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Random amplified polymorphic DNA analysis of kinship within host-associated populations of the symbiotic water mite *Unionicola foili* (Acari: Unionicolidae)

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Abstract. Kinship relations within populations of unionicolid water mites are not well known, owing to their complex life cycles and the fact that interactions between active and resting stages for some species are transitory. A number of species of unionicolid water mites are, however, obligate symbionts of freshwater mussels and spend most of their life cycle in association with these hosts. Among these species of mites, parents and offspring are more likely to co-occur and thus provide opportunities to address questions related to the structure of the mating system. The present study employs random amplified polymorphic DNA (RAPD) analysis to address kinship within populations of *Unionicola foili* living in symbiotic association with the host mussel *Utterbackia imbecillis*. DNA was amplified from adult mites and a representative number of eggs or larvae (n = 20–30) that were removed from mussels collected on three separate occasions (July, November, and March) over a 12-month period. Parsimony analyses of the molecular data for adults and progeny collected from mussels during July, November, and March revealed distinct groupings, that for the most part, corresponded to mites collected from each of the sampling periods. Many of the genetic markers obtained for male and female *U. foili* were not evident among the larvae or eggs, suggesting that adults obtained from a host mussel at the time of collection were not the parents of a majority of the progeny. However, female mites and eggs collected from mussels during March and November shared more markers than did females and progeny examined during July. Furthermore, many offspring in the July sampling period were found to have one or more parents absent from the sampled population. Overall, RAPD profiling appears to have limited usage in determining kinship within populations of *U. foili*, due to its recruitment patterns, and the relatively large number of adults and progeny per mussel. It may, however, prove to be a useful method for assessing genetic relatedness among unionicolid mussel-mites that have substantially lower population densities.

Introduction

The life cycle of water mites (Acarina: Hydrachnidia) includes egg, larva, protonymph, deutonymph, tritonymph, and adult. Larvae of most species undergo a brief parasitic association with aerial insects (Smith and Oliver 1986) and subsequently must return to the aquatic environment to complete their life cycle. The adults and deutonymphs of most water mite species are free-living predators (Gledhill 1985). There are, however, species from the family Pionidae

and Unionicolidae that are symbiotic with freshwater gastropods, mussels, and sponges (Mitchell 1955; Hevers 1980).

Water mites of the genus *Unionicola* (Haldeman) are common symbionts of freshwater mussels (Vidrine 1996). Some species are free-living predators as nymphs and adults, depending upon the host only for sites for oviposition and post-larval resting stages, while others are obligate symbionts of their host (Mitchell 1955). Adult mites presumably mate within the confines of the mantle cavity (Hevers 1978), with females subsequently depositing fertilized eggs in host gill tissues. Larvae emerge from the gills during spring and summer, leave the host mussel, and establish a parasitic association with pupae of the family Chironomidae (Jones 1978). When these insects molt, the larvae are dragged through the pupal exuvium and begin to engorge on host hemolymph (Böttger 1972; Hevers 1980; Gledhill 1985). Larvae that are attached to a host insect after it becomes aerial must return to the aquatic environment and re-enter a host mussel to complete their life cycle.

The North American unionicolid water mite *Unionicola foili* (Edwards and Vidrine) is an obligate symbiont that commonly occurs in permanent association with the freshwater mussel *Utterbackia imbecillis* (Say) (Vidrine 1996). Although the mite *Unionicola formosa* (Dana and Whelpley) traditionally has been described from freshwater mussels of the genera *Utterbackia* (Baker) and *Pyganodon* (Crosse and Fischer) (Vidrine 1996), Edwards and Vidrine (1994) have separated *U. formosa* into two species, *U. formosa* from mussels of the genus *Pyganodon* and *U. foili* from the genus *Utterbackia*. *Unionicola foili* is among the best studied water mites in North America, with published studies on its population dynamics (Dimock 1985; Edwards and Dimock 1988), genetic diversity (Edwards and Dimock 1997), patterns of host specificity (LaRochelle and Dimock 1981) and zoogeography (Dobson 1966; Vidrine 1996). Population studies indicate that *U. foili* exhibits a female-biased sex-ratio, with nearly 100% of host mussels harboring a single male and 40 or more females (Dimock 1985). Experimental and field evidence suggest that intra-sexual aggression and territoriality by males are, in part, responsible for this distribution (Dimock 1983). The territorial behavior displayed by males constitutes a female-defense polygynous mating system. Because male *U. foili* have exclusive access to numerous females within a host's mantle cavity, successfully defending that territory could potentially increase a male's fitness.

With the exception of the aforementioned studies, nothing else is known about the structure and dynamics of the mating system of *U. foili*. This unionicolid mussel-mite should, however, be well suited for addressing questions related to kinship, given its obligate relationship with mussels and the fact that adults, nymphs, and post-larval resting stages live concurrently in the same host. A number of species of unionicolid water mites are free-living predators as nymphs and adults, depending on a host only for oviposition and post-larval development (Mitchell 1955; Hevers 1980).

Over the past 10 years, molecular genetic approaches have become increasingly important to studies in behavioral and population ecology. In

particular, the ability to determine the genetic structure of populations using DNA fingerprinting technologies has enhanced our understanding of animal mating systems by permitting analyses of kinship relationships (Ross 2001). Unionicolid water mites present two important challenges to this type of analysis: (1) their genome is almost completely uncharacterized, and (2) individuals are small, limiting the amount of DNA available from a single individual. These constraints require that any genetic study involve amplification of template DNA without relying on extensive DNA sequence information. The use of random amplified polymorphic DNA (RAPD) markers addresses both of these concerns (Hadrys et al. 1992). The present study uses RAPD markers to address parentage among *U. foili* occurring in symbiotic association with its host mussel *U. imbecillis*.

Materials and methods

Study animals

Unionicola foili used for this study were obtained from a population of *Utterbackia imbecillis* collected from a 4-ha pond located in Perry Co., Indiana, U.S.A. (37° 56′N, 86° 43′W). A total of six mussels was collected, two each on three occasions over a 12-month period: summer (July 2002), autumn (November 2002), and winter (March 2003). Mussels were collected over different seasons because of the dynamic nature of displacement and recruitment of adult mites into the population (Dimock 1985). In the laboratory, all adults and a representative number of eggs or larvae (n = 20–30) were removed from a host mussel, washed several times in deionized water, and held in this medium for approximately 12 h. Mites were placed individually in 1.5 ml microcentrifuge tubes and stored at −70 °C, awaiting DNA extraction.

DNA extraction

Disposable pestles for grinding individuals were made by briefly heating 1000 μl pipette tips in a flame and pressing them into the bottom of a 1.5 ml centrifuge tube mold. The resulting pestles conformed to the shape of the storage tube, and allowed mites to be ground without being removed from the tube. After grinding, genomic DNA from individual mites was extracted using commercially available affinity methods: Geneclean™ or Qiagen DNeasy™ Tissue extraction kits.

PCR and electrophoresis

DNA amplification was carried out using a modification of the method of Williams et al. (1993). Twenty-five microliters reactions contained 2.5 units of

Taq DNA polymerase (Promega; Catalog number M1665), 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2 mM $MgCl_2$, 0.001% gelatin, 150 μM each dATP, dCTP, dGTP, and dTTP, 5 pmols of a single 10-base oligonucleotide primer, and 2 μl of template DNA (approximately 50 ng). The polymerase chain reaction used 45 cycles of denaturation at 94 °C for 1 min, annealing at 34 °C for 1 min, and extension at 72 °C for 2 min. PCR products were separated on 0.9% agarose gels and detected using ethidium bromide staining.

RAPD analysis

Parentage was determined by visual inspection of the markers present for each amplification reaction and assigning each marker as either present or absent in each individual. General relatedness among *U. foili* collected from mussels for each of the sampling periods was determined by parsimony analysis on genetic markers obtained from adult mites and offspring for three primers. The original data matrix is available from the corresponding author upon request. Parsimony analysis was done using heuristic search with nearest-neighbor interchange (NNI) branch swapping algorithm and MAXTREES set to 7200. Analysis was conducted using PAUP*4.0b10 (Swofford 2002). The shortest trees that were recovered were pooled and used to generate a majority rule consensus tree with majority-rule option set at 50%. Statistical support for the most parsimonious trees was assessed with bootstrap analysis on the original data matrix by generating 1000 pseudoreplicates.

Results

Individuals analyzed

All mussels from which mites were collected harbored one male and had a mean of 12.3 $\pm$ 1.7 (SE, range = 6–22) females/host. For mussels collected in July, offspring consisted mostly of larvae. In the autumn and winter sampling periods, there were no larvae present and all of the progeny from a mussel were eggs.

Preliminary data

To assess the suitability of RAPD profiling for determining parentage among *U. foili*, twelve 10-base oligonucleotide primers (Primer Kit A, Operon Technologies Inc.; Qiagen) were used individually to amplify DNA fragments for adult mites obtained from an individual mussel. Of those 12 primers, three (OPA03 [5′-AGTCAGCCAC], OPA09 [5′-GGGTAACGCC], and OPA11

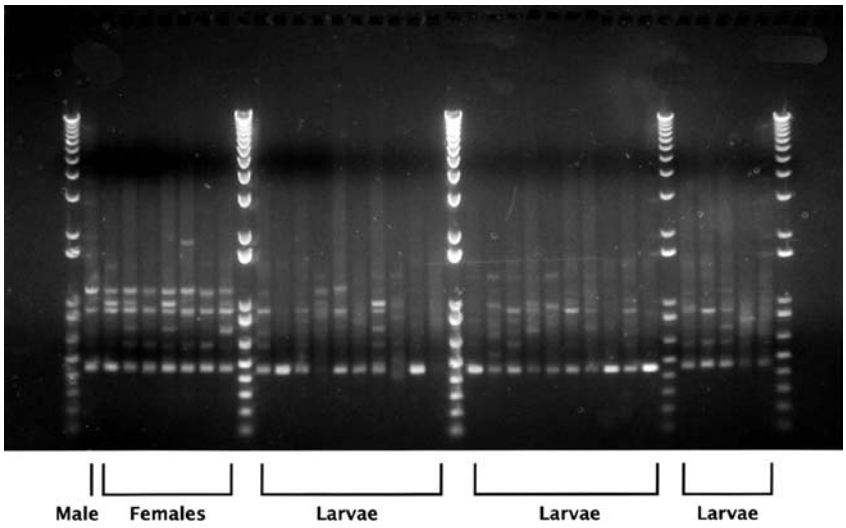

Figure 1. Agarose gel electrophoresis of RAPD products from *Unionicola foili* using primer OPA3. DNA was extracted from individuals isolated from a host mussel during the July sampling period. Five lanes contain molecular size standards. The remaining lanes contain RAPD products from all of the individuals isolated from this population. Male, females, and larvae are indicated at the bottom of the gel. Products are visualized by ethidium bromide staining.

[5′-CAATCGCCGT]) yielded a mixture of some PCR products that were present in most or all individuals and some that were present in a subset of individuals.

When RAPD amplifications were repeated on different days, using the same template DNA samples and the same primer, the number and molecular size of the markers were the same for most individuals. The reproducibility of RAPD markers was determined by comparing at least two profiles from the same population of *U. foili* for primers OPA03, OPA09, and OPA11. Where differences did occur, the bands that were not reproduced were very faint. For our determination of kinship relations, we analyzed the inheritance of RAPD markers that exhibited the same profiles when RAPD–PCR was repeated. All tissues including eggs yielded sufficient DNA for amplification using three primers. RAPD products for one primer from a population of mites removed from a host mussel collected in July are shown in Figure 1.

Parsimony analyses of RAPD profiles

A 50% majority rule consensus tree using RAPD profiles of individual *U. foili* from a representative mussel from July, November, and March is presented in Figure 2. The data set revealed 67 parsimony informative-characters. Heuristic searches identified 3280 equally parsimonious trees with a tree length of 322

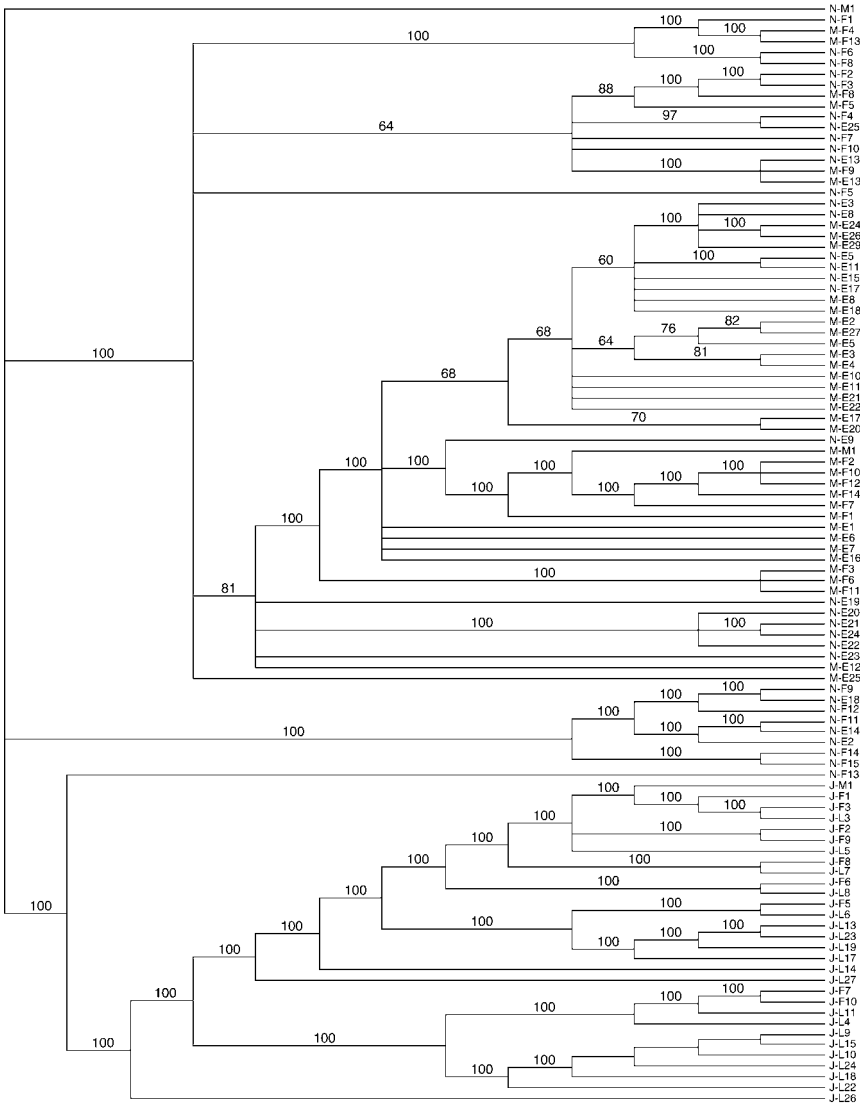

Figure 2. Majority rule consensus tree showing relatedness among *Unionicola foili* adults and progeny obtained from three *Utterbackia imbecillis* collected during July (J), November (N), and March (M). The tree was constructed using RAPD profiles for individuals using three 10-base oligonucleotide primers. Majority rule values > 50% are reported above the branches. M, male; F, female; L, larva; E, egg.

steps. Bootstrap analysis did not provide statistical support for most of the relationships presented in the 50% majority rule consensus tree. The majority rule consensus tree revealed distinct groupings, which with some exceptions, corresponded to mites obtained for each of the sampling periods.

RAPD profiles of U. foili *within host mussels*

Many of the genetic markers obtained for adult *U. foili* were not evident among larvae or eggs, suggesting that the male and females examined in a host mussel at the time of collection were not the parents of a majority of the progeny. However, adult mites and eggs collected from one mussel in March and from one mussel in November shared more markers than did adults and larvae obtained from other mussels. This became particularly evident when we examined which offspring (either larvae or eggs) from a single mussel contained one or more bands that were not present in any of the adults in the same host mussel (Table 1). Any band that was present in an offspring but absent in all adults excluded this individual offspring from being the progeny of the extant parents. In one representative June population, 90% of the offspring had one or both parents absent from the host's mantle cavity, with the excluded

Table 1. Number of exclusions for each offspring in three sampled populations of *U. foili.*

June		November		March	
Individual	Number of Exclusions	Individual	Number of Exclusions	Individual	Number of Exclusions
J-L3	1	N-E2	1	M-E1	0
J-L4	3	N-E3	1	M-E2	0
J-L5	3	N-E5	0	M-E3	0
J-L6	1	N-E8	1	M-E4	0
J-L7	0	N-E9	1	M-E5	0
J-L8	0	N-E11	0	M-E6	0
J-L9	6	N-E13	0	M-E7	0
J-L10	7	N-E14	0	M-E8	0
J-L11	7	N-E15	0	M-E9	0
J-L13	4	N-E17	0	M-E10	0
J-L14	1	N-E18	0	M-E11	0
J-L15	6	N-E19	0	M-E12	0
J-L17	3	N-E20	0	M-E13	0
J-L18	1	N-E21	0	M-E16	0
J-L19	1	N-E22	0	M-E17	0
J-L22	2	N-E23	0	M-E18	0
J-L23	5	N-E24	0	M-E20	1
J-L24	1	N-E25	0	M-E21	0
J-L26	3			M-E22	1
J-L27	1			M-E24	0
				M-E25	1
				M-E26	0
				M-E27	0
				M-E29	0
90% of offspring excluded		22% of offspring excluded		13% of offspring excluded	

Offspring were considered excluded if a marker present in an offspring was absent in all of the adults. J, July sampling period; N, November sampling period; M, March sampling period. L, larvae; E, eggs.

offspring having an average of 2.7 bands not present in any of the adults. During both November and March, one of the two sampled populations showed a much higher degree of relatedness, with 22% (November), and 13% (March) of offspring excluded in this way. Furthermore, during March and November, those offspring that were excluded all displayed a single band that was not present in the adults, in contrast to the multiple exclusions seen among summer populations.

Discussion

The results of the RAPD analyses indicate complex kinship relationships among the symbiotic water mite *U. foili*. Overall, a large percentage of RAPD markers found among female *U. foili* were not shared by larvae or eggs recovered from the same host mussel. In fact, most larvae and eggs were more closely related to each other than they were to adults collected from the same host.

With few exceptions, the results of the RAPD analysis showed that males had markers that were absent from most or all of the offspring. These results suggest that males that fathered the offspring may have been displaced by intruding males, perhaps through the intraspecific aggressive encounters that are characteristic of this species (Dimock 1983). The RAPD data also suggest that displacement of males may occur rather often. To determine the rate of turnover of resident males would require a more comprehensive sampling program than presented in this study and a better understanding of frequency of aggressive encounters between male mites in the field.

Despite overall low levels of similarity, there were higher degrees of genetic relatedness among female *U. foili* and immature stages that were collected during autumn (November) and winter (March) when compared to the summer (July) collecting period. This pattern of unrelated offspring and adults was observed in six populations of mites collected from mussels during the summer months, as well as in one of the two populations sampled in November, and one of the two populations sampled in March (data not shown). It was only during November and March that we observed any populations where the adults and offspring were closely related. In one of the two populations from each of these months, the majority of offspring present appear to be the offspring of the extant parents, and the parsimony analysis separates these individuals into closely related clusters. In general, the results of this study are consistent with seasonal changes in the population structure that have been reported for this mussel-mite. For example, Dimock (1985) reported annual minimum density of female *U. foili* per mussel in June and July but also found a higher proportion of small females in mussels during May and June. Dimock (1985) suggested that late spring–early summer represented a major period of recruitment into the adult population, with overwintering nymphs replacing the previous generation of females. The lack of genetic relatedness among

females and larvae from the summer sampling period may thus not be surprising if larvae emerging from the gills of host mussels during summer are the progeny of females that oviposited during winter and were subsequently replaced by nymphs that were recruiting into the population.

An increase in the genetic similarity between female *U. foili* and eggs collected during November is consistent with the data pertaining to oviposition and the size-frequency distribution of females at this time. Dimock (1985) reported an increase in the density of female mites among *U. imbecillis* in November and also observed an increase both in the number of eggs per female and occurrence of mite eggs in host gills during this period. It is possible that females recovered from mussels during November represent individuals that were recruited in to the host population over the summer and that these mites were responsible for the increase in the number of eggs in the gills of mussels during winter. Similarly, the relatively high degree of genetic relatedness among female *U. foili* and eggs examined from mussels collected in March correspond to the concomitant maxima in the density of eggs per female and density of eggs in the gill tissue of *U. imbecillis* (Dimock 1985). Gravid females present in mussels during late winter–early spring are likely responsible for the large increase in the density of eggs in the host's gill during this period.

The RAPD methods reported in this study represent the first time that molecular markers have been used to characterize aspects of the mating system of water mites. DNA extraction from isolated individuals yielded sufficient DNA for multiple RAPD assays even from individuals in early stages of development, and RAPD profiles of eggs were as reproducible as those generated from adults. Initially, we were concerned that DNA extractions from early stages of a small species living inside the host would introduce unacceptable levels of contamination with host tissue, leading to RAPD products that indicative of the genetic state of the host rather than the symbiotic mites. Overnight incubation after extraction from the host along with extensive washing and removal of any residual liquid that might contain host tissue were carried out with each individual to decrease the ratio of host DNA to mite DNA in the extracted samples. The reproducible variation between individuals suggests that contamination with host DNA is not a serious concern in this system.

There are, however, a number of reasons why the RAPD data presented in this study should be interpreted with some degree of caution. First, RAPD profiles were generated for mites from only two host mussels for each of the sampling periods. A larger number of host-associated populations of *U. foili* from each of the sampling periods will need to be examined before more definitive conclusions regarding kinship relations for this species can be made. Interestingly, we have RAPD profiles for individual *U. foili* from three additional mussels collected during July and the genetic relatedness among mites from these mussels is consistent with the RAPD data obtained for mites from the two host mussels collected in July presented in this study. Second, there are contrasting differences in the degree of genetic relatedness among individuals from the two host mussels collected during both the November and March

sampling periods, with one host-associated population of mites exhibiting a relatively high degree of similarity and the other showing relatively low degree of relatedness. Third, the bootstrap analysis did not provide support for the relationships generated by the 50% majority consensus trees. Finally, the anonymity of RAPD products, and our electrophoretic separation on the basis of product size raise the possibility of occasionally combining two non-identical markers that happen to be about the same molecular size as identical (Backeljau et al. 1995). This type of noise should, however, be rare, given the relatively small number of bands produced by any single RAPD primer, and the ability demonstrated by the wide separation of marker bands in the size range of the RAPD products in this assay to resolve size differences as small as a few percent. Furthermore, since the parsimony analysis is based on the shared inheritance of a large number of characters, it should be robust to the possibility of this type of occasional misidentification of non-identical characters as identical.

RAPD profiling has been useful in analyzing breeding system properties for an array of animal species, including ants, bees, and spiders (see Ross 2001 for a review), but it appears to have limited usage in addressing kinship within populations of the symbiotic water mite *U. foili*. The population dynamics of *U. foili*, coupled with extraordinary large numbers of eggs (maximum density of eggs/cm^2 = +300; Dimock 1985) and larvae (maximum density of larvae/cm^2 = +90; Dimock 1985) in the gills of host mussels, makes it particularly difficult to elucidate the precise nature of the genetic relationships between adults and progeny for this species. Future studies may, in part, circumvent these issues by removing eggs from gravid females and comparing the RAPD profiles of these eggs to those of their mothers and the resident male. Paternity relations among *U. foili* could be examined by removing a resident male and gravid females from a host mussel, introducing a new male, and determining whether the newly established male fathered the remaining females' offspring. Paternity in *U. foili* could also be addressed by inducing females to abort their eggs and determining whether the resident male was the sole father of future offspring. RAPD may prove to be a useful method for assessing the outcome of sperm competition for any species of water mite that will mate and develop eggs under laboratory conditions.

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References

Backeljau T., de Bruyn L., de Wolf H., Jordaens K., van Dongen S., Verhagen R. and Winnepenninckx B. 1995. Random amplified polymorphic DNA (RAPD) and parsimony methods. Cladistics 11: 119–130.

Böttger K. 1972. Vergleichende biologisch-ökologische Studien zum Entwicklungszyklus der Süsswassermilben (Hydrachnellae, Acari). II. Der Entwicklungzyklus von *Limnesia maculata* and *Unionicola crassipes*, International Revue Gesamten. Hydrobiologie 57: 263–319.

Dimock R.V. Jr. 1983. In defense of the harem: intraspecific aggression by male water mites (Acari: Unionicolidae). Ann. Entomol. Soc. Am. 76: 463–465.

Dimock R.V. Jr. 1985. Population dynamics of *Unionicola formosa* (Acari: Unionicolidae), a water mite with a harem. Am. Midl. Nat. 114: 168–179.

Dobson R. 1966. A survey of parasitic Unionicolidae (Arachnida: Acarina) of the Apalachicolan faunal region of the southern United States. Masters Thesis, Florida State University, Tallahassee, FL, 99 p.

Edwards D.D. and Dimock R.V. Jr. 1988. A comparison of the population dynamics of *Unionicola formosa* from two anodontine bivalves in a North Carolina farm pond. J. Elisha Mitch. Sci. Soc. 104: 90–98.

Edwards D.D. and Dimock R.V. Jr. 1997. Genetic differentiation between *Unionicola formosa* and *U. foili* (Acari: Unionicolidae): cryptic species of molluscan symbionts. Invertebr. Biol. 116: 124–133.

Edwards D.D. and Vidrine M.F. 1994. A new species in the water mite subgenus *Parasitatax* (Acari: Unionicolidae: *Unionicola*) from the North American freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae). J. Elisha Mitch. Sci. Soc. 110: 1–6.

Gledhill T. 1985. Water mites – predators and parasites. Freshw. Biol. Assoc. Ann. Rep. 53: 45–59.

Hadrys H., Balick M. and Schierwater B. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. 1: 55–63.

Hevers J. 1978. Interspezifische Beziehungen zwischen *Unionicola*-Larven (Hydrachnellae, Acari) und Chironomidae (Diptera, Insecta). Verhandlungen Gesellschraft für Oekologie 7: 211–217.

Hevers J. 1980. Biologisch-ökologische Untersuchungen zum Entwicklungszklus der in Deutschland auftretenden *Unionicola*-Arten (Hydrachnellae, Acari). Arch. Hydrobiol. Suppl. 57: 324–373.

Jones R.K.H. 1978. Parasitism by *Unionicola* spp. larvae on chironomids. Hydrobiologia 60: 81–87.

LaRochelle P.B. and Dimock R.V. Jr. 1981. Behavioral aspects of host recognition by the symbiotic water mite *Unionicola formosa* (Acarina: Unionicolidae). Oecologia 48: 257–259.

Mitchell R.D. 1955. Anatomy, life history, and evolution of the mites parasitizing freshwater mussels. Miscellaneous Publications Museum of Zoology, University of Michigan 89: 1–28.

Ross K.G. 2001. Molecular ecology of social behavior: analyses of breeding systems and genetic structure. Mol. Ecol. 10: 265–284.

Smith I.M. and Oliver D.R. 1986. Review of parasitic associations of larval water mites (Acari: Parasitengona: Hydrachnida) with insect hosts. Can. Entomol. 118: 407–472.

Swofford D.L. 2002. PAUP*4.0b10: Phylogenetic Analysis using Parsimony (* and other Methods). Sinauer Associates, Sunderland, MA.

Vidrine M.F. 1996. North American *Najadicola* and *Unionicola*: Diagnoses and Distributions. Gail Q. Vidrine Collectibles, Eunice, Louisiana, 356 p.

Williams J.G., Hanafey M.K., Rafalsky J.A. and Tingey S.V. 1993. Genetic analysis using random amplified polymorphic DNA markers. Meth. Enzymol. 51: 704–737.