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

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**A B S T R A C T**

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 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.

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1. Introduction

Genus *Dermanyssus* Dugès 1834 is currently composed of 24 hematophagous mite species, primarily parasitic on birds (Roy and Chauve, 2007; Knee, 2008). 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 prognephilius* 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 Tomkins (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. wutaensis* Gu and Ting, 1992, *Dermanyssus rwanda* Fain, 1993 and *Dermanyssus diphys* 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
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, 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. carpaticus 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. prognehophilus, Dermanyssus transvaalensis Evans and Till, 1962, D. brevirribulus, Dermanyssus triscutatus Krantz, 1959, Dermanyssus trochilinis Moss, 1978, D. mutaiensi 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 alaudea (Schrank, 1781), Dermanyssus americanus Ewing 1922, D. brevirribulus 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. alaudea, 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 lateral 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. alaudea). However, D. grochovskae occurs on two bird orders, Pici forms and Passeriformes, and some of these species have been found only once (D. brevis, D. brevirribulus), 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 nidiculous, 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. brevirribulus and D. wutaiensis were unavailable for examination (specimen dam-
aged for D. passerinus and institution housing types for D. brevivir-
ulus and D. wutaiensis was unresponsive), and we did not find any
other reference specimens for these species. The type specimen of
D. longipes was also damaged but we were able to collect speci-
mens in the field from the type locality near Avignon (France) as
well as examine specimens in a Slovak collection (Fend'a, P., Come-
nius University). D. diphyes has been described during revision of
present paper. Three species have been included as outgroups: 
Ornithonyssus bacoti (Hirst, 1913) (Mesostigmata: Dermanyso-
idea: Macronyssidae), Haemogamasus hirsutus Berlese, 1889 (Meso-
stigmata: Dermanyssidea: Haemogamasidae), Androlaelaps casalis
(Berlese, 1887) (Mesostigmata: Dermanyssidea: Laelapidae) and
Typhlodromus pyri Scheuten, 1857 (Mesostigmata: Ascoidea: Phy-
toseiidae). The family Dermanyssidae also includes Liponyssoides,
but unfortunately no specimens were available for morphological
or molecular study, despite efforts of the authors (several collec-
tions in which some types of Liponyssoides sp should have been
deposited have been contacted, without any success). This has
forced us to only include distant outgroups of Dermanyssus.

2.2.2. Character sampling

Forty six morphological characters are included in the matrix
(Appendix B and C). Due to very high variation (at the population
level) of traditional chaetotactic characters (Roy and Chauve,
2006), stringent coding of such characters appeared impossible in
many cases. Therefore, we strongly reduced our reliance on such
characters (only five traditional characters in present study), and
completely omitted leg chaetotaxy. We selected and coded 31
addional morphological characters and ten morphometric
characters.

- Five characters focus on chaetotaxy (K13, 14, 21, 34 and 35) of
  anal and dorsal shields including the soft integument.
- Twenty one characters describe diverse parts of the body with
  five morphological characters describing soft integument (K10,
  11, 18, 20 and 41), five describing shields (anal and dorsal
  shields; K8, 23, 24, 25 and 36), two describing peritremata (K9
  and 42), one describing the palps (K1), five describing internal
  organs (K5, 6, 7, 19 and 45), two describing chelicerae (K32
  and 33) and one describing cornicles (K46).
- Ten characters focus on the shape of some setae located on dor-
  sal shield, hypostome, legs, palps and soft integument of opis-
  thosoma (K4, 12, 15, 16, 17, 29, 30, 31, 43, and 44).
- Ten characters use relative morphometry, six of which describe
dorsal, epigynial and anal shields (K2, 3, 22, 28, 37 and 38) and
four of which describe the legs (K26, 27, 39 and 40).

2.2.3. Phylogenetic analysis based on morphological data

For the phylogenetic analysis, all characters were treated as
unordered and unweighted. A heuristic analysis was performed
under the parsimony criterion using PAUP* 4.0b10 (Swofford,
2001) with TBR branch swapping and 10,000 random additions
saving all most parsimonious trees. Heuristic searches in TNT
(Goloboff et al., 2008) were used to obtain relative Bremer (Golob-
off and Farris, 2001) and bootstrap support values. TNT searches
recovered the same topology and tree length as PAUP, but calcula-
tion of support values is much more efficient in TNT.

2.3. Molecular exploration

2.3.1. Biological material

Dermanyssus specimens were collected from November 2005 to
2008, mainly in France (some in USA), using two sampling
methods due to the different lifestyles found within the genus.

Most samples come directly from wild bird nests, which have
been treated following the method of De Lillo (2001) with slight
modifications. Overall, 327 nests were analyzed from 37 different
bird species distributed across eight different orders. Bird taxon-
omy follows Peterson (2007). Due to their diversity and ubiquity,
passeriforms were the most represented host group, accounting
for 248 nests, with most nests distributed across four families
(202 nests): Hirundinidae (46 nests), Parus sp. (Paridae; 120 nests),
Alaudidae (14 nests), Passer sp. (Passeridae; 22 nests). The remain-
ing 46 passeriform nests were from 12 different species (less than
ten nests/group). One species of aplodontia is strongly present here
(Apus apus L., 1758, Apodidae; 52 nests). Other nest samples exam-
ined were from the following bird groups: Columbiformes (13
nests), Ciconiiformes (six nests), Strigiformes (three nests), Anser-
iformes (two nests), Gruiformes (one nest), Piciformes (two nests).

Additional collections of living mites have been made directly
from birds captured during bird-banding and/or bird care activi-
ties. This provided specimens from an additional species represent-
ing a ninth order: Coracias garrulus L., 1758 (Coraciiformes: Coraciidae). Three complementary populations have been obtained from
other sampling activities (D. hirsutusADhirs, D. quintusADqiu,
D. hirundinisADhirun).

2.3.2. Taxon sampling

Due to requirements of preserved specimens for DNA studies, our
molecular dataset includes only those Dermanyssus species col-
lected freshly into ethanol (or simply dried) by the authors or col-
laborators. Due to these constraints, very few Microdermanyssus
+hirsutus-group have been included in this part of study: no Micro-
dermanyssus and only two species of the Moss' hirsutus-group (D.
quintus and D. hirsutus) have been collected. On the opposite, sig-
nificant sampling of the gallinae-group has been included in the
molecular dataset: four known and one unknown species of galli-
nae-group are included. Because of the noted lack of discriminating
characters found within the gallinae-group, several specimens
were sampled from separate populations resulting in the inclusion
of 45 gallinae-group OTUs in the combined molecular matrix, 29 of
which are included in the total evidence analyses.

Only three of the four outgroups used in the morphological
analysis (O. bacoti, A. casalis, and T. pyri) were available fresh for
molecular examination. Although efforts were made to collect
specimens of Liponyssoides, all attempts were unsuccessful.

2.3.3. Four different a priori morphs on field collected species

The key problems lie in the separation of the species of gallinae-
group due to variable characters within species and a general lack
of discriminating characteristics across species. Therefore, a first
and rough examination of material led to delimitation of four a pri-
ori morphogroups. Only species of gallinae-group are dealt with
here, as members of the “Microdermanyssus + hirsutus-group” are
easily defined. Initial examination focused on numerous popula-
tions across Europe and resulted in separation into the following
four morphogroups based upon preliminary successive compari-
sions of sequences and morphological data: DG-morph (type
population SK (Table 1) — all1 of palp genu lanceolate, sternal shield
with a deep central concave neckline, dorsal shield narrower than podo-
soma), GO-morph (type population GO1—all1 of palp genu lanceo-
late, sternal shield without a sharp postero-medial neckline, dorsal
shield as wide as podosoma, anterior pair of setae in hypostomal
parallelogram larger than in D. gallinae), RQ-morph (type popula-
tion RQ—all1 of palp genu spine-like) and DL-morph (type popula-
tion PAS—similar to DG-morph, but with a slightly different anal
shield, proportionally longer and slightly more angular—cf. above).

The following populations were sequenced during this study
however, not all were included in the final combined analyses, typ-
ically because of missing data (Table 1).
### Table 1

Taxonomic sampling and EMBL accession numbers for each sequence. Each record corresponds to one mite population belonging to a single species sampled from a single nest or a single bird. Locality and host information are also provided.

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**gallinae-group**: GO-morph, 11 populations; DC-morph, 25 populations; RQ-morph, 7 populations; DL-morph, 2 populations.

### 2.3.4. Character sampling

Three gene regions have been selected for sequencing: two mitochondrial markers (COI and 16S) and one nuclear marker (ITS). The molecular dataset includes a total of 1524 bp for each taxon (ng accession numbers in Table 1) and sequence data was aligned using MAFFT (Katoh et al., 2005) with the L-INS-i iterative refinement option on the MAFFT server at http://align.bmr.kyushu-u.ac.jp/mafft/online/server/. MAFFT with the L-INS-i option has been shown to be the most accurate and consistent method for sequences (Wilm et al., 2006; Carroll et al., 2007).

### 2.3.5. Amplification and sequencing of DNA

#### 2.3.5.1. Morphological preparation and DNA extraction

DNA was extracted from individual mites by cutting the cuticle at two points on the opisthosoma and pushing most of the internal elements out. This was done with a sterile pipet tip in the appropriate compound buffer containing proteinase K (Qiagen) and digestion was performed at 70 °C for 19–30 h. The cuticle was separated from the DNA mixture and mounted as a voucher and for microscopic observation. Specimens slide mounted directly from alcohol having not undergone DNA extraction were compared to these vouchers to determine the usefulness of the DNA voucher cuticles. The proteinase K digestion did not appear to have any adverse effects on the cuticle and all characteristics necessary for morphological examination remained intact. DNA was extracted following procedures in the Qiagen QIAamp DNA Mini Kit. When possible (most cases), two to three separate specimens from each tested population were extracted and sequenced.

#### 2.3.5.2. DNA amplification and sequencing

PCRs were separately performed in order to amplify two mitochondrial gene regions (part of COI gene and of 16S) and one nuclear region (fragment ITS1-5.8S-ITS2) in either a Biometra TGradient or a MWG AG Biotech Primus 96plus thermal cycler in typical buffer containing 2 µl of template DNA, 2.5 U of Taq polymerase, 10 nmol of dNTPs, 20 pmol of each primer and a variable volume of 50 mM MgCl2 depending of the target gene in accordance to Table 2 in a final volume of 50 µl. After an initial denaturation step (95 °C) for 10 min, followed by 40 cycles of: 20 s at 95 °C (denaturation), 30 s at the annealing temperature specified for each primer set (Table 2), and 90 s at 72 °C (extension). A final extension step was carried out for 10 min at 72 °C. Several primers have been designed for amplification in various species and are provided in Table 2.

Negative and positive controls were run with each round of amplification. PCR products were checked by electrophoresis in a 1% agarose gel. Depending on the brightness of the band either additional PCRs were run on the original template or reamplifications of the original PCR product were performed. PCR reamplifications using same primers were assessed on 1 µl of product and PCR conditions were as follows: initial denaturation at 95 °C for 3 min followed by 20 cycles of: 20 s at 95 °C, 45 s at specified annealing temperature (Table 2), and 2 min at 72 °C. A final extension step was carried out for 45 min at 60 °C. In both cases, a total of four reactions were run for each taxon sample and the resulting amplifications were pooled in order to obtain enough DNA for sequencing. The four PCR tubes from each sample were pooled together, submitted for electrophoresis in a 1% agarose gel, and PCR products were excised from the gel and purified using the Macherey-Nagel Nucleospin Extract-II kit. Purified PCR products were sequenced by Genoscreen (France, Lille) using a 96-capillary sequencer ABI3730XL.

<table>
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<th>Primer annealing T° (°C)</th>
<th>MgCl2 (mM)</th>
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</table>

| Primer sequence from De Rojas et al. (2002). |
| Primer sequence from De Rojas et al. (2001). |
2.3.6. Phylogenetic analyses based on combined morphological and molecular data

Phylogenetic analyses with Maximum Parsimony (MP) were run for the total evidence dataset, a combined molecular only analysis, and individual analyses of morphology and the separate gene regions. Heuristic searches were carried out in PAUP* 4.0b10 (Swofford, 2001) with TBR branch swapping and 10,000 random additions saving all most parsimonious trees. Heuristic searches in TNT (Goloboff et al., 2008) were used to obtain relative Bremer (Goloboff and Farris, 2001) and bootstrap support values.

For Bayesian analyses the total evidence data set (morphology and molecules), the combined molecular only data set, and the individual genes were all run using MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). MrBayes differs from other programs in allowing partitions within the data set to implement different models of evolution, presumably allowing for a more realistic analysis of the data. Models of evolution were applied to individual molecular partitions and determined for each gene by MrModeltest (Nylander, 2004) for Bayesian analyses. In the total evidence Bayesian analysis, the following models were applied to each partition: (1) Morphology used the standard discrete model (appropriate for likelihood approximations of morphological datasets; Lewis, 2001) and assumed gamma-shaped rate variation; (2) ITS used GTR + I (proportion of invariable sites without a gamma distribution); (3) COI used GTR + I + I; and (4) 16S used GTR + I. Each of the models for the molecular partitions was determined in MrModeltest using Akaike information criterion (Akaike, 1974). Parameters within each model were not specified (or fixed) and MrBayes was left to estimate these independently for each partition from the data during analysis. All analyses in MrBayes included two independent runs, each consisting of four chains and 5,000,000 generations. Appropriate burnins were determined based on stationarity being reached through the use of Tracer v1.4 (Rambaut and Drummond, 2007).

Two other Bayesian runs were completed in order to test the effects of 3rd position change in the COI analysis as well as missing data in the molecular only analysis. In many analyses involving COI, the majority of change or divergence between taxa or populations is found in the highly variable 3rd position. Results based solely on 3rd position change are often criticized and considered based upon random data. This analysis excluded 3rd positions from the COI single gene analysis to determine if any presently supported groups would still exist in the final tree. The analysis was run with all the same parameters as previous Bayesian runs and an appropriate model determined by MrModeltest.

The primary reason for using a subset of taxa in the combined analyses was to eliminate a large amount of missing data that would have to be incorporated in order to include every population sequenced for at least one gene region. In order to test whether these missing data would have had an impact on the final analyses, all taxa sequenced for at least one molecule were included in a final “all-taxa” molecular only dataset. The Bayesian analysis was run under the same parameters as the other molecular only combined analysis.

3. Results

3.1. Comprehensive phylogenetic reconstruction based on morphology alone

The MP heuristic analysis of 25 taxa and 46 morphological characters resulted in 12 most parsimonious trees ($L = 129, CI = 0.4264, RI = 0.6085$) and the strict consensus is represented in Fig. 1. The monophyly of tested species of Dermanyssus is supported by a very short sternal shield (K3), a strongly atrophied third cheliceral segment (K32), a strongly elongate and foliate second cheliceral segment (K33) and membranous cornicles (K46).

![Fig. 1. Strict consensus tree of 12 most parsimonious trees ($L = 129, CI = 0.4264, RI = 0.6085$) using matrix of 46 morphological characters. The numbers below nodes refer to the relative Bremer support and numbers above refer to bootstrap percentages from 1000 replicates. Additionally, mapping of the main morphological synapomorphies is figured by white (character state 0), grey (character state 1) and black (character state 2) dots, labeled with corresponding character number.](image-url)
Within *Dermanyssus*, *D. trochilinis* is a sister to all other *Dermanyssus* species, although support at this node is moderate (0.44 relative Bremer, 83% bootstrap). The remaining species share a strongly unresolved basal node and the clade grouping *Microdermanyssus* and the *hirsutus*-group is the only group with any kind of relative Bremer support and includes eight species (*D. alaudae*, *D. americanus*, *D. brevis*, *D. grochovskiae*, *D. hirsutus*, *D. quintus*, *D. rwandae*). Monophyly of the group is supported within *Dermanyssus* by one apomorphy: the proportions of tibia I (K39, with an evolution in distal OTUs in *D. alaudae* + *D. americanus*). Moreover, the character state 2 in K6 (shape of the principal pore on the post-stigmatic element) is an apomorphy of this group within *Dermanyssus*, but there is a homoplasy with one outgroup (*H. hirsutus* shares same character state in K6). Note that the *hirsutus*-group does not appear to be monophyletic here, the interrelationships between the three species keep unresolved. It should be interesting to test the monophyly of this group using molecular data in a further study. *D. carpathicus*, one of the *gallinae*-group species, appears as sister group of above clade (0.44 relative Bremer; 65% bootstrap). Two synapomorphies support this new clade: character state 1 in K17 (relative length of setae on dorsal side of femur I) and 2 in K40 (proportions of genu I), with an evolution in distal OTUs in *Microdermanyssus*, (transition to the third state 1). As for other species, interrelationships in the *gallinae*-group keep unresolved (forming a large polytomous), except for the sister group relationship of *D. antillarum* and *D. triscutatus* (0.42 relative Bremer; 50% bootstrap).

3.2. Combined analyses on the subset of field collected species

Molecular information has been obtained from one to three genes in 46 different populations (Table 1). Obtained topologies are shown in Figs. 2A and 3A. Distribution of pairwise divergence among tested populations in the three tested genes is provided in Fig. 4.

3.2.1. Combined molecular matrix

The MP heuristic analysis of 34 taxa and 1524 characters (643 bp of ITS, 540 bp of COI, 341 bp of 16S) resulted in 576 most parsimonious trees (*L* = 1357 CI = 0.6478 RI = 0.7641). A strict consensus (Fig. 2A) resulted in two main clades within *Dermanyssus*. The first groups most of the DG-morph populations (clade B) with fairly strong support (0.77 relative Bremer; 98% bootstrap). Within this clade, several internal clades are strongly supported. Of them, two successive sister clades (F and E) group strongly together three and two populations respectively (1.0 relative Bremer and 100% bootstrap scores) and distinction from the other DG-morphs and each other. The second clade groups populations with RQ-morph, DL-morph and the remaining DG-morph (clade 1) although with very little support at the basal nodes. The individual morphs are monophyletic group with good support, including RQ-morph (J, 0.55 relative Bremer; 100% bootstrap), DL-morph (N, 0.90 relative Bremer; 100% bootstrap), and the remaining DG-morphs (L, 0.45 relative Bremer; 98% bootstrap). The remaining groups are basally unresolved and show no affinities to either of the previously mentioned clades. This includes the *hirsutus*-group (*D. hirsutus* and *D. quintus*) which is entirely unresolved and the populations with GO-morph, which form a monophyletic group with strong support (1.0 relative Bremer; 100% bootstrap) but with relationships to other *Dermanyssus* unresolved.

In the Bayesian analysis different models of evolution were implemented for each partition (gene region) in the molecular dataset and resulted in a more resolved tree than the MP run, although many characteristics of the two analyses are similar. The topology obtained from MrBayes (Fig. 2B) is almost completely resolved and results also in two major clades of *Dermanyssus*. The clade G contains the RQ-morph, DL-morph, some of the populations with DG-morph, and unlike the MP analysis, the populations with GO-morph and the *hirsutus*-group are basally resolved and showing affinities to this first clade. Each of the morphs found in this large clade represents a monophyletic group of populations. Within this clade, the populations with GO-morphs are most basal and strongly supported (1.0 Bayesian Posterior Probabilities (BPP)), followed by the *hirsutus*-group (0.74 BPP), the RQ-morphs (1.0 BPP), then the populations with DL-morph (1.0 BPP), and finally a clade of DG-morphs (1.0 BPP). The other main clade (B) consists of the remaining populations with DG-morph and shows little sequence variation across the clade. This clade, like in the MP analysis, is very poorly supported (0.55 BPP) indicating the basal relationships within this clade are questionable. All other relationships within the clade B corroborate the results of the MP analysis.

The results of the “all-taxa” matrix represented in Appendix E show that the same groups are resolved, albeit with more members, and for all intents and purposes provides the same topology as the previously run molecular-only combined analysis. The large amount of missing data apparently had no effect on the final results, and in fact because the same major groups emerged, would indicate how strongly the individual genes converge on the same hypothesis.

3.2.2. Total evidence matrix

The MP heuristic analysis of 34 taxa and 1570 characters (46 morphological K, 643 bp of ITS, 540 bp of COI, 341 bp of 16S) resulted in 288 most parsimonious trees (*L* = 1426 CI = 0.6452 RI = 0.7634). Strict consensus (Fig. 3A) resulted in a hypothesis identical to the molecular only result.

The Bayesian analysis once again resulted in a well resolved tree (Fig. 3B) very similar to the molecular only tree (Fig. 2B). One major difference is the placement of the *hirsutus*-group. In the molecular only tree, the *hirsutus*-group was sister to the RQ-morph + DL-morph + DG-morph clade (clade H in Fig. 2B), whereas in the total evidence results, the *hirsutus*-group is sister to the RQ-morph clade and overall (clade 1’ in Fig. 3B), support values are much higher. All other groupings remain the same between the two results.

Removing 3rd positions from the COI analysis produced a less resolved phylogenetic hypothesis (Fig. 5) than the full COI analysis, as expected. However, the following major groups were still recovered: GO-morph (1.0 BPP), RQ-morph (1.0 BPP), RQ + DL-morphs group (0.76 BPP), GO + DG + *hirsutus* + DL + RQ-morphs group (0.82 BPP), and two DG-morph subgroups from the main DG-morph clade typically recovered (*COL* + *JGC* + *LC* + *PL*, corresponding to clade F: 0.88 BPP and *JBSO1* + *LB07* + *LB18*, corresponding to clade E: 0.95 BPP). These results indicate that while major change is present in the 3rd position, informative change (information) still exists in the more slowly evolving 1st and 2nd positions.

3.3. Single gene analyses

**COI**: The MP heuristic analysis of 41 taxa and 540 characters of COI resulted in 36 most parsimonious trees (*L* = 699 CI = 0.5622 RI = 0.8216). The strict consensus resulted in a similar topology to the results of the combined analyses, although more unresolved. Most notably is the lack of resolution between the DL-morph and the DG-morphs, normally resolved in the combined analyses.

The Bayesian results for COI are more resolved than the MP results and the only difference between the COI results and the combined results is the separation of *D. hirsutus* and *D. quintus*.

**16S**: The MP heuristic analysis of 34 taxa and 341 bp of 16S resulted in 28 most parsimonious trees (*L* = 309 CI = 0.6796 RI = 0.8467). The strict consensus resulted in a similar result to the combined analyses, although slightly less resolved. Unlike the COI analysis, relationships of RQ-, DL- and part of DG-morphs are
resolved, but with RQ-morph grouped with part of DG-morph, and with DL-morph as a sister group (different than results found in the combined analyses). Finally, interrelationships within the second main clade are less resolved than in previous analyses. Bayesian results are basically identical to the MP results, showing the same relationships.

Fig. 2. Molecular combined analysis using 1570 bp from cytochrome oxidase subunit I, rRNA 16S and rRNA 18S–28S, including ITS1, 5.8S and ITS2. (A) Maximum parsimony criterion, PAUP 4.0. Strict consensus of 576 most parsimonious trees ($L = 1357$ CI = 0.6478 RI = 0.7641). The numbers below nodes refer to the relative Bremer support and numbers above refer to bootstrap percentages from 1000 replicates. (B) Bayesian analysis from 5,000,000 generations using partitioned data and independent models of evolution for each partition. Numbers on nodes refer to Bayesian posterior probabilities.

ITS: The MP heuristic analysis of 35 taxa and 643 bp from ITS resulted in 1000 most parsimonious trees ($L = 413$ CI = 0.8184 RI = 0.7706). The strict consensus resulted in a conspicuously less resolved topology than in the combined analyses. The monophyly of populations with RQ-morph appears strongly supported (1.0 relative Bremer; 92% bootstrap) however no other relationships are
Fig. 3. Total evidence analyses using 46 morphological characters and 1570 bp from cytochrome oxidase subunit I, rRNA 16S and rRNA 18S–28S, including ITS1, 5.8S and ITS 2. (A) Maximum parsimony criterion, PAUP 4.0. Strict consensus of 288 most parsimonious trees ($L = 1426$ CI = 0.6452 RI = 0.7634). The numbers below nodes refer to the relative Bremer support and numbers above refer to bootstrap percentages from 1000 replicates. Additionally, mapping of the main morphological synapomorphies is figured by white (character state 0), grey (character state 1) and black (character state 2) dots, labeled with corresponding character number. (B) Bayesian analysis from 5,000,000 generations using partitioned data and independent models of evolution for each partition. Numbers on nodes refer to Bayesian posterior probabilities. Moreover, triangular signs indicate populations found in a human-shaped environment, internal color corresponding to different bird groups (grey triangle, pigeons breeding facilities, white triangle, fringillids breeding facilities, black triangle, layer hen houses).
Fig. 4. Distribution of percentages of pairwise divergence among populations of the eight OTUs used in molecular analyses. RSE = reference specific entities (cf. discussion, § species), ie D. carpathicus, D. hirsutus, D. quintus. L1, L2, L3 = hierarchical levels 1, 2, 3 noted on trees are discussed in the text.

Fig. 5. Bayesian analysis of the COI matrix, excluding 3rd positions of codons.
discernable. Bayesian analysis provided a slightly more resolved result including a weakly supported clade of DG- and DL-morphs as in other results and a strongly supported clade of RQ-morph. Additionally, the DG-morph clade F forms a strongly supported monophyletic group.

As individual units, none of the gene regions show high resolving power or large amounts of useful phylogenetic data. However, as a combined unit the resolving power greatly increases and understandable relationships emerge, thus reinforcing the utility of total evidence approaches.

3.4. Species boundaries

In all three single gene analyses, as in the total evidence and combined analyses, the same populations typically group together, and it's the more internal nodes where disagreements are found. Mitochondrial gene analyses resolve rather similarly to each other, with less resolution intra-morph in 16S than in COI-based topologies. The ITS-based topology is less resolved, but still recovers the RQ-morph group.

On the whole, phylogenetic analyses of the total evidence and molecular-only matrices, recovered a monophyletic grouping of the different expected species of the gallinae-group with strong support values (bootstrap values from 98 to 100% in all analyses; Table 3). One interesting result, however, was the consistent splitting of the DG-morphs into two clades. In the analyses, the GO-morph reveals an isolated entity along with the following species level delineations:

- Populations with DL-morph (ENVL08, PAS) group together with 1.0 BPP in Bayesian and 1.0 relative Bremer and 100% bootstrap, in MP combined analyses and correspond to D. longipes.
- Populations with RQ-morph (RQ, 5, JBO59, JMC10) group together with 1.0 BPP in Bayesian and 1.0 relative Bremer and 100% bootstrap, in MP combined analyses and correspond to D. carpathicus.

Population with DG-morph split into two different clades, corresponding to at least two species. These four populations (ADhi, Hir1, HR, OC) group together with 1.0 BPP in Bayesian and 0.28 relative Bremer and 98% bootstrap, in MP combined analyses and correspond to D. gallinae. These fifteen populations (COL, LC, PI, CANIT, Chab, DR, Fa1, Fa2, JBO51-7, LB18, P02, ROL1, ROL2, SK, Woodp) group together with 0.60 BPP in Bayesian and 1.0 relative Bremer and 100% bootstrap, in MP combined analyses and correspond to D. gallinae.

Populations with GO-morph (GO1, GO54, MAR) group together with 1.0 BPP in Bayesian and 1.0 relative Bremer and 100% bootstrap in MP combined analyses and correspond to D. apodis n. sp. described below.

Moreover, observations on the distribution of pairwise divergences between populations in mitochondrial genes allowed detection of three main levels of hierarchy represented in the trees (Fig. 4), which partially corroborate above cladistic delineations. Separation between level 1 (20–25% in COI, 22–31% in 16S and 19–25% in ITS) corresponds to separation between the ingroup and outgroup and level 2 (8–18% in COI, 9–16% in 16S and 0–8% in ITS) to separation between species (RSE, see below). Level 3 (0–6% in COI and 0–7% in 16S) is internal within ingroup (i.e. concerns differences within species). In the nuclear gene, level 3 does not generate a conspicuous gap and part of levels 2 and 3 are overlapping each other (1–2% pairwise divergence).

On the other hand, non-hirundinis populations with DG-morph do not clearly group together with strong support values, except for some subsets of populations. Populations of clade F appear clearly grouped together in the three single gene analyses and separated from other D. gallinae populations in the ITS analysis (sister group and to D. gallinae and to the GO-morph group). Other D. gallinae, together with this subset, form a monophyletic clade with the GO-morph clade in ITS single gene analyses, but with very weak bootstrap support (Table 3). Moreover, the populations of clade E in Figs. 2 and 3 form a strongly supported clade in COI analysis, but group in a weakly supported clade and together with the population CANIT in ITS single gene analysis (Table 3).

3.5. A posteriori observation of some traditional characters

Observation of traditional descriptive characters from the retained cuticles of sequenced individuals confirmed the strong variability of most of these traits. Closer examination in reference to phylogeny does indicate that some subtle characters do exist that may be useful for species distinction (cf. diagnostic characters in the key for identification below §4.5). Here are results on a posteriori tested characters.

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<th>Table 3</th>
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<td></td>
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<tr>
<td>DG-morph</td>
<td>D. gallinae except lineage 1(L1)</td>
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<tr>
<td>DG-morph</td>
<td>D. hirundinis</td>
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<tr>
<td>RQ-morph</td>
<td>D. carpathicus</td>
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<tr>
<td>DG-morph</td>
<td>D. gallinae special lineage 1(L1)</td>
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<tr>
<td>GO-morph</td>
<td>D. apodis</td>
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<tr>
<td>DG-morph</td>
<td>(D. gallinarum; JBO51)+ clade E</td>
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<td>DG-morph</td>
<td>(D. hirundinis; D. longipes)</td>
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<td>DG-morph</td>
<td>D. gallinae</td>
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<td>DG+GO- morph</td>
<td>(D. gallinarum; D. apodis)</td>
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<td>Genus Dermanyssus</td>
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3.5.1. Dorsal shield chaetotaxy

The dorsal shield has very rough and irregular contours in many specimens, and j1 and s1 are sometimes situated on the shield and other times off the shield. Several specimens have been found with identical DNA sequences but variable position of such setae.

This is especially conspicuous in *D. gallinae* where 21% of sequenced individuals had j1 clearly off shield, 16% clearly on, 53% along the edge and 11% exhibited clear asymmetry (off on one side, on the other side). In the clade B, 67% of sequenced individuals had j1 along the edge of the shield and 33% with clear asymmetry.

In other species, the number of sequenced individuals is lesser, so fewer variations have been noted. However, below is an overview of noted variations:

- In *D. carpathicus*, 57% of sequenced individuals had j1 off and 43% with clear asymmetry.
- In populations GO-n and MAR, which slightly differ morphologically and strongly group together, 67% of sequenced individuals had j1 off shield and 33% with clear asymmetry.

In some species, such as, *D. hirundinis* and *D. longipes*, 100% of sequenced individuals had j1 off the shield without variation.

Moreover, variations in the total number of setae present on the dorsal shield have been noted in every species: 21–25 setae in *D. hirundinis*, 17–24 setae in *D. carpathicus*, 21–30 setae in *D. gallinae*, 20–25 setae in *D. longipes*, 25–29 setae in populations GO-n and MAR.

3.5.2. Leg chaetotaxy

Leg chaetotaxy is highly variable in parasitic mite species (Evans, 1963), and especially within *Dermanyssus* (cf. Evans and Till, 1962; Moss, 1968). Within *Dermanyssus*, we found intraspecific and intra-individual variation across many species. In order to determine if there are any evolutionary tendencies or patterns in leg chaetotaxy, we mapped these characters onto the molecular phylogenetic hypothesis. Because of high mobility of setae on the legs, traditionally annotated setae (on femur I v, in genu I and II d, v and pl and in genu IV d and pl) in symmetrical individuals, intraspecific variation was found to be very high. Variation in the number of setae in homologous area between individuals has been noted on femur I v in 18% of sequenced individuals, in 11% on femur I pl, in 19% on genu II and III v, in 6% on genu IV d and v. This variation is found not only between populations, but also within single populations. Clearly leg chaetotaxy is not a phylogenetically informative character and appears amazingly plastic during the development of *D. gallinae*.

In other sequenced species of the *gallinae*-group, asymmetries (number of setae differing from one side to the other) were noted in 50% or more for sequenced individuals of *D. hirundinis* (mainly ventral faces of femur I, genu I, II and IV, and al and dorsal face in genu II). Additionally, variation between individuals of *D. hirundinis* was found in 14% of sequenced individuals on genu III al and in 50% on genu II v, genu III d, and genu IV d.

3.5.3. Pronotal scutella

*Moss* (1966) identifies the pronotal scutella as the anterior portion of the dorsal shield not rounded, but with two somewhat acute and laterally pointed angles, suggestive of shoulders. He states that this condition is present in some species and absent in some others, indicating its utility in species identification. However, upon investigation, we found several cases where individuals found in the same aggregation exhibited body types with and without these shoulders or pronotal scutella. Additionally, when tested genetically with the three gene regions used in this study, all produced identical sequences. Moss observed that the “prominent shoulders” of *D. gallinae* were formed “by the fusion of the dorsal shield with the platelet” (*Moss, 1966*). These platelets in fact seem to be present in most species (K41), but can be fused or unfused to the dorsal shield within a single population of one species. Furthermore, many cases of bilateral asymmetry were discovered in this study, especially in *D. carpathicus* (25% of sequenced individuals) and in *D. gallinae* (9.5%).

4. Discussion

On the whole, the morphology-based analysis does not provide adequate information to determine species delineation nor phylogenetic relationships within the *gallinae*-group. Additionally, this analysis only brings some information about possible relationships within *Microdermanyssus* + *hirsutus*-group (cf. below). On the other hand, analyses including molecular data, either with MP or Bayesian, provide valuable information regarding delineation of some species and species’ relationships (cf. below). All converge on similar results, with more or less resolution. The least resolved topologies result from ITS single gene analyses (MP, Bayesian) indicating that most of the resolving power is found in the mitochondrial genes. Total evidence and combined molecular only datasets offer much more resolution in the *gallinae*-group than the morphological.

The present study took into account mitochondrial and nuclear genes. As it has previously been observed at similar levels (Moore, 1995; Springer et al., 2001; Shaw, 2002), our results suggest that the tested nuclear gene (ITS) has less resolving power than the tested mitochondrial genes in recovering relationships within *Dermanyssus*. Therefore, our species delineation and evaluation of relationships are mainly based on mitochondrial data. It is recognized that this could be misleading for inferring species phylogenies due to the haploid character of mitochondrial origin, however, single gene analyses do not produce results highly contradictory to the total evidence and combined analyses.

4.1. Species

While the combined dataset does provide some interesting information about the *gallinae*-group phylogeny, the main purpose of incorporating molecules that tend to sort out closely related species, or even distinct populations, was to determine if the *gallinae*-group actually constitute a number of morphologically similar species or if they represent one homogenous population of *D. gallinae*. This is important in terms of dealing with pest species of domestic birds, which to this point have been continuously identified as *D. gallinae*.

4.1.1. Distribution of multi-population a priori morphs

In order to reveal species boundaries within morphologically similar entities, it is important to include several geographically distant populations in phylogenetic analyses (*Monaghan et al., 2005*). In the present study, several geographically distant populations (from various places in France) from the four *a priori* morphs have been tested in combined analyses (three genes and morphology, three genes only) and in single gene analyses (Appendix D) of both mitochondrial and nuclear sequences (GO-morph: 10 populations; DG-morph: 45 populations; RQ-morph: 5 populations). Populations of GO-morph and RQ-morph are, respectively, grouped...
together in strongly supported clades (Table 3 and Figs. 2 and 3), whereas DG-morph is clearly separated in two distinct clades: populations HR, OC, CHOV, HIR1 and ADhirun in the clade L (Figs. 2 and 3), and the other DG-morphs (Table 1) in the clade B (Figs. 2 and 3).

4.1.2. D. carpathicus, D. hirustus and D. quintus, clearly characterized described species, as reference specific entities

Three species appeared clearly characterized early in the study: D. carpathicus (sharp pairwise divergence with other entities, very little divergence between geographically distant populations within the species, phylogenetically grouped together with strong support in all analyses (Table 3), slight morphological but clear divergence); D. hirustus (pairwise divergence with other entities, sharp morphological divergence); and D. quintus (pairwise divergence with other entities, sharp morphological divergence). Of course, respective monophilies of D. quintus and D. hirustus have not been tested, due to lack of additional populations, but their DNA sequences are very divergent from each other and other populations and morphological characterization is obvious. For all these reasons, these three specific entities will be used in the present study as references for species status and are referred to as reference specific entities (RSE) in order to have a comparison of the distribution of pairwise divergence percentages (Fig. 4). Pairwise divergences located in hierarchical level 2 in Fig. 4 correspond to interspecific divergence.

4.1.3. D. hirundinis and D. longipes

Populations of D. hirundinis also form distinct clades separate from other Dermanyssus displaying large genetic divergence and some diagnostic morphological characters have been noted in the a posteriori feedback described in Section 4.3. D. longipes is a sister to D. hirundinis populations in all analyses, and this group as a whole is distinct from other Dermanyssus. There is a slight exception in 16S single gene Bayesian analysis, where it appears sister to a clade including D. carpathicus and D. hirundinis. These results provide confidence that D. hirundinis is a unique species. Moreover, as tested populations of D. longipes not only group together in all topologies and are separate from D. hirundinis in 16S gene analysis, but also it is 11.2% (16S) and 9.4% (COI) different from D. hirundinisAD, it is apparent that it also represents a good species concept. Additionally, slight morphological differences between D. longipes and all other Dermanyssus species have been noted. The anal plate is slightly more elongated (relative measures) and more or less subrectangular and the dorsal shield has a subapical shrinkage and ends with a quite rounded apex. In most cases, monophyly of tested populations in phylogenetic analyses supported not only by this amount of genetic divergence, but also by a few morphological characters typically constitute unique species status. It would be interesting to include more than two populations of this species in the future in order to firmly fix the specific status.

4.1.4. Non-hirundinis populations with DG-morph

The results from the analysis of the combined dataset indicate that what had been identified by the authors as D. gallinae is not as clear and does in fact group into several different lineages. These populations do not group together in a strongly supported clade in single gene analyses either (Table 3). Moreover, pairwise divergence between them is in some cases in level 2 and in some others in level 1. Populations LB18, JB051 (clade E in Figs. 2 and 3) and LB07-4 resolve monophyly in single gene COI analysis (94% bootstrap), but not in ITS analysis, where LB18 and JB051 branch with population D. gallinae CANIT (Table 3). This incongruence between mitochondrial and nuclear genes suggests that, even if these entities are partially isolated from each other, there is some gene flow between them and D. gallinae populations. This implies that it has to be deemed belonging to D. gallinae. Additionally, ITS sequences in these populations are almost identical to that of other D. gallinae populations, except for one site (common to CANIT).

On the other hand, populations of the clade F (Figs. 2 and 3) appear morphologically identical to D. gallinae populations, but resolve monophyletic in all analyses. They are divergent from other D. gallinae in all analyses, and may represent a cryptic species. Thus single gene analyses resulted in similar topologies, and grouped these populations in strongly supported clades both in mitochondrial and nuclear genes (Table 3), with identical ITS sequences between each other and differing by 2% from D. gallinae populations. There is likely no gene flow between populations COL, LC, PI, G08 and JGC1 (from various environments, with geographical distances between them from about 100 to 300 km) and other tested populations of D. gallinae, but since there are no clear diagnostic morphological characters, these populations may at best represent recent speciation or cryptic species. As Heethoff et al. (2007) concluded when studying potentially cryptic species of the orbibatid mite Platynothrus peltifer Koch, 1839, we have decided to make no decision regarding species status until more biological information is obtained. Thus, as recommended by DeSalle et al. (2005), it is necessary to get corroboration for more than one line of evidence for delineation of a new taxon. Here, DNA is the only line of evidence. In the case of a cryptic species, as no morphological clues are available (even subtle as in some species of Tectocepheus in Laumann et al., 2007, some geographical or ecological data is necessary to corroborate the DNA evidence. Future studies will try and obtain additional samples from pigeons of various geographical origins and various types of environment (breeding facilities, urban nests), as an ecological common trait seems to be the host group (cf. below). In present paper, this entity will be referred to as D. gallinae special lineage one.

4.1.5. Does population Woodp belong to D. gallinoides?

Moss (1966) described D. gallinoides as follows: (1) no prominent shoulders (anterior part of the dorsal shield not rounded, but with two somewhat acute and laterally pointed angles, suggestive of shoulders), (2) small platelet on the soft integument on each side of the dorsal shield, (3) dorsal shield scaling smooth, (4) j1 and s1 off the dorsal shield (D. gallinae: j1 always and s1 generally on the dorsal shield), (5) epigynial pores off the shield, (6) tibia IV pl with 2 setae (instead of one in D. gallinae), (7) genu II pl, III al and IV al with 2 setae (opposed to only one seta in D. prognephilus), (8) peritreme extending only to the middle or anterior margin of coxa II rather than to the middle of coxa I (different from D. prognephilus). Upon examination of numerous populations of D. gallinae and now recognizing the great amounts of variability in many of these characters, the sixth argument appears to be the only valid one. In terms of host associations, D. gallinoides, has been found on several different species of Picidae, a group not normally associated with D. gallinae. We collected one specimen originally identified as D. gallinae (labelled Woodp population) from an adult female Dendrocopos major (Picidae) that exhibits several characteristics resembling D. gallinoides.

The shape of dorsal shield fits arguments one and two depending on the observed side (bilateral asymmetry) and position of j1 is asymmetric (one side on, the other side off shield, 4th argument). Of course, depending on which side of the body you look at, these character states either direct you to D. gallinoides or D. gallinae. Tibia IV pl only has one seta, like in D. gallinae, but femur I pl only possesses one seta (instead of two in all other D. gallinae). Genetically, population Woodp falls within the other D. gallinae specimens indicating even more variation within that species, and if this does in fact represent what has been called D. gallinoides, it suggests that this should be synonymised with D. gallinae. However, many more specimens would be necessary to make this decision.
4.1.6. D. apodis n. sp

Strongly supported monophyly of populations GOn and MAR along with several subtle morphological characters separate these populations from other species of the galliniae-group. These populations constitute an entity which appears to be of specific status and which we describe here under the name *D. apodis*.

Female (holotype) (Fig. 6A–B):

Gnathosoma. Length of setae: anterior pair of hypostomal setae 37 μm long (range with 5 paratypes 31–39 μm), central pair 57 μm long (47–65 μm), lateral pair 26 μm long (24–31 μm), posterior pair 26 μm (26–28 μm). Capitulum 96 μm (96–137 μm) long (from its basis to apex of palp coxae), 151 μm (143–166 μm) wide basally and 117 μm (104–130 μm) wide distally, (i.e. between lateral margins of palp coxae’ apex).

Setae *al1* of palp genu lanceolate. Anterior hypostomal setae pair wider than other gnathosomal setae.

Idiosoma. 840 μm (735–1050 μm) long and 494 μm (420–693 μm) wide. Dorsum: dorsal shield length 714 μm (646–

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Fig. 6. *D. apodis* n. sp. (A) Venter of an adult female (holotype). (B) Dorsal shield of an adult female (holotype). (C) Venter of an adult male. (D) Dorsal shield of an adult male.
798 μm), width 286 μm (262–291 μm) at midlevel. Its anterior margin with a concave slit between the two anterior pores. These pores are often anteriorly located and separated from the shield. (Fig. 6B). Relative length of dorsal shield more than twice the podosoma area bounded by coxae (367 μm, 4 par. 346–451 μm) in length. Relative width of dorsal shield almost as wide as the podosoma area bounded by coxae, with lateral margin running across each coxa. Shape of ultrastructural network on dorsal shield slightly differing on anterior part and on middle and posterior part: grooves delimiting rather short areas (about as wide as long) in anterior part and longer areas around the middle of dorsal shield, which seem to converge toward posterior part via longitudinal axis. No major difference of length between central/peripheral setae of dorsal shield (series j4–6 and z5/j2, z2, z4 and s4). Prontal scutella present, separated from dorsal shield. Venter (Fig. 6A). Sternal shield 18 μm (18–29 μm) long and 148 μm (122–171 μm) wide. Genito-ventral shield 254 μm (234–260 μm) long and 140 μm (119–140 μm) wide at midlevel. Oviporal flap 130 μm (109–169 μm) long. Anal shield 153 μm (148–174 μm) long and 148 μm (137–156 μm) wide, with anterior margin’s outline very irregular. Post-stigmatic trachea (which extends posteri- orly from stigmata): principal pore large (ca. 3× setae base), a hole surrounded by a large raised chitinous ring, forming something like a neck.

Legs. Tibia I 99 μm long (94–101 μm) and 60 μm wide (52–65 μm). Tibia II 78 μm (73–75 μm) long and 52 μm wide (48–60 μm). Genu I 99 μm long (94–99 μm) and 73 μm wide (62–75 μm). Genu II 80 μm long (75–78 μm) and 68 μm wide (57–70 μm). Chaetotaxy of legs: Genu I 2–5/3–2; Genu II 2–4/2–2; Genu III 2–4/1–1. Variations in paratypes (6 examined): Genu I: one paratype 2–3/3–2 and two with a bilateral asymmetry on ventral face. Genu II: two paratypes with a bilateral asymmetry on ventral face. Genu III: one paratype with a bilateral asymmetry on ventral face, two not determined (legs III lost). Genu IV: one paratype 1–4/1–0, another with a bilateral asymmetry on ventral face.

Nucleic acids. Several amplicons from the three tested genes have been sequenced for different populations of D. apodis (accession numbers in Table 1: holotype belongs to population GO54 and paratype females to populations GO1, GO15, GO16, GO46, GO59, MAR). All obtained ITS and 16S sequences were exactly the same. Only two bases were different between sequences of COI obtained from the holotype population (holotype individuals and another individual found from the same swift nest) and the paratype popu- lation MAR (0.3% divergence).

Male (paratype) (Fig. 6C and D)

Gnathosoma. Length of setae: anterior pair of hypostomal setae 26 μm long (18 μm in a second male paratype), central pair 47 μm long (31 μm), lateral pair 33 μm long (not visible in second para- type), posterior pair 14 μm (13 μm). Capitulum 75 μm (75 μm) long (from its basis to apex of palp coxae), 127 μm (98 μm) wide basally and 109 μm (91 μm) wide distally, (i.e. between lateral margins of palp coxae) apical). Spermatodactyl 96 μm long (from its basis to apex of palp coxae’ apex). Spermatodactyl 96 μm long. Post-stigmatic trachea like a neck.

Remarks. Populations of D. apodis constitute a distal clade with strong bootstrap values in all combined analyses and in mitochondrial single gene analyses (Table 3, Figs. 2, 3 and 5, Appendix D). Moreover, branch lengths are much more important between this entity and others than within the cluster (Monaghan et al., 2005), which highlights the unique status of this particular hierarch- ily in the tree (idem in D. carpaticus and D. hirundinis).

Among the 24 species of Dermapyrus described so far, only three species have been recorded from some Apodiformes: D. hirundinis (on swifts, family Apodidae), D. rwanda (on swifts, family Apodidae) and D. trochilinis (on hummingbirds, family Trochilidae).

Closely related to D. gallinae (and especially the subset of popu- lations COL, JGC1, PL, LC), D. apodis morphologically differs from it mainly by the concave slit between the two anterior pores of dorsal shield (continuously rounded convex margin in D. gallinae). Moreover, the prontal scutella are not fused to dorsal shield (usu- ally fused in D. gallinae) and the anterior pair of hypostomal setae is slightly wider than in D. gallinae. It also clearly differs from D. hirundinis by the concave slit between the two anterior pores of dorsal shield (continuously rounded convex margin in D. hirundinis) and by some elements of leg chaetotaxy (pl of genu II and III with 2 setae in D. apodis, 1 in D. hirundinis). It also clearly differs from D. trochilinis by the concave slit between the two anterior pores of dorsal shield (continuously rounded convex margin in D. trochilinis), by the relative width of setae of anterior hypostomal pair (about as wide as other hypostomal setae) and by the absence of prontal scutella.

Etymology. The species name is derived from the specific name of host and is the genitive form of the word.

Material examined.

– Individuals ex nests and adult birds of A. apus (Apodiformes: Apodidae), Nîmes, France (Gard), June–July 2007:

Holotype female (one individual of population GO54, (n° MNHN Ac1111a, cf. Table 1). Seven paratype adult females from following populations: GO1 (n° MNHN Ac1112), GO15 (n° MNHN Ac1113b), GO46 (n° MNHN Ac1116a and n° MNHN Ac1111b), GO54 (n° MNHN Ac1111b), GO59 (n° MNHN Ac1117a and n° MNHN Ac1111b); 2 paratype deutonymphs: GO15 (n° MNHN Ac1131a), GO16 (n° MNHN Ac1131c); 2 paratype adult males: GO44 (n° MNHN Ac1115), GO59 (n° MNHN Ac117c).

Nest samples from which these mites have been isolated and samples directly caught from birds in this locality have kindly been provided by G. Gory (Muséum d’Histoire naturelle de Nîmes and Centre de Recherche sur la Biologie et les Populations d’Oiseaux).

– Individuals ex a young individual of A. apus, Francheville, France (Rhône), July 17th, 2007:

3 paratype adult females from population MAR (n° MNHN Ac1114a, n° MNHN Ac1114b, n° MNHN Ac1114c).

G. Lallemand sampled these mites during care activity in the Centre de Soins aux Oiseaux Sauvages du Lyonnais.

The holotype and paratype series are deposited in the Museum National d’Histoire Naturelle, Paris, France.

4.2. Different rates of evolution

The amount of genetic differentiation within and between spe- cies varied depending on the gene, however, we did not find any evidence of intrapopulation variation among any of the gene re- gions sampled. The nuclear marker (ITS region) provided few but sharp variations between each species, with the majority of varia- tion found in ITS1 and 5.8S and almost no difference in ITS2. This is contrary to findings concerning most other mites that have been sampled (Navajas and Fenton, 2000; Cruickshank, 2002), but sim- ilar to patterns found in Tetranychus species (Navajas et al.,
1998). Resulting Dermanyssus sequences were easy to align and displayed several differences useful for molecular identification at the species level. Differences clearly characterize our respective populations of D. carpathicus, D. hirundinis, D. longipes, D. hirsutus, D. quintus, D. apodis and clearly separate these species from each other. Anyway, some few differences also separate some of the D. gallinae (especially the special lineage one L1) and D. longipes populations.

The mitochondrial markers (COI and 16S rRNA) show much higher levels of genetic differentiation between species than ITS (hierarchical level 2; Fig. 4), and exhibit small amounts of change between populations of a given specific entity (hierarchical level 1 of pairwise divergence in 16S and COI, Fig. 4). Additionally, some populations with identical ITS sequences exhibit several differences in their 16S sequences (Fig. 4). COI is by far the most variable of the three genes tested and provides many changes between populations. Pairwise divergence percentages between several populations of D. gallinae collected from European layer houses show that this marker is likely appropriate for phylogeographic investigation concerning economically important species. Gene sequences of COI revealed it to be a pertinent marker for phylogeographic exploration at a low taxonomic level (between closely related species or even populations of the same species) and although it is a protein coding gene, pairwise divergence appears often sufficient, if not excessive in some organisms (DeSalle et al., 2005), for obtaining valuable phylogeographic information, even in some parthenogenetic species (Heethoff et al., 2007).

4.3. Feedback on primary hypothesis: a diagnostic key for identification of the gallinae-group species from France

As stated previously, many characters traditionally used for species identification have shown to be highly variable even within single individuals of the various species of the gallinae-group. To date, there are no clear elements available for determining whether these differences are real phenotypic plasticity or only pure variations, because the impact of environmental influence on these variations is very difficult to estimate. Thus, it appears that some confusion may have occurred in some of the previous records likely due to these variations. A very recent example is found in Brännström et al. (2008), who found differences in ITS1 between some D. gallinae from layer farms and some D. gallinae from wild avifauna. Conspicuously, the ITS1 sequence found from wild birds corresponds to our D. longipes.

Most of these characters are no longer useful for distinguishing species, however, in light of the phylogenetic results and closer examination of characters, the following key has been generated for use in discriminating several species of the gallinae-group. Thus, among leg chaetotactic characters, it turned out that lateral sides of some leg articles have very few intraspecific variations, in contrast to most of other sides.

1. al1 seta of palp genu spine-like, two setae on femur I d longer than the other three, pl of genu II and III usually each with one seta, pronotal scutella fused or not to dorsal shield (often asymmetric arrangement), anal plate D-shaped, anterior part of dorsal shield more or less convex. .......................... D. carpathicus

2. al1 seta of palp genu lanceolate, setae on femur I d without any conspicuous difference of length, other characters diversе. ... 2

2. pl of genu II and III usually each with one seta, anal plate D-shaped or elongate and more or less subrectangular, pronotal scutella usually unfused to dorsal shield and far from it, anterior part of dorsal shield more or less convex. .......................... 3

3. Anal plate D-shaped, dorsal shield without any conspicuous subapical shrinkage............................. D. hirundinis

4. Pronotal scutella usually separated from dorsal shield and far from it, anterior part of dorsal shield more or less concave, forming a neckline between the two anterior pores, anterior pores usually far from dorsal shield. .......................... D. longipes

4. Pronotal scutella in most cases touching dorsal shield, anterior part of dorsal shield more or less convex. .......................... D. hirundinis, including the special lineage one.

4.4. Phylogenetic relationships between species

Within Dermanyssidae, Liponyssoides possesses similar cheliceral and cornicular characters, but has a hexagonal-shaped sternal shield. Most species of Liponyssoides are also found on mammals instead of birds and it is unknown whether they are a sister group to Dermanyssus or originate from within the genus. Unfortunately, no specimens were available for inclusion in this study.

The results of the morphological analysis indicate that only two internal nodes provide a strong relative Bremer support (1.00). First, D. trochilinus is sister to the rest of tested Dermanyssus species. Secondly, the group Microdermanyssus + hirsutus-group appears monophyletic. Within the group Microdermanyssus + hirsutus-group, the relationships of the three species D. quintus, D. hirsutus and D. grochovskiae, which correspond to Moss’ (1968) hirsutus-group in the subgenus Dermanyssus, are unresolved. Three of the four remaining species correspond to Moss’ Microdermanyssus and D. rwandae is sister to these three. This species is one of the more recently described species, and has never been included in a generic review of the group. Present results suggest it may be a member of the subgenus Microdermanyssus. D. carpathicus, also described after Moss’ last review, is resolved as a sister group to the Microdermanyssus + hirsutus-group clade, but with rather weak support values. Our current morphological results coincide with Moss’ idealized phylogeny with the exception of the hirsutus-group placement, which clearly falls out within Moss’ Microdermanyssus. The subgenus Dermanyssus appears to be paraphyletic.

The total evidence and combined molecular only datasets offer much more resolution in the gallinae-group than the morphological analysis. These species appear more differentiated than expected on the single basis of morphology. Whereas the morphology-only topology results in a comb of gallinae-group species, with only Microdermanyssus + hirsutus-group forming a supported clade based on relative Bremer support, the combined tree shows a gallinae-group split into two different clades, one of them involving D. carpathicus, D. longipes and D. hirundinis (clade I), the second one a complex of D. gallinae lineages (clade B). The position of D. apodis is unresolved in the MP analyses, but is sister to the hirsutus-group + D. carpathicus + D. longipes + D. hirundinis (clade G) in the Bayesian analysis. As for the only two tested species of hirsutus-group, their mutual position is not strongly supported in these analyses. According to Bayesian analysis, they would be considered close to the clade I. This would be rather congruent with morphological topology, with D. carpathicus in a sister position to the hirsutus-group. But this is not supported by MP analysis.

4.5. Host specificity

Molecular results strongly confirm very low host specificity among D. gallinae, which has been previously suggested (Zemskaya, 1971; Nosek and Lichard, 1962; Zeman and Jurík, 1981; Fendá and Schniererová, 2004). Populations of this species were
collected in this study from eight different orders of birds (Passeriformes, Coraciiformes, Piciformes, Galliformes, Ciconiiformes, Columbiformes, Apodiformes, Strigiformes), including domestic and wild birds. This leads to the conclusion that parasite transfer between wild bird fauna and domestic fowl is not out of the question. Additionally, within it, the special lineage one appears also rather unspecific, having also been collected from three different orders of birds (Columbiformes, Apodiformes, Strigiformes, Table 1).

On the other hand, many species appear more host specific, at least in France, such as D. hirundinis (found only on Hirundinidae) and D. carpaticus (found only in nests of two genera, Passer and Phoenicurus) and D. longipes (found in nests of two genera, Passer and Passer). Additionally, D. apodis was collected many times from two different places in France (ca 300 km apart), from more than 50 nests of A. apus and numerous individuals caught for banding. This is the only host species it is known from for the moment.

Some of these results contradict the published literature. D. hirundinis has been recorded from roughly 40 different bird species, in 9 bird orders (for review, Roy and Chauve, 2007): Passeriformes (19 previously recorded genera), Anseriformes, Apodiformes, Sturniformes, Strigiformes, Coraciiformes, Ciconiiformes, Columbiformes, Piciformes. In the present study, representatives from six of the above bird orders have been tested, but individuals belonging to D. hirundinis have been found in only three species of Hirundinidae (Passeriformes: Delichon urbica and Hirundo rustica in France, and Tachycineta bicolor in the USA), which is the type host family. Moreover, it was present in 25% of analyzed hirundinid nests in France. This suggests that D. hirundinis is more specific in France than expected from published data. As no faunistic inventory of Dermanyssus species in France was available today, it is likely that “dermanyssofauna” (and host specificity) differs in Europe and in the USA.

4.6. Evolutionary hypothesis for tested species of Dermanyssus

Tested species of Dermanyssus split into two clades in the total evidence (B and G in Figs. 2B and 3B) and molecular—only analyses. No clear evolutionary hypothesis can be drawn on the basis of morphology from this topology. Thus, several characters change their state at the basis of the clade grouping tested species of Dermanyssus, but all revere in one clade or another more distally (K1, K17, K26, K27, K40, K43). These homoplasies obscure a clear view of morphological evolution. If we consider some ecological traits, some clues can be found.

No strict comparison with outgroups can be done, due to the different ways of life: A. casalis and T. pyri are predatory mites (and even A. casalis is not able to be occasionally parasitic; Lesna and Sabelis, pers. comm.) Only O. bacoti is a parasite, but is from an unrelated family of mites.

All strongly supported lineages here involve species found on a narrow host spectrum, except for the clade of D. gallinae. Even within this clade, an evolution of host spectrum seems to be visible, although host spectrum is not to be considered phylogenetically. Indeed, observation of obtained topologies in correlation with our bird data did not allow considering that there are any coevolution events (A. Cibois, pers. comm.). This suggests that this genus includes species with opportunistic habits. But phylogeny can be correlated to some ecological traits, difficult to clearly define, but inducing variations in adaptability to environment (farms vs natura, transferability from one to another bird species, etc.). D. hirundinis has been found in three species of Hirundinidae (D. urbica, H. rustica, T. bicolor). D. carpaticus was found in two different passeriform genera distributed in two different bird families (Phoenicurus and Passer, respectively, Muscicapidae and Paridae), as is the distribution found in the literature (Zeman, 1979). D. longipes has been found in two genera of Passeriformes distributed in two different bird families as well (Passer and Parus, respectively Passeridae and Paridae), the first being the genus of the type host. D. apodis has been found only on one species A. apus. All these species have been found only in wild avifauna.

As for lineages in the D. gallinae clade (B), not only have several of them been found in “human managed environment” (cf. Fig. 3B), but also several lineages group populations from disparate bird species (cf. above). Especially, the clade D that groups together populations from layer farms, canary breeding facilities, wild European Roller, and a Woodpecker with strong support values (internal clades not supported). Moreover, the clade E includes populations found only in wild avifauna (D. urbica, Hirundinidae and P. major, Paridae).

Finally, special lineage one, the more basal lineage of D. gallinae, has been found in pigeon breeding facilities, also in pigeon nests in town, but never in layer farms. This lineage does not appear absolutely specific, as it has been isolated from two other bird groups, in natura (a owl and a swift; cf. Table 1). But in these two cases, mites were not necessary infesting the inhabitant of nest (a single mite in each case, isolated dead and dried from the two nests). Pigeon are known to be concurrent with swift concerning nesting place, especially into a town, which could explain the presence of the single specimen GO8.

In short, host specificity may appear higher at the basis of the gallinae clade, with special lineage one (clade F) mainly on pigeons, with the intermediate clade E only found in wild passeriforms, and finally with remaining distal populations isolated from disparate bird groups. And synanthropicity appears to be proper to this clade B, the second one, clade G in Fig. 3B, being only found in wild avifauna. Intermediate ecological characteristics can maybe be seen in D. apodis and in some cases D. gallinae special lineage one, both having urban hosts (swift and pigeons).

4.7. Conclusion

The morphology-based phylogenetic hypothesis presented herein involves 20 of the 24 currently recognized species and a new species. The monophyly of Dermanyssus could not be tested due to the lack of Liponyssoides specimens and no assessment of relationships between the Microdermanyssus + hirsutus-group and the gallinae-group has been possible. Anyway relationships of species of the gallinae-group within the genus are robustly examined. These results suggest that Microdermanyssus + hirsutus-group contains species which are clearly distinguishable from one another solely on the basis of morphology, whereas the remaining species (gallinae-group) are sometimes indistinguishable from one another. Molecular data obtained from several populations of the gallinae-group indicates that many of these indiscernible species are clearly distinct species: D. gallinae and D. hirundinis are molecularly clearly divergent species, and D. gallinae contains several lineages. D. carpaticus is also a valid species, it is present in France and, by mapping morphological characters onto the molecular phylogeny, it appears there are two diagnostic characters (K4 and K17) for the species (Fig. 3A). A special lineage constituted by the subset of populations COL, JGC1, PI, LC may be a cryptic species closely related to D. gallinae. D. apodis is a new species to be linked to the gallinae-group, found on the black swift A. apus.

Based upon the way the tested populations of the gallinae-group sorted out, host specificity of D. gallinae appears very low, found on domestic birds as well as several orders of wild birds. On the other hand, D. hirundinis and D. carpaticus appear much more host specific, only found on a restricted set of hosts, at least in France. D. apodis has been found very often on A. apus, and only on this species in France. The fauna of Dermanyssus seems to strongly differ between USA and Europe.
Finally, two different clades were revealed within tested species of the *gallinae*-group, one of which seems to develop synanthropicity and proliferative capacity, with most derived OTUs present in hen farms. This also seems to be correlated with an adaptation to a wider host spectrum, populations with similar sequences being found on various bird orders in distal position in the *gallinae* clade on molecular tree.

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**Appendix A**

*List of taxa examined for morphological analysis. Note: Indicated individuals correspond to specimens considered here as references. Other specimens of the same species may have been examined. T = type material; NT = non-type material.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Loan from</th>
<th>Specimens' status</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dermanyssus alaudae</em></td>
<td>British museum of natural history (London, UK; neotype) and Belgian royal institute of natural sciences (Brussel, Belgium; NT, A. Fain’s collection)</td>
<td>T + NT</td>
</tr>
<tr>
<td><em>Dermanyssus americanus</em></td>
<td>British museum of natural history (London, UK; NT) and National museum of natural history (Washington, DC, USA)</td>
<td>T + NT</td>
</tr>
<tr>
<td><em>Dermanyssus antillarum</em></td>
<td>Institute of parasitology (Ceske Budejovice, Czech Republic)</td>
<td>T</td>
</tr>
<tr>
<td><em>Dermanyssus brevis</em></td>
<td>National museum of natural history (Washington, DC, USA)</td>
<td>T</td>
</tr>
<tr>
<td><em>Dermanyssus carpathicus</em></td>
<td>Institute of parasitology (Ceske Budejovice, Czech Republic)and P. Zeman (Czech Republic), specimens from several field collections of <em>Parus major</em> and <em>Phoenicurus phoenicurus</em> in France</td>
<td>T + NT</td>
</tr>
<tr>
<td><em>Dermanyssus chelidonis</em></td>
<td>British museum of natural history (London, UK;), and Agriculture and Agri-food Canada (Ottawa, Canada)</td>
<td>NT</td>
</tr>
<tr>
<td><em>Dermanyssus faralloni</em></td>
<td>National museum of natural history (Washington, DC, USA)</td>
<td>T</td>
</tr>
<tr>
<td><em>Dermanyssus gallinae</em></td>
<td>British museum of natural history (London, UK; neotype) — Museum Koenig (Bonn; Germany; NT)—Muséum National d’Histoire naturelle (Paris, France; NT)—field samples</td>
<td>T + NT</td>
</tr>
<tr>
<td><em>Dermanyssus gallinoides</em></td>
<td>Severtsov institute of ecology and evolution, Russian academy of sciences, (Moscow, Russia; NT, but specimens identified by A. Zemskaya herself)</td>
<td>T</td>
</tr>
<tr>
<td><em>Dermanyssus grochovskae</em></td>
<td>National museum of natural history (Washington, DC, USA; T)</td>
<td>T</td>
</tr>
<tr>
<td><em>Dermanyssus hirsutus</em></td>
<td>British museum of natural history (Bonn, Germany;neotype), Koenig museum (London, UK;NT), Belgian royal institute of natural sciences (Brussel, Belgium;NT, Fain’s collection), National museum of natural history Naturalis. (Leiden, The Netherlands; coll. Oudemans, NT, nr P-4632)</td>
<td>T + NT</td>
</tr>
<tr>
<td><em>Dermanyssus nipponensis</em></td>
<td>National science museum of Tokyo (Tokyo, Japan; TNSMT-Ac 12495)</td>
<td>T</td>
</tr>
<tr>
<td><em>Dermanyssus progrophilus</em></td>
<td>British museum of natural history (London, UK;NT). National museum of natural history (Washington, D.C., USA) (T, AL000244) and Ohio State University (Columbus, USA;NT)</td>
<td>T + NT</td>
</tr>
</tbody>
</table>
Appendix A (continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Loan from</th>
<th>Specimens' status</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dermanyssus quintus</em> Vitzthum, 1921</td>
<td>Agriculture and agri-food Canada (Ottawa, Canada; T), Museum of Zoology, University of Michigan (Ann Arbor, USA; NT), W. Knee (NI),</td>
<td>NT</td>
</tr>
<tr>
<td><em>Dermanyssus rwandae</em> Fain, 1993</td>
<td>Belgian royal institute of natural sciences (Brussel, Belgium; T)</td>
<td>T</td>
</tr>
<tr>
<td><em>Dermanyssus transvaalenisis</em> Evans and Till, 1962</td>
<td>British museum of natural history (London, Uk; T)</td>
<td>T</td>
</tr>
<tr>
<td><em>Dermanyssus triscutatus</em> Krantz, 1959</td>
<td>National museum of natural history (Washington, D.C., USA; T) and agriculture and agri-food Canada (Ottawa, Canada, NT)</td>
<td>NT</td>
</tr>
<tr>
<td><em>Dermanyssus trochilinis</em> Moss, 1978</td>
<td>National museum of natural history (Washington, D.C., USA; T)</td>
<td>T</td>
</tr>
<tr>
<td><em>Dermanyssus longipes</em> (Berlese and Trouessart, 1889)</td>
<td>Slovak national museum in Bratislava (Bratislava, Slovakia; 2 slides labelled <em>D. passerinus</em>, from specimens collected from prof. Milan Mrčiak from <em>Passer domesticus</em> (NT) and specimens collected from a nest of <em>Passer montanus</em> near Avignon (France; NT)</td>
<td>NT</td>
</tr>
<tr>
<td><em>Dermanyssus apodis</em> n. sp.</td>
<td>Specimens from several field collections from <em>A. apus</em> in France by G. Gory and G. Lallemand (T+NT)</td>
<td>NT</td>
</tr>
<tr>
<td><em>Haemogamasus hirsutus</em> Berlese, 1889</td>
<td>MNHN (3D7, 3E1-3E4, 3E6, 3E10)</td>
<td>NT</td>
</tr>
<tr>
<td><em>Ornithonyssus bacoti</em> (Hirst, 1913)</td>
<td>Specimens from a live lab strain in MNHN (O. Bain)</td>
<td>NT</td>
</tr>
<tr>
<td><em>Androlaelaps casalis</em> (Berlese, 1887)</td>
<td>Specimens from several field collections in France</td>
<td>NT</td>
</tr>
<tr>
<td><em>Typhlodromus pyri</em> Scheuten, 1857</td>
<td>Specimens from mite culture in the lab of S. Kreiter (Supagro, Montpellier)</td>
<td></td>
</tr>
</tbody>
</table>

Appendix B

List of morphological characters and states used in the analysis. The main source of the following characters was either direct observations or the following publications: Moss (1966, 1968, 1978) and Evans and Till (1962).

1. Lateral contours of palp coxae in ventral view-0-Straight-1-Convex.
2. Sternal shield shape: relative location of points c and d, with c medially located on anterior margin of sternal shield and d laterally located on anterior margin of sternal shield-0-at the same level as d (anterior margin rather straight)-1-c located above the line between both points d (anterior margin quite curved).
3. Sternal shield shape: ratio e/a with e = width at the largest point and a = central height -0-e/a>3-1-e/a=1≤2.
4. Shape of seta al1 of palp genu-0-Rounded, quite sclerotized-1-Wider than long, with lateral angles more rounded, D-shaped.
5. Shape of post-stigmatic trachea (a tube extending posteriorly from stigmata)-0-One curved tube, around coxa IV-1-in two separate pieces.
6. Principal pore on post-stigmatic trachea (a pore located on post-stigmatic trachea, which extends posteriorly from stigmata)-0-absent-1-present, large (ca. 3× setae base), a hole usually surrounded by a large raised chitinous ring (something like a neck)-2-present, small (diameter smaller than setae bases) and simple (without any neck).
7. Intermediate pore on post-stigmatic trachea (a small pore located between principal pore and stigmata, close to stigmata)-0-absent-1-present.
8. Ultrastructure of dorsal shield-0-grooves absent-1-grooves present.
9. Relative length of peritreme-0-<2×diameter of stigmate-1-4×diameter of stigmate.

The traditional character (peritreme length in relation with the coxa it reaches) is a character which appeared to us not to be reliable in any case as such, because of its own nature (see below K9 in K definitions). It is something soft, and superficial. It forms a narrow groove inserted in the integument along the podosoma. The position and length of this element vary from one to another mite from the same strain and traditionally used character states constitute a continuum, which suggest this is not valuable species specific character within *Dermanyssus*. Anyway, there exists very short peritremes in some species (type specimens), which appear really different than others (*D. chelidonis, D. alaudae, D. rwandae...*), being almost atrophied. Between this state of characters and all others, a gap is visible. That is the reason why we encoded it differently, with only two characters states (short, ie less than twice the diameter of stigmate and long, ie more than 4 times the diameter of stigmate).

10. Humeral paired simple pores as large as setae bases, on dorsal shield-0-absent-1-present.
11. Humeral paired large pores, about 4 times larger than setae bases and containing a central conical prominence (on or off dorsal shield)-0-absent-1-present.
12. Apico-opisthosomal setae width-0-similar with shape and width to other setae-1-much wider and more massive.
14. Vento-opisthosomal setae, located on areas lateral to anal shield-0-classical number-1-neotrichy.
15. Dorsal setae: comparison between central/peripheral setae of dorsal shield (series j4-6 and z5/j2, z2, z4 and s4)-0-no major difference-1-length of central setae less than 1/3 length of peripheral setae.
16. Relative length of setae on dorsal side of genu-0-all quite the same length-1-One apical and one basal setae much longer than others (> length of genu).
17. Relative length of setae on dorsal side of femur-0-all quite the same length-1-Two apical setae much longer than others (> length of genu).
18. Mesonotal scutella (are considered here only sclerotized areas which detour more than 3 grooves of soft integument)-0-absent-1-present.
19. Ampulla near internal margin of coxa IV-0-fuzzy outlines and sharp apex (as if it was some crumpled membrane)-1-roughly rounded, quite sclerotized.
20. Ultrastructure of leg segments’ cuticle-0-smooth-1-embossed with large circles (about 2-3 on each side of segments longitudinal).
21. Third seta on anal shield-0-absent-1-present.
22. Proportions of anal shield-0-as wide as long, apically rounded, D-shaped-1-wider than long, with lateral angles more...
or less prominent-2-D-shaped, close to state 0, but longer than wide and apically subrectangular.

23. Central longitudinal pillar within anal opening-0-present-1-absent.


25. Relative location of the largest part of anal shield-0-anterior-1-central.


27. Proportions of genu II-0-longer than wide-1-wider than large-2-as long as wide.

28. Morphometric comparison of oviporal flap (OF) and epigynial shield (ES): ratio length of OF/total length OF + ES-0-≤ 1/3-1-1-ca. = ½.

29. pv seta of palp trochanter-0-present, as narrow as next setae-1-present, massive compared to next setae (large base, appears full)-2-absent.

30. al seta of trochanter I-0-present, as narrow as next setae-1-present, massive compared to next setae (large base, appears full)-2-absent.

31. Anterior pair of setae within hypostomal parallelogram-0-empty-1-filled with a clear substance.

32. Chelae-0-Mobile digit clearly distinguishable with an optical microscope-1-Mobile digit reduced, undistinguishable with an optical microscope.

33. Shape of 2nd cheliceral segment section-0-as wide as or wider than palp genu-1-narrower than palp genu.

34. J3-0-absent-1-present.

35. J3 and J4-0-off shield-1-J3 and J4 on shield-2-J3 on and J4 off or on limit.

36. Dorsal shield-0-Rounded, apical contours fuzzy-1-Posteriorly subtruncate, with two rounded “angles”.

37. Relative length of dorsal shield according to podosoma-0-same length-1-Dorsal shield much longer than podosoma (extending posteriorly, far behind coxae IV).

38. Relative width of dorsal shield according to podosoma-0-same width-1-Dorsal shield less wide than podosoma (lateral margin of dorsal shield not running across each coxa).


40. Proportions of genu I-0-longer than wide-1-wider than large-2-as long as wide.

41. Pronotal scutella (sticky or not to dorsal shield, usually raspberry-shaped with 3-4 “berries”)-0-present-1-Pronotal scutella absent.

42. Shape of stigmata-0-roughly rounded-1-dewdrop shaped.

43. Relative width of anterior setae in hypostomal parallelogram according to al setae of palp femur-0-base of anterior setae (basal part of seta, not the pit receiving base of seta) quite as wide as base of palp setae-1-3× or more.

44. Outlines and shape of epigynial shield-0-Irregular contours, with a rather tapering apex-1-Sharp contours, with a rounded apex, following a rounded narrowing.

45. Ampoula near internal margin of coxa IV-0-Conspicuously visible, with a narrowing at the base-1-Non clearly discernable or simply a filiform element, such as a slight extension of poststigmatic trachea.


Appendix C

Matrix of 24 taxa and 46 morphological characters used in the analysis.
Appendix D

Single gene analyses (COI, 16S, ITS) using MP and Bayesian analyses.

COI MP

COI BA
Appendix E

Results of the molecular “all-taxa” matrix, involving all tested taxa, including those with only one gene sequenced. Bayesian analysis from 5,000,000 generations using partitioned data and independent models of evolution for each partition. Numbers on nodes refer to Bayesian posterior probabilities.

References


Dermanyssus


