Traditional and geometric morphometric analyses reveal homogeneity in European *Scutacarus acarorum* Goeze, 1780 populations (Acari: Scutacaridae: Heterostigmatina)

Julia Jagersbacher-Baumann*

Institute of Zoology, University of Graz, Graz, Austria

(Received 19 February 2014; accepted 6 October 2014; first published online 5 November 2014)

The mite *Scutacarus acarorum* (Scutacaridae, Heterostigmatina) is one of the dominant bumblebee inquilines in the Holarctic. The wide distribution and the host generalist behaviour of *S. acarorum* suggest that it could be a group of multiple cryptic species. European *S. acarorum* populations and a small population from New York were studied using traditional and geometric morphometric methods to detect possible geographical or host-dependent variation. The analyses revealed homogeneity of all populations, suggesting that the species experienced a bottleneck during the last ice age and that gene flow between the populations is maintained. High variability within populations indicates a high genetic diversity. No host-related morphological differences were detected, suggesting that *S. acarorum* is a true generalist. Fresh mite samples from locations all over the Holarctic are needed to draw further conclusions on the species’ phylogeography and also on its population genetic structure.

**Keywords:** morphometrics; phoresy; host generalists; *Bombus*

Introduction

Like all social hymenopterans, bumblebees (*Bombus*, Apidae) serve as hosts for a variety of mites (Chmielewski 1971; Eickwort 1994). One of the dominant bumblebee inquilines in Europe and North America is the scutacarid mite species *Scutacarus acarorum* Goeze, 1780 (Eickwort 1994). Contrary to Chmielewski’s (1971) report, *S. acarorum* has proven not to be a parasite of its host, but a fungivorous commensal (Schousboe 1986; Ebermann 1995; Jagersbacher-Baumann and Ebermann 2013). The species can indeed be found attached to bumblebees, but only for the purpose of phoretic dispersal. For attachment, these mites use large claws on leg I (Ebermann 1995). Nevertheless, the statements that *S. acarorum* uses its mouthparts for attachment and that it may be a parasite are still misleadingly circulating in the literature (e.g. in Chmielewski and Baker 2008).

*Scutacarus acarorum* shows a wide spatial distribution as well as a wide host range. The species has been reported from several countries: Austria (Ebermann 1980), Germany (Karafiat 1959), Italy (Karafiat 1959), Belgium (Fain et al. 1992; Fain and Baugnée 1996), the Netherlands (Schousboe 1986), Poland (Chmielewski 1971; Chmielewski and Baker 2008), Sweden (Larsson 2007), Denmark (Larsson 2007), the former Czechoslovakia (Mahunka 1967a), Russia (Klimov 1998), Turkey (Çankaya and Kaftanoglu 2006), Mongolia (Mahunka 1965, 1967b), Japan (Kurosa

*Email: julia.jagersbacher-baumann@uni-graz.at*
1980), Canada (Richards and Richards 1976) and the USA (Husband 1968; Cross and Bohart 1969; Delfinado et al. 1976). More recently, *S. acarorum* has also been reported from the Southern hemisphere, namely from Argentina (Maggi et al. 2011). At least eight bumblebee species have been identified as hosts (Schousboe 1986), and *S. acarorum* can preferably be found on large species like *Bombus terrestris*, *B. lapidarius* and *B. agrorum* (Chmielewski 1971). Reports of Halictidae and Formicidae as phoresy hosts (Fain and Baugnée 1996) are unusual and most likely erroneous due to misidentification of the scutacarids.

As more phylogeographic and genetic studies have been conducted in recent years, more cryptic species have been uncovered in several taxa (Knee et al. 2012a). The wide distribution and the generalist behaviour of *S. acarorum* suggest that it could actually be a group of multiple cryptic species as well, defined as species that cannot or can only hardly be distinguished by morphological differences. In the present paper, the landmarks and distance traits of European *S. acarorum* populations from different bumblebee host species were analysed to reveal whether any morphological differentiation correlated to geographic location or host species was present, which would point to the existence of cryptic species.

**Material and methods**

Nine populations of *S. acarorum* from different locations in Central Europe were analysed (Figure 1, Table 1). Microscopic slides were available mostly from the collection of E. Ebermann (EE), as well as from the collection of A. Khaustov (AK) and of the Manchester Museum (MM). Additionally, living specimens of

**Figure 1.** Sampling sites of the studied *Scutacarus acarorum* populations. (1) Thomatal, (2) Southern Styria, (3) Lienz, (4) Erlangen (5) Sandomierz, (6) Poznan, (7) Yalta, (8) Wales, (9) Cheshire and (10) New York.
Table 1. Collection dates of the analysed *Scutacarus acarorum* populations. The collection year of the slides from Erlangen is given as unknown because they are labelled with ‘1927?’.

| Country  | No. in Figure 1 | Location           | Host                                      | Number of mites | Year          | Collected by          | Collection |
|----------|-----------------|--------------------|-------------------------------------------|-----------------|---------------|-----------------------|------------|
| Austria  | 1               | Thomatal           | *Bombus terrestris* (nest)                | 36              | 1982          | Ebermann E.           | EE         |
|          | 2               | Southern Styria    | *B. terrestris*                           | 11              | 2007, 2008    | Jagersbacher-Baumann J. | JJB        |
|          | 3               | Lienz              | not known                                 | 3               | 2010          | not known             | EE         |
| Germany  | 4               | Erlangen           | *B. cognatus* (synonym of *B. pascuorum*) | 6               | not known     | Chmielewski W.        | EE         |
| Poland   | 5               | Sandomierz         | *B. lucorum/l*terrestris                  | 34              | 1984          | Chmielewski W.        | EE         |
|          | 6               | Poznan             | *B. lucorum/l*terrestris and *Bombus* (Psithyrus) sp. | 29              | 1984          | Chmielewski W.        | EE         |
| Ukraine  | 7               | Yalta              | *Bombus sp.*                               | 10              | 2004          | Khaustov A.           | AK         |
| Great Britain | 8        | Wales              | *Bombus sp.*                               | 16              | 1984/85       | Turk R.               | EE         |
|          | 9               | Cheshire           | *B. lucorum/l*terrestris and *Bombus* (Psithyrus) bohemanicus | 7               | 1935, 1941   | Radford C.D., Britten H. | MM         |
| USA      | 10              | New York           | *B. americanorum*                         | 3               | 1940, 1974    | Crabill R., O’Connor B. | EE         |

Note: Collection abbreviations: AK, Alexandr Khaustov; EE, Ernst Ebermann; JJB, Julia Jagersbacher-Baumann; MM, Manchester Museum.
mites were collected from bumblebee queens caught when visiting flowers in Southern Styria in spring 2007 and 2008 by the author (JJB). For collection of mites, the bumblebees were anaesthetized with carbon dioxide (CO₂) and investigated under a stereo microscope. Phoretic mites were removed using insect needles, and the bumblebees were released after removal of their phoronts. For slide preparation, Swan’s embedding medium was used (Swan 1936). Additional remarks on the infestation rate of the analysed Styrian bumblebees are available in the supplementary material.

Additional to the European populations, a small population consisting of only three specimens from New York, USA, was also included in the study. As the morphological distinction between B. lucorum and B. terrestris workers is extremely difficult (Huck et al. 1998; Wolf et al. 2010) and information on the caste was not available on all slides, the hosts are referred to as B. lucorum/terrestris in all uncertain cases.

Females of S. acarorum are dimorphic in terms of phoretic and non-phoretic specimens (Ebermann 1995). Non-phoretic females display a higher degree of intraspecific variability (Jagersbacher-Baumann 2014); therefore, only phoretic females were used for the analyses.

Fifty variables, mostly lengths of and distances between body setae as well as particular apodemata and articles of the legs (Supplementary Material, Table S1), were measured for the traditional morphometric analyses. Illustrations of the respective variables are available in Jagersbacher-Baumann and Ebermann (2012, 2013). The setal nomenclature follows that of Grandjean (1940), modified for Heterostigmatina by Lindquist (1986). Mean, standard deviation and coefficient of variation (CV) were calculated for each variable. Kruskal-Wallis test was used to compare the variables between the populations. Five variables (the ventral setae 1a, 1b, 2a, 3c and the kinner apodeme iap) were removed from the subsequent analyses because they could not be measured reliably. For size correction, each variable was divided through the geometric mean of all variables of the respective specimen (Klimov and OConnor 2004; Klimov et al. 2004, 2006; Jagersbacher-Baumann and Ebermann 2012). Principal component analyses (PCA) were performed on log₁₀-transformed raw and size-corrected data. Correlations between PCs and ‘size’ were calculated by contrasting the PC-values of each specimen against their geometric mean.

For the geometric morphometric analysis, 13 landmarks on the mites’ posterior sternal plate were used (Figure 2). Insertions of body setae as well as the crossing point of particular apodemata were used as landmarks. Data acquisition was performed as described in Jagersbacher-Baumann and Ebermann (2012). For eliminating size, rotation and location, Procrustes superimposition was performed using CoordGen6f (Sheets 2003), and PCA on the obtained shape coordinates was done using PCA Gen6n (Sheets 2003). Multivariate analysis of variance (MANOVA) was used for testing equality of means of all populations. The percentage of specimens correctly classified by all samples canonical variates analysis (CVA) and leave-one-out cross-validation CVA was calculated.

To determine phenetic similarities, unrooted neighbour-joining (NJ) trees were constructed using the Mahalanobis distances between the populations’ means gained by CVA (Gould and Johnston 1972; Kerschbaumer et al. 2011) on the values of the first three canonical variates. Accordingly, pairwise comparisons of populations for testing equality of means were performed on CV values using Bonferroni corrected Hotelling’s T² test. All multivariate analyses were performed with the program PAST (Hammer et al. 2001).
Results

Traditional morphometric analyses

A subjective ‘by eye’ revision during measuring of the continuous variables revealed no distinct qualitative or quantitative morphological differences between the populations. Univariate analysis of the metric data showed a moderately high intraspecific variability indicated by the CV values, which were around 0.10 for most variables in all populations (Table S1). High CVs (> 0.15) could almost exclusively be detected in the lengths of different setae. Most variables (80%) differed significantly ($p < 0.001$) between all populations. Using the geometric mean as reference for size, the specimens from Wales and Yalta turned out to be the largest by trend, whereas those from Sandomierz and Erlangen were the smallest (Figure 3).

PCA on log-transformed raw data resulted in in 45 components, with the first three explaining 57.2% of the total variation (Table 2). All populations clustered together, with some individuals lying slightly outside of the large ‘main cluster’ – for example, two of the three specimens from New York (Figure 4a). No separation of populations was recognizable on any principal component.
PC1, explaining 45.8% of the total variation, was highly correlated with size ($r = 0.96$). Variation on PC1 was extensive in all populations, most of all in the population from Thomatal, which indeed covered all other populations on this principal component. There were nine canonical functions; the first two jointly explained 49.4% of the total variation, the first accounting for 27.4% and the second for 22.0% of the variation. Though some populations were distinct from each other and the population from Thomatal was separated from the rest of the populations to a certain degree, they all generally formed one large cluster (Figure 5a).

The analysis of the log-transformed size-corrected data revealed a very similar result (Figure 4b). PCA resulted in 45 components, with the first three components accounting for 30.8% of the total variation (Table 2). The elimination of the size effect resulted in a decrease of 41.0% from the total variation. Like in the raw data, all populations clearly clustered together. The two individuals from New York placed outside of the cluster in the previous analysis were again slightly separated from the other specimens. The first two of the nine detected canonical functions jointly accounted for 50.2% of the total variation, the first explained 27.1% and the second 23.1% of the variation. Again, all populations together formed one large but scattered cluster, and the visible distances between the single populations appeared more distinct than in the analysis of the raw data (Figure 5b).
Table 2. First three principal components (PC) of log-transformed raw and size-corrected data of *Scutacarus acarorum* populations. High loadings with values > 0.3 are in italics.

|                          | Log-transformed raw data | Log-transformed size-corrected data |
|--------------------------|--------------------------|-----------------------------------|
|                          | PC 1  | PC 2  | PC 3  | PC 1  | PC 2  | PC 3  |
| Claw                     | 0.140 | -0.011| -0.026| -0.025| 0.008| -0.111|
| Solenidion, ω1           | 0.084 | 0.102 | 0.216 | 0.246 | -0.019| 0.121 |
| Solenidion, φ1           | 0.077 | 0.088 | 0.200 | 0.163 | -0.012| 0.100 |
| Tibiotarsus, TiTa1       | 0.133 | 0.017 | 0.060 | 0.051 | 0.006 | -0.043|
| Tarsus, Ta2              | 0.151 | 0.130 | 0.150 | 0.083 | -0.083| -0.002|
| Tarsus, Ta3              | 0.131 | 0.081 | 0.114 | 0.091 | -0.039| 0.064 |
| Trochanter, Tr4          | 0.136 | 0.048 | 0.038 | 0.047 | -0.026| 0.019 |
| Tibiotarsus, TiTa4       | 0.128 | 0.057 | 0.056 | 0.066 | -0.033| 0.015 |
| Tarsal seta, pv”         | 0.206 | 0.047 | 0.221 | -0.220| -0.109| -0.213|
| Tarsal seta, tc”         | 0.291 | -0.356| 0.382 | 0.533 | 0.216 | -0.062|
| Tarsal seta, tc’         | 0.141 | 0.001 | 0.127 | -0.069| -0.031| -0.140|
| Seta, c1                 | 0.204 | 0.025 | 0.001 | -1.080| -0.039| 0.062 |
| Distance between setae, c1-c1 | 0.091 | 0.026 | 0.064 | 0.094 | 0.018 | -0.009|
| Seta, c2                 | 0.158 | -0.036| -0.010 | -0.039| 0.026 | 0.175 |
| Seta, d                  | 0.201 | 0.008 | 0.025 | -0.086| -0.007| 0.116 |
| Distance between setae, d-d | 0.110 | 0.052 | 0.085 | 0.113 | -0.015| -0.062|
| Seta, f                  | 0.164 | 0.080 | 0.102 | -0.066| -0.099| 0.221 |
| Distance between setae, f-f | 0.100 | 0.066 | 0.086 | 0.143 | -0.018| -0.022|
| Seta, h1                 | 0.155 | 0.031 | -0.163| -0.090| -0.063| 0.374 |
| Distance between setae, h1-h1 | 0.139 | -0.067| 0.031 | 0.031 | 0.088 | -0.115|
| Seta, e                  | 0.116 | 0.115 | -0.043| 0.062 | -0.115| 0.389 |
| Seta, h2                 | 0.136 | -0.003| -0.180| -0.058| -0.031| 0.579 |
| Distance between setae, 1a-1a | 0.113 | 0.020 | 0.178 | 0.152 | 0.042 | 0.025 |
| Distance between setae, 1b-1b | 0.114 | 0.019 | 0.109 | 0.091 | 0.014 | -0.036|
| Distance between setae, 2a-2a | 0.121 | 0.027 | 0.088 | 0.083 | 0.000 | -0.024|
| Distance between setae, 2b-2b | 0.119 | 0.036 | 0.118 | 0.107 | 0.006 | -0.062|
| Seta, 2b                 | 0.146 | 0.011 | -0.087| -0.048| -0.020| -0.055|
| Distance between setae, 3c-3c | 0.130 | 0.034 | 0.083 | 0.078 | -0.001 | -0.015|
| Distance between setae, 3a-3a | 0.098 | 0.005 | 0.293 | 0.224 | 0.082 | -0.164|
| Distance between setae, 3b-3b | 0.123 | 0.039 | 0.145 | 0.124 | 0.013 | -0.045|
| Seta, 3a                 | 0.182 | 0.062 | 0.058 | -0.054| -0.054 | -0.012|
| Seta, 3b                 | 0.198 | 0.032 | 0.087 | -0.145| -0.042 | -0.013|
| Seta, 4c                 | 0.196 | -0.024| -0.166| -0.180| -0.026 | -0.027|
| Distance between setae, 4c-4c | 0.125 | 0.056 | 0.075 | 0.086 | -0.021 | -0.037|
| Seta, 4a                 | 0.261 | -0.032| -0.237| -0.365| -0.053 | 0.047 |
| Seta, 4b                 | 0.179 | -0.043| -0.148| -0.157| -0.001 | 0.009 |
| Distance between setae, 4a-4a | 0.126 | 0.145 | 0.173 | 0.150 | -0.082 | -0.071|
| Distance between setae, 4b-4b | 0.110 | 0.115 | 0.103 | 0.144 | -0.069 | -0.084|
| Trichobothrium, tb       | 0.080 | 0.011 | -0.025| 0.105 | 0.014 | 0.060 |
| Width of gnathosoma, wg   | 0.085 | -0.014| 0.175 | 0.201 | 0.074 | 0.186 |
| Width of anterior sternal plate, waStpl | 0.112 | 0.075 | 0.083 | 0.093 | -0.029 | -0.027|
| Apodeme, ap4              | 0.141 | 0.115 | 0.211 | 0.104 | -0.056 | 0.037 |
| Length of anterior sternal plate, laStpl | 0.182 | -0.028| 0.068 | -0.059| 0.078 | 0.211 |
| Length of posterior sternal plate, lpStpl | 0.161 | 0.073 | 0.041 | 0.006 | -0.050 | -0.005|
| Width of genital sclerite, wgsc | 0.109 | -0.844| 0.359 | 0.062 | 0.918 | 0.088 |
| Variance explained (%)    | 45.91 | 6.05  | 5.27  | 13.5  | 10.1  | 7.2   |
| Total variation           | 0.106 |       |       | 0.063 |       |       |
Geometric morphometric analysis

The geometric morphometric analysis revealed no separation between the populations at all (Figure 4c). PCA resulted in 22 principal components, with the first three explaining 46.7% of the total variation. In contrast to the traditional

Figure 4. Scatter plots from principal component analysis (PCA). (A) Log-transformed raw data; (B) log-transformed size corrected data; (C) shape coordinates (including deformation grids showing the shape deformation explained by PC1 and PC2).

*J. Jagersbacher-Baumann*
morphometrics, few outlying specimens were present. No principal component was correlated with ‘size’.

The first two canonical functions explained 53.3% of the total variation, the first one accounting for 33.9% and the second one for 19.4% of the variation. Again, nine canonical functions were found. All populations clearly clustered together and no distinct separation between pairs of populations was present (Figure 5c).

In all three morphometric analyses, MANOVA revealed significant ($p < 0.001$) differences between all populations, and post-hoc pairwise comparisons also showed significant differences between most populations (Table 3). However, it was not possible to sufficiently classify the populations by CVA (Table S2). The percentage of specimens correctly classified by leave-one-out cross-validation CVA was below
62.0% in all analyses. The worst classification results (only 34.3% of the specimens were correctly classified) were gained from analysis of shape variables.

The unrooted NJ trees based on the phenetic similarities between the populations (Figure 6) showed the following results: firstly, populations from geographically close-by locations didn’t cluster together on the trees. For example, the three populations from Austria (Lienz, Thomatal and Southern Styria) were found far from each other in all of the trees. Secondly, the population from New York always differed most clearly from the European populations and, thirdly, the differences between the populations were most pronounced in the analysis of size-corrected data, and least clear in the analysis of shape variables.

Discussion

European S. acarorum populations show morphological homogeneity

European populations of S. acarorum do not differ in qualitative variables like shapes of setae, and the present analyses revealed an overall morphological homogeneity shown by metric variables as well. The remarkable morphological similarity of the small population from New York to the European populations will be discussed below. The data sets and methods applied in the present study already proved their sensitivity in detecting morphological differences in other questions regarding scutacarid mites: for example, they revealed differences between morphologically similar scutacarid species belonging to the acarorum species-complex (Jagersbacher-Baumann 2014) and between populations of the African scutacarid species Heterodispus foveatus (Jagersbacher-Baumann and Ebermann 2012). The homogeneity detected in the present study thus is a reliable result and not an artefact of the applied methodology.

The homogeneous morphology of European S. acarorum is likely to be the result of a bottleneck the species experienced during the last ice age. Accordingly, redistribution supposedly started at the beginning of the last warm period and there wasn’t enough time for evolution of geographic variations on the European continent yet. The subtle differences between the populations are clearest in size-corrected data, which suggests that they represent genetic and not environmentally induced variation (Klimov et al. 2006). The genetic variability of S. acarorum is, however, totally unknown at present. DNA extraction and sequencing of the mitochondrial cytochrome oxidase I gene for DNA-barcoding have already been successfully performed in other Scutacaridae, but the mites’ small size may make enhanced extraction strategies necessary (Young et al. 2012; Jagersbacher-Baumann unpublished). Theoretically, the morphological homogeneity of European S. acarorum could also be a result of the global commercialisation of B. terrestris, which started about three decades ago (Dafni et al. 2010; see below). The almost worldwide export of bumblebees could have been the cause of a mixing between European bumblebee populations and consequently also of the associated mites. However, since several of the analysed mite populations were sampled before the time bumblebee commercialisation started (see Table 1), this explanation is void. As phenetic similarities between the mite populations do not reflect any geographical pattern and the within-population variability is high, the degree of genetic diversity within S. acarorum is also supposed to be high. Very similar results were found by Ebermann et al. (2013) for
Table 3. Results (p-values) of pairwise comparisons of coefficient of variation (CV) values of populations using Bonferroni corrected Hotelling’s $T^2$ test. Italics indicate significant differences.

|                   | Southern Styria | Erlangen  | Sandomierz | Poznan  | New York | Wales  | Yalta  | Cheshire | Lienz  |
|-------------------|-----------------|-----------|------------|---------|----------|--------|--------|----------|--------|
| **Raw data**      |                 |           |            |         |          |        |        |          |        |
| Thomatal          | $8.68E-15$      | $9.97E-12$| $7.48E-22$ | $1.19E-22$ | $3.89E-06$ | $3.74E-16$ | $1.77E-16$ | $1.42E-10$ | $8.35E-07$ |
| Southern Styria    | $0.00237577$    | $8.97E-15$| $1.51E-09$ | $0.00341269$ | $1$ | $3.48E-05$ | $5.20E-05$ | $1$ |        |
| Erlangen          | $3.54E-12$      | $0.00049569$ | $0.0319573$ | $0.00014701$ | $3.08E-05$ | $0.00118043$ | $1$ |         |        |
| Sandomierz        | $1.37E-14$      | $2.44E-07$ | $1.81E-14$ | $2.16E-07$ | $1$ | $9.24E-05$ |        |         |        |
| Poznan            |                 |           |            |         |          | $6.07E-09$ | $1.75E-09$ | $1.66E-09$ | $5.78E-06$ | $0.0579982$ |
| New York          |                 |           |            |         |          | $0.00033085$ | $0.0143265$ | $0.121568$ | $1$ |        |
| Wales             |                 |           |            |         |          | $4.13E-05$ | $3.24E-05$ | $1$ |         |        |
| Yalta             |                 |           |            |         |          |          | $0.245843$ | $0.620834$ |        |        |
| Cheshire          |                 |           |            |         |          |          |          | $0.600225$ |        |        |
| **Size corrected data** |                 |           |            |         |          |        |        |          |        |
| Thomatal          | $8.27E-15$      | $8.81E-11$| $1.02E-18$ | $3.11E-19$ | $1.02E-09$ | $6.51E-16$ | $3.08E-18$ | $1.49E-08$ | $1.30E-07$ |
| Southern Styria    | $0.00055885$    | $2.39E-14$| $1.34E-08$ | $0.00084094$ | $0.324446$ | $2.09E-06$ | $0.00019835$ | $1$ |        |
| Erlangen          | $1.93E-12$      | $0.0109821$ | $0.0189094$ | $4.63E-05$ | $6.39E-06$ | $0.00146893$ | $0.851367$ |        |        |
| Sandomierz        | $3.27E-17$      | $3.67E-12$ | $2.40E-12$ | $6.41E-09$ | $1$ | $5.14E-05$ |        |         |        |
| Poznan            | $7.04E-11$      | $4.67E-07$ | $6.81E-12$ | $1.60E-06$ | $0.143105$ |        |         |        |        |
| New York          |                 |           |            |         |          | $4.63E-06$ | $0.00011624$ | $0.0116705$ | $1$ |        |
| Wales             |                 |           |            |         |          | $4.00E-06$ | $0.00137494$ | $1$ |         |        |
| Yalta             |                 |           |            |         |          |          | $0.0705255$ | $0.193131$ |        |        |
| Cheshire          |                 |           |            |         |          |          |          | $0.829838$ |        |        |
| **Shape data**    |                 |           |            |         |          |        |        |          |        |
| Thomatal          | $9.51E-06$      | $0.00243083$ | $4.17E-10$ | $1.46E-08$ | $3.10E-05$ | $2.72E-07$ | $8.08E-06$ | $0.485339$ | $0.00485052$ |
| Southern Styria    | $1$             | $1.58E-06$ | $0.0150372$ | $0.238182$ | $0.100022$ | $0.359964$ | $0.42601$ | $1$ |        |
| Erlangen          | $0.288175$      | $1$       | $1$       | $1$       | $1$       | $1$       | $1$       | $1$ |        |
| Sandomierz        | $0.00982419$    | $0.00208223$ | $0.0929702$ | $0.0391525$ | $0.260725$ | $0.476796$ |        |        |        |
| Poznan            | $0.00267216$    | $1$       | $1$       | $1$       | $1$       | $1$       | $1$       | $1$ |        |
| New York          |                 |           |            |         |          | $0.069439$ | $1$       | $0.599805$ | $1$ |        |
| Wales             |                 |           |            |         |          | $1$       | $1$       | $1$ |        |        |
| Yalta             |                 |           |            |         |          | $1$       |        |        |        |        |
| Cheshire          |                 |           |            |         |          | $1$       |        |        |        |        |
the central European scutacarid *Imparipes burgeri*, which uses a variety of wild bees and digger wasps as hosts, and by Navia et al. (2009) for Brazilian populations of the coconut mite *Aceria guerreronis* (Eriophyidae).

The present results also suggest that gene flow between European *S. acarorum* populations is maintained. Most mites analysed in the present study were associated with the buff-tailed bumblebee *B. terrestris*, which is one of the preferred hosts or even the main host of (European) *S. acarorum* (Chmielewski 1971). The population structure of *S. acarorum* shows similarities to that of *B. terrestris*: European mainland populations of *B. terrestris* show morphological as well as genetic homogeneity, which is regarded to be the result of a bottleneck experience (Estoup et al. 1996; Widmer et al. 1998; Lecocq et al. 2013). Moreover, the homogeneity of *B. terrestris* can also be explained by the species' abundance in the Western Palaearctic, and its extreme mobility, which makes gene flow across the European mainland possible (Estoup et al. 1996; Widmer et al. 1998). By using their host for dispersal and by

---

**Figure 6.** Unrooted neighbour-joining (NJ) trees of *Scutacarus acarorum* populations based on squared Mahalanobis distances obtained from canonical variates analysis (CVA) on canonical variates values of (A) log-transformed raw data; (B) log-transformed size corrected data; (C) shape coordinates.
transferring horizontally between hosts, phoretic mites like *S. acarorum* can also sustain gene flow. A horizontal switch between conspecific host individuals or between host specimens belonging to different species can occur during copulation, when a bumblebee queen tries to usurp another queen’s nest, or it can happen on flowers bumblebees visit for foraging (Eickwort 1994; Schwarz and Huck 1997). As *S. acarorum* is supposed to be a host generalist, its dispersal abilities presumably are even higher than those of *B. terrestris*. While European mainland populations of *B. terrestris* display homogeneity, European island populations from the Canary Islands, Madeira, Tyrrhenian Islands and Mediterranean Islands differ morphologically as well genetically from mainland populations. Nevertheless, they all can be assigned to the same species (Estoup et al. 1996; Widmer et al. 1998; Rasmont et al. 2008; Lecocq et al. 2013). The same may apply to *S. acarorum* associated with these island populations, but so far, the mite has never been reported from the respective locations. For future studies, extended sampling of *S. acarorum* across Europe is planned to get fresh specimens not only for morphometric but also for molecular genetic analyses. European islands will also be included to check whether *S. acarorum* is present and, if so, to analyse whether the mites’ population structure correlates with that of its host or diverges from it due to the mites’ generalist behaviour and ability to thrive independently from any host in soil (Karafiat 1959; Kampmann 1991; Ebermann 1995).

To date, *S. acarorum* is regarded to be a true host generalist. Most specimens of the European populations used for the present study were found in association with *B. lucorum*/*terrestris* (Table 1); a few individuals were also collected from undetermined *Bombus* species and from cuckoo bumblebee *Bombus* (*Psithyrus*) species. None of the mite specimens from the latter host species were separated from the other mites from their respective locations in morphometric analyses. However, the sample sizes for these mites were too low to perform statistical tests on host-related morphological variation. The specimens from Erlangen were all found on *B. pascuorum* and were available in a reasonable sample size. They did not show morphological differences from the other populations either, which would have suggested host-specific (cryptic) speciation. Although the present results support the hypothesis of *S. acarorum* being a generalist, systematic sampling of different bumblebee species in sufficient sample sizes is needed. Thus, it will be possible to further evaluate the degree of morphological and genetic variation between specimens from different hosts and to detect whether the species really is a generalist or rather consists of multiple specialist species. Studies on beetle-associated uropodoid mites with apparent broad host ranges revealed both species that are really host generalists as well as species that actually are a complex of cryptic species (Knee et al. 2012a, 2012b).

**The population from New York**

The small population from New York differed only slightly from the European populations. In consideration of the large spatial distance between New York and Europe (about 6300 km), the low degree of morphological difference is surprising. It may be the result of a recent, unintended anthropogenic introduction of the mites in the Nearctic via the Atlantic Ocean. However, it is not clear how the mites could have been dragged into the Nearctic. The global bumblebee commercialisation of *B. terrestris* (see
below) is out of the question of being responsible for the spread of *S. acarorum* in North America: not only is the import of non-native bumblebees (particularly of *B. terrestris*) prohibited in the United States (Dafni et al. 2010), but the mite was also already present before the commercialisation started (the analysed slides are dated from 1940 and 1974; Table 1). Anthropogenic introduction of bumblebees or mites may also have happened earlier, for example via shipping of plants.

The morphological similarity of the North American population presumably indeed reflects a global homogeneity of *S. acarorum*. The colonisation of the Nearctic by *S. acarorum* thus temporally corresponded with the mites’ redistribution in Europe, and it most likely happened via Russia, Asia and Beringia, the land bridge which connected the Palearctic and Nearctic until the end of the last ice age. On the North American continent, a host shift to other species like *B. americanorum* (from which the investigated specimens had been collected) happened: *S. acarorum* can be found on presumably most boreal species of North American *Bombus* species (Cross and Bohart 1969). Unfortunately, no other specimens from Asia and North America were available for the present study. Again, further sampling is urgently needed for future studies on the biogeography and geographic variation of *S. acarorum* across the Holarctic. By analysing material from the whole area of its distribution, it will also be possible to gain information on the probable origin of the species.

**Worldwide spreading of *S. acarorum***

While other specialised bumblebee species are declining (Goulson et al. 2008), the *B. terrestris* stock is stable (Goulson et al. 2008; Rasmont et al. 2008; Dafni et al. 2010): the species can spread among new habitats at an extreme speed; it is a generalist and can thus easily adapt to new habitats. Due to worldwide bumblebee commercialisation for pollination mostly of tomatoes in greenhouses, *B. terrestris* is spreading in recent years and poses a serious threat to native bumblebee species (Dafni et al. 2010). Although the bumblebees are not supposed to be able to escape from greenhouses, they have proven to forage in the wild, interact with native species and spread their genes into feral conspecific populations (Otterstatter and Thomson 2008; Kraus et al. 2011).

Along with the bumblebees, associated pathogens and parasites (Goka et al. 2001; Otterstatter and Thomson 2008) as well as presumably harmless commensals are also likely to colonise new areas. Accordingly, the distribution of *S. acarorum* could therefore expand to regions beyond the Holarctic. So far, *S. acarorum* has not been reported either from New Zealand, where *B. terrestris* was introduced around the beginning of the nineteenth century (Donovan 1980; MacFarlane 2005), or from Tasmania, where it was first detected in 1992 and is supposed to have invaded via New Zealand (Allen et al. 2007). In South America, *B. terrestris* was introduced in Chile in 1998 and consequently invaded Argentina (Torretta et al. 2006). Few studies on the acarofauna associated with South American bumblebees are available, but Maggi et al. (2011) and Reavinerá et al. (2014) found *S. acarorum* in low abundance on native Argentinian bumblebee species, and these records may be the first clues to carry-over of scutacarids by *B. terrestris* and infestation of native bumblebees.
Acknowledgements

I am grateful to Alexandr Khaustov, Ernst Ebermann and Dmitri Logunov from the Manchester Museum for making specimens of *S. acarorum* available for my study. Special thanks to Tobias Pfingstl for his help.

Supplemental material

Supplemental material for this article can be accessed here: http://dx.doi.org/10.1080/00222933.2014.974705

References

Allen GR, Seeman OD, Schmid-Hempel P, Buttermore RE. 2007. Low parasite loads accompany the invading population of the bumblebee, *Bombus terrestris* in Tasmania. Insectes Soc. 54:56–63. doi:10.1007/s00040-007-0908-y

Çankaya NE, Kaftanoglu O. 2006. An investigation on some diseases and parasites of bumblebee queens (*Bombus terrestris* L.) in Turkey. Pak J Biol Sci. 9:1281–1286.

Chmielewski W. 1971. The mites (Acarina) found on bumble bees (*Bombus* Latr.) and in their nests. Ekol Pol. 19:57–71.

Chmielewski W, Baker RA. 2008. Mites (Acarina) phoretic on some common bumblebee species (*Bombus* spp.) from the Puławy area (South-Eastern Poland). J Apicult Sci. 52:37–47.

Cross EA, Bohart GE. 1969. Phoretic behavior of four species of alkali bee mites as influenced by season and host sex. J Kans Entomol Soc. 42:195–219.

Dafni A, Kevan P, Gross CL, Goka K. 2010. *Bombus terrestris*, pollinator, invasive and pest: an assessment of problems associated with its widespread introductions for commercial purposes. Appl Entomol Zool. 45:101–113. doi:10.1303/aez.2010.101

Delfinado EW, Baker MD, Abbatiello MJ. 1976. Terrestrial mites of New York III. The family Scutacaridae. J New York Entomol Soc. 84:106–145.

Donovan BJ. 1980. Interactions between native and introduced bees in New Zealand. New Zeal J Ecol. 3:104–116.

Ebermann E. 1980. Neue Funde bodenbewohnender Milben (Fam. Scutacaridae) aus Kärnten und benachbarten Gebieten [New findings of soil inhabiting mites (Fam. Scutacaridae) from Carinthia and neighbouring regions]. Carinthia II. 90:347–363. German.

Ebermann E. 1995. *Scutacarus acarorum* (Goeze, 1780); Heterostigmata, Scutacaridae- an example for the interrelationship between phoresy and polymorphism in mites. In: Kropecynska, D. et al., editors. The Acari: proceedings of the second symposium of EURAAC. Warsaw: Oficyna Dabor; p. 193–196.

Ebermann E, Hall M, Hausr-Hofstätter U, Jagersbacher-Baumann JM, Kirchner R, Pfingstl T, Plassnig E. 2013. A new phoretic mite species with remarks to the phenomenon “Sporothecae” (Acari, Scutacaridae; Hymenoptera, Aculeata). Zool Anz. 252:234–242. doi:10.1016/j.jcz.2012.06.003

Eickwort GC. 1994. Evolution and life-history patterns of mites associated with bees. In: Houck MA, editor. Mites. Ecological and evolutionary analyses of life-history patterns. New York (NY): Chapman & Hall; p. 218–251.

Estoup A, Solignac M, Cornuet JM, Goudet J, Scholl A. 1996. Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. Mol Ecol. 5:19–31. doi:10.1111/j.1365-294X.1996.tb00288.x

Fain A, Baugnée JY. 1996. Acariens phoretiques ou parasites recoltes sur des insectes du sud de la Belgique [Phoretic or parasitic mites found on insects in the South of Belgium]. Bull Ann Soc R Entomol Belg Entomol. 132:19–33. French
Fain A, Baugnée JY, Hidvegi F. 1992. Acariens phoretiques ou parasites recoltes sur des Hymenopteres et un Homoptere dans la region de Treignes, en Belgique [Phoretic or parasitic mites found on hymenopterans and one homoptere in the region of Treignes in Belgium]. Bull Ann Soc R Entomol Belg. 128:335–338. French

Goeze JAE. 1780. Herrn Pastor Gözens neuentdeckte Theile an einigen Insekten [Mister pastor Gőze’s newly discovered parts on some insects]. Der Naturforscher. 14:93–102. German.

Goka K, Okabe K, Yoneda M, Niwa S. 2001. Bumblebee commercialization will cause worldwide migration of parasitic mites. Mol Ecol. 10:2095–2099. doi:10.1046/j.0962-1083.2001.01323.x

Gould SJ, Johnston RF. 1972. Geographic variation. Annu Rev Ecol Syst. 3:457–498. doi:10.1146/annurev.es.03.110172.002325

Goulson D, Lye GC, Darvill B. 2008. Decline and conservation of bumble bees. Annu Rev Entomol. 53:191–208. doi:10.1146/annurev.ento.53.103106.093454

Grandjean F. 1940. Les poils et les organes sensitifs portés par les pattes et le palpe chez les Oribates. Deuxième partie [The hairs and sensitive organs on the legs and palps of oribatids. Part two]. Bull Soc Zool Fr. 65:32–44. French

Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: paleontological statistics software package for education and data analysis. Paleontologica Electronica. 4:9.

Huck K, Schwarz HH, Schmid-Hempel P. 1998. Host choice in the phoretic mite *Parasitellus fucorum* (Mesostigmata: Parasitidae): which bumblebee caste is the best? Oecologia. 115:385–390. doi:10.1007/s004420050532

Husband RW. 1968. Acarina associated with Michigan Bombinae. Pap Mich Acad Sci Arts Lett. 53:109–112.

Jagersbacher-Baumann J. 2014. Species differentiation of scutacarid mites (Heterostigmatina) using multivariate morphometric methods. Exp Appl Acarol. 62:279–292. doi: 10.1007/s10493-013-9747-x

Jagersbacher-Baumann J, Ebermann E. 2012. Fungal spore transfer and intraspecific variability of a newly described African soil mite (Heterostigmata, Scutacaridae, *Heterodispus*). Zool Anz. 251:101–114. doi: 10.1016/j.jcz.2011.05.008

Jagersbacher-Baumann J, Ebermann E. 2013. Methods for rearing scutacarid mites (Acari, Heterostigmatina) and the influence of laboratory cultures on morphometric variables. Exp Appl Acarol. 59:447–462. doi: 10.1007/s10493-012-9621-2

Kampmann T. 1991. The density of Tarsonemida in cropped arable soil in relation to fertilizer and crop-protection treatments. In: Schuster R, Murphy PW, editors. The Acari: reproduction, development and life-history strategies. London: Springer; p. 485–490.

Karafiat H. 1959. Systematik und Ökologie der Scutacariden [Systematics and ecology of scutacarid mites]. In: Stammer HJ, editor. Beiträge zur Systematik und Ökologie mitteleuropäischer Acarina. Band I, Tyroglyphidae und Tarsonemini. Leipzig: Akademische Verlagsgesellschaft Geest & Portig K.-G.; p. 627–712. German.

Kerschbaumer M, Postl L, Koch M, Wiedl T, Sturmbauer C. 2011. Morphological distinctness despite large-scale phenotypic plasticity- analysis of wild and pond-bred juveniles of allopatric populations of *Tropheus moorii*. Naturwissenschaften. 98:125–134. doi:10.1007/s00114-010-0751-2

Klimov PB. 1998. To the knowledge of mites and ticks (Acari) of Kuril Islands. Far East Ent. 63:1–36.

Klimov PB, Bochkov AV, OConnor BM. 2006. Host specificity and multivariate diagnostics of cryptic species in predaceous cheyletid mites of the genus *Cheletophyes* (Acari: Cheyletidae) associated with large carpenter bees. Biol J Linn Soc. 87:45–58. doi:10.1111/j.1095-8312.2006.00554.x

Klimov PB, Lekveishvili M, Dowling APG, OConnor BM. 2004. Multivariate analysis of morphological variation in two cryptic species of *Sancassania* (Acari: Acaridae) from
Klimov PB, O'Connor BM. 2004. Multivariate discrimination among cryptic species of the mite genus Chaetodactylus (Acari: Chaetodactylidae) associated with bees of the genus Lithurgus (Hymenoptera: Megachilidae) in North America. Exp Appl Acarol. 33:157–182. doi:10.1023/B:APPA.0000032927.78170.c1

Knee W, Beaulieu F, Skevington JH, Kelso S, Cognato AI, Forbes MR. 2012a. Species Boundaries and Host Range of Tortoise Mites (Uropodoidea) Phoretic on Bark Beetles (Scolytinae), Using Morphometric and Molecular Markers. PLoS ONE. 7:e47243. doi:10.1371/journal.pone.0047243

Knee W, Beaulieu F, Skevington JH, Kelso S, Forbes MR. 2012b. Cryptic species of mites (Uropodoidea: Uroobovella spp.) associated with burying beetles (Silphidae: Nicrophorus): the collapse of a host generalist revealed by molecular and morphological analyses. Mol Phylogenet Evol. 65:276–286. doi:10.1016/j.ympev.2012.06.013

Kraus FB, Szentgyörgyi H, Rożej E, Rhode M, Woyciechowski M, Moritz RFA. 2011. Greenhouse bumblebees (Bombus terrestris) spread their genes into the wild. Conserv Genet. 12:187–192. doi:10.1007/s10592-010-0131-7

Kurosa K. 1980. Caraboacaridae, Pygmephoridae, Scutacaridae. In: Ehara S, editor. Illustrations of the mites and ticks of Japan. Tokyo: Zenkoku Nôson Kyôiku Kyôkai Inc; p. 214–241.

Larsson JIR. 2007. Cytological variation and pathogenicity of the bumble bee parasite Nosema bomby (Microspora, Nosematidae). J Invertebr Pathol. 94:1–11. doi:10.1016/j.jip.2006.07.006

Lecocq T, Vereecken NJ, Michez D, Dellicour S, Lhomme P, Valterová I, Rasplus J, Rasmont P. 2013. Patterns of genetic and reproductive traits differentiation in mainland vs. Corsican populations of bumblebees. PLoS ONE. 8:e65642.

Lindquist EE. 1986. The world genera of Tarsonemidae (Acari: Heterostigmata): a morphological, phylogenetic, and systematic revision, with a reclassification of family-group taxa in the Heterostigmata. Mem Entomol Soc Can. 118:1–517. doi:10.4039/entm118136fv

MacFarlane RP. 2005. Mites associated with bumble bees (Bombus: Apidae) in New Zealand. Rec Canterbury Mus. 19:29–34.

Maggi M, Lucia M, Abrahamovich AH. 2011. Study of the acarofauna of native bumblebee species (Bombus) from Argentina. Apidologie. 42:280–292. doi:10.1007/s13592-011-0018-8

Mahunka S. 1965. Ergebnisse der zoologischen Forsuchen von Dr. Z. Kaszab in der Mongolei. 30. Acari: Pyemotidae and Scutacaridae. [Results of Dr. Z. Kaszab’s zoological research in Mongolia. 30. Acari: Pyemotidae and Scutacaridae]. Annls Hist-Nat Mus Nat Hung Pars Zool. 57:435–441. English, only title in German.

Mahunka S. 1967a. Beiträge zur Kenntnis der Tschechoslowakischen Tarsonemini-Fauna [Contributions to the knowledge about the tarsonemid fauna of Czechoslovakia]. Acta Soc Zool Bohemoslov. 3:240–244. German

Mahunka S. 1967b. 83. Acari: Pyemotidae and Scutacaridae. Ergebnisse der zoologischen Forsuchen von Dr. Z. Kaszab in der Mongolei. 83. Acari: Pyemotidae and Scutacaridae. Annls Hist-Nat Mus Nat Hung Pars Zool. 57:435–441. English, only title in German.

Navia D, Moraes GJ, Querino RB. 2009. Geographic pattern of morphological variation of the coconut mite, Aceria guerreronis Keifer (Acari: Eriophyidae), using multivariate morphometry. Braz J Biol. 69:773–783. doi:10.1590/S1519-69842009000400004

Otterstatter MC, Thomson JD. 2008. Does pathogen spillover from commercially reared bumble bees threaten wild pollinators? PLoS ONE. 3:e2771. doi:10.1371/journal.pone.0002771
Rasmont P, Coppée A, Michez D, de Meulemeester T. 2008. An overview of the *Bombus terrestris* (L. 1758) subspecies (Hymenoptera: Apidae). Ann Soc Entomol Fr (n.s.). 44:243–250.

Revainera P, Lucia M, Abrahamovich AH, Maggi M. 2014. Spatial aggregation of phoretic mites on *Bombus atratus* and *Bombus opifex* (Hymenoptera: Apidae) in Argentina. Apidologie. 45:579–589. doi:10.1007/s13592-014-0275-4

Richards LA, Richards KW. 1976. Parasitid mites associated with bumblebees in Alberta, Canada (Acarina: Parasitidae; Hymenoptera: Apidae) II. Biology. Univ Kans Sci Bull. 51:1–18.

Schousboe C. 1986. On the biology of *Scutacarus acarorum* Goeze (Acari: Trombidiformes). Acarologia. 27:151–158.

Schwarz HH, Huck K. 1997. Phoretic mites use flowers to transfer between foraging bumblebees. Insect Soc. 44:303–310. doi:10.1007/s000400050051

Sheets HD. 2003. IMP-Integrated Morphometrics Package. Department of Physics, Canisius College, Buffalo, NY; [cited 2013 July 31]. Available from: http://www3.canisius.edu/~sheets/morphsoft.html

Swan DC. 1936. Berlese’s fluid: remarks upon its preparation and use as a mounting medium. Bull Entomol Res. 27:389–391. doi:10.1017/S0007485300058259

Torretta JP, Medan D, Abrahamovich AH. 2006. First record of the invasive bumblebee *Bombus terrestris* (L.) (Hymenoptera, Apidae) in Argentina. T Am Entomol Soc. 132:285–289.

Widmer A, Schmid-Hempel P, Estoup A, Scholls A. 1998. Population genetic structure and colonization history of *Bombus terrestris* s.l. (Hymenoptera: Apidae) from the Canary Islands and Madeira. Heredity. 81:563–572. doi:10.1046/j.1365-2540.1998.00407.x

Wolf S, Rohde M, Moritz RFA. 2010. The reliability of morphological traits in the differentiation of *Bombus terrestris* and *B. lucorum* (Hymenoptera: Apidae). Apidologie. 41:45–53. doi:10.1051/apido/2009048

Young MR, Behan-Pelletier VM, Hebert PDN. 2012. Revealing the hyperdiverse mite fauna of subarctic Canada through DNA barcoding. PLoS ONE. 7:e48755. doi:10.1371/journal.pone.0048755