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Delimiting species boundaries within *Dermanyssus* Dugès, 1834 (Acari:Dermanyssidae) using a total evidence approach

L. Roy [a,*], A.P.G. Dowling [b], C.M. Chauve [a], T. Buronfosse [a]

[a] *Université de Lyon, Ecole Nationale Vétérinaire de Lyon, Laboratoire de Parasitologie et maladies parasitaires, 69280 Marcy l'Etoile, France*
[b] *University of Arkansas, Department of Entomology, Fayetteville, Arkansas, USA*

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ABSTRACT

The genus *Dermanyssus* is currently composed of 24 hematophagous mite species and includes the Poultry Red Mite, *Dermanyssus gallinae*, a serious pest in poultry houses. Morphologically, *Dermanyssus* species fall into two groups corresponding to Moss' *gallinae*-group and to *hirsutus*-group + *Microdermanyssus*. Species of the *gallinae*-group exhibit high levels of morphological variability, and are nearly impossible to distinguish. Species of the second group display consistent characters and host associations and are easily distinguishable. Species of the *gallinae*-group tend to be the major problems in poultry houses and it is unknown whether *D. gallinae* is the only pest, or if there are numerous cryptic species present in the system.

Twenty species of *Dermanyssus* were tested phylogenetically based on 46 morphological characters. A subset of species, mainly of the *gallinae*-group, represented each by several populations, was sequenced for two mitochondrial and one nuclear gene regions. This allowed testing their specific status and their interrelationships based and on morphological and molecular characters. The molecular data was analysed separately and in combination with morphological characters. As expected, morphology did a poor job resolving relationships.

Molecular data proved more informative. The resulting phylogenetic hypotheses brought some information about interrelationships among species of the gallinae-group showing a split into two main clades. The invasion of human managed environments seems to occur only in taxa within one of the two clades. The host spectrum seems to get enlarged in more derived taxa in the same clade. A delineation of six species within the gallinae-group is provided. Additionally, a key for morphological identification of these species is provided. *D. gallinae* appears to be the only pest in poultry houses, but is composed of several different and more or less strongly isolated lineages. A new species found from the black swift is described.

1. Introduction

Genus *Dermanyssus* Dugès 1834 is currently composed of 24 hematophagous mite species, primarily parasitic on birds (Roy and Chauve, 2007; Knee, 2008).[1] The Poultry Red Mite, *Dermanyssus gallinae* (De Geer, 1778), is a serious pest in poultry houses, and other *Dermanyssus* species have been shown to affect wild birds, such as *Dermanyssus prognephilus* Ewing, 1933 on Purple martin chicks (Moss and Camin, 1970) and *Dermanyssus hirundinis* (Hermann, 1804) on the offspring of House Wrens (Johnson and Albrecht, 1993; Pacejka et al., 1996, 1998). Additionally, Clayton and Tompkins (1995) showed that *D. gallinae* can induce adult Rock Doves *Columba livia* Gmelin, 1789 to spend less time incubating their eggs.

No complete taxonomic review of the genus has been completed since 1978 (Moss, 18 species), and six species have been described since that date: *Dermanyssus carpathicus* Zeman, 1979, *Dermanyssus nipponensis* Uchikawa and Kitaoka, 1981, *D. brevirivulus* Gu and Ting, 1992, *D. wutaiensis* Gu and Ting, 1992, *Dermanyssus rwandae* Fain, 1993 and *Dermanyssus diphyes* Knee, 2008. *Dermanyssus* is clearly defined compared to other genera due to its roughly crescent-shaped and particularly short sternal shield and characteristic chelicerae. Chelicerae possess strongly reduced chelae and a strongly elongate, flattened and medially concave second segment, which forms something like a gutter and allows the two chelicerae, once joined together, to form a tube through which blood is sucked up (Phillis, 2006). On the other hand, species limits are not clearly defined and morphological characters traditionally used for diagnosis are highly variable within a single population (Roy and Chauve, 2006) and even the same individual (bilateral asymmetries). Consequently, some species other than *D. gallinae* may infest farms, which, if confirmed, may have serious consequences on control strategies.

In order to better define species limits, elucidate *Dermanyssus* phylogeny, and develop molecular tools for applied use, we have conducted a phylogenetic study of a part of the genus. The dataset

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* Corresponding author. Fax: 33 478872577.
E-mail address: l.roy@vet-lyon.fr (L. Roy).

[1] *Dermanyssus diphyes* Knee, 2008 was published during paper revision, and therefore has not been included in present study.

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includes morphological characters, several of which have never been examined for any study on *Dermanyssus* species relationships, and molecular data from ITS1 and 2 (plus some few bases of flanking regions of 18S and 28S rRNA,[2] and including 5.8S rRNA), 16S rRNA, and coding gene for Cytochrome oxidase subunit I (COI).

Dermanyssus species morphologically fall into two groups; those possessing a soft body adapted for sporadic and large engorgement with reduced shielding and slender legs (14 species) and those possessing a compact, more heavily sclerotized body with shorter, stouter legs (9 species). Species possessing the soft-body type are the most common and most of them are nearly indistinguishable from each other; they constitute the *gallinae*-group: *Dermanyssus antillarum* Dusbábek and Černý, 1971, *D. carpathicus* Zeman, 1979, *Dermanyssus chelidonis* Oudemans, 1939, *Dermanyssus faralloni* Nelson and Furman, 1967, *D. gallinae*, *Dermanyssus gallinoides* Moss, 1966, *D. hirundinis*, *Dermanyssus longipes* (Berlese and Trouessart, 1889) (*nomen dubium*), *D. nipponensis* Uchikawa and Kitaoka, 1981, *D. prognephilus*, *Dermanyssus transvaalensis* Evans and Till, 1962, *Dermanyssus triscutatus* Krantz, 1959, *Dermanyssus trochilinis* Moss, 1978, *D. wutaiensis* Gu & Ting, 1992. Several of these species have very large host ranges, in particular *D. hirundinis* and *D. gallinae*, which have been collected from numerous bird species, distributed across eight to nine orders (Roy and Chauve, 2007).

Dermanyssus species possessing the second body type correspond to Moss' subgenus *Microdermanyssus* (*Dermanyssus alaudae* (Schrank, 1781), *Dermanyssus americanus* Ewing 1922, *D. brevirivulus* Gu & Ting, 1992, *Dermanyssus brevis* Ewing, 1936, *Dermanyssus grochovskae* Zemskaya, 1961, *Dermanyssus hirsutus*, *Dermanyssus passerinus* Berlese and Trouessart, 1889, *Dermanyssus quintus* Vitzthum, 1921 and *D. rwandae* Fain, 1993) and members of his *hirsutus*-group (*D. hirsutus* Moss and Radovsky 1967, *D. grochovskae* and *D. quintus*). All except *D. quintus* display a strong asymmetry in setae length between those situated centrally on the dorsal shield and those located on the perimeter. Several species possess conspicuous and distinctive morphological characters, such as a paired sclerotized porelike-structures on dorsum in *D. alaudae*, *D. americanus* and *D. brevis* (Moss' subgenus *Microdermanyssus*), a U-shaped row of large and deeply rooted setae on the opisthogaster in *D. quintus*, and ventral neotrichy in the form of a cluster of elongate, simple setae laterad of the anal shield in *D. hirsutus*. These species are more clearly distinguishable if compared to one another than species of the *gallinae*-group on the basis of morphology.

Moreover, available data on these species suggest they are more host specific than the *gallinae*-group, typically parasitizing a single bird family (Picidae for *D. quintus* and *D. hirsutus*, Alaudidae for *D. alaudae*). However, *D. grochovskae* occurs on two bird orders, Piciformes and Passeriformes, and some of these species have been found only once (*D. brevis*, *D. brevirivulus*), so the extent of their host specificity is unknown.

Morphological differences between *gallinae*- and *hirsutus*-groups have been suggested by Moss to be correlated to life-style. Most *Dermanyssus* species are known to be nidicolous, climbing onto the host only to obtain a meal before returning to their hiding-place in the host nest or roost. However, some species frequently remain on the host for extended periods of time and can deposit their eggs on its feathers. These species possess a morphology more adapted to clinging onto the host rather than to running around on it (e.g. *D. grochovskae* and *D. quintus*) (Moss, 1978).

Dermanyssus gallinae (*gallinae*-group) is of economic and veterinary importance and it possesses highly polymorphic morphological characters. Sclerotized areas, usually bearing most of the phylogenetically informative characters, are strongly reduced in these species and are often asymmetric on a single individual. The dorsal shield also displays irregularities, including contours that are asymmetric in an individual in almost all species of the *gallinae*-group and asymmetric setal patterns including numbers and position. Additionally, leg chaetotaxy is highly variable intraspecifically (Evans and Till, 1962; Moss, 1978), a characteristic common among mites that have formed parasitic associations (Evans, 1963).

Such phenotypic variability not only makes species identification difficult within a genus (Evans and Till, 1962; Moss, 1978), it also produces major problems for accurately coding morphological characters in a phylogenetic framework. Overall, this variability has led to confusion regarding species limits and evolution within *Dermanyssus* and until this study, molecular characters have not been consulted.

The aim of the present study is to explore relationships between *Dermanyssus* species using a phylogenetic framework based on morphological characters and between some species of the *gallinae*-group using and morphological and molecular characters. From obtained results, we plan (1) determining whether the *gallinae*-group includes distinct species or simply variants of populations, (2) estimating whether the *gallinae*-group body type is primitive or derived and examine its adaptive significance and (3) evaluating host specificity of field collected species.

2. Material and methods

In the aim of processing in a standardized manner, only adult female mites have been used in this study. Adult females have been selected as this is the only stage/sex described for all species. Adult males are less often found. Moreover, discriminant morphological characters appear to be mainly found in females.

2.1. Methodology for delineation of species boundaries

Primary hypotheses of alpha-taxonomy have been tested following Samadi and Barberousse (2006) recommendations for helping in species delimitation. Our objective was to identify reproductively isolated groups of organisms that warrant classification as distinct species by using phylogenetic tools. For such a purpose, successive validations of morphological characterization with correlation to molecular information have been processed in order to test primary hypotheses provided by α-taxonomy.

For testing primary hypotheses, two main actions have been carried out. First, a comprehensive analysis of *Dermanyssus* phylogeny at the species level based on reference material has been carried out, allowing us to obtain a set of discrete characters usable for phylogenetic exploration. Second, partial exploration of *Dermanyssus* phylogeny involving various populations of several field collected species, based on previously coded morphological characters and on molecular data. Several successive steps including comparisons between individual morphology and corresponding sequences was followed by phylogenetic analyses. Finally, some of the traditional species specific characters have been compared to the obtained phylogenetic hypotheses in order to assess their actual utility (*a posteriori* feedback).

2.2. Morphological study

2.2.1. Taxon sampling

In the present study, *Dermanyssus* is represented by 20 of the currently recognized 24 species and one unidentified taxon (Appendix A). Type specimens of *D. passerinus*, *D. brevirivulus* and *D. wutaiensis* were unavailable for examination (specimen dam-

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[2] *Abbreviations used:* 16S, rRNA 16S; bp, base pairs; BPP, Bayesian posterior probabilities; COI, cytochrome oxidase subunit I; ITS, rRNA 18S (partial sequence), internal transcibed spacer 1 (ITS1), 5.8S, internal transcribed spacer 2 (ITS2), 28S (partial sequence); K*x*, morphological character n°*x*; MP, maximum parsimony; OTU, operational taxonomic unit; RSE, reference specific entities.