Molecular Phylogenetics and Evolution 65 (2012) 276–286

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

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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ARTICLE INFO

Article history:
Received 17 February 2012
Revised 12 June 2012
Accepted 17 June 2012
Available online 22 June 2012

Keywords:
Uroobovella nova
Coevolution
Species boundaries
Host preferences
COI mtDNA
ITS2 rDNA

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.

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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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http://dx.doi.org/10.1016/j.ympev.2012.06.013

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.

Table 1
Collection location and host species records of uropodoid mites collected from silphid beetles (ingroup) and scolytines (outgroup) with GenBank accession no. for COI and ITS2.

Beetle no.	Beetle species	Collection location	Lat	Long	Coll date	Mite species*	COI	ITS2
1 – WKN078	*Nicrophorus vespilloides*	Netherlands, North Holland, Bloemendaal	52.406	4.623	17 iv 2009	*Uroob.* sp. 3	JN992098	JN992127
2 – WKN084	*N. sayi*	Can, QE, Pont-Rouge	46.806	−71.679	05 vi 2009	*Uroob.* sp. 2	JN992096	JN992126
3 – WKN085	*N. sayi*	Can, ON, Algonquin P.P. 1	45.902	−77.605	11 vi 2009	*Uroob.* sp. 2	JN992076	JN992118
4 – WKN114	*N. orbicollis*	Can, PEI, Wellington, Route 2	46.452	−63.949	06 vii 2009	*Uroob.* sp. 1	–	–
5 – WKN131	*N. defodiens*	Can, BC, Prince George, nr. UNBC	53.904	−122.783	12 vi 2009	*Uroob.* sp. 2	JN992077	JN992119
6 – WKN157	*N. orbicollis*	Can, MB, Vassar, Hwy 12	49.084	−95.875	12 vii 2009	*Uroob.* sp. 1	JN992048	JN992105
7 – WKN165	*N. orbicollis*	Can, ON, Carbine Rd.	45.330	−76.371	23 vii 2009	*Uroob.* sp. 1	JN992074	JN992117
8 – WKN184	*N. vespilloides*	Germany, Mooswald Forest, nr. Freiburg	48.0	7.85	vi 2009	*Uroob.* sp. 3	JN992102	JN992131
9 – WKN186	*N. vespillo*	Germany, Mooswald Forest, nr. Freiburg	48.0	7.85	vi 2009	*Uroob.* sp. 3	JN992099	JN992128
10 – WKN193	*N. sayi*	USA, CT, Bethany	41.462	−72.961	14 viii 2009	*Uroob.* sp. 2	JN992078	JN992120
11 – WKN211	*N. sayi*	USA, NH, Durham	43.134	−70.926	10 vi 2009	*Uroob.* sp. 2	–	–
12 – WKN224	*N. defodiens*	USA, NH, Durham	43.134	−70.926	07 vi 2009	*Uroob.* sp. 1	JN992049	JN992106
13 – WKN228	*N. orbicollis*	USA, NH, Durham	43.134	−70.926	07 vi 2009	*Uroob.* sp. 1	JN992050	JN992107
14 – WKN232	*N. defodiens*	Can, AB, nr. Drayton Valley	53.358	−114.984	05 vii 2009	*Uroob.* sp. 2	–	JN992121
15 – WKN305	*N. carolinus*	USA, NE, Kearney Co.			vii 2009	*Uroob.* sp. 1	JN992051	JN992108
16 – WKN307	*N. pustulatus*	USA, NE, Kearney Co.			vii 2009	*Uroob.* sp. 1	JN992052	JN992109
17 – WKN345	*N. quadripunctatus*	Japan, Niigata, Matsunoyama Matsuguchi			25 x 2009	*Uroob.* sp. 5	–	–
18 – WKN349	*N. orbicollis*	Can, NS, Juniper Lake	44.7	−63.6	2009	*Uroob.* sp. 1	JN992053	JN992110
19 – WKN355	*N. sayi*	Can, NS, McNabs Island	44.6	−63.517	2009	*Uroob.* sp. 2	JN992079	–
20 – WKN357	*N. defodiens*	Can, NS, McNabs Island	44.6	−63.517	2009	*Uroob.* sp. 2	–	JN992122
21 – WKN165	*N. orbicollis*	Can, ON, Carbine Rd.	45.330	−76.371	23 vii 2009	*Uroob.* sp. 1	JN992075	–
22 – WKN184	*N. vespilloides*	Germany, Mooswald Forest, nr. Freiburg	48.0	7.85	vi 2009	*Uroob.* sp. 3	JN992100	JN992129
23 – WKN079	*N. vespilloides*	Netherlands, North Holland, Bloemendaal	52.406	4.623	18 iv 2009	*Uroob.* sp. 3	JN992101	JN992130
24 – WKN082	*N. sayi*	Can, ON, Hwy 132, Dacre	45.369	−76.988	16 v 2009	*Uroob.* sp. 2	–	–
25 – WKN083	*N. sayi*	Can, PEI, Milburn, Route 176	46.677	−64.323	26 v 2009	*Uroob.* sp. 2	JN992080	–
26 – WKN084	*N. sayi*	Can, QE, Pont-Rouge	46.806	−71.679	05 vi 2009	*Uroob.* sp. 2	–	–
27 – WKN086	*N. orbicollis*	Can, ON, Windsor, Elgin St.	42.261	−83.057	18 vi 2009	*Uroob.* sp. 1	–	–
28 – WKN087	*N. sayi*	Can, ON, Algonquin P.P. 1	45.902	−77.605	25 vi 2009	*Uroob.* sp. 2	JN992081	–
29 – WKN088	*N. orbicollis*	Can, ON, Hwy 132, Dacre	45.369	−76.988	25 vi 2009	*Uroob.* sp. 1	JN992054	–
30 – WKN090	*N. nepalensis*	Taiwan, Nantou, nr. Mei feng	24.088	121.171	02 v 2007	*Uroob.* sp. 4	JN992103	JN992132
31 – WKN096	*N. quadripunctatus*	Japan, Nagano, Honshu, Koshimizu-ga-hara			04 vii 2008	*Uroob.* sp. 5	–	–
32 – WKN099	*N. sayi*	Can, NS, Hantsport	45.055	−64.176	19 vi 2009	*Uroob.* sp. 2	JN992082	–
33 – WKN100	*N. orbicollis*	Can, NS, Hantsport	45.055	−64.176	19 vi 2009	*Uroob.* sp. 1	JN992055	–
34 – WKN116	*N. orbicollis*	Can, PEI, Wellington, Route 2	46.452	−63.949	06 vii 2009	*Uroob.* sp. 2	JN992083	–
35 – WKN117	*N. sayi*	Can, QE, Pont-Rouge	46.562	−71.545	08 vi 2009	*Uroob.* sp. 2	JN992084	–
36 – WKN118	*N. defodiens*	Can, NS, Aylesford, Lake Rd.	44.981	−64.635	25 vi 2009	*Uroob.* sp. 2	–	–
37 – WKN119	*N. orbicollis*	Can, NS, Wolfville, Deep Hollow Rd.	45.047	−64.416	02 vii 2009	*Uroob.* sp. 1	JN992056	–
38 – WKN135	*N. defodiens*	Can, BC, Prince George, nr. UNBC	53.904	−122.783	12 vi 2009	*Uroob.* sp. 2	JN992085	JN992123
39 – WKN147	*N. orbicollis*	Can, ON, Hamilton, Site 4-7			07 vii 2009	*Uroob.* sp. 1	–	–
40 – WKN155	*N. defodiens*	Can, NS, Debert Industrial Park	45.428	−63.429	25 vi 2009	*Uroob.* sp. 2	JN992086	–
41 – WKN158	*N. sayi*	Can, MB, Sundown, Hwy 201	49.102	−96.277	09 vii 2009	*Uroob.* sp. 2	JN992087	JN992124
42 – WKN164	*N. orbicollis*	Can, ON, Waterloo	43.540	−80.211	07 vii 2009	*Uroob.* sp. 1	JN992057	–
43 – WKN167	*N. orbicollis*	Can, NS, Glenmont, Black Hole Rd.	45.111	−64.296	17 vii 2009	*Uroob.* sp. 1	JN992058	–
44 – WKN168	*N. orbicollis*	Can, NS, Cold Brook, Hwy 101	45.079	−64.592	13 vii 2009	*Uroob.* sp. 1	JN992059	JN992111
45 – WKN169	*N. orbicollis*	Can, NB, Chipman	46.174	−65.899	08 vii 2009	*Uroob.* sp. 1	JN992060	–
46 – WKN170	*N. defodiens*	Can, NB, Darktown, 200 South Rd.	46.553	−66.123	23 vii 2009	*Uroob.* sp. 2	JN992088	JN992125
47 – WKN171	*N. defodiens*	Can, NS, Goodwood	44.603	−63.677	13 vii 2009	*Uroob.* sp. 2	JN992089	–
48 – WKN176	*N. defodiens*	Can, NS, Sandy Cove, Liverpool Bay	44.053	−64.699	12 viii 2009	*Uroob.* sp. 2	JN992090	–
49 – WKN178	*N. orbicollis*	Can, NS, East River off Hwy 329	44.583	−64.164	10 viii 2009	*Uroob.* sp. 1	JN992061	–
50 – WKN179	*N. orbicollis*	Can, NS, Debert Industrial Park	45.428	−63.429	05 viii 2009	*Uroob.* sp. 1	–	–
51 – WKN192	*N. orbicollis*	USA, CT, Bethany	41.462	−72.961	14 viii 2009	*Uroob.* sp. 1	JN992062	–
52 – WKN193	*N. sayi*	USA, CT, Bethany	41.462	−72.961	14 viii 2009	*Uroob.* sp. 2	JN992091	–
53 – WKN203	*N. sayi*	USA, NH, Durham	43.134	−70.926	10 vi 2009	*Uroob.* sp. 2	–	–
54 – WKN222	*N. defodiens*	USA, NH, Durham	43.134	−70.926	07 vi 2009	*Uroob.* sp. 1	–	–
55 – WKN230	*N. orbicollis*	USA, NH, Durham	43.134	−70.926	07 vi 2009	*Uroob.* sp. 1	–	–
56 – WKN233	*N. defodiens*	Can, AB, nr. Drayton Valley	53.358	−114.984	05 vii 2009	*Uroob.* sp. 2	–	–
57 – WKN296	*N. carolinus*	USA, NE, Kearney Co.			03 vi 2009	Urop. orbicularis	–	JN992133
58 – WKN297	*N. pustulatus*	USA, NE, Kearney Co.			13 vii 2009	*Uroob.* sp. 1	JN992063	JN992112
59 – WKN299	*Necrodes surinamensis*	USA, NE, Kearney Co.			19 vii 2009	*Uroob.* sp. 1	JN992064	JN992113
60 – WKN300	*Heterosilpha ramosa*	USA, NE, Kearney Co.			11 vii 2009	*Uroob.* sp. 1	JN992065	JN992114
61 – WKN302	*N. marginatus*	USA, NE, Kearney Co.			06 vii 2009	Urop. orbicularis	JN992104	JN992134

Table 1 (*continued*)

Beetle no.	Beetle species	Collection location	Lat	Long	Coll date	Mite species*	COI	ITS2
62 – WKN337	*N. tomentosus*	USA, NE, Kearney Co.			vi 2009	Urop. orbicularis	–	–
63 – WKN344	*N. quadripunctatus*	Japan, Niigata, Matsunoyama Matsuguchi			25 x 2009	*Uroob.* sp. 5	–	–
64 – WKN348	*N. orbicollis*	Can, NS, Conrads	44.65	−63.6	2009	*Uroob.* sp. 1	–	–
65 – WKN350	*N. sayi*	Can, NS, Portobello	44.75	−63.6	2009	*Uroob.* sp. 2	JN992097	–
66 – WKN351	*N. orbicollis*	Can, NS, Portobello	44.75	−63.6	2009	*Uroob.* sp. 1	JN992066	–
67 – WKN361	*N. defodiens*	Can, NS, McNabs Island	44.6	−63.517	2009	*Uroob.* sp. 1	JN992067	–
68 – WKN364	*N. sayi*	Can, NS, Devon	44.9	−63.5	2009	*Uroob.* sp. 2	JN992092	–
69 – WKN368	*N. sayi*	Can, NS, McNabs Island	44.6	−63.517	2009	*Uroob.* sp. 2	–	–
70 – WKN082	*N. sayi*	Can, ON, Hwy 132, Dacre	45.369	−76.988	16 v 2009	*Uroob.* sp. 2	–	–
71 – WKN086	*N. orbicollis*	Can, ON, Windsor, Elgin St.	42.261	−83.057	18 vi 2009	*Uroob.* sp. 1	–	–
72 – WKN098	*N. quadripunctatus*	Japan, Nagano, Honshu, Koshimizu-gahara			04 vii 2008	*Uroob.* sp. 5	–	–
73 – WKN118	*N. defodiens*	Can, NS, Aylesford, Lake Rd.	44.981	−64.635	25 vi 2009	*Uroob.* sp. 2	–	–
74 – WKN145	*N. orbicollis*	Can, ON, Hamilton, Site 4-7			07 vii 2009	*Uroob.* sp. 1	–	–
75 – WKN159	*N. orbicollis*	Can, PEI, Wellington, Route 2	46.452	−63.949	17 vii 2009	*Uroob.* sp. 1	JN992068	–
76 – WKN196	*N. sayi*	USA, NH, Durham	43.134	−70.926	10 vi 2009	*Uroob.* sp. 2	–	–
77 – WKN232	*N. defodiens*	Can, AB, nr. Drayton Valley	53.358	−114.984	05 vii 2009	*Uroob.* sp. 2	–	–
78 – WKN260	*N. obscurus*	Can, AB, Onefour	49.121	−110.47	12 vi 2001	*Uroob.* sp. 1	–	–
79 – WKN261	*N. guttula*	Can, AB, Onefour	49.121	−110.47	12 vi 2001	*Uroob.* sp. 1	–	–
80 – WKN292	*N. marginatus*	Can, AB, Onefour	49.121	−110.47	17 vi 2004	*Uroob.* sp. 1	–	–
81 – WKN348	*N. orbicollis*	Can, NS, Conrads	44.65	−63.6	2009	*Uroob.* sp. 1	–	–
82 – WKN098	*N. quadripunctatus*	Japan, Nagano, Honshu, Koshimizu-gahara			04 vii 2008	*Uroob.* sp. 5	–	–
83 – WKN118	*N. defodiens*	Can, NS, Aylesford, Lake Rd.	44.981	−64.635	25 vi 2009	*Uroob.* sp. 2	JN992093	–
84 – WKN145	*N. orbicollis*	Can, ON, Hamilton, Site 4-7			07 vii 2009	*Uroob.* sp. 1	JN992069	–
85 – WKN196	*N. sayi*	USA, NH, Durham	43.134	−70.926	10 vi 2009	*Uroob.* sp. 2	JN992094	–
86 – WKN232	*N. defodiens*	Can, AB, nr. Drayton Valley	53.358	−114.984	05 vii 2009	*Uroob.* sp. 2	JN992095	–
87 – WKN260	*N. obscurus*	Can, AB, Onefour	49.121	−110.47	12 vi 2001	*Uroob.* sp. 1	JN992070	–
88 – WKN261	*N. guttula*	Can, AB, Onefour	49.121	−110.47	12 vi 2001	*Uroob.* sp. 1	–	–
89 – WKN282	*N. marginatus*	Can, AB, Onefour	49.121	−110.47	17 vi 2003	*Uroob.* sp. 1	–	–
90 – WKN348	*N. orbicollis*	Can, NS, Conrads	44.65	−63.6	2009	*Uroob.* sp. 1	JN992071	JN992115
91 – WKN375	*N. orbicollis*	USA, MD, Scientist's Cliffs	38.504	−76.518	26 vii 2009	*Uroob.* sp. 1	JN992072	–
92 – WKN376	*N. orbicollis*	USA, MD, Scientist's Cliffs	38.504	−76.518	26 vii 2009	*Uroob.* sp. 1	JN992073	JN992116
93 – WKN377	*N. nepalensis*	Taiwan, Nantou, nr. Mei feng	24.087	121.171	4 v 2007	*Uroob.* sp. 4	–	–
94 – WKN378	*N. nepalensis*	Taiwan, Nantou, nr. Mei feng	24.087	121.171	4 v 2007	*Uroob.* sp. 4	–	–
95 – WKN379	*N. nepalensis*	Taiwan, Nantou, nr. Mei feng	24.087	121.171	4 v 2007	*Uroob.* sp. 4	–	–
96 – WKN380	*N. nepalensis*	Taiwan, Nantou, nr. Mei feng	24.087	121.171	4 v 2007	*Uroob.* sp. 4	–	–
97 – WKN380	*N. nepalensis*	Taiwan, Nantou, nr. Mei feng	24.087	121.171	4 v 2007	*Uroob.* sp. 4	–	–
50 – WKHD080	*Dendroctonus rufipennis*	Can, NS, Victoria Beach	44.703	−65.747	22 vi 2009	*Uroob. orri*	JN992234	JQ316466
65 – WKHD142	*Dryocoetes affaber*	Can, NS, Sheet Harbour	44.909	−62.503	19 vi 2009	*Uroob. orri*	JN992238	JQ316467
77 – WKHD184	*Gnathotrichus materiarius*	Can, NS, Debert, Industrial Park	45.428	−63.429	25 vi 2009	*Trich. parisiana*	JN992188	–
78 – WKHD185	*Ips pini*	Can, NS, Debert, Industrial Park	45.428	−63.429	25 vi 2009	*Trich. australis*	JN992141	–
95 – WKB5929	*Dendroctonus valens*	Can, ON, Algonquin P.P. 2	45.895	−78.071	25 vi 2009	*Uroob. americana*	JN992205	–

* *Uroob.* = *Uroobovella*, *Urop.* = *Uropoda*, *Trich.* = *Trichouropoda*.

To ensure that mites used in molecular analyses could also be included in morphometric analyses without any incurred bias, the effect of DNA extraction on mite morphology was tested by comparing the aforementioned 15 morphological characters (standardised for body size) of specimens that underwent DNA extraction with specimens that did not undergo DNA extraction, using Wilcoxon signed rank tests performed in SPSS v17.

2.3. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from whole specimens for 24 h using a DNeasy Tissue kit (Qiagen Inc., Santa Clara, CA, USA). Following extraction, mites were removed from the extraction buffer, and genomic DNA was purified following the DNeasy Tissue kit protocol.

PCR amplifications were performed in a total volume of 25 μl, with 16 μl ddH_2O, 2.5 μl 10× PCR buffer, 2.5 μl 25 mM $MgCl_2$, 0.5 μl of each 10 μM primer, 0.5 μl 10 mM dNTPs, 0.5 μl Taq DNA polymerase (Promega Corp., Madison, WI, USA), and 2 μl genomic DNA template. PCR amplification cycles were performed on an Eppendorf ep Gradient S Mastercycler (Eppendorf AG, Hamburg, Germany). Primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al., 1994) were used to amplify a 709 bp fragment of the mitochondrial COI gene. Specimens that did not produce detectable PCR products using LCO1490 and HCO2198 were reattempted using LCO1490 and LoDog (5′-GGRTCAAAAAAAGAWGTRTTRAARTTTCG-3′) which amplified a 643 bp fragment of COI. Amplification of COI was attempted with 89 mites across 39 sites, from which only 56 mites, from five countries and 36 sites yielded COI sequence data; COI was also amplified from six outgroup individuals (Table 1). The thermocycler protocol for COI amplification was as follows: initial denaturation cycle at 94 °C for 3 min, followed by 40 cycles of 94 °C for 45 s, 45 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The entire nuclear ribosomal ITS2 (333 bp) marker, was amplified using ITS2-F1 and ITS2-R1 primers (5′-ATATGCTTAAATTCAGCGGG-3′, 5′-GGGTCGATGAAGAACGCAGC-3′) (Navajas et al., 1998), which are anchored in the highly conserved 5.8S and 28S regions. ITS2 was amplified from 28 mites from 19 sites, as well as from four outgroup specimens (Table 1). The PCR protocol for ITS2 amplification was as follows: initial denaturation cycle at 95 °C for 3 min, followed by 30 cycles of 95 °C for 1 min, 52 °C for 1.5 min, 72 °C for 2 min, and a final extension at 72 °C for 5 min.

Amplified products and negative controls were visualised on 1% agarose electrophoresis gels, and purified using pre-cast E-Gel CloneWell 0.8% SYBR Safe agarose gels (Invitrogen, Carlsbad, CA, USA) following the protocol of Gibson et al. (2010). Sequencing reactions were performed in a total reaction volume of 10 μl, with 3 μl ddH_2O, 1.5 μl of 5× sequencing buffer, 0.5 μl of primer, 1 μl of BigDye Terminator (PE Applied Biosystems, Foster City, CA, USA), and 4 μl of purified PCR product. Sequencing was performed at the Agriculture & Agri-Food Canada, Eastern Cereal and Oilseed Research Centre Core Sequencing Facility (Ottawa, ON, Canada). Purification of sequencing reactions was performed using the ABI ethanol/EDTA/sodium acetate precipitation protocol and reactions were analysed on an ABI 3130xl Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

2.4. Sequence alignment and phylogenetic analysis

Sequence chromatograms were edited and contiguous sequences were assembled using Sequencher v4.7 (Gene Codes Corp., Ann Arbor, MI, USA). COI sequences were aligned manually in Mesquite v2.74 (Maddison and Maddison, 2010) according to the translated amino acid sequence. ITS2 was initially aligned in ClustalX v2.0.12 (Larkin et al., 2007) with the default settings, and subsequently adjusted manually in Mesquite. Sequences have been submitted to GenBank (Table 1).

Pairwise distances were calculated using neighbour-joining (NJ) analyses with the Kimura-2-parameter (K2P) model in PAUP* v4.0b10 (Swofford, 2003). Phylogenetic reconstructions of COI, ITS2, and concatenated datasets were performed using Bayesian inference (BI) in MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), and parsimony analyses in TNT v1.1 (Goloboff et al., 2008). In each phylogeny, each specimen is labelled with a unique number, followed by the host species and abbreviated state, province or country (Table 1).

MrModeltest v2.3 (Nylander, 2004) was used to determine the best-fit model of molecular evolution for each molecular marker, which was determined to be GTR+I+G. Bayesian analysis was performed in MrBayes with a Markov Chain Monte Carlo (MCMC) method, two independent runs, with nucmodel = 4by4, N_{st} = 6, rates = invgamma, samplefreq = 1000, four chains = one cold and three heated. The COI dataset ran for 20 million generations, producing 380,002 trees after a burn-in of 1000 trees. ITS2 dataset ran for 10 million generations, producing 19,246 trees after a burn-in of 378. The concatenated dataset ran for 10 million generations, producing 18,002 trees after a burn-in of 1000. In Mesquite the remaining trees, excluding the burn-in, were used to generate a majority-rule consensus tree displaying the posterior probability supports for each node. Bayesian analyses were performed using the on-line Computational Biology Service Unit at Cornell University, and at the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal (Miller et al., 2010).

Parsimony analysis was performed using a heuristic search with tree bisection-reconnection (TBR) branch swapping and 1000 random addition sequence replicates, all characters were treated as unordered, equal weighting, and gaps were treated as missing. Gaps scored as a fifth state produced the same topology as that observed for gaps as missing for each of the analytical approaches. Analyses of the COI dataset excluding the third codon positions produced poorly supported reconstructions with similar topology to the analyses including the third codon position; hence analyses were performed including the 3rd codon. Multiple trees were obtained and these were presented in a semistrict consensus tree. Node support was assessed in TNT, using jackknife resampling with 36% of characters removed and 1000 replicates, Bremer supports and partitioned Bremer supports (PBS) were also determined using TNT. Parsimony analysis of the concatenated dataset only included those specimens with both COI and ITS2 genes, because the absence of either gene skews PBS values.

3. Results

3.1. Morphological analysis

Uropodoid deutonymphs were collected from 14 species and three genera of silphids, across six countries and 17 provinces or states (Table 1). Initial identifications based on descriptions from the literature suggested that the deutonymph uropodoids collected from *Nicrophorus* beetles were all the same species, *Uroobovella nova* (=*Uroseius novus*)[1] (sensu Hirschmann and Zirngiebl-Nicol, 1962). Prior to examining the molecular data, slide-mounted deutonymph specimens were closely examined for morphological variation, and this investigation revealed five subtle morphological characters which were used to delimit five putative cryptic species, *Uroobovella* sp. 1, *U.* sp. 2, *U.* sp. 3, *U.* sp. 4, and *U.* sp. 5 (Table 2). Herein, these putative cryptic species are treated as distinct species, and intraspecific and interspecific measurements correspond accordingly.

Based on morphometric measurements alone, the SSH MDS ordination (n = 68, stress = 0.1308) (Fig. 1) and ANOSIM indicate that each of the putative cryptic species are significantly distinct from each other, with the exception of *U.* sp. 3 and *U.* sp. 5. The ANOSIM findings indicate that *U.* sp. 1 is significantly distinct from *U.* sp. 2, *U.* sp. 3, *U.* sp. 4 and *U.* sp. 5 ($p < 0.0001$). In addition, *U.* sp. 2 is significantly distinct from *U.* sp. 3 ($p = 0.025$), *U.* sp. 4 and *U.* sp. 5 ($p < 0.0001$). The ANOSIM also indicates that *U.* sp. 3 is distinct from *U.* sp. 4 ($p = 0.005$), but is not distinct from *U.* sp. 5 ($p = 0.1762$). Lastly, *U.* sp. 4 is significantly distinct from *U.* sp. 5 ($p = 0.002$) (Fig. 1).

No significant differences in morphology were observed between mites that underwent DNA extraction versus those which did not undergo extraction, based on measurements of 15 characters from 22 mites with 11 pairwise comparisons (p = 0.062–0.79). Therefore, morphological analysis of mites that undergo DNA extraction can be included in statistical analyses with other specimens.

3.2. Gene sequence characteristics and pairwise divergence

COI was amplified from 62 individuals, with 673 characters in total, 410 constant, 38 parsimony-uninformative, and 225 parsimony-informative. Mean base pair frequencies (A: 0.298, C: 0.172, G: 0.158, T: 0.371) were found to be homogeneous across all specimens ($\chi^2 = 82$, $p = 1.0$). ITS2 was used to assess the branching patterns observed in the COI reconstructions, and to confirm species boundaries. ITS2 was amplified from 28 mites, with 377 characters in total, 180 constant, 9 parsimony-uninformative, and 188 parsimony-informative. Mean base pair frequencies (A: 0.33, C: 0.225, G: 0.183, T: 0.262) were also found to be homogeneous across all specimens ($\chi^2 = 15.7$, $p = 1.0$).

NJ analysis (K2P) of COI was performed on 62 uropodoid specimens. Average intraspecific pairwise distance was relatively low (1.5% ± 0.6), and the maximum intraspecific divergence was reported for *Uroobovella* sp. 2 (3.3%) (Table 3). Mean interspecific divergence was high (16.3% ± 0.6), and the maximum divergence was observed between *U.* sp. 1 and *U.* sp. 2 (18.7%) (Table 3). The range of intra- (0–3.3%) and interspecific (13.4–18.7%) pairwise distances did not overlap (Table 3).

[1] *Uroseius novus* was described by Oudemans in 1902 based on deutonymphs, and in 1903 Oudemans placed this species under the newly described genus *Neoseius* (Athias-Binche et al., 1993), which Hirschmann and Zirngiebl-Nicol (1962) then placed under the genus *Uroobovella*.

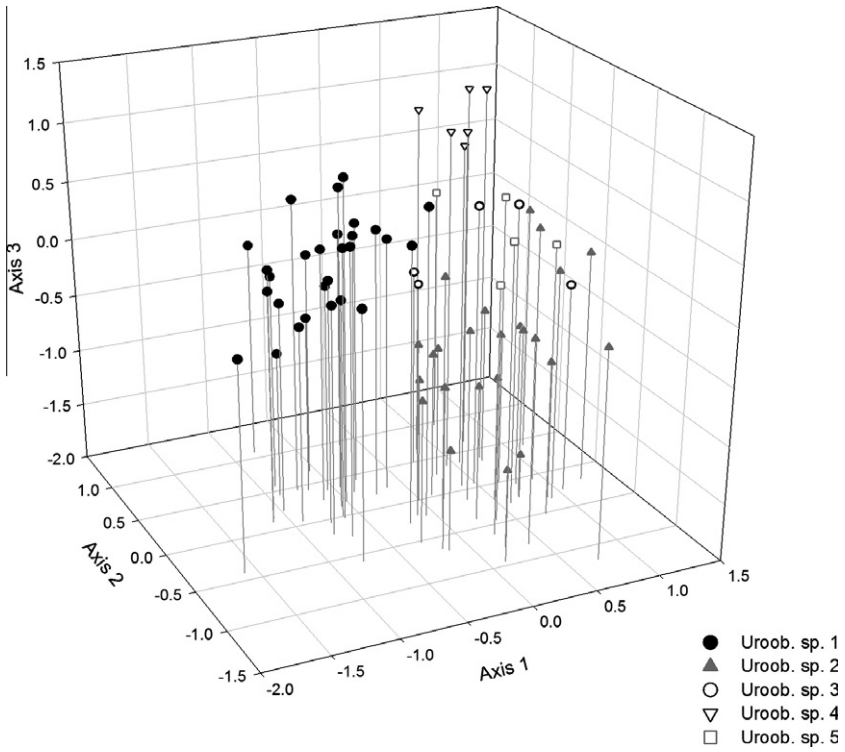

Fig. 1. Three-dimensional SSH MDS ordination with Bray–Curtis distance (1000 replicates, 1000 random starts) performed on measurements ((value − minimum)/range transformed) of 15 morphological characters from 68 uropodoids representing five species, *Uroobovella* sp. 1, *U.* sp. 2, *U.* sp. 3, *U.* sp. 4, and *U.* sp. 5 (stress = 0.1308).

Table 2
Morphological character states delineating between *Uroobovella* species collected from silphids.

Mite species	Seta J5 shape	Ventrianal shield shape	Sternal and ventrianal shield pattern	Light-refractant pits on endopodal shield	Anterodistal projection on femur II
U. sp. 1	Filamentous	Rounded corners, lateral margins slightly convex	Widely spaced pits absent or barely noticeable	No light-refractant pits	Broadly Rounded
U. sp. 2	Scalloped	Sharp corners, lateral margins parallel	Fine widely spaced pits	Widely spaced irregularly shaped light-refractant pits	Subtriangular
U. sp. 3	Scalloped	Sharp corners, lateral margins parallel	Fine widely spaced pits	Widely spaced irregularly shaped light-refractant pits	Broadly rounded
U. sp. 4	Scalloped	Sharp corners, lateral margins parallel	Fine widely spaced pits	Widely spaced irregularly shaped light-refractant pits	Hook-like
U. sp. 5	Scalloped	Very sharp corners, lateral margins parallel	Fine widely spaced pits	Widely spaced oval-shaped light-refractant pits	Subtriangular

Table 3
Intra- and interspecific nucleotide divergence (%) ± standard deviation (range) of COI and ITS2 amplified from *Uroobovella* mites associated with silphids.

	COI Mean (range)	ITS2 Mean (range)
Intraspecific		
U. sp. 1	1.2 ± 0.4 (0–2.4)	0 ± 0 (0)
U. sp. 2	1.6 ± 0.8 (0–3.3)	0.05 ± 0.1 (0–0.4)
U. sp. 3	1.8 ± 0.5 (0.8–2.5)	0.9 ± 1.2 (0–2.3)
Overall	1.5 ± 0.6 (0–3.3)	0.3 ± 0.4 (0–2.3)
Interspecific		
U. sp. 1–2	16.7 ± 0.5 (15.5–18.7)	10.05 ± 0.9 (8.0–11.6)
U. sp. 1–3	15.7 ± 0.4 (14.7–17.0)	6.7 ± 1.3 (5.7–9.9)
U. sp. 1–4	17.1 ± 0.4 (16.7–18.0)	7.5 ± 0.5 (7.0–8.3)
U. sp. 2–3	15.0 ± 0.9 (13.4–17.3)	6.4 ± 1.1 (4.2–8.8)
U. sp. 2–4	17.4 ± 0.5 (16.7–18.5)	5.3 ± 0.5 (4.2–5.8)
U. sp. 3–4	15.5 ± 1.0 (14.4–16.6)	2.7 ± 0.9 (2.2–4.4)
Overall	16.3 ± 0.6 (13.4–18.7)	6.4 ± 0.9 (2.2–11.6)

NJ analysis of ITS2 was performed on 32 uropodoid specimens. Average intraspecific pairwise divergence was low (0.3 ± 0.4%), and the maximum intraspecific divergence was observed for *U.* sp. 3 (2.3%) (Table 3). *Uroobovella* sp. 1 had 0% intraspecific divergence for the entire ITS2 marker. Average interspecific divergence (6.4 ± 0.9%) was markedly greater than intraspecific divergence, and the range of intra- (0–2.3%) and interspecific (2.2–11.6%) pairwise distances overlapped slightly. As seen in COI, the maximum divergence was observed between *U.* sp. 1 and *U.* sp. 2 (11.6%) (Table 3).

3.3. Bayesian inference

The majority rule consensus tree from the BI of COI was well supported, with most nodes having moderate to high posterior probabilities, and three nodes with 100% support, two of which are the basal nodes to two ingroup species (TL = 796, CI = 0.4799, RI = 0.8727) (Fig. 2). *Uroobovella* sp. 1 and *U.* sp. 2 had multiple

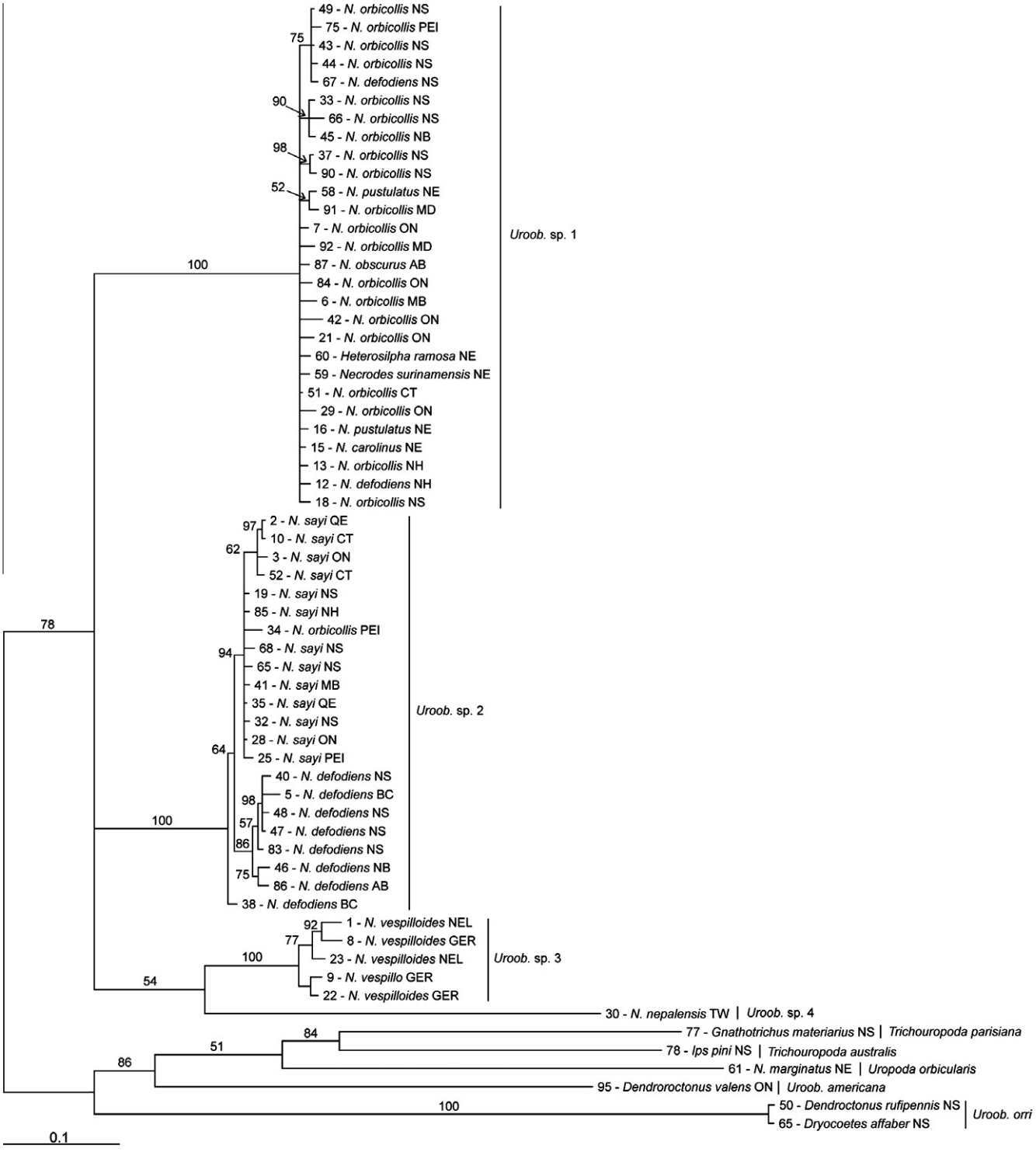

Fig. 2. Majority rule consensus tree of 38002 trees generated by Bayesian MCMC analysis (20 million generations) of 673 bp fragment of COI from 62 uropodoid specimens, 56 ingroup specimens representing four *Uroobovella* species (Uroob.), and six outgroup specimens representing five species, posterior probabilities >50% shown above branches (TL = 796, CI = 0.4799, RI = 0.8727).

unresolved nodes collapsing into a few intraspecific polytomies. The majority rule consensus tree from the BI of ITS2 was well supported: five nodes had 100% support, two of which are basal to two ingroup species, and one is basal to the ingroup (TL = 249, CI = 0.9478, RI = 0.9771) (tree not shown). The low level of intraspecific divergence observed in ITS2 resulted in each species collapsing into an intraspecific polytomy. The consensus tree from the BI of the concatenated dataset was well supported: six nodes had 100% support, three of which are basal to three ingroup species and one is basal to the ingroup (TL = 871, CI = 0.6441, RI = 0.9131) (Fig. 3).

3.4. Parsimony

The parsimony heuristic analysis of COI resulted in 54 most parsimonious trees (TL = 752, CI = 0.508, RI = 0.8863) presented in a

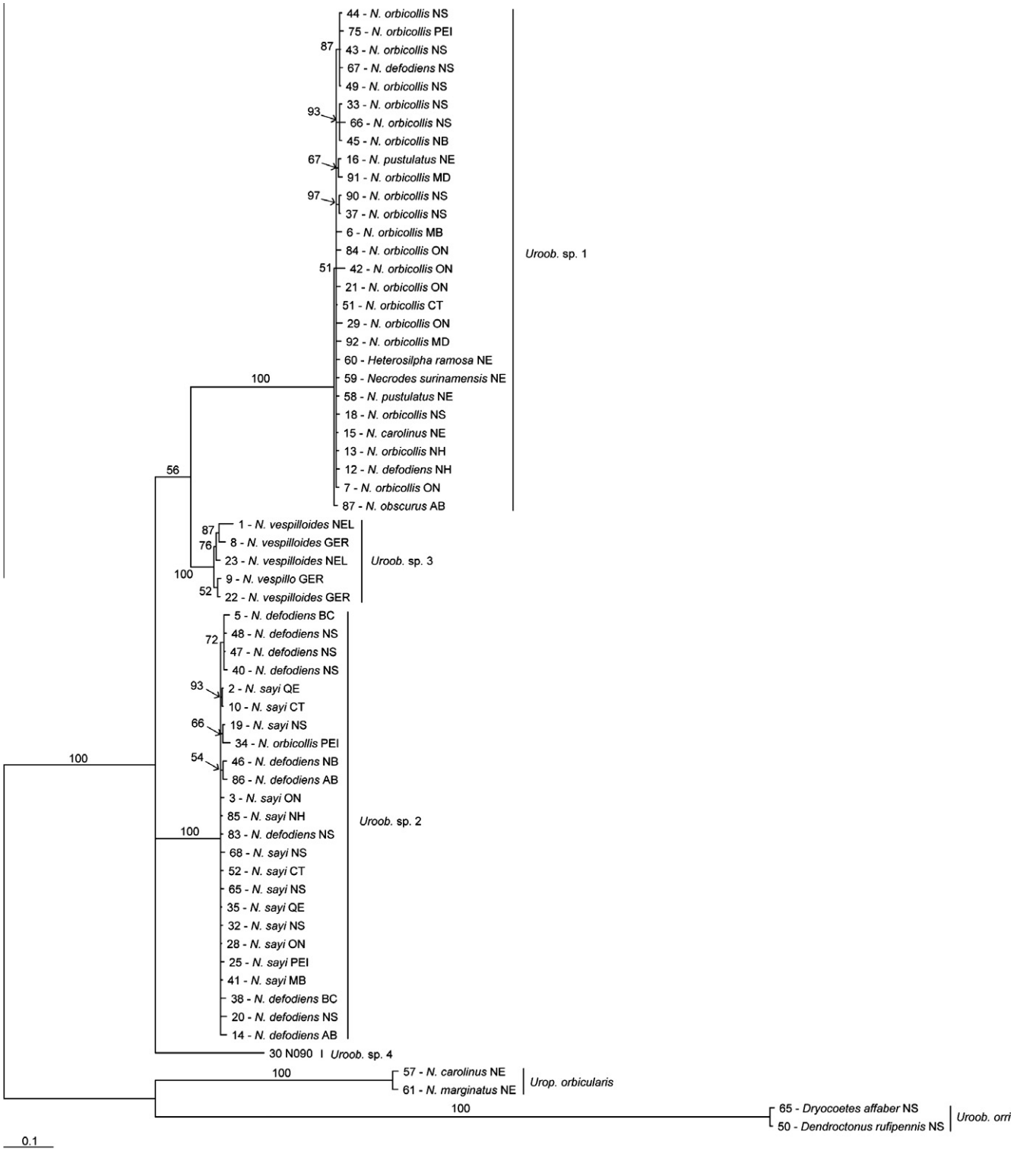

Fig. 3. Majority rule consensus tree of 18002 trees generated by Bayesian MCMC analysis (10 million generations) of the concatenated dataset of 673 bp fragment of COI and entire ITS2 (377 bp) from 62 uropodoid specimens, 58 ingroup specimens representing four *Uroobovella* species, and four outgroup specimens representing two species, posterior probabilities >50% shown above branches (TL = 871, CI = 0.6441, RI = 0.9131).

semistrict consensus tree (tree not shown). Multiple nodes had no JKS, a few nodes had moderate JKS, and only four nodes had 100% JKS. Most nodes had poor Bremer support, and only four nodes had relatively strong ($\geqslant$19) support. Three of the four well supported nodes are basal nodes to three of the four putative ingroup species. Similarly to the Bayesian analysis of COI, *U.* sp. 1 and *U.* sp. 2 had several unresolved nodes collapsing into a few intraspecific polytomies. The heuristic analysis of ITS2 produced two most parsimonious trees (TL = 248, CI = 0.9516, RI = 0.9789) presented in a semistrict consensus tree (tree not shown). All nodes had JKS, and five nodes had 100% support, two of which are the basal nodes to two ingroup species, and one is the basal node to the ingroup. Bremer support of each node was moderate, with only two nodes showing relatively strong Bremer support within the ingroup (tree

not shown). As seen in the BI of ITS2, each species collapsed into an intraspecific polytomy. The parsimony analysis of the concatenated dataset resulted in 44 most parsimonious trees (TL = 737, CI = 0.7503, RI = 0.8985) presented in a semistrict consensus tree (tree not shown). Most nodes had moderate to high JKS: five nodes had 100% JKS, three of which are basal to three ingroup species, and one is basal to the ingroup. The PBS varied from poor to strong, with the strongest PBS reported for the basal nodes for three of the ingroup species, and the basal node to the ingroup. The PBS revealed conflict between the COI and ITS2 datasets for two nodes within *U.* sp. 2, and two nodes within *U.* sp. 3, in which COI supported these nodes, and ITS2 displayed negative support.

3.5. Summary of molecular reconstructions

The BI and parsimony analyses of COI, ITS2 and concatenated datasets yielded similar results, indicating that *U. nova* is not a single species but instead at least four morphologically similar species. In most reconstructions the ingroup was shown to be monophyletic with relatively strong support. Additionally, in most reconstructions *U.* sp. 1, *U.* sp. 2 and *U.* sp. 3 were monophyletic with moderate to strong support. All analyses, except BI of COI and concatenated datasets, placed *U.* sp. 2 as the sister taxa to the rest of the ingroup, suggesting that *U.* sp. 1, *U.* sp. 3 and *U.* sp. 4 are more closely related to each other than to *U.* sp. 2; however, the basal placement of *U.* sp. 2 was not strongly supported.

4. Discussion

This study is the first to use the barcoding region of COI or ITS2 to examine the species boundaries and phylogenetic relationships among uropodoid mites. It is difficult to predict the relative effectiveness of a particular marker for a previously unstudied group. COI and ITS2, either alone or combined with other markers, have been successfully used to clarify species boundaries and to assess host specificity of mites (Kawazoe et al., 2008; Mahani et al., 2009; Morelli and Spicer, 2007; Schäffer et al., 2010; Webster et al., 2004). This study indicates that both COI and ITS2 are suitable markers for distinguishing between closely related uropodoid species, with 13.4–18.7% and 2.2–11.6% divergence between species, respectively. However, while COI showed 1.5% average intraspecific divergence, ITS2 varied minimally intraspecifically, with 0.3% average divergence.

4.1. Species boundaries and host specificity

COI and ITS2 as well as morphological characters indicate that an apparent generalist, *Uroobovella nova*, collected from 14 species of silphids in this study, is not a single species but rather multiple distinct species with differing degrees of host specificity. Morphological data from adults would likely reinforce the observed species boundaries. As a result of the relatively poor species description by Oudemans (1902), it is not known which of these cryptic species is closest to the original *U. nova* holotype. We suspect that *U.* sp. 3 is the closest (or even identical) to the original *U. nova* because they overlap in host species and geographic distribution. These issues will be addressed in a taxonomic revision of the species complex involving morphological descriptions by W.K.

Overall, the molecular reconstructions strongly support the monophyly of the three species (*U.* sp. 1, *U.* sp. 2 and *U.* sp. 3) that had adequate sampling. However, the phylogenetic relationships between *Uroobovella* species were poorly resolved in the molecular reconstructions. Examination of additional deutonymphs as well as adults, from more host species, would improve our understanding of their relationships, and almost certainly uncover more cryptic diversity in mites associated with burying beetles.

The divergence of COI between mite species was high (13.4–18.7%), especially given that these species are nearly identical morphologically. Such high levels of COI divergence have been reported across morphologically indistinguishable lineages in other groups of mites. The cereal rust mite *Abacarus hystrix* (Eriophyidae) species complex showed an average COI divergence of 22.6% between *A. hystrix* strains (Skoracka and Dabert, 2010); the water mite *Hygrobates nigromaculatus* (Hygrobatidae) exhibited an average COI divergence of 17.8% between stream- and lake-inhabiting populations, supporting the division into subspecies (Martin et al., 2010); *Stereotydeus mollis* (Penthalodidae), collected across the trans-Antarctic mountains, exhibited a maximum of 18% COI divergence across populations (Stevens and Hogg, 2006). The ITS region can also exhibit considerable divergence between cryptic lineages (2.2–11.6% in this study); for instances, the avian nasal mite, *Ptilonyssus sairae* (Rhinonyssidae) species complex, exhibited mean divergence of 10.4% (maximum 17.6%) across five species of hosts (Morelli and Spicer, 2007). Although not necessarily implied nor rigorously tested by the authors, some of these mite lineages may represent distinct species. The Acari is a particularly diverse group with at least 54,617 described species worldwide and an estimated diversity of 500,000–1,000,000 species (Walter and Proctor, 1999; Zhang, 2011). Such high levels of genetic divergence and cryptic diversity in mite taxa suggests that current estimates of global mite diversity are realistic or possibly even conservative.

Most of the cryptic species fettered from the apparent host generalist *U. nova* were found on a few host species. *Uroobovella* sp. 4 and *U.* sp. 5 were each associated with one host species (although *U.* sp. 4 was found on a single beetle specimen). *Uroobovella* sp. 2 and *U.* sp. 3 also have narrow host ranges, both largely associated with two host species. Moreover, the COI reconstructions suggest that *U.* sp. 2 represents two host races (i.e. genetically distinct populations associated with different hosts, between which gene flow may occur; Drès and Mallet, 2002), one on *N. sayi* and the other on *N. defodiens*. Even if *U.* sp. 1 was collected from nine host species it appears to be primarily associated with *N. orbicollis*. The association of *U.* sp. 1 with at least five host species (*N. pustulatus*, *N. carolinus, N. defodiens*, *Heterosilpha ramosa* and *Necrodes surinamensis*) was highly infrequent (i.e. 1–4 beetles with mites out of hundreds examined), and the geographic distribution of these species overlaps with *N. orbicollis* (Anderson and Peck, 1985), suggesting that these represent incidental host records. Occasionally more than one *Nicrophorus* species attempt to colonise the same carcass and these hosts compete over a carcass for brooding material (Trumbo, 1990). This interspecific co-occurrence may provide mites with an opportunity for host switching, which may explain in part the instances where a mite such as *U.* sp. 1 was on an atypical host. Overall, our data indicates that *Uroobovella* species are each associated with a relatively narrow range of *Nicrophorus* species, and certainly fewer than previously considered for *U. nova*.

4.2. Coevolution versus ecological fitting

Host species that are ecologically similar are more likely to co-occur spatially and temporally than are dissimilar host species. The co-occurrence of host species could provide more opportunities for host switching. Nevertheless, our study suggests that rather than tracking hosts with a shared ecology, silphid-associated mites are coevolving with their hosts. The association of *U.* sp. 2 with two ecologically distinct hosts (*N. sayi* and *N. defodiens*) rather than two similar ones (*N. sayi* and *N. orbicollis*), provides some evidence that these mites are not tracking ecologically similar hosts. *Nicrophorus sayi* and *N. orbicollis* have similar habitat preferences and behaviour; they are large, nocturnal beetles that dwell in forests

and open habitats, and they burry carcasses relatively deep in the ground; *N. sayi* overwintering adults emerge in mid-April, and *N. orbicollis* in mid-May (Anderson, 1982; Anderson and Peck, 1985). In contrast, *N. defodiens* is a small, crepuscular, forest-dwelling species, which buries carcasses shallowly beneath the leaf litter, and emerges in mid-May (Anderson, 1982; Anderson and Peck, 1985). Overall, *N. sayi* and *N. defodiens* are quite different ecologically, even though they share the same mite species (*U.* sp. 2). If mites were tracking hosts ecologically it would be expected that ecologically similar beetles (*N. sayi* and *N. orbicollis*) share the same mite species or sibling species. Plausibly, *U.* sp. 2 is found on both *N. sayi* and *N. defodiens* because these hosts are phylogenetically closely related (Peck and Anderson, 1985; Sikes, 2003). Conversely, *U.* sp. 1 may be distantly related to *U.* sp. 2 because its primary host, *N. orbicollis*, is a distant relative of *N. sayi* and *N. defodiens* (Peck and Anderson, 1985; Sikes, 2003). Clearly, additional taxon sampling and a more complete phylogeny of associated mites is needed in order to elucidate the mechanisms driving host associations of these silphid symbionts.

A more conspicuous silphid associate, *Poecilochirus* (Mesostigmata: Parasitidae), may also be tracking the phylogenetic history of their hosts. As *U. nova*, *Poecilochirus carabi* G. & R. Canestrini was historically considered to be an apparent generalist. Studies on the host preferences of deutonymphs of *P. carabi* revealed host-specific races in North America and Europe (Brown and Wilson, 1992; Müller and Schwarz, 1990), and subsequent enzyme electrophoresis and morphometric analysis suggested that these host races were actually distinct species (Baker and Schwarz, 1997; Schwarz et al., 1991). In Michigan (USA), two host races of the *P. carabi* complex were reported from three ecologically distinct hosts; one race was associated with *N. orbicollis*, and the other with *N. tomentosus* and *N. defodiens* (Anderson, 1982; Anderson and Peck, 1985; Brown and Wilson, 1992). It is possible that *N. tomentosus* and *N. defodiens* share the same host race of mites because they are phylogenetically more closely related to each other than to *N. orbicollis* (Sikes, 2003); however, this hypothesis was not proposed by Brown and Wilson (1992). *Poecilochirus* mites are vagile and they have been shown to switch between *Nicrophorus* species at larger carcasses, in which most *Nicrophorus* species do not reproduce since they prefer small carrion for breeding (Brown and Wilson, 1992). Despite their ease of host switching, which should favour gene flow and inhibit speciation, these mites have diverged genetically and morphologically, and this divergence seems to reflect the phylogenetic history, not the ecology, of their hosts. The apparent host preferences of *Poecilochirus* species, and possibly *Uroobovella* species, persisting despite the potential for host switching suggests that these mites are physiologically, phenologically or otherwise adapted to their preferred host.

Acknowledgments

We are grateful to D. Johnson, D. Sikes, J. Müller, J. Sweeney, M. Nishikawa, M. Schilthuizen, M. Scott, P. Smiseth, R. Dawson, S. Peck, S. Suzuki, S. Trumbo, M. Locke, W. Hoback, and W. Hunting for providing numerous specimens from around the world. We also thank H. Klompen for his advice on many of the molecular aspects of the study; M. Jackson for his input during analyses; A.I. Cognato and E. Lindquist for their comments on a previous version of the manuscript. T. Hartzenberg for her assistance in the field and the lab, as well as the private land owners who permitted sampling on their property. This research was conducted with a permit to collect in Provincial Parks issued by Ontario Parks and coordinated by B. Steinberg. This study was funded by an NSERC Discovery Grant to M.R. Forbes, and NSERC CGS D award to W. Knee.

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