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RESEARCH ARTICLE

Host range of the phoretic mite genus *Parasitellus* (Acariformes: Parasitidae) on wild bees (Hymenoptera: Apiformes), with specific notes on its morphological and genetic diversity in Northern Germany

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Abstract

Bees, as important pollinators, have received much attention in the recent past. Similarly, some of their associated invertebrates, such as *Varroa* Oudemans, 1904 mites, have been intensively studied. Especially in social bees, complex nests promote a large community of organisms associated with the bees, mites being one group commonly associated with them. Yet, surprisingly little is known about most of these mutualists and parasites. In this study, we examined 141 wild bee specimens collected in 2018 and 213 collected in 2021, on which a total of 29,161 mite specimens were found. The mites were morphologically sorted into five different genera: *Kuzinia* Zachvatkin, 1941, *Parasitellus* Willmann, 1939, *Pneumolaelaps* Berlese, 1920, *Proctolaelaps* Berlese, 1923 and *Scutacarus* Gros, 1845. However, here we focus only on the genus *Parasitellus*, for which *COI* sequences were generated to determine the number of mOTUs in the genus. Three different mOTUs were detected. *Parasitellus* specimens were also determined morphologically to assign the DNA sequences to specific species. Sequences could be assigned to *P. fucorum* de Geer, 1778, *P. ignotus* (Vitzthum, 1930) and *P. talparum* (Oudemans, 1913). Subsequently, host preferences of the individual *Parasitellus* species were examined. All detected species of *Parasitellus* seem to be host specific to the bee genus *Bombus* Latreille, 1802, from which *B. terrestris* (Linnaeus, 1758), *B. lapidarius* (Linnaeus, 1758) and *B. pascuorum* (Scopoli, 1763) were particularly preferred. This study provides a first attempt to combine morphological identification and DNA barcodes in bee mites, to facilitate future, more far-reaching ecological studies on this interesting system.

Keywords: *Bombus*, DNA barcoding, phoretic behavior, biological interactions, bee mites.

Zusammenfassung

Bienen sind wichtige Bestäuber und haben als solche in den letzten Jahren viel Aufmerksamkeit bekommen. In gleicher Weise wurden viele assoziierte Wirbellose, wie die *Varroa* Milbe intensiv untersucht. Vor allem die sozialen Bienen, mit ihren komplexen Nestern, haben viele vergesellschaftete Organismen; Milben sind eine der Gruppen, die hier besonders häufig vorkommen. Trotzdem ist über diese Mutualisten und Parasiten wenig bekannt. In dieser Arbeit haben wir 141 Wildbienen aus dem Jahr 2018 und 213 Individuen aus 2021 untersucht. Auf diesen haben wir insgesamt 29.161 Milben gefunden. Die Milben wurden morphologisch fünf verschiedenen Gattungen zugeordnet: *Kuzinia* Zachvatkin, 1941, *Parasitellus* Willmann, 1939, *Pneumolaelaps* Berlese, 1920, *Proctolaelaps* Berlese, 1923 und *Scutacarus* Gros, 1845. Hier haben wir uns speziell mit der Gattung *Parasitellus* beschäftigt und *COI* Sequenzen generiert um die Anzahl der mOTUs zu bestimmen – wir konnten drei verschiedene mOTUs feststellen. Zudem haben wir die Milben der Gattung *Parasitellus* auf Artebene bestimmt. Die Sequenzen konnten dadurch den Arten *P. fucorum* de Geer, 1778, *P. ignotus* (Vitzthum, 1930) und *P. talparum* (Oudemans, 1913) zugeordnet werden. Anhand der Daten haben wir auch die Wirtsspezifität der *Parasitellus* Arten bestimmt. Alle drei Arten scheinen spezifisch nur auf der Gattung *Bombus* Latreille, 1802 vorzukommen; die Arten *B. terrestris* (Linnaeus, 1758), *B. lapidarius* (Linnaeus, 1758) und *B. pascuorum* (Scopoli, 1763) wurden besonders bevorzugt. Diese Arbeit ist die erste, die genetische Barcodes morphologisch bestimmten Arten in der Gattung *Parasitellus* zuordnet. Hierdurch sollen zukünftige ökologische und evolutionsbiologische Arbeiten erleichtert werden.

Introduction

In recent years, climate change and environmental destruction have led to the extinction of numerous species of various animal groups (Wagner et al. 2021). In particular, insects have shown a decline in their biodiversity and biomass (Amendt 2021). The decline of insects directly impacts ecosystem functions that are important to human health and survival, and increasingly threatens nature (van der Sluijs 2020). A recent study revealed a 76% decline of flying insects over a 27-year period in several protected areas in Germany (Hallmann et al. 2017). Insect decline is even more dramatic than the decrease of birds or plants, and may cause sweeping effects within the world's ecosystems (Sánchez-Bayo & Wyckhuys 2019). For example, insects are indispensable for nutrient cycling and provide a nutritional basis for higher trophic levels occupied by mammals or birds (Hallmann et al. 2017). Furthermore, pollinators, by fertilizing flowering plants, play a central role in securing the world's food supply (Meeus et al. 2011). For instance, it has been calculated that animal pollination is responsible for 35% of global crop production (Klein et al. 2007). However, European monitoring programs have shown substantial declines in the populations of pollinators and the plants they pollinate (Biesmeijer et al. 2006).

Climate change, habitat loss, habitat fragmentation and pesticides have been identified among the main causes of insect decline (Sánchez-Bayo & Wyckhuys 2019). The spread of newly arising pathogens, such as viruses, has also been suggested as playing a role in the global extinction of pollinating insects (Ribière et al. 2008; Wagner et al. 2021). Honey bees are vulnerable to infection by a large variety of pathogens, resulting in notable colony losses. For example, viruses have been recognized as a major threat to the health and survival of honey bee populations (Chen & Siede 2007). To date, there are more than 18 different viruses known to infect honey bees worldwide, e.g., the deformed wing virus (DWV) or the acute bee paralysis virus (ABPV) (Allen & Ball 1996; Ellis & Munn 2005; Tehel et al. 2016). Symptoms caused by these viruses include deformed wings, hair loss and paralysis, as well as offspring and adult mortality (Bailey 1968; Chen & Siede 2007). The mite *Varroa destructor* Anderson & Trueman, 2000 is not only recognized as one of the main parasites of honey bees, but also transmits many of these viruses (Ellis & Munn 2005). Moreover, DWV and other viruses have now been identified in wild bee species such as bumble bees (*Bombus* Latreille, 1802 spp.) (McMahon et al. 2015; Tehel et al. 2016) and other pollinators (Pislak Ocepek et al. 2022). Since *Varroa* Oudemans, 1904 is strictly host specific to honey bees of the genus *Apis* Linnaeus, 1758, the transfer of pathogens to wild bees via *Varroa destructor* is very unlikely. However, many other mite species have been reported on a number of other bee species (e.g., Schwarz & Huck 1997; Klimov et al. 2007a, 2007b; Różej et al. 2012). Most of these mites are considered phoretic, but relatively little is known about their diversity and host specificity and their identification can be difficult.

Phoretic mites associate with bees for transport, to colonize their nests and exploit resources such as pollen, nectar or microorganisms, as commensals (Revainera et al. 2020). Moreover, most phoretic mites reproduce in bee nests, where they develop into deutonymphs capable of attaching to the bees for transportation. Bee colonies typically decline in autumn, and the phoretic mite stages associate with the young queens, hibernating throughout the winter (Chmielewski 1971; Goldblatt & Fell 1984). In spring, when the queen founds a new colony, the mites detach, mature and reproduce in the new nest (Eickwort 1994). Many different mite species can be found on wild bees, e.g., in the genera *Imparipes* Berlese, 1903, *Kuzinia* Zachvatkin, 1941, *Parasitellus* Willmann, 1939, *Pneumolaelaps* Berlese, 1920, *Proctolaelaps* Berlese, 1923, *Tyrophagus* Oudemans, 1924 and *Scutacarus* Gros, 1845 (Eickwort 1994; Schwarz & Huck 1997; Maggi et al., 2011; Kerl et al., in prep.). These species have been found primarily on bumble bees, but *Imparipes* and *Scutacarus* mites were also discovered in association with other apid bees (Eickwort 1994). The number of mites per bee individual can vary tremendously, from single specimens to more than 200 mite individuals per bee (Maggi et al., 2011). The abundance of the different mite genera seems to be variable. Eickwort (1994), for example, reported *Kuzinia* and *Parasitellus* as most abundant, whereas *Proctolaelaps* was relatively rare in that study and *Tyrophagus* was most abundant on commercial bumble bees from Poland (Różej et al. 2012). Other studies have reported different patterns (Maggi et al. 2011).

Generally, relatively little is known on the abundance, distribution and host associations of bee mites. Hence, to improve our knowledge, we started investigating the bee mite community in Northern Germany and focused on the morphological and genetic diversity of the genus *Parasitellus* (Mesostigmata: Parasitidae) (Koulianos & Schwarz 1999). To date, four species have been reported in Europe: *Parasitellus fucorum* De Geer, 1778, *P. talparum* (Oudemans, 1913), *P. crinitus* (Oudemans, 1903) and *P. ignotus* (Vitzthum, 1930) (Hyatt 1980; Schousboe 1987). They all live and reproduce in the nests of bumble bees and are sometimes found in beehives (Vitzthum 1930). The life cycle of *Parasitellus* mites consists of five stages: egg, larva, protonymph, deutonymph and adult. The deutonymphs are phoretic instars capable of attaching to adult bees for transport (Huck et al. 1998). When the bee colonies die in autumn, the mites overwinter on the young queens. Just after the queens establish new nests in spring, the deutonymphs detach and develop into adults to colonize the nest (Koulianos & Schwarz 1999). *Parasitellus* are found on bumble bees of all castes, but seem to prefer queens (Schwarz & Huck 1997). Morphologically, *Parasitellus* differs from all other genera of Parasitidae by its opisthogastric region bearing more than 40 pairs of setae, whereas other genera have less than 30 (Hrúzová & Fenďa 2018). Although *Parasitellus* seems to be a common phoretic mite on bees, little is known about its genetic diversity, and the potential for undiscovered cryptic species may be high. Even basic data such as genetic barcodes associated with identified specimens is lacking. Hence, in this study, we aimed to generate new knowledge about the host range of the mite genus *Parasitellus* and its morphological and genetic diversity, in order to examine its potential for virus spillover between different wild bee species.

Material and methods

Field studies

All wild bees which could be found were collected once a week for about two months by JK and FK, from the end of March to the end of May, at the Energieberg (a closed former waste disposal site) in Georgswerder, Hamburg, Germany (53°30′47.7″N 10°01′43.1″E). This 45 ha. area, located in the Wilhelmsburg district, is situated directly south-west of a motorway junction. Some sections reach an altitude of 40 m. The heterogeneous vegetation is artificially designed, showing mainly grassland with some shrubs and a few small trees. Scattered sandy regions with stones can also be found. As a marshland, the area has soil with a continuous layer of clay. Temperatures varied from 6 to 23 °C throughout the sampling period. The average wind velocity was 17.66 km/h. Cloud density was between 1 and 4 (based on https://www.timeanddate.de/wetter/). A transect of about 3.5 km was established and bee individuals were caught with the aid of an insect net; sampling lasted from 10 am to noon each time. After capture, each bee was individually transferred to a clean Falcon tube and later stored at -80 °C.

Morphological studies

Mites were detached from the wild bees with a dissecting needle and a paintbrush and stored in 99% ethanol at -80 °C until further examination. Five different genera of mites were determined morphologically with the aid of a light microscope (Leica Camera, Wetzlar, Germany) and the fact sheets of Bee Mite ID (https://idtools.org/id/mites/beemites/): *Kuzinia*, *Parasitellus*, *Pneumolaelaps*, *Proctolaelaps* and *Scutacarus*. The number and identity of the mites were noted for each bee individual. Individuals of the genus *Parasitellus* were further examined and species were determined with a modified identification key based on the original key of Hyatt (1980).

Genetic studies

From 221 wild bee individuals, 213 DNA extractions were successfully obtained by using the high salt extraction protocol (Paxton et al. 1996). The right hind leg of each wild bee was removed for DNA extraction and stored at -80 °C. Before DNA extraction, bee samples were rinsed twice with 200 µl autoclaved ultra-pure water and dried for 5 min at 45 °C to remove pollen or any other pollutants. Similarly, *Parasitellus* specimens were dried before DNA extraction to remove all ethanol. Some bee samples were extracted with a Chelex extraction protocol (Walsh et al. 1991); however, because there was little success with that method, samples were re-extracted with the high salt extraction method.

The barcode fragment of the mitochondrial *cytochrome oxidase subunit 1* (*COI*) gene of the wild bees was amplified using the primer pair LCO1492 and HCO2198 (Folmer et al. 1994). For *Parasitellus* specimens, the primers Uni-MinibarF1 and HCO2198 were used (Meusnier et al. 2008). The reaction mixture consisted of 6.75 µl ddH$_2$O, 1 µl Buffer (10x), 0.2 µl dNTPs, 0.5 µl forward primer, 0.5 µl reverse primer and 0.2 µl DreamTaq (ThermoFisher Scientific, Waltham, Massachusetts) for each reaction. The PCR protocol included an initial denaturation of 2 min at 95 °C followed by 35 cycles of 30 s at 95 °C (denaturation), 1 min at 50 °C (annealing) and 1 min at 72 °C (extension), with a final extension of 10 min at 72 °C. PCR products were checked by electrophoresis. Successfully amplified PCR products were purified with ExoSap (ThermoFisher Scientific, Waltham, Massachusetts) and sent to Macrogen (Amsterdam, The Netherlands) for sequencing.

Phylogenetic analyses

In addition to the 213 newly generated bee sequences, 141 bee sequences from a previous study were included, for a total of 354 sequences (Heller, unpublished M.Sc. thesis). Of these 141 old bee sequences, 65 were from specimens from the Energieberg in Georgswerder, Hamburg, Germany and an additional 75 sequences were from specimens from the Knick Bargteheide, Schleswig-Holstein, Germany (53°44′11.3″N 10°16′16.4″E). One sequence (H1) was from an additional specimen found dead in Hamburg.

For the mites, in addition to the seven newly-sequenced individuals identified at species level, we included 164 sequences of unidentified *Parasitellus* from a previous study (Heller, unpublished M.Sc. thesis), for a total of 171 sequences.

Sequences were edited and checked for correct amino acid translation using Geneious Prime® 2021.2.2 (Kearse et al. 2012). In case of unclear reads, the fragment was sequenced also for the reverse strand. Wild bee sequences were searched in NCBI BLAST and BOLD to determine the species. In the case of *Parasitellus* sequences, a BLAST search was also performed to check for potential contaminations. Two sequences of *Parasitus hyalinus* (Willmann, 1949) (MH983685, MH983817) from the NCBI database were retrieved and used as outgroup. The MUSCLE algorithm (Edgar 2004), with predefined settings as implemented in Geneious, was used to align all bee and mite sequences into two separate alignments, respectively; the alignments were further edited manually to exclude ambiguous regions.

Genetic distances were calculated in MEGA 11.0.10 (Tamura et al. 2021) with the Maximum Composite Likelihood Method. The best fitting substitution models were calculated in MEGA. For phylogenetic analyses, Bayesian inference was used as implemented in MrBayes 3.2.7a (Huelsenbeck & Ronquist 2001). Analyses were run for 10 million generations and a sampling frequency of 1,000. A burn-in of 25% of samples was discarded and the final phylogenetic tree was visualized and edited in FigTree v1.4.4 (Rambaut 2009).

For mites, we also performed statistical species delimitation. To delimit molecular operational taxonomic units (mOTUs) of *Parasitellus*, the Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012) was used. The alignment of the *Parasitellus* sequences without the outgroup was run online on the ABGD Homepage (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html), with default settings. Microsoft Excel v. 15.41 was used to analyze the relation between the mite genus *Parasitellus* and other mite genera, as well as the distribution of *Parasitellus* species on wild bee species.

Results

Wild bees

In total, we generated 354 wild bee sequences (213 newly generated and 141 from a previous study). Five bee families were detected: Apidae, Colletidae, Andrenidae, Halictidae and Megachilidae (Table 1). Species of the family Apidae were the most common, representing 71.8% of the total individuals collected. Within that family, *Bombus terrestris* (Linnaeus, 1758) appeared most often, with 107 individuals. *Bombus pascuorum* (Scopoli, 1763) also appeared frequently, with a total of 64 individuals. The family Colletidae was the second most abundant at 15.3%, represented by the species *Colletes cunicularius* (Linnaeus, 1761) with 54 individuals. Thirty-three individuals from the family Andrenidae were collected, *Andrena bicolor* Fabricius, 1775 being the most common species with 18 individuals. Only eight individuals representing two species, *Lasioglossum calceatum* (Scopoli, 1763) and *Sphecodes albilabris* (Fabricius, 1793), of the family Halictidae were found. The family Megachilidae was relatively rare, with only five individuals belonging to the species *Osmia bicornis* (Linnaeus, 1758). For *Andrena cineraria* (Linnaeus, 1758), *Andrena nigroaenea* (Kirby, 1802) and *Nomada ferruginata* (Linnaeus, 1767), only single individuals were recorded.

Phoretic mites

In total, 29,161 mite specimens were found on the 354 wild bees (213 bees from this study, 141 from Heller [2018]). Five different genera were identified: *Kuzinia*, *Parasitellus*, *Pneumolaelaps*, *Proctolaelaps* and *Scutacarus*. *Kuzinia* was the most common mite genus with 26,277 individuals, corresponding to approximately 90% of the total mite count. *Scutacarus* was the second most frequent genus with 1,585 individuals. *Parasitellus* comprised 640 individuals. *Pneumolaelaps* and *Proctolaelaps* were comparatively rare, with 279 and 126 individuals, respectively; together, they made up less than 2% of the total.

Table 1. Wild bee species found in Hamburg, Germany in 2018 and 2021, with number and percentage (%) of specimens.

Family	Genus	Species	Number of specimens	Percentage (%)
Andrenidae	*Andrena*	*Andrena cineraria*	1	0.3
		Andrena bicolor	18	5.1
		Andrena gravida	2	0.6
		Andrena haemorrhoa	2	0.6
		Andrena nigroaenea	1	0.3
		Andrena nitida	3	0.8
		Andrena praecox	6	1.7
Apidae	*Apis*	*Apis mellifera*	10	2.8
	Bombus	*Bombus bohemicus*	2	0.6
		Bombus hortorum	2	0.6
		Bombus hypnorum	2	0.6
		Bombus lapidarius	35	9.9
		Bombus lucorum	8	2.3
		Bombus pascuorum	64	18.1
		Bombus pratorum	19	5.4
		Bombus sylvestris	4	1.1
		Bombus terrestris	107	30.2
	Nomada	*Nomada ferruginata*	1	0.3
Colletidae	*Colletes*	*Colletes cunicularius*	54	15.3
Halictidae	*Lasioglossum*	*Lasioglossum calceatum*	2	0.6
	Sphecodes	*Sphecodes albilabris*	6	1.7
Megachilidae	*Osmia*	*Osmia bicornis*	5	1.4
Total number/quantity			**354**	**100**

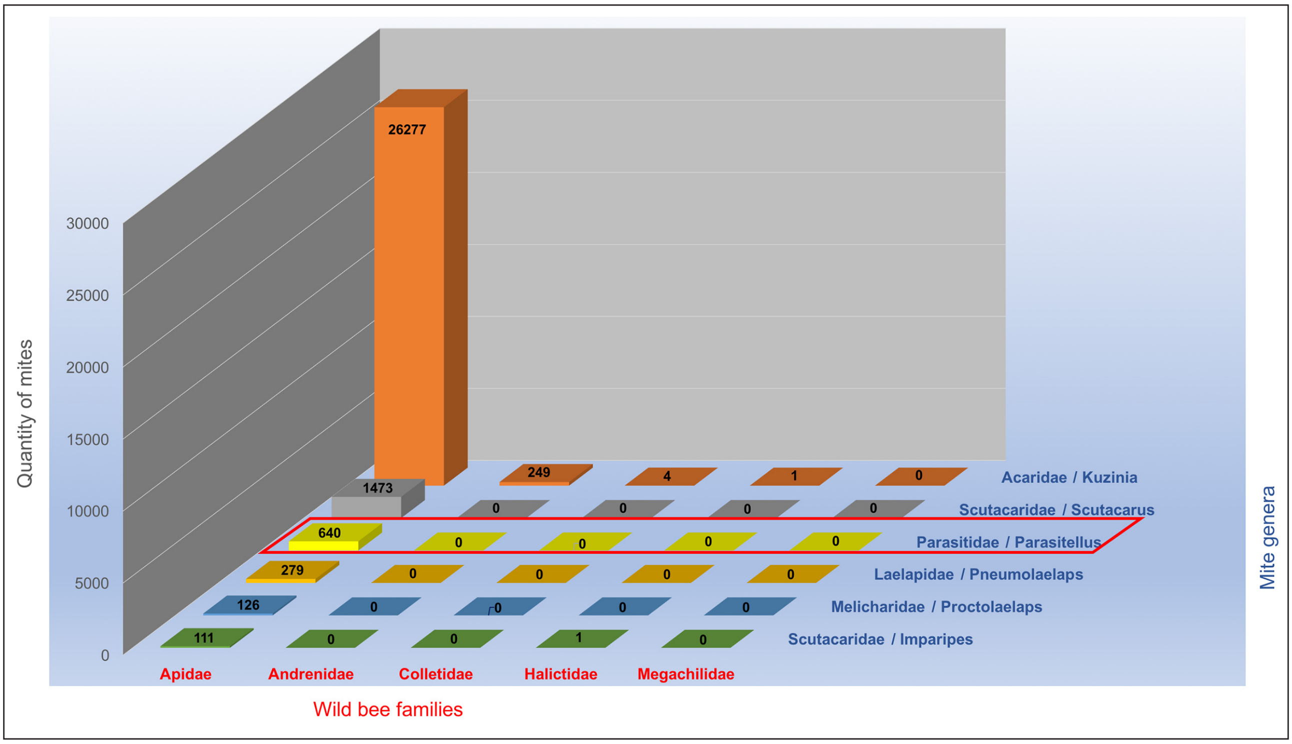

Fig. 1. Distribution of the various mite genera on wild bee families.

Parasitellus mites

The genus *Parasitellus* was only detected on wild bees of the family Apidae (Fig. 1). In total, we found 640 individuals of *Parasitellus* in association with wild bees. The average infestation was 3.9 mites per wild bee specimen. *Parasitellus* occurred most frequently on *Bombus terrestris*, on which we found 419 mites, whereas 143 individuals were detected on *B. lapidarius* (Linnaeus, 1758), 53 on *B. pascuorum*, 16 on *B. pratorum* (Linnaeus, 1761) and six on *B. lucorum* (Linnaeus, 1761). On *B. bohemicus* (Seidl, 1837), *B. hortorum* (Linnaeus, 1761) and *B. sylvestris* (Lepeletier, 1832), only single individuals of *Parasitellus* were found. We did not find *Parasitellus* on *A. mellifera*, *B. hypnorum* and *N. ferruginata* (Table 2). The highest number of *Parasitellus* individuals found on a single bee was 39, on *B. terrestris*.

In total, 427 *Parasitellus* specimens were determined morphologically at the species level. Three species were identified: *P. fucorum*, *P. ignotus* and *P. talparum*. Morphologically, no individuals of the fourth native German species, *P. crinitus*, could be detected. Also genetically, there were most likely no individuals of *P. crinitus* in the dataset, as we only found three well-supported lineages. However, when not all mites found on one bee were genetically determined, they were excluded from the host-parasite analysis. This resulted in a total of 334 securely identified *Parasitellus* specimens. *Parasitellus fucorum* was the most common species, making up approximately 90.1% of the total individuals analyzed. With 8.1%, *P. ignotus* was the second most common species, while *Parasitellus talparum* was relatively rare and only occurred in 1.8% of cases.

Table 2. Total number of *Parasitellus* individuals per wild bee species of the family Apidae.

Species	Number of *Parasitellus*
Bombus terrestris	419
Bombus lapidarius	143
Bombus pascuorum	53
Bombus pratorum	16
Bombus lucorum	6
Bombus bohemicus	1
Bombus hortorum	1
Bombus sylvestris	1
Apis mellifera	0
Bombus hypnorum	0
Nomada ferruginata	0

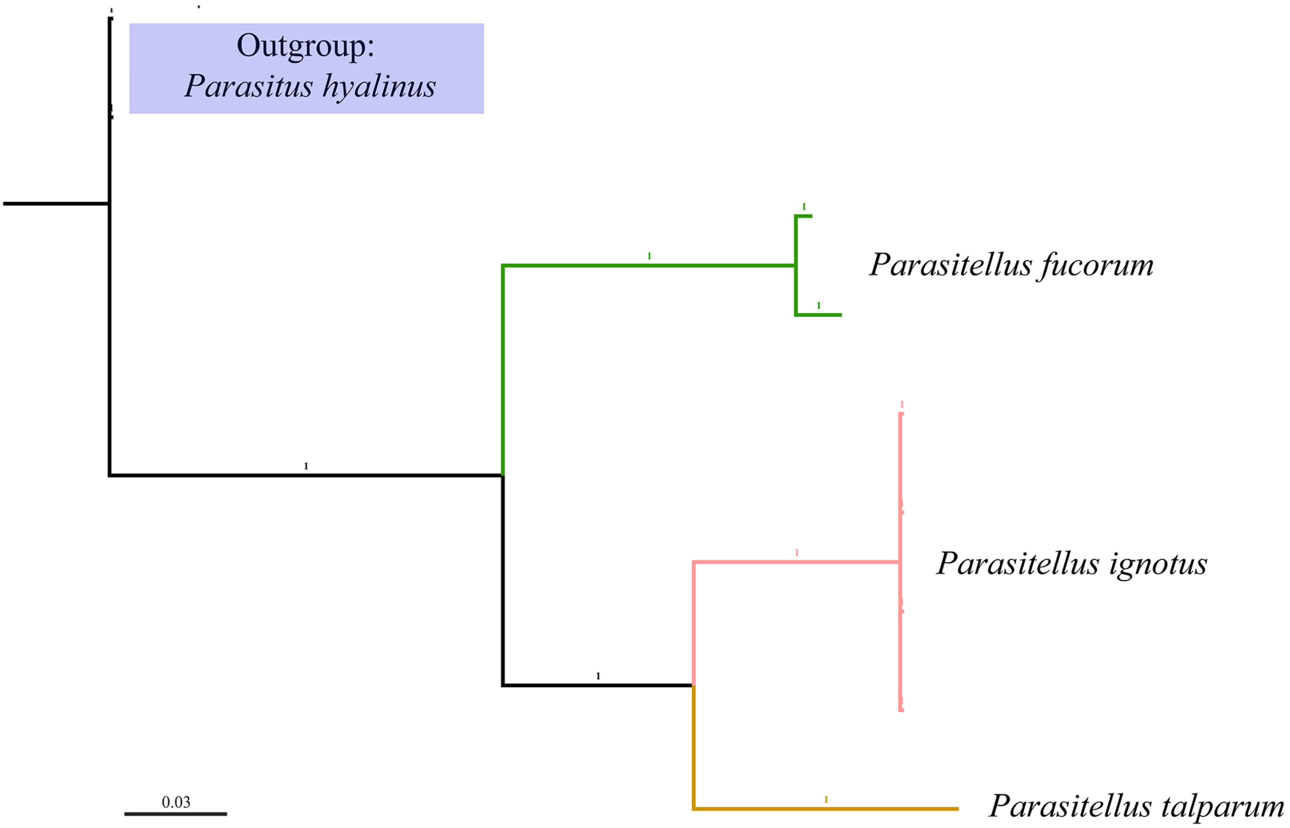

Fig. 2. Phylogenetic tree of the genetically determined *Parasitellus* species (*COI*) found in Hamburg, Germany in 2021. Numbers on branches represent posterior probability support.

Phylogenetic analyses

As only the mites collected in 2021 were identified at species level, they were those subjected to a first phylogenetic analysis. The phylogenetic reconstruction of these morphologically identified specimens yielded three clearly differentiated groups (Fig. 2). All morphologically identified species were recovered as monophyletic in the tree, with high support. The genetic distances of these *Parasitellus* sequences were calculated in MEGA (Table 3). The distance of *P. fucorum* to *P. ignotus* is 0.20. The distance of *P. talparum* to *P. ignotus* is smaller than the distance of *P. talparum* to *P. fucorum* (Table 3).

Samples from 2018 were only identified morphologically at the genus level. Hence, we used the 2021 samples as a reference in the tree for the 2018 samples (Fig. 3). We examined 164 specimens of *Parasitellus* from 2018. Again, three different clades were recovered, which were attributed to the three morphologically identified species. In addition, species delimitation based on ABGD determined the same three mOTUs. The barcode gap distance was 0.091.

Host-parasite network

A host-parasite network was constructed to understand the host range of *Parasitellus* species (Fig. 4). *Parasitellus fucorum* was found on seven different *Bombus* species: *B. terrestris*, *B. lapidarius*, *B. pascuorum*, *B. pratorum*, *B. lucorum* and *B. hortorum*. *Parasitellus ignotus* was associated with 10 bee specimens belonging to four different species: *B. terrestris*, *B. lapidarius*, *B. pratorum* and *B. bohemicus*. *Parasitellus talparum* was only found on two wild bee individuals, both belonging to the species *B. terrestris* (Fig. 4).

Discussion

With this study, we aimed to provide new information on the distribution, genetic makeup and host specificity of bee mites of the genus *Parasitellus*. Specifically, for the first time, we associated species-level morphological identification with genetic barcodes and used this data to understand if *Parasitellus* species are host-specific or gen-

Table 3. Genetic distances of *Parasitellus* specimens collected in 2021. PaFu – *Parasitellus fucorum*, PaIg – *Parasitellus ignotus*, PaTa – *Parasitellus talparum*.

Sequences	**[1] PaFuA**	**[2] PaFuB**	**[3] PaIgA**	**[4] PaIgC**	**[5] PaIgD**	**[6] PaIgE**
[1] PaFuA						
[2] PaFuB	0.0173					
[3] PaIgA	0.2005	0.2126				
[4] PaIgC	0.2009	0.2131	0.0000			
[5] PaIgD	0.2005	0.2126	0.0000	0.0000		
[6] PaIgE	0.2005	0.2126	0.0000	0.0000	0.0000	
[7] PaTaE	0.2214	0.2282	0.1461	0.1464	0.1461	0.1461

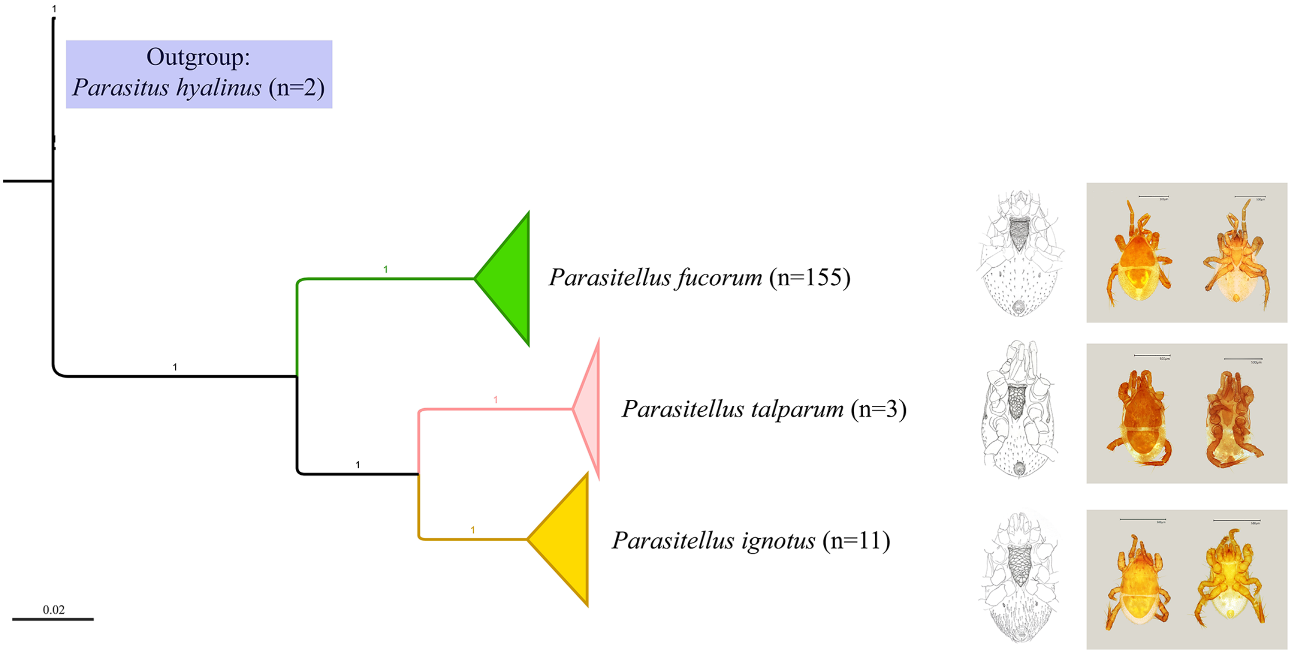

Fig. 3. Phylogenetic tree of all *Parasitellus* specimens (*CO1*) found in Hamburg, Germany in 2018 and 2021, with images and drawings of the three detected species. Numbers on branches represent posterior probability support.

eralists. We were able to detect three of the four species of the genus *Parasitellus* known for Germany and confirm that they are genetically distinct. Further, we showed that the mites are specific to bumble bees but not to any particular species. As such, they also may serve as cross-specific vectors for bee diseases, specifically viruses. In the following paragraphs, we discuss our results in more detail.

Host specificity of the mite genus *Parasitellus* and its potential as a vector

At least two studies have addressed the host specificity of the mite genus *Parasitellus* (Schousboe 1987; Huck et al. 1998). These studies showed the capability of phoretic mites to actively choose the best host individual (worker or queen) for their survival. However, there is little research on whether *Parasitellus* associates with wild bee species

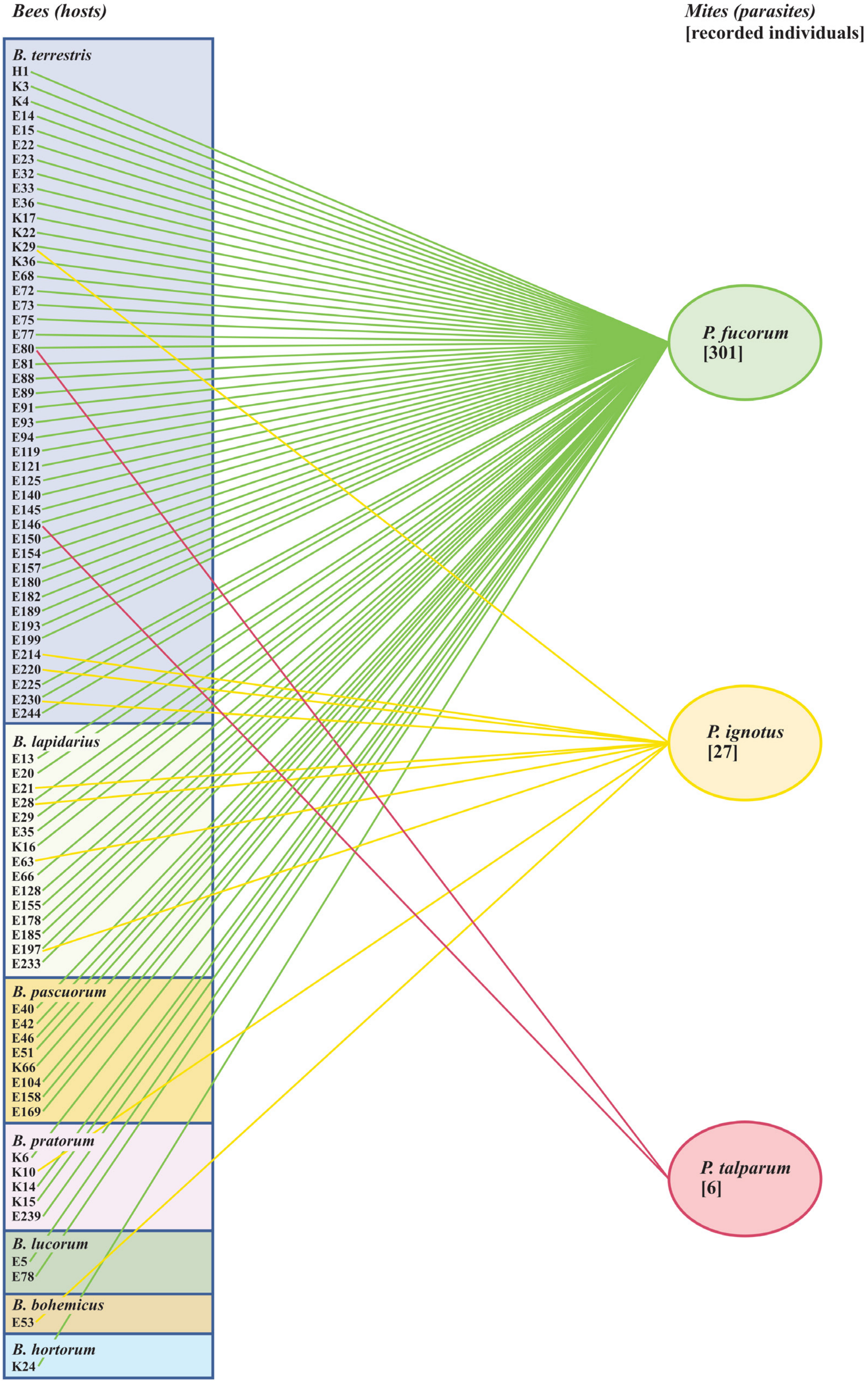

Fig. 4. Host-parasite network of wild bee individuals of the family Apidae and *Parasitellus* species found in Hamburg, Germany in 2018 and 2021.

other than bumble bees. In addition, most of these studies have focused on the species *P. fucorum*, and little is known about the host preferences of other *Parasitellus* species (Schousboe 1987; Huck et al. 1998; Koulianos & Schwarz 1999). The data collected in this study were used to check for any host specificity of the mite genus *Parasitellus* on wild bees.

First, we examined host specificity at the bee family level (Fig. 1). *Parasitellus* seems to be only associated with individuals of the family Apidae, and within it only with the genus *Bombus.* It appears that these phoretic mites prefer primitively social bees because spreading and breeding is easier within a nest, but the defense mechanisms in, e.g., *Bombus* are not as strict as in the eusocial honey bees. Our data suggest that mites of the genus *Parasitellus* may be specific to *Bombus* at the genus level (but not at the species level), because their life cycle is closely associated with the bumble bees' life cycle. Mites develop in five stages, including egg, larva, protonymph, deutonymph and adult (Evans 1992). All these stages live in bumble bee nests, where they feed on pollen (Richards 1976). The deutonymphs attach to the young queens in order to disperse; they overwinter on the queens to reproduce in a new nest in spring (Stebbing 1965). Probably, phoretic mites of the genus *Parasitellus* have the best chances of survival and reproduction within bumble bee nests.

Within the genus *Bombus*, three species were particularly preferred by the mites: *B. terrestris*, *B. pascuorum* and *B. lapidarius*, which are generally the most common *Bombus* species in the study area. The highest average infestation of 4.1 mites per bumble bee individual was calculated for *B. lapidarius*. Previous studies reported *B. terrestris* and *B. lapidarius* as having the highest *Parasitellus* infestation rates (Schousboe 1987; Huck et al. 1998). Host size may also play a role (Allen et al. 2007). This aspect was not studied here, but may indicate annual or localized differences in infestation rates at the local level (Schousboe 1987). The three most infested *Bombus* species were also the most common ones in the area, hence their sample size in our study was significantly larger than that of the rarer *Bombus* species. Moreover, biological differences among bumble bee species may play an important role. For instance, their nesting preferences differ with regard to soil type, position, sun exposure and rain protection (Fussell & Corbet 1992), and these different conditions may cause differences in microclimate within the bees' nests, influencing the mites' life cycle in various ways (Schousboe 1987). Also, while being mostly polylectic, some species may have preferences for specific pollen, which may in turn promote or restrict the presence of mites. These factors were not explicitly investigated here, but may be an interesting avenue for future studies.

Our data suggest that *Parasitellus* mites are specific to bumble bees, but with no or little preference for a particular species. This would allow the mites to potentially spread diseases from one bumble bee species to another. In fact, some preliminary analyses have shown that mites can carry bee viruses (Husemann, unpublished data). Specifically, we even found virus-carrying mites on a bumble bee that was not infected with the virus itself (Husemann, unpublished data). Should an infected mite enter the nest of an uninfected bumble bee and mature and reproduce there, an infection of the host-nest would seem possible. No data on this potential transmission pathway are available yet, but future studies should address this topic.

Species-level analyses

This represents the first study which associates barcodes with species-level morphological identifications within the genus *Parasitellus*. We most commonly found *P. fucorum*, but also *P. ignotus* and *P. talparum*, whereas no individuals of *P. crinitus* were detected and therefore the host specificity of this last species could not be determined. Generally, only very few records of this species are known (e.g., from Denmark [Schousboe 1987]) and further studies are needed (Schwarz et al. 1996).

The three sampled species, *P. fucorum*, *P. ignotus* and *P. talparum*, were examined for host preferences. *Parasitellus fucorum* and *P. talparum* were most common on *B. terrestris*; in contrast, *P. ignotus* seemed to prefer individuals of *B. lapidarius* and was also the only species associated with *B. bohemicus*. However, as the latter is a parasitic species, the mite may be more interested in its hosts, the species of the *B. terrestris* group, rather than the cuckoo bee itself. The life strategies of the three *Parasitellus* species likely differ in some significant points, which may be a reason to choose hosts with different conditions within the nests (Schousboe 1987). There may be preferences for the two different types of nesting behaviors in bumble bees, i.e., pocket makers vs. pollen storers, but it is up to further studies to determine which species prefers which particular conditions.

Phylogenetic analyses

Until now, no studies have systematically investigated the *Parasitellus* group with molecular tools and only a single DNA sequence of an identified specimen of the genus was available on NCBI and BOLD (as to 08.01.2024). Hence, we here provide the first phylogenetic analysis of the genus, providing baseline data for future investigation. All three morphologically identified species were recovered as distinct clades in the tree. These correspond to three of the four native species known in Europe: *P. fucorum*, *P. ignotus* and *P. talparum*; we did not find any evidence of *P. crinitus* in our data (Schousboe 1987; Schwarz et al. 1996). Therefore, DNA barcodes can be unequivocally assigned to morphologically identified species in this genus for the first time. This opens up the possibility of examining the degree of relatedness between the different species, but also provides important barcode data for future investigations, e.g., via meta-barcoding.

All species were clearly distinct and genetically well recognizable in our analysis. The genetic distance between *P. fucorum* and *P. ignotus* was approximately 20%, as was the distance between *P. fucorum* and *P. talparum*. *Parasitellus ignotus* and *P. talparum* exhibit a genetic distance of approximately 15%. These distances are high and compare to the genetic distances of astigmatid mites at the genus level. For example, the distance between the species *Aleuroglyohus ovatus* Troupeau, 1879 and *Blomia tropicalis* van Bronswijk & de Cock, 1973, also based on *COI* sequences, is 20% (Yang et al. 2011). Although these two species are from different genera and even families, they are genetically well studied and certainly closely related.

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