Zootaxa 2302: 61–68 (2009)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Re-evaluation of species allied to *Mithrax hispidus* (Decapoda: Brachyura: Majoidea: Mithracidae) based on three mitochondrial genes

AMANDA M. WINDSOR[1] & DARRYL L. FELDER[2]

[1]*The University of Louisiana at Lafayette, Department of Biology, P.O. Box 42451 Lafayette, Louisiana 70504, U.S.A. E-mail: amwindsor@gmail.com*

[2]*The University of Louisiana at Lafayette, Department of Biology, P.O. Box 42451 Lafayette, Louisiana 70504, U.S.A. E-mail: dlf4517@louisiana.edu*

Abstract

Mithrax hispidus, *M. caribbaeus*, *M. pleuracanthus*, and *M. tortugae* are closely related shallow-water crabs that are difficult to distinguish by morphology alone. This led to recent synonymy of the four species under *M. hispidus* (Herbst, 1790). The use of three mitochondrial genes (12s, 16s, and COI) nevertheless provides evidence for three distinct species (*M. hispidus*, *M. pleuracanthus*, and *M. tortugae*) and the synonymy of *M. caribbaeus* with *M. hispidus*. Morphological features of the merus and carpus of the chelipeds serve as characters to separate the three species.

Key words: Phylogenetics, Sequencing, Spider Crabs

Introduction

Mithrax hispidus (Herbst, 1790) and some of its close congeners form a species complex that exemplifies the problematic taxonomy for several subgroups in the genus *Mithrax* Desmarest, 1823. The four species that make up this complex, *M. hispidus*, *M. caribbaeus* (Rathbun, 1920), *M. pleuracanthus* (Stimpson, 1871), and *M. tortugae* (Rathbun, 1920), were originally distinguished by Rathbun (1925) on the shape of the rostral sinus and the degree of carapace tuberculation. Wagner (1990) however, did not consider that these characters were sufficient enough to separate the species and synonymized the four species treated here along with *M. laevimanus* Desbonne, in Desbonne & Schramm, 1867, which was not included in this study. He cited distinctive grooves on the distal half of the first gonopod as a unifying character not found in any other species of *Mithrax*. Ng *et al*. (2008) followed this synonymy under which *Mithrax hispidus* is the only recognized species within the complex.

Characters originally proposed to separate the four species are highly variable when a series of specimens is examined, especially when individuals are not closely comparable in size and age. For example, variation is seen in the shape of the rostral sinus, which was used in Rathbun's (1925) diagnoses of the species. *Mithrax hispidus, M. caribbaeus*, and adults of *M. pleuracanthus* were originally reported to have a U-shaped rostral sinus, while *M. tortugae* and juveniles of *M. pleuracanthus* were noted to exhibit a V-shaped sinus. However, among the specimens we observed, rostral sinuses intermediate between U- and V-shaped are commonplace. Relative development of tuberculation and spines on the posterolateral slope of the carapace was also used in these diagnoses. Rathbun (1925) concluded that *M. hispidus* and juveniles of *M. caribbaeus* exhibited a spine on or above the posterolateral slope of the carapace. The slope was noted to be tuberculate in *M. pleuracanthus*, while it was said to be smooth with only a single tubercle above in *M. tortugae*. *Mithrax caribbaeus* was diagnosed by Rathbun (1920; 1925) as possessing two transverse, parallel rows of tubercles on the posterolateral slope. For all four of these species, we find that the degree of tuberculation varies with

Accepted by P. Castro: 29 Oct. 2009; published: 2 Dec. 2009

age. As the juvenile molts, the carapace usually broadens and accessory spines and tubercles become apparent (pers. obs.). As a result, juveniles are all but impossible to identify given such ontogenetic changes in ornamentation.

Because the male first gonopods are not directly influenced by habitat in the same ways as carapace shape and because they can play a role in reproductive isolation, morphological convergence of the gonopod between two species is not expected solely because species share a similar habitat or lifestyle (Martin & Abele 1986). In some brachyuran crabs, the morphology of the first gonopods can provide valuable characters for the identification of species that are otherwise morphologically indistinct (Garth 1958; Guinot 1967; Harrison 2004; Martin & Abele 1986). This led Wagner (1990) to undertake comparative examination of the gonopods of several species of *Mithrax*. He determined that minute grooves on the side of the first gonopods were unique to the four species he synonymized with *M. hispidus* and cited this similarity as evidence for their synonymy.

Larval morphology has also been used to determine species relationships (Clark *et al*. 1998; Rice 1980), and several studies have used larval characteristics to construct family-level phylogenies within the Majoidea (Clark & Webber 1991; Marques & Pohle 1998; 2003; Marques *et al*. 2003; Pohle & Marques 2000; Santana *et al*. 2003). Santana *et al*. (2003) reared larvae of *M. hispidus* and then compared them to previously described larvae of other species belonging to *Mithrax* and *Mithraculus*. Special attention was focused on the larvae of *Mithrax pleuracanthus* and *Mithrax caribbaeus* because they had been synonymized with *Mithrax hispidus*. Larvae of *Mithrax tortugae* have yet to be described from laboratory-reared material, so they could not be included in their analyses. Larval comparisons did, however, include *Mithraculus coryphe* and *Mithraculus forceps*. The comparisons showed that mithracid larvae are morphologically similar to one another but differ in setation of the carapace and antennules. Santana *et al*. (2003) also found that the zoeae of *Mithrax hispidus* more closely resemble the zoeae of *Mithraculus coryphe* (Herbst, 1801), *Mithraculus forceps* (A. Milne-Edwards, 1875), and *Mithrax caribbaeus* than the zoeae of *Mithrax pleuracanthus*, a proposed synonym. Because larvae of *Mithrax* and *Mithraculus* are so similar, Santana *et al*. (2003) did not agree with Wagner's (1990) division of *Mithrax* into two genera based on adult characters, nor did they agree with Wagner's synonymy of *Mithrax pleuracanthus* and *Mithrax hispidus*. They argued that both the zoeal and megalopal characters between these species are too different to represent the same species. Santana *et al*. (2003), however, did concede that *Mithrax caribbaeus* and *Mithrax hispidus* are nearly identical in larval forms, exhibiting nothing more than expected levels of intraspecific variation.

This study applies molecular phylogenetic methods to address taxonomic uncertainty within the complex of species allied to *Mithrax hispidus*. Mitochondrial DNA sequences for the 12s, 16s, and Cytochrome Oxidase Subunit-1 (COI) genes are used because these genes have proven particularly useful in resolving species-level relationships within the Crustacea (Harrison 2004; Schubart *et al*. 2000). Additional morphological characters are re-examined to determine whether they support separations of species within this complex.

Materials and methods

Specimens collected from throughout the Gulf of Mexico and Caribbean Sea were available among tissue and specimen holdings in the University of Louisiana at Lafayette Zoological collections (ULLZ), or were obtained on loan from Texas A&M University, College Station (TCWC) and the Florida Museum of Natural History (FLMNH) (Table 1). Type materials were obtained on loan from the Smithsonian Institution, National Museum of Natural History, Washington, D.C. (USNM) for morphological comparisons to the specimens held in the ULLZ collections. Specimens utilized for genetic sequencing were either frozen and subsequently transferred to 80% ethanol or preserved directly in 80% ethanol. A total of 12 specimens tentatively assigned to species within the complex were included in genetic analyses. Specimens of *Mithraculus forceps* and *M. sculptus* were included as outgroups.

Muscle tissue was removed from inside pereopods or from the exposed ends of coxae where limbs had autotomized. Total genomic DNA was extracted using the Cartagen Genomic DNA Extraction Kit for Arthropods (Cartagen Cat. No. 20810-050), the Qiagen DNeasy Blood and Tissue extraction kit (Cat. No. 69504), or the ethanol precipitation extraction protocol detailed in Robles *et al*. (2007). DNA was quantified and assessed for quality using a Nanodrop ND-1000 Spectrophotometer. Three mitochondrial genes were amplified separately using the Polymerase Chain Reaction (PCR) on a Stratagene Robocycler Gradient 96. The primers for the amplified genes were as follows: 12SsF (5'–GAAACCAGGATTAGATACCC–3'), 12S1R (5'–AGCGACGGGCGATATGTAC–3'), 16SF (5'–TATTTTGACCGTGCAAAGGTAG–3'), 16SR (5'–ATTTAAAGGTCGAACAGACCCT–3') (Hultgren & Stachowicz 2008), LCO1490 (5'–GGTCAACAAATCATAAAGATATTGG–3'), and HCO2198 (5'–TAAACTTCAGGGTGACCAAAAAATCA–3') (Folmer *et al*. 1994). Successful amplifications were purified using a GenCatch ™ PCR Cleanup Kit (Epoch Biolabs Catalog No. 1360250). Cycle sequencing reactions were completed using the ABI BigDye terminator mix (Applied Biosystems Catalog No. 4337456). Both strands were then sequenced using an Applied Biosystems 3100 automated sequencer.

TABLE 1. Species names, catalog numbers, collection sites, and GenBank accession numbers for specimens included in this study (TCWC = Texas A&M University, Texas Cooperative Wildlife Collection, College Station; FLMNH = Florida Museum of Natural History; ULLZ = University of Louisiana at Lafayette Zoological Collections; GMx = Gulf of Mexico).

Taxon Name	Catalog No.	Collection Site	Acc. No. 16S	Acc. No. 12S	Acc. No. COI
OUTGROUP					
Mithraculus forceps	ULLZ 6922	Florida HBOI	GU144541	GU144524	GU144554
Mithraculus sculptus	ULLZ 6915	Florida Keys-3	GU144540	GU144525	GU144553
Mithraculus sculptus	ULLZ 8774	Florida Keys-4	GU144539	GU144526	GU144555
INGROUP					
Mithrax hispidus	TCWC 2-6261	GMx-1; Off TX	GU144551	GU144530	
Mithrax hispidus	TCWC 2-2235	WGMx-2; off TX	GU144552	GU144531	
Mithrax caribbaeus	FLMNH 11383	Florida Keys-1	GU144549	GU144533	GU144556
Mithrax sp.	ULLZ 4572	Florida Keys-2	GU144548	GU144535	GU144558
Mithrax pleuracanthus	ULLZ 5694	Ft. Pierce, FL	GU144544	GU144537	GU144560
Mithrax sp.	ULLZ 6751	SGMx-1; off Yucatán	GU144545	GU144534	
Mithrax sp.	ULLZ 6792	SGMx-2; off Yucatán	GU144543	GU144529	GU144564
Mithrax tortugae	ULLZ 6980	Bocas del Toro, Panamá	GU144542	GU144527	GU144562
Mithrax pleuracanthus	ULLZ 6995	SGMx-3; off Yucatán	GU144547	GU144538	GU144559
Mithrax sp.	ULLZ 7353	SGMx-4; off Yucatán		GU144528	GU144563
Mithrax pleuracanthus	ULLZ 7714	EGMx; off FL	GU144546	GU144536	GU144561
Mithrax hispidus	ULLZ 8619	NEGMx; off FL	GU144550	GU144532	GU144557

Sequences of each gene were aligned separately using the MUSCLE (Edgar 2004) alignment algorithm set to 20 iterations. We determined the model of evolution appropriate for the dataset using the MrAIC Perl script (Nylander 2004). The MrAIC program determined that the Hasegawa–Kishino–Yano model (Hasegawa *et al*. 1985) was the most appropriate model for each of the three genes. We concatenated the three genes into a single alignment of 1530 base pairs using Mesquite (Maddison & Maddison 2008). We then conducted a partition test of homogeneity (incongruence length difference test) (Bull *et al*. 1993), and the results indicated that the genes could be concatenated. Phylograms were built for each gene using MrBayes 3.2 and PAUP*. Bayesian Analysis (BA) was run in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) on the individual datasets

using a Markov Chain Monte Carlo (MCMC) algorigthm with 4 chains for 10,000 generations with a sampling frequency of every 10 trees. The first 2,500 generations were discarded as burn-in. For the concatenated dataset, MCMC was run for 10,000,000 generations and with a sampling frequency of every 1000 trees and a burn-in of the first 2,500,000 generations. The remaining trees were used to create a 50% majority rule consensus tree. Posterior probabilities were obtained to determine nodal support in the consensus tree. Maximum Parsimony (MP) was run in PAUP* (Swofford 2003) and Maximum Likelihood (ML) was run using PhyML3.0 (Guindon & Gascuel 2003). Maximum Parsimony was run as a heuristic search with random sequence addition, with tree bisection and reconnection as the branch swapping option. Bootstrap support values were calculated for both MP and ML with 1000 bootstrap replicates each.

The external morphology was examined in detail for each of the specimens that were sequenced and for other specimens that were not suitable for DNA sequencing. Measurements of the carapace and articles of appendages were made to examine trends that may facilitate identification of the individual species (data not shown). Tuberculation of the appendages was also examined for possible use as a diagnostic character.

Results

The tree topology for the three individual mitochondrial genes and the concatenated data set each resulted in the same tree topology, so only the concatenated phylogram is presented (Figure 1). The phylogram shows three distinct and well-supported clades within the *Mithrax hispidus* species complex. The clades identified as *M. hispidus* and *M. pleuracanthus* are sister groups (BA=97, MP= 65, ML=60) to the clade of *M. tortugae*.

The clade containing *M. tortugae* is 100% supported under Bayesian analysis, Maximum Parsimony, and Maximum Likelihood. Within this clade is a subclade (BA=80, MP=76, ML=64) representing two juveniles (ULLZ 7353, ULLZ 6792) that cannot presently be identified with characters used for adults. Both of these specimens are from offshore rubble habitats on the Campeche Banks in the southern Gulf of Mexico off the northern Yucatán Peninsula. The other individual (ULLZ 6980) within this clade is an adult collected from the coastal Caribbean at Bocas del Toro, Panamá.

The clade for *M. hispidus* (BA=100, MP=100, ML=100) contains three specimens originally identified as *M. hispidus* and one that had been identified as *M. caribbaeus* (FLMNH-11383). Two of the specimens (TCWC 2-2235 and FLMNH 11383) appeared to exhibit the two transverse rows of tubercles on the posterolateral slope of the carapace that Rathbun (1920) applied as a defining character in *M. caribbaeus*.

The clade containing specimens of *M. pleuracanthus* (BA=100, MP=100, ML=100) is the most geographically diverse clade, with only two specimens collected from the same geographic region within the Gulf of Mexico. This clade also showed the most variation with respect to the shape of the rostral sinus and the degree of tuberculation on the posterolateral slope of the carapace.

Discussion

The species complex including *Mithrax hispidus* and its closest congeners has been questionable and problematic for nearly two decades. Our findings contradict those of Wagner (1990) and show that grouping all four species of this complex into one is not warranted. Molecular evidence reveals at least three species within the complex on the basis of the available samples: *M. hispidus* sensu stricto, *M. pleuracanthus*, and *M. tortugae*. The specimen identified as *M. caribbaeus* (FLMNH-11838) clearly falls within the clade of *M. hispidus*. A second specimen that fits the description of *M. caribbaeus* (TCWC-2-2235) also grouped within the clade for *M. hispidus*. We therefore support the synonymy of *M. caribbaeus* with *M. hispidus*. The present findings also support those of Santana *et al*. (2003) by synonymizing *M. caribbaeus* with *M. hispidus* and maintaining *M. pleuracanthus* as a separate species.

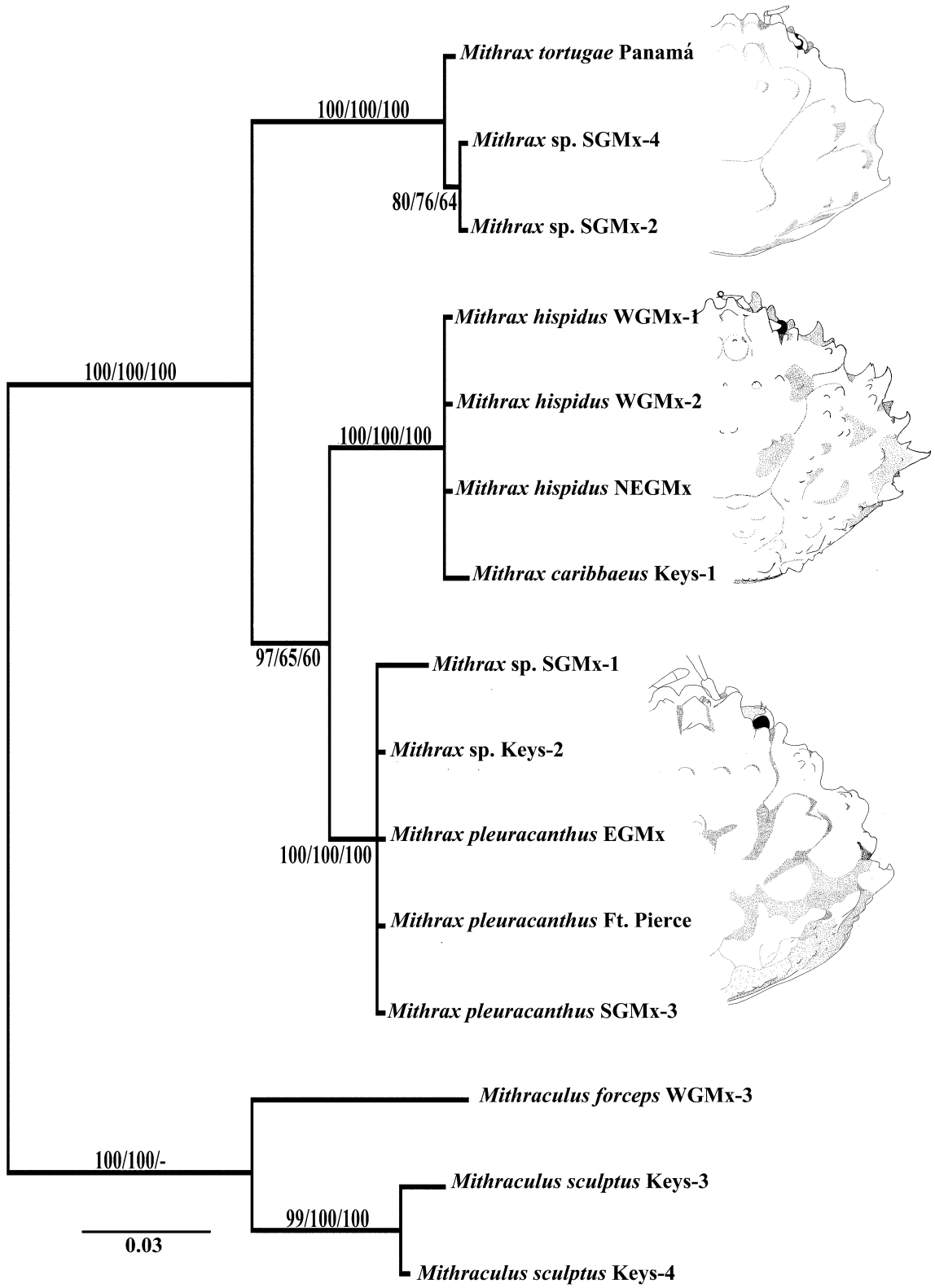

FIGURE 1. 50% majority-rule consensus tree inferred from Bayesian analysis of 12s, 16s, and COI DNA data. Support from left to right: BA, MP, and ML. Keys = Florida Keys, NEGMx = northeast Gulf of Mexico, SGMx = southern gulf of Mexico, EGMx = eastern Gulf of Mexico, WGMx = western Gulf of Mexico.

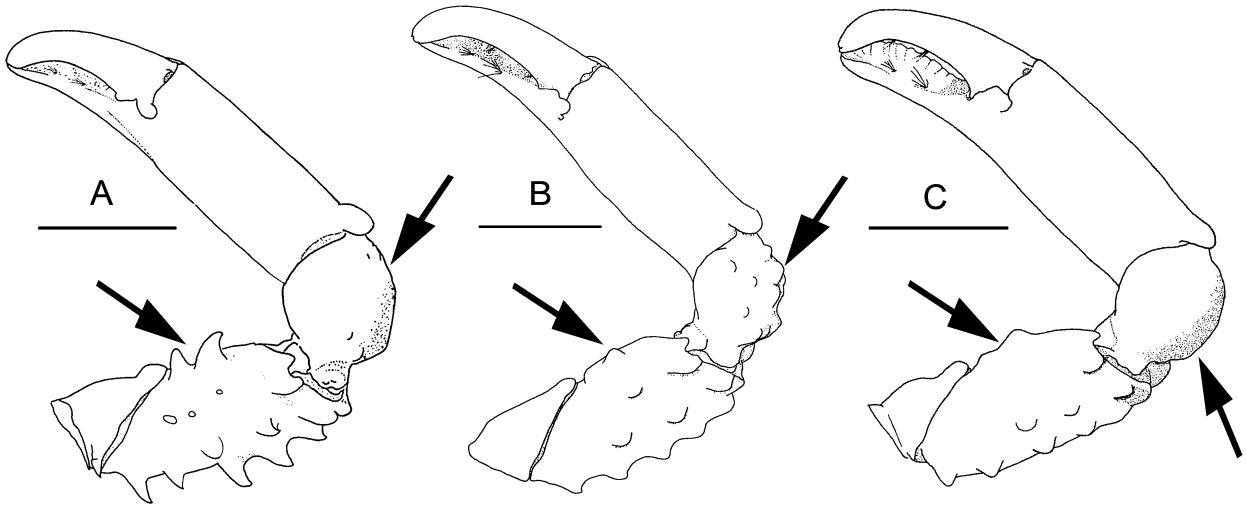

FIGURE 2. Chelipeds of: A) *Mithrax hispidus* ULLZ 11041, B) *Mithrax pleuracanthus* ULLZ 6751 C) *Mithrax tortugae* ULLZ 6980. Arrows indicate diagnostic characters on carpus and merus.

We examined the type specimen of *M. caribbaeus*, which has a carapace width of 78.4 mm, including spines, and was considered by Rathbun (1920) to be a very large specimen for this species. The description of the type specimen of *M. hispidus* has a carapace width of 146 mm, so *M. caribbaeus* would fall well within the expected size range of *M. hispidus*. Two specimens included in this study (TCWC 2-2235, FLMNH 11383) exhibit two transverse rows of three tubercles on the posterolateral margin, which is ostensibly the diagnostic character of *M. caribbaeus*. As these specimens unquestionably grouped with the clade containing *M. hispidus* on the basis of three molecular markers, we must conclude that this character does not support the recognition of *M. caribbaeus* as a separate species. The character is ambiguous in larger specimens due to wear of the carapace and tubercles after the terminal molt. Williams (1965) noted that *Mithrax hispidus* is smooth with some rounded tubercles, and the illustrations of *M. hispidus* in (Williams 1965: fig 236; 1984: fig 268) clearly shows these two rows of tubercles on one side, but only obscurely on the other.

Wagner (1990) cited first gonopod morphology as a unifying character for *M. hispidus* and its close relatives. While we agree that the first gonopods cannot be effectively used to distinguish between these four species, this alone does not justify synonymizing these four species especially given the molecular data. Illustrations by Williams (1965) show the same striations discussed by Wagner (1990) in *M. hispidus* and *M. pleuracanthus*, but in *Mithrax verrucosus* H. Milne Edwards, 1832 as well. These minute striations are not unique to the four species of the complex and thus are not useful in species identification. While gonopod morphology may provide valuable characters for separation of brachyuran species, many cases are also known in which obviously different species share indistinguishably similar gonopods. One excellent example can be found among six separate species of the genus *Panopeus* that exhibit no consistent differences in gonopods (Williams 1983). For Majoidea in particular, Garth (1958: 13) found gonopod morphology more useful at the family and generic level than species level, and urged caution before utilizing the male gonopod as a taxonomic character in this group.

The specimens within the clade attributed to *M. pleuracanthus* exhibit the highest degree of morphological variation among any of the clades. Shape of the rostral sinus ranges from a distinct V-shape to a broad V-shape, but is never U-shaped in the specimens available. Tuberculation on the carapace is also variable between specimens. One of the specimens examined was heavily tuberculate (ULLZ 6751), while others had a few, low tubercles (ULLZ 5694, 6995, 7714), and a third was nearly smooth (ULLZ 4572). This degree of variation within one species exemplifies how uninformative these characters are.

The specimens within the clade for *M. tortugae* require further examination. The adult specimen within this clade (ULLZ 6980) is clearly assignable to *M. tortugae* on the basis of our careful comparison of its

morphology to that of the type specimen of *M. tortugae* (USNM 50442). However, two juveniles (ULLZ 7353, 6792) included in our study also fall within this clade even though present adult morphological characters alone would not definitively place them there. Both are from the southern Gulf of Mexico and were collected within the same area as two specimens of *M. pleuracanthus* (ULLZ 6751, 6995), confirming that there are geographically overlapping, genetically separate populations of closely related species within this complex in the Gulf of Mexico. Because juveniles of *Mithrax* are very difficult to identify to the species level, more juveniles must be sequenced and examined to search for defining morphological characters that conform to our genetically defined clades. Again, the shape of the rostral sinus and carapace tuberculation does not appear to be as informative as formerly reported by Rathbun (1925).

A set of characters for differentiating between the three genetically established clades is proposed. Ornamentation of the cheliped merus and carpus is consistent within each clade but is recognizably different between clades (Figure 2).

The cheliped merus is armed with prominent spines on the superior and lateral margins in *M. hispidus*. The mesial margin is also armed with one or two sharp tubercles, and the cheliped carpus is obscurely tuberculate. This agrees with the description of the cheliped by Wagner (1990). The type specimen of *M. caribbaeus* also shares these characters. The cheliped merus of *M. pleuracanthus* is armed with blunt tubercles, never spines. There is only one blunt tubercle above the mesial margin of the cheliped merus. The cheliped carpus of *M. pleuracanthus* is tuberculate to obscurely tuberculate, but there is always some indication of tuberculation present, and it is more obvious than in *M. hispidus*. *Mithrax tortugae* has a cheliped merus armed with two low, blunt tubercles on the mesial surface of the cheliped merus, but there are overall fewer tubercles on the merus than observed in either *M. hispidus* or *M. pleuracanthus*. The cheliped carpus is unique from the other two species in that it is completely smooth with no indication of tuberculation.

Thorough studies of the ecology and behavior for these species may explain why such close relatives have speciated and remained genetically isolated, even though they inhabit broadly overlapping geographic ranges.

Acknowledgements

We thank H. Bracken, R. Lemaitre, G. Paulay, A. Anker, E. Palacios-Theil, R. Robles, B. Thoma, and M. Wicksten for assistance in collection of specimens or for providing museum materials for analysis. This study is part of the Assembling the Tree of Life, Decapoda project supported by U.S. National Science Foundation grants NSF/BS&1 DEB-0315995 and NSF/AToL EF-0531603 to D. Felder. The Smithsonian Marine Station, Ft. Pierce, Florida and the Smithsonian Caribbean Coral Reef Ecosystem Program, Belize provided additional travel grants. This is University of Louisiana Laboratory for Crustacean Research contribution no. 137

References

Bull, J.J., Huelsenbeck, J.P., Cunningham, C.W., Swofford, D.L. & Waddell, P.J. (1993) Partitioning and Combining Data in Phylogenetic Analysis. *Systematic Biology,* 42, 384–397.

Clark, P.F., Calazans, D.K. & Pohle, G.W. (1998) Accuracy and standardization of brachyuran larval descriptions. *Invertebrate Reproduction and Development,* 33, 127–144.

Clark, P.F. & Webber, W.R. (1991) A redescription of *Macrocheira kaempferi* (Temminck, 1836) zoeas with a discussion of the classification of the Majoidea Samouelle, 1819 (Crustacea: Brachyura). *Journal of Natural History,* 25, 1259–1279.

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research,* 32, 1792–1797.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* 3, 294–297.

Garth, J.S. (1958) Brachyura of the Pacific coast of America: Oxyrhyncha. *Allan Hancock Pacific Expeditions,* 21, 1–499.

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology,* 52, 696–704.

Guinot, D. (1967) Recherches préliminaires sur les groupements naturels chez les Crustacés Décapodes Brachyoures. IV. Observations sur quelques genres de Xanthidae. *Bulletin du Muséum National d'Histoire Naturelle, 2e Série,* 39, 695–727.

Harrison, J.S. (2004) Evolution, biogeography, and the utility of mitochondrial 16s and COI genes in phylogenetic analysis of the crab genus *Austinixa* (Decapoda : Pinnotheridae). *Molecular Phylogenetics and Evolution,* 30, 743–754.

Hasegawa, M., Kishino, K. & Yano, T. (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution,* 22, 160–174.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics,* 17, 754–755.

Hultgren, K.M. & Stachowicz, J.J. (2008) Molecular phylogeny of the brachyuran crab superfamily Majoidea indicates close congruence with trees based on larval morphology. *Molecular Phylogenentics and Evolution,* 48, 986–996.

Maddison, W.P. & Maddison, D.R. (2008) Mesquite: a modular system for evolutionary analysis Version 2.6. http://mesquiteproject.org.

Marques, F.P.L. & Pohle, G. (1998) The use of structural reduction in phylogenetic reconstruction of decapods and a phylogenetic hypothesis for fifteen genera of Majidae: testing previous hypotheses and assumptions. *Invertebrate Reproduction and Development,* 33, 241–262.

Marques, F.P.L. & Pohle, G. (2003) Searching for larval support for majoid families (Crustacea : Brachyura) with particular reference to Inachoididae Dana, 1851. *Invertebrate Reproduction & Development,* 43, 71–82.

Marques, F.P.L., Pohle, G.W. & Vrbova, L. (2003) On the larval stages of *Macrocoeloma diplacanthum* (Decapoda: Brachyura: Majidae), with a review of mithracine phylogenetic aspects. *Journal of Crustacean Biology,* 23, 187–200.

Martin, J.W. & Abele, L.G. (1986) Notes on male pleopod morphology in the brachyuran crab family Panopeidae Ortmann, 1893, sensu Guinot (1978) (Decapoda). *Crustaceana,* 50, 182–198.

Ng, P.K.L., Guinot, D. & Davie, P.J.F. (2008) Systema Brachyurorum: Part I. An annotated checklist of extant brachyuran crabs of the world. *The Raffles Bulletin of Zoology,* 17, 1–286.

Nylander, J.A.A. (2004) MrAIC.pl Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pohle, G. & Marques, F.P.L. (2000) Larval stages of *Paradasygyius depressus* (Bell, 1835) (Crustacea: Decapoda: Brachyura: Majidae) and a phylogenetic analysis for 21 genera of Majidae. *Proceedings of the Biological Society of Washington,* 113, 739–760.

Rathbun, M.J. (1920) New species of spider crabs from the Straits of Florida and Caribbean Sea. *Proceedings of the Biological Society of Washington,* 33, 23–24.

Rathbun, M.J. (1925) *The spider crabs of America*. Smithsonian Institution, Washington, D.C., 613 pp.

Rice, A.L. (1980) Crab zoeal morphology and its bearing on the classification of the Brachyura. *Transactions of the Zoological Society of London,* 35, 271–424.

Robles, R., Schubart, C.D., Conde, J.E., Carmona-Suarez, C., Alvarez, F., Villalobos, J., *et al*. (2007) Molecular phylogeny of the American *Callinectes* Stimpson, 1860 (Brachyura: Portunidae), based on two partial mitochondrial genes. *Marine Biology,* 150, 1265–1274.

Santana, W., Pohle, G. & Marques, F.P.L. (2003) Zoeal stages and megalopa of *Mithrax hispidus* (Herbst, 1790) (Decapoda: Brachyura: Majoidea: Mithracidae): a reappraisal of larval characters from laboratory cultured material and a review of larvae of the *Mithrax-Mithraculus* species complex. *Invertebrate Reproduction & Development,* 44, 17–32.

Schubart, C.D., Neigel, J.E. & Felder, D.L. (2000) Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *In:* J. C. von Vaupel Klein & F. R. Schram (Eds), *The Biodiversity Crisis and Crustacea: Proceedings of the Fourth International Crustacean Congress, Amsterdam, Netherlands, 20-24 July 1998, volume 2*. A.A. Balkema, Rotterdam, pp. 817–830.

Swofford, D.L. (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0. Sinauer Associates, Sunderland, Massachusetts.

Williams, A.B. (1965) Marine decapod crustaceans of the Carolinas. *Fishery Bulletin,* 65, 1–298.

Williams, A.B. (1983) The mud crab, *Panopeus herbstii*, s.l. partition into six species (Decapoda: Xanthidae). *Fishery Bulletin,* 81, 863–882.

Williams, A.B. (1984) *Shrimps, Lobsters, and Crabs of the Atlantic Coast of the Eastern United States, Maine to Florida*. Smithsonian Institution Press, Washington, D.C., 550 pp.