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Cryptic species of mites (Uropodoidea: *Uroobovella* spp.) associated with burying beetles (Silphidae: *Nicrophorus*): The collapse of a host generalist revealed by molecular and morphological analyses

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ABSTRACT

Uroobovella (Mesostigmata: Uropodoidea: Urodinychidae) species are among the most common mites associated with carrion-feeding *Nicrophorus* (Silphidae) beetles. Previous taxonomic understanding suggests that a single host generalist, *U. nova*, disperses and lives with *Nicrophorus* species worldwide (reported from at least seven host species). Using morphometrics and morphological characteristics, as well as partial cytochrome oxidase I (COI) and the entire internal transcribed spacer 2 (ITS2) markers, we tested whether this apparent generalist is truly a generalist or rather a complex of cryptic species with narrower host ranges. Based on deutonymph mites collected from 14 host species across six countries and 17 provinces or states, we show that *U. nova* represents at least five morphologically similar species with relatively restricted host ranges. Except for one species which yielded no molecular data (but did exhibit morphological differences), both molecular and morphological datasets were congruent in delimiting species boundaries. Moreover, comparing the mite phylogeny with the known ecology and phylogenetic relationships of their host species suggests that these mites are coevolving with their silphid hosts rather than tracking ecologically similar species.

1. Introduction

Cryptic species, defined as species distinguishable by no or subtle morphological differences, have been reported across numerous invertebrate taxa, and more cryptic species are being uncovered as more genetic and phylogeographical studies are conducted (Bensch et al., 2004; Miura et al., 2005; Williams et al., 2006). There has been an exponential increase in research on cryptic species over the last two decades, primarily as a result of the increased availability of nucleotide sequencing (Bickford et al., 2007). In order to accurately assess the biodiversity, as well as the ecology and evolutionary history of a given group, a thorough understanding of the extent of cryptic diversity in that group is essential.

The use of molecular markers to elucidate previously unrecognized species boundaries can significantly facilitate our understanding of host specificity. For instance, DNA markers have often revealed that a putative host generalist is actually multiple specialist species. The avian nasal mite, *Ptilonyssus sairae* (Mesostigmata: Rhinonyssidae), associated with numerous passerine species worldwide, was shown to be a complex of cryptic species, each restricted to a single bird species (Morelli and Spicer, 2007). In Costa Rica, the 5′-end of mitochondrial cytochrome oxidase I (COI) (barcoding region, sensu Hebert et al., 2004) revealed that 16 apparent generalist morphospecies of parasitoid tachinid flies were actually a complex of 64 host specialist and 9 generalist species (Smith et al., 2007).

In addition to detecting cryptic species, modern molecular techniques have been used to assess the extent to which symbionts are coevolving with their hosts. Rhinonyssid nasal mites have been shown to track the phylogeny of their passerine hosts: strict cospeciation was observed between five lineages of the *Ptilonyssus sairae* species complex and five host species, based on the internal transcribed spacer region (Morelli and Spicer, 2007). Similarly, the phylogenetic relationships of pocket gophers and their associated lice, based on partial COI, have shown considerable congruence (Page, 1996). On the other hand, the evolution of symbionts may reflect a history of ecological fitting, where a symbiont is associated with phylogenetically unrelated hosts that are ecologically similar (Brooks et al., 2006; Kethley and Johnston, 1975). Phylogenetically unrelated but ecologically distant host species of sparid

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fish living in sympatry, often shared the same species of monogenean gill parasites (Desdevises et al., 2002).

Mites associated with *Nicrophorus* (Silphidae: Nicrophorinae) beetles provide a model system to explore species boundaries and cryptic diversity, as well as host specificity and coevolution. *Nicrophorus* species are large-bodied beetles, which breed and feed on decaying organic matter, most often vertebrate carcasses (Anderson and Peck, 1985). *Nicrophorus* beetles are cosmopolitan, with at least 60 extant species worldwide, 21 of which occur in the New World (Sikes et al., 2008). *Nicrophorus* beetles have been of interest to behavioural ecologists because most species provide biparental care (see Anderson and Peck (1985) for a summary of their life cycle). *Nicrophorus* beetles are associated with at least 14 species of mites representing four families, and these mites can occur at high prevalences, with up to 95% of a given beetle population carrying mites (Wilson and Knollenberg, 1987). The beetles provide a means of phoretic dispersal for mites to and from carcasses. Overall, the symbiotic relationship between mites and their silphid hosts are poorly understood; however, their association appears to be a blend of commensalism and mutualism, as some mite species will actively prey on the eggs of carrion-feeding flies which compete with *Nicrophorus* (Wilson and Knollenberg, 1987).

Uroobovella nova (=*Uroseius novus*) (Oudemans, 1902) (Uropodoidea: Urodinychidae) is only found on silphids, it is a common associate of *Nicrophorus* beetles and has been reported from at least seven *Nicrophorus* species in Germany, Poland and the United States (Athias-Binche et al., 1993; H. Klompen unpubl. res.). The nature of the symbiotic relationship between *U. nova* and its hosts, as well as the feeding ecology, have never been investigated. There are over 2000 described species of uropodoid mites worldwide, occurring in forest litter, as well as patchy habitats such as carcasses, nests, dead wood, and dung (Błoszyk et al., 2003). Uropodoids known to be phoretic, including *U. nova*, disperse as deutonymphs by gluing themselves to their host with an anally-secreted pedicel. Silphid-associated mites occupy patchy and ephemeral habitats, and this may have reinforced barriers to gene flow and selected for the evolution of host-specific preferences. Athias-Binche et al. (1993) studied the morphology of *U. nova* deutonymphs collected from three host species in Bielefeld Germany, and found that *U. nova* exhibited two significantly different host-dependent size classes: a small morph associated with *N. vespillo* and *N. vespilloides*, and a slightly larger morph on *N. humator* (Athias-Binche et al., 1993). Although size alone is a weak criterion, the discrete variation in body size across host species could reflect the existence of at least to two morphologically similar species that evolved sympatrically through adaptations to their hosts.

Numerous acarological studies have used COI and internal transcribed spacer 2 (ITS2) either alone or combined with other markers to elucidate species boundaries in mites, often resulting in the dissolution of putative host generalists into cryptic species complexes with narrower host, habitat or geographic range (Anderson and Trueman, 2000; Kawazoe et al., 2008; Mahani et al., 2009; Morelli and Spicer, 2007; Schäffer et al., 2010; Webster et al., 2004). Although, COI and ITS2 do not always uncover cryptic diversity, as shown for the spider mites *Tetranychus cinnabarinus* and *T. urticae* (Xie et al., 2008). In this study, we employed morphometrics and morphological characters, as well as mitochondrial (COI) and nuclear (ITS2) markers to test whether *Uroobovella nova*, associated with *Nicrophorus* beetles worldwide, is indeed a single species with a broad host range, or instead a complex of cryptic species with restricted host associations. We then explored whether mites are tracking the ecology (tend to switch hosts) or the phylogeny (tend to coevolve) of their hosts by comparing the *U. nova* phylogeny with the known ecology and phylogenetic relationships of *Nicrophorus* species.

2. Materials and methods

2.1. Biological material

Silphids were collected by various researchers across eight countries and 21 provinces or states (see acknowledgments). In Canada, most silphids were collected as bycatch from xylophagous beetle trapping by W.K. Specimens from other countries were mostly collected in pitfall traps, and others were hand collected. Beetle specimens preserved in ethanol were shipped to Carleton University, and upon receipt specimens were placed in 95% ethanol and stored at −20 °C. Silphids were identified to species using keys from Anderson and Peck (1985). Using a dissecting microscope, *Nicrophorus* beetles were examined for uropodoid deutonymphs, and all mites were removed and placed in a 0.5 ml microfuge tube with 95% ethanol and stored at −20 °C. Four species of uropodoids (*Trichouropoda australis*, *T. parisiana*, *Uroobovella americana*, *U. orri*) collected from bark beetles (Scolytinae), and one uropodoid species (*Uropoda orbicularis*) collected from *Nicrophorus* beetles were used as outgroup specimens. Following DNA extraction, mites were recovered from the extraction buffer and slide-mounted in a polyvinyl alcohol medium, and slides were cured on a slide warmer at about 40 °C for 3–4 days. Slide-mounted deutonymph specimens were examined using a compound microscope (Leica DM 5500B or Nikon 80I) and sorted to hypothetical species-level taxa (morphospecies) by examining taxonomically informative morphological characters based on published species descriptions (Hirschmann and Zirngiebl-Nicol, 1962). Five putative morphospecies were identified prior to examining the molecular phylogenies. Voucher specimens are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes, in Ottawa, Canada (Table 1).

2.2. Morphological analysis

In order to assess the extent of morphological divergence among putative cryptic species, 68 slide-mounted specimens were examined using a Leica DM 5500B compound microscope, and 15 characters were measured using Leica Application Suite, Live and Interactive Measurements Modules v3.5. Characters from different body regions were selected based on their relative ease of measurement and prominence, as well as previously observed variation across specimens. The 15 characters measured were: maximal length and width of the dorsal shield and ventrianal shield; sternal shield (SS) median length; SS width at five regions (from anterior to posterior): maximal width of SS anterior margin, the maximum width at level of coxa III, the maximum width of SS posterior margin, and the minimum width of the two lateral constrictions level with coxa II and with coxa IV; the length of tarsus I; and the length of the following setae: dorsal seta *J5* (Hirschmann and Zirngiebl-Nicol, 1962), opisthogastric seta *V3* (*JV3* sensu Evans and Till, 1965), the longest of the anterodorsal setae of tarsus I, and palp-trochanter seta *Pa1* (*v1* sensu Evans and Till, 1965). Morphological divergence was visualised using an ordination based on semistrong hybrid multidimensional scaling (SSH MDS) generated in PATN v2.27 (Belbin, 2003). A distance matrix between mite specimens was created using morphometric data standardised for body size to eliminate possible bias linked to body size, then transformed ((value − minimum)/range) to balance the weight of all measured characters, and visualised in a three-dimensional SSH MDS ordination based on Bray-Curtis distance. The ordination was generated using 1000 replicates and 1000 random starts. Significant differences among putative species were tested using ANOSIM (analysis of similarity) with 1000 iterations.