Acarologia

A quarterly journal of acarology, since 1959
Publishing on all aspects of the Acari

All information:

<http://www1.montpellier.inra.fr/CBGP/acarologia/>
acarologia-contact@supagro.fr

**Acarologia is proudly non-profit,
with no page charges and free open access**

Please help us maintain this system by
encouraging your institutes to subscribe to the print version of the journal
and by sending us your high quality research on the Acari.

Subscriptions: Year 2023 (Volume 63): 450 €

<http://www1.montpellier.inra.fr/CBGP/acarologia/subscribe.php>

Previous volumes (2010-2021): 250 € / year (4 issues)

Acarologia, CBGP, CS 30016, 34988 MONTFERRIER-sur-LEZ Cedex, France

ISSN 0044-586X (print), ISSN 2107-7207 (electronic)

The digitalization of Acarologia papers prior to 2000 was supported by Agropolis Fondation under the reference ID 1500-024 through the « Investissements d'avenir » programme (Labex Agro: ANR-10-LABX-0001-01)

Acarologia is under free license and distributed under the terms of the
Creative Commons-BY

CONTRASTED ECOLOGICAL REPARTITION OF THE NORTHERN FOWL MITE
ORNITHONYSSUS SYLVIARUM (MESOSTIGMATA: MACRONYSSIDAE) AND THE
CHICKEN RED MITE *DERMANYSSUS GALLINAE* (MESOSTIGMATA:
DERMANYSSIDAE)

Lise ROY, Claude Marie CHAUVÉ and Thierry BURONFOSSE

(Received 12 January 2009; accepted 23 February 2010; published online 30 June 2010)

Université de Lyon - Ecole Nationale Vétérinaire de Lyon - Laboratoire de Parasitologie - 1 av. Bourgelat - 69280 Marcy l'Etoile - France.
l.roy@vet-lyon.fr

ABSTRACT — The two hematophagous mite species, the Northern Fowl Mite *Ornithonyssus sylviarum* (Macronyssidae) and the Chicken Red Mite *Dermanyssus gallinae* (Dermanyssidae), are considered serious pests in fowl farms in the USA and in Europe respectively. However, neither of them seems to be restricted to either of these two continents according to sparse records in the literature. The aim here was to explore the respective ecological repartitions of these two species in France and to compare them with data from the USA. We thus analyzed hematophagous mesostigmatid mites collected in France *in natura*, in orchards agroecosystems, in pet bird and fowl farms and compared DNA sequences from some North American and French mites in both species. It is remarkable that *O. sylviarum* has been recurrently encountered in bird nests from France and yet been absent from French layer farms, whereas it is a serious pest in layer farms in the USA. On the other hand, *O. sylviarum* has been isolated from ornamental bird farms in France (canary, pheasant). This suggests either a strong impact of different farming practices between continents or a colonization in process in Europe. It remains to be explored whether the opposite applies to *D. gallinae* in the USA. Lastly, mites belonging to the special lineage *D. gallinae* L1 have been isolated from North American pigeons providing mt and nDNA sequences very close to French L1 isolates'. This confirms the specific status of this cryptic entity.

KEYWORDS — *Ornithonyssus sylviarum*; *Dermanyssus gallinae*; poultry farms; wild avifauna; lineage L1

INTRODUCTION

The two hematophagous mite species, the Northern Fowl Mite *Ornithonyssus sylviarum* (Canestrini and Fanzago, 1877) (Mesostigmata: Dermanyssoidea: Macronyssidae) and the Chicken Red Mite *Dermanyssus gallinae* (De Geer, 1778) (Mesostigmata: Dermanyssoidea: Dermanyssidae) are obligatory ectoparasites primarily parasitizing birds. They are serious pests in fowl farms in the USA and

in Europe respectively. Neither of them seems to be restricted to either of these two continents according to sparse records in the literature.

Ornithonyssus sylviarum has been recorded in nests of various birds in several European countries (Ambros *et al.*, 1992; Krištofik *et al.*, 2007) as well as in some French layer farms (Bruneau *et al.*, 2002), but is not considered a problem in layer farms in France. In contrast, it is a well-known pest in

layer farms in the USA (Axtell and Arends, 1990; Mullens *et al.*, 2001). It is also largely present in wild avifauna in the North America (Hogsette *et al.*, 1991; Knee and Proctor, 2007).

Dermanyssus gallinae is an important pest in European layer farms (Guy *et al.*, 2004; Sparagano *et al.*, 2009) and has been recorded in the wild avifauna in both continents (Roy and Chauve, 2007), but is not currently recorded as an important pest in North American layer farms. In contrast, this species represents an increasing problem in South American layer farms (Tucci *et al.*, 2008).

Both species are especially bothersome in layer farms, since flocks in egg industry are maintained long enough to allow mite populations to increase up to very high levels, as opposed to flocks in meat-type fowl which stay only a few weeks. Protonymphs and adults of *O. sylviarum* feed on blood. These, plus the deutonymphal instar, do the same in *D. gallinae*. A second important difference between both species is their way of life at least in poultry: *O. sylviarum* acts as a typical ectoparasite, completing its entire life cycle on the host, so that a direct examination of the bird host allows detecting a large number of mites (Mullens *et al.*, 2001). However, contrarily to most permanent ectoparasites, it is able to move off hosts and cross empty cages reaching clean birds (Mullens *et al.*, 2001), as well as being able survive up to at least several weeks in the environment (Sikes and Chamberlain, 1955). In contrast, *D. gallinae* is more of a micropredator, having exclusively nidicolous habits and being much more indifferent to its host individual, similar to mosquitoes or even more appropriately to wingless bed bugs. Its blood meals are fast and rare and once the meal is completed, the mite quickly goes back to its hiding-place (Wood, 1917). Eggs are laid and molting is completed in hiding-places, never on the host bird (Moss, 1978). Note that some particular populations have been reported by Nakamae *et al.* (1997), as spending more time on chicken host in some Japanese sites, but these are likely to belong to a distinct species, albeit morphologically indistinguishable (see below). Indeed, micropredator habits are not common to all *Dermanyssus* species, some of them having been reported behaving like

permanent parasites (Moss, 1978). Likely due to this difference between the two economic pest species, although both are able to be starved over long periods, Kirkwood (1963) has shown that marked difference in the duration of their starvation ability separated them, since in identical conditions and in the absence of any host, *D. gallinae* lived for as long as 34 weeks, compared to only 3 weeks for *O. sylviarum*. Chen and Mullens (2008) have shown that the on-host survival duration in *O. sylviarum* may increase with respect to ambient temperature and humidity as well as to the mite's development stage, but nonetheless remaining far more inferior to the range noted in *D. gallinae*.

Most of the repartition data of these species are based upon morphological identifications. These appeared to be partly irrelevant concerning *D. gallinae* according to Moss *et al.* (1970). These authors have shown that non *gallinae* species of *Dermanyssus* had likely been previously misidentified and confused with *D. gallinae* in some North American records. And yet, Roy *et al.* (2009a, b), by using both morphological and molecular data, have shown that previous European records might have been incorrect as well, since non *gallinae* species revealed to be much more host specific than previously believed. This is also probably the key to the amazing and parentally transmitted behaviour differences noted by Nakamae *et al.* (1997) between two assumed *D. gallinae* populations.

As a result, one might wonder whether recorded distribution of populations in both species and for both continents are relevant or not. Second, for each species are mites parasitizing wild and domestic birds in each species are truly conspecific respectively?

Our aim is to establish the respective ecological segregation of these two species in France and to compare these findings with some data from the USA, at the strictly specific level and regardless of intraspecific population differentiation. Especially, we test whether *O. sylviarum* is present in the French wild avifauna and in French layer farms, as the presence of *D. gallinae* in French wild and domestic birds has already been shown by Roy *et al.* (2009b).

To this end, an inventory of hematophagous

mesostigmatid mites in birds at the specific level has been carried out in France and based on both morphological and DNA data. The obtained DNA sequences for each species are compared with sequences obtained from other countries, including the USA.

MATERIAL AND METHODS

Biological material

A detailed list of the analyzed French samples is available in Appendix 1. Additionally, a few samples collected in other countries, including the USA, are detailed in Appendix 2. Mites were sampled between 2005 and 2009. Two types of samples are considered here:

FIGURE 1: Location of samples collected in France and containing either *Dermanyssus gallinae* s. str. (ie non L1) (solid dots) or *Ornithonyssus sylviarum* (open dots). The shape of dots represents environmental information: circle for industrial fowls, triangle for amateur fowl houses, trapezoid for pet bird breeding facilities, square for game bird breeding facilities, 5-branch star for wild avifauna not sampled in orchard/field agroecosystems, 8-branch star for wild avifauna sampled in orchard/field agroecosystems. IF industrial fowl farms, AF amateur breeding facilities, fowl, PB amateur breeding facilities, pet birds, BG amateur breeding facilities, game birds, WA wild avifauna sampled in orchard or field agroecosystems, WNA wild avifauna not sampled in orchard or field agroecosystem.

1) Material sampled in the bird's immediate environment (nest or litter). This mainly concerns wild avifauna.

2) Some mites have been directly sampled as such: mite aggregates or individuals found in the host environment (crevices, under dried droppings...) or on host. This mainly concerns birds which are reared or kept by humans.

In order to get an overview of the different bird ecologies, samples have been collected in a diversity of environments, both in wild avifauna and in domestic birds. Within each of these two categories, several different types may be distinguished. The wild avifauna has been sampled in crop agroecosystems or not, and some on urban systems. Domestic birds' samples come from industrial layers, industrial broilers, amateur fowl, pet bird facilities, and even one isolate collected in game bird breeding facilities. Finally, a diversity of geographical origins within France has been sampled, in order to avoid artifacts due to endemism.

Additionally, mites have been sampled in several other countries throughout Europe, as well as in the USA, Brazil, Australia (see Appendix 2).

Nests/litter analysis

Mites were isolated from nests following the method described in de Lillo (2001), slightly modified as described in (Roy *et al.*, 2009b). This method is based on the immersion of the nest or litter sample in a fixed quantity of water and subsequent filtration using stacked sieves. Next, the obtained filtrate is reimmersed into water and observed using a dissecting microscope. Compared to the Berlese funnel method used in Burt *et al.* (1991), de Lillo's method allows detection of both living and dead mites. All detected hematophagous Mesostigmata were isolated, since their heterogeneous coloration due to blood ingestion and digestion makes them highly recognizable using stereoscopic magnifying glasses. They then were digested by proteinase K, then mounted into Hoyer's medium and identified using a photonic microscope. In some individuals, DNA contained into the digestion product was purified and used in molecular analyses (see below).

Mites are not counted in the present study. A simple occurrence assessment is used per sample (presence/absence of each species).

A Chi-square test was used to assess the association between species (*D. gallinae*, *O. sylviarum*) and their ecological distribution assuming as the null hypothesis that ecological distribution of hematophagous Mesostigmata species is homogeneous. The distribution between the observed occurrence of *D. gallinae* and *O. sylviarum* in Mesostigmata containing nest/litter and their theoretical distribution was also tested under the same null hypothesis.

DNA analyses

DNA sequencing of some gene portions (16S rRNA, COI coding gene, Tropomyosin) was performed in some isolates following Roy *et al.* (2009a, b) and Roy (2009) in order to check the specific identity and/or assess conspecificity. One to five individuals were sequenced per isolate. Phylogenetic analyses of obtained partial sequences of the mitochondrial coding gene for cytochrome oxidase I were run using both maximum-likelihood and maximum-parsimony methods. Maximum-likelihood (ML) analysis has been performed using the online software PHYML (Guindon *et al.*, 2005) with the substitution model GTR. Branch supports were estimated by approximate Likelihood Ratio Tests (aLRT) following Anisimova and Gascuel (2006). Maximum-parsimony (MP) analysis has been performed using PAUP* 4.0b10 (Swofford, 2001): a heuristic analysis has been run with TBR branch swapping and 10,000 random additions saving all most parsimonious trees. Heuristic searches in TNT 1.1 (Goloboff *et al.*, 2008, program available with the sponsorship of the Willi Hennig Society from <http://www.cladistics.org/tnt.html>) were used to obtain relative Bremer (Goloboff and Farris, 2001) and bootstrap support values. TNT searches recovered the same topology and tree length as PAUP, but calculation of support values is much more efficient in TNT.

RESULTS

French samples

Overall, 729 French samples were analyzed, of which 361 contained at least one hematophagous

Mesostigmata individual (see Table 1). The eight following species of hematophagous Mesostigmata have been isolated: *D. gallinae*, *D. carpathicus* Zeman, 1979, *D. longipes* Berlese & Trouessart, 1889, *D. hirundinis* (Hermann, 1804), *D. apodis* Roy, Dowling, Chauve & Buronfosse, 2009 (Dermanyssidae), *O. sylviarum*, *Ornithonyssus* sp. 1 (Macronyssidae), *Myonyssus* sp. (Laleapidae). The last two of these eight species occurred very rarely (see Table 1). Within *D. gallinae*, the special lineage L1 (Roy *et al.*, 2009a) has been shown strongly isolated on a reproductive point of view (Roy, 2009). Therefore, it is noted *D. gallinae* L1 all along present paper, and non L1 *D. gallinae* mites are labelled *D. gallinae* s. str.

FIGURE 2: Percentage of samples containing identified hematophagous mites, according to the type of environment. Solid bar: wild avifauna (n=166), grey bar: industrial fowl (layer and chicken farms) (n=132), open bar: other domestic birds (n=12).

Concerning the specific identification of *Ornithonyssus*, only two species have been isolated in present samples. *O. sylviarum* individuals provided rather clear morphological characteristics *sensu* Micherdziński (1980), similar to the North American isolate OSBM's. *Ornithonyssus* sp. 1 was very similar to, but slightly differed from *O. bacoti* (Hirst, 1913), according to Micherdziński (1980) and compared with individuals of *O. bacoti* from a lab strain cultured in the French Museum of Natural History (O. Bain). It did not key to another of the *Ornithonyssus* species *sensu* Micherdziński (1980).

Concerning the specific identification within *Dermanyssus*, all mites from domestic birds have been successfully identified at the specific level. As for samples from wild avifauna, some mites were too damaged for morphological identification, and/or DNA characterization was not yet fully de-

TABLE 1: Number of samples which have allowed isolating hematophagous mesostigmatid mites, according to the mite species and the environment.

Mite species		<i>Dermanyssus</i> <i>apodis</i>	<i>Dermanyssus</i> <i>carpathicus</i>	<i>Dermanyssus</i> <i>gallinae</i> s. str.	<i>Dermanyssus</i> <i>gallinae</i> L1	<i>Dermanyssus</i> <i>hirundinis</i>	<i>Dermanyssus</i> <i>longipes</i>	Unid. <i>Dermanyssus</i>	<i>Ornithonyssus</i> <i>sylviarum</i>	<i>Ornithonyssus</i> sp. 1	<i>Myonyssus</i> sp.
Farms	industrial farms (IF)	0	0	132	0	0	0	0	0	0	0
	amateur farms	0	0	5	1	0	0	0	0	0	0
	amateur fowl (AF)	0	0	0	4	0	0	0	1	0	0
	pet birds (PB)	0	0	0	0	0	0	0	1	0	0
Wild avifauna in crop agroecosystems (WA)	game birds (BG)	0	0	0	0	0	0	0	1	0	0
	not in crop agroecosystems (WNA)	0	9	7	0	0	3	0	16	2	1
		50	18	15	4	20	4	51	17	1	0
Total		50	27	159	9	20	7	51	35	3	1

veloped at the moment of sample exploration (before or at the beginning of the study of Roy *et al.* 2009a), thus 51 samples contained *Dermanyssus* mites not identified at the specific level.

Only 12 samples (*ie* 3.3% hematophagous Mesostigmata positive samples) allowed for detection of two different species in a single nest, eight of which grouped together species of different families (Macronyssidae + Dermanyssidae or Macronyssidae + Lalepididae). The four remaining cases (1.1% hematophagous Mesostigmata positive samples) grouped together *D. gallinae* either with *D. apodis* (3 cases) or with *D. carpathicus* (1 case) (see Appendix 1).

As in Roy *et al.* (2009b), *D. gallinae* s. str. appeared to be the most generalist species in France, not only from the host point of view but also with regard to the environment (see Figure 2). Note that it was present in free-range as well as in caged layer farms, and in layer as well as in broiler farms (see Appendix 1). The special lineage *D. gallinae* L1 (Roy *et al.*, 2009a, b) was present not only in wild but also in farm environments, almost exclusively on pigeons and never on hens. Other *Dermanyssus* species have been found only in wild avifauna. *O. sylviarum* has been found in almost all situations, but never in poultry farms in France (see Appendix 1), which is significant given the number of farms sampled (n=132). Moreover, only *D. gallinae* s. str. has been isolated from other European farm samples under test (see Appendix 2), but the number of samples (n=8) is insufficient to estimate the pres-

ence/absence of *O. sylviarum*.

Lastly, the percentage of samples containing hematophagous Mesostigmata is presented in Table 2. The presence of mites in direct samples (collection of mites directly in the bird's environment or on the host) was not significant as such with regards to a prevalence estimate. Most farms had been visited as a result of farmers' complaints. As for the wild avifauna, the exact number of wild birds examined is not known. Consequently, only nests or litters are considered here. At the generic level, *Dermanyssus* appeared present more often than *Ornithonyssus*. But most of the *Dermanyssus* positive nest samples contained non *gallinae* species. On the contrary, when considering solely *D. gallinae* and *O. sylviarum*, the latter appeared slightly more often than the former ($P < 0.001$). It is important to keep in mind that these data are mainly composed of wild bird nests. Overall, both *D. gallinae* and *O. sylviarum* have been regularly encountered in the wild avifauna, and in distant points in France (see Figure 1).

Other samples

In addition to the French samples, 24 different mite samples of non French origin are listed in Appendix 2. Hematophagous mesostigmatid mites from layer farms collected in other European countries, as well as from Australia and from Brazil, all belong to *D. gallinae* s. str. In contrast, none of the three North American *gallinae*-like isolates belong to *D. gallinae* s. str. The only isolate from a North American

TABLE 2: Percentage of occurrence of hematophagous Mesostigmata isolated from the analysis of French nest/litter material. Of 576 samples under test here, 213 allowed detecting any hematophagous Mesostigmata. Considering these positive samples, the null hypothesis of a homogenous distribution of *Dermanyssus gallinae* and *Ornithonyssus sylviarum* is rejected ($P < 0.001$).

Mite taxa	<i>Dermanyssus gallinae</i> s. str.	<i>Dermanyssus gallinae</i> L1	other	<i>Dermanyssus</i> unidentified	<i>Dermanyssus</i> whole	<i>Dermanyssus</i>	<i>Ornithonyssus sylviarum</i>	<i>Ornithonyssus</i> sp. 1	whole <i>Ornithonyssus</i>	<i>Myonyssus</i>
Percentage of occurrence	3.6	0.9	17.9	8.2	30.6	5.9	0.5	6.4	0.1	

poultry farm does not belong to *D. gallinae*, (neither s. str., nor L1). It appears clearly isolated from *D. gallinae*, branching from within a clade grouping together *D. carpathicus*, *D. hirundinis*, *D. longipes*, *D. quintus*, *D. hirsutus* in the mitochondrial COI topologies (accession numbers FN646522-5, see Figure 3). Naturally, exclusively mtDNA-based analyses are usually insufficient to establish the reproductive isolation of any isolate. Anyway, species boundaries based on both mitochondrial and nuclear markers have been robustly established (Roy *et al.*, 2009a; Roy, 2009) and have shown COI being a powerful factor in species delineation within *Dermanyssus* while not as powerful for interspecific relationships' resolution. Moreover, on the morphological basis, mites in this isolate key to *D. gallinae*, except that setae *j1* and *s1* are off the dorsal shield, *sensu* Moss (1978). These characters make it closer to *D. gallinoides*, however, leg chaetotaxy is typical of *D. gallinae*. As a result, the single-molecule analysis is considered here to be a sufficient in order to estimate that isolate JOW is strongly isolated from *D. gallinae* s. str. as well as L1 on the reproductive point of view. From this point, here will be referred to this entity as *D. sp.* JOW.

Two other *gallinae*-like isolates have been sampled in some pigeons in the USA (JOW_N33 and JOW_123). Both revealed to belong to the special lineage L1 of *D. gallinae* (Roy *et al.*, 2009a, b). This special lineage has been recorded almost exclusively on pigeons at several locations in France and proves here to be also present on North American pigeons. COI (accession numbers FN646512-21) and Tropomyosin (accession numbers FN646507-8)

sequences obtained from these two isolates are extremely close to the L1 French isolates haplotypes (1-2% divergence in COI, 0-1% in Tropomyosin intron *n* between American and French isolates, as well as between French isolates).

Finally, the DNA sequencing of some mitochondrial regions showed that isolates of *O. sylviarum* from French bird nests under test in the present study were conspecific to the North American poultry farm isolate OSBM. Based on an rRNA 16S region isolated following Roy *et al.* (2009a), isolates sampled in France (EMBL accession numbers: FN599080, FN646506) and isolate OSBM (accession number: FN599081) are diverging by 2-3% from each other, and by 1-2% from an isolate sampled from African Gold Breasted Starlings (*Cosmopsarus regius*) (accession number AY185362) (Schrenzel *et al.*, 2003). Based on the COI coding region, OSBM (accession number FN599077) and isolates sampled in France (accession numbers FM179677, FN432541, FN432544, FN432545, FN432547, FN432548, FN599074) were diverging by 2-3% from each other. Within these French isolates, individuals sampled in tit nests located in the South of France from orchards were diverging by 1% from the two isolates sampled in nests of Montagu's Harrier, *Circus pygargus* (Linnaeus, 1758) in the Western North of France (FS5 and FS6, accession numbers FN432544, FN432545, FN432547, FN432548) and from individuals sampled in a canary cage in the east-central France (Rh1, accession number FN646526). FS5, FS6 and Rh1 provided exactly the same haplotype. In contrast, 14-15% divergence separates all these North American and

FIGURE 3: COI-based phylogenetic topology involving all haplotypes isolated in Roy *et al.* (2009b), plus sequences newly obtained from French and North American *Ornithonyssus sylviarum* and from North American *Dermanyssus* individuals. Maximum-likelihood (PHYML). Numbers at nodes represent aLRT values.

French isolates of *O. sylviarum* from representatives of the close relative species *O. bacoti* (OBAC, accession number FM179677). From a phylogenetic point of view, French individuals group together as a sister to the American isolate (OSBM) according to COI-based ML and MP phylogenetic topologies.

Branch length are much shorter (more than 3 times shorter) between each other than between their common clade and their common ancestor with *O. bacoti* (Figure 3). All that strongly suggests that variations between included *O. sylviarum* samples do be intraspecific variations, not interspecific ones.

DISCUSSION

Among the eight species of hematophagous Mesostigmata recorded in the present study, the *Dermanyssus* species have been encountered more frequently than the *Ornithonyssus* species. Nevertheless, *O. sylviarum* is the only species of *Ornithonyssus* which has been repeatedly isolated, whereas each of the five species of *Dermanyssus* have been isolated rather frequently. The laelapid *Myonyssus* is a genus known to parasitize shrews and its presence is likely to be fortuitous (a single individual in a single nest).

FIGURE 4: COI-based phylogenetic topology involving all haplotypes isolated in Roy *et al.* (2009b), plus sequences newly obtained from French and North American *Ornithonyssus sylviarum* and from North American *Dermanyssus* individuals. 50% majority rule consensus of the 867 equiparsimonious trees obtained under maximum-parsimony. Numbers at nodes represent bootstrap values for 1000 replicates/Bremer relative indices.

It is obvious that *O. sylviarum* and *D. gallinae* are both present in the wild avifauna in France, and in some non-industrial bird breeding facilities (pet birds, game birds), but the former is absent to date from industrial fowl farms in France, especially from layer farms.

This contradicts the report of Bruneau *et al.* (2002). And yet, arguments advanced by these authors for mite identification are not discriminating

at the specific level, nor even at the generic level, but are characters allowing a family-level discrimination.

Phylogenetic topologies based on mtDNA sequences do not highlight important distances between populations from layer farms (American isolate OSBM) and from the wild avifauna (French and African samples). Present data do not show that populations from farms and from the wild avi-

fauna are different. However, this does not either mean that these populations are closely related and what's the more that transfers are at all possible. A close relationship between poultry and wild populations of *D. gallinae* is also established in Roy *et al.* (2009b), but no common COI haplotype has been isolated between wild and domestic birds, although mites collected in some very distant farms share the same haplotypes. In both cases, much larger sample batches along with analyses using population genetic tools are needed in order to assess the real closeness of populations parasitizing wild and domestic birds.

In any case, it is worth noting that *O. sylviarum* is present in environments where synthetic molecules targeting arthropods are largely applied such as conventional orchards and wheat fields (see Appendix 1). *O. sylviarum* has shown being able to develop resistance to inhibitors of acetylcholinesterase as well as to pyrethroids in the USA (Mullens *et al.*, 2004). Populations in this species are regularly exposed to organophosphates (Ravap EC®) or carbamates (sevin-80s®) in North American poultry farms, not only by spraying, but also in some cases by digging (Mullens *et al.*, 2009; Rubinoff I., pers. comm.), due to the typical parasitic habits of *O. sylviarum* described above. And yet, it is also exposed to various pesticides in French pet birds breeding facilities, including organophosphates and carbamates (M. Serre, Association Ornithologique Rhodanienne, pers. comm.), which may have resulted in a selection of resistant populations in France too. This might explain its presence in conventionally controlled crop farm agroecosystems. European layer farms are regularly treated with organophosphates and carbamates too, as well as other synthetic pesticides (such as amitraz, for instance). In France, in particular, an organophosphate-based pesticide (phoxim, Bye-Mite®/Bye-Pou®) is allowed for usage not only between, but also during flocks since 2007. And many products composed of various molecules, including organophosphates (e.g. dichlorvos-based products) or carbamates (e.g. Carbyl®), have long been used illegally during flocks, as a consequence of the lack of allowed products for this period. All these prod-

ucts are sprayed in the farm buildings, which does not differ so much from North American use. As a result, it is very unlikely that pesticide use might be the cause of the disbalance in the red mites' distribution between North American and European layer farms.

It is not impossible that *O. sylviarum* is currently in the process of colonizing European farms, since a few cases of infestations in layer farms have been recently reported in Northern Europe (J. Chirico, pers. comm.). And some pet bird as well as pheasant breeders provided accounts suggesting that *O. sylviarum* is in the process of invading their facilities (red mites staying on host since a few years). Some population genetics would be needed in order to determine whether populations found in farms in Europe come from wild birds or correspond to different populations, which might have potentially been transferred by human activities.

In the USA, no mite belonging to *D. gallinae* s. str. has been isolated in this study. The two samples from North American wild avifauna containing mites belonging to *D. gallinae* clearly belong to the special lineage L1. This confirms the specific status of this cryptic entity as already suggested in Roy *et al.* (2009a) and Roy (2009). Indeed, cryptic species are species which do not possess any morphological divergence with their closest sister(s) and are usually the result of a recent speciation event. Establishing the specific status of cryptic entities is not so easy. Overall, reproductive isolation corroborations need the congruence of more than one line of evidence, for instance morphology and DNA sequences. In the case of cryptic species, morphological characters do not help. Consequently, it was necessary to explore some other characters (DeSalle *et al.*, 2005), such as life histories or ecological associations. In the case of lineage L1, molecular assessment has been checked (Roy, 2009) by comparing topologies based on mt- and nDNA and including isolates of several different origins in France. Isolates under test in this study were almost solely pigeons. In the present study, the close association of L1 with pigeons is confirmed. This characteristic, along with specific COI (mtDNA) and Tropomyosin (nDNA) haplotypes is maintained throughout Eu-

rope and America. This is a considerable insight in favour of a complete reproductive isolation between this lineage and other *D. gallinae* entities, which appears to be more a cryptic species complex.

As for mite samples from layer farms collected in the USA, no *D. gallinae* s. str. has been isolated, but the number of samples is far too small to establish the presence/absence of this species in North American layer farms. J. Owen and B. Mullens, although having performed recurrent experiments in layer farms (Mullens *et al.*, 2001, 2009; Owen and Mullens, 2004, Owen *et al.*, 2009, ...), only sampled mites belonging to *O. sylviarum* with one exception (J.O. and B.M. pers. comm.), which provided mites belonging to a non *gallinae* species (*D. sp.* JOW). Their studies did not target *D. gallinae*, which should be searched for by different means, due to its nidicolous habits. As a result, it is difficult to establish the presence/absence of *D. gallinae* in North American layer farms, due to the lack of sampling data in such environments. Nevertheless, the isolation of a non *gallinae* species in a North American layer farm is very interesting. Roy *et al.* (2009a, b) have shown that previous European records based on morphological identification alone were at least partly erroneous in the morphologically plastic species composing the *gallinae* group *sensu* Moss (1968, 1978). And yet, Moss *et al.* (1970) have highlighted the likely frequent misidentifications which might have led to consider *D. gallinae* more often present than it was in the USA. These authors have shown that non *gallinae* species in the genus *Dermanyssus* have likely been repeatedly confused with *D. gallinae*. Consequently, given that no study involving morphological along with molecular analyses in the North American *Dermanyssus* acaroфаuna have been performed, the ecological distribution of *D. gallinae* in this continent is to be considered unknown. *D. gallinae* s. str. is the only species of *Dermanyssus* recorded in layer farms in other parts of the world, including South America according to present data. Moreover, in France, non *gallinae* species of *Dermanyssus* appear strictly restricted to the wild avifauna. Is *D. sp.* JOW a second *Dermanyssus* species adapted to layer farm environments? Or was its presence in a small free range

hen house a fortuitous one? It is worth noting that *D. gallinae* s. str. is a relatively recent problem in Brazilian layers, since *O. bursa* was the predominant ectoparasite around 1939 and *D. gallinae* was established being more common in 1997 (Tucci *et al.*, 2008). The causes of this colonization in process in Brazilian farms remain unclear. It is not known whether *D. gallinae* s. str. was present in the wild avifauna in Brazil before.

Many questions remain unsolved here. It would be especially very useful to further explore the "dermanyssofauna" in North American layer farms as well as in the wild avifauna, to check whether *D. sp.* JOW is recurrently present or not, and whether *D. gallinae* s. str. is also present or not.

CONCLUSION

It is remarkable that *O. sylviarum* is so commonly encountered in French bird nests under test, yet absent from French layer farms, whereas it is a serious pest in layer farms in the USA. This suggests that different farming practices between both continents might explain the paradox of the omnipresence of both species in wild avifauna and a selective presence in French layer farms. Nevertheless, this difference does not seem to be attributable to pesticides commonly used in these farms. In any case, it remains to be checked whether the opposite applies to *D. gallinae* s. str. in the USA. It will be also interesting to look for additional samples of *D. sp.* JOW and characterize it using some other DNA sequences, in order to check its specific status and establish whether it is recurrently present in North American layers.

In any case, it is not excluded that populations under test in either *O. sylviarum* (France and USA) or *D. gallinae* (Europe, Brazil, Australia), although respectively conspecific, are genetically different and do not possess the same ability to parasitize hens in farms. Some population genetics in both species are needed in order to assess it. And more generally further surveys of ectoparasites in both wild nests and domestic fowl farms are lacking in the USA and are desperately needed in order to answer such important questions.

ACKNOWLEDGMENTS

Authors want to warmly thank persons who provided or actively helped providing biological material: C. Basset (Avibresse, France), F. Boléat et coll. (Ecopôle du Forez, Chambéon, France), Damien and Marion Buronfosse (Lyon, France), O. Caparros, L. Brucy, L. Carrier, J.C. Bouvier, N. Vincent-Martin, Y. Beauvallet, O. Dehorter, F. Salmon (Centre de Recherche sur la Biologie et les Populations d'Oiseaux, MNHN, France), Calabro (Association Ornithologique Bcs Crochus Centre Est, Relevant, France), T. Cencek (Państwowy Instytut Weterynaryjny, Puławy, Poland), P. Chabrol (veterinary office, Bourg-en-Bresse, France), M. De Rojas (Universidad de Sevilla, Sevilla, Spain), G. Gory (Museum d'Histoire naturelle, Nîmes, France), P.A. Heuch and A.G. Gjevre (National Veterinary Institute of Norway, Oslo, Norway), J. Kellaway (Australian Egg Corporation, North Sydney, Australia), O. Kilpinen (Danish Institute of Agricultural Sciences, Lyngby, Denmark), A. Marchal and M. Serre (Association Ornithologique Rhodanienne, Lyon, France), B.M. O'Connor (Museum of Zoology, University of Michigan, Ann Arbor, USA), J. Owen (Washington State University, USA), B. Paoletti (University of Teramo, Teramo, Italy), M. Sabelis and I. Lesna (University of Amsterdam, IBED, Amsterdam, The Netherlands), J.J. Sage (Club Français du Montauban, France), P. Tavernier (Centre de Soins aux Oiseaux sauvages du Lyonnais, Francheville, France), E.C. Tucci (Instituto Biológico, Centro de Pesquisa e Desenvolvimento em Sanidade Animal, São Paulo, Brazil), F. Veau (CORA Ardèche, France).

We also want to sincerely thank S. Lubac (Institut Technique de l'Aviculture, Lyon, France), V. Gonnier (Comité National pour la Promotion de l'œuf, France), I. Rubinoff (Hy-Line International, Dallas Center, USA) for very useful information about the poultry industries. Many thanks to B. Mullens (University of California, Riverside, USA), Claire Mottet and Scott for valuable comments which greatly improved the manuscript.

The study has been partly funded by the Region Rhône-Alpes (Pôle d'Expérimentation et de Progrès Avicole).

Finally, LR would like to offer her most sincere thanks for their technical participation to S. Bonnet and N. Guichard (LEGTA Saint-Genis-Laval, France), M. Rigaux (IUT A, Université Lyon 1, France), S. Merlin (Lycée Jean-Baptiste de la Salle, Lyon, France), S. El Ouartiti (IUT GBGE, Université Jean Monnet, Saint-Etienne, France), G. Lallemand (Lycée des Mandailles, Châteauneuf de Galaure, France).

SUPPLEMENTARY MATERIALS

Supplementary materials are available online at <http://www1.montpellier.inra.fr/CBGP/acarologia/>.

Appendix 1. Detailed information for samples collected in France

Appendix 2. Detailed information for samples collected in other countries

REFERENCES

- Ambros M., Křištofik J., Šustek Z. 1992 — The mites (Acari: Mesostigmata) in the birds' nests in Slovakia — *Biologia*, 47: 369-381.
- Anisimova M., Gascuel O. 2006 — Approximate likelihood ratio test for branches: A fast, accurate and powerful alternative — *Syst. Biol.*, 55: 539-552.
- Axtell R.C., Arends J.J. 1990 — Ecology and management of arthropod pests of poultry — *Annu. Rev. Entomol.*, 35: 101-126.
- Bruneau A., Dernburg, A., Chauve, C.M., Zenner, L. 2002 — First report of the northern fowl mite *Ornithonyssus sylviarum* in France — *Vet. Rec.*, 150: 413-414.
- Burt E.H. Jr., Chow W., Babbitt G.A. 1991 — Occurrence and demography of mites of Tree Swallow, House Wren, and Eastern Bluebird nests — In: Loye J.E. and Zuk M. (Eds). *Bird-parasite interactions: ecology, evolution, and behaviour*. Oxford, UK: Oxford University Press. p. 104-122
- Chen B.L., Mullens B.A. 2008 — Temperature and Humidity Effects on Off-Host Survival of the Northern Fowl Mite (Acari: Macronyssidae) and the Chicken Body Louse (Phthiraptera: Menoponidae) — *J. Econ. Entomol.*, 101: 637-646
- De Lillo E. 2001 — A modified method for Eriophyoid mite extraction (Acari: Eriophyoidea) — *Int. J. Acarol.*, 27: 67-70.

- DeSalle R., Egan M.G., Siddall M. 2005 — The unholy trinity: taxonomy, species delimitation and DNA barcoding — *Philos. trans. - R. Soc. Biol. Sci.*, 360: 1905-1916.
- Goloboff P.A., Farris J.S. 2001 — Methods for quick consensus estimation — *Cladistics*, 17: S26-S34.
- Goloboff P., Farris J., Nixon K. 2008 — TNT, a free program for phylogenetic analysis — *Cladistics*, 24: 774-786.
- Guindon S., Lethiec F., Duroux P., Gascuel O. 2005 — PHYML Online, a web server for fast maximum likelihood-based phylogenetic inference — *Nucleic Acids Res.*, 33: W557-559.
- Guy J.H., Khajavi M., Hlalel M.M., Sparagano O. 2004 — Red mite (*Dermanyssus gallinae*) prevalence in laying units in Northern England — *Br. Poult. Sci.*, 45: 15-16.
- Hogsette J.A., Butler J.F., Miller W.V., Hall R.D. 1991 — Annotated bibliography of the northern fowl mite, *Ornithonyssus sylviarum* (Canestrini and Fanzago) (Acari: Macronyssidae) — *Misc. Publ. Entomol. Soc. Am.*, 76.
- Kirkwood A. 1963 — Longevity of the mites *Dermanyssus gallinae* and *Liponyssus sylviarum* — *Exp. Parasitol.*, 14: 358-366.
- Knee W., Proctor H. 2007 — Host records for *Ornithonyssus sylviarum* (Mesostigmata: Macronyssidae) from birds of North America (Canada, United States and Mexico) — *J. Med. Entomol.*, 44: 709-713.
- Křištofik J., Mašán P., Šustek Z. 2007 — Arthropods (Pseudoscorpionidea, Acarina, Coleoptera, Siphonaptera) in nests of the bearded tit *Panurus biarmicus*) — *Biología*, 62: 749-755.
- Micherdziński W. 1980 — Eine taxonomische Analyse der Familie Macronyssidae Oudemans, 1936 — Warszawa Kraków: Polska Akademia Nauk, Zakład Zoologii Systematycznej I Doświadczalnej. pp. 263
- Moss W.W. 1968 — An Illustrated Key to the Species of the Acarine Genus *Dermanyssus* (Mesostigmata: Laelapoidea: Dermanyssidae) *J. Med. Entomol.*, 1: 67-84.
- Moss W.W. 1978 — The mite genus *Dermanyssus*: a survey, with description of *Dermanyssus trochilinis*, n. sp., and a revised key to the species (Acari: Mesostigmata: Dermanyssidae) — *J. Med. Entomol.*, 14: 627-640.
- Moss W.W., Mitchell C.J., Johnston D.E. 1970 — New north American host and distribution records for the mite genus *Dermanyssus* (Acari: Mesostigmata: Dermanyssidae) — *J. Med. Entomol.*, 7: 589-593.
- Mullens B.A., Hinkle N.C., Robinson L.J., Szijj C.E. 2001 — Dispersal of Northern Fowl Mites, *Ornithonyssus sylviarum*, Among Hens in an Experimental Poultry House — *J. Appl. Poult. Res.*, 10: 60-64.
- Mullens B.A., Owen J.P., Kuney D.R., Szijj C.E., Klingler K.A. 2009 — Temporal changes in distribution, prevalence and intensity of northern fowl mite (*Ornithonyssus sylviarum*) parasitism in commercial caged laying hens, with a comprehensive economic analysis of parasite impact — *Vet. Parasitol.*, 160: 116-33.
- Mullens BA, Velten RK, Hinkle NC, Kuney DR, Szijj CE. 2004 — Acaricide resistance in northern fowl mite (*Ornithonyssus sylviarum*) populations on caged layer operations in Southern California — *Poult. Sci.*, 83: 365-374
- Nakamae H., Kishi S., Fujisaki K., Oshiro S., Furuta K. 1997 — Incidence of the Parasitism of Chicken Mite *Dermanyssus gallinae* Parasitizing and Propagating on Chicken Even in the Daytime and Their Life Cycle — *Japan. Poult. Sci.*, 34: 240-247
- Owen J.P., Delany M.E., Cardona C.J., Bickford A.A., Mullens B.A. 2009 — Host inflammatory response governs fitness in an avian ectoparasite, the northern fowl mite (*Ornithonyssus sylviarum*) — *Int. J. Parasitol.*, 39: 789-799.
- Owen J.P., Mullens B.A. 2004 — Influence of Heat and Vibration on the Movement of the Northern Fowl Mite (Acari: Macronyssidae) — *J. Med. Entomol.*, 41: 865-872.
- Roy L. 2009 — Evolutionary ecology of a hematophagous mite genus: Phylogeny-based approach for interspecific delineations and comparative characterization of populations in five species of the genus *Dermanyssus* (Acari: Mesostigmata) [PhD thesis] — Institut des Sciences et Industries du Vivant et de l'Environnement (Agro Paris Tech). pp. 297.
- Roy L., Chauve C.M. 2007 — Historical review of the genus *Dermanyssus* Dugès, 1834 (Acari: Mesostigmata: Dermanyssidae) — *Parasite*, 14: 87-100.
- Roy L., Dowling A.P.G., Chauve C.M., Buronfosse T. 2009a — Delimiting species boundaries within *Dermanyssus* Dugès, 1834 (Acari: Dermanyssidae) using a total evidence approach — *Mol. Phylogenet. Evol.*, 50: 446-470.
- Roy L., Dowling A.P.G., Chauve C.M., Lesna I., Sabelis M.W., Buronfosse T. 2009b — Molecular phylogenetic assessment of host range in five *Dermanyssus* species — *Exp. Appl. Acarol.*, 48: 115-142.
- Schrenzel M.D., Maalouf G.A., Keener L.L., Gaffney P.M. 2003 — Molecular characterization of malarial parasites in captive passerine birds — *J. Parasitol.*, 89: 1025-1033.
- Sikes R.K., Chamberlain R.W. 1955 — Laboratory observations on three species of bird mites — *J. Parasitol.*, 40: 691-697.
- Sparagano O., Pavličević A., Murano T., Camarda A., Sahibi H., Kilpinen O., Mul M., van Emous R., le Bouquin S., Hoel K., Cafiero M. 2009 — Prevalence

and key figures for the poultry red mite *Dermanyssus gallinae* infections in poultry farm systems — Exp. Appl. Acarol., 48: 3-10.

Swofford D.L. 2001 — PAUP* Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0B 10. [Computer software and manual] — Sunderland, Massachusetts: Sinauer Associates.

Tucci E.C., Prado A.P., Araujo R.P. 2008. — Development of *Dermanyssus gallinae* (Acari: Dermanyssidae) at different temperatures — Vet. Parasitol., 155: 127-32.

Wood H.P. 1917 — The chicken mite: its life history

and habits — United States Department of Agriculture Washington DC. Bulletin, 553: 1-14.

COPYRIGHT

Roy *et al.*. Acarologia is under free license. This open-access article is distributed under the terms of the Creative Commons-BY-NC-ND which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.