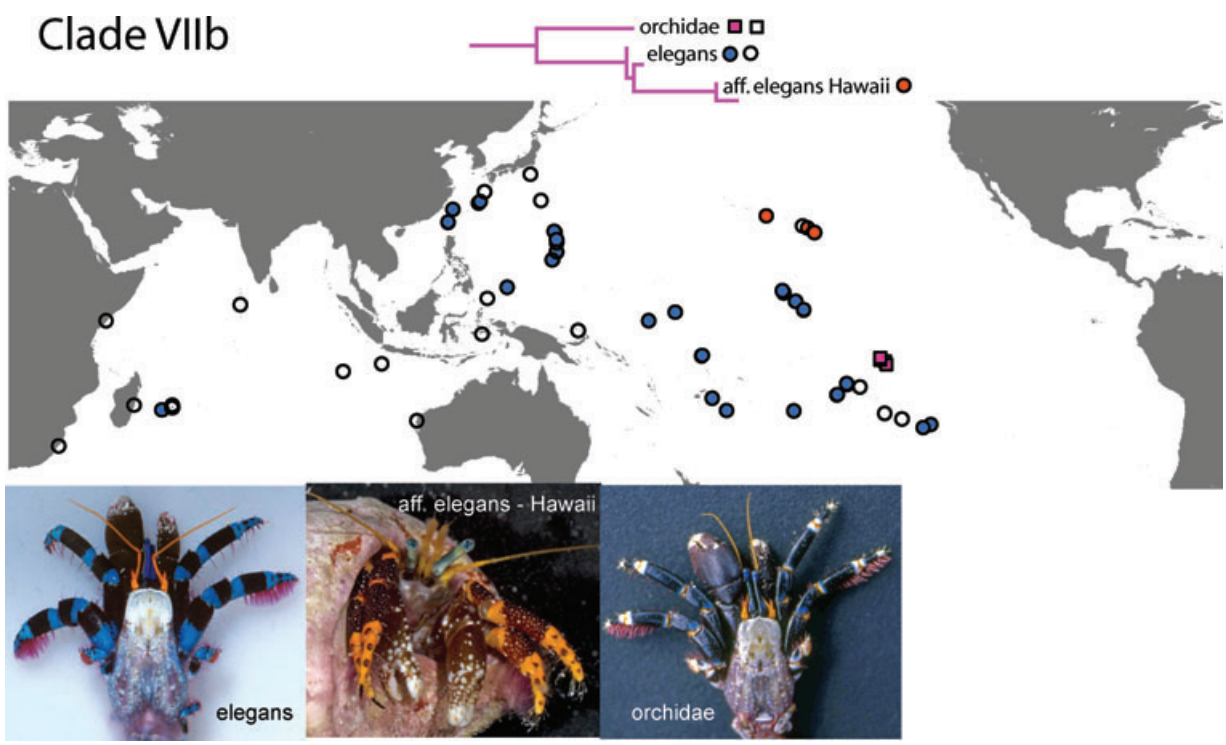


Figure 9. Distributions, color patterns, and COI phylogeny of Clade VIIb *Calcinus* species. Symbols follow Fig. 3.

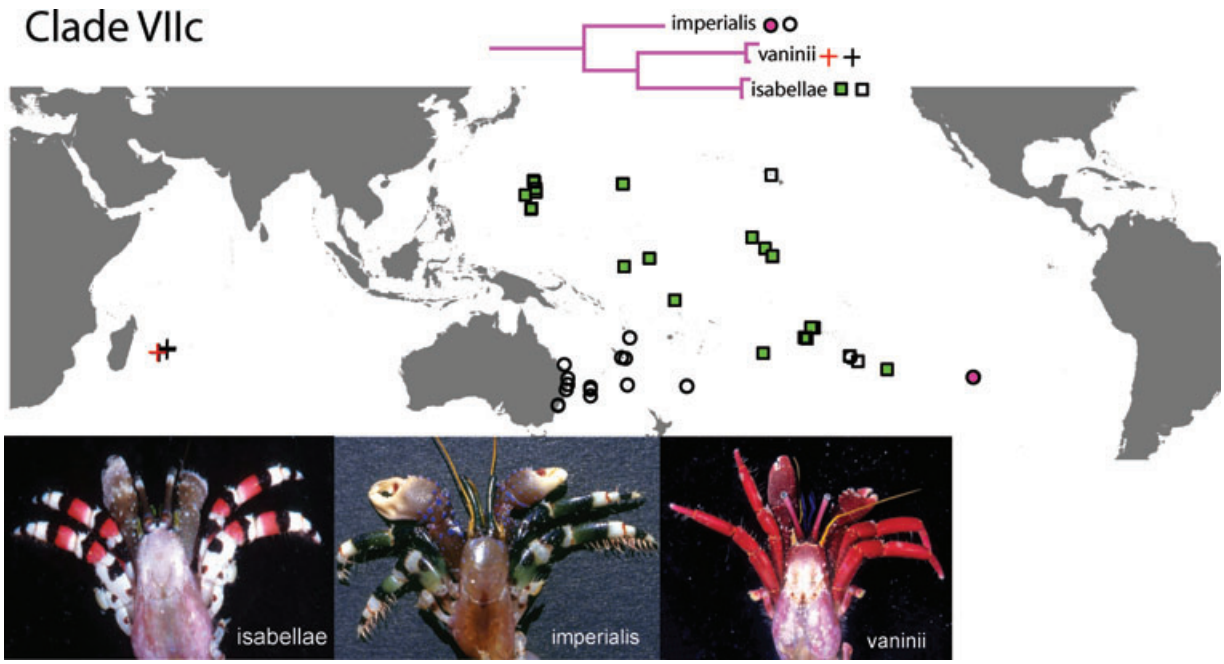


Figure 10. Distributions, color patterns, and COI phylogeny of Clade VIIc *Calcinus* species. Symbols follow Fig. 3.

reciprocal; i.e., expansion in the range of one ESU was associated with contraction in the range of the other. Although the latter hypothesis is difficult to falsify, the former is much more parsimonious and also more likely because boundaries between sister ESUs tend to fall at recognized zones of transition associated with major dispersal or ecological barriers.

Narrowly allopatric ranges also imply that localized endemics are the result of speciation rather than reliction. Endemism can be high on peripheral island groups, but endemics can result from either local (typically peripatric) speciation (e.g., neoendemics) or reliction (paleoendemics; see Ladd 1960; Stehli and Wells 1971; Newman and Foster 1987). Reliction refers to the survival of formerly widespread taxa; often in remote, biologically less-intense, "safe" places (Vermeij 1987). As relicts are generally older taxa that have undergone substantial reduction in their range, they are not expected to be narrowly allopatric with their

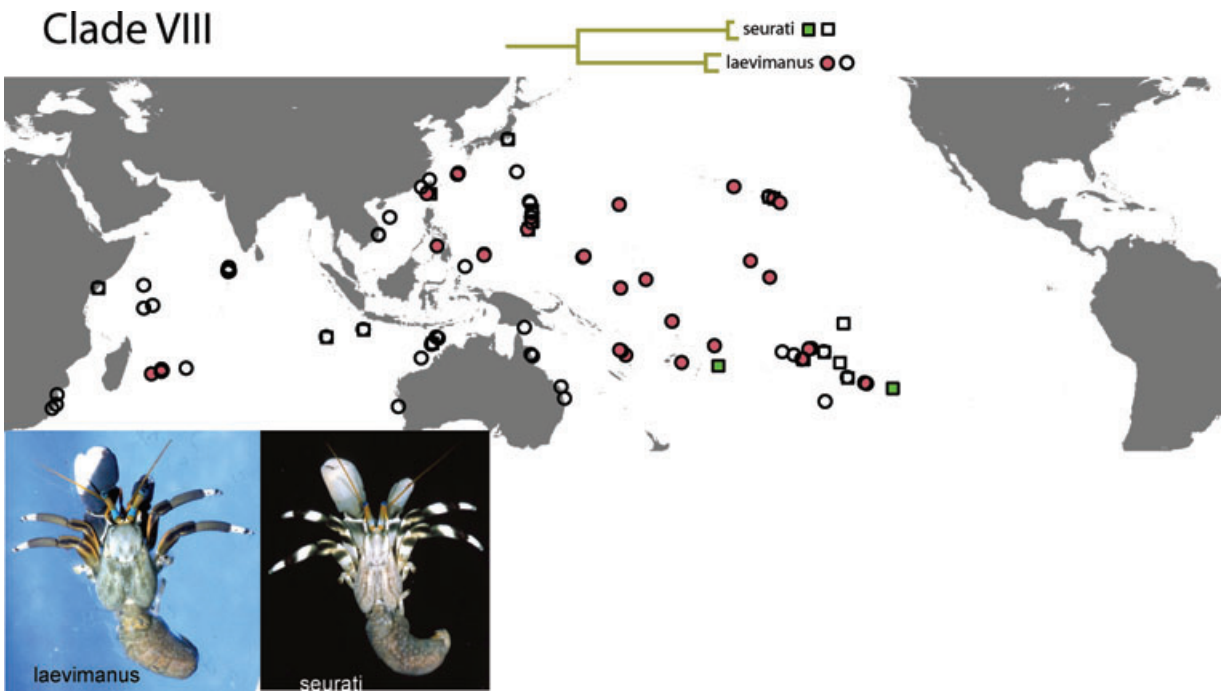


Figure 11. Distributions, color patterns, and COI phylogeny of Clade VIII *Calcinus* species. Symbols follow Fig. 3.

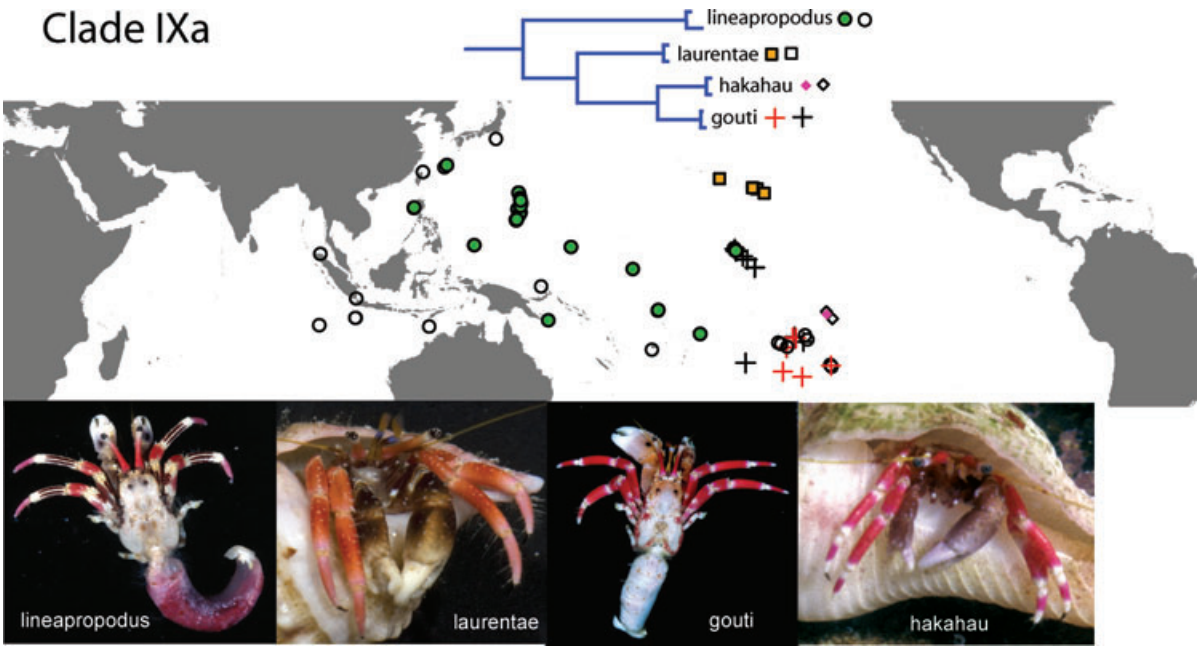


Figure 12. Distributions, color patterns, and COI phylogeny of Clade IXa *Calcinus* species. Symbols follow Fig. 3.

sister taxa, but to show disjunct or sympatric ranges. None (except *C. albengai*; Fig. 14) of the insular endemics are sympatric or have disjunct distribution with their sister taxa.

The location of geographic speciation events span the globe, but are not randomly distributed. Peripatric speciation on remote islands is most prevalent, whereas speciation events between the Indian and Pacific ocean basins or within the Indo-Malayan triangle are rare/absent. Speciation across ecological gradients, such as latitude and depth (Fig. 16), and between the four tropical regions is also evident.

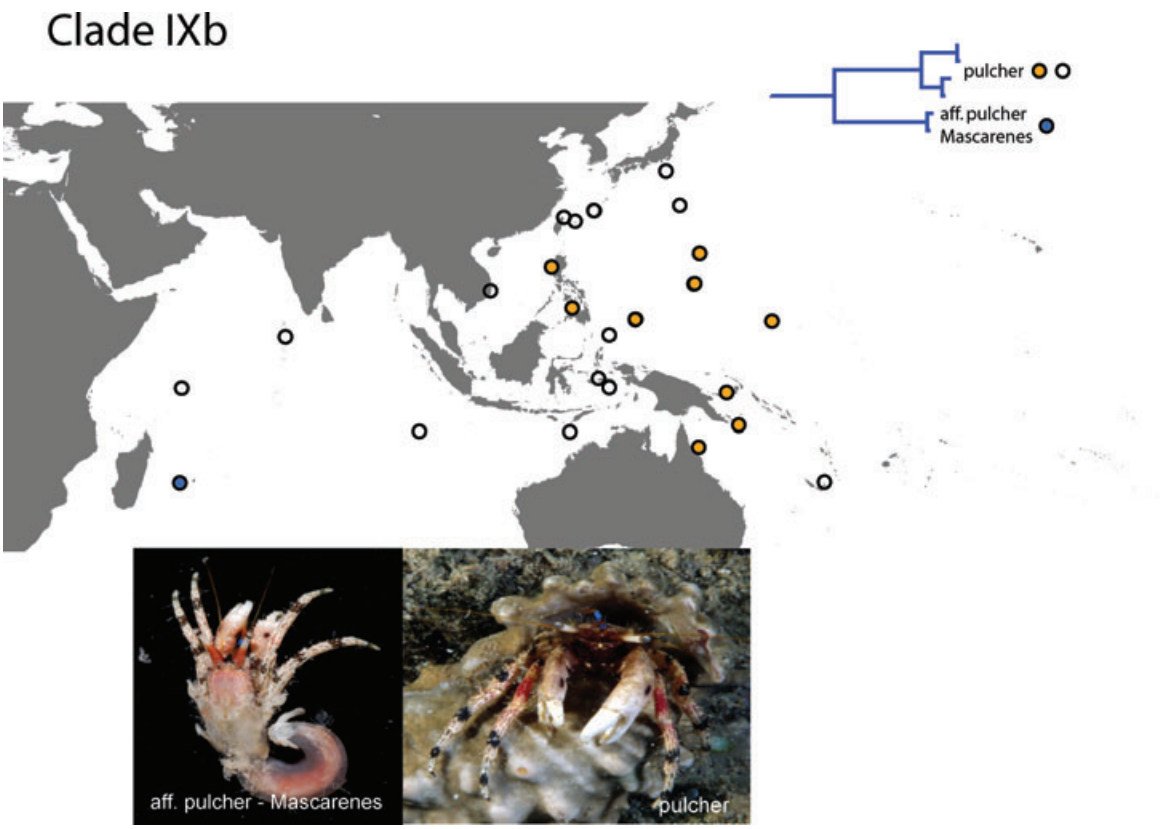


Figure 13. Distributions, color patterns, and COI phylogeny of Clade IXb *Calcinus* species. Symbols follow Fig. 3.

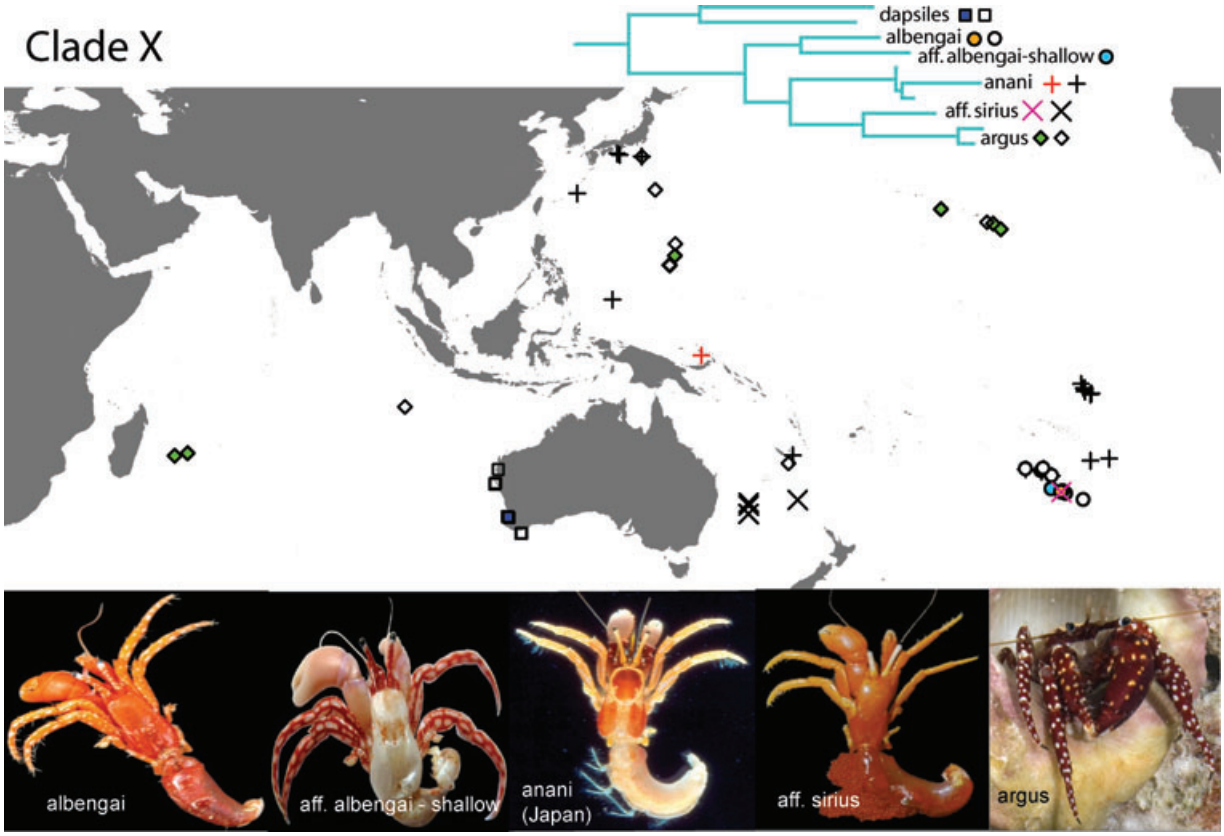


Figure 14. Distributions, color patterns, and COI phylogeny of Clade X *Calcinus* species. Symbols follow Fig. 3.

Isolation on remote islands and archipelagos appears to be the most prevalent cause of speciation in *Calcinus*: 60% of the ESEs have resulted in at least one of the sister taxa becoming restricted to a remote island group, lending some support to the center of accumulation hypothesis. This hypothesis posits that species predominantly originate in peripheral areas, and subsequently accumulate in Indo-Malaya by distributional expansion across the IWP, followed by reliction to Indo-Malaya (Ladd 1960; Jokiel and Martinelli 1992). Peripheral endemics may follow one of two trajectories: (1) range expansion after establishment of reproductive barriers with their sister species, leading to buildup of regional species diversity, or (2) maintenance of restriction until eventual extinction in their isolated ranges. It is difficult to test the importance of these two alternatives, although the predominance

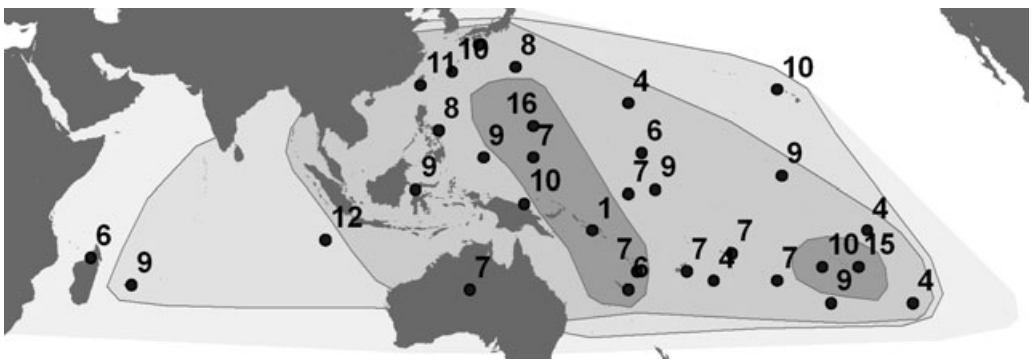


Figure 15. Spatial distribution of species richness. Contour lines represent number of species expected in area based on overlay of species ranges (Figs. 3–14, see methods). Contours are drawn for 4, 10, 13, and 17 species in increasingly darker shades. Numbers represent number of documented species within the following regions and archipelagoes: Australia, Austral-Rapa, Caroline, Cocos-Christmas, Cook, Fiji, Gilbert, Hawaii, Indonesia, Japan, Line, Madagascar, Mariana, Marquesas, Marshall, Mascarene, Nauru, New Caledonia, New Guinea, Ogasawara, Palau, Philippine, Pitcairn, Ryukyu, Samoa, Society, Solomon, Taiwan, Tonga, Tuamotu, Vanuatu, and Wake. Differences between the contour line and numbered estimates are presumably the result of local undersampling.

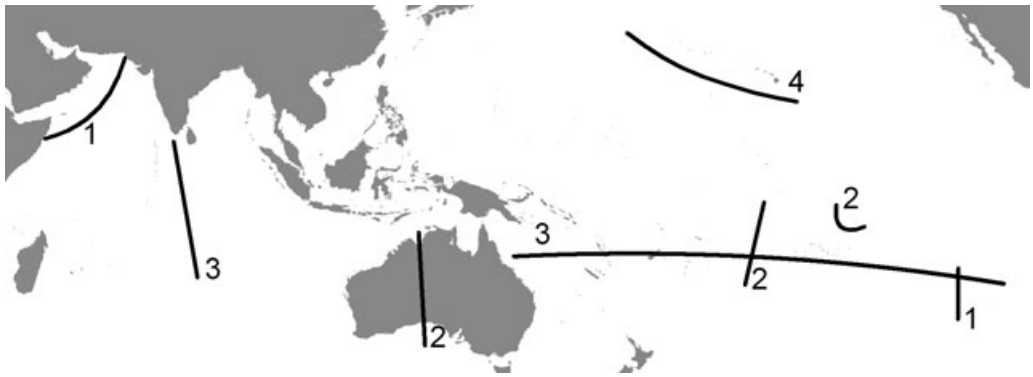


Figure 16. Approximate distribution of IWP ESEs. Boundaries separating the ranges of sister taxa—and thus the location of ESEs—are drawn as lines. Numbers indicate how many ESEs occur across each of these zones.

of peripatric speciation in *Calcinus*, combined with high diversity of widespread taxa and substantially greater age of sympatric sister species, suggest that the first trajectory may occur reasonably frequently. In contrast, reliction to Indo-Malaya has not been an important process, as demonstrated by the paucity of Indo-Malayan *Calcinus* endemics.

Insular *Calcinus* endemics have evolved in Hawaii (4), Marquesas (2), SE Polynesia (2), Mascarenes (2), Easter Island (1), Clipperton (1), and Bermuda (1); an additional endemic putatively assigned to the genus (*C. paradoxus*; see above) in the Azores has not been sampled. These are some of the most remote islands in the world, renowned for high endemism (e.g., Briggs 1974; Randall 1998), thus it is not surprising that they also host endemic *Calcinus*. A similar pattern of predominantly peripatric speciation has been found in *Thalassoma* wrasses (Bernardi et al. 2004). Among fish, the highest levels of endemism in the IWP are encountered in peripheral areas: Easter, Hawaiian, Marquesas, Mascarene islands, and the Red Sea (4.4–23% endemics; Randall 1998; Allen 2007). Among these remote island groups, we have sampled the Hawaiian Islands most thoroughly, and have sequenced nine of 10 species known from there. Of the nine, four (44%) are endemic: *C. laurentae,* and endemic ESUs of the widespread *C. hazletti, C. latens,* and *C. elegans*. *Calcinus isabellae* (known from two Hawaiian records) remains untested. In the Marquesas, two of four recorded species are endemic whereas one of three from Easter Island are. However the status of widespread species in the Marquesas and Easter remain to be genetically evaluated.

Four clades appear to have given rise to multiple peripheral endemics. In two, peripatric speciation from a widespread form appears to have been the source of these endemics whereas in two others, insular endemics appear to have undergone local diversification within a basin. *Calcinus elegans* (Fig. 1B Clade VII, Fig. 9) and *C. latens* (Fig. 1B Clade II, Fig. 4), both ranging from East Africa to Polynesia, gave rise to four peripatric endemics: three on remote islands and one on the Arabian peninsula. The wide-ranging ESU is a terminal branch in both clades, implying it was the source of successive peripheral endemics. In contrast the insular-endemic sister species *C. laurentae-C. hakahau-C. gouti*

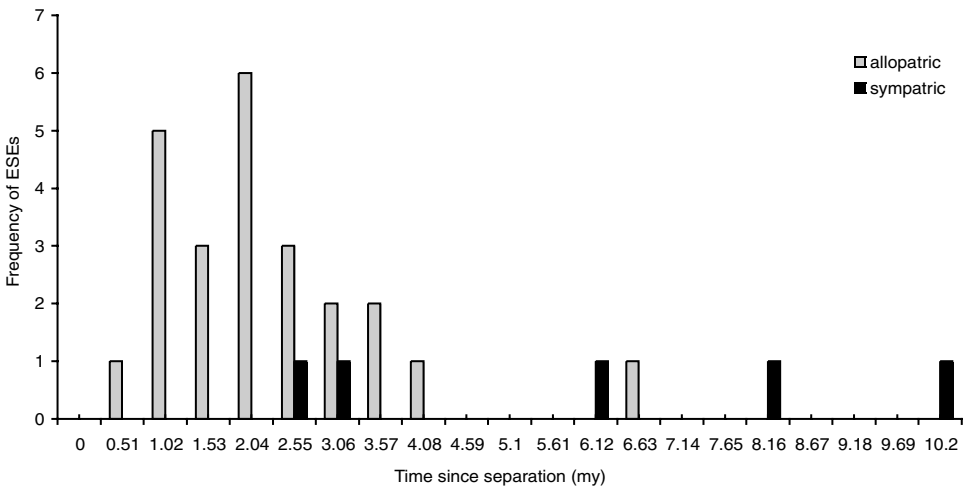


Figure 17. Age distribution (in million years, my) of *Calcinus* sister species pairs.

(Fig. 1B Clade IX, Fig. 12) and Hawaiian-Micronesian ESUs of *C. hazletti* (Fig. 1B Clade III, Fig. 5) represent lineages diversifying within the central Pacific, and are only more distantly related to widespread taxa (*C. lineapropodus-pulcher* and *C. minutus*-complex, respectively).

In contrast to the abundance of peripheral speciation, *Calcinus* show no diversification within the Indo-Malayan area: no ESEs are identified within the area, and only one species, *C. gaimardii,* is (largely) confined to it (Fig. 8). This contrasts with many marine taxa that have numerous endemics in Indo-Malaya, some with substantial in situ diversification (e.g., Paulay 1997; Meyer et al. 2005; Barber et al. 2006; Williams and Reid 2004), as predicted by the center of origin hypothesis. Overall, speciation along continental shorelines appears to be uncommon in *Calcinus*, with the divergence between the EP species *C. californiensis* (Gulf of California to El Salvador) and *C. obscurus* (El Salvador to Peru) the only known example (Fig. 1B Clade VI, Fig. 7).

Calcinus species show little differentiation between the Indian and Pacific Ocean basins. In contrast the restricted seaway between the Indian and Pacific basins is one of the most important sites of speciation for other marine taxa, with numerous well-known as well as cryptic species-pairs differentiating across the boundary between these great basins (e.g., Randall 1998; Read et al. 2006; Barber et al. 2000), as predicted by the center of overlap hypothesis. In *Calcinus* only two ESEs are known that may fall in this area, i.e., in the *C. pulcher* (Clade IX, Fig. 13) and *C. vachoni* (Clade IV, Fig. 6) complexes. However the location of the boundary between western and eastern ESUs of both species is poorly constrained, as no samples have been genetically tested between the Philippines/Ryukyus and Mascarenes. In contrast none of the other five widespread species tested (*C. laevimanus, C. argus, C. elegans, C. guamensis, C. latens*) show much genetic differentiation between populations in the Indian and Pacific Ocean basins. The genetic homogeneity of such wide-ranging species, prevalence of peripatric speciation on remote archipelagos, and diverse *Calcinus* assemblages on the world's most isolated islands imply that these crabs have great powers of dispersal, and that this has influenced their modes of speciation.

INTERREGIONAL COMPARISONS

Although *Calcinus* diversity in the IWP and EP are largely the result of in situ radiation, interregional speciation was the source of Atlantic diversity. All non-IWP species studied are in two clades (I and VI). Clade I (Fig. 3) is comprised of *C. tubularis* (EA) and *C. verrilli* (Bermuda). The eastward relationship of the Bermudan endemic is unusual, as the majority of marine organisms in Bermuda originated from the WA, a result of the Gulf Stream facilitating dispersal (Sterrer 1986; Smith-Vaniz et al. 1999; Floeter et al. 2008).

Clade VI (Fig. 7) is comprised of four EP, one WA, and one EA species. Close connections between the EP, WA, and EA is a common pattern among marine organisms (Briggs 1974; Paulay 1997). Species in this clade are nearly morphologically identical, but are readily distinguished by color pattern. *Calcinus tibicen* (WA) is sister to *C. talismani* (EA), and this Atlantic-species pair is sister to *C. explorator* (EP), a geminate species likely isolated by the emergence of the Isthmus of Panama. The other subclade is comprised of EP species only (*C. californiensis, C. obscurus,* and *C. mclaughlinae*). *Calcinus mclaughlinae* is endemic to Clipperton Island whereas *C. californiensis* and *C. obscurus* have parapatric ranges along the central American coast and are absent from EP oceanic islands. Offshore EP islands mostly harbor *C. explorator*, a species also present in and near the Gulf of California, but not along the continental coast further south (Fig. 7).

ECOLOGY

Species distributional boundaries can be set by ecological limitations as well as dispersal barriers (with dispersal barriers themselves a type of ecological limitation). A prevalent form of distributional restriction in the IWP is to "continental" or "oceanic" habitats (Abbott 1960; George 1974; Paulay 1994; Reid et al. 2006). Although both can be caused by dispersal as well as ecological limitations, ecological restriction is implied for species that range widely among remote islands, but are absent from nearby continents. Pacific-plate endemism (Springer 1982; Kay 1984) is a well-documented example of such ecological oceanic restriction (Paulay 1997). Continental and oceanic habitats differ in many ways, including levels of primary productivity, terrigenous influence, habitat diversity, and presence/absence of predators and competitors that are restricted to continental shores by dispersal limitations.

Oceanic restriction is prevalent in *Calcinus*. Thus only seven of 17 species of *Calcinus* recorded from Australian territories are known from the continent, the remaining species are recorded only from offshore islands (Morgan 1991). Although 12 species are recorded from Cocos Keeling and Christmas Islands, small oceanic islands just SW of Indonesia, only nine species have been recorded from all of Indonesia, the most diverse marine archipelago in the world (Fig. 15). Similar continental avoidance (including greater diversity on nearby islands than in Australia or Indonesia) occurs in several groups of terrestrial crabs and hermit crabs (Paulay and Starmer, in press). *Calcinus isabellae* is a classic, widespread Pacific-plate endemic (Fig. 10), and five other species appear to be regionally widespread, yet largely confined to islands: *C. sirius* in the South Pacific (Fig. 14), *C. argus* (Fig. 14) and *C. seurati* (Fig. 11) across the IWP, *C. explorator* in the EP (Fig. 7), and *C. talismani* in the EA (Fig. 7). An additional 16 species are restricted to one or a few neighboring oceanic island

groups, but could be so restricted by dispersal limitation as well as ecology. Conversely, only three species show largely continental restriction: *C. gaimardii* in the IWP (Fig. 8) and *C. californiensis* and *C. obscurus* in the EP (Fig. 7).

Calcinus also includes several species restricted to relatively cool, subtropical or moderately deep (100–300 m) waters. The following species are known only from subtropical latitudes in the southern IWP: *C. sirius*, *C. aff. sirius*, *C. albengai, C. aff. albengai, C. dapsiles* (clade X, Fig. 14), *C. spicatus, C. pascuensis* (clade V, Fig. 7), *C. imperialis, and C. vanninii* (clade VII; Fig. 10). The origin of these taxa is predominantly by in situ diversification within the subtropics. Similar latitude-based niche conservatism has been demonstrated in gastropods (Frey and Vermeij 2008; Williams et al. 2003; Williams 2007). Only the last clade has a relatively recent and thus readily identifiable origin in the tropics, sister to the parapatric *C. isabellae*.

All deep water species investigated (*C. anani, C. albengai*, *C. aff. sirius*) are members of clade X (Fig. 14), suggesting that invasion of deep reef habitats may have occurred only once. Interestingly this clade also includes a large portion of subtropically restricted *Calcinus*, implying that temperature may be an important factor limiting their distribution. Our field observations show that even the two clade X members known from relatively shallow, tropical waters (*C. argus* and *C. anani*) are rare in those habitats, but also occur in the subtropics or deep water. Sequence and/or morphological data suggest incipient differentiation in four of five described species in this clade (*C. anani, C. argus*, *C. sirius, C. albengai*), the only exception being the geographically restricted W Australian endemic *C. dapsiles*. Moreover, subtropical and deep reef habitats remain substantially undersampled for *Calcinus*, and future explorations will likely result in discovery of numerous new forms and document additional radiation. The small number of samples on hand prevents detailed analysis of speciation in this clade.

Another example of niche conservatism are the sister species *C. tubularis* and *C. verrilli* (Clade I, Fig. 3). These are the only *Calcinus* known with sexually dimorphic behavior; with females commonly adopting a sessile habit, living in tubes of sessile turritellid and vermetid gastropods (Markham 1977; Gherardi 2004). Although there is substantial niche conservatism in *Calcinus*, there is also evidence for interesting ecological shifts between sister species. Sister species *C. seurati* and *C. laevimanus* (Clade VIII, Fig. 11) are the only high intertidal/supratidal *Calcinus*, a habitat otherwise occupied by the related (but competitively inferior—Hazlett 1981) diogenid genus *Clibanarius*. However although *C. laevimanus* lives in the upper intertidal, *C. seurati* is restricted to supratidal splash pools. Two ESUs of *C. albengai* (clade X, Fig. 14) separate by depth: one ranging from shore to $<$50 m depths whereas the other is exclusively deep-water (50–280 m; Poupin and Lemaitre 2003). The role of ecology versus geography in the divergence of these species deserves further attention; the latter, with both forms only known from one small island, is potentially a case of sympatric speciation through niche differentiation. Such depth-related sympatric differentiation has also been reported in cnidarians (Carlon and Budd 2002; Prada et al. 2008; Eytan et al. 2009).

DIVERSITY PATTERNS

Calcinus species diversity is about an order of magnitude higher in the IWP than other regions, a pattern typical for reef organisms (Paulay 1997). The IWP is home to 40 ESUs, the EP, WA, and EA to four, three, and two respectively. Local diversity shows similar interregional differences, with up to 16 species coexisting in one archipelago (Marianas) in the IWP, but at most two in other regions.

Calcinus species richness does not peak within Indo-Malaya, but is highest in Oceania, peaking at two locations: the Mariana and Tuamotu Islands, with 16 and 15 species, respectively (Fig. 15). This contrasts with the majority of marine taxa that reach their diversity peak in Indo-Malaya, from where richness decreases in all directions, but most conspicuously across the Pacific basin (Stehli et al. 1967; Briggs 1974; Hoeksema 2007). Nevertheless, diversity patterns, especially the steepness of the diversity increase toward Indo-Malaya varies greatly among taxa, and in large groups it arises from the composite of varied, clade-specific patterns (see Fig. 4 in Paulay and Meyer 2006). Similarly, the unusual diversity pattern in *Calcinus* contrasts with hermit crabs as a whole, which show the typical diversity pattern, with diversity much higher in Indo-Malaya than in the oceanic Pacific (compare McLaughlin et al. 2007 [133 paguroid species in Taiwan] with Paulay et al. 2003 [64 spp. in Guam] and Poupin 1996 [45 spp. in French Polynesia]).

We propose that the diversity pattern of *Calcinus* is a reflection of the genus' affinity to oceanic conditions, combined with substantial dispersal ability that has allowed species to reach remote islands. The 10 species diversity-contour extends from the Mascarene to the Hawaiian Islands, and although 13 species are recorded from the total SE Asian area, only 10 are known from any one country in the Indo-Malayan archipelago. As noted above, several *Calcinus* species avoid continental habitats, such that diversity is higher on oceanic islands immediately outside the IWP diversity center than on the more terrigenous and continental settings of Indo-Malaya and Australia. Finally, the predominance of peripatric speciation has lead to local diversity hot spots in peripheral locations, like in SE Polynesia, where 21 species are known, with up to 15 species recorded from a single archipelago (Tuamotus).

The distribution of diversity in *Calcinus* contrasts with hermit crabs as a whole, implying that the typical diversity pattern of hermit crabs is a composite of different clade-specific

patterns. Similar variance in the distribution of diversity and implied modes of speciation were demonstrated among groups of cowries by Paulay and Meyer (2006). Such variance in patterns of diversity and diversification among related clades implies that multiple processes are involved in generating diversity in larger taxa. Thus, although hypotheses such as the center of origin, overlap, or accumulation may be supported in small groups, they are not general or exclusive explanations for diversification in the IWP.

Conclusions

Our study has uncovered a wealth of unrecognized diversity in a relatively well-known reef dweller, *Calcinus*. The number of ESUs in the IWP was augmented by 22%. This large increase in ESUs was made possible by our approach of intensively sampling populations of every accessible species across their range. Through photo-documentation of live specimens, we conclude that differences in coloration correspond to boundaries between ESUs, thus color patterns are very important in species delineations. We show that differences in color pattern evolve rapidly, and hypothesize that coloration may serve an adaptive purpose, such as species recognition or mate selection. This hypothesis deserves further investigation, for instance by studying the evolution of genes responsible for differences in decapod coloration.

The geographic distributions of *Calcinus* species are now well documented and illustrate several patterns atypical for reef fauna. Non-IWP species fall into two clades. One clade connects a Bermudan endemic with an EA species, a rarely observed pattern. The second non-IWP clade groups together species from EP, WA, and EA (including one geminate species pair). This clade contains the only known instances in *Calcinus* of speciation along a continental margin.

Among IWP species, we show that the center of species diversity is not in the Indo-Malayan triangle, but further east in the Mariana Islands, with a second peak in SE Polynesia. This may be the result of a tendency in *Calcinus* to prefer oceanic habitats. We found no support for either center of origin or center of overlap theories. Instead, our results show generally high-dispersal abilities coupled with peripatric speciation in remote areas. The youngest sister species pairs all have narrowly allopatric distributions, and a substantial amount of time (>2 million years, usually much longer) is needed for sister species to develop sympatric distributions.

Ecological factors have also played a role in *Calcinus* distribution and speciation. Distributions are shaped in part by restriction of species to oceanic environments (common) or continental environments (rare). Phylogenetic conservatism of ecological niches is common, however there are also a few cases of large ecological shifts between sister species. In one instance, a shift between a shallow-water and a deep-water morph may have occurred in sympatry.

A diverse range of factors, including both historical and ecological mechanisms, influence species distributions in *Calcinus*. Given this complexity, biogeographers should expect different taxa to show nonidentical biogeographic patterns, reflecting the unique histories and ecological adaptations of different groups. The overall top-down picture of marine biodiversity is a summation of all these individual histories.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Calcinus_records_L_S_v2.xls

Supporting Information may be found in the online version of this article.

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