**Title: High diversity and low genetic structure of feather mites associated with a phenotypically variable bird host**

**Short title** Within-host genetic diversity of symbionts

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SUMMARY

Obligate symbionts may be genetically structured among host individuals and among phenotypically distinct host populations. Such processes may in turn determine within-host genetic diversity of symbionts, which is relevant for understanding symbiont population dynamics. We analysed the population genetic structure of two species of feather mites (*Proctophyllodes sylviae* and *Trouessartia bifurcata*) in migratory and resident blackcaps *Sylvia atricapilla* that winter sympatrically. Resident and migratory hosts may provide mites with habitats of different qualities, what might promote specialization of mite populations. We found high genetic diversity of within-host populations for both mite species, but no sign of genetic structure of mites between migratory and resident hosts. Our results suggest that, although dispersal mechanisms between hosts during the non-breeding season are unclear, mite populations are not limited by transmission bottlenecks that would reduce genetic diversity among individuals that share a host. Additionally, there is no evidence that host phenotypic divergence (associated with the evolution of migration and residency) has promoted the evolution of host-specialist mite populations. Unrestricted dispersal among host types may allow symbiotic organisms to avoid inbreeding and to persist in the face of habitat heterogeneity in phenotypically diverse host populations.

Key words: Astigmata, COI gene, DNA-barcoding, migratory behaviour, symbiont dispersal, symbiont genetic diversity

KEY FINDINGS

1. Feather mites of blackcaps have a great genetic diversity

2. Mite genetic diversity is independent of host phenotypic races

3. Mites are not limited by bottlenecks that would reduce within-host genetic diversity

4. There is no proof that diverse host phenotypes promote the evolution of specialist mites

5. The cost of settling in suboptimal habitat is not equally shared by competing mite lines

## INTRODUCTION

Genetic structuring is the outcome of restricted gene flow and lineage divergence during periods of population isolation (Hartl and Clark, 2007). For some organisms, such as obligate symbionts (parasites, mutualists and commensals), population isolation events may take place at very small spatial and temporal scales, because individual hosts represent a patchy and ephemeral habitat (Poulin, 2007; Barrett *et al.* 2008). This forces symbionts to colonize continuously new habitat patches, thereby creating opportunities for population structuring via founder effects, especially if populations established on one individual host originate in a low number of colonizers (Hedrick, 2000). Host population size and symbiont dispersal ability can also influence symbiont genetic structuring (Johnson *et al.* 2002; Whiteman *et al.* 2007; Dabert *et al.* 2015; Martinu *et al.* 2015). Moreover, geographic structuring of host populations themselves, either because of low host vagility, or because of host population isolation in discrete habitat patches, may result in further genetic structuring of symbiont populations (Harper *et al.* 2015).

The genetic structuring of symbiont infrapopulations (the stock of symbionts that become temporarily isolated in a single host individual; Poulin, 2007) can determine several aspects of their ecological and evolutionary interactions. Regarding within-host population dynamics, individual symbionts may differ in their ability to access host resources, or to occupy the best habitat within the host (Mideo, 2009). Poor competitors may be displaced to areas of inferior quality and have reduced individual fitness (Fretwell and Lucas, 1970). However, within-host fitness differences among symbionts will have evolutionary consequences only in genetically diverse symbiont infrapopulations (Rigaud *et al.* 2010): if all symbionts are close kin because of intense transmission bottlenecks, individuals that occupy poorer microhabitats within the host may still obtain fitness returns from close kin

occupying the best habitat (Emlen, 1995). Among-host population dynamics can also be dependent on genetic structuring, because reduced infrapopulation genetic diversity might entail inbreeding costs (Keller and Waller, 2002), while too much gene flow might hamper local adaptation to specific host types (Lenormand, 2002). Host specificity is thus a further element driving the genetic structuring of symbiont populations: some symbionts may exploit different host types (either different host species, or host populations of the same species occupying contrasting environments), which may differ in their spatio-temporal distribution or in their suitability for the symbionts. For example, alternative host types may offer different quantity or quality of resources to the symbiont (Fernández-González *et al.* 2013), which may promote specialization of symbionts, and ultimately genetic isolation among populations of symbionts associated with different host types (Nadler, 1995; Rigaud *et al.* 2010).

Population genetic structure of symbionts should inform our understanding of their ecology and evolution (Hewitt, 2001), but the genetic composition of symbiont populations within host individuals, and the genetic structuring of such populations among individual hosts and host types, remain obscure for most host-symbiont systems (Nadler, 1995; McCoy *et al.* 2003; Doña *et al.* 2015*a*). We studied the genetic structuring of two species of astigmatan feather mites, *Proctophyllodes sylviae* Gaud (Proctophyllodidae) and *Trouessartia bifurcata* (Trouessart) (Trouessartiidae) sampled from resident and migratory blackcaps, *Sylvia atricapilla* (L.) (Sylviidae), coexisting in winter in Southern Spain. Feather mites are a very diverse and broadly distributed group of avian mutualists, composed mostly of host specialists (Proctor, 2003). Estimates of genetic diversity calculated for mitochondrial DNA show that it depends to some extent on average infrapopulation size (Doña *et al.* 2015*a*), which is in turn roughly repeatable for a given species of feather mite (Díaz-Real *et al.* 2014).

As for most feather mites, *P. sylviae* and *T. bifurcata* are only known to be transmitted by direct contact between hosts (Proctor, 2003), so that their genetic diversity on a single host individual might be very reduced if the number of founders is low.

Blackcaps are the main host of the two studied species of feather mite, which live on the flight feathers of the wing (Doña *et al.* 2016). Blackcaps breeding in medium and high latitudes of Western Europe typically winter around the Mediterranean region, where they coexist with resident blackcap populations (Pérez-Tris and Tellería, 2002*a*). The extreme SW of Spain, where this study was conducted, offers an exceptional opportunity to study the symbiont communities of birds that, although temporally sympatric during winter, are effectively segregated during the breeding period and have undergone contrasting evolutionary paths (Pérez-Tris *et al.* 2004). Resident birds provide mites with more stable environmental conditions, while migrants move between contrasting regions biannually. Resident and migratory blackcaps also differ as 'habitats' for feather mite infracommunities. Residents are longer-lived (Pérez-Tris and Tellería, 2002*b*), have differently shaped wings and structurally different feathers (De la Hera *et al.* 2009). They also possess larger uropygial glands, which likely make them more rewarding hosts from a nutritional perspective (vane-dwelling feather mites feed on the oil of the uropygial gland that covers the feathers, and on the particles embedded within; Proctor, 2003).

In bird species with no social behaviour (as blackcaps), feather mite transmission is posited to be reduced to male-female and parent-nestling contact during the breeding season (Proctor 2003; Doña *et al.* 2017a). It is thus likely that if the differences between migratory and resident blackcaps have promoted mite specialization, mite populations may have detectable genetic structure related to host type. However, the coexistence of migratory and

resident blackcaps may provide opportunities for mite dispersal among hosts with different phenotype and geographic origins, thereby preventing genetic structuring of mite populations.

These processes may further differ between the two species studied, which are unequally distributed between host types: whereas *P. sylviae* occurs with similar prevalence on migratory and resident blackcaps, it is more abundant on migratory blackcaps. In contrast, *T. bifurcata* is rarely found on migratory blackcaps (Fernández-González *et al.* 2013). Such a pattern of segregated distribution may be associated with differences between mite species in host preference, dispersal capabilities, or in-host population dynamics, all of which could lead to variation in the patterns of genetic structure between mite species. In addition, coexistence of both mite species happens at the expense of the smaller species (*P. sylviae*), which on resident hosts is displaced by *T. bifurcata* from preferred wing areas and attains lower population size (Fernández-González *et al.* 2015). Because of these interspecific interactions, if within-host genetic diversity of mites depends on population size (Doña *et al.* 2015*a*), the genetic diversity of *P. sylviae* could be lower on resident than on migratory hosts, where infrapopulation size is not limited by the existence of the other species.

Our study aims to elucidate (1) the genetic implications for symbiotic feather mites of the coexistence of different host phenotypes in the same habitat (i.e., sharing of wintering grounds by migratory and resident blackcaps), and (2) whether the pattern of genetic diversity differs between the two species of symbionts. To this end, we analysed genetic structure among mite infrapopulations (with the host individual as the habitat patch for mites), both within and between host groups (with migratory or resident blackcaps as types of hosts that may harbour genetically distinct mite infrapopulations).

## MATERIALS AND METHODS

*Study site and field methods*

Blackcaps were captured during two winter seasons (February and December 2010) in a forest of the Campo de Gibraltar region (extreme south of Spain: 36° 9' 33.98'' N, 5° 34' 50.0'' W). A total of 160 birds were mist-netted and individualised with metal rings. We measured the length of the eighth primary, tail length and the difference between the distances from primary feathers 1 and 9 to the wing tip to classify blackcaps as migratory or resident, using a discriminant function that correctly assigns > 97% of individuals (De la Hera *et al.* 2012).

Blackcaps were held individually until processing in bird bags to prevent mite cross-contamination. Mites were retrieved by immersing several wing feathers in tubes filled with absolute ethanol, trying to sample from the whole area of the wing that was populated by mites. Blackcaps were subsequently released unharmed. The samples were stored at -20 ºC until analysed. We aimed to obtain five individuals of each mite species from each host that could be typed for genetic analyses and slide-mounted for species identification by microscopy. *Proctophyllodes* females cannot be reliably determined to the species level based on morphology (Atyeo and Braasch, 1966), so that although no other species of *Proctophyllodes* than *P. sylviae* are known from *S. atricapilla*, we erred on the side of caution by only sampling male mites. We sampled as many males as it was possible for *Trouessartia* (which was far less abundant), but some female individuals were included in our analyses as females can be identified to species (Santana, 1976). Among the blackcaps that harboured a sufficient number of male *Proctophyllodes* mites, we selected 24 individuals (12 migratory and 12 resident). These birds included nine resident blackcaps that also harboured *Trouessartia* mites (so that the sample of hosts used to test for population structure of the two mite species overlapped as much as possible). We also included three migratory blackcaps

that harboured *Trouessartia* mites (we did not have any migratory individuals with a sufficient number of mites of both species). Therefore, the total number of hosts sampled was 27.

*DNA extraction, PCR and sequencing*

Total genomic DNA was extracted from individual mites using a DNeasy Blood and Tissue Kit (Qiagen, USA), following a specific protocol that modified the manufacturer's instructions (Dabert *et al.* 2008; M. Dabert pers. comm.). Individual mites were transferred from the original stock to tubes containing 180 µl of ATL lysis buffer with 20 µl of Proteinase K (Qiagen, USA), which were then incubated at 57 °C with 500 rpm shaking in a thermoshaker (GRANT ®) for 72 h, vortexing thoroughly and spinning down the samples every day. After digestion, the sample was mixed by vortexing for 10 s and spun down. The supernatant was transferred to a new tube for DNA isolation, and the exoskeleton of the mite was stored in 80% ethanol at -20 ºC until mounted on polivynil alcohol (PVA) and used for species identification with a Leica DM 2500/BF with Differential Interference Contrast, following the keys of Atyeo and Braasch (1966) and Santana (1976). All mites employed in our analyses were morphologically confirmed to be either *P. sylviae* or *T. bifurcata*.

The metazoan DNA barcoding fragment (661 bp near the 5' end of the cytochrome oxidase I [COI] mitochondrial gene) was amplified by PCR with the degenerated primers bcdF05 and bcdR04 (Dabert *et al.* 2008). PCR reactions were carried out in 10 µl total volume, and contained 5 µl of Type-it Microsatellite PCR Kit (Qiagen, USA), 5 pmoles of each primer, and 4 µl of template DNA (undiluted DNA extract). Reaction conditions consisted of one initial step of 5 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 60 s at 50 °C, 60 s at 72 °C, with a final extension step of 5 min at 72 °C. After amplification, 5 µl of

purified water was added to PCR products, and 5 µl of the diluted PCR product was visualized on 2% agarose gels stained with GelRed™ (Biotium, USA) under UV light. After electrophoresis, 5 µl of purified water was added to the remaining PCR product. Bands of sufficient quality were sequenced from both ends with an ABI 3730 XL automated sequencer (Applied Biosystems) using 1-1.5 µl of diluted PCR product and 50 pmoles of each primer. Sequences were edited manually using BioEdit 7.0.5.3 (Hall, 1999) and aligned using ClustalW as implemented in the same program.

*Genetic analyses*

Since there are several cases of cryptic speciation described among feather mites (Doña *et al.* 2015*b*), before performing our analyses of population genetic structure, we wanted to ensure that we were working with samples pertaining to a single species. To do so, we compared our mite sequences with all the other haplotypes of COI amplified from feather mites of the same two species available in GenBank (downloaded the 27 September 2016). We included as well COI haplotypes of the mite species most closely related to our two focal ones (according to Doña *et al.* 2017b). After discarding the sequences with low coverage or ambiguous nucleotides, our final working file (including our sequences) was composed of 150 sequences of *Proctophyllodes* spp. belonging to five morphospecies, and of 46 sequences of *Trouessartia* spp. belonging to four morphospecies. Sequences were trimmed to a final length of 505 bp, which included the hypervariable minibarcoding sequence of 200 bp identified by Doña et al. (2015*b*) as a good marker of feather mite species limits. To estimate the relationships among sequences, we conducted a neighbour-joining phylogenetic analysis using MEGA7 (Kumar *et al.* 2016) using the Kimura 2-parameter (K2P) substitution model. Support for internal nodes was derived from a bootstrap resampling with 1,000 replications.

After establishing that our sequences of *P. sylviae* and *T. bifurcata* pertained to well-defined species (see Results), to estimate population genetic structure of mites between migratory and resident blackcaps, and among host individuals within blackcap populations, we conducted simple and hierarchical Analyses of Molecular Variance (AMOVA) using Arlequin 3.5.1.2 (Excoffier and Lischer, 2010). We used jModelTest 2.1.4 (Darriba *et al.* 2012) to infer the most appropriate model of nucleotide substitution for the COI gene in each mite species (TPM2uf+I+G for *P. sylviae* and HKY+I for *T. bifurcata*). However, given that the Arlequin software does not implement these models, we used the Tamura and Nei model for both mite species (with $\alpha = 0.24$ for *P. sylviae*). This was the 6th best model according to the Akaike Information Criterion implemented in jModelTest, and according to model parameters it was the closest to the best models among the available in Arlequin. We tested statistical significance of population genetic structure using 1,000 permutations. To be sure that potential differences between mite species were not affected by the mix of *T. bifurcata* males and females if mite dispersal is somehow sex-biased, we also repeated the AMOVA analysis using only male mites (although at the expense of sample size and statistical power).

The evolutionary relationships among all our unique haplotypes of *P. sylviae* and *T. bifurcata* were reassessed separately for each species (alignments of our sequences were 661 bp long). We performed a neighbour-joining analysis using MEGA7 (Kumar *et al.* 2016) and the Kimura 2-parameter (K2P) substitution model. Support for internal nodes was derived from a bootstrap resampling with 1,000 replications.. Furthermore, to better visualize the patterns of population structure of mites among host groups, a haplotype network was built for each mite species with the software NETWORK (Fluxus Technology), using the Median-Joining algorithm. For each mite species, we computed the mean genetic distance between

haplotypes on the same host individual in order to better assess the degree of within-host genetic resemblance among mites.

## RESULTS

We found 72 haplotypes among 93 sequenced *P. sylviae* individuals, and 29 haplotypes among the 58 *T. bifurcata* individuals (Table S1, on Supporting Information). For an alignment length of 661 bp., the number of polymorphic sites was 108 for *P. sylviae* and 58 for *T. bifurcata*, and nucleotide diversity was 0.020 and 0.014, respectively. Sequence data met the assumption of selective neutrality for both species, as shown by non-significant Tajima's D statistics (in both cases with $P > 0.05$). All haplotypes were deposited in GenBank with accession numbers KF613605-KF613676 (*P. sylviae*), and KF613684-KF613716 (*T. bifurcata*).

In relation to the sequences assigned to other feather mite morphospecies, our sequences of *P. sylviae* were recovered as components of a single clade, amongst sequences of the same morphospecies retrieved from other studies (99% bootstrap support) and with no evident internal structure. The same was true for *T. bifurcata* (73% bootstrap support), although there were only two sequences available for comparison.

Genetic diversity among hosts was very high: for *P. sylviae*, we only found one haplotype shared by mites on two host individuals, whereas for *T. bifurcata* we found one haplotype shared by mites on three hosts and another shared by mites on two hosts. In contrast, individual mites more frequently shared the same haplotype at the infrapopulation level (Fig. 1). This trend was stronger for *T. bifurcata*: an AMOVA analysis revealed that, whereas in *P. sylviae* more than 80% of genetic variance could be explained by differences

within mite infrapopulations, this value was only roughly 50% for *T. bifurcata* (Table 1). This pattern held true when analysing only *T. bifurcata* males (Table 1).

When the same test was conducted with individual hosts classified as migratory or resident in a hierarchical AMOVA, a similar amount of genetic variance was explained by differences among infrapopulations of the same host group (Table 1). However, no genetic structure was detected between populations of mites sampled on migratory or resident blackcaps (Table 1). This was supported also by the phylogenetic reconstructions at the mite species level and the haplotype networks, where haplotypes coming from mites sampled on resident or migratory hosts do not form any sort of clustering (Figs. 1, 2). The distribution of average within-host pairwise genetic distances among feather mites revealed that blackcaps harboured unrelated mites as a rule (Fig. 3).

## DISCUSSION

Our analyses unveiled high within-host genetic diversity of two feather mite species living in migratory and resident blackcaps. Remarkably, this genetic diversity was not structured between host types, despite the fact that resident and migratory birds have contrasting population histories and phenotypic attributes. The specific identity of all the sampled mites was ascertained both by microscopy and by molecular means, ruling out the possibility that our samples contained a mix of cryptic mite species that could distort our estimates of genetic diversity. At the infrapopulation level, genetic diversity was significantly greater for one of the two mite species (*P. sylviae*), what shows that the pattern of genetic structuring of mite populations may differ among mite species that share hosts in the same environment.

Large levels of intraspecific COI gene genetic diversity had already been reported by Doña *et al*. (2015*a*) for *P. sylviae*, as well as several other species of European feather mites,

284 although their sampling was not designed to detect genetic structuring among discrete bird
285 populations (samples of *P. sylviae* included in the analyses, for instance, came from breeding
286 birds sampled in Russia and from wintering birds of unknown origin captured in Spain; R.
287 Jovani, pers. com.). It is important to recall that the resident blackcaps sampled in this study
288 represent a distinctive population within the host's range. They show genetic evidence of
289 reproductive isolation from other populations, and a number of phenotypical traits of potential
290 relevance for feather mite biology that make them different from migratory blackcaps (Pérez-
291 Tris and Tellería, 2002*a*; Pérez-Tris *et al.* 2004; Fernández-González *et al.* 2013). Contrary to
292 our expectations, we did not find any genetic structuring of mites inhabiting resident
293 blackcaps in relation to those sampled from migratory hosts: terminal nodes within each mite
294 species' COI tree were randomly distributed among host individuals and host types. Neither
295 was there any trace of founder effect in the haplotype networks, what on the contrary is
296 clearly shown by mites sampled elsewhere (Dabert et al. 2015, Doña et al. 2015a).
297 The absence of mite genetic structure between host types suggest that the coexistence
298 of blackcaps from different geographic origins in sympatric wintering areas promotes the
299 interchange of mites outside the host's breeding season. Nevertheless, mechanisms of
300 transmission different from parent-offspring transmission, which could explain the exchange
301 of mites between hosts outside the breeding season, are yet to be explored. Several groups of
302 feather symbionts (some groups of feather lice and mites) are known to be transmitted
303 phoretically by louse flies (Hippoboscidae), but this behaviour has not been found for the
304 mites under study (Jovani *et al.* 2001). In our study region blackcaps do protect small feeding
305 territories around particularly attractive bushes, and do engage in the odd fight (pers. obs.).
306 Fighting has been posited by Dabert *et al*. (2015) as a potential mechanism of mite exchange
307 between skua species, despite the fact that feather mites display a number of morphological

adaptations directed to avoid the dislodging of feather mites from their hosts (Doña *et al.* 2017a).

Incomplete lineage sorting could also explain this pattern, because the actual haplotype sharing between resident and migratory blackcaps is reduced to one haplotype of *P. sylviae* and two of *T. bifurcata*. Still, a few dispersal events each winter might represent a number of migrant mites per generation large enough to erase any structure associated to host population (Slatkin, 1987). On the other hand, the lack of genetic clustering found among the mites of some fully sedentary birds species studied by Doña *et al*. (2015b) suggest that in spite of the apparent genetic isolation of sedentary blackcaps, a stepwise transmission of mites following short-distance host dispersal could be sufficient to dilute the genetic structuring of mite populations. Actually, differences between the effective population sizes of both blackcaps and mites might explain that the degree of genetic structuring found among blackcaps is not mirrored by their symbionts (Criscione, 2008).

Once acquired, the high genetic diversity of mite infrapopulations revealed in our study seems incompatible with the existence of severe bottlenecks during mite transmission. Fledglings are colonized by large numbers of mite nymphs coming from their parents (Doña *et al.* 2017a), so that mite infrapopulation genetic diversity would likely be preserved across bird generations. At the infrapopulation level, some individuals sampled on the same host shared COI haplotypes, which is to be expected if related mites from the same founder stock are sampled. Some degree of genetic homogeneity at the infrapopulation level has been described recently in other feather mite species (Dabert *et al.* 2015; Doña *et al.* 2015*a*); however, we still found a great genetic diversity of mite infrapopulations, even if our small sample size (five mites per host) somewhat limits our capacity to detect many different haplotypes in the same infrapopulation.

Differences on infrapopulation genetic diversity between both mite species also call for an explanation. *Trouessartia bifurcata* is much more mobile along the feathers than *P. sylviae* (pers. obs.), so that in theory it could switch between individual hosts more easily, yet it is the species with the lower infrapopulation genetic diversity. In line with the findings of Dabert *et al*. (2015), differences between the infrapopulation genetic diversity of the two mite species could be related to their degree of host specificity. *Trouessartia bifurcata* occurs very rarely on migratory hosts, while *P. sylviae* is equally frequent in both host types (Fernández-González *et al.* 2013; 2015). Even though *P. sylviae* is much more abundant in migratory hosts (Fernández-González *et al.* 2013, 2015), its infrapopulation genetic diversity did not change between host types. Furthermore, even in sedentary birds the average number of *T. bifurcata* individuals per host is lower than that of *P. sylviae*, so that differences between the effective population size could be behind these differences in genetic diversity (Criscione, 2008). Although we are aware of the problems of interpreting two-species comparisons from an adaptive perspective (e.g. Garland and Adolph, 1994), our results show that peculiarities of each symbiont species may shape the patterns of genetic structuring of different symbionts that share host species.

Since feather mites are obligate symbionts, their populations are subjected to environmental changes associated with host phenotypic diversity and host habitat use (Proctor 2003). Our results suggest a scenario in which a mite that lives on a resident blackcap may sometimes have its offspring living on a migratory host, which differs in evolutionary history, geographic origins and ecological attributes. This scenario in which mites with different ancestry can end up sharing a host may have important implications in intra-host mite interactions. Previous research on the same study system analysed here has revealed a non-random distribution of feather mites among wing feathers and sectors of the same feather in

blackcaps (Jovani and Serrano 2004; Fernández-González *et al.* 2015), suggesting that some areas of the bird plumage may be preferred and others may be avoided by different species. If competition determines the distribution of mites among host microhabitats of different quality, our results suggest a scenario in which mites are unable to compensate for the costs of occupying poor sectors through inclusive fitness returns, because in genetically diverse mite populations there is no guarantee that the best sectors will always be occupied by close kin. Still, whether different mite families segregate among sectors of the host plumage, or freely mix among host microhabitats, remains an open question for future research.

As a final remark, it should be taken into account that both *P. sylviae* and *T. bifurcata* have been reported from several other host species (see records in Doña *et al.* 2016). Whether the amount of genetic variation observed in our study may be associated with their degree of host specialization can only be answered with broader comparative analyses. Since the very few studies analysing genetic diversity within feather mite species have documented cases of both cryptic speciation and of mites with no genetic structuring whatsoever between different hosts (Dabert *et al.* 2015; Doña *et al.* 2015*a;* Szudarek *et al.*, 2017), the exploration of how population structure varies among different mite species on different host species is a promising research field. This study makes thus a significant contribution to our understanding of the evolutionary implications of competitive asymmetries among symbionts that share an individual host, as well as of the factors that may promote or hamper the genetic structuring of symbiont communities among different host populations.

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SUPPORTING INFORMATION

**Figure S1.** Complete phylogeny of cytochrome oxidase I (COI) haplotypes of feather mites.

**Table S1.** Feather mite COI haplotypes found in this study and host identity.

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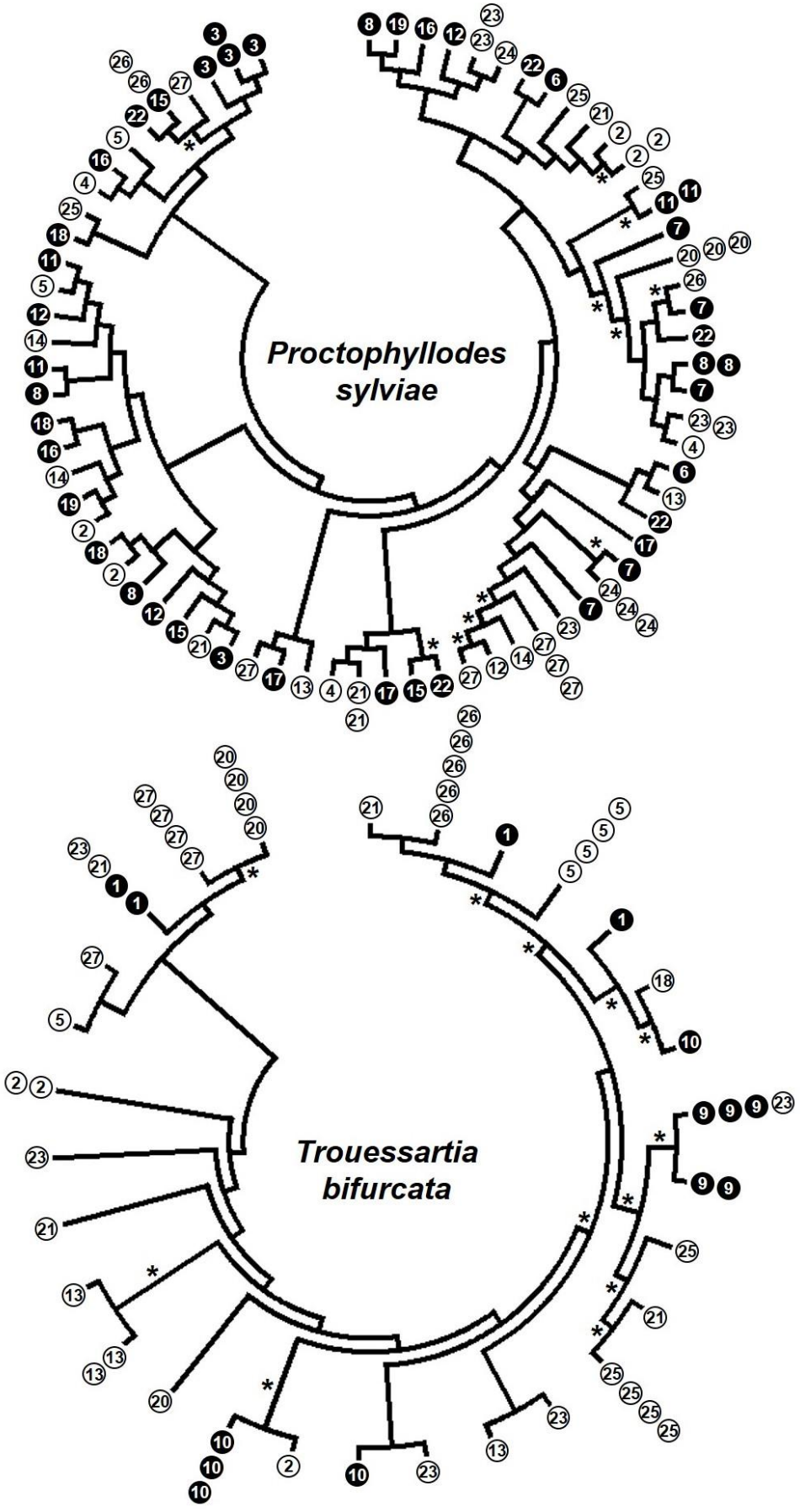
**Table 1.** Results of AMOVA for population genetic structure of the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* in migratory and resident blackcap populations. The analyses partition total molecular variance into different components, whose significance was obtained by randomization after 1000 permutations.

| | *P. sylviae* | | | | *T. bifurcata* | | | | *T. bifurcata* males only | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population structure tested | d.f. | Var. comp. | % Var. | P | d.f. | Var. comp. | % Var. | P | d.f. | Var. comp. | % Var. | P |
| **No grouping:** | | | | | | | | | | | | |
| Among infrapopulations | 23 | 1.431 | 18.79 | < 0.001 | 11 | 2.241 | 48.29 | < 0.001 | 10 | 1.881 | 36.81 | < 0.001 |
| Within infrapopulations | 69 | 6.184 | 81.21 | | 46 | 2.400 | 51.71 | | 16 | 3.229 | 63.19 | |
| **Between host populations:** | | | | | | | | | | | | |
| Between host types | 1 | -0.011 | 0 | 0.421 | 1 | -0.137 | 0 | 0.663 | 1 | -0.201 | -4.03 | 0.663 |
| Among | 22 | 1.437 | 18.88 | < 0.001 | 10 | 2.296 | 50.36 | < 0.001 | 9 | 1.980 | 39.54 | < 0.001 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| infrapopulations | | | | | | | | | |
| Within | | | | | | | | | |
| infrapopulations | 69 | 6.184 | 81.27 | 46 | 2.400 | 52.64 | 16 | 3.229 | 64.49 |

**Figure 1.** Evolutionary relationships among the cytochrome oxidase I (COI) haplotypes of feather mites sampled from wintering blackcaps. Optimal NJ trees of the haplotypes of *Proctophyllodes sylviae* and *Trouessartia bifurcata* found on this study, calculated after 1,000 bootstrap replications. * indicates bootstrap support on nodes greater than 50%. The numbers in the circles indicate individual hosts of origin of each haplotype (open circles represent resident blackcaps and filled circles migratory blackcaps). The connections between haplotype and host ID's can be seen in Table S1.




550

551

**Figure 2.** Haplotype networks for the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* sampled from wintering blackcaps. Shading in the circles indicate resident (white) or migratory (black) blackcaps. The shortest link between haplotypes sets the scale for 1 bp sequence difference, and the size of circles is proportional to haplotype frequency.

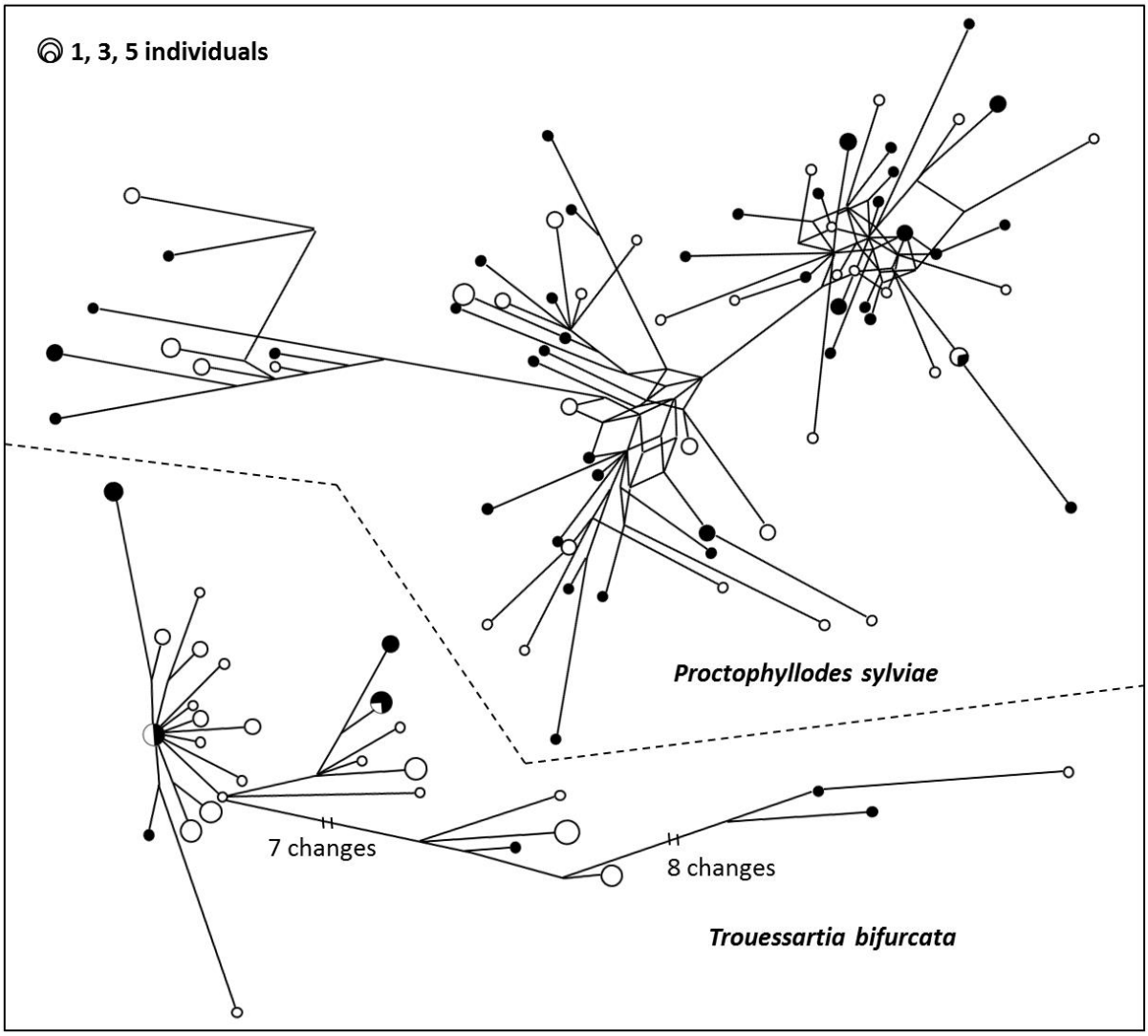


**Figure 3.** Frequency distribution of average within-host pairwise genetic distances among haplotypes of the feather mites *Proctophyllodes sylviae* and *Trouessartia bifurcata* sampled from blackcaps.

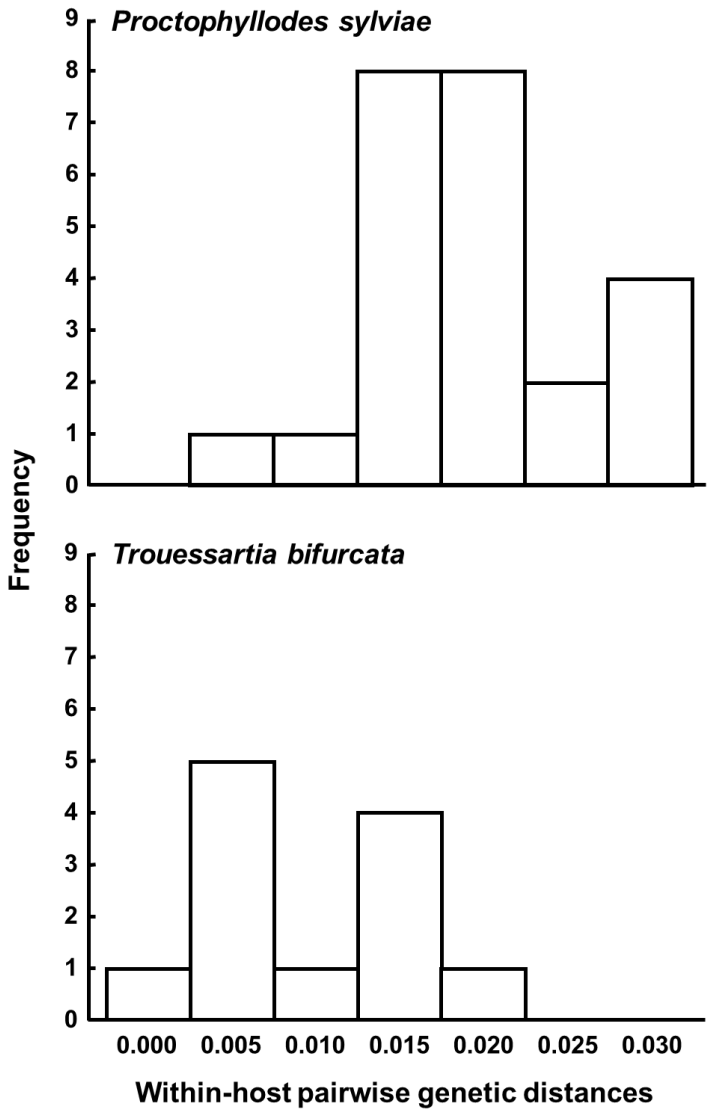


**Table S1.** Feather mite COI haplotypes found in this study and identity of their hosts. Host species: blackcap *Sylvia atricapilla*.

The table shows COI haplotype names and feather mite species. Individual host ID and phenotype (migratory or resident) are also presented. Host ID numbers match those of Figure 1 in the main text of the manuscript.

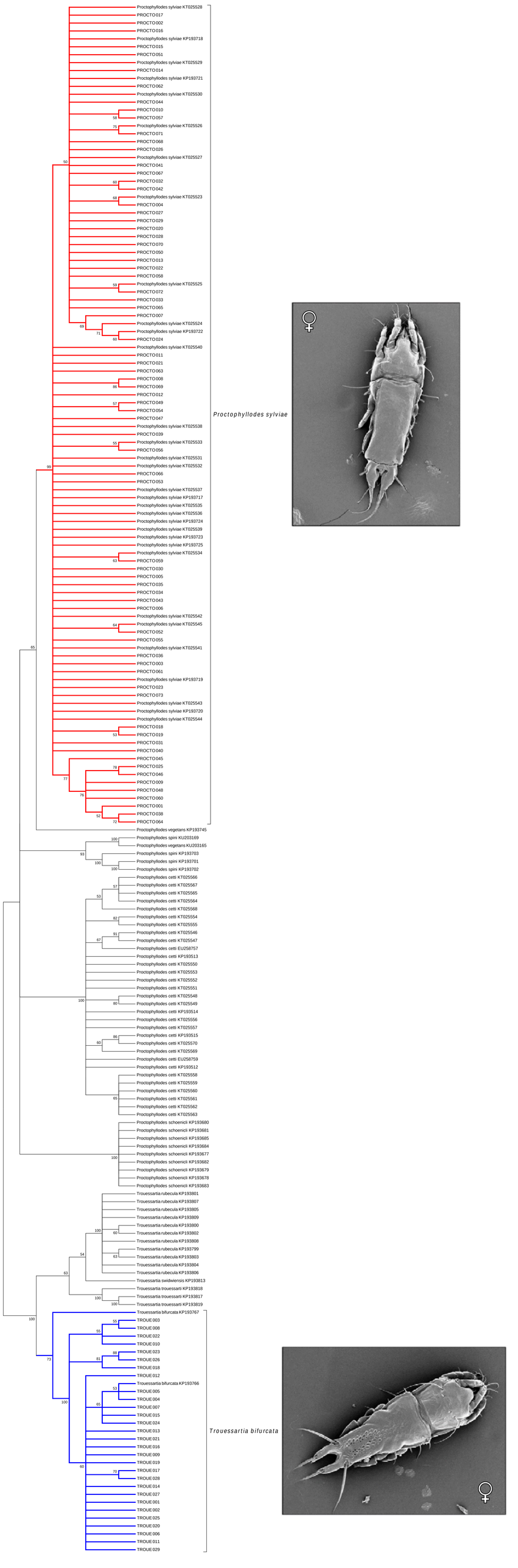
| Feather mite haplotype | Feather mite species | Blackcap ID | Host type |
|---|---|---|---|
| PROCTO_001 | *Proctophyllodes sylviae* | 8 | Migratory |
| PROCTO_001 | *Proctophyllodes sylviae* | 8 | Migratory |
| PROCTO_002 | *Proctophyllodes sylviae* | 8 | Migratory |
| PROCTO_003 | *Proctophyllodes sylviae* | 8 | Migratory |
| PROCTO_004 | *Proctophyllodes sylviae* | 8 | Migratory |
| PROCTO_005 | *Proctophyllodes sylviae* | 22 | Migratory |
| PROCTO_006 | *Proctophyllodes sylviae* | 22 | Migratory |
| PROCTO_007 | *Proctophyllodes sylviae* | 22 | Migratory |
| PROCTO_008 | *Proctophyllodes sylviae* | 22 | Migratory |
| PROCTO_009 | *Proctophyllodes sylviae* | 22 | Migratory |
| PROCTO_010 | *Proctophyllodes sylviae* | 17 | Migratory |
| PROCTO_010 | *Proctophyllodes sylviae* | 17 | Migratory |
| PROCTO_011 | *Proctophyllodes sylviae* | 17 | Migratory |
| PROCTO_012 | *Proctophyllodes sylviae* | 17 | Migratory |
| PROCTO_013 | *Proctophyllodes sylviae* | 3 | Migratory |
| PROCTO_014 | *Proctophyllodes sylviae* | 3 | Migratory |
| PROCTO_015 | *Proctophyllodes sylviae* | 3 | Migratory |
| PROCTO_015 | *Proctophyllodes sylviae* | 3 | Migratory |
| PROCTO_016 | *Proctophyllodes sylviae* | 3 | Migratory |
| PROCTO_017 | *Proctophyllodes sylviae* | 2 | Resident |
| PROCTO_018 | *Proctophyllodes sylviae* | 2 | Resident |
| PROCTO_019 | *Proctophyllodes sylviae* | 2 | Resident |
| PROCTO_019 | *Proctophyllodes sylviae* | 2 | Resident |
| PROCTO_020 | *Proctophyllodes sylviae* | 2 | Resident |
| PROCTO_021 | *Proctophyllodes sylviae* | 21 | Resident |
| PROCTO_021 | *Proctophyllodes sylviae* | 21 | Resident |
| PROCTO_022 | *Proctophyllodes sylviae* | 21 | Resident |
| PROCTO_023 | *Proctophyllodes sylviae* | 21 | Resident |
| PROCTO_024 | *Proctophyllodes sylviae* | 15 | Migratory |
| PROCTO_024 | *Proctophyllodes sylviae* | 26 | Resident |
| PROCTO_024 | *Proctophyllodes sylviae* | 26 | Resident |
| PROCTO_025 | *Proctophyllodes sylviae* | 26 | Resident |
| PROCTO_025 | *Proctophyllodes sylviae* | 26 | Resident |
| PROCTO_026 | *Proctophyllodes sylviae* | 18 | Migratory |
| PROCTO_027 | *Proctophyllodes sylviae* | 18 | Migratory |
| PROCTO_028 | *Proctophyllodes sylviae* | 18 | Migratory |
| PROCTO_029 | *Proctophyllodes sylviae* | 25 | Resident |
| PROCTO_030 | *Proctophyllodes sylviae* | 25 | Resident |
| PROCTO_031 | *Proctophyllodes sylviae* | 25 | Resident |

| | | | |
|---|---|---|---|
| PROCTO_032 | *Proctophyllodes sylviae* | 5 | Resident |
| PROCTO_033 | *Proctophyllodes sylviae* | 5 | Resident |
| PROCTO_034 | *Proctophyllodes sylviae* | 6 | Migratory |
| PROCTO_035 | *Proctophyllodes sylviae* | 6 | Migratory |
| PROCTO_036 | *Proctophyllodes sylviae* | 6 | Migratory |
| PROCTO_038 | *Proctophyllodes sylviae* | 23 | Resident |
| PROCTO_038 | *Proctophyllodes sylviae* | 23 | Resident |
| PROCTO_039 | *Proctophyllodes sylviae* | 23 | Resident |
| PROCTO_039 | *Proctophyllodes sylviae* | 23 | Resident |
| PROCTO_040 | *Proctophyllodes sylviae* | 11 | Migratory |
| PROCTO_040 | *Proctophyllodes sylviae* | 11 | Migratory |
| PROCTO_041 | *Proctophyllodes sylviae* | 11 | Migratory |
| PROCTO_041 | *Proctophyllodes sylviae* | 11 | Migratory |
| PROCTO_042 | *Proctophyllodes sylviae* | 11 | Migratory |
| PROCTO_043 | *Proctophyllodes sylviae* | 13 | Resident |
| PROCTO_043 | *Proctophyllodes sylviae* | 13 | Resident |
| PROCTO_044 | *Proctophyllodes sylviae* | 13 | Resident |
| PROCTO_045 | *Proctophyllodes sylviae* | 7 | Migratory |
| PROCTO_046 | *Proctophyllodes sylviae* | 7 | Migratory |
| PROCTO_047 | *Proctophyllodes sylviae* | 7 | Migratory |
| PROCTO_048 | *Proctophyllodes sylviae* | 7 | Migratory |
| PROCTO_049 | *Proctophyllodes sylviae* | 7 | Migratory |
| PROCTO_050 | *Proctophyllodes sylviae* | 12 | Migratory |
| PROCTO_051 | *Proctophyllodes sylviae* | 12 | Migratory |
| PROCTO_052 | *Proctophyllodes sylviae* | 12 | Migratory |
| PROCTO_053 | *Proctophyllodes sylviae* | 12 | Migratory |
| PROCTO_054 | *Proctophyllodes sylviae* | 24 | Resident |
| PROCTO_054 | *Proctophyllodes sylviae* | 24 | Resident |
| PROCTO_054 | *Proctophyllodes sylviae* | 24 | Resident |
| PROCTO_054 | *Proctophyllodes sylviae* | 24 | Resident |
| PROCTO_055 | *Proctophyllodes sylviae* | 24 | Resident |
| PROCTO_056 | *Proctophyllodes sylviae* | 27 | Resident |
| PROCTO_056 | *Proctophyllodes sylviae* | 27 | Resident |
| PROCTO_057 | *Proctophyllodes sylviae* | 27 | Resident |
| PROCTO_058 | *Proctophyllodes sylviae* | 27 | Resident |
| PROCTO_059 | *Proctophyllodes sylviae* | 27 | Resident |
| PROCTO_060 | *Proctophyllodes sylviae* | 20 | Resident |
| PROCTO_060 | *Proctophyllodes sylviae* | 20 | Resident |
| PROCTO_060 | *Proctophyllodes sylviae* | 20 | Resident |
| PROCTO_061 | *Proctophyllodes sylviae* | 19 | Migratory |
| PROCTO_062 | *Proctophyllodes sylviae* | 19 | Migratory |
| PROCTO_063 | *Proctophyllodes sylviae* | 4 | Resident |
| PROCTO_063 | *Proctophyllodes sylviae* | 4 | Resident |
| PROCTO_064 | *Proctophyllodes sylviae* | 4 | Resident |
| PROCTO_065 | *Proctophyllodes sylviae* | 4 | Resident |
| PROCTO_066 | *Proctophyllodes sylviae* | 14 | Resident |
| PROCTO_067 | *Proctophyllodes sylviae* | 14 | Resident |
| PROCTO_068 | *Proctophyllodes sylviae* | 14 | Resident |

| | | | |
|---|---|---|---|
| PROCTO_069 | *Proctophyllodes sylviae* | 15 | Migratory |
| PROCTO_070 | *Proctophyllodes sylviae* | 15 | Migratory |
| PROCTO_071 | *Proctophyllodes sylviae* | 16 | Migratory |
| PROCTO_072 | *Proctophyllodes sylviae* | 16 | Migratory |
| PROCTO_072 | *Proctophyllodes sylviae* | 16 | Migratory |
| PROCTO_073 | *Proctophyllodes sylviae* | 16 | Migratory |
| TROUE_001 | *Trouessartia bifurcata* | 2 | Resident |
| TROUE_001 | *Trouessartia bifurcata* | 2 | Resident |
| TROUE_002 | *Trouessartia bifurcata* | 2 | Resident |
| TROUE_002 | *Trouessartia bifurcata* | 2 | Resident |
| TROUE_003 | *Trouessartia bifurcata* | 26 | Resident |
| TROUE_003 | *Trouessartia bifurcata* | 26 | Resident |
| TROUE_003 | *Trouessartia bifurcata* | 26 | Resident |
| TROUE_003 | *Trouessartia bifurcata* | 26 | Resident |
| TROUE_003 | *Trouessartia bifurcata* | 26 | Resident |
| TROUE_004 | *Trouessartia bifurcata* | 25 | Resident |
| TROUE_004 | *Trouessartia bifurcata* | 25 | Resident |
| TROUE_004 | *Trouessartia bifurcata* | 25 | Resident |
| TROUE_004 | *Trouessartia bifurcata* | 25 | Resident |
| TROUE_005 | *Trouessartia bifurcata* | 25 | Resident |
| TROUE_006 | *Trouessartia bifurcata* | 1 | Migratory |
| TROUE_006 | *Trouessartia bifurcata* | 1 | Migratory |
| TROUE_006 | *Trouessartia bifurcata* | 21 | Resident |
| TROUE_006 | *Trouessartia bifurcata* | 23 | Resident |
| TROUE_007 | *Trouessartia bifurcata* | 21 | Resident |
| TROUE_008 | *Trouessartia bifurcata* | 21 | Resident |
| TROUE_009 | *Trouessartia bifurcata* | 21 | Resident |
| TROUE_009 | *Trouessartia bifurcata* | 21 | Resident |
| TROUE_010 | *Trouessartia bifurcata* | 5 | Resident |
| TROUE_010 | *Trouessartia bifurcata* | 5 | Resident |
| TROUE_010 | *Trouessartia bifurcata* | 5 | Resident |
| TROUE_010 | *Trouessartia bifurcata* | 5 | Resident |
| TROUE_011 | *Trouessartia bifurcata* | 5 | Resident |
| TROUE_012 | *Trouessartia bifurcata* | 23 | Resident |
| TROUE_013 | *Trouessartia bifurcata* | 23 | Resident |
| TROUE_014 | *Trouessartia bifurcata* | 23 | Resident |
| TROUE_015 | *Trouessartia bifurcata* | 9 | Migratory |
| TROUE_015 | *Trouessartia bifurcata* | 9 | Migratory |
| TROUE_015 | *Trouessartia bifurcata* | 9 | Migratory |
| TROUE_015 | *Trouessartia bifurcata* | 23 | Resident |
| TROUE_016 | *Trouessartia bifurcata* | 27 | Resident |
| TROUE_017 | *Trouessartia bifurcata* | 27 | Resident |
| TROUE_017 | *Trouessartia bifurcata* | 27 | Resident |
| TROUE_017 | *Trouessartia bifurcata* | 27 | Resident |
| TROUE_017 | *Trouessartia bifurcata* | 27 | Resident |
| TROUE_018 | *Trouessartia bifurcata* | 13 | Resident |
| TROUE_019 | *Trouessartia bifurcata* | 13 | Resident |
| TROUE_019 | *Trouessartia bifurcata* | 13 | Resident |

| | | | |
|---|---|---|---|
| TROUE_020 | *Trouessartia bifurcata* | 13 | Resident |
| TROUE_021 | *Trouessartia bifurcata* | 13 | Resident |
| TROUE_022 | *Trouessartia bifurcata* | 1 | Migratory |
| TROUE_023 | *Trouessartia bifurcata* | 1 | Migratory |
| TROUE_024 | *Trouessartia bifurcata* | 9 | Migratory |
| TROUE_024 | *Trouessartia bifurcata* | 9 | Migratory |
| TROUE_025 | *Trouessartia bifurcata* | 10 | Migratory |
| TROUE_025 | *Trouessartia bifurcata* | 10 | Migratory |
| TROUE_025 | *Trouessartia bifurcata* | 10 | Migratory |
| TROUE_026 | *Trouessartia bifurcata* | 10 | Migratory |
| TROUE_027 | *Trouessartia bifurcata* | 10 | Migratory |
| TROUE_028 | *Trouessartia bifurcata* | 20 | Resident |
| TROUE_028 | *Trouessartia bifurcata* | 20 | Resident |
| TROUE_028 | *Trouessartia bifurcata* | 20 | Resident |
| TROUE_028 | *Trouessartia bifurcata* | 20 | Resident |
| TROUE_029 | *Trouessartia bifurcata* | 20 | Resident |

**Figure S1. Assessment of specific identity of the sampled mites.** Consensus bootstrap tree with cut-off value 50% of 196 haplotypes of 9 morphospecies of feather mite of the genera *Proctophyllodes* and *Trouessartia*, run to confirm the specific assignation of our samples. Numbers on branches represent % branch support as calculated by 1,000 bootstrap replications. The clades including ours as well as previously available sequences of the two mite species under study are marked in red (*P. sylviae*) and blue (*T. bifurcata*). © *Images: the authors*.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PROCTO 017
PROCTO 002
PROCTO 016
Proctophyllodes sylviae KP193718
PROCTO 015
PROCTO 051
Proctophyllodes sylviae KT025529
PROCTO 014
Proctophyllodes sylviae KP193721
PROCTO 062
Proctophyllodes sylviae KT025530
PROCTO 044
PROCTO 010
PROCTO 057
Proctophyllodes sylviae KT025526
PROCTO 071
PROCTO 068
PROCTO 026
Proctophyllodes sylviae KT025527
PROCTO 041
PROCTO 067
PROCTO 032
PROCTO 042
Proctophyllodes sylviae KT025523
PROCTO 004
PROCTO 027
PROCTO 029
PROCTO 020
PROCTO 028
PROCTO 070
PROCTO 050
PROCTO 013
PROCTO 022
PROCTO 058
Proctophyllodes sylviae KT025525
PROCTO 072
PROCTO 033
PROCTO 065
PROCTO 007
Proctophyllodes sylviae KT025524
Proctophyllodes sylviae KP193722
PROCTO 024
Proctophyllodes sylviae KT025540
PROCTO 011
PROCTO 021
PROCTO 063
PROCTO 008
PROCTO 069
PROCTO 012
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PROCTO 054
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PROCTO 056
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Proctophyllodes sylviae KT025532
PROCTO 066
PROCTO 053
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Proctophyllodes sylviae KP193717
Proctophyllodes sylviae KT025535
Proctophyllodes sylviae KT025536
Proctophyllodes sylviae KP193724
Proctophyllodes sylviae KT025539
Proctophyllodes sylviae KP193723
Proctophyllodes sylviae KP193725
Proctophyllodes sylviae KT025534
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PROCTO 030
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PROCTO 035
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PROCTO 043
PROCTO 006
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Proctophyllodes sylviae KT025545
PROCTO 052
PROCTO 055
Proctophyllodes sylviae KT025541
PROCTO 036
PROCTO 003
PROCTO 061
Proctophyllodes sylviae KP193719
PROCTO 023
PROCTO 073
Proctophyllodes sylviae KT025543
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PROCTO 031
PROCTO 040
PROCTO 045
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PROCTO 046
PROCTO 009
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PROCTO 038
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Proctophyllodes vegetans KP193745
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Proctophyllodes vegetans KU203165
Proctophyllodes spini KP193703
Proctophyllodes spini KP193701
Proctophyllodes spini KP193702
Proctophyllodes cetti KT025566
Proctophyllodes cetti KT025567
Proctophyllodes cetti KT025565
Proctophyllodes cetti KT025564
Proctophyllodes cetti KT025568
Proctophyllodes cetti KT025554
Proctophyllodes cetti KT025555
Proctophyllodes cetti KT025546
Proctophyllodes cetti KT025547
Proctophyllodes cetti EU258757
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Proctophyllodes cetti KT025553
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Proctophyllodes schoenicli KP193681
Proctophyllodes schoenicli KP193685
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Proctophyllodes schoenicli KP193677
Proctophyllodes schoenicli KP193682
Proctophyllodes schoenicli KP193679
Proctophyllodes schoenicli KP193678
Proctophyllodes schoenicli KP193683
Trouessartia rubecula KP193801
Trouessartia rubecula KP193807
Trouessartia rubecula KP193805
Trouessartia rubecula KP193809
Trouessartia rubecula KP193800
Trouessartia rubecula KP193802
Trouessartia rubecula KP193808
Trouessartia rubecula KP193799
Trouessartia rubecula KP193803
Trouessartia rubecula KP193804
Trouessartia rubecula KP193806
Trouessartia swidwiensis KP193813
Trouessartia trouessarti KP193818
Trouessartia trouessarti KP193817
Trouessartia trouessarti KP193819
Trouessartia bifurcata KP193767
TROUE 003
TROUE 008
TROUE 022
TROUE 010
TROUE 023
TROUE 026
TROUE 018
TROUE 012
Trouessartia bifurcata KP193766
TROUE 005
TROUE 004
TROUE 007
TROUE 015
TROUE 024
TROUE 013
TROUE 021
TROUE 016
TROUE 009
TROUE 019
TROUE 017
TROUE 028
TROUE 014
TROUE 027
TROUE 001
TROUE 002
TROUE 025
TROUE 020
TROUE 006
TROUE 011
TROUE 029
Trouessartia bifurcata