PHYLOGENETIC RELATIONSHIPS AMONG SPECIES OF THE SUBGENUS *PARASITATAX* (ACARI: UNIONICOLIDAE: *UNIONICOLA*) BASED ON DNA SEQUENCE OF THE MITOCHONDRIAL CYTOCHROME OXIDASE I GENE

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ABSTRACT - Morphological differences among species of parasitic water mites that comprise subgenus *Parasitatax* (Unionicolidae: *Unionicola*) are comparatively minor, with taxonomic distinctions among most members of the taxon based on subtle differences in one or two morphological characters. Two species of the subgenus, *Unionicola formosa* Dana and Whelpley 1836 and *U. foili* Edwards and Vidrine 1994 are morphologically indistinguishable and have been designated separate species on the basis of host specificity and allozyme analysis. The present study examines heterogeneity in sequence data of the mitochondrial cytochrome oxidase subunit I (COI) gene among four putative species of the subgenus *Parasitatax*: *U. dimocki* Vidrine 1986, *U. foili*, *U. formosa*, and *U. ypsilophora* Bonz 1783. Because populations of *U. formosa* from different species of host mussels are genetically divergent, intraspecific diversity of the COI gene among host-associated populations of *U. formosa* was also examined. DNA sequence data was used to construct a phylogenetic hypothesis for the group and assess the degree to which a phylogeny based on molecular data is consistent with traditional taxonomy. Maximum parsimony and maximum likelihood analysis each yielded a single tree with the same topology and high bootstrap support. The gene tree indicates two major clades, with *U. dimocki* and *U. ypsilophora* forming one clade and *U. foili* along with host-associated populations of *U. formosa* forming the other. The latter clade resolved into two branches and indicated that one host-associated population of *U. formosa* is more closely related to *U. foili* than it is to other host-associated populations of *U. formosa.*

Key words - Acari, Unionicolidae, *Unionicola, Parasitatax,* COI gene, phylogeny, clam mites, water mites, USA.

INTRODUCTION

Water mites of the genus *Unionicola* Haldeman 1842 (Acari: Unionicolidae) are a diverse group of acariformes with more than 200 named species in some 50 subgenera (Gledhill and Vidrine, 2002). Members of the genus commonly occur in parasitic association with sponges or mollusks during one or more stages of their life cycle. More than half of the described species of *Unionicola* parasitize freshwater mussels, living on the gills or mantle and foot of their hosts and using these tissues as sites of oviposition (Vidrine, 1996a).

Although the identification and classification of *Unionicola* have been reasonably documented (Vidrine, 1986, 1996a), phylogenetic relationships among members that comprise the group are less well known. The evolution of unionicolid mites appears to be closely tied to the evolutionary history of their hosts, given that major clades of mollusks are parasitized by unique assemblages of *Unionicola* subgenera (Vidrine, 1996b). Vidrine (1986a, 1996b) provided a general framework regarding relationships among *Unionicola* subgenera based upon general anatomical comparisons, but a reconstruction of the evolutionary history among subgenera or among species that are included in subgeneric groupings has not been attempted using phylogenetic approaches.

Addressing evolutionary relationships among species of *Unionicola* within subgenera using morphological criteria may prove to be problematical for at least two reasons. First, morphological differences among species that comprise subgenera are often comparatively minor, making it rather difficult to delineate species on the basis of morphological characters (Vidrine, 1996b; Edwards and Dimock, 1997). Second, there have been suggestions that

Table 1. Names and localities of collection sites of host mussels of representative species of *Parasitatax* and *U. parkeri* (outgroup).

Species of mites	Species of host mussels	Name of collecting site	Location of collecting site	Coordinates
Unionicola dimocki	*Strophitus subvexus*	Calcasieu River	Rapides Parish, Louisiana	92° 42' W, 31° 05' N
U. foili	*Utterbackia imbecillis*	Pangburn Pond	Vanderburgh County, Indiana	87° 41' W, 39° 24' N
*U. formosa**	*Anodonta suborbiculata*	Birch Lake	Vanderburgh County, Indiana	87° 25' W, 38° 04' N
U. formosa	*Pyganodon cataracta*	Meyer's Pond	Forsyth County, North Carolina	80° 16' W, 36° 03' N
U. formosa	*P. grandis*	Heckel's Pond	Vanderburgh County, Indiana	87° 54' W, 38° 12' N
U. ypsilophora	*A. cygnea*	Harlaxton Manor Pond	Lincolnshire County, England	00° 41' W, 52° 53' N
U. parkeri	*Fusconaia askewi*	Calcasieu River	Rapides Parish, Louisiana	92° 42' W, 31° 05' N

* Now designated as *U. foili* based on DNA sequence results presented in this study.

morphological characters that have been used to distinguish between certain species of *Unionicola* (i.e., species from the subgenus *Unionicolides* Lundblad 1937) are plastic and influenced by the host species in which these mites metamorphose (Downes, 1990). These issues underscore the need for accurate and reliable means of delineating among species of *Unionicola* collected in the field, before we can begin to elucidate their phylogenetic relationships.

Nowhere has it proven more difficult to delineate unionicolid mites using morphological criteria than among the six species that comprise the subgenus *Parasitatax* Viets 1949: *Unionicola ypsilophora* Bonz 1783, *U. foili* Edwards and Vidrine 1994, *U. formosa* Dana and Whelpley 1836, *U. dimocki* Vidrine 1986, *U. uchidai* Imamura 1953, and *U. thienemanni* Viets 1957. Taxonomic distinctions among members of the taxon are often based on subtle differences in one or two morphological characters (Vidrine, 1986b). For example, *U. ypsilophora* is extremely similar to *U. foili* and *U. formosa,* with the former being distinguished from the latter two on the basis of slight differences in the posterior coxal group that is evident only among males (Vidrine, 1986b; Vidrine, 1996a). *Unionicola formosa* (*sensu lato*) traditionally has been reported from several species of freshwater mussels of the genera *Pyganodon* Crosse and Fischer 1893, *Utterbackia* Baker 1927, and *Anodonta* Lamarck 1799 (Vidrine, 1996a). However, an examination of the genetic structure of *U. formosa* from different host genera has revealed high levels of genetic differentiation among some host-associated populations. Edwards and Dimock (1997) reported fixed allelic differences for three enzyme loci between *U. formosa* from *Pyganodon cataracta* Say 1817 and *Utterbackia imbecillis* Say, 1829. Based on these data, Edwards and Vidrine (1994) recognized mites from *P. cataracta* as *U. formosa sensu stricto* and designated mites from *U. imbecillis* as a new taxon, *U. foili.* An additional electrophoretic analysis of *U. formosa (s.l.)* from *Anodonta suborbiculata* Say, 1831 and two species of *Pyganodon* (*P. cataracta* and *P. grandis* Say, 1829) revealed that mites from these genera were reproductively isolated (Edwards *et al.*, 1998). The aforementioned studies emphasize the need for molecular approaches in an effort to recognize cryptic species of mites from the subgenus *Parasitatax.*

The present study examines heterogeneity in sequence data of the mitochondrial cytochrome oxidase subunit I (COI) gene among a number of putative species and host-associated species populations of unionicolid mites of the subgenus *Parasitatax*. Sequence data from the COI gene was used for the analysis because it enables ready alignment of its bases among closely related species of acari (Navajas *et al*., 1996; SÖller *et al*., 2001; Toda *et al.*, 2001). Moreover, the COI gene is known to have a faster rate of sequence divergence than nuclear ribosomal genes (Otto and Wilson, 1998) and thus may be more likely to resolve relationships among closely related species. The DNA sequence data was used to construct a phylogenetic hypothesis among representative members of the group and assess the degree to which a phylogeny based on molecular data is consistent with traditional taxonomy.

MATERIALS AND METHODS

Study animals - The species of *Parasitatax* used in this study, along with their host mussels and collection localities are presented in Table 1. Because *U. formosa*

Table 2. The primers used to amplify fragments of the COI gene for species of *Unionicola* of the subgenus *Parasitatax*. Estimated and T_m annealing temperature for each of the primers is also presented.

Primer Set Name	Primer name	Sequence (5' to 3')	Estimated T_m (°C)	Annealing Temperature (°C)
A	FB	GCACCAGATATAGCTTTTCCACG	62	52
	RC-2	GGATAATCTGAATAACGACGAGG	59	52
B	FC-4	GGAGCAGGAACAGGATGAACA	67	52
	RA-3	GATAAACATAGTGAAAATGAGC	56	52
C	FC-3	GGAACAGGATGAACAGTTTATCC	62	50
	RA-3	GATAAACATAGTGAAAATGAGC	56	50
D	FW-2	GCAGGAATCTCCTCAATTTTAGG	64	50
	RW-2	AATAACTCCTGTTTATCCCTCC	58	50

from different species of *Pyganodon* and *Anodonta* are known to exhibit considerable genetic differentiation (Edwards *et al.,* 1998), individual mites from both host genera were examined. *Unionicola parkeri* Vidrine 1987, a member of a distinctively different subgenus of gill mites (*Unioincolides),* was designated as an outgroup and COI sequence data obtained from this mite species was included in the phylogenetic analysis. Voucher specimens of all species used for DNA analysis have been deposited in the National Museum of Natural History of the Smithsonian Institution.

Species of *Parasitatax* obtained from mussels in Indiana were removed from their hosts within 24 hr of being collected in the field. Mites from North Carolina and Louisiana were removed from host mussels at these collection localities, placed in 1 cm i.d.. glass vials containing pond water, and shipped in insulated coolers packed with ice. All individuals were washed several times in deionized water, placed individually in 1.5 ml microcentrifuge tubes, and stored at -70° C, awaiting DNA extraction. *Unionicola ypsilophora* obtained from England were washed as above, preserved in 100% ethyl alcohol

Table 3. The species of *Unionicola* and their respective hosts from which COI DNA sequences were obtained. The accession numbers of DNA sequences submitted to Genbank, original sequence lengths, and number of sequence nucleotide polymorphisms (SNPs) are also provided.

Mite species	Host species	Accession number	# individuals sequenced	Primer set	Original sequence length (bases)	Number of SNPs in in 593-base accession
U. dimocki	*S. subvexus*	DQ222465	4	D	593	1
U. foili	*U. imbecillis*	DQ222460	4	A	947	1
*U. formosa**	*A. suborbiculata*	DQ222461	4	A	964	0
U. formosa	*P. cataracta*	DQ222462	4	B	753	0
U. formosa	*P. grandis*	DQ222463	4	D	607	1
U. ypsilophora	*A. cygnea*	DQ222464	4	C	703	0
U. parkeri	*F. askewi*	DQ222466	6	C	700	2

* Now designated as *U. foili* based on DNA sequence results presented in this study.

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U. dimocki(S. subvexus)      CAATTTTAGGTTCCATTAACTTTATAGCAACCATTTTAAATATAAAACCC 50
U. foili (U. imbecillis)     CAATTTTAGGAGCCATCAATTTCATAGCAACCATTTTTAACATAAAACCC 50
U. foili (A. suborbiculata)  CAATTTTAGGAGCCATCAATTTCATAGCAACCATTTTTAACATAAAACCC 50
U. formosa (P. cataracta)    CAATTTTAGGATCTATTAATTTTATAGCAACTATTCTAAATATAAAACCC 50
U. formosa (P. grandis)      CAATTTTAGGATCTATTAATTTTATAGCAACTATTCTAAATATAAAACCC 50
U. ypsilophora (A. cygnea)   CTATTCTAGGGTCTATTAATTTCATAGCCACTATTTTAAATATAAAACCC 50
U. parkeri (F. askewi)       CCATTTTAGGAGCAATCAACTTCCTAGTGACGATTCTAAATATAAAACCT 50
                             * *** ****  * ** ** **  ***  ** *** * ** ********

U. dimocki (S. subvexus)     AAACGGATAATAATAGAACAAATACCCCTATTTGTTTGATCAATTCTAAT 100
U. foili (U. imbecillis)     AAACGAATATTAATAGAACAAGTGCCTTTATTTGTCTGATCTATTTTAAT 100
U. foili (A. suborbiculata)  AAACGAATATTAATAGAACAAGTGCCTTTATTTGTCTGATCTATTTTAAT 100
U. formosa (P. cataracta)    AAACGAATAATAATAGAACAAATACCATTATTTGTATGATCAATCCTTAT 100
U. formosa (P. grandis)      AAACGAATAATAATAGAACAAATACCATTATTTGTATGATCAATCCTTAT 100
U. ypsilophora (A. cygnea)   AAACACATAATAATAGAACAAATACCCCTCTTCGTTTGATCTATTCTAAT 100
U. parkeri (F. askewi)       AAGTATATACAAAAAGAACAAATACCGTTATTCGTATATTCAATCCTAGT 100
                             **    ***  ** ******* * **  * ** ** *  ** **  *  *

U. dimocki (S. subvexus)     TACTGTTATCCTACTTTTATTATCTTTRCCTGTTTTAGCAGGAGCTATTA 150
U. foili (U. imbecillis)     TACAGTAATCCTACTATTACTTTCATTACCTGTTTTAGCTGGGGCTATTA 150
U. foili (A. suborbiculata)  TACAGTAATCCTACTATTACTTTCATTACCTGTTTTAGCTGGGGCTATTA 150
U. formosa (P. cataracta)    TACAGTAGTTTTACTGTTACTCTCTTTACCCGTTTTAGCCGGAGCCATTA 150
U. formosa (P. grandis)      TACAGTAGTTTTACTGTTACTCTCTTTACCCGTTTTAGCCGGAGCCATTA 150
U. ypsilophora (A. cygnea)   CACTGTAGTTTTACTCTTACTCTCTTTACCTGTTTTAGCAGGAGCCATTA 150
U. parkeri (F. askewi)       AACAGTAATCCTTCTTTTACTATCTTTACCTGTATTAGCAGGAGCTATTA 150
                             ** **  *  * ** *** * ** ** ** ** ***** ** ** ****

U. dimocki (S. subvexus)     CAATACTTCTGACAGATCGAAATTTTAATACATCATTTTTCGACCCTGCT 200
U. foili (U. imbecillis)     CTATACTTTTAACAGACCGAAATTTTAACACCTCCTTCTTCGACCCCGCC 200
U. foili (A. suborbiculata)  CTATACTTTTAACAGACCGAAATTTTAACACCTCCTTCTTCGACCCCGCC 200
U. formosa (P. cataracta)    CCATACTATTAACAGACCGAAACTTTAACACTTCTTTTTTCGACCCTGCT 200
U. formosa (P. grandis)      CCATACTATTAACAGACCGAAACTTTAACACTTCTTTTTTCGACCCTGCT 200
U. ypsilophora (A. cygnea)   CAATACTTTTAACAGATCGTAATTTTAACACATCTTTCTTCGATCCCGCA 200
U. parkeri (F. askewi)       CCATACTATTAACTGATCGAAATTTCAATACATCATTTTTTGACCCAGCA 200
                             * *****  * ** ** ** ** ** ** ** ** ** ** ** ** ** **

U. dimocki (S. subvexus)     GGTGGAGGGGACCCAATCCTCTATCAACACCTATTTTGATTTTTTGGGCA 250
U. foili (U. imbecillis)     GGGGGAGGAGATCCAATTCTTTATCAACATTTATTTTGATTTTTCGGTCA 250
U. foili (A. suborbiculata)  GGGGGAGGAGATCCAATTCTTTATCAACATTTATTTTGATTTTTCGGTCA 250
U. formosa (P. cataracta)    GGAGGAGGAGATCCCATTTTATACCAACATCTATTTTGATTTTTCGGACA 250
U. formosa (P. grandis)      GGAGGAGGAGATCCCATTTTATACCAACATCTATTTTGATTTTTCGGACA 250
U. ypsilophora (A. cygnea)   GGGGGTGGAGACCCCATCCTTTATCAACATCTATTCTGATTTTTTGGACA 250
U. parkeri (F. askewi)       GGAGGAGGAGACCCTATCCTTTATCAACACCTATTTTGATTTTTCGGGCA 250
                             ** ** ** ** ** **  * ** *****  **** ******** ** **

U. dimocki (S. subvexus)     CCCAGAAGTATACATTTTAATTCTACCAGGATTTGGTATGATCTCTCATA 300
U. foili (U. imbecillis)     CCCAGAAGTTTATATTTTAATTTTACCAGGATTTGGAATGATTTCTCATA 300
U. foili (A. suborbiculata)  CCCAGAAGTTTATATTTTAATTTTACCAGGATTTGGAATGATTTCTCATA 300
U. formosa (P. cataracta)    CCCTGAAGTTTATATTTTAATCCTACCAGGATTTGGAATAATCTCTCATA 300
U. formosa (P. grandis)      CCCTGAAGTTTATATTTTAATCCTACCAGGATTTGGAATAATCTCTCATA 300
U. ypsilophora (A. cygnea)   CCCAGAAGTATACATTTTAATTTTACCAGGATTTGGTATAATCTCCCATA 300
U. parkeri (F. askewi)       CCCTGAAGTATATATTTTAATTTTACCAGGATTTGGAATAATTTCTCATA 300
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Fig. 1. Aligned partial COI sequences (5'–3') for *Parasitatax* species and *Unionicola parkeri*. Hosts for each species of mite are in parentheses. Asterisks (*) indicate nucleotides that are shared by all species and host-associated populations. *Unionicola foili* from *A. suborbiculata* was previously recognized as *U. formosa.* (See discussion for explanation of designation change) (continued on next page).

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U. dimocki (S. subvexus)      TCGTAAGCTTTTACTCAGGTAAAAAAGAACCTTTAGGAGCATTAGGTATA 350
U. foili (U. imbecillis)      TCGTAAGATTTTACTCAGGGAAAAAAGAACCTCTAGGATCTCTAGGAATA 350
U. foili (A. suborbiculata)   TCGTAAGATTTTACTCAGGGAAAAAAGAACCTCTAGGATCTCTAGGAATA 350
U. formosa (P. cataracta)     TAGTAAGATTTTATTCAGGAAAAAAAGAACCGCTAGGTTCACTAGGAATA 350
U. formosa (P. grandis)       TAGTAAGATTTTATTCAGGAAAAAAAGAACCGCTAGGTTCACTAGGAATA 350
U. ypsilophora (A. cygnea)    TCGTAAGATTCTATTCAGGAAAAAAAGAACCTTTAGGTCCATTAGGGATA 350
U. parkeri (F. askewi)        TCGTTAGCTTCTACTCAGGAAAAAAGGAACCCTTAGGACCACTAAGAATA 350
                              * ** ** ** ** ***** ***** *****  ****  *  ** * ***

U. dimocki (S. subvexus)      ATCTTTGCTATAGCAGCAATCGGATTTTTAGGTTTTATTGTTTGGGCCCA 400
U. foili (U. imbecillis)      ATTTACGCAATAGTAGCAATCGGATTCTTAGGTTTTATCGTATGAGCTCA 400
U. foili (A. suborbiculata)   ATTTACGCAATAGTAGCAATCGGATTCTTAGGTTTTATCGTATGAGCTCA 400
U. formosa (P. cataracta)     ATTTATGCAATAGTAGCAATCGGATTTTTAGGATTTATTGTATGAGCCCA 400
U. formosa (P. grandis)       ATTTATGCAATAGTAGCAATCGGATTTTTAGGATTTATTGTATGAGCCCA 400
U. ypsilophora (A. cygnea)    ATTTATGCAATAGCAGCAATCGGATTTCTCGGTTTTATCGTTTGGGCTCA 400
U. parkeri (F. askewi)        ACTTACGCTATGTTTGCCATCGGACTTTTAGGATTCCTAGTATGAGCACA 400
                              *  *  ** **    ** ****** *  * ** **  * ** ** ** **

U. dimocki (S. subvexus)      TCACATATTTACAGTAGGTTTAGATGTAGATACACGAGCTTACTTCACTG 450
U. foili (U. imbecillis)      TCACATATTCACAGTAGGTTTAGATGTTGATACACGAGCCTATTTTACAG 450
U. foili (A. suborbiculata)   TCACATATTCACAGTAGGTTTAGATGTTGATACACGAGCCTATTTTACAG 450
U. formosa (P. cataracta)     CCATATATTTACAGTAGGATTAGATGTTGATACACGAGCTTATTTCACAG 450
U. formosa (P. grandis)       CCATATATTTACAGTAGGATTAGATGTTGATACACGAGCTTATTTCACAG 450
U. ypsilophora (A. cygnea)    CCATATATTCACAGTAGGATTAGATGTAGATACACGGGCCTACTTTACTG 450
U. parkeri (F. askewi)        TCATATATTTACTGTAGGTATAGACGTAGATACACGAGCATAMTTCACAG 450
                              ** ***** ** *****  **** ** ******** ** ** ** ** *

U. dimocki (S. subvexus)      CCGCAACAATAGTTATCGCCATCCCAACAGGAATTAAAATTTTTAGCTGA 500
U. foili (U. imbecillis)      CCGCAACAATAGTTATTGCTATCCCAACYGGAATTAAAATTTTTAGCTGA 500
U. foili (A. suborbiculata)   CCGCAACAATAGTTATTGCTATCCCAACCGGAATTAAAATTTTTAGCTGA 500
U. formosa (P. cataracta)     CTGCAACAATAGTAATTGCCATTCCAACAGGAATTAAAATTTTTAGATGA 500
U. formosa (P. grandis)       CTGCAACAATAGTAATTGCCATTCCAACAGGAATTAAAATYTTTAGATGA 500
U. ypsilophora (A. cygnea)    CTGCAACAATAGTTATTGCTATCCCAACAGGAATCAAAATCTTCAGATGA 500
U. parkeri (F. askewi)        CTGCAACCATAATCATTGCCATCCCCACAGGAATTAAAATTTTCAGCTGA 500
                              * ***** *** * ** ** ** ** ** ***** ***** ** ** ***

U. dimocki (S. subvexus)      ATAGCTACCTTTTCAGGATCCCCTATCTCCTTCGACACTCCCACCTTATG 550
U. foili (U. imbecillis)      ATAGCTACTTACTCAGGATCCCCCATCTCTTTTGATACACCAACCCTCTG 550
U. foili (A. suborbiculata)   ATAGCTACTTACTCAGGATCCCCCATCTCTTTTGATACACCAACCCTCTG 550
U. formosa (P. cataracta)     TTAGCTACCTATTCAGGGTCCCCAATCACTTTTGACACACCAACTTTATG 550
U. formosa (P. grandis)       TTAGCCACCTATTCAGGGTCCCCAATCACTTTTGACACACCAACTTTATG 550
U. ypsilophora (A. cygnea)    ATAGCTACTATCTCCGGGTCTTTCATCTCCTTTGATACCCCAACCCTTTG 550
U. parkeri (F. askewi)        ATAGCCACAATCGCAGGATCCCCAATCTCATTTGACCCCCCAGTATTATG 550
                              **** **     * ** **    *** * ** **  * **     * **

U. dimocki (S. subvexus)      GGCGTTTGGGTTTATTTTTTTATTCACCATAGGAGGGATAACA 593
U. foili (U. imbecillis)      ATCCTTCGGTTTTATTTTTCTATTCACCATAGGAGGTATAACA 593
U. foili (A. suborbiculata)   ATCCTTCGGTTTTATTTTTCTATTCACCATAGGAGGTATAACA 593
U. formosa (P. cataracta)     GTCATTTGGGTTTATTTTTTTATTTACAATAGGAGGGATAACA 593
U. formosa (P. grandis)       GTCATTTGGATTTATTTTTTTATTTACAATAGGAGGGATAACA 593
U. ypsilophora (A. cygnea)    AGCCTTAGGTTTTATTTTTCTATTCACTGTAGGGGGGATAACA 593
U. parkeri (F. askewi)        ATCTTTAGGHTTTATTTTTCTATTCACAGTAGGAGGAATAACA 593
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and placed in 1.5 ml polypropylene microcentrifuge tubes prior to shipment and storage.

Primer design - PCR and sequencing primers used in this work are shown in Table 2. Because of the possibility that universal primers used at low annealing temperatures might amplify either nuclear pseudogenes or sequences from the host mussel, we designed several forward and reverse primers based on the mitochondrial COI sequence of the honeybee tracheal mite *Varroa destructor* Anderson & Trueman 2000 (Evans, 2002; primer Sets A, B, and C in Table 2). Later, sequence data from unionicolid mites were used to design new primers (primer set D in Table2). Characteristics of individual primers and primer pairs were evaluated using NetPrimer (Premier Biosoft International).

DNA extraction, PCR and sequencing - Total cellular DNA was extracted from individual mites using Qiagen DNeasy^{TM} Tissue Kits (Qiagen). 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.

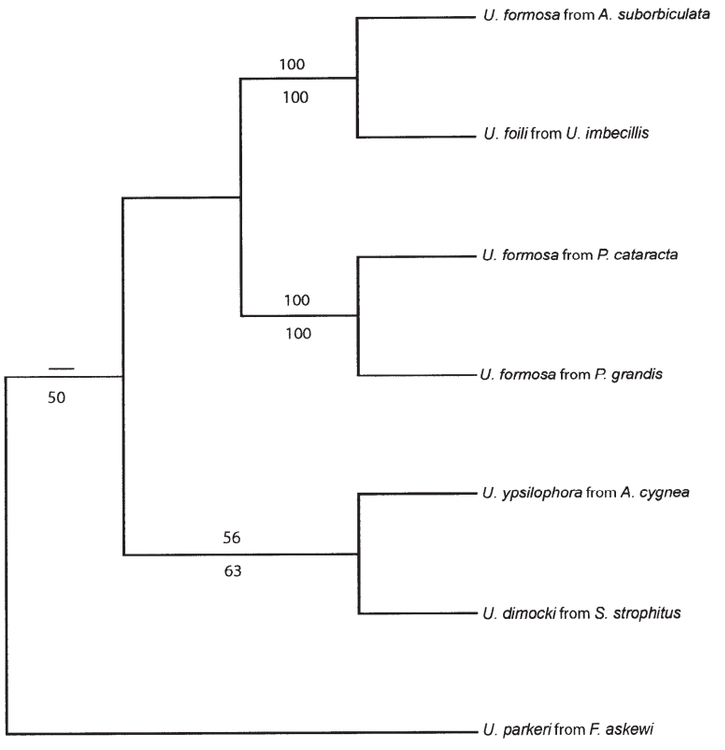

Fig. 2. Maximum-parsimony (MP) tree showing phylogenetic relationships among species of *Unionicola* of the subgenus *Parasitatax* based on partial sequence data of the COI gene. Maximum-likelihood (ML) tree (not shown) indicates the same topology. Bootstrap support values >50% from MP and ML analysis are reported above and below the branches, respectively.

PCR reactions (50 µl) were set up using PCR Master Mix (Promega; 1.5 mM $MgCl_2$, 200 µM dNTPs), 1 µM each primer, and 1µl of DNA template per reaction. PCR annealing temperature varied (Table 2), but all 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 at least four individuals were sequenced using the same forward and reverse primers used for the PCR amplification.

DNA sequence analysis - Forward and reverse sequence reads for all individuals representing a species were assembled using CodonCode Aligner (CodonCode), generating a single sequence for each species, with any discrepancies resolved using the sequencing chromatograms. Sequences were aligned using ClustalX (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/) alignment suite (Thompson *et al*., 1997).

Phylogenetic analysis - Phylogenetic analyses of aligned sequence data among species and host-associated populations *Parasitatax* and *U. parkeri* were performed using maximum-parsimony (MP) and maximum-likelihood (ML) search criteria with PAUP*4.0b10 (Swofford, 2002). Sequence data from *U. parkeri* served as the outgroup. MP and ML analyses were done using heuristic searches with nearest-neighbor interchange (NNI) branch swapping algorithms and MAXTREES set to 100. For MP analysis, the shortest trees that were recovered were pooled and used to generate strict consensus trees with majority-rule option set at 50%. ML analysis used the GTR+G model recommended by Modeltest 3.04 (Posada and Crandall, 1998), with empirical base frequencies. Statistical support for trees generated by the analyses was estimated by 100 bootstrap iterations on the original data matrix.

RESULTS

DNA sequences - For each species, DNA sequence was determined for four or more individual mites, using - both forward and reverse primers for each individual. A summary of the DNA sequencing data is shown in Table

3. For most species, all sequencing reads indicated the same sequence for this region. Where differences between the sequences of individuals within a species were observed, these bases are reported as ambiguous in the accessions, and are likely to represent single nucleotide polymorphisms within the species. The 593-base sequence alignment (Fig. 1) has no gaps for any of the taxa aligned, and contains 192 variable nucleotides. Sequence variation clusters at the third position of codons (74%), 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 COI sequence, rather than nuclear mitochondrial pseudogenes.

Phylogenetic analysis - Maximum-parsimony analysis revealed 122 parsimony informative characters. Heuristic searches yielded a single tree (Figure 2) with a tree length of 299 steps (CI = 0.78). Maximum-likelihood analysis resulted a single most likely tree with a score of -ln 2054.59. The topology of the ML tree (not shown) was identical to the tree generated by the MP analysis. Bootstrap analysis (100 pseudoreplicates) provided statistical support for most relationships presented in the tree (Fig. 2).

The typology of the MP and ML trees indicate two major clades, with *U. dimocki* and *U. ypsilophora* forming one clade and *U. foili* along with host-associated populations of *U. formosa* forming the other. The latter clade clearly resolved into two branches: one with *U. foili* and *U. formosa* from *A. suborbiculata* and the other with *U. formosa* from the two species of *Pyganodon.*

DISCUSSION

The gene tree for representative species of *Parasitatax* based on COI sequence data revealed that *U. dimocki* and *U. ypsilophora* were more closely related to each other than they were to *U. foil* or populations of *U. formosa.* In addition, the tree indicates a high degree of relatedness among *U. foili* and host-associated populations of *U. formosa.* A clade that has *U. ypsilophora* grouped with *U. dimocki* rather than *U. foili* and *U. formosa* is somewhat surprising given that *U. ypsilophora* is morphologically more similar to the two latter species (Vidrine, 1996a). Moreover, the genera of host mussels utilized by *U. dimocki* (*Lasmigona* Rafinesque 1931 and *Strophitus* Rafinesque, 1920) are phylogenetically distinct from those used by *U. ypsilophora, U. formosa,* and *U. foili,* (*Anodonta, Pyganodon,* and *Utterbackia,* respectively) (Hoeh, 1990), predicting a higher degree of affinity among the three latter species. The clustering together of *U. dimocki* and *U. ypsilophora* does, however, reflect the fact that *U. dimocki* is morphologically more similar to *U. ypsilophora* than it is to any other species of the subgenus *Parasitatax.* While the posterior coxal group of male *U. dimocki* and *U. ypsilophora* is well sclerotized, it is weakly sclerotized among male *U. foili* and *U. formosa* (Vidrine, 1996a).

The gene tree generated in this study also suggests that *U. formosa* from *A. suborbiculata* is more closely related to *U. foili* from *U. imbecillis* than it is to populations of *U. formosa* from *P. cataracta* and *P. grandis.* In fact, the partial COI sequences for *U. foili* and *U. formosa* from *A. suborbiculata* were identical. These results are consistent with those of Edwards *et al.* (1998) who found, based on an electrophoretic analysis, a high degree of genetic similarity among unionicolid mites from these two species of hosts. Edwards *et al.* (1998) also provided genetic evidence to suggest that *U. foili* from *U. imbecillis* and *U. formosa* from *A. suborbiculata* were reproductively isolated from populations of *U. formosa* associated with host mussels of the genus *Pyganodon.* The results of the molecular analysis from the present study, in conjunction with the genetic data from Edwards *et al.* (1998), argue that mites from *A. suborbiculata* should be designated as *U. foili* rather than *U. formosa.* A molecular characterization of *U. formosa s.l.* that occur in symbiotic association with other species *Anodonta* should be conducted to determine whether these mite populations should also be recognized as *U. foili*.

With one exception (*U. foili* from *A. suborbiculata)*, the monophyletic clades for the gene tree presented in this study do not conflict with traditional classifications of species comprising the subgenus *Parasitatax.* Whether or not the gene tree for species of *Parasitatax* presented in this study accurately reflects the evolutionary history of the group remains open to question. The degree of congruence between the gene tree and a phylogenetic hypothesis that incorporates morphological data cannot be compared adequately due to the fact that most of the species that comprise the subgenus are morphologically similar (Vidrine, 1996a). A more robust evolutionary history among species that comprise this taxon will require an analysis that incorporates sequence data from additional mitochondrial and nuclear genes. Future studies will use molecular sequence data to construct a multi-locus gene tree for *Parasitatax* and species of *Unionicola* from other subgenera for which morphological differences among species are comparatively conservative. Once hypotheses regarding the evolutionary relationships among species from various subgenera have been constructed, rates and patterns of character evolution and diversification among species that comprise these taxa can be elucidated.

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