Patiriella pseudoexigua (Asteroidea: Asterinidae): a cryptic species complex revealed by molecular and embryological analyses

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Cryptic lineages were identified within a morphologically uniform group of sea stars distributed from Australia to Japan. Among eight populations, all of which have been referred to *Patiriella pseudoexigua*, we found seven unique mitochondrial DNA sequences clustered into four distinct lineages. These four lineages formed a monophyletic group in which sister clades were separated by small genetic distances but could be differentiated from each other on the basis of reproductive differences. The four lineages thus appear to be separate but very closely related species. Examination of reproduction in several Queensland populations revealed that one population (Statue Bay) consisted of hermaphroditic intragonadal brooders with live-born offspring while other populations (Townsville, Bowen, Airlie Beach) consisted of dioecious free-spawners with a planktonic larva. The brooded larvae from central Queensland populations closely resembled brooded embryos and larvae of a Japanese lineage, while the planktonic larvae from northern Queensland were similar to the original description of planktonic larvae from a Taiwan population. However, each of the viviparous lineages was more closely related to a lineage with planktonic larval development than the viviparous lineages were to each other. *Patiriella pseudoexigua* thus comprises at least four species with different reproductive phenotypes in which viviparous brooding appears to have evolved in parallel. Based on previous taxonomic work we propose the following names for these four lineages: the dioecious free-spawner from northern Queensland (including the *P. pseudoexigua* type locality) is *P. pseudoexigua sensu stricto*; the viviparous brooder from central Queensland is undescribed and here referred to as *Patiriella* sp. nov; the dioecious free-spawner from Taiwan is temporarily referred to as *Patiriella* sp. (a senior name for this species may be *P. pentagonus*); and the hermaphrodite brooder from Japan should be raised to specific status and referred to by the new combination *P. pacifica*.

INTRODUCTION

Speciation in marine animals is frequently accompanied by little obvious morphological variation among divergent lineages (Palumbi, 1992, 1994; Knowlton, 1993, 2000). Many cases of cryptic species have recently been identified through molecular studies of geographically widespread taxa (e.g. Baric & Sturmbauer, 1999; Dawson & Jacobs, 2001; Williams et al., 2001; Quattro et al., 2001; Lazoski et al., 2001; Tarjuelo et al., 2001; Rocha-Olivares et al., 2001; Larsen, 2001). Diagnostic morphological traits for these cryptic species may be difficult to find even in hindsight. The presence of long-undetected cryptic macrofaunal species is exemplified by recent studies of several sea star lineages in which new species have been discovered based on differences in reproductive and molecular characters (Hart et al., 1997; Williams, 2000; Flowers & Foltz, 2001). Some of the most striking species divergences, apparently tied to evolution of development, are seen in the sea star family Asterinidae which includes the widest diversity of life histories among all living Asteroidea (Chia & Walker, 1991; Byrne & Cerra, 1996; Hart et al., 1997).

Species of *Asterina*, *Patiriella*, and their relatives include at least five different styles of reproduction and modes of larval development: planktonic dispersal by feeding larvae; planktonic nonfeeding larvae; benthic development in egg masses without brood protection; external brooding of egg masses; and internal brooding with viviparous juveniles. Some brooders develop from large, yolk-rich eggs like those of species with planktonic nonfeeding development, while others develop from small eggs and are nourished by brood cannibalism (Komatsu et al., 1990; Byrne & Cerra, 1996; Byrne et al., 1999b). Molecular phylogenetic analyses, based on mitochondrial DNA (mtDNA) sequences of 12 nominal *Patiriella* and *Asterina* species with divergent life histories, suggested both widespread convergent evolution of modified ontogenies and diverse life histories among closely related species (Hart et al., 1997).

The southern Australian marine region is characterized by a highly endemic invertebrate fauna (Wilson & Allen, 1987) and is the centre of diversity for the genus *Patiriella*. New *Patiriella* species from southern Australia await description (O'Loughlin et al., 2002; M. O'Loughlin, personal communication). Northern Australia shares its species-rich tropical reef fauna with much of the rest of the Indo-Pacific, although a significant endemic element is associated with the coast (Wilson & Allen, 1987).

Recent studies along the coast of Queensland indicate the presence of previously undocumented endemic biodiversity (Pape et al., 2000), as indeed we describe here for *Patiriella*. Prompted by the apparently wide distribution of *P. pseudoexigua* from Queensland through Borneo and the Philippines to Taiwan and Japan, and the propensity of life history evolution in *Patiriella* (Dartnall, 1971; Marsh, 1977; Chen & Chen, 1992; Byrne & Cerra, 1996), we examined the molecular and reproductive characteristics of northern and southern populations of this species for the presence of cryptic biodiversity outside the highly endemic southern Australian region.

Taxonomic history of Patiriella pseudoexigua

Dartnall (1971) described two small, pentagonal *Patiriella* species from the mid-intertidal zone of rocky or muddy shores in north-eastern Australia: *P. pseudoexigua* and *P. obscura*. These sea stars resemble the temperate species *P. exigua* which lays eggs and has oral gonopores. In contrast to *P. exigua,* however, *P. pseudoexigua* has aboral gonopores and occurs north of 28°S latitude. Dartnall noted that a senior name, *P. pentagonus*, was available for one of these species but he was unable to determine which. He suggested that later work might prove either that one of his new species (*pseudoexigua* or *obscura*) is a junior synonym of *pentagonus*, or that *pentagonus* is an invalid species.

Hayashi (1977) described a Japanese subspecies of *P. pseudoexigua pacifica* (=*Asterina pseudoexigua pacifica*) and Komatsu et al. (1990) showed that this subspecies is a viviparous brooder. This contrasts with the planktonic development of *P. pseudoexigua* populations in Taiwan (Chen & Chen, 1992).

Clark (1993) reassigned Hayashi's new subspecies to *Patiriella* as *P. pseudoexigua pacifica*. Rowe & Gates (1995) synonymized *P. obscura* under *P. pseudoexigua*. Finally, Byrne et al. (1999a) pointed out the existence of viviparous *P. pseudoexigua* individuals in north-eastern Australia.

Thus the combination of taxonomic and embryological methods has combined several divergent populations into *Patiriella pseudoexigua* as a single widespread species in which some populations reproduce as planktonic larvae and others as viviparous brooders. Here we provide molecular and phenotypic evidence for cryptic species in this taxon. The life history and larval development of *P. pseudoexigua* from Australia are compared to life histories of other asterinids. We found some relatively large genetic distances among 11 mtDNA haplotypes from eight *P. pseudoexigua* populations in Japan, Taiwan, and Queensland. These haplotypes formed a monophyletic clade in phylogenetic analyses. Some sister groups within this clade consisted of lineages with different larval forms and life histories. The three results together suggest the existence of four closely-related species that can be identified by a combination of molecular and reproductive differences.

MATERIALS AND METHODS

Life history traits of Patiriella pseudoexigua in Queensland

Adult *Patiriella pseudoexigua* were collected from four sites in northern Queensland at Kissing Point near Townsville

(19°13′S 146°48′W), Dalrymple Point and Rose Bay near Bowen (20°1′S 148°16′E), and Airlie Beach (20°30′S 148°45′E); and from a fifth site in central Queensland at Statue Bay (23°15′S 150°45′E). The Airlie Beach site is near the type locality for *P. pseudoexigua* (Dartnall, 1971). We examined 15–20 individuals from each of the northern locations in October 2002; we examined approximately 20 individuals per year from 1998 through 2002 at the Statue Bay site. Adults were kept in laboratory aquaria and dissected periodically to assess the stage of development of their gonads and offspring. Mature ovaries from the northern specimens were placed in a 10^{-4} M solution of the ovulatory hormone 1-methyladenine in filtered seawater (FSW) and sperm were collected from the testes. After fertilization the embryos and larvae were raised in bowls of $0.45\,\mu m$ FSW at 24°C. Sea stars from the Statue Bay population were intragonadal brooders. The larvae of this species were freed from the gonadal brood space by dissection and raised in bowls of FSW at room temperature (22–24°C).

Mitochondrial DNA sequences

For identification of *P. pseudoexigua* lineages we used a single pair of polymerase chain reaction (PCR) primers to amplify and sequence a portion of the mitochondrial genome. The primer pair Pat1 (5′ ATCATGATTGGCAT TACC 3′) and Pat2 (5′ CTTTGAAGGCTTTTAGTTTA GATTAAC 3′) amplify five transfer RNA (tRNA) genes (for alanine, leucine, asparagine, glutamine, and proline), a short promoter region, and the 5′ end of the cytochrome *c* oxidase I (COI) gene. The amplified fragment, from the 5′ end of Pat2 to the 5′ end of Pat1, corresponds to positions 11725 to 12377 in the complete *Asterina pectinifera* mitochondrial genome (Asakawa et al., 1995). The PCR products were directly sequenced (using the PCR primers) or cloned into the TOPO-TA cloning vector (Invitrogen) before sequencing (using M13 primers). The PCR products were cloned only in cases where one of the PCR primers failed to give unambiguous sequences when used in direct sequencing. Templates were sequenced with Thermosequenase (Amersham) and dye-labelled terminators (Li-Cor).

We collected new sequences for eight individuals from five sites: Kissing Point (N=2), Dalrymple Point (N=2), Rose Bay (N=1), Airlie Beach (N=2), and Statue Bay (N=1). These sequences were 473–564 bp in length (excluding the non-coding promoter region). We found four unique haplotypes among these eight individuals (GenBank accession numbers AF509224, AF509225, AY230268, AY246546).

These new sequence data were compared to sequences available for other *Asterina* and *Patiriella* species. These older sequences included a haplotype (U50052) 357 bp in length, consisting of tRNA but no COI sequence, for a *P. pseudoexigua* specimen from a second site in central Queensland at Yeppoon (23°10′S 150°50′E). Other older sequences (U50045–U50058) consisted of both tRNAs and complete coding sequences for COI (totalling about 1915 bp), including a haplotype for a *P. pseudoexigua* specimen from Wanlitung (21°29′N 120°41′E) in Taiwan (U50051), and a haplotype for a specimen of the subspecies *P. pseudoexigua pacifica* from Kushimoto (33°28′N 135°48′E) in Japan (U50057).

The tRNA sequences were aligned using ClustalW (Higgins & Sharp, 1988). For specimens from which we obtained no COI sequence or only partial COI sequence, the unsequenced nucleotide sites were coded as missing. We calculated Kimura two-parameter genetic distances between *P. pseudoexigua* mtDNA haplotypes, and inferred phylogenies for these haplotypes, using the computer software PAUP* (Swofford, 2002). We treated the nucleotide characters as unordered and weighted all characters equally. We used only the tRNA and COI nucleotide sites and did not include the short promoter region in any analyses. Details of the specific phylogenetic methods are given below.

RESULTS

Life history traits of Patiriella pseudoexigua *in Queensland*

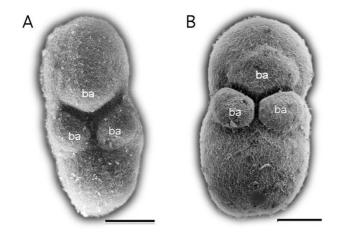The populations from northern Queensland consisted of dioecious free-spawners. The eggs had a mean diameter of

Figure 1. Brachiolaria larvae of *Patiriella pseudoexigua*. (A) Planktonic brachiolaria larva of the dioecious free spawning specimens collected from Dalrymple Point, Queensland (=*Patiriella pseudoexigua*); (B) internally brooded brachiolaria larva dissected from the gonads of specimens collected from Statue Bay, Queensland (=*Patiriella* sp. nov.) with functional brachiolar arms (ba). Note the similarity of the larvae from the two species, despite contrasting location of development. Scale bars: 100 μm.

413 μm (SE=6.4, N=20) and were positively buoyant. Fertilization and development of these sea stars was typical of asteroids with planktonic non-feeding larvae. The embryos developed into yolky brachiolaria larvae in two days (Figure 1A). These larvae swam in the culture dishes propelled by the cilia which were scattered over the epithelial surface (Figure 1A). The larvae did not feed. The attachment complex of three brachiolar arms and a central adhesive disc formed in an anterior, mid-ventral position on the larva. In preparation for metamorphosis advanced larvae attached to the culture dishes with their sticky brachiolar arms and disc. Metamorphosis occurred in approximately nine days. Development of these specimens generally resembled the development of embryos and larvae of *P. pseudoexigua* from populations in Taiwan (Chen & Chen, 1992) (Table 1).

The population at Statue Bay consisted of simultaneous hermaphrodites. Most gonads were a mosaic of oogenic and spermatogenic tissue. The eggs had a mean diameter of 440 μm (SE=6.6, N=8) and were positively buoyant. Fertilization and development of the Statue Bay sea stars occurred in the gonads. It is not known whether self- or cross-fertilization (or both) occurs. The embryos developed through the wrinkled blastula, gastrula, and brachiolaria stages characteristic of *Patiriella* species with planktonic, lecithotrophic development (Byrne et al., 1999a). Early embryos removed from the gonads at the blastula stage completed their development in laboratory culture in 16 days, thus the intragonadal environment was not essential for development. Interestingly, the brachiolaria larvae (Figure 1B) were similar in appearance and behaviour to the planktonic non-feeding larvae of the northern Queensland specimens. They were covered in cilia and swam in the culture dishes. These larvae had three brachiolar arms in an anterior, mid-ventral position and a central adhesive disc. The arms and disc were sticky like those of lecithotrophic planktonic larvae of other asterinids. Larvae *in vitro* attached to the surface of the culture dishes as they became competent to metamorphose in a fashion similar to that of planktonic brachiolaria larvae. Although settlement behaviour was observed *in vitro*, metamorphosis occurs in the gonads and it is unlikely to involve the adhesive features of the brachiolar apparatus.

Juveniles were observed emerging from the gonopores of several individual parents in several different years of

Table 1. *Life history traits for four* Patiriella *species, including three lineages formerly known as* P. pseudoexigua.

Location and site	Proposed taxon name	Breeding system	Egg diameter	Fertilization site	Larval habitat
Japan					
Kushimoto	*Patiriella pacifica*	hermaphrodite	450 μm	internal	benthic
Taiwan					
Wanlitung	*Patiriella* sp.	dioecious	320 μm	external	planktonic
Queensland (north)					
Kissing Point, Rose Bay, Dalrymple Point, Airlie Beach[1]	*Patiriella pseudoexigua sensu stricto*	dioecious	413 μm	external	planktonic
Queensland (central)					
Statue Bay[2]	*Patiriella* sp. nov.	hermaphrodite	440 μm	internal	benthic

[1], Airlie Beach is the type locality for *P. pseudoexigua sensu stricto*. [2], Populations at Yeppoon are probably conspecific with the Statue Bay population but their life histories have not been examined.

laboratory culture. The young stars were about $600\,\mu m$ diameter with two pairs of tube feet per radius. We never observed premetamorphic larval stages in the water of these cultures, but frequently observed newly released metamorphic juvenile stars. Therefore, it seems unlikely that eggs or ciliated swimming larvae are released for planktonic dispersal but we cannot completely rule out this possibility.

This large difference in mode of reproduction, spawning ecology, and larval dispersal suggests that the two sets of Queensland populations are not conspecific. No other asteroid (or any other echinoderm) includes such dramatically different life histories within a single species.

Genetic distances among Patiriella pseudoexigua *haplotypes*

We found large variation in pairwise genetic distances among *P. pseudoexigua* individuals (Table 2). We found five unique haplotypes with different frequencies among the Queensland sites: one (AY246546) occurred five times at Kissing Point, Rose Bay, and Dalrymple Point, while the others occurred just once each. The two haplotypes at Airlie Beach differed from each other by 0.6%, and this was the largest genetic distance found among haplotypes from the northern Queensland sites. Similarly, the two central Queensland haplotypes differed by just 0.6%. These differences are comparable to conspecific haplotype differences in other echinoderms (Palumbi & Wilson, 1990; Arndt & Smith, 1998; Williams, 2000; Flowers & Foltz, 2001).

Genetic distances were larger (1.1–2.1%) between northern and central Queensland haplotypes (Table 2). These distances are comparable to genetic distances between closely related sister species pairs (e.g. *P. vivipara* and *P. parvivipara*), though the latter distances include a large number of rapidly evolving COI nucleotide sites

while the former distances are based mostly on slowly evolving tRNA nucleotide sites (see Hart et al., 1997).

The largest genetic distances among *P. pseudoexigua* lineages were those between the Kushimoto and Wanlitung haplotypes (2.4%) and between these two haplotypes and haplotypes from the Queensland sites (2.7–4.3%). These latter distances are similar to genetic distances between *P. pseudoexigua* haplotypes and other *Patiriella* species from south-eastern Australia (*P. regularis*, *P. exigua*, *P. vivipara*, *P. parvivipara*). The contrast in genetic distances, especially the close genetic distances among Queensland haplotypes from populations with different life histories, suggests that the *P. pseudoexigua* lineages are closely related to each other and probably diverged recently from each other.

Phylogenetic relationships among Patiriella pseudoexigua *and other haplotypes*

We performed heuristic searches of most parsimonious trees, with *Patiriella exigua*, *P. vivipara*, and *P. parvivipara* as the outgroup, gaps coded as a fifth base, and 1000 random taxon addition sequences. This outgroup rooting is consistent with other more comprehensive analyses of mtDNA relationships among asterinid species (Hart et al., 1997), and we obtained the same results when we included a much broader diversity of *Patiriella* and *Asterina* species (Hart et al., 1997; results not shown). Taxon addition order did not affect the phylogenetic result, and we obtained the same result when gap sites were excluded. We found a single most parsimonious tree (Figure 2). This tree is 730 steps long (including insertions and deletions), with a consistency index of 0.87. *Patiriella pseudoexigua* haplotypes formed a monophyletic clade with strong bootstrap support (100%). The Wanlitung haplotype was sister group to the Kushimoto haplotype. The Australian haplotypes formed a weakly supported monophyletic

Table 2. *Kimura two-parameter genetic distances between nine mtDNA haplotypes from six Queensland sites (*Patiriella pseudoexigua sensu stricto *and* Patiriella sp. nov.*),* Patiriella *sp. from Wanlitung, Taiwan,* P. pacifica *from Kushimoto, Japan, and four closely related* Patiriella *species from south-eastern Australia. Genetic distances between lineages thought to be conspecific based on geographic range and life history traits are shown in bold.*

	AB1	AB2	RB1	KP1	KP2	DP1	DP2	SB	Ye	Wa	Ku	*reg*	*exi*	*viv*
Airlie Beach 2	**0.006**													
Rose Bay	**0.000**[1]	**0.006**												
Kissing Point 1	**0.000**[1]	**0.006**	**0.000**[2]											
Kissing Point 2	**0.000**[1]	**0.006**	**0.000**[2]	**0.000**										
Dalrymple Point 1	**0.000**[1]	**0.006**	**0.000**[2]	**0.000**	**0.000**									
Dalrymple Point 2	**0.000**[1]	**0.006**	**0.000**[2]	**0.000**	**0.000**	**0.000**								
Statue Bay	0.020	0.013	0.020	0.020	0.021	0.020	0.020							
Yeppoon	0.021	0.011	0.021	0.021	0.021	0.021	0.021	**0.006**						
Wanlitung[3]	0.036	0.033	0.036	0.036	0.039	0.035	0.035	0.043	0.039					
Kushimoto[3]	0.029	0.027	0.029	0.029	0.030	0.028	0.028	0.036	0.029	*0.024*				
regularis[3]	0.162	0.176	0.162	0.162	0.160	0.163	0.163	0.183	0.148	*0.252*	*0.245*			
exigua[3]	0.151	0.164	0.150	0.150	0.153	0.148	0.148	0.165	0.140	*0.254*	*0.262*	*0.251*		
vivipara[3]	0.148	0.158	0.148	0.148	0.150	0.149	0.149	0.163	0.130	*0.270*	*0.280*	*0.248*	*0.098*	
parvivipara[3]	0.145	0.150	0.145	0.145	0.144	0.142	0.142	0.154	0.141	*0.254*	*0.264*	*0.249*	*0.110*	*0.064*

[1], Among *Patiriella pseudoexigua sensu stricto* haplotypes, the Airlie Beach 1 sequence (AY230268) differed from the Rose Bay, Kissing Point, and Dalrymple Point haplotypes by a single deletion in tRNA-Ala. [2], The Rose Bay sequence (AY246546) was identical to the four *Patiriella pseudoexigua sensu stricto* haplotypes from Kissing Point and Dalrymple Point. [3], Genetic distances among these six haplotypes (in italics) include complete sequences for COI (1554 bp); all other genetic distances include no COI sequence (Yeppoon) or partial COI sequences (all other Queensland haplotypes).
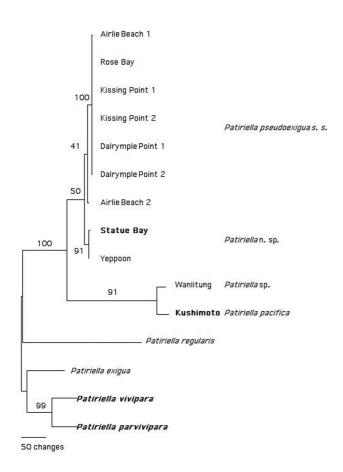
Figure 2. Phylogram of the single most parsimonious tree inferred from mtDNA sequences for *Patiriella pseudoexigua* (identified by collection site) and other closely related asterinid sea stars. Proposed taxon names for four *P. pseudoexigua* lineages are shown to the right of the phylogram. Bold names indicate viviparous brooders (*Patiriella* sp. nov. from Statue Bay; *P. pacifica* from Kushimoto; and *P. vivipara* and *P. parvivipara* from south-eastern Australia). Individuals from the Yeppoon site appear to be conspecific with *Patiriella* sp. nov., but larval forms and life histories at the Yeppoon site have not been directly examined. Numbers above internal branches show bootstrap percentages out of 1000 bootstrap replicates. Branch lengths are proportional to number of nucleotide changes. Scale bar: 50 changes.

group (50%) in which the central Queensland haplotypes from Statue Bay and Yeppoon formed a clade (91%) that was sister group to the northern Queensland haplotypes. Within this latter group, two of the three unique haplotypes formed a strongly supported clade (100%) that was sister group to one of the two haplotypes from Airlie Beach.

These results are consistent with the comparisons of larval form and life history. The *P. pseudoexigua* haplotypes fall into two pairs of reciprocally monophyletic lineages: (Wanlitung + Kushimoto), (northern Queensland + central Queensland). Within each pair, one lineage (Wanlitung or northern Queensland) develops as a planktonic lecithotrophic larva and the other (Kushimoto or central Queensland) is a viviparous brooder (Figure 2; Table 1).

The lone possible exception to this pattern is the Airlie Beach 2 haplotype (AF509224). Bootstrap support for grouping this haplotype with other northern Queensland

haplotypes was low (41%). We used the Kishino–Hasegawa test for log likelihoods in PAUP* (with the HKY85 + G + I substitution model) to compare this most parsimonious tree to two alternative trees: one in which the relationships among the *Patiriella pseudoexigua* lineages are collapsed into four well-supported clades (Airlie Beach 2, other northern Queensland haplotypes, central Queensland haplotypes, and Wanlitung + Kushimoto) arising from a four-way polytomy; and a second in which the Airlie Beach 2 haplotype is sister group to a clade consisting of all other northern plus central Queensland haplotypes (rendering the northern Queensland group paraphyletic). These alternative trees were only 1–4 steps longer than the most parsimonious tree, and the differences in −ln(likelihoods) were small (0.62–1.99), but both alternative trees were significantly less likely than the most parsimonious tree ($P < 0.001$). This result suggests that, in spite of the weak parsimony support for a monophyletic northern Queensland clade, *P. pseudoexigua* lineages in Queensland do consist of a viviparous brooder and its sister species with planktonic lecithotrophic larval development. The diversity of mtDNA sequences from Airlie Beach, and the relatively weak support for a monophyletic northern Queensland clade, may reflect retention of ancestral mtDNA sequence polymorphisms in Queensland that have not yet been resolved into distinctive and monophyletic clades that correspond to species with distinctive morphological and reproductive phenotypes.

DISCUSSION

Our understanding of speciation in the sea has been advanced by recent molecular studies demonstrating the importance of mechanisms that either promote or limit species divergences. Species-specific fertilization mechanisms lead to genetic isolation in some taxa while hybridization and reticulate evolution are common in others (Palumbi, 1994; van Oppen et al., 2001). Closer examination of geographically widespread morphospecies has frequently revealed cryptic species diversity that may be accompanied by reproductive isolation but is not accompanied by obvious morphological diversification (Knowlton, 1993, 2000). As seen here for *Patiriella*, cryptic species have been identified in other sea star taxa through molecular analysis (Williams, 2000; Flowers & Foltz, 2001). Williams (2000) found deep divergences among Indo-Pacific *Linckia guildingi* mtDNA haplotypes that have broadly overlapping geographic distributions, and suggested these lineages probably correspond to biological species. Flowers & Foltz (2001) found cryptic speciation within the north-east Pacific *Hexactis* subgenus of *Leptasterias*, including significant haplotype differences that corresponded with reproductive incompatibility among individuals within nominal morphospecies (Foltz, 1997).

The striking difference in life history mode and divergent phylogenetic relationships among *Patiriella pseudoexigua* specimens strongly suggest that more than one species is lumped into this taxon. No other interpretation seems to account for our observations. Recent hybridization events could distribute similar mtDNA haplotypes among lineages that are not sister taxa, which would result in close relationships between haplotypes but

not between morphospecies (van Oppen et al., 2001). This mechanism could in principle explain relationships such as the one between the Wanlitung and Kushimoto haplotypes. However, outcrossing (whether with conspecifics or with other species) has not been confirmed for any of the internally fertilizing brooders (including the Kushimoto and Statue Bay populations; Table 1) so the potential for hybridization is not known but seems low. Alternatively, some of the genetic variation could represent ancestral polymorphisms that have failed to be sorted into monophyletic lineages since the recent divergence of some clades. Although we cannot rule out this possibility, it seems unlikely because some of the genetic distances between *P. pseudoexigua* haplotypes are similar to or smaller than those between asterinid sister species that have evolved distinctive morphological or reproductive phenotypes (Hart et al., 1997). The genetic distances and divergent phylogenetic relationships among the *P. pseudoexigua* investigated here reflect the evolution of cryptic biological species.

The discovery of cryptic species of *P. pseudoexigua* is perhaps not surprising in light of the broad geographic range of this taxon (Dartnall, 1971; Marsh, 1977; Chen & Chen, 1992; Byrne & Cerra, 1996; Byrne et al., 1999a) and the difficulties experienced by systematists in using adult morphological variation to delineate asterinid species and genera (Dartnall, 1971; Clark & Downey, 1992; Rowe & Gates, 1995). The taxonomy of this family is dynamic: new species are regularly described from new and old collections (Emson & Crump, 1979; Clark, 1983; Campbell & Rowe, 1997; O'Loughlin et al., 2002; M. O'Loughlin, personal communication) while others are synonymized (Rowe & Gates, 1995). However, the extent of undescribed and highly divergent lineages within *P. pseudoexigua* is surprising. Among just seven unique mtDNA haplotypes we found four lineages that must be considered to be distinct species. Moreover, key life history traits such as the location of fertilization and larval development differ among the Queensland populations. The populations in northern Queensland are similar to other asteroids with lecithotrophic development in being dioecious, spawning gametes for external fertilization, and having planktonic development through a non-feeding brachiolaria. In contrast, all individuals at the Statue Bay site are simultaneous hermaphrodites, have internal fertilization and larval development, and give birth directly to crawl-away juveniles, a highly derived form of parental care.

Our data show that the taxonomy of *Patiriella pseudoexigua* must be revised (Table 1). First, *P. pseudoexigua sensu stricto (s. s.)* as defined by Dartnall (1971) should probably be restricted to sea stars with mtDNA haplotypes and life histories similar to those at Airlie Beach. One fortunate corollary of this change is that the well-known planktonic lecithotrophic larval biology of *P. pseudoexigua* specimens from Wanlitung (Chen & Chen, 1992) are still characteristic of the original taxon name, though that original description of larval development came from populations that are not conspecific with the individuals at the type locality (Table 1). Our sampling of Queensland populations suggests that *P. pseudoexigua s. s.* is restricted to northern Queensland, but further sampling will be needed to determine whether its geographic range overlaps with the viviparous brooder. The most southern site at which we

have found planktonic lecithotrophic larval development in *P. pseudoexigua s. s.* (Airlie Beach) is about 350 km north of the site at which we have found viviparous brooders (Statue Bay). We have no data so far to indicate that any of these four species are presently sympatric.

Second, the Taiwan lineage requires a new species name because this lineage is clearly not conspecific with *P. pseudoexigua s. s.* (though adults of both lineages are morphologically similar and the life histories and larval forms are remarkably alike; Table 1). We temporarily refer to this lineage with planktonic lecithotrophic larvae as *Patiriella* sp.

Third, the central Queensland lineage appears to be an undescribed species of viviparous brooders for which no senior name is available. *Patiriella obscura*, described from Queensland at the same time as *P. pseudoexigua* (Dartnall, 1971), is probably not a valid taxon (Rowe & Gates, 1995; A.J. Dartnall, personal communication). We temporarily refer to this undescribed species as *Patiriella* sp. nov.

Fourth, the subspecific status of *P. pseudoexigua pacifica* must be changed: this taxon is reproductively distinct from and not a phylogenetically close relative of *P. pseudoexigua s. s.* The subspecific name should be raised to specific status and this viviparous brooding species should be known as *Patiriella pacifica* (comb. nov.) (Clark, 1983, 1993).

The taxonomic fluidity and reproductive diversity of these morphologically similar sea stars invites a more comprehensive re-examination of species and reproductive diversity within *P. pseudoexigua*. First, we need a broader analysis of genetic diversity within and among species (e.g. Flowers & Foltz, 2001). Multiple nuclear DNA markers may be especially useful for this survey because such markers may be more effective in testing species designations. Second, we need a careful survey of adult morphological variation with respect to type specimens of *Patiriella pseudoexigua* (e.g. Rowe & Gates, 1995). Our four taxonomic suggestions above are contingent on this re-examination of types. Third, we need wherever possible to test the reproductive compatibility of individuals assigned to these taxa because the recent divergence of these species may not necessarily be accompanied by complete reproductive isolation (Byrne & Anderson, 1994; Foltz, 1997). Such tests will be difficult for lineages of self-fertilizing hermaphrodite brooders. The combination of these approaches may eventually help to define more clearly the phylogenetic, morphological, or biological species formerly incorporated in *P. pseudoexigua*.

Finally, we need surveys of modes of larval development within species (however the species are eventually defined by morphological and genetic characters). The morphological similarities between planktonic and brooded larvae in *Patiriella pseudoexigua* and *Patiriella* sp. nov., the small genetic distances between these two lineages, and the ability of the brooded larvae to develop and metamorphose *in vitro*, suggest that brooding could be phenotypically plastic in *Patiriella* sp. nov. Such plasticity is rare but known for other asterinids: *Asterina phylactica* is an external brooder of egg masses, but Strathmann et al. (1984) observed occasional development of embryos and larvae in the gonad. Such plasticity could provide the variation on which selection acts to promote the evolution of planktonic or brooded development.

Intraspecific surveys might also reveal more unrecognized species diversity associated with life history variation. Our results suggest that more unrecognized reproductive and species diversity might be found among lineages formerly known as *Patiriella pseudoexigua*. It is likely that other cryptic species exist, for instance through the Indonesian Archipelago, a region of high marine invertebrate diversity (Briggs, 1974).

Our results increase the number of known parallel derivations of intragonadal viviparous brooding among *Patiriella* species (the only sea star genus in which such brooding is known). The failure of *Patiriella* sp. nov. haplotypes to form a clade with other hermaphrodite intragonadal brooders suggests the parallel derivation of this life history mode in *Patiriella* sp. nov. and *P. pacifica*. The close phylogenetic relationships among species with planktonic (*Patiriella* sp., *P. pseudoexigua s. s.*) and brooded (*Patiriella* sp. nov., *P. pacifica*) larvae, and the morphological similarities between these larvae (Figure 1), are consistent with a direct origin of brooding in *Patiriella* sp. nov. and *P. pacifica*, via the internal fertilization of large eggs. Other species (*P. vivipara*, *P. parvivipara*) may have evolved brooding indirectly via an intermediate form with external fertilization of benthic egg masses (as in *P. exigua*, the sister group to *vivipara + parvivipara*).

Alternatively, if brooding is homologous in *Patiriella* sp. nov. and in *P. pacifica*, then the closely related species with lecithotrophic larvae (*Patiriella* sp., *P. pseudoexigua s. s.*) may have secondarily evolved planktonic dispersal from a recent brooding ancestor. The presence of functional brachiolaria larvae in the gonads of brooders, which can be reared to the juvenile stage without parental care, suggests that a reversal to planktonic development may be possible. Reversals from brooding to planktonic dispersal have probably evolved in other sea star lineages (McEdward, 1992). Some derived life history traits, such as the large and yolk-rich eggs of planktonic or brooded lecithotrophic species, are reversible in asterinids and can give rise to secondarily small eggs in viviparous species that have other modes of nutrition of offspring (Byrne et al., 1999b).

Other patterns of homology and reversal might be inferred from this phylogeny but seem less likely. For example, within the small group of *Patiriella* species shown in Figure 1 (called the 'exigua group' by Dartnall, 1971), only *P. regularis* has the planktotrophic larval form that is widespread among asteroids. An inference of homology of brooding among *Patiriella* sp. nov., *P. pacifica*, *P. vivipara*, and *P. parvivipara* would imply recent reversal to planktonic dispersal and planktotrophic nutrition for *P. regularis*. We doubt that such reversals have occurred among asterinid species because a more comprehensive molecular phylogeny of asterinids (Hart et al., 1997; M. Hart, S. Johnson, J. Addison, M. Byrne, unpublished data), which includes more planktotrophic species, is not consistent with reversals to planktotrophy, and because the planktotrophic larvae of asterinids are so similar in morphology, behaviour, and other details to the planktotrophic larvae of distantly related sea stars. Reversals from nonfeeding, brooded larvae to functionally complex planktotrophic development might not be expected to reproduce the conserved feeding larval form in such exact detail (Chia & Walker, 1991; Strathmann & Eernisse, 1994).

Comparisons of cell lineages, gene expression, or other aspects of the development of viviparous brooding *Patiriella* species (and similar comparisons among asterinids with planktonic or planktotrophic larval forms) might shed light on the possibility that some lineages share brooding as a homology (and that others have become secondarily planktonic). Why this extreme form of viviparity seems to have evolved in *Patiriella* and no other asteroids is an interesting and unanswered question.

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