

Molecular phylogeny of enigmatic Caribbean spider crabs from the *Mithrax*–*Mithraculus* species complex (Brachyura: Majidae: Mithracinae): ecological diversity and a formal test of genera monophyly

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Crabs from the Mithrax–Mithraculus species complex are known for their diversity of lifestyles, habitats, and coloration. This group includes small, colourful, symbiotic species and much larger, reef-dwelling crabs targeted by fishermen. The evolutionary relationships between the species within this complex are not well-defined. Previous studies based upon morphological characters have proposed the separation of this complex into two genera (Mithrax and Mithraculus), but cladistic analyses based upon larval characters do not support this division. A molecular phylogeny of the group may help to resolve this long-standing taxonomic question and shed light on the ecological conditions driving the diversity of these crabs. Using a 550-bp alignment of the 16S rRNA mitochondrial DNA segment we examined the phylogenetic relationships between 8 species within the Mithrax–Mithraculus complex native to the Caribbean. The resulting phylogeny indicates that this complex is paraphyletic, as it includes the genus Microphrys. The analyses revealed a well-supported, monophyletic group containing four species of Mithraculus (M. cinctimanus, M. coryphe, M. sculptus and M. forceps) and supported one pair of sister species from the genus Mithrax (M. caribbaeus and M. spinosissimus). No complete segregation of species, according to genera, was evident, however, from tree topologies. Bayesian-factor analyses revealed strong support for the unconstrained tree instead of alternative trees in which monophyly of the two genera was forced. Thus, the present molecular phylogeny does not support the separation of the species within this complex into the genera Mithrax and Mithraculus. A review of the literature demonstrated considerable phenotypic variation within monophyletic clades in this group.

Keywords: *Mithrax*, *Mithraculus*, Mithracinae, emerald crab, spiny crab, spider crab, Venezuela

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INTRODUCTION

Among the Decapoda, crabs from the infraorder Brachyura are recognized for their astonishing anatomical, ecological, and behavioural diversity (Martin & Davis, 2001; Ng *et al.*, 2008). During the last decade, various phylogenetic studies have supported monophyly of the Brachyura and have begun to reveal internal relationships and clarify the position of this clade relative to other major, decapod lineages (Scholtz & Richter, 1995; Sturmbauer *et al.*, 1996; Ah Yong & O'Meally, 2004; Porter *et al.*, 2005; Schubart *et al.*, 2006). Recent studies also have uncovered an evolutionary history much more complex than originally recognized (Ah Yong & O'Meally, 2004; Porter *et al.*, 2005; Schubart *et al.*, 2006; Ah Yong *et al.*,

2007; Hultgren & Stachowicz, 2008). Furthermore, some systematic studies, combined with behavioural and ecological observations, have exposed the evolutionary basis for most peculiar behaviours and the conditions favouring them (land colonization and subsequent evolution of cooperative behaviour in bromeliad dwelling crabs—Schubart *et al.*, 1998; independent evolutionary origins of tree climbing behaviours—Fratini *et al.*, 2005; increasing complexity of courtship through time—Sturmbauer *et al.*, 1996; origins and evolutionary trade-offs associated with camouflage—Hultgren & Stachowicz, 2009). Albeit, our knowledge of the evolutionary history of true crabs has increased substantially, the internal relationships between many genera and families still remain unknown. This remaining ignorance is mostly a consequence of high morphological diversity, convergence of adult features at high taxonomic levels, difficulties sampling extreme environments colonized by some groups (e.g. hydrothermal vents), and the burden of taxonomic expertise and costs of molecular techniques (see Ah Yong *et al.*, 2007).

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Among spider crabs of the superfamily Majoidea (*sensu* Ng *et al.*, 2008), one of the most species-rich clades of brachyurans, the *Mithrax*–*Mithraculus* species complex is of particular interest. Crabs from these two genera demonstrate a considerable diversity of lifestyles, body sizes, habitats, and coloration. The 25 recognized species (Ng *et al.*, 2008; but see Boschi, 2000) are restricted to the Americas and inhabit shallow or relatively deep, warm-temperate, subtropical, and tropical, rocky and coral reefs. Some species live in groups (small aggregations); whereas, others remain solitarily within shelters (*M. spinosissimus*—Rathbun, 1925; personal observations). Several species with cryptic coloration dwell under rocks or in crevices, but other, more-colourful species inhabit sea anemones in shallow reefs (*M. commensalis*—Manning, 1970). These colourful species, and other less-conspicuous, congenic crabs, are capable of controlling aquarium pests, and thus, are heavily traded in the marine aquarium industry (Rhyne *et al.*, 2004). Lastly, most species are small (e.g. *M. forceps*, *M. sculptus*, *M. cinctimanus*, *M. coryphe* and *M. ruber*) but some grow up to ~20 cm carapace length (e.g. *M. spinosissimus*—Rathbun, 1925). These large species are of economic importance as targets of local fisheries in developing countries. The ecological diversity of crabs from the genera *Mithrax* and *Mithraculus* has already attracted the attention of systematists, fisheries biologists and aquaculturists. The same diversity suggests that these crabs are ideal model systems to explore the role of environmental conditions in explaining evolutionary innovations in the marine environment. Phylogenetic studies in this *Mithrax*–*Mithraculus* species complex are warranted because of the implications for conservation biology and biodiversity.

The genus *Mithrax* (Desmarest, 1823) was originally designated to contain various species of majids (currently comprising the subfamily Mithracinae McLeay 1838) characterized, among other traits, by an ovate or oblong carapace (either broader than long or slightly longer than broad) with anterolateral margins having four or three spines or lobes behind the orbital front, two small horns at the rostrum, and robust legs (Rathbun, 1925). Later, White (1847) proposed the separation of this genus in two subgenera, *Mithrax* and *Mithraculus*. *Mithraculus* contains species, including the type *M. sculptus*, which differ from members of *Mithrax* by having a smooth carapace with oblique branchial sulci, very short rostral horns, and minor teeth at the orbits that are inconspicuous and tubercle-like (Rathbun, 1925). All species but one (*M. cinctimanus*) in this subgenus are characterized by having a carapace broader than long. White's suggestion was followed by scientists throughout the 20th Century, including Rathbun (1925), and these two subgenera were elevated to the genus level by Wagner (1990), a view supported by Ng *et al.* (2008). Most recently, detailed morphological examination of larval characters (e.g. setae distribution) revealed only minor differences among species from *Mithrax* (e.g. *M. caribbaeus*, *M. hispidus* and *M. pleuracanthus*) and *Mithraculus* (e.g. *M. sculptus*, *M. coryphe*, and *M. forceps*) (Provenzano & Brownell, 1977; Scotto & Gore, 1980; Bolaños & Scelzo, 1981; Goy *et al.*, 1981; Fransozo & Hebling, 1982; Bolaños *et al.*, 1990, 2000; Santana *et al.*, 2003; Rhyne *et al.*, 2004). The strong similarities in larval characters do not support the separation of this species complex into two genera, as proposed by studies based upon adult morphology (Wagner, 1990). Furthermore, additional cladistic analyses with larval characters have been unable to

support the monophyly of the Mithracinae and its position among other majoid lineages (Pohle & Marques, 2000). Comprehensive taxonomic studies, including adult morphology, larval characters, and molecular markers are needed to resolve outstanding systematic problems in the *Mithrax*–*Mithraculus* species complex and the Mithracinae (Hultgren & Stachowicz, 2008).

This study represents the first contribution to the phylogeny of crabs from the genera *Mithrax* and *Mithraculus* that uses molecular characters. We have focused specifically on addressing the hypothesis of monophyly of these two genera. We predicted that, a molecular phylogeny of the species included within the two genera should segregate the species into two well-supported monophyletic clades. We present a molecular phylogeny of this species complex based upon the large-subunit, 16S mitochondrial rRNA gene applied to eight available species from the two genera plus outgroups.

MATERIALS AND METHODS

A total of 18 specimens from eight species of crabs from the genera *Mithrax* and *Mithraculus* were included in the present study (Table 1). Two specimens of *Microphrys bicornutus*, one specimen of *Pitho lherminieri* (Mithracinae) and another of *Herbstia parvifrons* (Pisinae) were also included as outgroups during the phylogenetic analyses. Most crab species were collected between 2005 and 2008 from different localities at Isla Margarita, Venezuela, and Carrie Bow Caye, Belize. Immediately after collection, specimens were preserved in 95–99% ethanol. In the laboratory, the different species were identified using descriptions of Rathbun (1925), Rodríguez (1980), Williams (1984), Abele & Kim (1986), Wagner (1990), Melo (1996) and Bolaños *et al.* (2000).

Total genomic DNA was extracted from leg-muscle tissue using the QIAGEN® DNeasy® Blood and Tissue Kit following the manufacturer's protocol. The polymerase chain reaction (PCR) was used to amplify an approximately 550 base-pair (bp) region (excluding primers) of the 16S rRNA, using the primers 16ar (5'-CGCCTGTTTAACAAAAACAT-3') and 16br (5'-CCGGTTTGAAGTCAGATCACGT-3') (Palumbi *et al.*, 1991), or 16L2 (5'-TGCCTGTTTATCAAAAACAT-3') and 16L472 (5'-AGATAGAAACCAACCTGG-3') (Schubart *et al.*, 2000; Baeza *et al.*, 2009). Standard PCR 25- μ l reactions (2.5 μ l of 10 \times Taq buffer, 2 μ l of 50 mM MgCl₂, 2.5 μ l of 10 mM dNTPs, 2.5 μ l each of the two primers (10 mM), 0.625 U Taq, 1.25 μ l of 20 mM BSI and 8.625 μ l double distilled water) were performed on a Peltier Thermal Cycler (DYAD®) under the following conditions: initial denaturation at 96°C for 4 minutes followed by 40 cycles of 94°C for 45 seconds, 52–57°C (depending on the species) for 1 minute, and 72°C for 1 minute, followed by chain extension at 72°C for 10 minutes. PCR products were purified with ExoSapIT (a mixture of exonuclease and shrimp alkali phosphatase, Amersham Pharmacia) and then sent for sequencing with the ABI Big Dye Terminator Mix (Applied Biosystems) to the Laboratory of Analytical Biology of the National Museum of Natural History (LAB-NMNH, Maryland), which is equipped with an ABI Prism 3730xl Genetic Analyser (Applied Biosystems automated sequencer). All sequences were confirmed by sequencing both strands, and a consensus sequence for the two strands was obtained using the software

Table 1. *Mithrax*–*Mithraculus* species and other majoid crabs used for the phylogeny reconstruction. The sites of collection, museum catalogue number (CN; MOBR: Museo Oceanológico Hermano Benigno Roman, Estación de Investigaciones Marinas de Margarita, Fundación La Salle de Ciencias Naturales, Venezuela; UMML, University of Miami Marine Laboratories, Rosenstiel School of Marine Science, University of Miami) and the Genbank accession numbers (GenBank) are shown in bold for each species. NA, not available.

Species	Collection site	CN/GenBank
<i>Mithraculus cinctimanus</i>	Carrie Bow Key, Belize	GQ438762 /UMML32.9623
<i>Mithraculus coryphe</i> 01	Isla Margarita, Venezuela	GQ438771 /MOBR-C-1535
<i>Mithraculus coryphe</i> 02	Isla Margarita, Venezuela	GQ438778 /MOBR-C-1535
<i>Mithraculus forceps</i>	Bocas del Toro, Panama	EU682782/NA
<i>Mithraculus forceps</i> 01	Isla Margarita, Venezuela	GQ438761 /MOBR-C-1533
<i>Mithraculus forceps</i> 02	Isla Margarita, Venezuela	GQ438764 /MOBR-C-1537
<i>Mithraculus sculptus</i>	Florida, USA	EU682783/NA
<i>Mithraculus sculptus</i>	Bocas del Toro, Panama	EU682784/NA
<i>Mithraculus sculptus</i>	Bocas del Toro, Panama	EU682785/NA
<i>Mithraculus ruber</i> 01	Isla Margarita, Venezuela	GQ438769 /MOBR-C-1531
<i>Mithraculus ruber</i> 02	Isla Margarita, Venezuela	GQ438770 /MOBR-C-1532
<i>Mithrax caribbaeus</i> Large 01	Isla Margarita, Venezuela	GQ438773 /MOBR-C-1528
<i>Mithrax caribbaeus</i> Large 02	Isla Margarita, Venezuela	GQ438794 /MOBR-C-1528
<i>Mithrax caribbaeus</i> Small 01	Isla Margarita, Venezuela	GQ438765 /MOBR-C-1529
<i>Mithrax caribbaeus</i> Small 02	Isla Margarita, Venezuela	GQ438766 /MOBR-C-1529
<i>Mithrax verrucosus</i>	Isla Margarita, Venezuela	GQ438767 /MOBR-C-1534
<i>Mithrax verrucosus</i>	Isla Margarita, Venezuela	GQ438768 /MOBR-C-1536
<i>Mithrax spinosissimus</i>	Carrie Bow Key, Belize	GQ438763 /UMML
<i>Herbstia parvifrons</i>	Catalina Island, CA, USA	EU682792/NA
<i>Microphrys bicornutus</i>	Isla Margarita, Venezuela	GQ438760 /MOBR-C-1530
<i>Microphrys bicornutus</i>	Bocas del Toro, Panama	EU682781/NA
<i>Pitho lherminieri</i>	Bocas del Toro, Panama	EU682789/NA

Sequencher 4.5 (Gene Codes Corp). The sequences for another six specimens from four species were retrieved from Genbank (Hultgren & Stachowicz, 2008; Table 1).

The final set of consensus sequences was aligned with the integrated ClustalW, corrected manually with MEGA4 (Tamura *et al.*, 2007), and then exported to PAUP* (Swofford, 2002) and MrBayes 3.1.2 (Huelsenbeck, 2000; Ronquist & Huelsenbeck, 2003). This dataset was analysed with the software Modeltest v. 3.7 (Posada & Crandall, 1998) in PAUP*, which compares different models of DNA substitution in a hierarchical, hypothesis-testing framework to select a base-substitution model that best fit the data. The optimal model found by Modeltest (selected by both the hierarchical likelihood ratio test and the Akaike information criteria) was a TVM + G evolutionary model ($-\ln L = -2207.9871$). The calculated parameters were as follows: assumed nucleotide frequencies A = 0.3614, G = 0.1795, T = 0.3350, C = 0.1242; substitution rate matrix with A–C substitution = 0.6795, A–G = 6.7878, A–T = 2.5647, C–G = 0.2506, C–T = 6.7878, G–T = 1.0; rates for variable sites assumed to follow a gamma distribution (G) with shape parameter = 0.2056.

Phylogenetic analyses conducted herein were maximum parsimony (MP) and maximum likelihood (ML, in PAUP*) and Bayesian inference (BI, in MrBayes). We applied the TVM + G model of substitution to our ML and Bayesian analyses. Maximum parsimony analysis was performed as a heuristic search, with a starting tree obtained by stepwise addition, random addition of sequences, random replicates, and TBR (tree-bisection-reconnection) branch swapping. For ML, the specifications were the same as in MP. Branch swapping, however, was performed in the starting tree and all other parameters used were those of the default option in PAUP*. For BI, we used unique, random-starting trees in the Metropolis-coupled Markov Monte Carlo Chain (MCMC)

(Huelsenbeck, 2000). The analysis was performed for 6,000,000 generations. Every 100th tree was sampled from the MCMC analysis, resulting in a total of 60,000 trees. We determined a burn-in period of less than 10,000 generations after examining a plot of the log likelihood values against the number of generations. Next, we calculated a consensus tree with the 50% majority rule for the last 59,900 sampled trees. We assessed the robustness of the MP and ML tree topologies by bootstrap reiterations of the observed data 2000 and 1000 times, respectively, and reconstructing trees using each resampled data set. Support for nodes in the BI tree topology was obtained by posterior probability values that represent the frequency that each clade occurred within the collection of trees provided by the analysis.

We tested if the different species of the genera *Mithrax* and *Mithraculus* segregated and formed different genus-specific monophyletic clades. For this purpose, constrained trees (in which the monophyly of a particular genus was enforced) were obtained in MrBayes with the command *constraint*. We ran MCMC searches and obtained the harmonic mean of tree-likelihood values by sampling the post burn-in, posterior distribution as above. Next, Bayes factors were used to evaluate whether or not there was evidence against monophyly (constrained versus unconstrained trees) according to the criteria of Kass & Raftery (1995). Bayes factors compare the total harmonic mean of the marginal likelihood of unconstrained versus monophyly-constrained models. The higher the value of the Bayes factor statistic implies stronger support against the monophyly of a particular group (Kass & Raftery, 1995). Specifically, a value for the test statistic $2 \log_e(B_{10})$ between 0 and 2 indicates no evidence against H_0 ; values from 2 to 6 indicate positive evidence against H_0 ; values from 6 to 10 indicate strong evidence against H_0 ; and values >10 indicate very strong evidence against H_0 (Kass & Raftery, 1995; Nylander *et al.*, 2004).

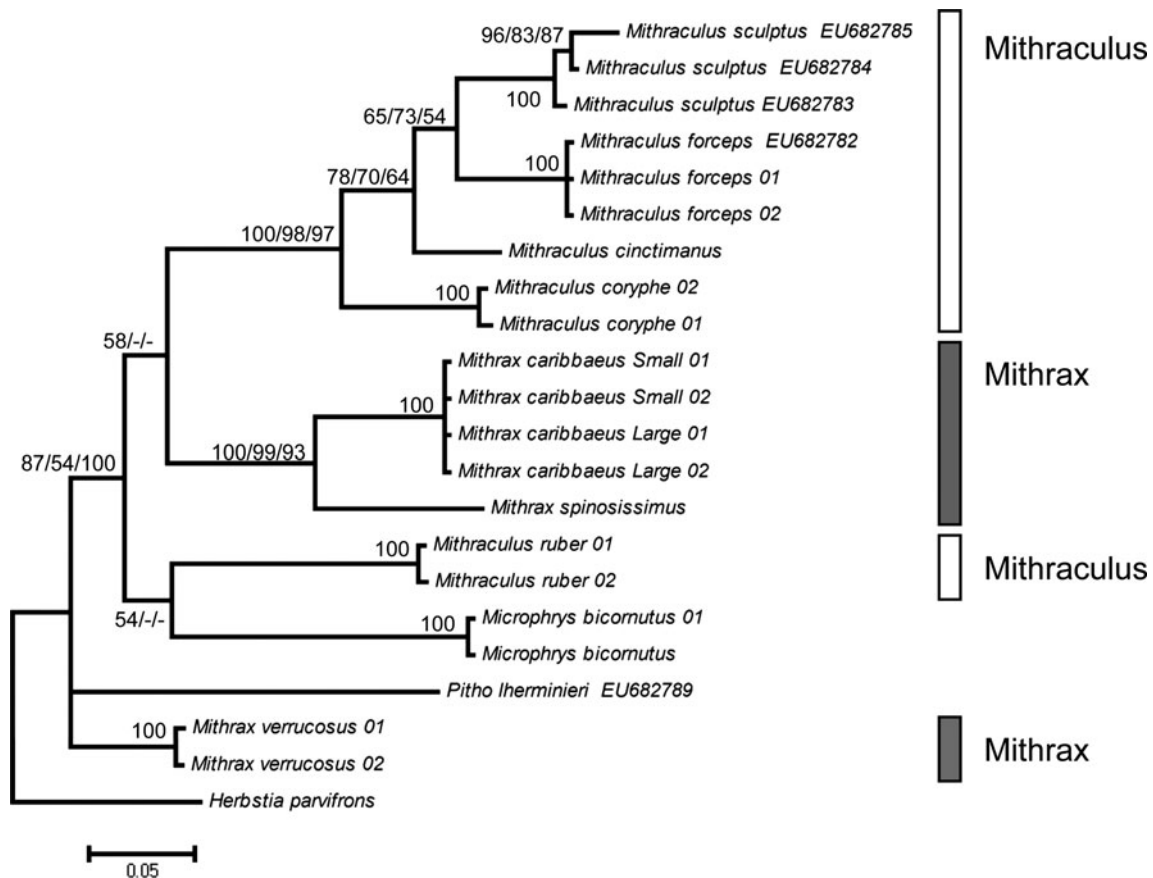


Fig. 1. Phylogenetic tree obtained from BI analysis of the partial 16S rRNA gene for crabs from the *Mithrax*–*Mithraculus* species complex, and other selected taxa from the family Majidae. Numbers above or below the branches represent the posterior probabilities from the BI analysis and bootstrap values obtained from ML and MP analyses in PAUP* (BI/ML/MP). The general topology of the trees obtained from MP and ML analyses was the same.

RESULTS

A total of 550 homologous alignment positions were used during the present phylogenetic analysis; 140 of these were found to be parsimony-informative positions. All phylogenetic trees obtained with the different inference methods (BI, ML and MP), resulted in the same general topology (Figure 1). Considering our limited number of outgroup species, belonging to three different genera within the Majoidea, the *Mithrax*–*Mithraculus* species complex is not a monophyletic group (Figure 1).

The overall topology of the trees indicated one well-supported, monophyletic clade that includes four of five species of *Mithraculus* (*M. cinctimanus*, *M. coryphe*, *M. forceps* and *M. sculptus*). The basal position of *M. coryphe* within this clade was well supported. The position of the fourth species of *Mithraculus* (*M. ruber*) was not resolved in the trees. The tree topology also indicated a pair of sister species (well supported) that included two of the three species of *Mithrax* (*M. spinosissimus* and *M. caribbaeus*). Reciprocal monophyly, that would have indicated putatively-cryptic species, was not observed between large and small specimens of *M. caribbaeus* collected from different localities in Venezuela. The position of *M. spinosissimus* and *M. caribbaeus* among the other representatives of the complex was not resolved. Similarly, the position of the third species of *Mithrax* (*M. verrucosus*) that did not group together with the other two congeners was not resolved.

The tree topologies demonstrated that species within this complex did not segregate according to genus and formed well-supported, monophyletic clades, as should be expected according to adult morphology. Similarly, Bayes factor analyses revealed no support for the separation of species within the complex into two different genera (*Mithrax* and *Mithraculus*). Comparisons of the unconstrained tree (harmonic mean = -3258.48) versus the trees wherein *Mithrax* or *Mithraculus* were imposed as monophyletic clades, indicated strong support for the unconstrained tree (*Mithrax*: harmonic mean = -3216.16 , $2\ln(B_{10}) = 7.49$; *Mithraculus*: harmonic mean = -3117.80 , $2\ln(B_{10}) = 9.89$).

The literature review of the 8 species included in our analyses revealed that the coloration, lifestyle (habit), habitat, sizes, and bathymetric distributions of these species are relatively well known. Caribbean crabs from the *Mithrax*–*Mithraculus* species complex can be found among rocks or fossilized-coral terraces, live corals, under rocks or stones in muddy and/shell bottoms, and associated with seaweeds and sea anemones in the intertidal or subtidal (up to ~ 60 m depth). Most species are colourful, having plane greenish, brownish or reddish coloration. Only one species (*Mithraculus cinctimanus*) has a striking colour pattern (white yellowish with reddish spots in the carapace). This crab is one of two symbiotic species in the Caribbean (if *M. commensalis* is not considered synonymous—see Ng *et al.*, 2008). Interestingly, *M. sculptus* and *M. forceps* appear to form occasional facultative associations, respectively, with

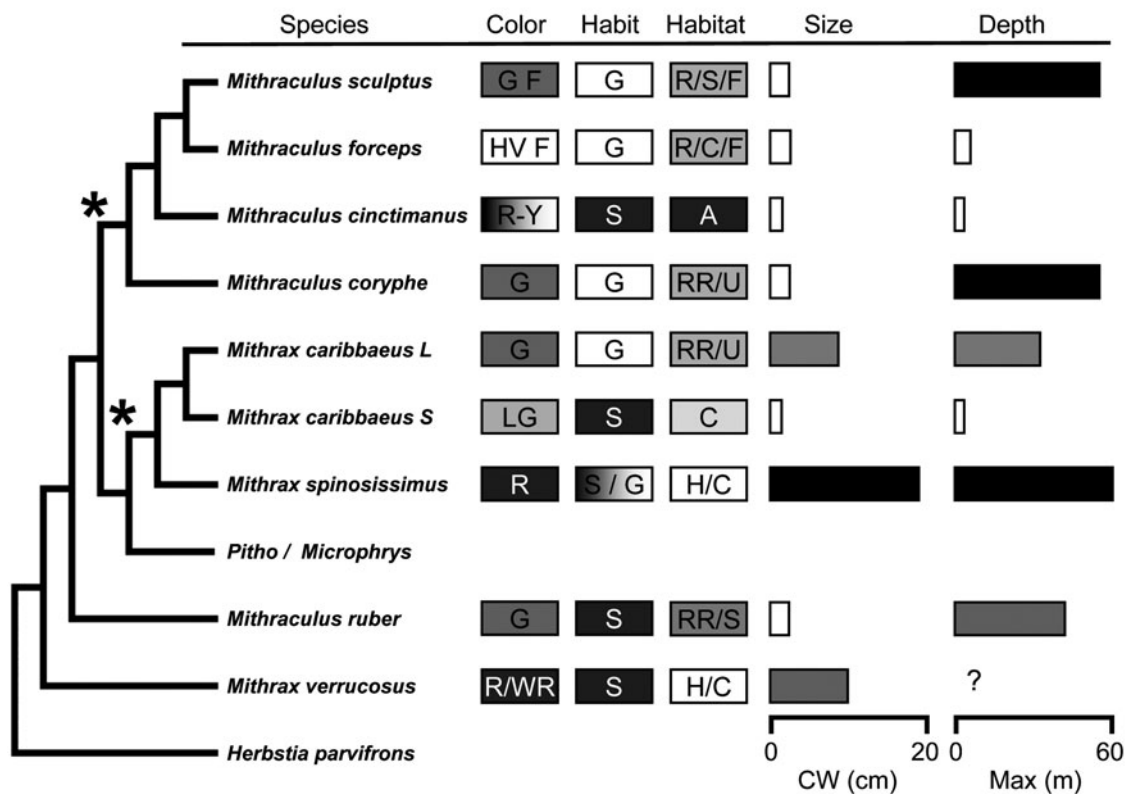


Fig. 2. Ecology and lifestyle in crabs from the *Mithrax–Mithraculus* species complex. Colour: G, green; HV, highly variable, including reddish, reddish-brown, green, bluish-green; LG, light green; R, reddish; R-Y, reddish-yellow; Y, yellow; WR, wine red. Habit: G, gregarious, S, solitary. Habitat: A, symbiotic with sea anemones and sponges, C, among clumps of pearl oysters or other bivalves (*Arca zebra*), F, facultatively symbiotic with coralline algae or branching corals, H/C, holes/crevices on reefs or brain corals, R, rubble, RR, rock and rubble, U, under stones; S, sand bottom with red algae. Size: bars represent the range in size reported for the species. Depth: bars represent the range of depth from where specimens have been collected. Asterisk indicates node of well supported monophyletic clades or sister species. Information is based on Williams (1984), Wagner (1990), Stachowicz & Hay (1996, 1999), Bolaños *et al.* (2000) and personal observations (J.A.Bs., J.E.H., R.L.).

the coralline algae *Neogoniolithon strictum* and the coral *Oculina arbuscula* (Stachowicz & Hay, 1996, 1999). Unfortunately little is known about the costs and benefits experienced by *M. cinctimanus* as consequences of association with sea anemones. On the other hand, *M. forceps* obtains food (lipid-rich mucus), shelter and protection against fish predators from its coral host (Stachowicz & Hay, 1999). Similarly, *M. sculptus* obtain protection against predators from its algae host and, at the same time, this species appears to clean its algal host of fouling seaweeds (Stachowicz & Hay, 1996). Information regarding the natural diet of most of the species is lacking, although all species are considered to be herbivorous or omnivorous (e.g. *M. sculptus*—Stachowicz & Hay, 1996) (Figure 2). Information on the lifestyle (distribution) is available for most species. Three species live solitarily (*M. cinctimanus* in sea anemones, and *M. verrucosus* and *M. caribbaeus* on holes or crevices), and the remaining species live in aggregations (Rathbun, 1925; personal observations). Interestingly, *M. spinosissimus* have been seen living both solitarily and in aggregations at different localities in the Caribbean (Carrie Bow Caye, Belize and Los Roques, Venezuela, respectively). This revision confirms that the lifestyle of crabs from this species complex is diverse and demonstrates that this high diversity might be found within small, monophyletic clades (see below).

DISCUSSION

This study presents for the first time a molecular phylogeny of crabs from the genera *Mithrax* and *Mithraculus*, based upon a segment of the 16S rRNA mitochondrial gene. Considering our limited pool of species (only eight out of the 25 valid species within the complex—Ng *et al.*, 2008), these analyses with three different phylogenetic reconstruction methods recognized only one monophyletic group consisting of four species of *Mithraculus* (*M. cinctimanus*, *M. coryphe*, *M. forceps* and *M. sculptus*) and also supported two members from the genus *Mithrax* as sister species (*M. spinosissimus* and *M. caribbaeus*). The positions of the remaining species from the complex (*Mithraculus ruber* and *Mithrax verrucosus*) were not resolved. In disagreement with the hypothesis based solely upon adult morphology, a well-resolved grouping of all of the species from a particular genus was not revealed by our analyses. Also, Bayesian factors analyses strongly supported unconstrained trees over trees in which monophyly (either of *Mithrax* or *Mithraculus*) was imposed. The present results do not support the separation of these species into two different genera as proposed by Wagner (1990) that was based upon adult morphology alone. Instead, the present study agrees with cladistic analyses based upon larval characters, indicating that the *Mithrax–Mithraculus* divide within the complex is not natural (Bolaños & Scelzo, 1981; Bolaños *et al.*, 1990; Santana

et al., 2003). We argue in favour of future phylogenetic studies using various types of evidence (molecular, adult morphology and larval anatomy) to improve our knowledge of the natural relationships within this species complex and its position in the Mithracinae.

Mithrax verrucosus and *Mithraculus ruber* did not group together with the other members of their respective genera. *Mithrax verrucosus* differs from most of its congeners by having a carapace paved with closely set granules (Wagner, 1990). *Mithrax hemphilli* Rathbun, 1892 and *M. pilosus* Rathbun 1892 are the only other species from the genus that also have the carapace covered with granules or tubercles (Wagner, 1990). Given the basal position of *M. verrucosus*, as indicated by our phylogenetic analyses, the presence of granules/tubercles in the carapace might represent an ancestral condition lost in some members from the studied species complex (e.g. *Mithrax spinosissimus* and *M. caribbaeus*). Future phylogenetic studies including *M. hemphilli* and *M. pilosus* will help in determining whether or not the presence/absence of tubercles/granules on the carapace is a character with phylogenetic value in *Mithrax*–*Mithraculus* crabs. *Mithrax verrucosus* features the entire set of characters that define the genus *Mithrax* (*sensu* Desmarest, 1823); ovate or oblong carapace (either broader than long or slightly longer than broad) with antero-lateral margins having four or three spines or lobes behind the orbital front, two small horns at the rostrum, and robust legs (Rathbun, 1925). That this species did not group together with the other congeners also suggests that carapace shape and the presence/absence of lateral spines in the carapace might not be phylogenetically informative characters within the studied species complex.

With regards to *Mithraculus ruber*, this species is similar to *M. forceps*. Importantly, adults of *Mithraculus ruber* differ from all of its congeners by lacking a large tooth on the propodus of the cheliped (Wagner, 1990). Also, *Mithraculus ruber* is the only species from the genus having the last tubercle on the lateral margin of the carapace situated at a posterolateral angle and not at a position that separates the posterolateral from the anterolateral carapace margins as occurs in the remaining species from the genus (Wagner, 1990). The presence/absence and position of this tooth/tubercle might be a character of phylogenetic relevance. New studies combining molecular and morphological characters will help in determining whether or not carapace shape, the position of the lateral spines or tubercles on the carapace as well as other morphological traits are phylogenetically informative characters within the studied species complex.

The results in the present study suggest that the *Mithrax*–*Mithraculus* complex is paraphyletic; *Microphrys bicornutus* clusters together with *Mithraculus ruber* and has a derived position compared to *Mithrax verrucosus* (although not well supported). This observation agrees with previous phylogenetic analyses based upon larval characters, revealing that the monophyletic status of the Mithracinae is still uncertain, as well as its position within the family Majidae (Marques et al., 2003).

The set of species considered in the present study does not allow us to address other relevant systematic questions for the group. For instance, an unresolved question is whether or not the two sea anemone associates *M. cinctimanus*, recorded from several localities in the Caribbean, and *M. commensalis*, from Dominica (West Indies) are separate species or constitute a single, pan-Caribbean species (Rathbun, 1925; Manning, 1970; Bolaños et al., 2000; Ng et al., 2008).

Similarly, *Mithrax hispidus* and *Mithrax caribbaeus*, once considered different species, have been recently proposed as synonyms based upon studies of larval and adult morphological traits (Santana et al., 2003; Ng et al., 2008). It must be highlighted that, in contrast to other subfamilies of the Majidae where larvae from different species are relatively easy to differentiate (e.g. Pisinae—see Santana et al., 2004), larval morphology is highly consistent within the Mithracinae (especially in zoeal stages—Santana et al., 2003). Future phylogenies combining molecular, adult, and larval characters might reveal an evolutionary history much more complex than previously thought in this group (Hultgren & Stachowicz, 2008).

The ecology of the *Mithrax*–*Mithraculus* species complex is diverse, as confirmed by a thorough literature review. Most strongly, our study demonstrates that small, monophyletic groups within this complex can have considerable diversity in terms of coloration, body size, habitat, and lifestyle. For instance, the small and solitary, yellow and reddish-coloured, symbiotic crab, *M. cinctimanus*, was included in the same group in which *M. coryphe* was placed, a larger greenish-blue species that lives in small aggregations and inhabits deeper waters (Rathbun, 1925; personal observations). The solitary habit of *M. cinctimanus* might be a consequence of a symbiotic lifestyle, as suggested for other crustaceans associated with sea anemones (Baeza et al., 2001; Baeza & Thiel, 2007). Similarly, the large, reef-dwelling crab, *M. spinosissimus*, is the sister species to *M. caribbaeus*, with populations composed of small (or large) individuals that inhabit shallow waters. The absence of reciprocal monophyly between large and small specimens of *M. caribbaeus* also suggests that body size is a highly plastic phenotypic trait in this species. *Mithrax caribbaeus* might be used to test the role of environmental conditions (e.g. food, habitat constraints and predation pressure) in explaining striking size differences in the field (including dwarfism) and possible relationships to life history schedules.

In general, this study has shown that the separation of the *Mithrax*–*Mithraculus* species complex into two genera is not supported by molecular characters. Crabs from these two genera demonstrate a considerable diversity of lifestyles, body sizes, microhabitats, and coloration. Studies describing life history and ecology within a phylogenetic framework are underway. This approach is expected to prove most useful in understanding the role of environmental conditions in driving the evolution of morphological, ecological, and behavioural traits in the marine environment. The present study included only species from the Caribbean Sea; nevertheless, the ampho-American nature of the *Mithrax*–*Mithraculus* species complex (see Rathbun, 1925) suggests that this group might also be a model to study speciation mechanisms, as in other trans-isthmian clades of shrimps (Williams et al., 2001), fish (Birmingham et al., 1997), and sea urchins (Lessios, 2008).

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