TWO CRYPTIC AND SYMPATRIC SPECIES OF *PHILINOPSIS* (CEPHALASPIDEA: AGLAJIDAE) IN THE BAHAMAS DISTINGUISHED USING MOLECULAR AND ANATOMICAL DATA

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**ABSTRACT**

Sequence data of nuclear and mitochondrial genes obtained from specimens of *Philinopsis* collected in the Bahamas revealed two genetically distinct groups. Further examination of the shell and male reproductive morphology revealed consistent anatomical differences between them, corroborating that they are distinct species. The two species also display consistent differences in external morphology and colour pattern that became evident only after the molecular information was analysed. A review of the literature suggests that one of the species is *Philinopsis petra*, a widespread species in the Caribbean and northern Brazil, whereas the other is described here as a new species. Although both are sympatric in the Bahamas, they appear to be ecologically analogous, suggesting that they might have speciated allopatrically.

**INTRODUCTION**

*Philinopsis* is a group of aglajid opisthobranchs (Cephalaspidea: Aglajidae) containing about 19 described species. Like most aglajids, species of *Philinopsis* are carnivorous hunters lacking a radula, usually found crawling or burrowing on sand or sandy mud. Rudman (1972) distinguished two groups of *Philinopsis* with different digestive anatomies, the ‘typical’ group includes species with a large, partially eversible buccal bulb that is used to catch bubble shells and other opisthobranchs upon which they prey. The ‘atypical’ group includes species with a long, muscular, tubular buccal bulb. Rudman (1972) suggested that species of this second group could feed using the buccal bulb as a long eversible tube that can catch prey, possibly polychaete worms. Ortea, Moro & Espinosa (2007) recognized a third group of Atlantic species assigned to *Philinopsis*, including animals with a non-eversible buccal bulb and conical spines on the fully calcified shell. They named this third group *Spinoaglaja*, but provided no evidence of monophyly.

In recent years, we have been sequencing a large collection of opisthobranch specimens collected around Stocking Island, Exumas, Bahamas, and comparing the sequences with those of other western Atlantic populations of the same species. The first published paper (Ornelas-Gatdula, DuPont & Valdés, 2011) revealed the existence of a new cryptic species of *Chelidonura* among the Bahamas material. This new species (*C. normani* Ornelas-Gatdula, DuPont & Valdés, 2011) was almost indistinguishable from the common and widespread western Atlantic *Chelidonura berolina* Marcus & Marcus, 1970 (based on external morphological traits) and the two appear to be ecologically analogous (i.e. they occupy the same niche). Differences between *C. berolina* and *C. normani* include a different developmental mode and penial anatomy, as well as a number of molecular apomorphies in mitochondrial and nuclear genes.

In this paper, we examine a similar case, this time with species of *Philinopsis* (*Spinoaglaja sensu* Ortea et al., 2007). The objectives of this paper are to describe a new cryptic species of *Philinopsis* and to provide further data on the intriguing pattern of speciation in the Bahamas first detected by Ornelas-Gatdula et al. (2011).

**MATERIAL AND METHODS**

**Source of specimens**

Thirteen specimens initially identified as *Philinopsis petra* and two specimens of *P. pusa* (Ev. Marcus & Er. Marcus, 1976) were used in this study (Table 1). Most specimens were collected at Stocking Island, Exumas, Bahamas (Fig. 1). Specimens were photographed alive, narcotized using a 1 M solution of MgCl₂, and preserved in 70% ethanol. Two additional specimens were collected in Tobago and Martinique and preserved in 10% formalin. All the specimens examined are deposited at the Natural History Museum of Los Angeles County, USA (abbreviated LACM).
Table 1. Material of Philinopsis examined in this study including locality information, collection voucher numbers and GenBank accession numbers.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Date</th>
<th>Collection number</th>
<th>GenBank accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16S</td>
</tr>
<tr>
<td>P. petra</td>
<td>Monument Beach, Stocking I</td>
<td>9 Mar 2005</td>
<td>LACM 172268</td>
<td>JN825137 – JN825064</td>
</tr>
<tr>
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<td>LACM 172269</td>
<td>JN825138 – JN825065</td>
</tr>
<tr>
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<td>9 Mar 2005</td>
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<td>JN825139 – JN825066</td>
</tr>
<tr>
<td>P. petra</td>
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<td>9 Mar 2005</td>
<td>LACM 172271</td>
<td>JX188004 – –</td>
</tr>
<tr>
<td>P. petra</td>
<td>Hole 1, Stocking I</td>
<td>8 Feb 2010</td>
<td>LACM 178469</td>
<td>JX188009 – –</td>
</tr>
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<td>19 Jan 2010</td>
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<td>JX188007 – –</td>
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<td>8 Feb 2010</td>
<td>LACM 178471</td>
<td>JX188010 – –</td>
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<td>P. petra</td>
<td>Store Bay, Tobago</td>
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<td>LACM 178472</td>
<td>–</td>
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<tr>
<td>P. petra</td>
<td>Cave Point, Martinique</td>
<td>Jun 1986</td>
<td>LACM 178473</td>
<td>–</td>
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<tr>
<td>P. anneae</td>
<td>Sand Dollar Beach, Stocking I</td>
<td>21 Jan 2004</td>
<td>LACM 3227</td>
<td>JX188005 – –</td>
</tr>
<tr>
<td>P. anneae</td>
<td>Sand Dollar Beach, Stocking I</td>
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<td>LACM 3226</td>
<td>JX188006 – JX188012 –</td>
</tr>
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<td>P. anneae</td>
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<td>– – – – – –</td>
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<td>P. anneae</td>
<td>Hole 1, Stocking I</td>
<td>8 Feb 2010</td>
<td>LACM 3224</td>
<td>JX188008 – –</td>
</tr>
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<td>P. pusa</td>
<td>Gaviota Bay, Stocking I</td>
<td>20 Dec 2005</td>
<td>LACM 173220</td>
<td>JN825144 – JN825072</td>
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<td>P. pusa</td>
<td>Hurricane Hole, Stocking I</td>
<td>11 Jan 2006</td>
<td>LACM 173221</td>
<td>JN825145 – JN825200 – JN825073</td>
</tr>
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</table>

*Specimens examined morphologically.

Figure 1. Map of localities where the specimens included in this study were collected.
Morphological examination

Ten specimens were dissected and the features of the male reproductive system were examined and photographed using a dissecting microscope Leica EZ4D. The shell of each specimen was dissected and the surrounding tissue dissolved with NaOH. Shells were rinsed in water, dried, mounted and sputter coated for examination with a scanning electron microscope (SEM) Hitachi S-3000N at the LACM.

DNA extraction

DNA extraction was performed using either a hot Chelex® protocol or the DNeasy® Blood & Tissue Kit (Qiagen). C. 1–3 mg of the foot was cut into small pieces for extraction for both protocols. For the Chelex® extraction, the foot tissue was rinsed and rehydrated using 1.0 ml of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 20 min. A 10% (w/v) Chelex® 100 (100–200 mesh, sodium form, Bio-Rad) solution was prepared using TE buffer. After rehydration, the tissue mixture was centrifuged, 975.00 μl of the supernatant was removed and 175.00 μl of the Chelex® solution was added. Samples were then heated in a water bath at 56°C for 20 min, heated in a 100°C heating block for 8 min and the supernatant was used for polymerase chain reaction (PCR). The DNeasy protocol supplied by the manufacturer was followed, with some modifications. The elution step was modified such that the first elution was collected using 100.00 μl of Buffer AE and was also allowed to incubate at room temperature for 5 min before centrifugation. In a new test tube, a second elution step was conducted using 200.00 μl of Buffer AE and was also allowed to incubate at room temperature for 5 min before centrifugation. The first elution was used for PCR.

PCR amplification and sequencing

Histone-3 universal primers (H3AF 5′-ATATCCCTTGCGGATGATGTCGAC-3′, H3AR 5′-ATATCCCTTGCGGATGATGTCGAC-3′ developed by Colgan et al., 1998), 16S rRNA universal primers (16S ar-L 5′-GCGCTGTTATCAAAAACAT-3′, 16S br-H 5′-CCGGTCTGAACATCAGCTCGT-3′ developed by Palumbi, 1996) and COI universal primers (LCO1490 5′-GGTCAACAAATATCAAAAAATATGG-3′, HCO2198, 5′-TAAATCTTCAGGGTACCAAAAAATATC-3′ developed by Folmer et al., 1994) were used to amplify the regions of interest for all specimens. The master mix was prepared using 34.75 μl H2O2, 5.00 μl Buffer B (ExACTGene, Fisher Scientific), 5.00 μl 25 mM MgCl2, 1.00 μl 40 mM dNTPs, 1.00 μl 10 mM primer 1, 1.00 μl primer 2, 0.25 μl 5 mM/mg Taq and 2.00 μl extracted DNA. Reaction conditions for H3 and 16S were as follows: an initial denaturation of 94°C for 2 min, 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1 min, followed by a final elongation of 72°C for 7 min. Reaction conditions for COI consisted of an initial denaturation of 95°C for 3 min, 35 cycles of 94°C for 45 s, 45°C for 45 s and 72°C for 2 min, followed by a final elongation step of 72°C for 10 min.

PCR products yielding bands of appropriate size (c. 375 bp for H3, 475 bp for 16S and 700 bp for COI) were purified using the Montage PCR Cleanup Kit (Millipore). Cleaned PCR samples were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Each primer was diluted to 2.0 pmol/μl to send out for sequencing with the PCR products. PCR products were diluted to 6.0, 7.5 and 11.5 ng/μl for H3, 16S and COI, respectively. Samples were sequenced at the City of Hope DNA Sequencing Laboratory (Duarte, CA) using chemistry types BigDye v. 1.1 for fragments less than 500 bp and BigDye v. 3.1 for fragments larger than 500 bp.

Analyses

Sequences for each gene were assembled and edited using Geneious Pro v. 4.7.4 (Drummond, Ashton & Cheung, 2009). Geneious was also used to extract the consensus sequence between the primer regions, to construct the alignment for each gene using the default parameters and to concatenate the alignments. The sequences were not trimmed after alignment. A total of 328 bp for H3, 409–410 bp for 16S and 658 bp for COI were used for the phylogenetic analyses.

To assess whether H3, 16S and COI have significantly conflicting signals, the incongruence length difference (ILD) test (Mickevich & Farris, 1981; Farris et al., 1994), implemented in PAUP® v. 4.0 as the partition homogeneity test (Swoford, 2002), was conducted for all genes combined.

The levels of saturation for each gene and for the first and second versus third codon positions of COI and H3 were investigated using the substitution saturation test developed by Xia et al. (2003) and Xia & Lemey (2009), implemented in the program DAMBE (Xia & Xie, 2001).

The Akaike information criterion (Akaike, 1974) was executed in MrModeltest (Nylander, 2004) to determine the best-fit model of evolution (Table 2). Maximum likelihood analyses were conducted with the program GARLI v0.96b8 (Zwickl, 2006). Default parameters were used to run three different GARLI searches of 10 replicates each and a total of 2000 bootstrap replicates were performed to assess the robustness of each clade (Felsenstein, 1985). Bayesian analyses were executed in MrBayes v. 3.2.1 (Huelsenbeck & Ronquist, 2001), partitioned by gene (unlinked). The Markov-chain Monte Carlo analysis was run with two runs of six chains for 10 million generations, with sampling every 100 generations. The default 25% burn-in was applied before constructing majority-rule consensus trees.

Diagnostic nucleotides for each clade were identified visually in the alignments after collapsing identical haplotypes using the program Collapse v. 1.2 (Posada, 2004).

The Species Delimitation plugin (Masters, Fan & Ross, 2011) for Geneious was used to provide a statistical framework to help determine whether clades obtained in the phylogenetic analyses were genetically distinct from one another.

Table 2. Summary of each dataset used for the Bayesian and maximum likelihood analysis of all species, with the best-fit evolutionary models and estimated parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>H3</th>
<th>16S</th>
<th>COI</th>
<th>H3+16S+COI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of included characters</td>
<td>328</td>
<td>413</td>
<td>658</td>
<td>1399</td>
</tr>
<tr>
<td>Best-fit model</td>
<td>HKY+I</td>
<td>HKY</td>
<td>GTR+I</td>
<td>GTR+I+G</td>
</tr>
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<td>Frequency A</td>
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<td>Frequency C</td>
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<td>0.1510</td>
<td>0.1554</td>
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<td>Frequency G</td>
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<tr>
<td>Frequency T</td>
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<td>0.4146</td>
<td>0.3312</td>
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<tr>
<td>R-matrix [A-C]</td>
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<td>0.0000</td>
<td>0.6074</td>
</tr>
<tr>
<td>R-matrix [C-G]</td>
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<td>–</td>
<td>0.0000</td>
<td>0.2661</td>
</tr>
<tr>
<td>R-matrix [C-T]</td>
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<td>–</td>
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<td>8.3427</td>
</tr>
<tr>
<td>R-matrix [G-T]</td>
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<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>Proportion of invariant sites (I)</td>
<td>0.8446</td>
<td>0.6836</td>
<td>0.6043</td>
<td></td>
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</tbody>
</table>
analysis have identity as distinct species. The statistics implemented were the ratio between the mean distance within the members of the clade and the mean distance of those individuals to the nearest clade (the larger the value of this ratio is, the more distinct is the identity of the group) and the pID, which represents the mean probability, 95% confidence interval, for a member of the putative species to fit inside (strict pID), or at least to be the sister group (liberal pID) of the clade made up by the other individuals belonging to this species.

RESULTS

Molecular data

The saturation analysis showed insignificant levels of saturation for all three genes: COI: Iss (0.4351) < Iss.c (0.7738), $P = 0.000$; 16S: Iss (0.0665) < Iss.c (0.7123), $P = 0.000$; H3: Iss (0.3091) < Iss.c (0.7363), $P = 0.000$, even when the third codon positions of COI: Iss (0.4167) < Iss.c (0.7409), $P = 0.000$ and H3: Iss (0.0103) < Iss.c (0.7141), $P = 0.000$ were analysed independently. The ILD test showed no significant conflicting signals between the three genes combined ($P = 1.0$), COI vs. H3 ($P = 0.688$), COI vs. 16S ($P = 0.229$) and 16S vs. H3 ($P = 0.606$).

The combined analysis of the three genes (H3, 16S and COI) produced consensus bootstrap and Bayesian trees in which two main clades are well supported (Fig. 2). One of the clades (clade 1), with a posterior probability of 1 and bootstrap value of 100, includes four specimens. The other clade (clade 2), with a posterior probability of 0.98 and a bootstrap value of 72, includes seven specimens. A number of substitutions unique for each clade in all three genes (3 in H3, 11 in 16S and 69 in COI) provided a strong phylogenetic signal.

Morphology

The observation of the shell and penial morphology, as well as the external coloration and body shape, revealed consistent differences between the two clades distinguished using molecular data (Fig. 2). Clade 1 includes animals generally yellow-orange with elongate, smooth bodies and a series of denser concentrations of white pigment on the head (where it forms three large spots arranged on a transverse line), the posterior end of the cephalic shield, the posterior end of the visceral mass (where it forms an inverted triangle) and the edge of the parapodia. In contrast specimens in clade 2 vary from dark brown to light yellow-orange, their bodies are wider and have a visible transverse lateral expansion of the body on each side, near the end of the cephalic shield; the white pigment concentrations are located on the head, the posterior end of the cephalic shield, the posterior end of the visceral mass and on the lateral expansions of the body. All specimens examined in clade 1 have a well-calcified shell with a single conical spine on the apex, whereas all animals of clade 2 examined have two conical spines on the apex of the shell (Fig. 2). The male reproductive organs of members of clade 1 have a prostate much larger than that of specimens in clade 2 and a comparatively smaller penis (Fig. 3). Additional specimens collected from Tobago and Martinique were preserved in formalin, thus they were unsuitable for molecular work and the shells were dissolved. However, examination of the male reproductive

Figure 2. Bayesian tree of Philinopsis specimens examined, including posterior probabilities and bootstrap values from the maximum likelihood analysis. The shell apical morphology and male reproductive anatomy for the main clades is illustrated as well as the external morphology of all specimens.
morphology of the reproductive male anatomy (Marcus, 1976) and the external body shape and coloration (Ortea & Espinosa, 2001; Ortea et al., 2007). The possible synonymy between P. petra and P. aeci and the taxonomic placement of the two nominal species are discussed below. On the contrary, there are no published records of any western Atlantic species of Aglajidae with the characteristics of members of clade 1, thus a new species name (Philinopsis anneae) is here introduced for this species.

Valdés et al. (2006) illustrated specimens of P. petra from Martinique, Cayman Islands and Honduras, all matching the colouration and body morphology of P. petra. Additional photos from Florida provided by Anne DuPont also match the P. petra colour pattern and body shape. No illustrations of specimens with the colour pattern of P. anneae appear to exist from locations other than the Bahamas. This suggests that P. anneae is likely endemic to the Bahamas.

**SYSTEMATIC DESCRIPTION**

*Philinopsis anneae* new species (Figs 3C, 4)

*Type material:* Holotype: Hole 1 (23°31’20”N, 75°45’20”W), Stocking I., Exumas, Bahamas, 8 February 2010, leg. Anne DuPont (LACM 3224). Paratypes: Sand Dollar Beach (23°30’55”N, 75°44’36”W), Stocking I., Exumas, Bahamas, 26 December 2003, 1 specimen, leg. Anne DuPont (LACM 3225); 21 January 2004, 2 specimens, leg. Anne DuPont (LACM 3226, LACM 3227).

*Etymology:* This species is named in honour of our friend and colleague Anne DuPont, who collected all the specimens studied in this paper.

*Diagnosis:* Body cylindrical, elongate, smooth (Fig. 4A). Cephalic shield slightly longer than visceral mass when animal is in motion. Posterior end of body with two triangular extensions (tails) similar in size and shape. Posterior edge of cephalic shield with a low crest. Parapodia short, covering sides of body. Background colour varies from dark orange to yellow, with white pigment distributed over entire body. Denser concentrations of white pigment occur consistently on several parts of body including head, where it forms three large spots arranged on a transverse line; tapering crest at posterior end of cephalic shield; posterior end of visceral mass, where it forms an inverted triangle; and edge of parapodia. Shell calcified (Fig. 4B), elongate with a conspicuous conical spine on apical end. Digestive system with large, non-eversible buccal bulb. Penis conical, directly connected to elongate prostate (Fig. 3C).

* Biology: Specimens were found crawling on sand during the day. When disturbed they released an orange substance.

**DISCUSSION**

*Taxonomic status of Philinopsis petra and Spinoaglaja*

*Philinopsis petra* was originally described from northern Brazil (Pernambuco) by Marcus (1976) as *Chelidonura petra*. The original description was based on a preserved specimen and there is no information on the colour of the live animal. However, Marcus (1976) described the internal anatomy in detail, including drawings of the shell, nervous system and male and female reproductive systems. The main diagnostic characteristic of this species is the presence of a well-calcified shell with two spines on the apex. Additionally, the penis is wider and about as long as the prostate. Ortea & Espinosa (2001)
described \( P. \text{acci} \) from Costa Rica, based only on external characteristics. They compared their new species with \( P. \text{pusa} \) Ev. Marcus & Er. Marcus, 1967 but not with \( P. \text{petra} \).

Valdés et al. (2006) suggested that \( P. \text{petra} \) should be transferred to \( 
\text{Philinopsis} \) and \( P. \text{acci} \) should be synonymized with it, as these two names probably refer to the same species. In a subsequent paper, Ortea et al. (2007) redescribed \( P. \text{acci} \) including additional specimens from the Bahamas and Cuba and provided a drawing and description of the shell. The shell of \( P. \text{acci} \) is very similar to that described for \( P. \text{petra} \) and Ortea et al. (2007) recognized the possibility that these two species could be synonyms, but preferred to maintain them as different species until new material became available, particularly specimens with shells of intermediate size between 4.5 \( \times \) 3 mm (\( P. \text{petra} \) holotype) and 1.6 \( \times \) 1 mm (specimen of \( P. \text{acci} \) from Cuba). One of the specimens from Bahamas here examined and assigned to \( P. \text{petra} \) has a shell measuring 2.5 \( \times \) 1.7 mm and its morphology is very similar to that of the shells of \( P. \text{petra} \) and \( P. \text{acci} \), including the presence of two apical spines. Thus we consider these two species to be synonymous. In addition, Ortea et al. (2007) synonymized \( \text{Melanochlamys} \ \text{wildpetri} \) Ortea, Moro & Bacallado, 2003, an eastern Atlantic species, with \( P. \text{acci} \). Evidence from molecular studies of other species of Aglajidae (Ornelas-Gatdula et al., 2011) suggests the absence of gene flow across the Atlantic Ocean. The synonymization of \( M. \ \text{wildpetri} \) with \( P. \text{acci} \) or \( P. \text{petra} \) could have been premature until molecular data for the former become available.

Ortea et al. (2007) introduced the new genus name \( 
\text{Spinounglaja} \) for species of Aglajidae previously included in \( 
\text{Philinopsis} \) with apical spines on the shell and lacking a raised posterior edge of the cephalic shield (\( P. \text{petra} \) and \( P. \text{acci} \)). A preliminary molecular phylogeny of the Aglajidae (in preparation) shows that \( P. \text{petra} \) and \( P. \text{anneae} \) appear to be sister species (which is also supported by the presence of the unique shell and cephalic shield features mentioned above), but \( 
\text{Spinounglaja} \ \{P. \text{petra} + P. \text{anneae}\} \) is nested within \( 
\text{Philinopsis} \) and is therefore considered a synonym of the latter.

Cryptic species

We consider \( P. \text{petra} \) and \( P. \text{anneae} \) to be cryptic species because, even though \( P. \text{anneae} \) can be distinguished from \( P. \text{petra} \) by some external traits, these differences only became evident in light of molecular data. The main differences between these two species are molecular and internal (shell and reproductive morphology) and therefore not visible in live animals.

Mode of speciation and biogeography

One of the most interesting aspects of the biology of \( P. \text{anneae} \) is the fact that it is completely sympatric with the more widespread \( P. \text{petra} \). Coyne & Orr (2004) and Bolnick & Fitzpatrick (2007) proposed that sister species with overlapping ranges, inhabiting areas in which there is no evidence of present or past biogeographic barriers to dispersal, could be potential cases of sympatric speciation. Friesen et al. (2007) listed various sympatric models in which reproductive isolation could evolve in the absence of geographic isolation through polyploidization (such as in flowering plants), nonrandom mating driven by ecological isolation (e.g. host preference, differential migratory route, allochrony) and disruptive selection (e.g. frequency-dependent competition, divergent sexual selection). Thus, evidence of competitive exclusion (character displacement), divergent sexual selection or allochrony between the two species should be necessary to identify cases of sympatric speciation in animals.

Field observations suggest that \( P. \text{petra} \) and \( P. \text{anneae} \) in the Bahamas are ecologically analogous. Although the specific diet of these two species is unknown, adult animals of both species were found in the same environment, at the same time of the day and sometimes together (Table 4) and display a similar behaviour. This seems to reject any hypotheses of niche partitioning or allochrony.

Differences in the male reproductively anatomy between \( P. \text{petra} \) and \( P. \text{anneae} \), particularly the larger prostate size of \( P. \text{anneae} \) in relation to the penis, would suggest the action of sexual selection. Anthes et al. (2008) found that reproductive trait variation in Aglajidae is mediated by sexual coevolution and low gregariousness was associated with long, reciprocal copulations. It is conceivable that a larger prostate could be an adaptation to longer copulations or sperm competition in the less common, geographically restricted \( P. \text{anneae} \), but because this scenario requires initial populations with different degrees of gregariousness it is unlikely it could occur sympatrically.

All available evidence suggests that sympatric speciation is not a likely explanation for the morphological and behavioral traits observed in \( P. \text{petra} \) and \( P. \text{anneae} \).

Ornelas-Gatdula et al. (2011) found a similar situation in another aglajid group. \( 
\text{Chelidonura} \ \text{normani} \) is a Bahamas endemic closely related to two other widespread tropical Atlantic species, \( 
\text{C. berolina} \) (also found in the Bahamas) and \( 
\text{C. africana} \) Pruvot-Fol, 1953 (from the eastern Atlantic), \( 
\text{Chelidonura} \ \text{normani} \) and \( 
\text{C. berolina} \) differ in their developmental modes, suggesting that the nonplanktrophic \( 
\text{C. normani} \) is adapted to recruit locally, maintaining a restricted range. \( 
\text{Chelidonura} \ \text{normani} \) and \( 
\text{C. berolina} \) are likewise ecological analogues and were often found together in the same environment at the same time of the day; however no evidence of gene flow (hybridization) between them was found, which could have suggested reinforcement.
Several studies have investigated the genetic structure (Mitton, Berg & Orr, 1989; Díaz-Ferguson et al., 2010, 2012) and patterns of diversification (Lee & O’Foighil, 2004, 2005) of coastal tropical western Atlantic marine molluscs. Some of these studies revealed the existence of genetically distinct clades in the Bahamas (Lee & O’Foighil, 2004, 2005; Díaz-Ferguson et al., 2010), which have also been detected in reef fishes and corals (Taylor & Hellberg, 2003; Galindo, Olson & Palumbi, 2006). However, other Western Atlantic molluscs show little to no population structure (Mitton, Berg & Orr, 1989) or patterns of genetic differentiation not including distinct Bahamas clades (Díaz-Ferguson et al., 2012). Most authors agree that it is difficult to make generalizations about connectivity and diversification patterns of marine organisms in the tropical western Atlantic (Lee & O’Foighil, 2005; Vollmer & Palumbi, 2007; Díaz-Ferguson et al., 2012). The complex patterns of connectivity in the western tropical Atlantic and the existence of oceanographic barriers to gene flow (Cowen et al., 2006) are a possible explanation for the maintenance of allopatric distributions of genetically distinct species or populations over evolutionary timescales. The Bahamas are largely isolated from the rest of the Caribbean, except for a minor exchange from the north coast of Cuba and Haiti (Cowen et al., 2006), which would predict a certain level of genetic isolation in Bahamas populations. Galindo et al. (2006) developed a genetic model that uses connectivity estimates from oceanographic models. This model predicts empirical genetic patterns resulting from larval dispersal in a western Atlantic coral, including the isolation of the Bahamas and an east–west divergence near Puerto Rico. On the contrary, other authors have argued that post-recruitment ecological factors rather than limited connectivity are the primary cause of allopatric ranges of genetically distinct species or populations (Lee & O’Foighil, 2005). Much less attention has been paid to historical biogeography and the potential role of the formation and disappearance of barriers to gene flow in the divergence of allopatric clades over geological timescales.

Our data seem to indicate that the Bahamas endemics *P. anneae* and *C. normani* have evolved allopatrically from their sister taxa *P. pusa* and *C. berolina*. Because the geographic range of the widespread species *P. petra* and *C. berolina* include the western tropical Atlantic from Brazil to the Bahamas, whereas *P. anneae* and *C. normani* have only been found in the Bahamas, it seems logical to assume that a partial or complete interruption of gene flow between the Bahamas and other western Atlantic regions in the past has caused this pattern, with a subsequent recolonization of the Bahamas by *P. petra* and *C. berolina*. Hypotheses on the isolation of the Bahamas based on low connectivity or post-recruitment ecological factors do not fully explain the pattern of genetic differentiation and range overlap here observed. At present we can only speculate as to the causes of this pattern, although the hypothesis of formation of a barrier between the Bahamas and the rest of the Caribbean for long enough to result in speciation, followed by the subsequent relaxation of the barrier allowing recolonization by widespread species seems a good fit for our observations. A much broader sampling effort in the western Atlantic including other species with a similar ecology is necessary to test this hypothesis properly.

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