

GENETIC DIFFERENCES AMONG SIBLING SPECIES OF THE SUBGENUS *DIMOCKATA* (ACARI: UNIONICOLIDAE: *UNIONICOLA*): HETEROGENEITY IN DNA SEQUENCE DATA SUPPORTS MORPHOLOGICAL DIFFERENTIATION

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ABSTRACT - Water mites of the subgenus *Dimockatax* (Unionicolidae: *Unionicola*) are common inhabitants of anodontine bivalve molluscs. Morphological differences among the five species that comprise the subgenus are minor and based primarily on subtle differences in one or two characters. The newly described species *Unionicola ernstingi* (Edwards *et al.*, 2008) is distinguished from its sibling species *U. tumida* (Wolcott, 1898) by the number of clawlets on the tarsus of the pedipalps. These mites have been reported from the same locality, although they inhabit distinct species of host mussels. *Unionicola ernstingi* occurs with *Anodontooides radiatus* (Conrad, 1834), and *U. tumida* is found in association with *Strophitus subvexus* (Conrad, 1834). Taxonomic delineations among *Unionicola* mussel-mites parasitizing different species of hosts have, however, been challenged by the suggestion that morphological differences between mites may be influenced by the host species in which they metamorphosed. To test the validity of the species status for *Dimockatax* mites from *A. radiatus* and *S. subvexus*, heterogeneity in sequence data of the *cox1* gene was examined. A comparison of *cox1* sequences for *U. tumida* from *S. subvexus* and *U. ernstingi* from *A. radiatus* revealed a high degree of differentiation (12.7%). The genetic differences observed for *U. ernstingi* and *U. tumida* are consistent with those observed for other closely related, yet morphologically distinct species of *Unionicola* reported from different species of host mussel and thus complement morphological data recognizing them as valid species.

Key words - Acari, Unionicolidae, *cox1*, *Unionicola*, *Dimockatax*, genetic differences.

INTRODUCTION

Water mites of the genus *Unionicola* Haldeman, 1842 (Acari: Unionicolidae) are a cosmopolitan group of acariformes, commonly occurring in parasitic association with sponges or molluscs during one or more stages of their life cycle (Mitchell, 1955). More than half of the described species that comprise the group occur in association with freshwater mussels, living on the gills or mantle and foot of their hosts and using these tissues as sites of oviposition (Vidrine, 1996a).

The genus has undergone extensive revisions over the past 25 years (Vidrine 1986a, 1996a, 1996b) and currently contains more than 230 described species in some 56 subgenera (Gledhill and Vidrine, 2002; Vidrine *et al.*, 2007, 2008). Subgeneric distinctions are based primarily on variations in the structure of the genital field (Cook, 1974; Vidrine, 1986a), while species within subgenera

are distinguished by chaetotaxy or the shapes of claws on the walking legs (Vidrine, 1996a). There are, however, certain subgenera of *Unionicola* that contain groups of species that are extremely difficult to separate using morphological criteria. For example, *Unionicola bakeri* Vidrine, 1986 and *U. fossulata* Koenike, 1895 of the subgenus *Unionicolides* Lundblad, 1937 are morphologically very similar, except that *Unionicola bakeri* possesses minutely bifid tarsal claws on the three posterior pairs of walking legs (Vidrine, 1996a). The subgenus *Unionicola* Haldeman, 1842 (formerly *Parasitatax* Viets, 1948) has historically included the following species: *U. ypsilophora* Bonz, 1783, *U. formosa* Dana and Whelpley, 1836, *U. dimocki* Vidrine 1986, *U. uchidai* Imamura, 1953, and *U. thienemanni* Viets, 1957 (Vidrine, 1986b), and while some of these species can be identified by the unique structure of their dorsal plates, *U. ypsilophora* and *U. formosa* are separated on the basis of slight differences in

the posterior coxal group that is evident only among males (Vidrine, 1986b). Interestingly, an examination of allozyme variation (Edwards and Dimock, 1997) and heterogeneity in the sequence data of the mitochondrial cytochrome oxidase subunit I (*cox1*) gene (Ernsting *et al.*, 2006) among populations of *U. formosa* from different species of host mussels revealed high levels of genetic differentiation among some host-associated populations. The genetic findings of Edwards and Dimock (1997) resulted in the designation of a new species within the subgenus *Unionicola*, *U. foilli* Edwards and Vidrine, 1994.

There are 5 known species of *Unionicola* from the subgenus *Dimockatax* Vidrine, 1992, four of which occur in North America in association with anodontine bivalve molluscs (Vidrine, 1986b). Morphological differences among species from this subgenus are minor and based primarily on the number and relative size of clawlets on the palpal tarsus. For example, *U. neocooki* Vidrine, 1987, *U. bishopi* Vidrine, 1986, and *U. tumida* Wolcott, 1898 all possess 2 clawlets, but they differ from one another depending on whether the clawlets are moderately large and distinct (*U. bishopi*), small but distinct (*U. neocooki*), or small and indistinct (*U. tumida*) (Vidrine, 1996a). The Asian mite *U. tumidoides* Vidrine, 1986 (1986b) and the newly described species *U. ernstingi* Edwards *et al.*, 2008 (Edwards *et al.*, 2008) from North America have one moderately large clawlet inserted on the palp tarsus. The chaetotaxy of leg I of *U. ernstingi* is diagnostically more densely setose than the Asian *U. tumidoides*. The American species is much larger, nearly twice the size of its Asian counterpart (Edwards *et al.*, 2008).

The reliability of subtle differences in one or two morphological character states in differentiating among closely related species of *Unionicola* has been called into question by Downes (1990). Specifically, Downes (1990) argued that the minor differences in the shape of the tarsal claws of legs II-IV that were used to differentiate between *U. poundsi* Vidrine, 1986 from the host mussel *Villosa villosa* Wright, 1898 and *U. lasallei* Vidrine, 1986 from *Unio merus declivis* Say, 1831 were plastic and influenced by the species of host mussel in which these mites metamorphosed. If Downes's (1990) contention is correct, then *U. poundsi* and *U. lasallei* are simply variants of the same species. The conclusions of Downes (1990) were, however, challenged by Edwards and Labhart (2000) who questioned the experimental evidence used to support the notion of host-induced changes in morphology among these mites and provided electrophoretic data to suggest that mite populations from *V. villosa* and *U. declivis* were reproductively isolated.

Because molecular genetic data have been used to uncover sibling species in the genus *Unionicola* and test hypotheses about species delineations among morphological similar species, the present study addresses heteroge-

neity in sequence data of the *cox1* gene among *U. tumida* and the newly described species *U. ernstingi*. These mites occur in association with two distinct species of host mussels, and differ from one another morphologically on the basis of a single character state (Edwards *et al.*, 2008).

MATERIALS AND METHODS

The host mussels, *Strophitus subvexus* Conrad, 1834 (harboring *U. tumida*) and *Anodontoides radiatus* Conrad, 1834 (harboring *U. ernstingi*) were collected in September 2006 and July 2007 from Twelve Mile Creek, St. Helena Parish, Louisiana (30°45'N, 90°40'W). Voucher specimen of males for *U. tumida* and *U. ernstingi* have been deposited in the Smithsonian Institution's National Museum of Natural History (USNMNH gift # 2046995 and 2046207, respectively). Mussels collected in September 2006 were placed in an insulated cooler packed with ice and shipped to the University of Evansville (UE). In the laboratory, *U. tumida* and *U. ernstingi* were removed from their respective host mussels and washed several times in deionized water. Mites obtained from mussels collected in July 2007 were removed from their hosts at the Louisiana collecting locality. They were subsequently washed several times in deionized water, placed in 1 cm i.d. glass vials containing 100% ethyl alcohol, and shipped to UE in an insulated cooler packed with ice. All mites were placed individually in 1.5 ml microcentrifuge tubes and stored at -80°C, awaiting DNA extraction.

PCR and sequencing primers used for this study are presented in Table 1. Primers were designed based on sequence data from closely related species of *Unionicola* (Ernsting *et al.*, 2006). Characteristics of the primer pairs were evaluated using NetPrimer (Premier Biosoft International, Palo Alto, California, USA).

Total cellular DNA was extracted from individual mites (N = 6 for each species) using Qiagen DNeasyTM Tissue Kits (Qiagen, Valencia, California, USA). Mites were thawed and ground with disposable pestles (Edwards *et al.*, 2004) before overnight incubation in the presence of proteinase K. Extracted DNA was stored in nuclease-free water at -20°C.

PCR reactions (50 µl) were set up using PCR Master Mix (Promega, Madison, Wisconsin, USA); 1.5 mM MgCl₂, 200 µM dNTPs, 1 µM of each primer, and 1 µl of DNA template per reaction. PCR amplifications consisted of an initial 5 minute denaturation at 94°C followed by thirty cycles of 94°C for 1 minute, annealing for 1 minute, and extension at 72°C for 1 minute. PCR products were visualized on a 1% agarose gel and product size was estimated by visual comparison to molecular weight markers. PCR products were purified using QIAquick PCR purification kits (Qiagen) and submitted to commercial sequencing services. For each species, PCR products from

Table 1. The primers used to amplify fragments of the *cox1* gene for *Unionicola ernstingi* and *U. tumida*. Estimated T_m and annealing temperature for the primers are also presented.

Primer name	Sequence (5' to 3')	Estimated T_m (°C)	Annealing Temperature (°C)
FY-1	GGAGCAGGAACAGGATGAAC	56	48
RY-2	AAAACATAGTGAAAATGAGCTAC	49	48

among individuals (N = 6) for each species were identical. The 694-base alignments for *U. ernstingi* and *U. tumida* have no gaps and contain 88 (12.7%) variable nucleotides. Sequence variation clusters at the third position of codons (69%), based on the predicted amino acid sequence of the open reading frame in this region, and the majority of this variation is synonymous when translated using the invertebrate mitochondrial genetic code. The overrepresentation of synonymous variation strongly suggests that these sequences represent the actual mitochondrial *cox1* sequence, rather than nuclear mitochondrial pseudogenes.

DISCUSSION

A comparison of partial *cox1* sequences for *U. tumida* from *S. subvexus* and *U. ernstingi* from *A. radiatus* revealed a high degree of differentiation in sequence data between these two species of mites. The results of this molecular analysis are consistent with the heterogeneity in sequence data of the *cox1* gene observed for closely related species of *Unionicola* from the subgenus *Unionicola* (then *Parasitatax*) (Ernsting *et al.*, 2006). For example, a comparison of the 593-base sequence alignment of this gene for *U. dimocki* and *U. ypsilophora*, two species that are distinguished from one another based on subtle differences in the posterior coxal group that is evident only among males, reveals that these mites have 93 (15.7%) variable nucleotide sites. Two sibling species of this subgenus, *U. foili* and *U. formosa*, that have been designated separate species on the basis of host specificity and allozyme analysis (Edwards and Vidrine, 1994), display 88 (14.8%) variable nucleotide sites. The molecular data presented in this study support the recent findings of Edwards *et al.* (2008) who, based on differences in a few morphological character states, designated *Dimockatax* mites from *S. subvexus* and *A. radiatus* as separate species. The degree of sequence divergence reported among sibling species of *Unionicola* is consistent with sequence variation among other morphologically distinct species of Trombidiformes mites. For example, a comparison of DNA sequence data for the same region of the *cox1* gene among the scrub typhus mites *Leptotrombidium akamushi* (Genbank accession number: AB194045) and *L. deliense*

(Genbank accession number: AB194044) revealed a sequence divergence of 16.8%.

In addition to displaying heterogeneity in DNA sequence data and subtle differences in morphology, populations of *U. tumida* and *U. ernstingi* from Twelve Mile Creek differ from one another in their patterns of host association. *Unionicola tumida* occurs exclusively with *S. subvexus*, whereas *U. ernstingi* is found only in association with *A. radiatus*. Host associations exhibited by these mites are consistent with several studies of *Unionicola* mussel-mites indicating that closely related, and in some cases morphologically indistinguishable, species are often highly host specific (Edwards and Dimock, 1995; Edwards *et al.*, 1998). An examination of the specificity of the host recognition behavior of *U. tumida* and *U. ernstingi* will be required before we can conclude that these mites exhibit species-specific associations toward their mussel hosts. Collectively, North American *Unionicola* mussel-mites are known to exhibit highly variable patterns of host specificity, with some species occurring in association with a long list of host species and others utilizing one or at most a few species of hosts (Vidrine 1996c; Edwards and Vidrine, 2006). An examination of both interspecific and intraspecific diversity of molecular sequence data among host-associated populations of these mites will play a valuable role in testing hypotheses about current species designations and potentially uncovering sibling species of *Unionicola* mussel-mites.

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