School of Doctoral Studies in Biological Sciences
University of South Bohemia in České Budějovice
Faculty of Science

Host specificity, genetic variability and genealogy in populations of model parasite species

Ph.D. Thesis

RNDr. Jana Martinů

Supervisor: doc. RNDr. Jan Štefka, Ph.D.

University of South Bohemia in České Budějovice, Faculty of Science
and
Laboratory of Molecular Ecology and Evolution,
Institute of Parasitology, Biology Centre, CAS

České Budějovice 2019

This thesis should be cited as:
Martinů, J., 2019: Host specificity, genetic variability and genealogy in populations of model parasite species. Ph.D. Thesis. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 66 pp.

Annotation

Parasites represent one of the most common ecological strategies and host-parasite coevolution belongs among the major processes governing evolution of biodiversity on the global scale. Genetic structure and diversity of populations of parasites and their hosts, and their genetic connectivity are the key elements in long-term population survival and evolution.
Host switches often disturb the parallel evolution of interacting taxa, even in highly host-specific parasites. Evaluation of importance of the degree of intimacy between parasites and hosts is not a trivial task, because evolutionary patterns observed today were formed by an interplay of many (sometimes previously unforeseen) historical and ecological factors. To reveal the mechanisms of coevolution between parasites and their hosts, inter- and intra-specific genealogical structures in three model systems were analyzed: namely, the sucking lice *Polyplax serrata* and *Apodemus* hosts, chewing lice of the genus *Menacanthus* and endoparasitic coccidean genus *Eimeria* from *Apodemus* mice.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.
Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 30.8.2019

Jana Martinů

This thesis originated from a partnership of Faculty of Science, University of South Bohemia, and Institute of Parasitology, Biology Centre of the ASCR, supporting doctoral studies in the Parasitology study programme.

Financial support

Czech Science Foundation, project nos. P506/12/P529, P505/12/1620, 14-07004S

Grant Agency of the Academy of Sciences of the Czech Republic, project no. IAA601690901

National Grid Infrastructure MetaCentrum, Grant/Award Number: CESNET LM2015042 and CERIT Scientific Cloud LM2015

Bilateral Mobility Project (MAD), project no. SAV-16-22, and the Slovak Research and Development Agency, project no. APVV-14-0274

Acknowledgements

I would like to thank my supervisor Jan Štefka for the leadership, support, valuable advices and a lot of patience during my studies. Special thanks belong to Václav Hypša for his support and new impulses in our research and also thanks to collaborators from the Veterinary University of Brno (Oldřich Sychra and Ivan Literák) who provided us *Menacanthus* material with precise morphological determination. I am really grateful to the members of the Laboratory of Molecular Ecology and Evolution for friendly atmosphere and help. Thanks also belong to my family, especially Roman Chovanec, for endless patience and support.

I would like to thank all my colleagues and friends in the Czech Republic and abroad, who participated in field studies and provided us samples connected with the research presented in this thesis. Namely Jana Kvičerová, Roman Hrdlička, Anna Mácová, Jana Říhová, Michaela Matějková, Eva Nováková, Aneta Trefancová and Mišo Stanko and his colleagues from Košice, Slovakia, for help with obtaining *Polyplax serrata* louse and feacal material. Then thanks to Daniel R. Gustafsson, Ali Halajian and other phthirapterists, who provided us chewing lice material for *Menacanthus* project. Thanks belong also to Veronika Roubová, Milena Nováková, Lukáš Drag and Jaroslav Šoun, who helped me with laboratory work.

List of papers and author's contribution

The thesis is based on the following papers:

I. **Martinů, J.**, Roubová, V., Nováková, M., Smith, V. S., Hypša, V., Štefka, J. 2015. Characterisation of microsatellite loci in two species of lice, *Polyplax serrata* (Phthiraptera: Anoplura: Polyplacidae) and *Myrsidea nesomimi* (Phthiraptera: Amblycera: Menoponidae). Folia Parasitologica, 62: 1–6. (IF = 1.405).
Jana Martinů was responsible for the isolation of polymorphic microsatellite loci in Polyplax serrata, determinig genotypes of P. serrata from fragmentation analyses, population analysis of microsatellite data of P. serrata and drafting of the manuscript.

II. **Martinů, J.**, Hypša, V., Štefka, J. 2018. Host specificity driving genetic structure and diversity in ectoparasite populations: Coevolutionary patterns in *Apodemus* mice and their lice. Ecology and Evolution, 8: 10008–10022. (IF = 2.415).

Jana Martinů was responsible for obtaining part of the sampled material of mice and lice, for part of the laboratory analyses (DNA extraction, PCR of mtDNA, nuclear DNA and microsatellite data of both parasites and their hosts), exclusively for the assessment of the genotypes from fragmentation analyses, for all data analyses and graphical outputs and drafting the manuscript.

III. **Martinů, J.**, Sychra, O., Literák, I., Čapek, M., Gustafsson, D.L., Štefka, J. 2015. Host generalists and specialists emerging side by side: an analysis of evolutionary patterns in the cosmopolitan chewing louse genus *Menacanthus*. International Journal for Parasitology, 45: 63–73. (IF = 3.478).

Jana Martinů was responsible for DNA extraction, PCR, sequence assembling and aligning, phylogenetic analyses and drafting the manuscript.

IV. Mácová, A., Hoblíková, A., Hypša, V., Stanko, M., **Martinů, J**., Kvičerová, J. 2018. Mysteries of host switching: Diversification and host specificity in rodent-coccidia associations. Molecular Phylogenetics and Evolution, 127: 179–189. (IF = 3.992).

Jana Martinů participated in catching rodents across European localities and collecting their faeces, then was responsible for extracting DNA of Apodemus sylvaticus and A. flavicollis hosts and for molecular determination of both species. JM participated in editing the drafted manuscript.

Contents

1. Introduction

1.1 Coevolution of parasites and hosts

Host-parasite co-evolution belongs among the major processes governing evolution of biodiversity on the global scale. Initially, cospeciation of both interacting organisms was considered as the main driver of reciprocal evolution (Hafner a Nadler, 1990). With increasing numbers of reconstructed genealogies congruence between topologies of parasites and their hosts occured, but many discrepancies were detected (Paterson and Banks, 2001; Ricklefs et al., 2004; Hoberg and Brooks, 2008; Agosta et al., 2010). At inter-specific level researchers revealed a variety of patterns from strict co-speciation to the lack of co-divergence and frequent host-switching, even in species tightly linked to their hosts. The notion of parasites as passive partners entirely following evolutionary history of their hosts changed and parasite´s own biological traits and other factors independent of hosts have been taken into account (Paterson and Banks, 2001; Ricklefs et al., 2004; Hoberg and Brooks, 2008). Four main mechanisms generating incongruencies have been proposed (Page, 1994): 1) During *host switching* parasites are transmitted to a new host species. 2) *Duplication* of parasites happens when parasite speciates without speciation of its host. 3) In the case of *sorting event* one lineage of parasite is lost on a shared host species. Alternative explanation of the same evolutionary pattern is *missing the boat*, when the host speciates without infestation of the parasite. 4) the last mechanism, known as *colonization*, presumes speciation of the host without speciation of the parasite. Even though all evolutionary explanations of discordance were intuitive and clearly described, in many cases it was not trivial to differentiate between them and to identify the correct mechanism via comparing phylogenies of interacting taxa, in the absence of well-founded empirical data. Authors mostly decided from supposed tendency of the parasite to incline to one mechanism based on the knowledge of its bionomical traits.

Host-parasite structure mainly reflected combination of two aspects; geographic range of both parasites and their hosts and host-specificity. Current geographic distribution of many species was influenced by climatic changes during their evolution, particularly, quaternary glaciation had crucial impact on geographic ranges of organisms in the Palearctic (Webb et al., 1992). Most parts of Europe were forestless and uninhabitable for many temperate species. Organisms survived harsh conditions in isolated refugia, especially in Iberia, Apennines and Balkans in the south and in western Russia in the east (Bilton et al., 1998), where independent diversification and speciation processes took place. After the glaciation species recolonized previously inaccessible central and northern parts of the continent (Avise, 1997). Thus, present genealogical structure of many mammalian species as well as their parasites was formed by these historical, nonuniform evolutionary events.

Host-specificity played important role in host-parasite interactions as one of the bionomy dependent parameters, together with direct vs. indirect life cycle and the presence or absence of free-living life stages (Page et al., 2003). It was expected that parasites with direct life cycles (and often highly adapted to their hosts) developed narrow host specificity (i.e. Nieberding 2004; Hosokawa et al., 2006), whereas multihost parasites were an artefact of insufficient sampling or assumed to be split into cryptic lineages when analysed with molecular methods (i.e. Jousson et al., 2000; Demanche et al., 2001; Dowling et al., 2003; Brooks et al., 2004). Another traditional hypothesis postulated that parasites evolved from host generalists to host specialists (Eichler et al., 1941). Studies of ectoparasites demonstrated that even in parasites with high level of intimacy frequent host switches disturbed cophylogenetic signal. In addition to that, existence of generalists in phylogenies was documented as well as the ability of host

specialists to diversify several times independently (Johnson et al., 2009, 2011). Evidence of specialization as being not a one-way street process (or an evolutionary dead-end) with parasites highly adapted to their hosts at the expense of ability to harbour another host lead to establishment of new theories. One hypothesis, named „oscillation theory", proposed that diversification of an organism was maintained via phases of host expansion across geographic range followed by specialization/host race formation during periods of geographic isolation (Janz and Nylin, 2008). Phenotypic plasticity of the parasite maintained by temporary oscillations in the host population enabled parasite to become generalist again if ecological opportunities occurred.

1.2 Population genetics of parasites and their hosts

To understand the mechanisms influencing host-parasite interactions with precision, studying population genetics and dynamics became necessary. Populations are formed and maintained by various factors such as geographic distribution, climatic changes and life strategies. „Geographic mosaic theory" predicted an interplay between local adaptation and gene flow connecting geographically structured populations. Parasites could interact in different ways with different hosts in different geographic parts of its range. The mosaic distribution enabled local adaptations to emerge or disappear depending on the level of gene flow and the presence or absence of interacting species/hosts (Thompson and Fernandez, 2006). The theory could work hand in hand with „ecological fitting" hypothesis in the sense that ecological fitting elevated diversification established via local adaptions to new hosts and in some cases promoted isolation from other populations (Agosta et al., 2010). Overall diversification in parasites was driven by host „oscillations" and range shifts together with the mixing of host populations. All mentioned aspects provided opportunities for ecological fitting of parasites. The theory presented a dynamic process rather than an end point one, a process influenced by current level of gene flow between populations of parasites and hosts.
Genetic structure and diversity of populations, and their genetic connectivity are the key elements in long-term population survival and evolution, and in the origin of new species. Despite the fact that parasites represent one of the most common ecological strategies (Price 1980), majority of our knowledge on the population processes generating genetic diversity is derived from the studies on free-living organisms. The generally accepted view holds that species occupying large interconnected habitats tend to possess larger, more diverse populations, whereas species with isolated populations and/or recently bottlenecked species show reduced diversity (Allendorf et al. 2013). However, even in continental, highly mobile species, the level of local genetic diversity may differ and gene flow between the populations may be affected by moderate environmental differences, such as in the case of populations of passerines wintering in Europe(Lemoine et al. 2016).

In parasites, particularly in those with life-cycles closely bound to their hosts, as for example the parasitic lice, the host represents the sole parasite's environment. In such cases, the parasites typically develop a strong narrow host specificity, and their population structure, diversity, and speciation events are supposed to be strongly, or even entirely, influenced by their host. At phylogenetic level, this results in a parallel evolution, which may result in almost perfect fit between the host's and parasite's genealogies (Hughes et al. 2007; Light and Hafner, 2008). Mostly, host switches supressed the co-evolutionary signal, a phenomenon found even in highly host-specific parasites (Ricklefs et al. 2004; Banks et al. 2006). For example, in the sucking lice *Polyplax arvicanthis* and mice of the genus *Rhabdomys* in South Africa, du Toit et al. (2013) found that two sympatric lineages of *Polyplax arvicanthis* showed only limited congruencies with their hosts.

Similarly to discordances in host-parasite co-phylogenies, possible mechanisms causing genealogical discordances between populations of parasites and their hosts have also been discussed among parasitologists and key aspects were formulated (Toon and Hughes 2008; Lion and Gandon 2015, Wasimuddin et al. 2016). Several significant factors affecting the level of congruence in host-parasite interactions are connected with the host; especially its geographic range, vagility and social behaviour. On the other hand, the prevalence, life history and ecological traits of the parasites are supposed to play the same or sometimes even greater role in the coevolution. Degree of intimacy of every host-parasite model system could not be unambiguously predicted, because factors that were previously assumed as irrelevant performed an important task. This was for instance illustrated by Engelbrecht et al. (2016) in their study on a temporary parasite *Laelaps giganteus*, where authors found significant co-diversification pattern between mites and *Rhabdomys* mice, even though *Laelaps* mites spend most of their life off the host in their nests (Mullen and O'Connor 2002). Among the reasons, why *Laelaps* mites show seemingly higher level of intimacy than the permanent *Polyplax* lice, could be the limited dispersal abilities of mites due to their low abundance and prevalence on the hosts. Recent studies indicated the relevance to focus on parasites' population genetics and dynamics, and their main aspects for revealing coevolutionary patterns. Population structure and diversity of parasites determined several elements, such as effective population size or life history traits (Keeney et al., 2009), host dispersal abilities (e.g. Štefka et al. 2009; van Schaik et al. 2014), shared demographic history (e.g. Štefka et al. 2011, Kváč et al. 2013), feeding strategy (Jousson et al., 2000), and host-specificity (e.g. Archie and Ezenwa 2011; Booth et al. 2015).

Nadler (1995) emphasized the consequence of host spectra on forming populations of parasites in his work. According to him, generalists possessed larger effective populations due to greater "habitat" size than the host specific parasites occupying only single host species. In other words, multihost parasites were supposed to possess shallower population structure due to more opportunities to disperse. Despite the Nadler's idea could be seen in parasitological literature for decades (whether in the form of a formulated theory or as a rather vague statement), only a few empirical studies were performed to support the theory to date, such as a study of two pinworm species from the Carribean possessing different widths of host spectra (Falk and Perkins, 2013).

Koop et al. (2014) postulated another interesting idea formulated in their "hosts as islands" hypothesis. Authors concentrated on a shallow level of population structure in parasites (structure between host individuals). They predicted that limited contact between host's individuals creates a barrier restricting gene flow between infra-populations of their parasites. As a result populations of the parasites were fragmented to a greater degree than those of their hosts and more prone to the effect of genetic drift.

To provide satisfactory evidence to support or disapprove coevolutionary concepts described above, more studies performed on population level are necessary. The few models examined in detail indicate that coevolutionary processes in host-parasite systems may display a surprisingly high complexity: even in closely related lineages the genealogy and population structure may not reflect the most apparent biological features. This has been shown, for example, in a genealogical study on the human associated lice of the genus *Pediculus* (i.e. Reed et al. 2004). *Pediculus* formed two genetically distant lineages, but the division did not correspond to the two species based on morphology - *Pediculus humanus corporis* and *Pediculus humanus capitis*. While one lineage represented a ''capitis'' form dispersed mainly in the New World, the other lineage composed of admixture of both ''capitis'' and ''corporis'' forms with a worldwide distribution. A similar pattern of "random" changes of bionomical features have

been confirmed during a previous research in our laboratory on a phylogenetic/genealogy scale in two additional host-parasite associations, the lice of the genus *Polyplax* (Štefka and Hypša 2008) and the tapeworm *Ligula intestinalis* (Štefka et al. 2009). In addition to the genealogical component, the population level of host-parasite coevolution also involves many demographic aspects, such as estimation of migration rate and gene exchange, or tracing of the population size changes. During the last decade, methodological approaches in this research area experienced rapid advancement (Johnson et al., 2013; Sweet et al., 2018; Hooper et al., 2019) and they now allow for inferring complex demographic and phylogeographic scenarios form various types of genomics data. Although proved to be extremely useful in general host-parasite system, these concepts have only rarely been interconnected for parasites, hosts and symbiotic taxa.

1.3 Model organisms

1.3.1 Apodemus mice

Apodemus mice are considered amongst the most common small mammals in Palearctic region. Out of the 20 recognized species about one half occupies a variety of habitats in Europe. Interestingly, two (or even more) species of the genus often coinhabit and show resource partitioning (Suzuki et al., 2008). This phenomenon is also typical for the two most prevalent European species: *Apodemus flavicollis* and *A. sylvaticus*. Even though their ecological preferences slightly differ, they live syntopically across large part of their distribution. Furthermore, they possess similar phenotypes sometimes causing precise species determination a problem. Despite many shared features between both species, they reacted on Quarternary glaciation in a notably different manner. Iberian Peninsula served as a refugium for *A. sylvaticus* from which it recolonized western and northern Europe. *A. flavicollis* did not survive there, but it spent glaciation era in fragmented areas of Balkan region, where *A. sylvaticus* suffered genetic bottleneck (Michaux et al., 2005). *A. agrarius* is another generalist forest inhabitant common in the temperatate zone of Palearctic with an interesting evolutionary history. The species originated in Asia, then it expanded during the last 200 000 years from Far East Russia to Germany in the west, Finland in the north and Greece and Italy in the south (Suzuki et al., 2008). Such complex history of *Apodemus* mice influenced not only population genetics of particular *Apodemus* species themselves, but, at the same time, also the genealogies of their parasites.

1.3.2 Polyplax serrata lice

Lice of the genus *Polyplax* parasitize a wide range of rodent hosts throughout the world. The species of *Polyplax serrata* was described from two rodent genera and one genus of insectivores (e.g. Durden and Musser 1994), but it was predominantly found on *Apodemus* mice across Palearctic region (e.g. Krištofík 1999, Krištofík and Dudich 2000). According to coevolutionary principles the phylogenies of lice, as permanent ectoparasites, should mirror that of their hosts. From the pilot study of Štefka and Hypša (2008) it emerged that *Apodemus* mice influenced relationships among haplotypes of *Polyplax*, but not in an easily interpretable way. Population structure of the parasite based on 1 mtDNA and 1 nuclear gene was much more complex than was previously estimated and consisted of 3 clades diverging in the degree of host-specificity and geography. The authors described a host-specific clade B with 40 sequences from a single host, *A. flavicollis*, then a less specific clade A with haplotypes frequently collected from both *A. flavicollis* and *A. sylvaticus*, and a clade C with *A. agrarius* as the major host, less frequently found on *A. uralensis*. Clade B occurred in the Czech Republic and Slovakia, clade A partly

overlapped in distribution, but expanded to western Europe as well, and clade C had area restricted to the eastern part of Czech Republic and to Slovakia. Age of the separation of clades A and B was estimated to 1.5 Mya, approximately 2.5 Mya after the split of *A. sylvaticus* and *A. flavicollis*. Hence, the speciation event probably happened as a consequence of parasite's lineage duplication during fragmentation of populations during quaternary glaciations. Whether the three clades represent different species or whether the authors only uncovered an event of past isolation with possibility of recent admixture of lineages remained a question, which only more extensive sampling across Europe and more informative genetic tools could resolve.

1.3.3 Menacanthus eurysternus

Cosmopolitan chewing lice genus *Menacanthus* comprises of 98 species that parasitize more than 460 bird species belonging to seven orders (Price et al., 2003; Bansal et al., 2012). More than one third of the lice (36 species) live on passeriform birds and possess quite narrow host-specificity. Among them 10 species are monoxenous (restricted to single host species), 25 stenoxenous (narrow range of potentional host) and the only exception is *Menacanthus eurysternus* known from 170 passerines and 8 woodpeckers (Price et al., 2003). Whereas majority of *Menacanthus* louse species feed on detritus of skin and feathers of birds, *M. eurysternus* belongs among haematophagous insects, which, together with its high prevalence on hosts (about 60 % according to Chandra et al., 1990), could influence health condition of its hosts (Agarwal et al., 1983). Phthirapterists are not uniform about taxonomic status of *M. eurysternus*. On one hand, several authors consider *M. eurysternus* a complex of several species (Fedorenko, 1983; Banks and Paterson, 2005), on the other hand, Price et al., (2003) described it as one euryxenous (multihost) species.

At the time this thesis was commenced, only a few mtDNA gene sequences of *Menacathus eurysternus* were available in Genebank, with relatively low level of differences among nucleotide positions (4-7%). That was surprising in respect of the distant geographic origin of samples and general unrelatedness of their hosts. This pattern was counterintuitive in amblycerans, in which louse lineages with unrelated hosts typically showed higher levels of divergence. Similarity of sequences does not exlude a possibility of deeper population structure in *M. eurysternus* influenced more by other key factors than host-specificity. Genetic reconstruction of the genus, with particular attention to the multi-host species of *M. eurysternus* was required not only from the taxonomic point of view, but at the same time it had a potential to serve as a rare model for studying the evolution of host-specificity.

1.3.4 Genus Eimeria as frequent parasite of Apodemus mice

Genus *Eimeria* was considered as the most numerous coccidian genus with more than 1700 species described from various hosts across the world, about 400 harbour rodents (Duszynski and Upton, 2001). It has paraphyletic origin with respect to several *Cyclospora* and *Isospora* species (Carreno and Barta, 1999; Franzen et al., 2000; Modrý et al., 2001). Eimerians were often monoxenous parasites that infest gastrointestinal tract and were transmitted by faeces of the parasitized animals. New hosts were infected by ingestion of sporulated oocysts (Duszynski et al., 1999).

Previous work revealed complex patterns between *Eimerians* and *Apodemus* mice, with several species found on broader range of hosts while others parasitize only one host species (Kvicerova and Hypsa, 2013). Together with clearly polyphyletic origin all the explicit facts predestined them as a suitable model for studying host-switching and evolution of host-specificity.

1.4. Aims

The main objective of the thesis is the exploration genealogical structure, intraspecific variability and genealogical relationships within populations of the selected models. The influence of host-specificity and geographic factors on gene flow and structure of populations of the parasites will be evaluated. Main aims of the thesis are as follows:

1. To characterise of new polymorphic microsatellite loci in *Polyplax serrata*

2. a) To Approve/disapprove mtDNA integrity and host specificity of *Polyplax* lineages according to Štefka and Hypša, 2008 with extended sampling material and multilocus markers b) to compare population structures of *Polyplax lice* and *Apodemus* hosts c) to evaluate differences in population structure of sister S and N clades in the sense of Nadler´s rule

3. a) To reconstruct genealogy of *Menacanthus* chewing lice and compare it with morhological determination of species b) to concentrate on two *Menacanthus* species differed in the width of host spectra and evalute possible mechanisms influenced population structure of both taxa

4. To reconstruct the topology of *Apodemus*-associated eimerians together with eimerians from Arvicolinae rodents and interpret observed patterns with respect to host-specificity and geography

2. Summary of results

2.1 The *Polyplax serrata* and *Apodemus* mice model

To elucidate recent events connected with the emergence of louse population structure, multilocus markers were considered as appropriate tools. Enriched microsatellite libraries were constructed with two different approaches, initially the protocol of Fleisher and Loew (1995) was used, later on supplemented with the commercial NGS service (Genoscreen, France). Altogether 16 loci polymorphic in one louse population were composed to 4 multiplexes and tested on two populations from the host-specific lineage of *P. serrata* (S) and two populations from the nonspecific lineage (N) (**MS no.1; Table 1).** Eleven loci deviated from Hardy Weinberg equilibrium (HWE) in at least one tested population, one locus (PS 42) was excluded from further testing because of deviations across the studied set. Linkeage disequilibrium (LD) was found in 6 and 7 loci in German populations of the S and N lineages apparently due to hidden genetic structure caused by the usage of an elevated number of louse specimens per host individual (restricted gene flow between infrapopulations). Deviations from HWE and observed LD could be caused by inbreeding of multiple generations of lice on single host individual followed by reduction of heterozygotes and lowered diversity where genetic loci are inherited in linked blocks. In some cases null alleles could stand behind lower heterozygosity as well. Pairwise FST distances reflected mtDNA subdivision of *Polyplax serrata* with lower differences between populations within S or N lineages in comparison to higher distances between populations from different lineages. Apparent connectivity between patterns seen in mtDNA and microsatellite data determined microsatellites as the appropriate genetic marker for further population study of the lice.

Following the pilot study of Štefka and Hypša (2008) mitochondrial cytochrome oxidase I (COI) from another 430 specimens of lice sampled across 14 European countries (**MS no.2; Fig. 1**) were added to the dataset gained by the authors cited above. Resulting 126 haplotypes

split into 3 well supported lineages already described in previous work (**MS no.2; Fig. 2**). Relationships between the specific S (previously lineage B), nonspecific N (lineage A) and *A. agrarius* Aa (lineage C) clusters remained unresolved with the short 381 bp fragment of COI, therefore longer COI sequence together with 3 nuclear genes were used and confirmed S and N lineages as sister taxa (**MS no.2; Fig. S1)**. Even though the lice from S and N clusters lived sympatrically across large geographic area, N lineage did not show clear geographic substructuring (**MS no.2; Figs. 2, S3)**, wheras strong structure was detected in the S lineage. The S cluster was divided into two subgroups, named S_{EAST} and S_{WEST}, with almost exclusive geographic areas except for a narrow suture zone in the nort-west of the Czech Republic (**MS no.2; Figs. 2, S4)**. The third, Aa, lineage was found only in Eastern Europe mostly on *Apodemus agrarius* and *A. uralensis* (**MS no.2; Figs. 2)**.

To complement genetic information on the parasites with information on the hosts, mitochondrial Dloop from 229 *Apodemus flavicollis* and 92 *A. sylvaticus* were sequenced. *A. flavicollis* tree was split into two distinct lineages Af_1 and Af_2, which largely overlapped in their distribution, but differed in abundance (**MS no.2; Figs. 3, S5)**. *A. sylvaticus* composed of 3 clusters; As_1 and As_2 cooccurred across western Europe and Iberian Peninsula, and As_3 (paraphyletic with respect to As_2) was concentrated on Italian-Balkan peninsulas (**MS no.2; Figs. 3, S6)**.

Nucleotide diversity statistics corroborated the geographic differentiation visible within louse lineages using phylogenies and haplotype networks. Diversity statistics suggested that *Polyplax* populations spread from glacial refugia experienced initial genetic decline and expansion afterwards. On the other hand, demographic fluctuations within host species were much less pronounced. Major lineages of *A. flavicollis* and *A.sylvaticus* had high levels of haplotype diversities and haplotypes within clades of both species were largely geographically admixed and created panmictic populations (**MS no.2; Tables S2, S3)**.

Microsatellite multiplexes constructed for *P. serrata* in MS no.1 were used as an alternative source of information. All loci were polymorphic in at least 15 out of 32 screened populations, with up to 11 alleles per locus and population (**MS no.2; Table S5)**. As expected, lice showed low levels of heterozygosity (**MS no.2; Table 2)** and absence of heterozygotes lead to deviations from HWE (**MS no.2; Table S6)**, more frequent in the specific lineage S than nonspecific one (N). Possible presence of null alleles in some loci was indicated, but even revised values of genetic diversity differed only marginally in chosen populations (**MS no.2; Table S7)**, so all loci were retained for further use. Pairwise F_{ST} displayed wide range of genetic differences between populations with values 0.04 – 0.65 measured in the S lineage and 0.1 – 0.39 in the N lineage (**MS no.2; Table S8).**

Multilocus markers were applied for host taxa as well. For *A. flavicollis* 12 loci were adopted from Harr, Musolf, and Gerlach (2000) and Aurelle et al. (2010), 7 of them were shared with *A. sylvaticus*. For the latter species 17 loci were analyzed (10 exclusively for *A. sylvaticus*). All loci showed sufficient rate of polymorphism in both species (**MS no.2; Table S9)**. In most populations 1-4 loci deviated from HWE (**MS no.2; Table S10)**. Pairwise F_{ST} values ranged in *A. flavicollis* 0.03 – 0.47; in *A.sylvaticus* 0.04 – 0.59 (**MS no.2; Table S8)**.

PCoA analysis of microsatellites of louse and host populations detected much deeper population structure in *P. serrata* compared to *Apodemus* mice. Lice split to clusters according to main mtDNA lineages with the exception of one population, CZLi05N, that belonged to nonspecific lineage N mitochondrially, but PCoA on nuclear data showed its affiliation to the S lineage

(**MS no.2; Fig. 4)**. Distance based methods and Bayesian clustering confirmed the same patterns (**MS no.2; Figs. S8, S9)**. In PCoA analyses of individual genotypes, individuals within S and N lineages from geographically neighbouring samples were often considered as genetically closely related ones, and lice from the same localities usually created compact clusters. On the other hand suture zone within S lineage apparent from mtDNA genealogy was not detected. Multilocus analyses of seven loci shared by both host species confirmed clear genetic separation of *A. flavicollis* and *A. sylvaticus*. Pattern revealed by intraspecific PCoA did not follow clustering into mtDNA lineages and mouse individuals were much more admixed when compared with lice. The same analysis performed using population data instead of individuals showed several clusters that not reflect either mitochondrial tree subclades or biogeographical patterns (**MS no.2; Fig. S11)**. Bayesian and distance based methods revealed similar results (**MS no.2; Figs. S9, S12)**.

Results of isolation by distance (IBD) performed with Mantel test differed with the statistics used and lineage (in lice) or species (in hosts) analysed. IBD measured using pairwise F_{ST} was statistically significant only for *A. sylvaticus*, G_{ST} showed significant values in *A. sylvaticus* and *Polyplax* S lineage and D_{JOST} in *Polyplax* S (**MS no.2; Figs. S13, S14)**. Comparing correlation between pairwise Euclidean distances of individuals within S and N lineages and their geography, significant IBD was revealed in *Polyplax* S (**MS no.2; Fig. 5)**. Autocorrelation coefficient r, estimated the impact of IBD in dependence on different geographic scales, was calculated separately for *Polyplax* S and N and *A. flavicollis* and *A. sylvaticus*. Both parasites and hosts were influenced with positive significant autocorrelation that declined with increasing distances (**MS no.2; Fig. S15)**. The highest coefficient measured between neighbouring populations were two times greater in parasites than in hosts and ten times lower in *A. flavicollis* compared to *A. sylvaticus*.

Lineages of *P. serrata* were used as represetatives of generalist (N lineage) and specialist (S lineage) parasites for the verification of Nadler´s rule. The hypothesis assumes specialistsshow deeper genetic structure (higher values of F_{ST}) and limited chances to disperse (lower level of H index) compared to generalists. F_{ST} index showed significantly higher values for the S lineage (0,46) than N lineage (0,241) and, conversely, the H index was markedly lower in the S (0,389) than N (0,587) lineage. More detailed examination of genetic diversity of the parasites, performed on seven pairs of sympatric S and N populations collected across Europe, revealed the same pattern as was visible in the summary data for each lineage (**MS no.2; Fig. 6)**.

All results obtained for the parasites and hosts supported the general view that parasite's genealogy was strongly determined by that of their hosts, but also that parasites possessed lower genetic connectivity and deeper structure. On the other hand, the model system of *Apodemus*/*Polyplax* revealed a variety of other complex and intriguing patterns. In lice, even the closely related sister lineages N and S, living sympatrically across many European countries, differed in many aspects. The most striking was the absence of S lineage on *A. sylvaticus* that seemed to be the consequence of adaptive contstraints rather than insufficiency of chances to harbour (host switch) another host. Differences between host specificity in sister species impacted their uneven population structures.

Current distribution of *Apodemus sylvaticus* and *A. flavicollis* corresponded to the well described recolonization processes in Europe after Quaternary glaciation. Mice spread to northern and western parts of the continent from their southern and easten refuia and formed admixed populations at most places. Counterintuitively, *Polyplax* lice did not always mirror the tendencies of their hosts despite the intimacy of their relationship. The most conspicuous

conflict arose between the genealogies of *A. flavicollis* and *Polyplax S* lineage. Despite mice created panmictic population across the sampled area, lice from different refugia met in Central Europe where the expansion stopped forming a narrow mtDNA contact zone. The inability to identify the same suture zone with microsatellites could be explained by different level of information captured by the highly polymorphic loci that mirror the current degree of gene flow across the zone, but do not detect more ancient events.

S and N lineages should be considered as different taxonomic units due to clear mtDNA and nuclear genetic separation. Despite this fact the two lineages are not entirely resistant to occasional gene flow. One case of probable mitochondrial introgression was found at one locality, where two mitochondrially separated populations from S and N lineages clustered together to the S lineage using multilocus nuclear data. Since lice possess small genomes and fragmented populations, they are susceptible to genetic drift that leads to faster fixation of unoriginal haplotypes. On the other hand, introgression could be hardly traced with genetic markers after a few generations of back crossing which could explain the uniqueness of the observed event.

Overall deficiency of heterozygotes in *Polyplax* lice, mainly caused by limited levels of gene flow, even over short distances, supported the idea that host dispersal is the main driver of genetic diversity in this host/parasite system. Statistics comparing S and N lineages supported Nadler‘s rule. Lice from the N lineage, the representative of a generalist parasite, possess better chances to find convenient hosts and disperse more easily than a specialist parasite, the single host S lineage. Apparently even these relatively mild differences in host specificity can lead to significant changes in genetic characteristics between populations of parasites.

2.2 The *Menacanthus* model

In the study we demonstrated the existence of big differences in host-specificity between species of *Menacanthus* lice that could be only partially explained with ecology of their hosts. On population structure of multihost species *M. eurysternus* we provided evidence that geography served as the main driver of the genealogical structure rather than relatedness of bird hosts. Two convenient markers, mitochondrial cytochrome oxidase I and nuclear EF-1α, were used for the reconstruction of phylogeny and genealogy in *Menacanthus* lice. Phylogeny of mtDNA gene resulted in well supported clades with *Menacanthus cornutus* from domestic chicken nested at the base of the tree together with a sequence of *Menacanthus sp*. from a cracid host obtained from Genebank (**MS no.3; Fig. 1**). The rest of the ingroup divided into two supported units, named after the earliest described species as „curuccae“ and „alaudae“. Morphologically determined species mostly corresponded with monophyletic lineages, with one exception of the *Menacanthus takayamai*, which was paraphyletic with respect to *M. sinuatus* and *M. eurysternus* (**MS no.3; Fig. 2**). Altogether 14 louse species differed markedly in the width of their host specificity. Whereas most of the species were harboured by only one bird family, *Menacanthus obrteli* and *M. eurysternus* differed from the rest with their wide variety of hosts. The former species not only confirmed its legitimacy as valid taxon (Sychra et al., 2008) with its basal position in the „curuccae“ clade, but more importantly it was shown to be capable of infesting an even wider host spectrum than was previously known. The *M. obrteli* cluster consisted of haplotypes from turdid, parid, laniid and locustelid birds on top of the Savi´s warbler as the only previously described host. *M. eurysternus,* as expected by Price et al. (2003), created a monophyletic lineage located terminally in the „curuccae“ clade. Its lice were found on 63 bird species from 12 families of passeriform and piciform orders sampled at 15 localities across Europe, Asia, the Neotropics and Africa. Thirteen new host associations were found.

Topology of EF-1α showed weaker phylogenetic signal that lead to lower resolution between clades. *M. stramineus* branched at the base of the tree together with two haplotypes from turdid and picid hosts (**MS no.3; Supplementary Fig. S1**). Chicken body louse (*M. stramineus*) had been before determined only from phasianid birds (Price et al., 2003). Compared to the mtDNA tree, *M. cornutus* created sister clade to „alaudae" group and the positions inside „alaudae" clade differed, but both dissimilarities lacked support. More importantly, terminal clades formed the same species lineages as in COI phylogeny.Concatenated alignment (**MS no.3, Fig. 3**) resembled COI topology with the exception of a different branching inside „alaudae" group, which was, however, without any statistical support.

Haplotype networks were reconstructed for the two most numerous species, *M. eurysternus* and *M. camelinus*, to compare their intraclade (intraspecific) population structures. Diversities of their host spectra (**MS no.3; Fig. 4 A,B**) and geographic origin (**MS no.3; Fig. 4 C,D**) were mapped on networks to assess their influence on genealogies. *M. camelinus*, as a representative of monoxenous parasite, showed little sequence variation, with majority of haplotypes from one bird species, *Lanius collurio*, sampled from two European countries, Bulgaria and Sweden. One specimen found on an atypical host, *Turdus merula*, shared the same haplotype with three lice from *L. collurio*. With regard to the rarity of such host switch we considered the louse from *T. merula* an accidental straggler. We did not find any correlation between genetic structure and geographic origin of the samples, probably because the lice homogenize on wintering grounds of their bird hosts, where the birds share relatively small place. Contradict to the expectancy, *M. eurysternus* as euryxenous parasite showed only moderately higher sequence diversity than *M. camelinus*. *M. eurysternus* haplotype network was split into two distant lineages, one from *Prinia subflava*, of African origin, and the second formed by the rest of lice from Europe, Asia, Central America and 2 other hosts from Africa. Genealogy of the haplotypes did not mirror relationships between host taxa. Moreover, 2-3 families of hosts shared the same haplotypes in one third of the cases. The complex structure was rather influenced by geographic origin of the lice than by host taxonomy. Except 1 haplotype from 2 distant localities in Palearctic, the majority of haplotypes consisted of 1 or 2 countries from the same sub-continent. NCPA analysis confirmed that several intraspecific events in *M. eurysternus* were connected to geographic-determined origin (**MS no.3; Fig. 5**). Obtained information suggests that overlapping distribution and similar habitat preferences of the birds play major role in forming the genealogy of louse.

Evolution of *Menacanthus* lice did not undergo a co-speciation process, which is described in some amblyceran taxa that parasitize narrow spectra of birds (e.g. Hughes et al., 2007). Instead, frequent host switching occurring in the evolution of *Menacanthus*, contradicts together with several other studies (i. e. Johnson et al. 2011, Booth et al. 2015) the traditional dogma about parasites - their tendency to specialize on their host(s) further and futher in evolution. Processes with opposite directory (from specialist to generalist) evolved at least in 3 lineages; *M. eurysternus*, *M. obrteli* and *M. stramineus*. *Menacanthus* lice formed complex species genealogies with representatives of all spectra of host-specificity; from monoxenous *M. camelinus* to the multihost *M. eurysternus*. This nonuniformity of *Menacanthus* lice predetermines them to serve as model organism for further research.

2.3 *Eimeria* from *Apodemus*

Eimerians from *Apodemus* mice supplemented with specimens from Arvicolinae rodents served as suitable model for studying relatively recent host switching events and for investigating possible directions of evolution in host-specificity.

Phylogeny based on COI sequences from175 *Apodemus* mice, 36 Arvicolinae representatives and several specimens from other small rodents resulted in a well resolved tree with supported inner relationships (**MS no. 4; Fig. 2)**. Eimerians from *Apodemus*, *Microtus* and *Clethrionomys* created several clusters, where three main lineages were in concordance with morphological determination of the taxa and named after them as *apionodes, uptoni* and *jerfinica*. Another two clusters of subspecific structure of *apionodes* were assigned according to morphology as *kaunensis* and *alorani,* and the rest was named with Roman numerals I-IV. Host specificity of the lineages differed markedly, with *uptoni* and *jerfinica* as examples of specialists with affinity only to *Apodemus* genus and *apionodes* group with variety of relationships. *Apionodes* group showed the tendency of conversion from Arvicolid to *Apodemus* mice. Samples of the most basal *Apionodes* I parasitize only arvicolid mice, clusters *kaunensis*, *apionodes* III and IV were formed by a mixture of samples from various host groups and IV differed with only *Apodemus* samples. *Apionodes* II and *alorani* as derived offshoots had affinity only to *Apodemus* mice, and the latter lineage went further and was harboured by only 1 species of *Apodemus*, *Apodemus agrarius*.

In geberal, the tree of 18S rDNA showed the same pattern as COI phylogeny, with only a few samples contradicting the mtDNA tree (**MS no. 4; Fig. 3)**. All of them came from mixed infections, so the main obvious explanation was amplification of different *Eimerian* lineages with each marker.

Interesting geographic aspect was found between lineages with the same host specificity but distant phylogenetic origin. Many of them were gathered at sympatric localities. On the other hand, geographic contrast was observed among lineages, where *Eimeria* switched from Af/As to *A. agrarius* host. While in *apionodes* II, *A. agrarius* samples were nested inside Af/As localities, in case of *apionodes* IV with affinity to Af/As and *alorani* specific for Aa both lineages had exclusive distributions without overlap. Haplotype network of *apionodes* group nicely depicted the differences described above (**MS no. 4; Fig. 5)**. Relative ages in BEAST topology indicated recent origins of both host-switches from Af/As to Aa (**MS no. 4; Fig. 4)**. GammaST statistics revealed genetic barriers between Af/As and Aa within *Apodemus* specific clusters with highest values within the *jerfinica* lineage (**MS no. 4; Fig. 4)**.

The study confirmed polyphyletic nature of *Eimeria* harboured by *Apodemus* mice and revealed cases of frequent host switches between specific taxa, followed by fast fixation. Part of *apionodes* group indicated sequential restriction of host-specificity within the inner structure terminated with *alorani* lineage as the representative of a specialist. On the contrary, some taxa showed inverse direction of the extension of hosts (i.e. lineages *apionodes* III, *apionodes* IV). Eimerian genealogy gave us another piece of evidence that host-parasite systems are rather maintained by a dynamic process than long - termed stationary coevolution.

Quantification of the genetic diversification between Af/As and Aa in Eimerians restricted to *Apodemus* hosts revealed interesting pattern of emerging genetic barrier in *jerfinica* samples connected to each host species, in contradiction to the picture of mixed samples from Af/As/Aa seen in phylogenies. GammaST was markedly higher between Af/Aa and As/Aa (0,46; 0,22) than Af/As (0,14). The *alorani* went further with its separation from Af/As and created morphological adaptations that distinguished them from *apionodes* IV. Evident repetitive tendency of *Eimeria* to switch towards Aa evoked questions about the mechanism standing behind these observed events. The mechanism remained unknown with the current knowledge, but ecological or physiological differences between hosts seemed the most intuitive.

3. Conclusions and future goals

Presented research of *Polyplax* and *Menacanthus* lice and *Eimeria* coccidenas helped to untangle several interesting problems in the field of co-evolution of parasites and hosts, particularly the role of host specificity and dispersal affecting population processes at the finer genealogical scale. However, new crucial questions have arisen, for example: what stands behind the allopatric mtDNA distribution of S_{EAST} and S_{WEST} Polyplax lineages and prevents the lice to admix, unlike their host *A. sylvaticus*, which is panmictic ? Financial accessibility of Next Generation Sequencing (NGS) methods allowing in depth genomic screening of the populations should help us to reveal mechanisms underlying co-evolutionary processes of the lice and their hosts and at the same time concentrate on the concept of microbiome, treating host and associated microorganisms as a single unit influenced by selection. With the discovery of *Legionella polyplacis* as the symbiont of *P. serrata* and its biotin operon as the source of vitamins for lice, the possibility of occurrence of symbiont-mediated barrier exists. The idea is based on the known phenomenon that symbiotic bacteria can play important role in genetic incompatibility between insect populations. Preliminary amplicon sequencing results, performed by laboratory colleagues, found deep inter-population microbiome differences in *Polyplax*, with particularly aberrant microbiome composition at the "contact zone".
Microbiome theory would be useful also in the *Menacanthus* model, in the sense of screening all possible symbiotic candidates playing role in the adaptation processes towards the ability of some species to successfully feed on blood.
In Eimerians only the west-most region of *A. agrarius* distribution area were sampled and the results might only reflect the change of eimerian fauna at the contact of this species with the most common European species, *A. flavicollis* and *A. sylvaticus*. Investigation of *A. agrarius* from further localities in the east will be necessary to track possible shifts to the original fauna (be it abrupt change or more gradual mixing).

4. References

Agarwal, G.P., Saxena, A.K., Chandra, S., 1983. Haematophagous behaviour of *Menacanthus eurysternus* (Mallophaga, Amblycera). Angewandte Parasitologie, 24: 55–59.

Agosta, S.J., Janz, N., Brooks, D.R. 2010. How specialists can be generalists: resolving the "parasite paradox" and implications for emerging infectious disease. Zoologia, 27: 151–162.

Allendorf, F.W., Hohenlohe, P.A., Luikart, G. 2010. Genomics and the future of conservation genetics. Nature Reviews Genetics 11: 697–709.

Archie, E.A., Ezenwa, V.O. 2011. Population genetic structure and history of a generalist parasite infecting multiple sympatric host species. International Journal for Parasitology, 41: 89–98.

Aurelle, D., Baker, A.J., Bottin, L., Brouat, C., Caccone, A., Chaix, A., Yuan, Q.Y. 2010. Permanent genetic resources added to the molecular ecology resources database. Molecular Ecology Resources, 10: 751–754.

Avise, J.C. 2000. Phylogeography. The history and formation of species. Cambridge, MA: Harvard University Press.

Banks, J. C., Palma, R. L., Paterson, A. M. 2006. Cophylogenetic relationships between penguins and their chewing lice. Journal of Evolutionary Biology, 19: 156–166.

Banks, J.C., Paterson, A.M. 2005. Multi-host parasite species in cophylogenetic studies. International Journal for Parasitology 35: 741–746.

Bansal, N., Ahmad, A., Arya, G., Khan, V., Saxena, A.K., 2012. *Menacanthus palmai*,a new species of chewing louse (Menoponidae: Amblycera: Phthiraptera) from the *Coturnix coromandelica*. Journal of parasitic diseases, 37: 276–280.

Bilton, D.T., Mirol, P.M., Mascheretti, S. 1998. Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonization. Proceedings of the Royal Society of London. Series B, Biological Sciences, 265: 1219–1226.

Booth, W., Balvín, O., Vargo, E.L., Vilímová, J., Schal, C. 2015. Host type differentiation drives deep divergence in the common bed bug, *Cimex lectularius*. Molecular Ecology, 24: 980-992.

Brooks, D.R., Dowling, A.P.G., van Veller, M.G.P., Hoberg, E.P. 2004. Ending a decade of deception: a valiant failure, a not-so-valiant failure and a success story. Cladistics, 20: 32–46.

Carreno, R.A., Barta, J.R., 1999: An eimeriid origin of isosporoid coccidia with stieda bodies as shown by phylogenetic analysis of small subunit ribosomal RNA gene sequences. Journal of Parasitology 85: 77–83.

Chandra, S., Agarwal, G.P., Singh, S.P.N., Saxena, A.K. 1990. Seasonal changes in a population of *Menacanthus eurysternus* (Mallophaga, Amblycera) on the Common Myna *Acridotheres tristis*. International Journal for Parasitology, 20: 1063–1065.

Demanche, C., Berthelemy, M., Petit, T., Polack, B., Wakefield, A.E., Dei-Cas, E., Guillot, J. 2001. Phylogeny of *Pneumocystis carinii* from 18 primate species confirms host specificity and suggests coevolution. Journal of Clinical Microbiology, 39: 2126– 2133.

Dowling, A.P.G., van Veller, M.G.P., Hoberg, E.P., Brooks, D.R. 2003. A priori and a posteriori methods in comparative evolutionary studies of host–parasite associations. associations. Cladistics, 19: 240–253.

Durden, L.A., Musser, G.G. 1994. The sucking lice (Insecta, Anoplura) of the world: a taxonomic checklist with records of Mammalian hosts and geographical distributions. Bulletin of the American Museum of Natural History, 218: 1–90.

Duszynski, D.W., Upton S. J., 2001. *Cyclospora*, *Eimeria*, *Isospora*, and *Cryptosporidium* spp. In: Samuel, W.M., Pybus, M.J., Kocan A.A. (Eds.): Parasitic Diseases of Wild Mammals, 2nd Edition. Iowa State Press, Iowa City: 416–433.

Duszynski, D.W., Wilson, W.D., Upton, S.J., Levine N.D. 1999. *Coccidia* (Apicomplexa: Eimeriidae) in the Primates and the Scandentia. International Journal of Primatology, 20: 761–797.

du Toit, N., Van Vuuren, B. J., Matthee, S., Matthee, C. A. 2013. Biogeography and host-related factors trump parasite life history: Limited congruence among the genetic structures of specific ectoparasitic lice and their rodent hosts. Molecular Ecology, 22: 5185–5204.

Eichler, W. 1941. Wirtspezifität und stammesgeschichtliche Gleichläufigkeit (Fahrenholzsche Regel) bei Parasiten im allgemein und bei Mallophagen im besonderen. Zoologischer Anzeiger, 132: 254–262.

Engelbrecht, A., Matthee, S., du Toit, N., Matthee, C.A. 2016. Limited dispersal in an ectoparasitic mite, *Laelaps giganteus*, contributes to significant phylogeographic congruence with the rodent host. Molecular Ecology, 25: 1006–1021.

Falk, B.G., Perkins, S. L. 2013. Host specificity shapes population structure of pinworm parasites in Caribbean reptiles. Molecular Ecology, 22: 4576–4590.

Fedorenko, I.A., 1983. Chewing Lice – Menoponidae. Fauna of Ukraine, 22.

Fleischer, R.C., Loew, S. 1995. Construction and screening of microsatellite-enriched genomic libraries. In: J. Ferraris and S. Palumbi (Eds.), Molecular Zoology: Advances, Strategies and Protocols. Wiley-Liss, New York: 459–468.

Franzen, C., Müller, A., Bialek, R., Diehl, V., Salzberger, B., Fätkenheuer, G. 2000. Taxonomic position of the human intestinal protozoan parasite *Isospora belli* as based on ribosomal RNA sequences. Parasitology Research. 86: 669–676.

Hafner, M. S., Nadler, S. A. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. Nature, 332: 258–259.

Harr, B., Musolf, K., Gerlach, G. 2000. Characterization and isolation of DNA microsatellite primers in wood mice (*Apodemus sylvaticus*, Rodentia). Molecular Ecology, 9: 1664–1665.

Hoberg, E. P., Brooks, D. R. 2008. A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host-parasite systems. Journal of Biogeography, 35:1533–1550.

Hooper, R., Brealey, J.C., van der Valk, T., Alberdi, A, Durban, J.W., Fearnbach, H., Robertson, K.M., Baird, R.W., Bradley Hanson, M., Wade, P., Gilbert, M.T.P., Morin, P.A., Wolf, J.B.W., Foote, A.D., Guschanski, K 2019. Host-derived population genomics data provides insights into bacterial and diatom composition of the killer whale skin. Molecular Ecology, 28: 484–502.

Hosokawa, T., Kikuchi, Y., Nikoh, N., Shimada, M., Fukatsu, T. 2006. Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. PLoS Biology, 4: e337.

Hughes, J., Kennedy, M., Johnson, K.P., Palma, R.L., Page, R.D.M. 2007. Multiple cophylogenetic analyses reveal frequent cospeciation between pelecaniform birds and *Pectinopygus* lice. Systematic Biology 56: 232–251.

Janz, N., Nylin, S. The Oscillation Hypothesis of Host-Plant Range and Speciation In: Tilmon, K.J., editor. Specialization, speciation, and radiation: the evolution of herbivorous insects. 2008. pp. 203–215.

Johnson, K.P., Malenke, J. R., Clayton, D. H. 2009. Competition promotes the evolution of host generalists in obligate parasites. Proceedings of the Royal Society of London, B 276: 3921–3926.

Johnson, K.P., Walden, K.K., Robertson, H.M. 2013. Next-generation phylogenomics using a target restricted assembly method. Molecular Phylogenetics and Evolution, 66:417–422.

Johnson, K.P., Weckstein, J.D., Bush, S.E., Clayton, D.H. 2011. The evolution of host specificity in dove body lice. Parasitology, 138: 1730–1736.

Jousson, O., Bartoli, J., Pawlowski, J. 2000. Cryptic speciation among intestinal parasites (Trematoda: Digenea) infecting sympatric host fishes (Sparidae). Journal of Evolutionary Biology, 13: 778–785.

Keeney, D.B., King, T.M., Rowe, D.L., Poulin, R. 2009. Contrasting mtDNA diversity and population structure in a direct-developing marine gastropod and its trematode parasites. Molecular Ecology 18: 4591–4603.

Koop, J.A.H., DeMatteo, K.E., Parker, P.G., Whiteman, N.K. 2014. Birds are islands for parasites. Biology Letters, 10: 6–10.

Krištofík, J. 1999. Sucking lice (Phthiraptera) from Mongolian mammals. Biológia, 54: 143–149.

Krištofík, J., Dudich, A. 2000. Sucking lice of the *Enderleinellus*, *Hoplopleura*, *Schizophthirus* and *Neohaematopinus* genera (Phthiraptera) on small mammals (Insectivora, Rodentia) in Slovakia. Biológia, 55: 487–499.

Kváč M., McEvoy J., Loudová M., Stenger B., Sak B., Květoňová D. 2013. Coevolution of *Cryptosporidium tyzzeri* and the house mouse (*Mus musculus*). International Journal Parasitology, 43: 805–817.

Kvičerová, J., Hypša, V. 2013. Host-parasite incongruences in rodent *Eimeria* suggest significant role of adaptation rather than cophylogeny in maintenance of host specificity. PLoS ONE 8: e63601.

Lemoine, M., Lucek, K., Perrier, C., Saladin, V., Adriaensen, F., Barba, E., Richner, H. 2016. Low but contrasting neutral genetic differentiation shaped by winter temperature in European great tits. Biological Journal of the Linnean Society, 118: 668–685.

Light, J. E., Hafner, M. S. 2008. Codivergence in heteromyid rodents (Rodentia: Heteromyidae) and their sucking lice of the genus *Fahrenholzia* (Phthiraptera: Anoplura). Systematic Biology, 57: 449– 465.

Lion, S., Gandon, S. 2015. Evolution of spatially structured host-parasite interactions. Journal of Evolutionary Biology, 28: 10–28.

Michaux, J.R., Libois, R.. Filippucci, M.G. 2005. So close and so different: comparative phylogeography of two small mammal species, the yellow-necked fieldmouse (*Apodemus flavicollis*) and the woodmouse (*Apodemus sylvaticus*) in the western Palearctic region. Heredity 94:52–63.

Modrý, D., Šlapeta, J.R., Jirků, M., Oborník, M., Lukeš, J., Koudela, B. 2001. Phylogenetic position of a renal coccidium of the European green frogs, *Isospora lieberkuehni* Labbé, 1894 (Apicomplexa: Sarcocystidae) and its taxonomic implications. International Journal of Systematic and Evolutionary Microbiology, 51: 767–772.

Mullen, G.R., O'Connor, B.M. 2002. Mites (Acari). Medical and Veterinary Entomology. Elsevier Science (USA), San Diego, California, 478 p.

Nadler, S.A. 1995. Microevolution and the genetic structure of parasite populations. Journal of Parasitology, 81: 395–403.

Nieberding, C., Morand, S., Libois, R., Michaux, J.R. 2004. A parasite reveals cryptic phylogeographic history of its host. Proceedings of the Royal Society of London. Series B, Biological Sciences, 271: 2559–2568.

Page, R.D.M. 1994. Parallel phylogenies: reconstructing the history of host–parasite assemblages. Cladistics, 10: 155–173.

Page, R.D.M., 2003. Tangled Trees: Phylogeny, Cospeciation, and Coevolution. University of Chicago Press.

Paterson, A.M., Banks, J. 2001. Analytical approaches to measuring cospeciation of host and parasites: through a looking glass, darkly. International Journal for Parasitology, 31: 1012–1022.

Price, P. W. 1980. Evolutionary biology of parasites. Princeton University Press, Princeton, NJ.

Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H. 2003. The Chewing Lice: World Checklist and Biology Overview. Champaign-Urbana, Illinois: Illinois Natural History Survey.

Reed, D.L., Smith, V.S., Hammond, S.L., Rogers, A.R., Clayton, D.H. 2004. Genetic analysis of lice supports direct contact between modern and archaic humans. PLoS Biology, 2: 1972–1983.

Ricklefs, R. E., Fallon, S. M., Bermingham E. 2004. Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. Systematic Biology, 53:111–119.

Suzuki, H., Filippucci, M.G., Chelomina, G.N., Sato, J.J., Serizawa, K., Nevo, E. 2008. A biogeographic view of *Apodemus* in Asia and Europe inferred from Nuclear and Mitochondrial gene sequences. Biochemical Genetics, 46: 329–346.

Sychra, O., Sychrová, V., Literák, I. 2008. Identity of Menacanthus obrteli Balát (Phthiraptera: Menoponidae) from the Savi's warbler (Passeriformes: Sylviidae). Biologia, 63: 686–688.

Sweet, A.D., Johnson, K.P. 2018. The role of parasite dispersal in shaping a host–parasite system at multiple evolutionary scales. Molecular Ecology, 27: 5104–5119.

Štefka, J., Hoeck, P.E., Keller, L.F., Smith, V.S. 2011. A hitchhikers guide to the Galapagos: co-phylogeography of Galapagos mockingbirds and their parasites. BMC Evolutionary Biology 11: 284.

Štefka, J., Hypša, V., 2008. Host specificity and genealogy of the louse *Polyplax serrata* on field mice, *Apodemus* species: a case of parasite duplication or colonisation? International Journal of Parasitology, 38: 731–741.

Štefka, J., Hypša, V., Scholz, T., 2009. Interplay of host specificity and biogeography in the population structure of a cosmopolitan endoparasite: microsatellite study of *Ligula intestinalis* (Cestoda). Molecular Ecology 18: 1187–1206.

Thompson, J.N., Fernandez, C.C. 2006. Temporal dynamics of antagonism and mutualism in a geographically variable plant-insect interaction. Ecology, 87: 103–112.

Toon, A., Hughes, J.M. 2008. Are lice good proxies for host history? A comparative analysis of the Australian magpie, *Gymnorhina tibicen* and two species of feather louse. Heredity, 101:127–135.

van Schaik, J., Kerth, G., Bruyndonckx, N., Christe, P. 2014. The effect of host social system on parasite population genetic structure: Comparative population genetics of two ectoparasitic mites and their bat hosts. BMC Evolutionary Biology, 14: 18.

Wasimuddin, W., Bryja, J., Ribas, A., Baird, S.J.E., Piálek, J., Goüy de Bellocq, J. 2016. Testing parasite 'intimacy': the whipworm *Trichuris muris* in the European house mouse hybrid zone. Ecology and Evolution, 6: 2688–2701.

Webb, T., Bartlein, P.J. 1992. Global changcs during the last 3 million years: climatic controls and biotic rcsponses. Annual Reviews of Ecology and Systematics, 23: 141–173.

Manuscript No. 1:
Martinů, J., Roubová, V., Nováková, M., Smith, V. S., Hypša, V., Štefka, J. 2015. Characterisation of microsatellite loci in two species of lice, *Polyplax serrata* (Phthiraptera: Anoplura: Polyplacidae) and *Myrsidea nesomimi* (Phthiraptera: Amblycera: Menoponidae). Folia Parasitologica, 62: 1–6.

Folia Parasitologica 2015, 62: 016
doi: 10.14411/fp.2015.016

Folia Parasitologica
http://folia.paru.cas.cz

Research Article OPEN ACCESS

Characterisation of microsatellite loci in two species of lice, *Polyplax serrata* (Phthiraptera: Anoplura: Polyplacidae) and *Myrsidea nesomimi* (Phthiraptera: Amblycera: Menoponidae)

Jana Martinů[1,2], **Veronika Roubová**[1], **Milena Nováková**[1], **Vincent S. Smith**[3], **Václav Hypša**[1,2] and **Jan Štefka**[1,2]

[1] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic;

[2] Institute of Parasitology, Biology Centre of the Czech Academy of Science, České Budějovice, Czech Republic;

[3] Life Sciences Department, Natural History Museum, London, United Kingdom

Abstract: Polymorphic microsatellite loci were characterised for two louse species, the anopluran *Polyplax serrata* Burmeister, 1839, parasitising Eurasian field mice of the genus *Apodemus* Kaup, and the amblyceran *Myrsidea nesomimi* Palma et Price, 2010, found on mocking birds endemic to the Galápagos Islands. Evolutionary histories of the two parasites show complex patterns influenced both by their geographic distribution and through coevolution with their respective hosts, which renders them prospective evolutionary models. In *P. serrata*, 16 polymorphic loci were characterised and screened across 72 individuals from four European populations that belong to two sympatric mitochondrial lineages differing in their breadth of host-specificity. In *M. nesomimi*, 66 individuals from three island populations and two host species were genotyped for 15 polymorphic loci. The observed heterozygosity varied from 0.05 to 0.9 in *P. serrata* and from 0.0 to 0.96 in *M. nesomimi.* Deviations from the Hardy-Weinberg equilibrium were frequently observed in the populations of both parasites. Fst distances between tested populations correspond with previous phylogenetic data, suggesting the microsatellite loci are an informative resource for ecological and evolutionary studies of the two parasites.

Keywords: ectoparasite, population genetics, coevolution, *Polyplax*, *Myrsidea*, evolution, Europe, Galápagos

Parasitic lice (Phthiraptera) represent long-standing models for the study of host-parasite co-speciation. Many species of sucking lice (Anoplura) and two chewing louse suborders (Amblycera and Ischnocera) have been scrutinised with molecular methods to reveal complex evolutionary histories. These range from strict co-speciation to completely decoupled host-parasite phylogenies (e.g. Johnson and Clayton 2004, Demastes et al. 2012, Mizukoshi et al. 2012) and offer a unique opportunity to study the microevolutionary processes associated with the parasite's dependence on its host and geographic distribution.

Multilocus markers are a critical source of population genetic data necessary to tease apart the different patterns generated by these processes. With the exception of human louse (Leo et al. 2005, Ascunce et al. 2013), such data are still scarce and little is known about the genetic background of louse populations. Recent microsatellite studies include work on *Degeeriella regalis* Giebel, 1866, an ischnoceran louse parasitising Galapagos hawks (Koop et al. 2014) and preliminary data on two additional chewing louse species, *Colpocephalum turbinatum* Denny, 1842 and *Geomydoecus ewingi* Price et Emerson, 1971 (Peters et al. 2009, Nessner et al. 2014). In the present study, we extend this set by characterising polymorphic microsatellite loci in two more louse species, the anopluran *Polyplax serrata* Burmeister, 1839 and the amblyceran *Myrsidea nesomimi* Palma et Price, 2010. These lice have been the subjects of recent co-evolutionary research (Štefka and Hypša 2008, Štefka et al. 2011) and have the potential to serve as a model for microevolutionary studies of host-parasite interactions.

Polyplax serrata belongs to a cosmopolitan family Polyplacidae parasitising rodents (Light et al. 2010). It is found almost exclusively on the field mice genus *Apodemus* Kaup, and exceptionally on some other rodent taxa such as *Clethrionomys glareolus* (Schreber) or *Microtus arvalis* (Pallas) – Krištofík and Lysý (1992). *Polyplax serrata,* together with its hosts, occurs in the whole of Eurasia and has a complex population-genetic pattern that is partly influenced by geographical structure and partly by the host. According to Štefka and Hypša (2008), *P. serrata* is composed of three mitochondrial lineages, one parasitising two host species, *Apodemus agrarius* (Pallas) and *A. uralensis* (Pallas) (lineage C), and two sympatric lineages that differ in the extent of their host specificity: lineage A lives on two host species, *A. flavicollis* (Melchior)

Address for correspondence: J. Martinů, Institute of Parasitology, Biology Centre of the Czech Academy of Science, Branišovská 31, 370 05 České Budějovice, Czech Republic. Phone: (+420) 387775448; Fax: (+420) 385310388; E-mail: martinu@paru.cas.cz

and *A. sylvaticus* (Linneaus), whereas lineage B possesses high affinity to *A. flavicollis*. Given that lice from the lineages A and B occur in sympatry throughout Europe and are composed of clearly distinct lineages, they likely represent cryptic species. However, the original reconstruction of their evolutionary history was based on short fragments of mitochondrial *cox1* and nuclear *EF-1α* genes (Štefka and Hypša 2008). These provide reliable information on past isolation events, but may not be informative enough to indicate recent processes (e.g. post-glacial admixture of the lineages or recent fluctuations in population sizes).

The second model species, *Myrsidea nesomimi*, parasitises all four species of mockingbirds allopatrically distributed on the Galápagos islands (*Mimus parvulus* Gould, *M. trifasciatus* Gould, *M. melanotis* Gould and *M. macdonaldi* Ridgway). Studying mitochondrial DNA diversity of mockingbird lice from 11 islands, Štefka et al. (2011) found co-phylogeographic patterns shared by the louse and most of its host populations. The study, however, also revealed several incongruences. For example, despite a recent common origin of the neighbouring populations of an endangered Floreana mockingbird, surviving on the islets of Champion and Gardner by Floreana (Hoeck et al. 2010), their louse populations were not related. Instead, the *M. nesomimi* population from Champion showed a closer relationship to populations from Santa Fe in the central part of the archipelago.

For both model species, multilocus loci such as microsatellites could provide additional data critical for tracking recent migrations of these parasites and help to delineate the shape of the population structure in the parasite and its host. The present study focused on the development and characterisation of multiplex panels of genetically informative, polymorphic microsatellite loci (STR – short tandem repeats) in both species of lice. Such tools will help to extend our knowledge on the population genetics of parasites and detect historically recent events in the evolution of these two louse species.

MATERIALS AND METHODS

Two strategies were employed to obtain sequences of candidate microsatellite loci from the two louse genomes. First, the protocol of Fleischer and Loew (1995) was followed to construct the microsatellite-enriched libraries, clone them into *E. coli* plasmids and sequence them using Sanger sequencing. Due to a relatively low number of loci obtained in this way, a second strategy based on Next Generation Sequencing (NGS) was employed. Pooled extracts of louse DNA were used for constructing enriched libraries and sequenced with NGS technology (454, Roche, Brandford, USA) in a commercial laboratory (GenoScreen, Lille, France).

The protocol described by Fleischer and Loew (1995) was adopted with minor modifications (available upon request). Two oligonucleotides, comprising 13 CA and 13 AT repeats, were used to produce GT and TA microsatellite-enriched libraries. Both oligos were biotin-labelled at the 5'end and a three-carbon spacer was added to the 3'end (manufactured by Generi-Biotech, Hradec Králové, Czech Republic) to prevent the oligos from acting as a primer in the subsequent polymerase chain reaction (PCR) steps of the subtraction (Koblížková et al. 1998). Following this protocol, 120 and 68 microsatellite sequences were isolated for *P. serrata* and *M. nesomimi*, respectively.

Sixty and 24 sequences (for *P. serrata* and *M. nesomimi*, respectively), for which stable primer pairs could be designed, were selected for PCR testing. Structure of the microsatellite and surrounding regions were used as guidelines when selecting the candidates. The primers were constructed manually in PrimerSelect (DNASTAR, Madison, USA). The testing sample included populations representing two sympatric lineages, A and B, delimited by Štefka and Hypša (2008) for *P. serrata* and five Galápagos islands (Santa Cruz, Santa Fe, Española, Champion and San Cristobal) for *M. nesomimi*. One *P. serrata* individual per population (French isolate from lineage A, Slovakian isolate from eastern mitochondrial cluster of the lineage B and Czech isolate from western cluster of the lineage B) were used to test successful PCR amplification with the designed microsatellite primers.

The total volume of reaction was 10 µl, containing approximately 15 ng of template DNA, 5 µl of 2x QIAGEN Multiplex PCR Master Mix (QIAGEN Multiplex PCR Kit, Qiagen, Hilden, Germany), 2 µl 5x Q-Solution, and H_2O. Forward primers were labelled with 6-FAM fluorescent dye at the 5′end (Sigma Aldrich, Praha, Czech Republic). The thermal profile for *P. serrata* was as follows: 3 min at 94 °C followed by 29 cycles of 15 s at 94 °C, 30 s at 58 °C and 1 min at 72 °C; the duration time of the final extension was 15 min at 72 °C. For *M. nesomimi*, the annealing temperature was set to 55 °C and three individuals per each of the five islands were tested. Five microlitres of each PCR product were checked on a 2% agarose gel along with a 100-bp marker. PCR products of loci that showed satisfactory amplification were diluted to 1 : 10 and 1 : 100 and analysed on the ABI 3100 genetic analyser (Applied Biosystems, Life Technologies Czech Republic s.r.o., Praha, Czech Republic). Using the PCR conditions described above, five and four of the loci were found to be polymorphic in at least one of the tested *P. serrata* and *M. nesomimi* populations, respectively (see Tables 1 and 2 for primer description).

For NGS technology and selection of additional candidate microsatellite loci, DNA pools were prepared from (a) a total of 15 individuals of *P. serrata* from A and B lineages, and (b) nine individuals of *M. nesomimi* from the Santa Cruz population. The genomic DNA was isolated using the QIAamp® DNA Micro Kit (QIAGEN). The concentration and purity of genomic DNA was verified spectrophotometrically by NanoDrop 3 (NanoDrop Technologies, Inc., Wilmington, Delaware, USA). Obtained DNA was sent to GenoScreen for commercial NGS service GenoSat®. The NGS service analysis resulted in 455 microsatellite loci candidates in each species, containing di-, tri-, tetra-, penta-, and hexanucleotide repeats of length varying between 90–320 bp. Ninety loci containing various di- and trinucleotide repeat motifs were selected for tests of successful PCR amplification on *P. serrata* individuals from lineages A and B, as well as 34 loci in *M. nesomimi* on individuals from 5 islands. Conditions of PCR reactions and visualisation of samples were the same as described above.

Out of 90 and 34 loci tested in PCR, 23 and 17 loci provided PCR product of the expected size in individuals of *P. serrata* and *M. nesomimi*, respectively. Louse extracts typically produce low DNA yields allowing a limited number of PCRs, thus different louse specimens were used to determine STR allele polymorphism for 454 obtained loci compared to the tests above. The

Table 1. Characteristics of 16 microsatellite loci utilised for *Polyplax serrata* genotyping and tested on 72 individuals of 4 populations from 3 European localities.

Locus	Primer sequence	Repeat motif	Size range (bp)	GenBank Accession number	Dye*	FR A			GeB A			GeB B			GeT B		
						A	Ho/He	HWE	A	Ho/He	HWE	A	Ho/He	HWE	A	Ho/He	HWE
PS-84	F: TTTAATGTAAAACTCAAGTTCCCAA R: TTACTTTAGTTGAGGAAGAAGATCG	$(tct)_n$	186–200	KM086741	FAM[a]	6	0.71/0.73	ns	2	0.13/0.31	ns	2	0.05/0.05	ns	2	0.05/0.05	ns
PS-GT72	F: GCGAAAGGTGCGACGAG R: TCAACTGGACAACTACAATAAAC	$(gt)_n$	156–205	KM086756	NED[a]	4	0.41/0.52	*	4	0.75/0.59	ns	7	0.25/0.36	*	2	0.25/0.36	*
PS-53	F: TAGTGCTTTTACAGTGAACTGCC R: GTCGGAGCCATGGATTTTC	$(ct)_n$	245–284	KM086742	FAM[a]	4	0.35/0.48	ns	3	0.69/0.62	ns	1	x	x	1	x	x
PS-GT8	F: CATCGAATTGTCGGAGTCAAAGGAA R: GAAGAAAATCTCAAATTCGTCACGGAA	$(gt)_n$	180–220	KM086754	FAM[b]	6	0.59/0.74	ns	8	0.56/0.72	ns	8	0.8/0.85	ns	4	0.8/0.85	ns
PS-GT48	F: CGTCAGCTTGCAACTCGTCTCC R: CCCGGTCCCTGCCTACTGC	$(gt)_n$	196–208	KM086752	PET[b]	5	0.53/0.72	ns	5	0.13/0.63	***	3	0.25/0.61	***	3	0.25/0.61	***
PS-65	F: CCGTTTCGGACGATGTAGTA R: TGCCCGTGTATGTGTTGTTT	$(ga)_n$	214–232	KM086743	VIC[b]	4	0.24/0.36	ns	3	0.38/0.41	ns	2	0.45/0.45	ns	2	0.45/0.45	ns
PS-42	F: AAGAGACAAGTCAAACCCCG R: ACCTGCTACGTTTCCTCGTG	$(ac)_n$	260–287	KM086744	VIC[b]	6	0.59/0.81	**	4	0.06/0.51	***	7	0.65/0.87	**	4	0.65/0.87	**
PS-43	F: GAGCCGAACAATACGGAGAA R: CAGCGTATGACATTGTAAAAGAGAA	$(ct)_n$	256–298	KM086745	PET[b]	6	0.71/0.81	*	5	0.5/0.68	*	2	0.05/0.05	ns	2	0.05/0.05	ns
PS-22	F: CTCTTGCGAAACTTAAACTCG R: ACAGTTTCTCAACTTGGGGC	$(ga)_n$	207–239	KM086746	NED[c]	5	0.41/0.77	***	3	0.5/0.58	ns	3	0.3/0.55	***	2	0.3/0.55	**
PS-12	F: CCGTGAAACAAACGTTCCAA R: TCATCATCGGTTCTCCGTACT	$(aca)_n$	138–145	KM086747	FAM[c]	3	0.12/0.12	ns	2	0.38/0.31	ns	1	x	x	2	x	x
PS-16	F: AGTGACCAGCTTTTCAAAATAGA R: TGCGGAACTAATCTCGGAAC	$(ag)_n$	203–215	KM086748	PET[c]	7	0.88/0.81	ns	6	0.38/0.63	**	5	0.45/0.58	ns	2	0.45/0.58	ns
PS-41	F: ATTTCGGGGTGACTTCTTCC R: TGACTCAACGGCCTAGACAA	$(ac)_n$	258–262	KM086749	VIC[c]	4	0.47/0.54	ns	3	0.13/0.47	***	2	0.15/0.14	ns	2	0.15/0.14	ns
PS-CT57	F: TTCCAAATGTGAGGAGAAATCC R: GGAAATCACCGGGGAGAGG	$(ct)_n$	163–196	KM086755	NED[d]	6	0.53/0.52	ns	6	0.44/0.39	ns	7	0.9/0.77	ns	3	0.9/0.77	ns
PS-101	F: ATGAAAGAGCATCGAACATCG R: AGAGAAGTGGCAATTGCAGAG	$(gt)_n$	238–258	KM086750	FAM[d]	4	0.53/0.55	ns	4	0.75/0.67	ns	6	0.2/0.53	***	1	0.2/0.53	***
PS-GT43	F: TGGGATCAAAAAGACCTG R: TCCAACCTCACCCGAAAAC	$(gt)_n$	233–263	KM086753	FAM[d]	5	0.18/0.64	***	3	0.44/0.45	ns	6	0.35/0.49	ns	2	0.35/0.49	ns
PS-56	F: TTCAGTTACTCAATGACCCGTT R: TCATGTGGATTTCGGGCTA	$(ac)_n$	162–190	KM086751	NED[d]	6	0.35/0.77	***	4	0.19/0.23	ns	4	0.2/0.38	*	2	0.2/0.38	*

* superscripts indicate the multiplex PCR set in which the primer pair was used; FR A − France lineage A; GeB A − Germany Baiersbronn lineage A; GeB B − Germany Baiersbronn lineage B; GeT B − Germany Torgau lineage B; A − number of alleles; Ho − observed heterozygosity; He − expected heterozygosity; HWE – significance values for Hardy−Weinberg equilibrium test (ns – not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; x – monomorphic – not evaluated).

Table 2. Characteristics of 15 microsatellite loci utilised for *Myrsidea nesomimi* genotyping and tested on 66 individuals of 3 island populations.

Locus	Primer sequence	Repeat motif	Size range (bp)	GenBank Accession number	Dye*	SCr			Sfe			Esp		
						A	Ho/He	HWE	A	Ho/He	HWE	A	Ho/He	HWE
MNCT3	F: CTCGAGGTAATGAGGAAGTTTTC R: ATCTAGTTCCCAATCGGTCTTATCC	$(ga)_n$	188–222	KM08768	FAM[a]	11	0.79/0.88	*	1	x	x	9	0.74/0.84	ns
MNCT15	F: GAGCATTTCGCCACACG R: TTGCCAACTCCGATGACTT	$(ga)_n$	166–210	KM08770	FAM[b]	10	0.75/0.86	***	3	0.50/0.58	*	10	0.74/0.74	ns
MNGT10	F: CTCCGGTGGGTACAATAGCATCA R: CTCCGGTGGGTACAATAGCATCA	$(ac)_n$	186–222	KM08771	PET[b]	8	0.89/0.74	ns	3	1.00/0.53	**	9	0.74/0.78	*
MNCT8	F: CTACGTAAGGCTAGCAGTCACAAG R: GTACAGACATTACAATACCTTCCCC	$(ga)_n$	205–243	KM08769	NED[b]	5	0.36/0.52	ns	4	0.60/0.64	ns	4	0.22/0.20	ns
MNA	F: TCCGTGCCGTTTATATTGAG R:CGTCCGGGAGTTAGGAAGTT	$(aac)_n$	103–112	KM086757	VIC[a]	4	0.26/0.56	***	3	0.00/0.24	***	3	0.96/0.50	ns
MNB	F: CGGCTCAAATTATCCGTGAC R: TGAAATGGCGTGTGACAAAT	$(aac)_n$	99–111	KM086758	VIC[b]	4	0.43/0.49	ns	2	0.13/0.12	ns	3	0.43/0.51	***
MNM	F: TACAATGCATGGTTAACGGC R: TAGGGTTGCCTCACGTTTTC	$(ct)_n$	160–200	KM08762	NED[a]	19	0.75/0.91	ns	2	0.07/0.28	**	11	0.70/0.86	**
MNX	F: GCACTGGTAATTCCGTGGAT R: CCGAGCCGGTTAATATCAGA	$(gt)_n$	129–135	KM08764	PET[a]	4	0.61/0.59	ns	1	x	x	1	x	x
MNCH	F: AAACTTTGTAACAGGTTTTAGGGG R: ACAGTTTCTCAACTTGGGGC	$(agg)_n$	152–173	KM086760	FAM[c]	5	0.18/0.32	**	3	0.40/0.50	ns	1	x	x
MNH	F: CGTATTAACGAGAGCGGAGG R: CGACAGTTTACGCTGACGAT	$(agg)_n$	159–162	KM086759	PET[c]	3	0.07/0.07	ns	1	x	x	1	x	x
MNZ	F: CAGCTGTTCCTACATGCACC R: GCGTCACAAACAAGGCCTAA	$(gt)_n$	150–168	KM08765	FAM[d]	9	0.71/0.83	ns	2	0.13/0.12	ns	2	0.17/0.23	ns
MNZZ	F: ATCCGGATTCGGAAGAGTTC R: CGAGTTTTCTCCTGTTTCGC	$(gt)_n$	169–195	KM08766	VIC[c]	11	0.89/0.85	ns	7	0.53/0.66	ns	3	0.43/0.42	ns
MNJ	F: TTGGCATATGTCACGTTTACCT R: GAGGGGATTCACCCTTCATT	$(at)_n$	132–140	KM08761	NED[c]	6	0.61/0.67	**	2	0.13/0.32	*	1	x	x
MNAB	F: CGACTCCTCCCGTGATTAAA R: CGAAGCCTGTTCCGTAAAAT	$(gt)_n$	143–149	KM08767	NED[d]	3	0.25/0.41	***	1	x	x	2	0.04/0.04	ns
MNU	F: ACTTTCTATCGGATGCCCTC R: AGTGCACACGTTAAGCCAAA	$(gga)_n$	139–151	KM08763	PET[d]	3	0.43/0.53	**	2	0.47/0.49	ns	1	x	x

* superscripts indicate the multiplex PCR set in which the primer pair was used; island abbreviations: SCr – Santa Cruz, Sfe – Santa Fe, Esp – Española; A – number of alleles; Ho – observed heterozygosity; He – expected heterozygosity; HWE – significance values for Hardy–Weinberg equilibrium test (ns – not significant; * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$; x – monomorphic – not evaluated).

candidate loci were tested using a fragment analysis with fluorescently labelled primers for ten *P. serrata* individuals from one German locality (lineage B) and ten individuals from one French locality (lineage A). Out of these loci, 12 were excluded, either due to failure to amplify PCR product in several individuals (potential occurrence of null alleles), or because some loci were detected to be monomorphic. In *M. nesomimi,* candidate loci were tested using the same approach with ten individuals from the Santa Cruz and Española populations, respectively.

Finally, loci found to be polymorphic in at least one of the screened populations were subjected to the tests of Hardy-Weinberg equilibrium (HWE) and Linkage Disequilibrium (LD) using population sets of representative sizes. Eleven NGS obtained loci polymorphic in *P. serrata* (2–11 alleles per locus), together with five polymorphic loci gained from GT and TA libraries, were amplified in four multiplex PCR assays (the same conditions as for the STR loci polymorphism above). Similarly in *M. nesomimi,* four multiplex panels were created for 15 polymorphic loci (11 loci obtained from the NGS screening and four loci from the GA and TA libraries). Combinations of the markers used in the multiplexed assays are specified in Tables 1 and 2. For each panel, either different dyes were used for loci that overlapped in size, or a single dye was applied to PCR products of different sizes. PCR reactions were sent to the commercial service (Macrogen Inc. Korea and Netherlands, Seoul, Korea) for fragment analysis. The GeneMapper v.3.7 software (Applied Biosystems) was used for genotyping. In *P. serrata,* the 16 loci were tested for genotypic equilibrium and deviations from Hardy-Weinberg proportions. Seventy-two individuals coming from two localities from lineage A and two localities from lineage B (17 individuals from French lineage A, 15 from Baiersbronn German lineage A, 21 from Baiersbronn German lineage B and 19 from Torgau German lineage B) were analysed to evaluate performance of the markers (Table 1). In *M. nesomimi*, 66 individuals from three island populations (28 individuals from Santa Cruz, 15 from Santa Fe and 23 from Española) were used (Table 2). Tests for HWE and LD were performed online in the Genepop website (http://genepop.curtin.edu.au/). Fst values between all population pairs were calculated in Genalex 6.5 (Peakall and Smouse 2012), using 9999 permutations to test the significance of the results.

All microsatellites were also tested with extracts of pure host DNA to exclude possible cross-amplification with contaminant host DNA from a bloodmeal or skin and other host tissues in the louse gut.

RESULTS AND DISCUSSION

Levels of polymorphism detected in the tested populations (number of alleles, heterozygosity) and results of the HWE tests are listed in Tables 1 and 2. In *P. serrata*, all 16 loci were found to be polymorphic in populations from the lineage A (Germany Baiersbronn, France) with the number of alleles ranging from two to seven. Populations from lineage B (Germany Baiersbronn, Germany Torgau) were monomorphic in two loci (PS-12, PS-53). Significant deviations from HWE ($P < 0.05$) were observed in all four populations: five loci deviated in Germany Baiersbronn (lineage A) and six loci in Germany Baiersbronn (lineage B), Germany Torgau (lineage B) and France (lineage A; Table 1) populations. Overall, four loci were in HWE in all test populations and only one locus (PS-42) deviated from HWE across the studied set (Table 1). We therefore suggest that the PS-42 locus is excluded from further application.

All 16 loci were tested for LD, applying sequential Bonferroni correction for multiple comparisons. The tests were not significant for two populations, France lineage A and Germany Torgau lineage B. Out of the 120 locus-pair combinations tested, seven showed significant deviation in the Germany Baiersbronn lineage A (PS-GT72 and PS-53; PS-42 and PS-41; PS-GT72 and PS-101; PS-53 and PS-101; PS-42 and PS-12; PS-12 and PS-41; PS-GT72 and PS-42) and six in the Baiersbronn lineage B (PS-GT43 and PS-16; PS-GT43 and PS-101; PS-16 and PS-56; PS-101 and PS-56; PS-GT43 and PS-56; PS-16 and PS-101). Positive LD tests in the two populations may reflect a hidden genetic structure caused by using up to seven louse individuals per host individual (Koop et al. 2014). Louse populations inbreed for multiple generations on a single host individual, which may result in a sub-population with low diversity and genetic loci inherited in linked blocks. In the populations from France and Germany (Torgau), a maximum of two individuals per host were used, thus lessening this kind of artifact. Whilst deviation from HWE due to a reduced number of heterozygotes is still present, the level of LD in these populations is not significant. Fst pairwise distances among the four populations correspond to the mtDNA-based delimitation of the lineages: in all comparisons the within-lineage distances were lower (Fst = 0.206 and 0.230) than distances between the lineages, even for the two sympatric Baiersbronn populations (Fst = 0.283 to 0.351). All Fst values were significant at $P \leq 0.001$.

In *M. nesomimi*, all 15 loci were found polymorphic in the Santa Cruz population and eight loci were polymorphic across all three populations (Table 2). The levels of heterozygosity were low in many loci and deviations from HWE were frequent. Three loci (MNCT8, MNZ and MNZZ) were in HWE across all populations and six to eight loci were in HWE in different individual populations (Table 2). Interestingly, only one locus (MNCH) deviated from HWE in all populations. Such non-corresponding distribution of polymorphism in the loci between populations indicates a high degree of isolation between the three islands. We assume that different loci were fixed for one allele or gained mutations in the primer binding sites causing allelic drop-out independently in the affected populations.

Five out of 105 locus-pair combinations calculated across all *M. nesomimi* populations of showed moderate deviation from LD ($P = 0.05–0.01$), but none of the tests were significant after Bonferroni correction. Fst pairwise distances between the populations correspond with the geographic distribution of the host species. The distance between Santa Cruz and Santa Fe populations sharing the same host (*Mimus parvulus*) was lower (Fst = 0.179) than distances between each of the two islands and the Española population from *Mimus macdonaldi* (Fst = 0.393 and 0.214). All Fst values were significant at $P \leq 0.001$.

In conclusion, we characterised four multiplexed panels of polymorphic microsatellite loci in each of the two louse species and demonstrated their suitability for studies of population history. Low heterozygosity, observed in

some loci-and-population combinations, points to possible occurrence of null alleles. The impact of inner population subdivision on HWE cannot be fully excluded; but we find the null allele explanation more likely given the distribution of positive LD tests across populations. Samples in each test population were isolated from the same host species and, with the exception of the *P. serrata* Baiersbronn populations showing positive LD tests, only one to two lice per host individual were analysed to avoid inbred or highly related individuals (see Koop et al. 2014). In each species, different loci were usually out of HWE across test populations indicating independent evolution of the populations for many generations. Fst distances between the test populations reflected their respective evolutionary history and/or host origin. Thus the microsatellite loci isolated for *P. serrata* and *M. nesomimi* were shown to provide an appropriate tool for investigation of population substructuring and genealogical relationships. These data provide a foundation for further studies of the biogeography and host distribution of these ecologically interesting louse.

Acknowledgements. Research presented in this study was funded by the Czech Science Foundation (project nos. P506/12/P529 and P505/12/1620) and the Marie Curie Fellowship (project Galápagos, no. 235123, FP7-PEOPLE-IEF-2008). V. Roubová was supported by a Team Grant of the Grant Agency of the University of South Bohemia (GAJU, no. 159/2013/P). The material of *Polyplax serrata* used in the study originates from a louse collection created thanks to a long-term effort of many colleagues from the Laboratory of Molecular Phylogeny and Evolution of Parasites in České Budějovice. We thank to Lukas Keller (University of Zurich) and Paquita Hoeck (Institute for Conservation Research, San Diego) for providing material of *Myrsidea nesomimi*. The research on *M. nesomimi* was conducted under permit (no. PC-48-10) from the Galápagos National Park.

REFERENCES

Ascunce M.S., Toups M.A., Kassu G., Fane J., Scholl K., Reed D.L. 2013: Nuclear genetic diversity in human lice (*Pediculus humanus*) reveals continental differences and high inbreeding among worldwide populations. PLoS ONE 8: e57619.

Demastes J.W., Spradling T.A., Hafner M.S., Spies G.R., Hafner D.J., Light J.E. 2012: Cophylogeny on a fine scale: *Geomydoecus* chewing lice and their pocket gopher hosts, *Pappogeomys bulleri*. J. Parasitol. 98: 262–270.

Fleischer R.C., Loew S., 1995: Construction and screening of microsatellite-enriched genomic libraries. In: J. Ferraris and S. Palumbi (Eds.), Molecular Zoology: Advances, Strategies and Protocols. Wiley-Liss, New York, pp. 459–468.

Hoeck P.E.A., Beaumont M.A., James K.E., Grant R.B., Grant P.R., Keller L.F. 2010: Saving Darwin's muse: evolutionary genetics for the recovery of the Floreana mockingbird. Biol. Lett. 6: 212–215.

Johnson K.P., Clayton D.H. 2004: Untangling coevolutionary history. Syst. Biol. 53: 92–94.

Koblížková A., Doležel J., Macas J. 1998: Subtraction with 3' modified oligonucleotides eliminates amplification artifacts in DNA libraries enriched for microsatellites. Biotechniques 25: 32–34.

Koop J.A., DeMatteo K.E., Parker P.G., Whiteman N.K. 2014: Birds are islands for parasites. Biol. Lett. 10: 20140255.

Krištofík J., Lysý J. 1992: Seasonal dynamics of sucking lice (Anoplura) in small mammals (Insectivora, Rodentia) in the natural foci of infections in south-west Slovakia. Biologia 47: 605–617.

Leo N.P., Hughes J.M., Yang X., Poudel S.K.S., Brogdon W.G., Barker S.C. 2005: The head and body lice of humans are genetically distinct (Insecta: Phthiraptera, Pediculidae): evidence from double infestations. Heredity 95: 34–40.

Light J.E., Smith V.S., Allen J.M., Durden L.A., Reed D.L. 2010: Evolutionary history of mammalian sucking lice (Phthiraptera: Anoplura). BMC Evol. Biol. 10: 292.

Mizukoshi A., Johnson K.P., Yoshizawa K. 2012: Co-phylogeography and morphological evolution of sika deer lice (*Damalinia sika*) with their hosts (*Cervus nippon*). Parasitology 139: 1614–1629.

Nessner C.E., Andersen J.J., Renshaw M.A., Giresi M.M., Light J.E. 2014: Characterization of 17 novel polymorphic microsatellite loci in the mammal chewing louse *Geomydoecus ewingi* (Insecta: Phthiraptera) for population genetic analyses. J. Parasitol. 100: 873–877.

Peakall R., Smouse P.E. 2012: GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics 28: 2537–2539.

Peters M.B., Whiteman N.K., Hagen C., Parker P.G., Glenn T.C. 2009: Eight polymorphic microsatellite markers isolated from the widespread avian louse *Colpocephalum turbinatum* (Phthiraptera: Amblycera: Menoponidae). Mol. Ecol. Resour. 9: 910–912.

Štefka J., Hoeck P.E., Keller L.F., Smith V.S. 2011: A hitchhikers guide to the Galapagos: co-phylogeography of Galapagos mockingbirds and their parasites. BMC Evol. Biol. 11: 284.

Štefka J., Hypša V. 2008: Host specificity and genealogy of the louse *Polyplax serrata* on field mice, *Apodemus* species: a case of parasite duplication or colonisation? Int. J. Parasitol. 38: 731–741

Received 6 October 2014 Accepted 9 January 2015 Published online 13 February 2015

Cite this article as: Martinů J., Roubová V., Nováková M., Smith V.S., Hypša V., Štefka J. 2015: Characterisation of microsatellite loci in two species of lice, *Polyplax serrata* (Phthiraptera: Anoplura: Polyplacidae) and *Myrsidea nesomimi* (Phthiraptera: Amblycera: Menoponidae). Folia Parasitol. 62: 016.

Manuscript No. 2:
Martinů, J., Hypša, V., Štefka, J. 2018. Host specificity driving genetic structure and diversity in ectoparasite populations: Coevolutionary patterns in *Apodemus* mice and their lice. Ecology and Evolution, 8: 10008–10022.

Received: 25 April 2018 | Revised: 3 July 2018 | Accepted: 9 July 2018

DOI: 10.1002/ece3.4424

ORIGINAL RESEARCH

WILEY Ecology and Evolution Open Access

Host specificity driving genetic structure and diversity in ectoparasite populations: Coevolutionary patterns in *Apodemus* mice and their lice

Jana Martinů[1,2] | Václav Hypša[1,2] | Jan Štefka[1,2]

[1]Biology Centre CAS, Institute of Parasitology, České Budějovice, Czech Republic

[2]Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Correspondence
Jana Martinů, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.
Email: janamartinu84@gmail.com

Funding information
Grantová Agentura České Republiky, Grant/Award Number: 14-07004S; National Grid Infrastructure MetaCentrum, Grant/Award Number: CESNET LM2015042 and CERIT Scientific Cloud LM2015

Abstract

A degree of host specificity, manifested by the processes of host–parasite cospeciations and host switches, is assumed to be a major determinant of parasites' evolution. To understand these patterns and formulate appropriate ecological hypotheses, we need better insight into the coevolutionary processes at the intraspecific level, including the maintenance of genetic diversity and population structure of parasites and their hosts. Here, we address these questions by analyzing large-scale molecular data on the louse *Polyplax serrata* and its hosts, mice of the genus *Apodemus*, across a broad range of European localities. Using mitochondrial DNA sequences and microsatellite data, we demonstrate the general genetic correspondence of the *Apodemus*/*Polyplax* system to the scenario of the postglacial recolonization of Europe, but we also show several striking discrepancies. Among the most interesting are the evolution of different degrees of host specificity in closely related louse lineages in sympatry, or decoupled population structures of the host and parasites in central Europe. We also find strong support for the prediction that parasites with narrower host specificity possess a lower level of genetic diversity and a deeper pattern of interpopulation structure as a result of limited dispersal and smaller effective population size.

KEYWORDS

Apodemus, coevolution, dispersal, genetic diversity, host specificity, *Polyplax*

1 | INTRODUCTION

The formation and maintenance of genetic structure within populations are contingent upon an interplay of various factors, such as environment, geographic distribution, life strategy, population history. In parasites, particularly in those with life cycles closely bound to their hosts (e.g., parasitic lice), the host represents the parasite-only environment. In such cases, parasites typically develop a strong narrow host specificity, and their population structure, diversity, and speciation processes are assumed to be strongly determined by their host.

At an interspecific level, this results in a parallel evolution, which may lead to an almost perfect fit between the host's and the parasite's phylogenies (Hughes, Kennedy, Johnson, Palma, & Page, 2007; Light & Hafner, 2008). In most cases, however, host switches blur the cophylogenetic signal, even in highly host-specific parasites (Banks, Palma, & Paterson, 2006; Ricklefs, Fallon, & Bermingham, 2004; du Toit, Van Vuuren, Matthee, & Matthee, 2013).

Possible processes causing such incongruences have often been discussed in the parasitological literature, and a complex conceptual background has been developed (Clayton, Bush, & Johnson, 2004; Lion & Gandon, 2015; Page, 2003). For example, it has been suggested that the biogeography, social behavior, and vagility of the hosts affect the level of congruence in host–parasite equally or even to a greater extent than the physiology and life history traits of the parasite. However, estimating the degree of intimacy for a particular host–parasite association is not a simple task. It may even be counterintuitive, if previously unforeseen factors are involved in the interaction (e.g., the host abundance determining the parasite's dispersal ability; Engelbrecht, Matthee, du Toit, & Matthee, 2016). The key to understanding a coevolutionary pattern is the investigation of the parasites' population genetics and dynamics and their main determinants. At this intraspecific level, current research has shown that parasite diversity and population structure are affected by several factors, mainly shared demographic history (Nieberding, Morand, Libois, & Michaux, 2004; Štefka, Hoeck, Keller, & Smith, 2011), host dispersal capabilities affecting parasite gene flow (McCoy, Boulinier, Tirard, & Michalakis, 2003; Štefka, Hypša, & Scholz, 2009; van Schaik, Kerth, Bruyndonckx, & Christe, 2014), and the spectrum of parasitized hosts (Archie & Ezenwa, 2011; Barrett, Thrall, Burdon, & Linde, 2009). Nadler (1995) stressed the role of host specificity, predicting that multihost parasites display a shallower population structure due to having a better chance to disperse.

Several studies on the natural populations of parasite species sharing sympatric hosts have addressed these issues, for example the coevolutionary reconstruction of feather lice species with extremely different host specificities (Johnson, Williams, Drown, Adams, & Clayton, 2002) or the investigation of two generalist pinworms from Caribbean reptiles (Falk & Perkins, 2013) or the analysis of population sizes and selection in the bacterium *Anaplasma* (Aardema & von Loewenich, 2015). These works often support Nadler's hypothesis by showing that parasites with a stronger host specificity possessed a more pronounced genetic structure. Research on a related topic using generalist flea parasites (van der Mescht, Matthee, & Matthee, 2015) suggested that the tightness of the association between a host and its parasite represents an important factor. However, while in free-living organisms the effect of the ecological parameters and their shifts on population genetics are well explored (Lemoine et al., 2016), the extent to which even moderate changes in host specificity shape the structure and genetic diversity of parasites remains largely unknown.

In this study, we address the impact of host specificity on the genetics of parasite populations using the sucking louse *Polyplax serrata* and its hosts, mice of the genus *Apodemus*. The *Apodemus* model possesses representatives with a different geographic and ecological structure. The two most widespread species, *Apodemus flavicollis* and *A. sylvaticus*, co-occur throughout the majority of their European distribution in sympatry or even syntopy (Darvish, Mohammadi, Ghorbani, Mahmoudi, & Dubey, 2015; Demanche et al., 2015; Michaux, Libois, & Filippucci, 2005). They separated more than 4 million years ago (mya) (Michaux & Pasquier, 1974) and responded differently to the Quaternary climatic oscillations (Michaux et al., 2005). The nonuniform evolutionary history of the two species also had an impact on the genealogies of their parasites, including endoparasitic helminths (Nieberding, Libois, Douady, Morand, & Michaux, 2005; Nieberding et al., 2004), and ectoparasites such as the sucking lice of the genus *Polyplax* (Štefka & Hypša, 2008).

The basic genetic structure of the *Polyplax/Apodemus* system (Štefka & Hypša, 2008) shows this system to be a useful model for studying coevolution through the analysis of population-level codivergence and raises several interesting questions/hypotheses. At the general level, Štefka and Hypša (2008) showed that the genealogy and current geographic distribution of the lice were clearly coupled with the evolutionary history and distribution of *Apodemus* hosts. However, host specificity and phylogeographic patterns varied across three main mtDNA-based lineages of the parasite (designated as A, B, and C in Štefka and Hypša (2008)). Two lineages, A and B, were more ubiquitous in their distribution and occurred in sympatry, but differed in their degree of host specificities. Both clades shared *A. flavicollis* as a common host and mostly occupied sympatric localities in central Europe. However, Lineage A also parasitized another species, *A. sylvaticus*, and was also found in western Europe (France and United Kingdom). Due to the differences in host specificity, in this study we refer to the two lineages as *N* (nonspecific, Lineage A) and *S* (specific, Lineage B). The lice of Lineage C inhabited mainly *A. agrarius* and *A. uralensis* occurring in the central and eastern regions of Europe, and here, we refer to it as Lineage *Aa*. Štefka and Hypša (2008) also uncovered a lineage from *A. peninsulae* from central Asia (Baikal Lake locality), hereafter referred to as the *Ape* lineage. Here, using mtDNA and multilocus data we analyze the phylogeographic and population genetic structures of an extensive sample from multiple European countries to answer the following questions: (a) Do the mtDNA *Polyplax* lineages (Štefka & Hypša, 2008) retain their integrity and host specificity if analyzed with multilocus data from considerably extended geographic sampling? (b) Do *Polyplax* parasites possess a stronger pattern of population structure compared to their hosts as a result of increased mutation rates and small effective population sizes (N_e)? (c) Is host dispersal the determining factor of the parasite gene flow? That is, do the parasitic lineages *N* and *S*, with different levels of host specificity, follow Nadler's hypothesis (Nadler, 1995) in the sense of (a) deeper population structure in the more host-specific lineage caused by lower dispersal opportunities, and (b) significant differences in genetic diversity between sympatric *N* and *S* populations?

2 | MATERIALS AND METHODS

2.1 | Host sampling and DNA isolation

Mice were captured in wooden snap traps. *Apodemus* tissue samples (ear or fingertips) were preserved in ethanol, and the mice were examined for lice by visual checking and combing. Lice were stored

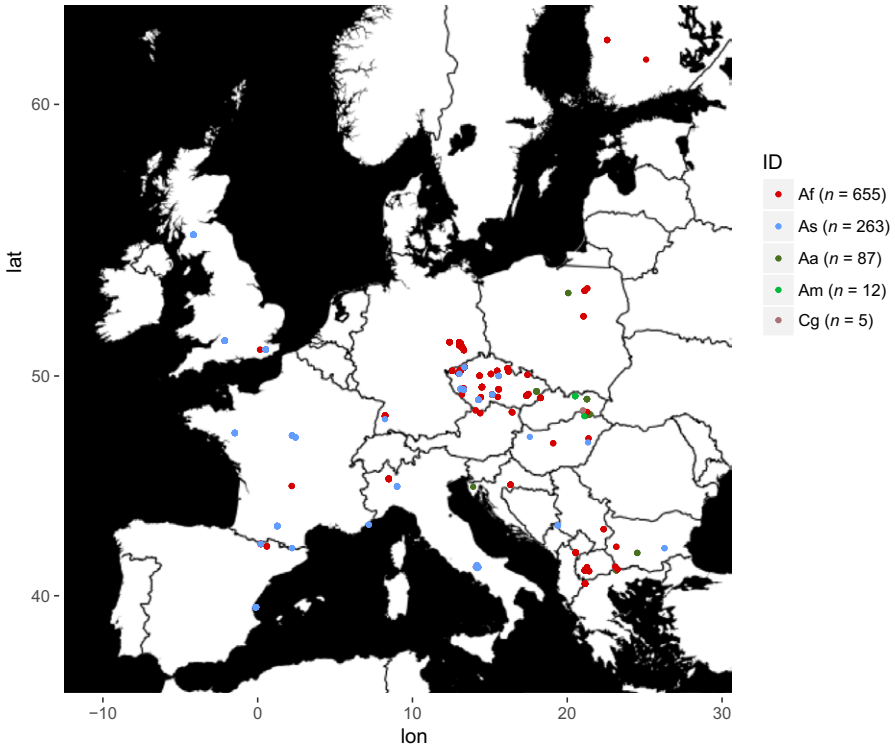


FIGURE 1 Map of sampling localities. Abbreviations: Af—*Apodemus flavicollis*, As—*A. sylvaticus*, Aa—*A. agrarius*, Am—*A. mystacinus*, Cg—*Clethrionomys glareolus*, lat—latitude, lon—longitude

in 100% ethanol in the freezer. Field studies were carried out with permits listed in the Supporting information Document S1. A total of 2,352 specimens of *Apodemus* hosts were collected across 14 European countries during the years 2005–2015. A total of 216 mice were infected with *P. serrata* resulting in a 9.18% prevalence. Host and parasite samples of infected mice and a subset of noninfected hosts covering a large part of the European continent (Figure 1, Table 1 and Supporting information Table S1) were analyzed genetically. DNA extractions were performed with a QIAamp DNA Micro Kit (Qiagen) into 30 µl of AE buffer. Louse skeletons were preserved in 70% ethanol as vouchers. Host DNA was isolated from the host tissue with a DNeasy Blood & Tissue Kit (Qiagen).

2.2 | DNA sequencing and population analysis

A fragment of the mitochondrial cytochrome oxidase subunit I gene (COI, 379 bp) was amplified for 430 specimens of *Polyplax serrata* lice from 216 *Apodemus* hosts using primers L6625 and H7005 (Hafner et al., 1994). These primers, reliably amplifying louse DNA samples, were selected to provide a gross picture of population structure across the whole sample set. For a better understanding of the relationships among the main mtDNA lineages of lice, a longer fragment of COI (1,027 bp), together with three nuclear genes VATP21 (304 bp), hyp (380 bp), and TMEDE6 (215 bp), was obtained for selected specimens of *Polyplax* (n = 25), using COI primers LCO1490 and H7005 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) and nuclear primers published by Sweet, Allen, and Johnson (2014). A description of the PCR reactions, thermal cycling conditions, and sequencing is provided in Supporting information Document S1. A mitochondrial D-loop region with the entire $tRNA^{Thr}$, $tRNA^{Pro}$, and the beginning of the 12S tRNA region (1,002 bp) was gained for 229 individuals of *A. flavicollis* and 92 specimens of *A. sylvaticus* with primers 1, 2bis, 3, and 4 (Bellinvia, 2004) using the PCR conditions described in Supporting information Document S1.

Obtained sequences were assembled in GENEIOUS 8.0.2 (Biomatters, Ltd), collapsed into haplotypes using ALTER (Glez-Peña, Gómez-Blanco, Reboiro-Jato, Fdez-Riverola, & Posada, 2010) and submitted to GenBank under accession numbers MH723758-MH724187. Phylogenies were reconstructed by maximum likelihood (ML) and Bayesian inference (BI). For all analyses, the best-fit models (listed in Supporting information Document S1) were selected according to a corrected Akaike information criterion using jModelTest2 (Darriba, Taboada, Doallo, & Posada, 2012). For the lice, *Polyplax spinulosa* was used as outgroup. For the hosts, *Apodemus sylvaticus* and *A. flavicollis* phylogenies were rooted with three individuals of the other species (three of *A. sylvaticus* with *A. flavicollis* and vice versa). Bayesian (BI) analyses conducted in MrBayes 3.2.4 (Ronquist et al., 2012) consisted of two parallel Markov chain Monte Carlo simulations with four chains run for 10 million generations with sampling frequency of 1,000 generations. The convergence of parameter estimates and their ESS values was checked in software TRACER 1.6 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). Two and a half million generations (25%) were discarded as burn-in. Maximum likelihood analyses were computed using PhyML 3.0 (Guindon et al., 2010) with 1000 bootstrap replicates to obtain nodal support.

To explore population genetic patterns and compare them with phylogeny derived results, we reconstructed haplotype networks, calculated standard diversity measures, and performed hierarchical AMOVA as detailed in Supporting information Document S1.

TABLE 1 List of sampling localities providing numbers of samples analyzed for each organism and marker

Country	Abbreviation	Polyplax lineage	No. of individuals analyzed per gene			Host species	No. of host individuals analyzed per gene	
			COI	Micro	Concat		D-loop	Micro
Bulgaria	BG	Aa	3	6		*Aa*		
		N	1			*Af*	3	
Croatia	HR	Aa	4		2	*Aa*		
		S	4	4	1	*Af*	2	2
Czech Republic	CZ	Aa	18	5		*Af*	78	83
		N	44	36	1	*As*	18	15
		S	164	106	4			
Finland	FIN	–				*Af*	1	4
France	F	N	22	27	3	*Af*	7	7
		S	7	8	1	*As*	22	22
Germany	D	N	26	11		*Af*	55	50
		S	70	41	2	*As*	5	5
Hungary	H	–				*Af*	2	
						As		2
Italy	I	N	10	5	2	*Af*	7	8
		S	18	14	1	*As*	8	5
Macedonia	MK	S	51	44	2	*Af*	35	25
Poland	PL	Aa	3		1	*Af*	5	2
		N	4		2			
Russia	Ru	Ape		5	1			
Slovakia	SK	Aa	38	31		*Af*	23	5
		N	7	4		*Aa*		
		S	27	11		*Au*, *Cg*		
Serbia	Srb	N	1			*Af*		3
		S	9	4		*As*	1	2
Spain	SP	–				*As*	26	17
United Kingdom	GB	N	22	18	2	*Af*	1	
		S	3			*Af*	5	6
						As	17	9

Notes. Abbreviations for genetic markers: Concat: concatenated dataset (COI+ three nuclear loci); Micro: microsatellites; N: nonspecific lineage; S: specific lineage; Aa: lineage with affinity to *Apodemus agrarius*; *Af*: *Apodemus flavicollis*; *As*: *Apodemus sylvaticus*; *Aa*: *Apodemus agrarius*; *Au*: *Apodemus uralensis*; *Ape*: *Apodemus peninsulae*; *Cg*: *Clethrionomys glareolus*.

2.3 | Microsatellite genotyping and population structure

To analyze population structure and level of diversity in individual populations of the parasite and two of its hosts, microsatellite loci were incorporated into the study. For 380 individuals of *Polyplax serrata* included into the mtDNA analysis, sixteen microsatellite loci were amplified in four multiplex PCR assays developed by Martinů et al. (2015). All microsatellite loci were tested for departure from the Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between loci pairs for all populations (with $n \geq 5$ individuals) in GenAlEx 6.5 (Peakall & Smouse, 2012). Micro-checker 2.2.3 was used to evaluate whether the observed heterozygote deficiencies could be explained by the occurrence of null alleles (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). For *Apodemus flavicollis* and *A. sylvaticus*, seven microsatellite loci were amplified in two multiplex assays, following Harr, Musolf, and Gerlach (2000) and Aurelle et al. (2010).The additional five loci exclusively specific to *A. flavicollis*, using multiplexes according to Aurelle et al. (2010), and 10 loci exclusively specific to *A. sylvaticus* (Harr et al., 2000) were amplified to complement datasets of each species. Altogether, 229 individuals of *A. flavicollis* and 92

individuals of *A. sylvaticus* were genotyped and all sampled specimens were also included in the mtDNA phylogenies. All loci were tested for departure from HWE and for LD between pairs of loci in GenAlEx 6.5 (Peakall & Smouse, 2012).

To determine whether populations of the parasite belonging to the *S*, *N*, *Aa*, and *Ape* mtDNA lineages form matching clusters in their nuclear data, or whether they admix, the multivariate technique of principal coordinate analysis (PCoA) was computed from the genetic distance matrix calculated across multiple loci for each pair of individuals. The same analysis was performed also on the population level. PCoA together with an assignment test of *S* and *N* lineages was performed in GenAlEx 6.5 (Peakall & Smouse, 2012). The PCoA as described above for *Polyplax* was performed also for both *Apodemus* species to reconstruct their population structure and to reveal the level of integrity/mixing of individual mtDNA lineages within and between populations. PCoA-based picture of population structure was checked using other distance-based methods and Bayesian clustering methods described in Supporting information Document S1 in detail.

2.4 | Distribution of genetic diversity in *Polyplax* and *Apodemus*

To assess the influence of geographic distance on genetic relatedness, Mantel tests (Mantel, 1967) were used to test for isolation by distance (IBD) using microsatellite estimates of genetic differentiation (F_{ST}, G_{ST}, and D_{JOST}) and geographic distances separately for both *Polyplax* lineages and both *Apodemus* species in the R package adegenet (Jombart, 2008). Statistical significance was computed by 10,000 random permutations. Because the effect of IBD may play different roles at different geographic scales, we analyzed the spatial autocorrelation coefficient (*r*) for *Polyplax S* and *N* lineages and both *Apodemus* hosts. The analyses were performed in GenAlEx 6.5 (Peakall & Smouse, 2012), where *r* was calculated for increasing distance classes with a 95% confidence interval obtained by 1,000 bootstrap replicates and 10 000 permuted *r* values (Peakall, Ruibal, & Lindenmayer, 2003; Smouse & Peakall, 1999).

The impact of host genealogy on the genetic structure of the parasite was evaluated by correlating the F_{ST} (and G_{ST}) matrixes of

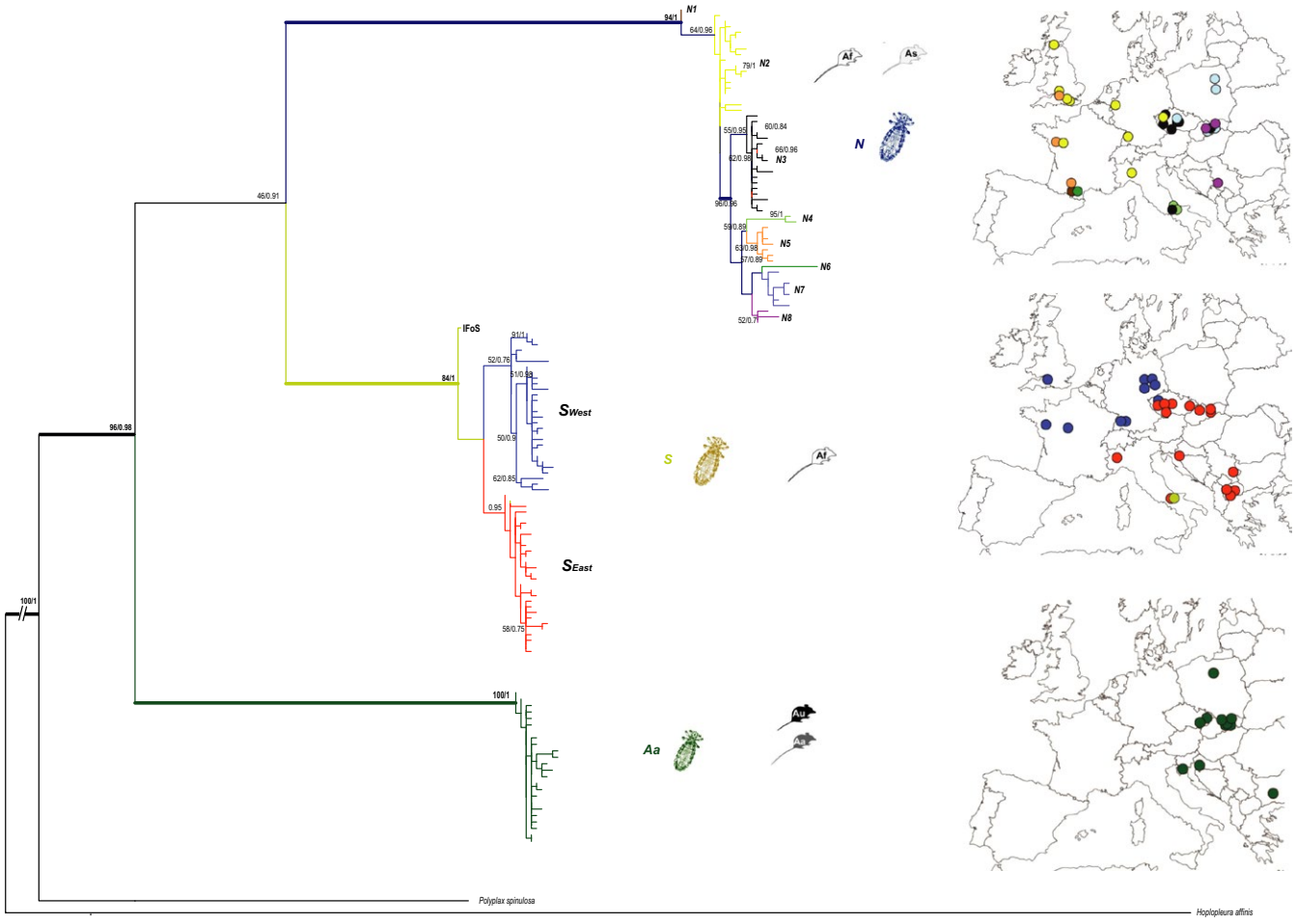


FIGURE 2 Mitochondrial DNA phylogeny for 556 specimens of *Polyplax serrata*. Maximum likelihood phylogeny was obtained with PHYML, statistical support (ML bootstrap/Bayesian posterior probability) is provided above nodes, supported clades (ML bootstrap higher than 80%/Bayesian posterior probability above 0.95) in bold. Geographic distribution of Subclades *N* and *S* is provided using matching colors. Abbreviations of clades and host species: *N*—nonspecific clade; *S*—specific clade; S_{West}—western lineage of specific clade; S_{East}—eastern lineage of specific clade; *Aa*—*Apodemus agrarius* and *uralensis* clade; Aa—*A. agrarius*; Af—*A. flavicollis*; As—*A. sylvaticus*; Au—*A. uralensis*

each of the *Polyplax* lineages and its host species using Mantel tests in R package adegenet and GenAlEx 6.5 (Jombart, 2008; Peakall & Smouse, 2012).

To determine the possible impact of host width (specificity) on population diversity of the parasites, we analyzed differences in the level of genetic diversity between *S* and *N* lineages of *Polyplax* using microsatellite data. F_{ST} and gene diversity (*H*) indices were calculated for pairs for *S* and *N* populations that were collected at identical sites (sympatric populations) or at closely placed sites (within 30 km from each other). Seven population pairs from five European countries matched these criteria and contained a sufficient number of genotyped individuals ($n > 3$). F_{ST} calculations were performed in FSTAT 2.9.3.2 (Goudet 2002) with *p*-values determined by 10,000 permutations. *H* estimates were obtained in GenAlEx 6.5 (Peakall & Smouse, 2012).

3 | RESULTS

3.1 | Phylogeny of *Polyplax serrata* and the *Apodemus* species

Partial COI genes were sequenced for 430 louse specimens and aligned with 126 sequences obtained by Štefka and Hypša (2008). Final mitochondrial dataset contained sequences of 556 *Polyplax* specimens (Table 1 and Supporting information Table S1). Phylogenetic analyses of the short matrix (379 bp, 138 haplotypes) clustered the lice into three well-supported lineages (Figure 2) described previously by Štefka and Hypša (2008).

The *S* and *N* lineages were found in sympatry or at adjacent localities across a large geographic area (Figure 2). However, while the *N* lineage did not show any clear geography dependent structure, an intriguing geographic pattern was detected for the *S* lineage. This lineage split into two well-supported subgroups with different, almost exclusive geographic distributions (except for a narrow overlap). These two subgroups are therefore designated as *Specific East* (S_{East}) and *Specific West* (S_{West}). The third main lineage (*Aa*) was only found in the eastern part of Europe, concurrently with its primary hosts (*A. agrarius* and *A. uralensis*).

The relationships between the *N*, *S*, and *Aa* lineages were not well supported in the analysis of short COI sequences, but could be reliably established by analyzing 25 representative samples for which longer COI sequences (1,027 bp) were concatenated with three nuclear genes. This analysis clustered the *S* and *N* lineages as sister groups (Supporting information Figure S1).

For the host, we obtained D-loop sequences from 229 *A. flavicollis* and 92 *A. sylvaticus* samples. *A. flavicollis* phylogeny revealed two phylogenetically distinct clusters (Af_1 and Af_2) largely overlapping in their geographic distribution (Figure 3) but differing in their abundance. For *A. sylvaticus*, phylogenetic tree contained three clusters (Figure 3). Two of them, As_1 and As_3, overlapped in their distributions across western Europe; however, As_3 was found more frequently across the whole area and extended also to central Europe and the Iberian Peninsula. As_3 was paraphyletic with respect to the third lineage, the Italian-Balkan clade As_2.

Genetic differentiation between the western and southeastern samples of the lice demonstrated by the phylogenetic trees (Figure 2 and Supporting information Figure S1) and haplotype networks (Supporting information Figure S2) is in accord with the nucleotide diversity statistics (Supporting information Tables S2 and S3), suggesting a recent spread of *Polyplax* populations from glacial refugia, accompanied by population decline and subsequent expansion in several lineages. The demographic pattern in the hosts was less pronounced. Haplotypes belonging to major clades within *A. flavicollis* and *A. sylvaticus* were geographically admixed, high levels of haplotype diversities

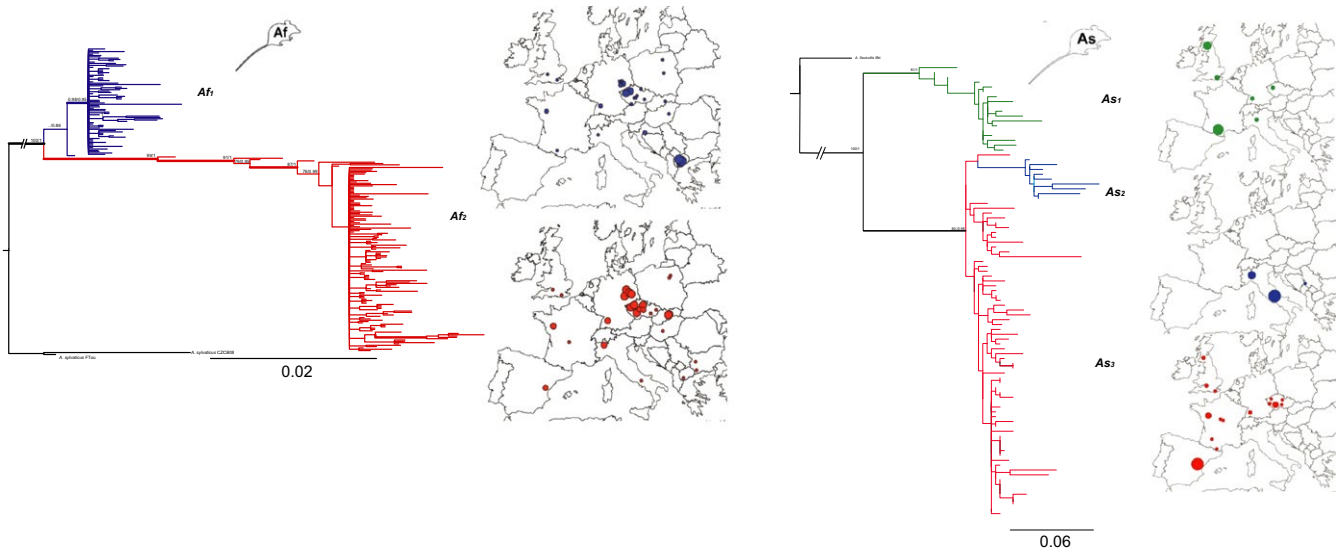


FIGURE 3 Mitochondrial DNA phylogeny for 229 specimens of *Apodemus flavicollis* and 92 specimens of *Apodemus sylvaticus*. Maximum likelihood phylogeny was obtained with PHYML, statistical support (ML bootstrap higher than 50% Bayesian posterior probability above 0.6) is provided above nodes, supported clades (ML bootstrap higher than 80%/Bayesian posterior probability above 0.95) in bold. Geographic distribution of subclades Af_1, Af_2, As_1, As_2, and As_3 is provided using matching colors

were obtained for lineages within both species, and fewer cases of past demographic fluctuations were revealed (Supporting information Figures S5 and S6, and Supporting information Tables S2 and S3).

3.2 | Microsatellite diversity and structure in the *Polyplax-Apodemus* system

The overall microsatellite diversity obtained for parasite and host samples are summarized in Table 2; Supporting information Table S5 and S8. For the lice, each of the microsatellite loci was polymorphic in at least 15 of the 32 populations, with up to 11 alleles per locus and population (Supporting information Table S5). Correspondingly to the low average heterozygosity (H_e, Table 2), all louse populations showed significant deviations from the Hardy–Weinberg equilibrium due to heterozygote deficiencies in at least one locus, but none of the loci was out of HWE across all populations (Supporting information Table S6). The deviations were more frequent in the *S* lineage than in the *N* lineage. Micro-checker

TABLE 2 Observed and expected heterozygosities for populations of *Polyplax serrata S*, *N* lineages, *Apodemus flavicollis*, and *A. sylvaticus*

	PS S lineage		*PS N* lineage		*A. flavicollis*		*A. sylvaticus*	
Pop	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e
CZBen	0.131	0.417			0.667	0.552		
CZCB			0.484	0.495				
CZCM1	0.072	0.162	0.435	0.569	0.563	0.622		
CZDou	0.219	0.285			0.583	0.709		
CZJach	0.200	0.256						
CZLi05	0.229	0.481	0.348	0.383	0.638	0.818		
CZPl			0.323	0.508			0.571	0.679
CZStr	0.199	0.299	0.354	0.465	0.670	0.763		
CZVyk	0.202	0.335						
DBa	0.353	0.420	0.335	0.554	0.600	0.738	0.718	0.729
DKot	0.050	0.073			0.625	0.630		
DKrei					0.741	0.741		
DLau	0.088	0.181			0.660	0.752		
DPin					0.604	0.641		
DSol	0.161	0.274			0.735	0.722		
DTor	0.218	0.269			0.740	0.734		
EBa							0.687	0.848
FGu	0.110	0.348	0.472	0.608	0.639	0.767	0.574	0.631
FTou			0.451	0.545			0.638	0.793
Fin					0.542	0.503		
GBAs			0.343	0.459			0.750	0.664
GBSc			0.250	0.297				
GBSt			0.539	0.625			0.594	0.663
HRVS	0.328	0.363						
IBri	0.174	0.403			0.668	0.748		
IBu			0.396	0.405				
ICiS			0.500	0.477				
MK8	0.425	0.602						
MK9	0.436	0.672			0.732	0.809		
MK10	0.469	0.636			0.764	0.799		
MK12	0.000	0.455						
PLPu			0.141	0.373				
SKPo	0.136	0.174						
SKRuz			0.422	0.547				
SrbSP	0.141	0.324						
Average	0.207	0.354	0.402	0.492	0.657	0.709	0.647	0.715

Note. Population abbreviations as in Supporting information Table S1.

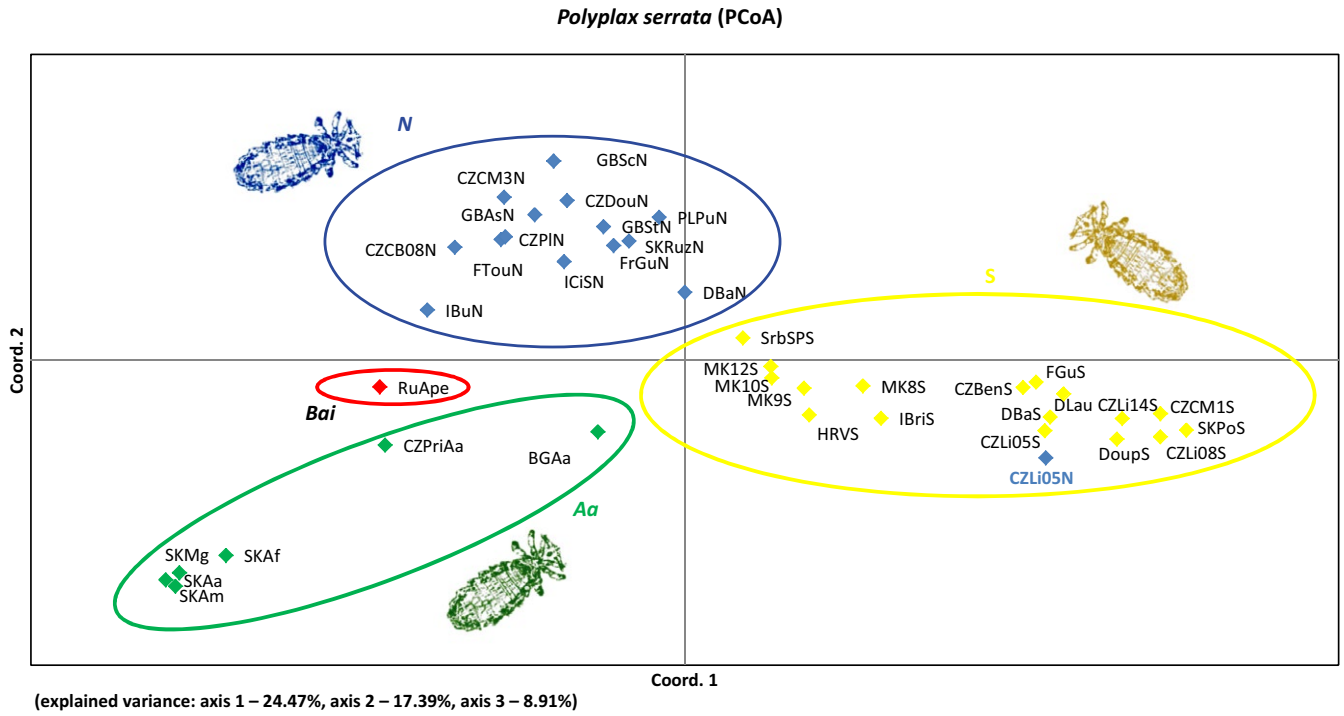


FIGURE 4 Principal coordinate analysis (PCoA) of *Polyplax serrata* populations using microsatellite data. Colors match major lineages used in Figure 2. Population sample containing mtDNA introgressed from the N lineage (CZLi05N) is highlighted in blue. Population abbreviations as in Supporting information Table S1

analysis indicated possible occurrence of null alleles in several cases; however, adjusted estimates of gene diversity of few populations differed only marginally (Supporting information Table S7), and we thus decided to keep all data for the subsequent analyses. Pairwise F_{ST} values indicated considerable degree of genetic differentiation between populations (with $n \geq 5$), ranging from 0.04 to 0.65 in the *S* lineage and 0.10 to 0.39 in the *N* lineage (Supporting information Table S8).

In the hosts, *A. flavicollis* and *A. sylvaticus*, the number of alleles per locus varied from one to 15 alleles with an average of four alleles per locus and population (Supporting information Table S9). In *A. flavicollis*, for which 12 loci were analyzed, two populations were in HWE, the rest showed deviations from HWE in one to four loci, and the German population DLau had six loci of HWE (Supporting information Table S10). In *A. sylvaticus*, with 17 loci analyzed, the British population GBA showed no deviations from HWE, the majority of other populations had one to four loci of HWE, the French population FTou had five loci, and the Spanish population EBa had 11 loci of HWE. Pairwise F_{ST} values showed considerable genetic structure, ranging from 0.03 to 0.47 in *A. flavicollis* and 0.04 to 0.59 in *A. sylvaticus* (Supporting information Table S8).

PCoA of the microsatellite datasets revealed deep genetic structure in the parasite and, on the contrary, a relatively shallow divergence in the hosts. In *Polyplax*, the analysis divided the populations into clusters corresponding to the main mtDNA lineages (Figure 4). The only discrepancy was found for the Czech population Litvínov (CZLi05N; blue in Figure 4), which belongs to the *N* lineage according to the mtDNA data, but clusters together with *S* populations in the microsatellite analysis. Genetic differentiation between the *S* and *N* lineages was also obvious from the assignment test performed in GenAlEx (results not shown) and from the Bayesian and distance-based clustering (Supporting information Figures S8 and S9). On the intralineage level, PCoA of individuals from *S* and *N* lineages showed in most cases that lice sampled from the same locality formed compact structures, and geographically close populations often showed genetic proximity (Supporting information Figure S10a, b). This trend was more pronounced in the *S* lineage compared to the *N*. PCoA based on data for the whole populations revealed further differences between the *S* and *N* lineages (Supporting information Figure S10c, d). While within *S* lineage the populations clearly clustered according to their geographic origin, a fractional geographic clustering was also discernible in the *N* lineage, but it did not create such explicit clusters as in the *S* lineage.

For the hosts, analyses performed on a set of seven microsatellite loci shared by both host species (PCoA, Bayesian and distance-based clustering—Document S1) agreed with the mtDNA pattern confirming that *A. flavicollis* and *A. sylvaticus* represent two separated species. On the intraspecific level, despite analyzing more loci, the PCoA results demonstrated in both species that host individuals from different mtDNA subclades did not form separated clusters when retrieved from sympatric localities (Supporting information Figure S11a, b). Geographically delimited populations (localities) were more admixed than in the parasites and did not cluster together. On the population level, PCoA (Supporting information Figure S11c, d) showed formation of several genetic lineages, which,

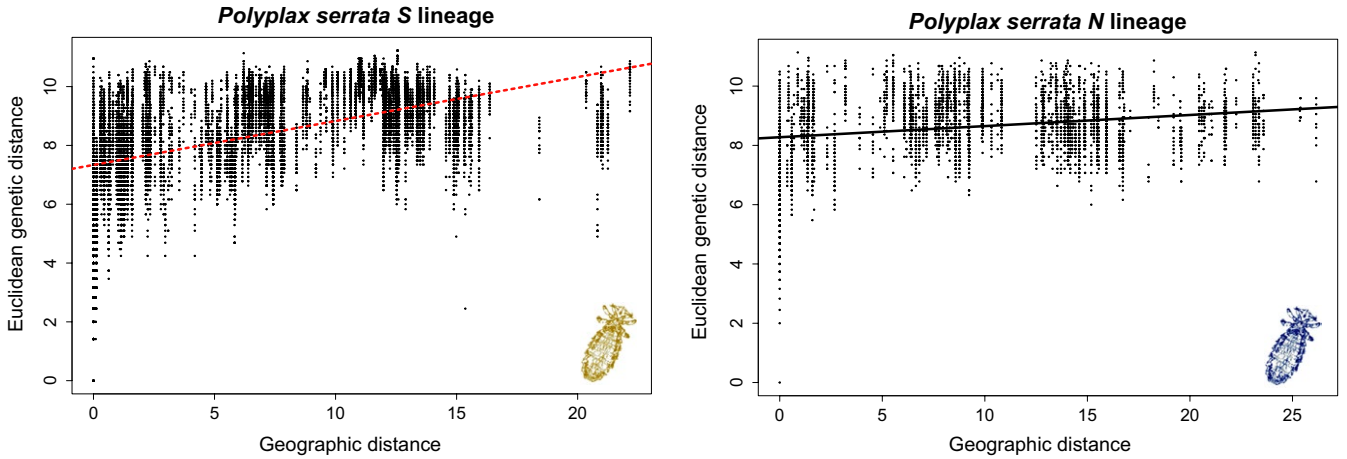


FIGURE 5 Correlation between Euclidean genetic distances and geographic distances for pairs of *Polyplax serrata* individuals. Plots were generated separately for S and N lineages in adegenet. Correlation was significant (red dashed line) for the S lineage and nonsignificant (black line) for the N lineage (10,000 permutations)

however, did not correspond to the mtDNA genealogy and showed only a limited correspondence to geography (e.g., GB and FR populations in *A. sylvaticus*, Supporting information Figure S11d). Similar results were obtained also from the Bayesian and distance-based clustering analyses (Document S1, Supporting information Figures S9 and S12).

3.3 | Spatial structure of the parasites and hosts

Correlations between genetic pairwise matrices and geographic distances, as analyzed by Mantel tests, varied in dependence on both the species/lineage of the host/parasite and the exact statistics used. F_{ST} tests found significant IBD only within *A. sylvaticus* (Supporting information Figure S13). G_{ST} tests were statistically significant for *Polyplax S* lineage (Supporting information Figure S14) and for *A. sylvaticus* (Supporting information Figure S13), whereas D_{JOST} test was significant only for the *Polyplax S* lineage (Supporting information Figure S14). When assessed as the correlation between Euclidean distances (performed on the level of individuals) and geographic distances, the IBD was only significant for the *S* lineage, with a markedly larger correlation than for the *N* lineage (Figure 5).

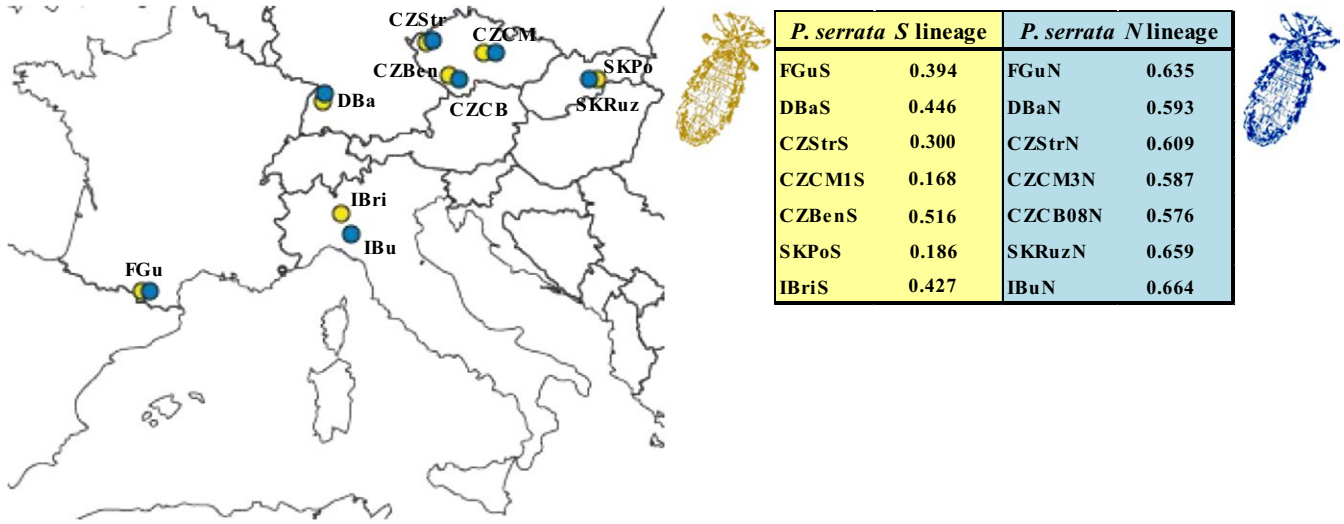
The autocorrelation coefficient (*r*), used to evaluate the effect of IBD on different geographic scales, revealed in all evaluated organisms (*Polyplax* lineages *S* and *N*, *A. sylvaticus*, and *A. flavicollis*; Supporting information Figure S15) a positive significant autocorrelation, which was declining with increase in the distance between populations. This pattern indicates that IBD is strongest between the neighboring populations in both hosts and parasites. However, the spatial extent and the strength of the autocorrelation differed between organisms, showing stronger signal at short distances for the parasite compared to the hosts. The highest values of autocorrelation coefficient (*r*) in *Polyplax* lineages were two times greater than those of the hosts. In the hosts, the *r* value was 10 times lower at the shortest distance range in *A. flavicollis* than in *A. sylvaticus*, which corresponded with the nonsignificant results of Mantel tests in *A. flavicollis*.

3.4 | Differences in population diversities between *S* and *N* lineages of Polyplax

Microsatellite data were used to verify Nadler's hypothesis using populations of the *S* and *N* lineages as representatives of the specialist and generalist parasitic strategies. According to the prediction, F_{ST} and *H* indices calculated for each of the two lineages revealed a lower genetic diversity and a stronger population structure for the *S* lineage. The F_{ST} index was statistically lower for the *N* lineage (0.241) than for the *S* lineage (0.460) (15 000 permutations). On the contrary, the *H* index was markedly higher for populations of the *N* lineage (0.587) than for the *S* populations (0.389) (15,000 permutations). A more detailed study of both lineages performed on seven pairs of sympatric (or closely located populations) showed, in all pairwise comparisons, higher values of *H* for *N* populations than for *S* (Figure 6).

4 | DISCUSSION

Using the *Apodemus*/*Polyplax* model, we demonstrate that coevolutionary processes, when viewed from a broad-scale population perspective, may produce surprisingly complex and intriguing patterns (Figures 2 and 3). At the most general level, the obtained patterns conform to the traditionally held views that parasites phylogenies and genealogies are strongly determined by their hosts and that populations of parasites have a lower genetic connectivity and are more structured than those of the hosts (Engelbrecht et al., 2016; Koop, DeMatteo, Parker, & Whiteman, 2014; Nieberding & Olivieri, 2007). However, at a more subtle level, the structure, genetic diversity, and host specificity of the parasite populations differ even between closely related sister



P. serrata S lineage		*P. serrata N* lineage	
FGuS	0.394	FGuN	0.635
DBaS	0.446	DBaN	0.593
CZStrS	0.300	CZStrN	0.609
CZCM1S	0.168	CZCM3N	0.587
CZBenS	0.516	CZCB08N	0.576
SKPoS	0.186	SKRuzN	0.659
IBriS	0.427	IBuN	0.664

FIGURE 6 Gene diversity (*H*) and geographic distribution for seven pairs of sympatric *S* and *N* lineage populations of *Polyplax serrata*. Color codes as in Figure 2. Population abbreviations as in Supporting information Table S1

clades. For example, although the two main sister lineages of the parasite (*S* and *N*) are widely distributed and share an identical host, *A. flavicollis*, only the *S* lineage is strictly specific, while lice of the *N* lineage can also be found on the other host species, *A. sylvaticus*. As the specific and nonspecific samples were collected in sympatry, sometimes even from identical host individuals, we suppose that the absence of the *S* lineage on *A. sylvaticus* is due to adaptive constraints rather than lack of opportunity to switch hosts. However, the most striking instance of the observed irregularities is probably provided by the sharp difference seen in the postglacial colonization process between *A. flavicollis* and its specific parasite, the *S* lineage of *Polyplax*. In this host/parasite association, the encounter of populations from different refugia resulted in a largely admixed European population of the host, while the louse populations remained genetically separated, with only a narrow contact zone (discussed below). This remarkable complexity of the whole system is further increased by various unique genetic events, such as a mitochondrial introgression of the *N* louse clade into a single population of the other clade (e.g., Figure 4). At last, we demonstrated that the effect of the level of host specificity on population structure and diversity of ectoparasite populations follows Nadler's predictions. We document this by a comparison between the specific lineage *S*, with low genetic diversity and a higher level of isolation by distance between its populations, and the more generalist *N* lineage found on two host species (Figure 5 and Supporting information Figure S14).

4.1 | Decoupled process of postglacial recolonization in host and parasite populations

The observed distribution of the clades and haplotypes within the *Apodemus/Polyplax* system corresponds in general to the presumed (re)colonization processes of Europe, determined by the biogeographic and climatic changes of the Quaternary glaciation. The host species likely recolonized Europe from several refugia (Russian Ukrainian and Balkan for *A. flavicollis*, Iberian peninsula/southern France for *A. sylvaticus*) and formed panmictic populations covering most of the territory of European (Figure 3; Supporting information Figures S5 and S6). It is interesting that while the lice accompanied the two host species during their retreat to refugia and subsequent expansion, they have not mirrored straightforwardly their recolonization process. A striking discrepancy was detected between the distribution of the *A. flavicollis* mtDNA lineages (Supporting information Figure S5) and the *A. flavicollis* specific lice (*S* lineage) (Supporting information Figure S3). As shown in the Supporting information Figures S3 and S5, after their expansion from different refugia, the two mtDNA lineages of *A. flavicollis* spread across the whole sampled area and can be now be found in sympatry at identical localities. Multilocus analyses show that this secondary postglacial encounter has been followed by frequent gene flow, resulting in (re)constitution of a single highly admixed population (Supporting information Figure S12). In contrast, the two mtDNA haplotype clusters (S_{East} and S_{West}) of the *P. serrata S* lineage stopped their expansion from the glacial refugia at the narrow contact zone in central Europe (Supporting information Figure S3). This incongruence is unexpected, as due to their intimate relationship, lice and their hosts are expected to share identical patterns of geographic expansion, unless the association is disrupted by a host switch. In other words, the geographic distribution of a louse species/population is believed to be entirely determined by the host(s) (Marshall, 1981). The incapability of the two louse populations to cross the contact zone thus indicates that factors other than host-mediated distribution, or a mere within-refugia speciation, have played a role during the recolonization process. Based on the presented data, it is difficult to hypothesize on the probable cause of this discrepancy. However, an interesting possibility is presented by the symbiotic bacteria known to inhabit the lice (Hypša & Křížek, 2007; Říhová, Nováková, Husník, & Hypša,

2017). The viability and/or reproduction of many blood feeding insects depend on various bacterial symbionts, and the intimacy of the host–symbiont association in such cases results in a metabolic cooperation between their genomes (Kirkness et al., 2010; Snyder & Rio, 2013). The long-term isolation in refugia (potentially lasting 0.4 to 0.6 My, see Michaux, Libois, Paradis, & Filippucci, 2004) could thus lead to specific louse-genome vs. symbiont-genome adaptations that prevent an "incorrect" genome–genome combination.

4.2 | Different level of resolution in mitochondrial and microsatellite data

In contrast to the mtDNA, microsatellites did not show any apparent suture between the S_{West} populations on the one hand and the S_{East} populations on the other hand. As the mtDNA-based picture is based on extensive sampling and is well supported (Figure 2 and Supporting information Figure S3), this discrepancy may reflect the different level of historical information preserved in the microsatellite data. As shown in Supporting information Figures S9 and S10, based on the microsatellite-derived signal, the analyses were able to recognize and cluster together geographically proximate populations, but did not provide information on the higher hierarchical structure across Europe. This picture is not entirely surprising. Due to a smaller N_e and quicker coalescence compared to nuclear loci, mtDNA is considered to be the leading indicator of speciation processes (Zink & Barrowclough, 2008). Instead, the mitonuclear discrepancy may also be the result of a selection caused by different lineages of a bacterial endosymbiont, as was shown in other insects (Kodandaramaiah, Simonsen, Bromilow, Wahlberg, & Sperling, 2013). Nevertheless, microsatellites could in future provide an appropriate tool for quantifying the volume of gene flow across the contact zone, after it is sampled more densely than in our current dataset.

4.3 | Occasional mitochondrial introgression

Apart from this general difference, we also observed rare assignment discrepancies between the two types of data for the CZLi louse population of *A. flavicollis*. Approximately half of the specimens sampled in 2005 (CZLi05N) clustered within the *N* lineage according to mtDNA (clustered with Subclade N2 in Figure 2), whereas microsatellites placed the whole sample CZLi05 within the S lineage (Figure 4 and Supporting information Figure S10a, c). The rest of the population sample (CZLi05S) was placed within the *S* lineage by both mtDNA (cluster S_{WEST} in Figure 2) and microsatellites (Figure 4; Supporting information Figure S10a, c). Such discrepancies are usually explained either by the incomplete sorting of an ancestral polymorphism or by introgression after a secondary contact (Hochkirch, 2013; Toews & Brelsford, 2012). As we only found a single instance of such shared haplotypes between the two louse lineages across the whole dataset, and the repeated sampling at the locality in 2008 and 2014 did not reveal any shared haplotypes, we conclude that a recent and short-lived mitochondrial introgression from the *N* lineage to the *S* lineage provides a more plausible explanation. Such a dynamic development, where genetic information is quickly lost (or fixed) after introgression, is in agreement with the biology of louse populations. Small, fragmented populations of lice are prone to rapid changes in their size and genetic composition. It was also demonstrated that after several generations of backcrossing, it is often difficult to trace introgression using microsatellites, and genomic tools allowing extensive screening of the genome are required (Oliveira et al., 2015).

It has recently been demonstrated in different systems that species boundaries may not be as resistant to the gene flow of either mtDNA or nuclear DNA as previously thought (Harrison & Larson, 2014). Although mitochondrial introgressions occurring together with a very low or even zero introgression of nuclear genes are rare, they were shown to occasionally happen, for example in Galapagos mockingbirds (Nietlisbach et al., 2013) and North American chipmunks (Good, Vanderpool, Keeble, & Bi, 2015). Because the N_e of mtDNA genes is four times lower than of autosomal genes, genetic drift influences mitochondrial haplotypes to a larger extent and can lead to a faster fixation of unoriginal mitochondrial haplotypes (Funk & Omland, 2003; Zink & Barrowclough, 2008). Parasites without free-living stages and intermediate hosts generally possess a female-biased sex ratio (Criscione, Poulin, & Blouin, 2005), which can also affect the introgression process after contact. By accident, a female-biased sex ratio was also found in a related louse species, the *Polyplax arvicanthis* lice from the South African *Rhabdomys* (Matthee et al., 2007).

4.4 | Host specificity governs parasite dispersal and population size: test of the Nadler's hypothesis

The dispersal capacity of parasites is to a great extent influenced by host sociality and vagility (Criscione et al., 2005; Mazé-Guilmo, Blanchet, Mccoy, & Loot, 2016; van Schaik et al., 2014). As parasitic lice inhabit a single host during their entire life cycle, their opportunities to spread are limited to direct host contact or to shared host shelters (Marshall, 1981). Likewise, populations of host-specific ectoparasites were recently shown to be more genetically fragmented than their hosts (Harper, Spradling, Demastes, & Calhoun, 2015; Koop et al., 2014). When comparing the dispersal activities of sucking lice and their hosts, one should expect a higher level of historical gene flow in mice and a lower level for lice because of the life history traits of the parasites, such as the lack of other vectors and occasional "missing the boat" events during the host's migration (Clayton et al., 2004; Page, 2003). In our system, we found markedly higher values of autocorrelation coefficients for both *Polyplax* lineages compared with *Apodemus* hosts, especially over shorter distances (Supporting information Figure S15), which is consistent with the expected lower level of gene flow in the parasite. Furthermore, the high rate of H_e deficiency in louse populations (Table 2) indicates that the gene flow is limited even within a single host population

among the lice from different host individuals. This is in agreement with earlier reports (Harper et al., 2015; Koop et al., 2014) and supports our expectations that host dispersal is the general factor driving parasite gene flow.

In contrast to the general pattern of a more pronounced population structure in the parasite compared to its host, a lower level of differentiation in the parasites was reported by du Toit et al. (2013) in the system of *Rhabdomys* mice and *Polyplax arvicanthis* lice in South Africa. As revealed by the authors, two factors seem to have caused the discrepancy. First, the *Rhabdomys* hosts comprise four species with a parapatric distribution, forming narrow contact zones, which allow occasional host switching followed by genetic admixture of the parasites. Second, *P. arvicanthis* has approximately five times higher prevalence (60%) than *P. serrata*, and thus reaches a high N_e potentially slowing down the rate of differentiation between populations. On the contrary, despite the fact that the sympatric occurrence of *A. sylvaticus* and *A. flavicollis* should allow for a higher rate of host switching in *Apodemus* parasites than in the case of *Rhabdomys*, the evolutionarily old origin of the *S* and *N* lineages and their long-term separation in different refugia led to an accumulation of changes that prevents successful host switching in the *S* lineage. The *N* and *S* lineages of *P. serrata* diverged ~1.5 mya (Štefka & Hypša, 2008), and their hosts were isolated in several refugia, some of them specific to only a single species, some of them shared (Michaux et al., 2004). Furthermore, the relatively low prevalence of the *P. serrata* (9%) results in small N_e that accelerates genetic drift and fragmentation of the populations.

In addition to the differences in gene flow between the hosts and the parasites, our system provided a unique opportunity to test specific predictions of Nadler's hypothesis (Nadler, 1995) by a comparison of two closely related parasites with different degrees of host specificity. According to the hypothesis, the less specialized *N* lineage should experience a higher degree of gene flow than the strictly specific *S* lineage, due to having more opportunities to find suitable hosts and hence a stronger dispersion capability. In agreement with this expectation, our IBD analysis of genetic and geographic distances among individual lice detected a steeper and statistically significant correlation in the *S* lineage in contrast to a weak and nonsignificant dependence in the *N* lineage (Figure 5).

Yet, another piece of evidence corroborating Nadler's hypothesis was provided by the comparison of genetic diversities between sympatric populations of the two louse lineages. In an overall statistical analysis, the *N* lineage populations showed a significantly lower F_{ST} index indicating that the *S* lineage lice (specialists) have a smaller N_e and more fragmented populations, expressed by the low frequency of heterozygotes as a result of the Wahlund effect. More important, the comparison of gene diversities between seven sympatric pairs of *N* and *S* populations (Figure 6) reached the same conclusions as the indexes calculated for the whole lineages. This multiple population comparison provides a strong body of evidence that even a moderate shift in host specificity translates into significant differences in genetic characteristics of parasite populations.

5 | CONCLUSION

The evolutionary history of the *Apodemus–Polyplax* association across a large area of Europe is more complicated that could be expected for such a "simple" relationship between a host and its permanent ectoparasite. The traditional coevolutionary view, holding that the distribution and genetic structure of a parasite populations are determined by host phylogeography, is here reflected by the overall genetic structure of the parasite, which corresponds to the presumed (re)colonization processes of the *Apodemus* species in Europe. This, however, is not a complete picture. Some of the patterns indicate that even a strong population structure and changes in the genetic background of the parasite's populations may be driven by forces independent of the host(s). This finding warns us against simplifying tendencies when studying host–parasite coevolution and underestimation of intrinsic genetic processes in parasitic organisms. To show this, we generated and analyzed the largest and most complex body of molecular data (mitochondrial haplotypes and microsatellites) available on this host–parasite association. This also allowed us to address in detail several other issues, such as Nadler's hypothesis for parasite genetic diversity or genetic introgression in temporal parasite populations.

ACKNOWLEDGEMENTS

We thank numerous colleagues from CZ and abroad and students of the University of South Bohemia, CZ, for their help with obtaining study material.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

This study forms part of the PhD research of J.M., who performed laboratory and data analyses under the supervision of J.Š., with V.H. and J.Š. conceiving the study of *Apodemus/Polyplax* coevolution. All three authors contributed toward the design of the study and drafted the manuscript.

DATA ACCESSIBILITY

DNA sequences obtained in the frame of the study will be submitted to GenBank upon acceptance of the MS. DNA alignments and microsatellite datasets are submitted to Dryad database (https://doi.org/10.5061/dryad.5jh39).

ORCID

Jana Martinů http://orcid.org/0000-0003-0296-7867

REFERENCES

Aardema, M., & von Loewenich, F. D. (2015). Varying influences of selection and demography in host-adapted populations of the tick-transmitted bacterium, *Anaplasma phagocytophilum*. *BMC Evolutionary Biology*, *15*, 58. https://doi.org/10.1186/s12862-015-0335-z

Archie, E. A., & Ezenwa, V. O. (2011). Population genetic structure and history of a generalist parasite infecting multiple sympatric host species. *International Journal for Parasitology*, *41*, 89–98. https://doi.org/10.1016/j.ijpara.2010.07.014

Aurelle, D., Baker, A. J., Bottin, L., Brouat, C., Caccone, A., Chaix, A., … Yuan, Q. Y. (2010). Permanent genetic resources added to the molecular ecology resources database. *Molecular Ecology Resources*, *10*, 751–754.

Banks, J. C., Palma, R. L., & Paterson, A. M. (2006). Cophylogenetic relationships between penguins and their chewing lice. *Journal of Evolutionary Biology*, *19*, 156–166. https://doi.org/10.1111/j.1420-9101.2005.00983.x

Barrett, L. G., Thrall, P. H., Burdon, J. J., & Linde, C. C. (2009). Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends in Ecology & Evolution*, *23*, 678–685.

Bellinvia, E. (2004). A phylogenetic study of the genus *Apodemus* by sequencing the mitochondrial DNA control region. *Journal of Zoological Systematics and Evolutionary Research*, *42*, 289–297. https://doi.org/10.1111/j.1439-0469.2004.00270.x

Clayton, D., Bush, S., & Johnson, K. (2004). Ecology of congruence: Past meets present. *Systematic Biology*, *53*, 165–173. https://doi.org/10.1080/10635150490265102

Criscione, C. D., Poulin, R., & Blouin, M. S. (2005). Molecular ecology of parasites: Elucidating ecological and microevolutionary processes. *Molecular Ecology*, *14*, 2247–2257. https://doi.org/10.1111/j.1365-294X.2005.02587.x

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772–772. https://doi.org/10.1038/nmeth.2109

Darvish, J., Mohammadi, Z., Ghorbani, F., Mahmoudi, A., & Dubey, S. (2015). Phylogenetic relationships of *Apodemus* Kaup, 1829 (Rodentia: Muridae) species in the Eastern Mediterranean inferred from mitochondrial DNA, with emphasis on Iranian species. *Journal of Mammalian Evolution*, *22*, 583–595. https://doi.org/10.1007/s10914-015-9294-9

Demanche, C., Deville, M., Michaux, J., Barriel, V., Pinçon, C., Aliouat-Denis, C. M., … Guillot, J. (2015). What do *Pneumocystis* organisms tell us about the phylogeography of their hosts? The case of the woodmouse *Apodemus sylvaticus* in continental Europe and Western Mediterranean Islands. *PLoS ONE*, *10*, 1–17.

du Toit, N., Van Vuuren, B. J., Matthee, S., & Matthee, C. A. (2013). Biogeography and host-related factors trump parasite life history: Limited congruence among the genetic structures of specific ectoparasitic lice and their rodent hosts. *Molecular Ecology*, *22*, 5185–5204. https://doi.org/10.1111/mec.12459

Engelbrecht, A., Matthee, S., du Toit, N., & Matthee, C. A. (2016). Limited dispersal in an ectoparasitic mite, *Laelaps giganteus*, contributes to significant phylogeographic congruence with the rodent host. *Molecular Ecology*, *25*, 1006–1021. https://doi.org/10.1111/mec.13523

Falk, B. G., & Perkins, S. L. (2013). Host specificity shapes population structure of pinworm parasites in Caribbean reptiles. *Molecular Ecology*, *22*, 4576–4590. https://doi.org/10.1111/mec.12410

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*, 294–299.

Funk, D. J., & Omland, K. E. (2003). Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics*, *34*, 397–423. https://doi.org/10.1146/annurev.ecolsys.34.011802.132421

Glez-Peña, D., Gómez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola, F., & Posada, D. (2010). ALTER: Program-oriented conversion of DNA and protein alignments. *Nucleic Acids Research*, *38*, 14–18. https://doi.org/10.1093/nar/gkq321

Good, J. M., Vanderpool, D., Keeble, S., & Bi, K. (2015). Negligible nuclear introgression despite complete mitochondrial capture between two species of chipmunks. *Evolution*, *69*, 1961–1972. https://doi.org/10.1111/evo.12712

Goudet, J. 2002. FSTAT 2.9.3.2, A Program to Estimate and Test Gene Diversities and Fixation Indices (updated from Goudet 1995). Lausanne, Switzerland, Available at: http:// www2.unil.ch/popgen/softwares/fstat.htm

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, *59*, 307–321. https://doi.org/10.1093/sysbio/syq010

Hafner, M. S., Sudman, P. D., Villablanca, F. X., Spradling, T. A., Demastes, J. W., & Nadler, S. A. (1994). Disparate rates of molecular evolution in cospeciating host and parasites. *Science*, *265*, 1087–1090. https://doi.org/10.1126/science.8066445

Harper, S. E., Spradling, T. A., Demastes, J. W., & Calhoun, C. S. (2015). Host behavior drives parasite genetics at multiple geographic scales: Population genetics of the chewing louse, *Thomomydoecus minor*. *Molecular Ecology*, *24*(16), 4129–4144. https://doi.org/10.1111/mec.13306

Harr, B., Musolf, K., & Gerlach, G. (2000). Characterization and isolation of DNA microsatellite primers in wood mice (*Apodemus sylvaticus*, Rodentia). *Molecular Ecology*, *9*, 1664–1665. https://doi.org/10.1046/j.1365-294x.2000.01043-3.x

Harrison, R. G., & Larson, E. L. (2014). Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity*, *105*, 795–809. https://doi.org/10.1093/jhered/esu033

Hochkirch, A. (2013). Hybridization and the origin of species. *Journal of Evolutionary Biology*, *26*, 247–251. https://doi.org/10.1111/j.1420-9101.2012.02623.x

Hughes, J., Kennedy, M., Johnson, K. P., Palma, R. L., & Page, R. D. M. (2007). Multiple cophylogenetic analyses reveal frequent cospeciation between pelecaniform birds and *Pectinopygus* lice. *Systematic Biology*, *56*, 232–251. https://doi.org/10.1080/10635150701311370

Hypša, V., & Křížek, J. (2007). Molecular evidence for polyphyletic origin of the primary symbionts of sucking lice (Phthiraptera, Anoplura). *Microbial Ecology*, *54*, 242–251. https://doi.org/10.1007/s00248-006-9194-x

Johnson, K. P., Williams, B. L., Drown, D. M., Adams, R. J., & Clayton, D. H. (2002). The population genetics of host specificity: Genetic differentiation in dove lice (Insecta: Phthiraptera). *Molecular Ecology*, *11*, 25–38. https://doi.org/10.1046/j.0962-1083.2001.01412.x

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Kirkness, E. F., Haas, B. J., Sun, W., Braig, H. R., Perotti, M. A., Clark, J. M., … Pittendrigh, B. R. (2010). Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 12168–12173. https://doi.org/10.1073/pnas.1003379107

Kodandaramaiah, U., Simonsen, T. J., Bromilow, S., Wahlberg, N., & Sperling, F. (2013). Deceptive single-locus taxonomy and phylogeography: *Wolbachia*-associated divergence in mitochondrial DNA is not reflected in morphology and nuclear markers in a

butterfly species. *Ecology and Evolution*, *3*, 5167–5176. https://doi.org/10.1002/ece3.886

Koop, J. A. H., DeMatteo, K. E., Parker, P. G., & Whiteman, N. K. (2014). Birds are islands for parasites. *Biology Letters*, *10*, 6–10.

Lemoine, M., Lucek, K., Perrier, C., Saladin, V., Adriaensen, F., Barba, E., … Richner, H. (2016). Low but contrasting neutral genetic differentiation shaped by winter temperature in European great tits. *Biological Journal of the Linnean Society*, *118*, 668–685. https://doi.org/10.1111/bij.12745

Light, J. E., & Hafner, M. S. (2008). Codivergence in heteromyid rodents (Rodentia: Heteromyidae) and their sucking lice of the genus *Fahrenholzia* (Phthiraptera: Anoplura). *Systematic Biology*, *57*, 449–465. https://doi.org/10.1080/10635150802169610

Lion, S., & Gandon, S. (2015). Evolution of spatially structured host-parasite interactions. *Journal of Evolutionary Biology*, *28*, 10–28. https://doi.org/10.1111/jeb.12551

Mantel, N. (1967). The Detection of Disease Clustering and a Generalized Regression Approach. *Cancer Research*, *27*, 209–220.

Marshall, A. G. (1981). *The ecology of ectoparasitic insects*. London, UK: Academic Press.

Martinů, J., Roubová, V., Nováková, M., Smith, V. S., Hypša, V., & Štefka, J. (2015). Characterisation of microsatellite loci in two species of lice, *Polyplax serrata* (Phthiraptera: Anoplura: Polyplacidae) and *Myrsidea nesomimi* (Phthiraptera: Amblycera: Menoponidae). *Folia Parasitologica (Praha)*, *62*, 1–6.

Matthee, S., Horak, I. G., Beaucournu, J.-C., Durden, L. A., Ueckermann, E. A., & McGeoch, M. A. (2007). Epifaunistic arthropod parasites of the four-striped mouse, *Rhabdomys pumilio*, in the Western Cape Province, South Africa. *Journal of Parasitology*, *93*, 47–59. https://doi.org/10.1645/GE-819R2.1

Mazé-Guilmo, E., Blanchet, S., Mccoy, K. D., & Loot, G. (2016). Host dispersal as the driver of parasite genetic structure: A paradigm lost? *Ecology Letters*, *19*, 336–347. https://doi.org/10.1111/ele.12564

McCoy, K. D., Boulinier, T., Tirard, C., & Michalakis, Y. (2003). Host-dependent genetic structure of parasite populations: Differential dispersal of seabird tick host races. *Evolution*, *57*, 288–296. https://doi.org/10.1111/j.0014-3820.2003.tb00263.x

Michaux, J. R., Libois, R., & Filippucci, M.-G. (2005). So close and so different: Comparative phylogeography of two small mammal species, the yellow-necked fieldmouse (*Apodemus flavicollis*) and the woodmouse (*Apodemus sylvaticus*) in the Western Palearctic region. *Heredity (Edinburgh)*, *94*, 52–63. https://doi.org/10.1038/sj.hdy.6800561

Michaux, J. R., Libois, R., Paradis, E., & Filippucci, M. G. (2004). Phylogeographic history of the yellow-necked fieldmouse (*Apodemus flavicollis*) in Europe and in the near and Middle East. *Molecular Phylogenetics and Evolution*, *32*, 788–798. https://doi.org/10.1016/j.ympev.2004.02.018

Michaux, J. R., & Pasquier, L. (1974). Dynamique des populations de mulots (Rodentia, *Apodemus*) en Europe durant le Quaternaire. *Premières Données Bull. Société Géologique Fr.*, *7*, 431–439. https://doi.org/10.2113/gssgfbull.S7-XVI.4.431

Nadler, S. A. (1995). Microevolution and the genetic structure of parasite populations. *Journal of Parasitology*, *81*, 395–403. https://doi.org/10.2307/3283821

Nieberding, C., Libois, R., Douady, C. J., Morand, S., & Michaux, J. R. (2005). Phylogeography of a nematode (*Heligmosomoides polygyrus*) in the western Palearctic region: Persistence of northern cryptic populations during ice ages? *Molecular Ecology*, *14*, 765–779. https://doi.org/10.1111/j.1365-294X.2005.02440.x

Nieberding, C., Morand, S., Libois, R., & Michaux, J. R. (2004). A parasite reveals cryptic phylogeographic history of its host. *Proceedings of the Royal Society B-Biological Sciences*, *271*, 2559–2568. https://doi.org/10.1098/rspb.2004.2930

Nieberding, C. M., & Olivieri, I. (2007). Parasites: Proxies for host genealogy and ecology?. *Trends in Ecology & Evolution*, *22*, 156–165. https://doi.org/10.1016/j.tree.2006.11.012

Nietlisbach, P., Wandeler, P., Parker, P. G., Grant, P. R., Grant, B. R., Keller, L. F., & Hoeck, P. E. A. (2013). Hybrid ancestry of an island subspecies of Galápagos mockingbird explains discordant gene trees. *Molecular Phylogenetics and Evolution*, *69*, 581–592. https://doi.org/10.1016/j.ympev.2013.07.020

Oliveira, R., Randi, E., Mattucci, F., Kurushima, J. D., Lyons, L. A., & Alves, P. C. (2015). Toward a genome-wide approach for detecting hybrids: Informative SNPs to detect introgression between domestic cats and European wildcats (*Felis silvestris*). *Heredity (Edinb).*, *115*, 195–205. https://doi.org/10.1038/hdy.2015.25

Page, R. D. M. (2003). *Tangled trees: Phylogeny, cospeciation and coevolution*. Chicago, IL: University of Chicago Press.

Peakall, R., Ruibal, M., & Lindenmayer, D. B. (2003). Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution*, *57*, 1182–1195. https://doi.org/10.1111/j.0014-3820.2003.tb00327.x

Peakall, R., & Smouse, P. E. (2012). GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, *28*, 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018) Tracer v1.7. Retrieved from http://tree.bio.ed.ac.uk/software/tracer

Ricklefs, R. E., Fallon, S. M., & Bermingham, E. (2004). Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology*, *53*, 111–119. https://doi.org/10.1080/10635150490264987

Říhová, J., Nováková, E., Husník, F., & Hypša, V. (2017). *Legionella* becoming a mutualist: Adaptive processes shaping the genome of symbiont in the louse *Polyplax serrata*. *Genome Biology and Evolution*, *9*, 2946–2957.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. https://doi.org/10.1093/sysbio/sys029

Smouse, P. E., & Peakall, R. (1999). Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity (Edinb).*, *82*(Pt 5), 561–573. https://doi.org/10.1038/sj.hdy.6885180

Snyder, A. K., & Rio, R. V. M. (2013). Interwoven biology of the tsetse holobiont. *Journal of Bacteriology*, *195*, 4322–4330. https://doi.org/10.1128/JB.00487-13

Štefka, J., Hoeck, P. E. A., Keller, L. F., & Smith, V. S. (2011). A hitchhikers guide to the Galápagos: Co-phylogeography of Galápagos mockingbirds and their parasites. *BMC Evolutionary Biology*, *11*, 284.

Štefka, J., & Hypša, V. (2008). Host specificity and genealogy of the louse *Polyplax serrata* on field mice, *Apodemus* species: A case of parasite duplication or colonisation? *International Journal for Parasitology*, *38*, 731–741.

Štefka, J., Hypša, V., & Scholz, T. (2009). Interplay of host specificity and biogeography in the population structure of a cosmopolitan endoparasite: Microsatellite study of *Ligula intestinalis* (Cestoda). *Molecular Ecology*, *18*, 1187–1206.

Sweet, A. D., Allen, J. M., & Johnson, K. P. (2014). Novel primers from informative nuclear loci for louse molecular phylogenetics (Insecta: Phthiraptera). *Journal of Medical Entomology*, *51*, 1122–1126. https://doi.org/10.1603/ME13218

Toews, D. P. L., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, *21*, 3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x

van der Mescht, L., Matthee, S., & Matthee, C. A. (2015). Comparative phylogeography between two generalist flea species reveal a

complex interaction between parasite life history and host vicariance: Parasite-host association matters. *BMC Evolutionary Biology*, *15*, 105. https://doi.org/10.1186/s12862-015-0389-y

van Schaik, J., Kerth, G., Bruyndonckx, N., & Christe, P. (2014). The effect of host social system on parasite population genetic structure: Comparative population genetics of two ectoparasitic mites and their bat hosts. *BMC Evolutionary Biology*, *14*, 18. https://doi.org/10.1186/1471-2148-14-18

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). Micro-Checker: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*, 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Zink, R. M., & Barrowclough, G. F. (2008). Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*, *17*, 2107–2121. https://doi.org/10.1111/j.1365-294X.2008.03737.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Martinů J, Hypša V, Štefka J. Host specificity driving genetic structure and diversity in ectoparasite populations: Coevolutionary patterns in *Apodemus* mice and their lice. *Ecol Evol*. 2018;00:1–15. https://doi.org/10.1002/ece3.4424

Manuscript No. 3:
Martinů, J., Sychra, O., Literák, I., Čapek, M., Gustafsson, D.L., Štefka, J. 2015. Host generalists and specialists emerging side by side: an analysis of evolutionary patterns in the cosmopolitan chewing louse genus *Menacanthus*. International Journal for Parasitology, 45: 63–73.

International Journal for Parasitology 45 (2015) 63–73

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Host generalists and specialists emerging side by side: an analysis of evolutionary patterns in the cosmopolitan chewing louse genus *Menacanthus* ☆

Jana Martinů [a,*], Oldřich Sychra [b], Ivan Literák [b], Miroslav Čapek [c], Daniel L. Gustafsson [d], Jan Štefka [a]

[a] Faculty of Science, University of South Bohemia and Biology Centre ASCR, Institute of Parasitology, Branisovska 31, 37005 Ceske Budejovice, Czech Republic
[b] Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackeho tr. 1/3, 61242 Brno, Czech Republic
[c] Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Kvetna 8, 60365 Brno, Czech Republic
[d] Department of Biology, University of Utah, Salt Lake City, UT, USA

ARTICLE INFO

Article history:
Received 16 May 2014
Received in revised form 26 August 2014
Accepted 4 September 2014
Available online 13 October 2014

Keywords:
Host specificity
Specialist
Generalist
Population structure
Geographic distribution
Menacanthus

ABSTRACT

Parasites with wide host spectra provide opportunities to study the ecological parameters of speciation, as well as the process of the evolution of host specificity. The speciose and cosmopolitan louse genus *Menacanthus* comprises both multi-host and specialised species, allowing exploration of the ecological and historical factors affecting the evolution of parasites using a comparative approach. We used phylogenetic analysis to reconstruct evolutionary relationships in 14 species of *Menacanthus* based on the sequences of one mitochondrial and one nuclear gene. The results allowed us to validate species identification based on morphology, as well as to explore host distribution by assumed generalist and specialist species. Our analyses confirmed a narrow host use for several species, however in some cases, the supposed host specialists had a wider host spectrum than anticipated. In one case a host generalist (*Menacanthus eurysternus*) was clustered terminally on a clade almost exclusively containing host specialists. Such a clade topology indicates that the process of host specialisation may not be irreversible in parasite evolution. Finally, we compared patterns of population genetic structure, geographic distribution and host spectra between two selected species, *M. eurysternus* and *Menacanthus camelinus*, using haplotype networks. *Menacanthus camelinus* showed limited geographical distribution in combination with monoxenous host use, whereas *M. eurysternus* showed a global distribution and lack of host specificity. It is suggested that frequent host switching maintains gene flow between *M. eurysternus* populations on unrelated hosts in local populations. However, gene flow between geographically distant localities was restricted, suggesting that geography rather than host-specificity is the main factor defining the global genetic diversity of *M. eurysternus*.

1. Introduction

The coevolutionary process in host–parasite systems may display a surprisingly high complexity: even in closely related lineages the genealogy and population structure may not reflect the most apparent biological features. Such a situation has been found, for example, in a genealogical study on the human associated lice of the genus *Pediculus* (i.e. Reed et al., 2004) and a similar pattern of "random" changes of ecological features has been confirmed on a phylogenetical/genealogical scale in two additional host–parasite associations, the lice of the genus *Polyplax* (Štefka and Hypša, 2008; du Toit et al., 2013) and the tapeworm *Ligula intestinalis* (Štefka et al., 2009). In host–parasite systems, the combination of geographical distribution and host specificity creates a complex background for genetic diversification and population structuring.

In chewing lice, long evolutionary periods of tight coexistence with their hosts and relatively few opportunities for dispersing among other host species were traditionally believed to constrain these parasites, causing them to show a high degree of codivergence and parallel evolution with their hosts (Eichler, 1941, 1942; Page and Hafner, 1996). Lice infesting multiple unrelated hosts were long thought to constitute cryptic species (Eichler, 1941), which resulted in the erection of new species, and even

☆ *Note:* Nucleotide sequence data reported in this paper are available in the GenBank database under accession numbers **KJ730527**–**KJ730843**.

* Corresponding author. Tel.: +420 38 5310351.
E-mail address: martinu@paru.cas.cz (J. Martinů).

http://dx.doi.org/10.1016/j.ijpara.2014.09.001
0020-7519/

genera, based primarily on host relationships; many of these names have subsequently found little acceptance (see e.g., Price et al., 2003). A similar problem exists with many described species of *Menacanthus* (Price, 1975, 1977; Pilgrim and Palma, 1982; Palma et al., 1998; Krištofík, 2000).

When analysed genetically, euryxenous (broad host range) parasite species are frequently revealed to constitute an assemblage of cryptic species (e.g. Jousson et al., 2000; Demanche et al., 2001; Smith et al., 2006). On the other hand, an increasing number of studies on chewing lice at the lower taxonomic level have revealed that multi-host (generalist) louse species are quite common, especially in the Ischnocera (e.g. Johnson et al., 2002a, 2003; Clayton and Johnson, 2003; Gustafsson and Olsson, 2012). Host generalists evidently do occur and, more importantly, contrary to the presumed idea of continuous host specialisation in evolution (Fahrenholz, 1913; Eichler, 1941), generalist lice have been derived from host specialists several times independently (Johnson et al., 2009, 2011).

Dispersal capabilities are probably among the most important factors affecting the level of host specificity in lice, and in parasites in general. Bueter et al. (2009) compared general phylogenetic patterns in the ischnoceran genus *Brueelia* and the amblyceran *Myrsidea*, and found fewer host specialists in *Brueelia* than in *Myrsidea*. Similarly, when comparing levels of intraspecific genetic variability and codivergence with their hosts across the Galapagos archipelago, Štefka et al. (2011) found a tighter correlation with hosts in *Myrsidea* than in *Brueelia*. In both studies phoresy, or "hitchhiking", on hippoboscid flies was suggested to explain the differences, as it is relatively common in the ischnoceran lice (Keirans, 1975). However, some amblyceran lice, particularly the genus *Myrsidea*, are probably also able to switch between distantly related hosts that share similar habitats and geographic distributions (Bueter et al., 2009). Whether or not host switching occurs by phoresy is presently unknown, but phoresy is not unknown in amblyceran lice (Hopkins, 1946). Similar results have been arrived at in the case of *Myrsidea* elsewhere (Clay and Meinertzhagen, 1943; Kounek et al., 2011; Sychra et al., 2014).

Host specificity and dispersal abilities in multi-host amblyceran lice have not previously been explored using molecular methods. However, taxonomists have long cast doubts on the actual numbers of species in several multi-host genera, for example in the genus *Colpocephalum* from the Corvidae (Price and Beer, 1965) or *Trochiliphagus* from the Trochilidae (Rheinwald, 2007). In this study we focused on the phylogenetic patterns of the amblyceran genus *Menacanthus*, and in particular on the genetic variability of *Menacanthus eurysternus*. *Menacanthus* is a speciose and cosmopolitan louse genus, comprising 98 species parasitising approximately 460 species of birds belonging to seven orders of birds (Cicchino, 2003; Price et al., 2003; Palma and Price, 2005; Bansal et al., 2012); however, despite the wide host range, they are most numerous on wildfowl (Galliformes), woodpeckers (Piciformes) and passerines (Passeriformes).

In the case of *Menacanthus* from passerines, 10 of the 36 recognised species are monoxenous (a single host parasite), while 25 are stenoxenous (with a narrow host range) with 2–22 closely related host species that usually belong to the same family (Price et al., 2003). The most euryxenous and cosmopolitan species within the genus is *M. eurysternus*, which has been recorded from eight species of woodpeckers and 170 species of passerines belonging to 20 families (Price et al., 2003). *Menacanthus eurysternus* often shows a relatively high prevalence (e.g., 56.4%, Boyd (1951); 68.4%, Chandra et al. (1990)) and can reach high intensities of infestation. It is haematophagous and can thus impact the condition of its hosts (Agarwal et al., 1983), and its population dynamics are synchronised with the reproduction cycle of the host (Foster, 1969; Srivastava et al., 2003).

Opinions are divided on the complex of species represented by *M. eurysternus* sensu lato. While the checklist of Price et al. (2003) considered *M. eurysternus* to be one widely distributed species, some authors (for example Fedorenko, 1983) consider it to be a complex of several remarkably similar species (sensu Banks and Paterson, 2005). Mey (2003) considered the various proposed species to be subspecies of *M. eurysternus*. Only a few *M. eurysternus* sequences have been published to date, all of which are mitochondrial (mt)DNA sequences (hosts *Lybius torquatus*, Piciformes, from Africa, *Zosterops japonicus*, *Pycnonotus blanfordi*, *Pycnonotus finlaysoni*, Passeriformes, from Vietnam). These samples possess a relatively low level of differentiation, with sequences differing only in approximately 4–7% of nucleotide positions (Najer et al., 2014). Such low genetic differentiation is surprising, given the diverse geographic and host origin of the samples. If the same trend was confirmed using a larger sampling size, it would represent a unique situation among lice, which typically possess narrow host specificity limited to one or a few related hosts (see the checklist of Price et al., 2003) and show higher levels of divergence between louse lineages or species from distantly related hosts (Johnson et al., 2003; Bueter et al., 2009).

However, even such low genetic divergence as seen in the six *M. eurysternus* samples does not a priori exclude the existence of distinct populations, where moderate levels of host specificity or geographic fragmentation have evolved. Thus, apart from presenting an interesting taxonomical problem, the lice of the genus *Menacanthus* (and *M. eurysternus* in particular) provide a rare opportunity to study the evolution of host specificity in parasites. Given the complicated taxonomy of the genus and somewhat ambiguous morphological determination of several species, we first reconstructed the phylogenetic relationships between *M. eurysternus* and 13 other species (10 species from passerines, one from a woodpecker and two from *Gallus gallus*) to validate species determinations and their relationships. Then selected lineages (euryxenous versus stenoxenous) were studied in more detail. We analysed the population genetic structure in two selected lineages of *Menacanthus* which differed in the width of their host spectrum and contrasted the patterns obtained through these analyses with the morphological traits and bionomy (ecology and physiology) of *Menacanthus* spp. to test the contribution of host generalist or specialist parasitic strategy on the formation of genetic structure and speciation in lice. Using the phylogenetic approach with wider taxon sampling, we were able to identify two more *Menacanthus* spp. with potential multi-host distribution (e.g. *Menacanthus obrteli*).

2. Materials and methods

2.1. Sampling of lice

Chewing lice of the genus *Menacanthus* were collected from 29 localities across a broad geographic range covering 12 countries (Supplementary Table S1). Samples were either collected by the authors or provided by collaborators listed in the Acknowledgments. Lice were collected from birds captured in mist nets using the fumigation chamber method (Clayton and Drown, 2001) with a visual examination of the head. Collected specimens were preserved in pure 95% or denatured 70% ethanol and stored in a refrigerator. Lice were cut between the thorax and the abdomen, and genomic DNA was extracted from individual specimens using the QIAamp DNA Micro Kit (Qiagen, Germany). Following DNA extraction, remaining exoskeletons were mounted in Canada balsam onto microscope slides and stored as vouchers at University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic or

Yamashina Institute for Ornithology, Chiba, Japan (see Supplementary Table S1). Specimens were identified based on Price (1977).

2.2. PCR amplification and DNA sequencing

Partial sequences of the nuclear coding gene for elongation factor 1-α (EF-1α, 347 bp) and the mitochondrial gene for cytochrome oxidase subunit I (COI, 381 bp) were amplified using PCR. PCR products of EF-1α were obtained using primers EF1-For3 and Cho10 (Danforth and Ji, 1998). Primers L6625 and H7005 (Hafner et al., 1994) were used for COI amplification. PCRs were carried out in a 20 μl volume using 1 μl of extracted DNA, 5 pM of each primer, 15 mM $MgCl_2$, 10 mM dNTPs, 10× PCR buffer and 0.25 U of High Fidelity PCR Enzyme Mix (Fermentas, United Kingdom). The amplification protocol consisted of one denaturation step at 95 °C for 3 min, then 30 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C (COI)/45 °C (EF-1α) for 45 s and an extension step at 72 °C for 1.5 min, followed by the last elongation step at 72 °C for 10 min. PCR products were cleaned up in a single-step enzymatic reaction using 0.2 μl of Exonuclease I (ExoI) and 0.2 μl of Calf Intestinal Alkaline Phosphatase (CIP) enzymes (New England Biolabs Inc., USA). Purified PCR products were sequenced using the PCR primers in a commercial laboratory (Macrogen Inc., Korea and the Netherlands). Obtained sequences were deposited in GenBank (GB) (see Supplementary Table S1).

2.3. Phylogenetic analysis

Datasets containing mitochondrial, nuclear and concatenated sequences of the two genes were aligned in BioEdit 7.05 (Hall, 1999). Sequences were collapsed to haplotypes using the Collapse 1.2 program (http://darwin.uvigo.es/software/collapse.html). Molecular phylogenies were reconstructed using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches. The analyses were performed individually for each dataset (mitochondrial, nuclear and concatenated). PhyML software (Guindon et al., 2005) was used to obtain ML phylogenies with a TVM + I + G model for COI, TIM3ef + G for EF-1α and HKY85 + I + G for the concatenated alignment. Substitution models of the molecular evolution for each dataset were selected in jModeltest 2 using the Akaike Information Criterion (AIC) (Guindon and Gascuel, 2003; Posada, 2008; Darriba et al., 2012). Parameters were estimated from the data and bootstrap supports were obtained by 1,000 replications. BI analyses were conducted with Mr. Bayes version 3.2.2 (Ronquist and Huelsenbeck, 2003) for COI and EF-1α datasets separately and with the concatenated dataset divided into two partitions, using the same models as in the ML analysis, separately for each gene partition. For each BI analysis we ran two parallel runs for 10 million generations, each with four Markov chains (Huelsenbeck and Bollback, 2001). Markov chains were sampled every 1,000 generations, yielding 10,000 parameter point estimates. The first 2,500 trees (25%) were discarded as burn-in when summarising phylogenies and Bayesian posterior probabilities. Convergence between estimated values of model parameters obtained in independent BI runs and their effective sampling sizes were checked using Tracer 1.6 (http://tree.bio.ed.ac.uk/software/tracer/). Convergence of inferred BI topologies was inspected using the 'are we there yet' (AWTY) method (Nylander et al., 2008). COI and EF-1α sequences of the chewing lice *Dennyus hirundinis* (GenBank Accession Nos. **AF385013** and **AF385032**) and *Myrsidea marksi* (GenBank Accession Nos. **DQ366669** and **FJ171315**) were used as outgroups.

2.3.1. Analysis of intra-clade diversity and population history

Haplotype networks were constructed for two species clades, "*eurysternus*" and "*camelinus*" (*Menacanthus camelinus*), in the TCS 1.21 program (Clement et al., 2000) using COI data. Information about the biogeographic history of the *M. eurysternus* clade was inferred using Nested Clade Phylogeographic Analysis (NCPA) (Templeton et al., 1987; Templeton and Sing, 1993) and used as input data for the analysis of the geographical dependence of genetic variability in Geodis (Posada et al., 2000) in ANeCAv1.2 software (Panchal, 2007). The program implements TCS and GeoDis algorithms (Clement et al., 2000; Posada et al., 2000) for testing the congruence between the population genetic structure and geographic distribution of haplotypes. The inference key of Templeton (2004) was implemented to evaluate possible historical and geographical events. The probability of the null hypothesis (no association between genetic structure and geography) was estimated by 1 million permutations. According to the suggestions of Posada et al. (2000) and Panchal (2007), four regions with large gaps between sampled populations were indicated to prevent a false inference of isolation by distance. These regions cover unsampled areas in North America, central Africa, eastern Europe and China where *M. eurysternus* probably occurs.

3. Results

In total 168 sequences of the COI gene and 151 sequences of EF-1α were obtained from lice of the genus *Menacanthus*. Amplification of either COI or, more commonly, EF-1α failed in some of the samples stored in denatured 70% ethanol. Such samples were removed from the concatenated dataset, resulting in 129 combined mitochondrial and nuclear sequences, which thus only includes samples which were sequenced for both markers. The sequences were collapsed into 61 haplotypes in COI, 28 haplotypes in EF-1α and 51 concatenated haplotypes. The list of sequenced specimens with their geographical origin, morphological identification and associated haplotype numbers are available in Supplementary Table S1.

3.1. Phylogenetic analysis

3.1.1. COI

The topology of the resulting ML tree was compatible with the topology obtained with BI analysis. The most basal division is between one clade ("*cornutus*" in Fig. 1) containing *Menacanthus cornutus* from domestic chickens clustered together with GenBank sequences of *Menacanthus* sp. from Cracidae (Figs. 1 and 3), and the rest of the ingroup; however the division received poor support. The larger clade, in turn, was split into two major clades, each with relatively high bootstrap support and posterior probabilities. We provisionally named these clades the "*curuccae*" and "*alaudae*" clades (see Fig. 1), after the earliest described species in each clade: *Menacanthus curuccae* and *Menacanthus alaudae*, respectively.

The distribution of haplotypes between the two major clades was mostly consistent with morphological identification with, however, some exceptions. A well-supported "*obrteli*" lineage was recovered (see Figs. 1 and 2) which contained lice from four families of hosts from central Europe, identified as *M. obrteli* and *Menacanthus* sp. (morphological identification of *Menacanthus* sp. specimens was not possible due to the excessive damage to those vouchers during DNA extraction or because they were represented only by nymphs). According to the literature (Sychra et al., 2008), *M. obrteli* is specific to *Locustella luscinioides* from the family Locustellidae. In our analyses the samples from *Locustella* clustered with samples from Turdidae, Laniidae and Paridae.

Another conflict between the morphological and genetic data occurred in a group of specimens identified on a morphological basis as *Menacanthus takayamai*. Although these specimens were morphologically homogeneous, they did not form a monophyletic

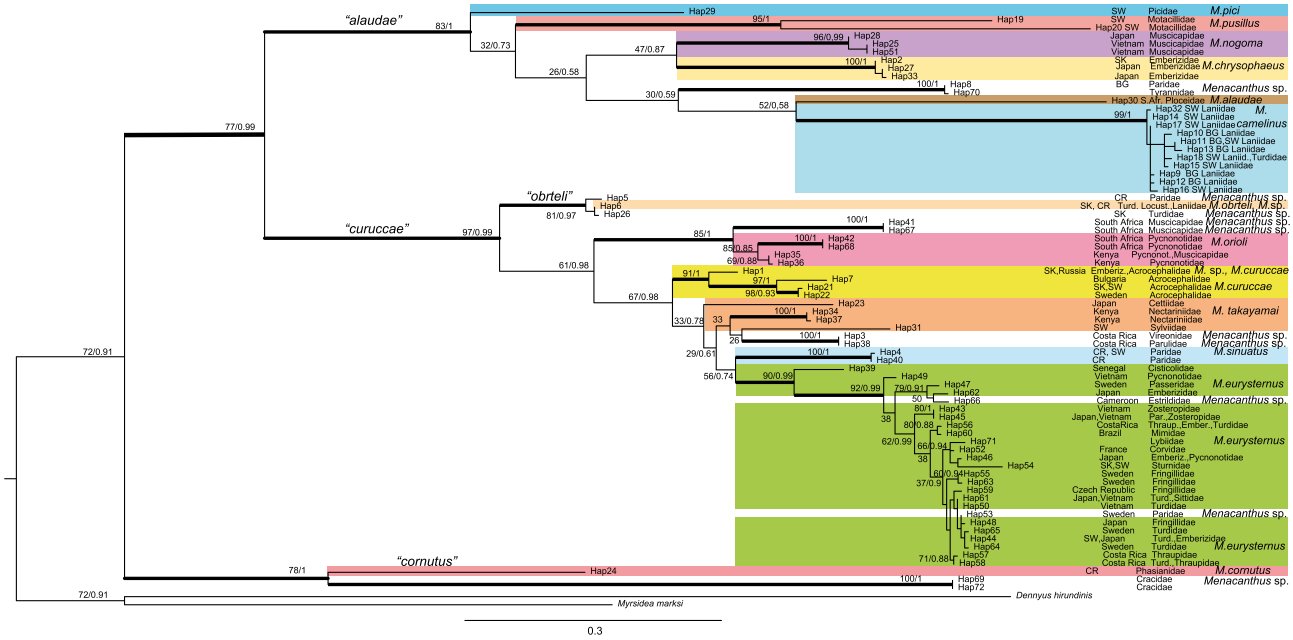


Fig. 1. Maximum Likelihood tree topology of *Menacanthus* based on cytochrome oxidase subunit I sequence data. Maximum Likelihood bootstrap values for 1,000 replicates and Bayesian Inference posterior probabilities are provided. Clades highlighted in bold indicate bootstrap values >70% and posterior probabilities >0.95. The tree was rooted with *Dennyus hirundinis* and *Myrsidea marksi* sequences from GenBank. Colours (shades of grey) mark morphological determinations of the species. BG, Bulgaria; CR, Costa Rica; Ember, Emberizidae; Hap, haplotype; Laniid, Laniidae; Locust, Locustelidae; SK, Slovakia; S. Afr, South Africa; SW, Sweden; Turd, Turdidae; Par, Paridae; Pycnonot, Pycnonotidae; Thraup, Thraupidae.

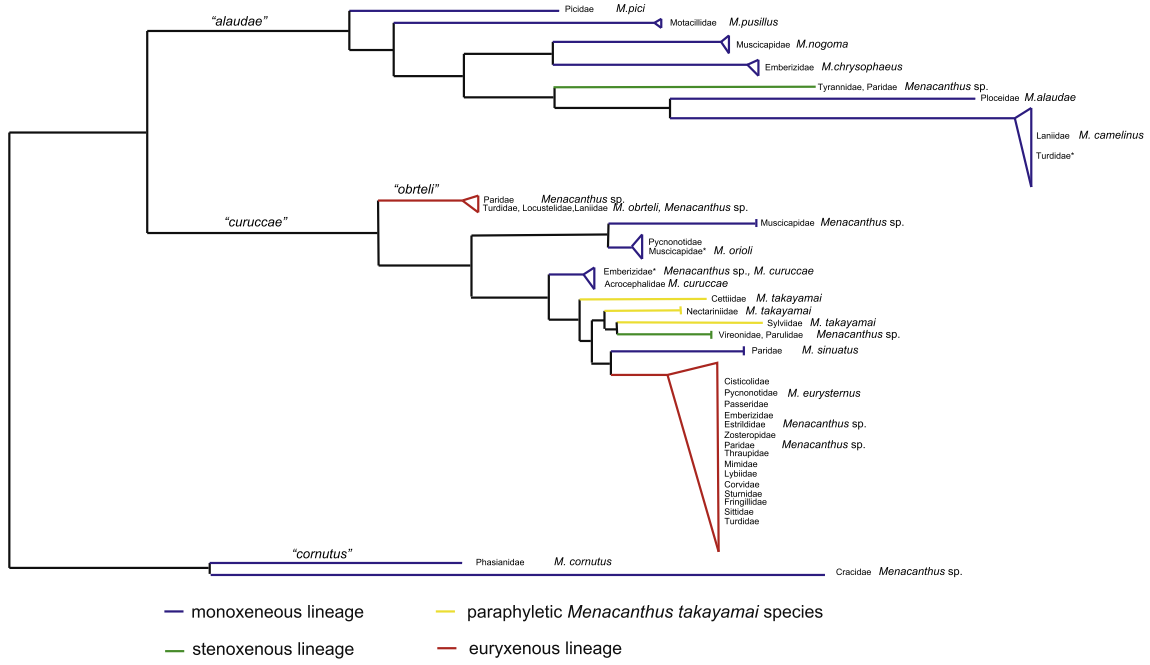


Fig. 2. Scheme of the evolution of the *Menacanthus* lineages. The topology was adapted from the cytochrome oxidase subunit I phylogeny in Fig. 1. Blue (dark grey) colour marks monoxeneous lineages (single host family), green colour (dashed line) stenoxenous lineages (two host families) and red colour (dotted line) euryxenous lineages (multi-host). Yellow (light grey) clades mark the paraphyletic *Menacanthus takayamai* species. *A record of a non-specific straggler. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

group in either the BI or ML analyses (see Figs. 1 and 2), but were paraphyletic with respect to *M. eurysternus* and *Menacanthus sinuatus*, as well as an unidentified lineage from two host families from Costa Rica. However, the topology of *M. takayamai* and the lineages related to it did not receive robust clade support and thus is not stable enough to draw further conclusions.

Most of the supported "species" lineages (clades where morphological determination of species correlated with genetic lineages) consisted of lice sampled from only one (blue clades in Fig. 2), or less commonly two (green clades in Fig. 2), host families. However, the "*eurysternus*" and "*obrteli*" lineages differed from the other lineages in their wide variety of hosts (red clades in Fig. 2)

and geographic locations (Fig. 1). The "*eurysternus*" lineage represented the most diverse monophyletic species lineage, comprising specimens from 63 host species in 13 families of passerine birds sampled from 15 localities in Europe, Asia the Neotropics and Africa, including 13 new louse-host associations (Supplementary Table S1).

3.1.2. EF-1α

The EF-1α sequences provided a weaker phylogenetic signal, resulting in lower topological resolution (Supplementary Fig. S1). The EF-1α topology recovered two highly supported sister clades. One contained *Menacanthus stramineus* from domestic chickens and *Menacanthus* sp. from picid and turdid hosts, both collected at the same locality (Supplementary Table S1), whereas the other contained the rest of the ingroup.

Despite generally lower clade support for the EF-1α topology and fewer samples analysed compared with the COI dataset (due to occasional amplification failures), in most cases the lice that were morphologically identified as a single species formed monophyletic groups in the EF-1α phylogeny, and the "*curuccae*" and "*alaudae*" clades of the COI phylogeny were also obtained in the EF-1α analysis (Supplementary Fig. S1). However, the "*cornutus*" group was placed as sister to the "*alaudae*" clade, although this placement received no support and may be spurious.

As in the COI analysis, the "*obrteli*" clade is sister to the "*curuccae*" clade, and the specimens identified as *M. takayamai* are paraphyletic with regards to *M. sinuatus* and *M. eurysternus*; however in the EF-1α phylogeny, these three morphological groups form a polytomy which also includes *M. curuccae* (Supplementary Fig. S1).

3.1.3. Concatenated alignment

The results of the phylogenetic analysis of the concatenated data set were largely similar to those obtained from the COI data set, but with some differences (Fig. 3). In both datasets the most basal division is between "*cornutus*" and the rest of the ingroup. Also, the topology of the "*curuccae*" clade is more or less the same as in the COI phylogeny, except for clades included in the COI data set but not included in the concatenated data set (due to failure to amplify EF-1α for these individuals). The topology within the "*alaudae*" clade differs between the COI and concatenated data sets. However, in both phylogenies much of the structure within this clade received no or little support. Furthermore, despite these conflicts the terminal clades formed the same "species" lineages with high support in both datasets (Figs. 1 and 3).

3.2. Analysis of intra-clade diversity and population history

mtDNA haplotype networks were prepared for the two most prevalent clades among the sampled lice (*M. camelinus* and *M. eurysternus*). Notable differences in the character of population structure between the two clades were found in the diversity of host spectra and geographic distribution of the haplotypes (Fig. 4).

The "*camelinus*" clade network showed little sequence variation (Fig. 4A), and the majority of haplotypes belonged to lice from only one host species, *Lanius collurio*, sampled in two European countries, Sweden and Bulgaria. The "*camelinus*" network contained several haplotypes without clear correlation between their genetic relationship and geographic origin in the two countries (Fig. 4C). The only host species other than *L. collurio* found in the network was *Turdus merula*. The single specimen from this host shared its haplotype with three other lice from *L. collurio* from the same locality.

By comparison, the "*eurysternus*" network (Fig. 4B) contained two strongly differentiated lineages. One lineage comprised a single specimen from *Prinia subflava* sampled in Africa, whereas the second lineage was almost global in its distribution and contained samples from the rest of the world (Europe, Asia, central America) as well as samples from two other African hosts (*L. torquatus* and *Lagonosticta rara*, Fig. 4D). Despite its wide geographical

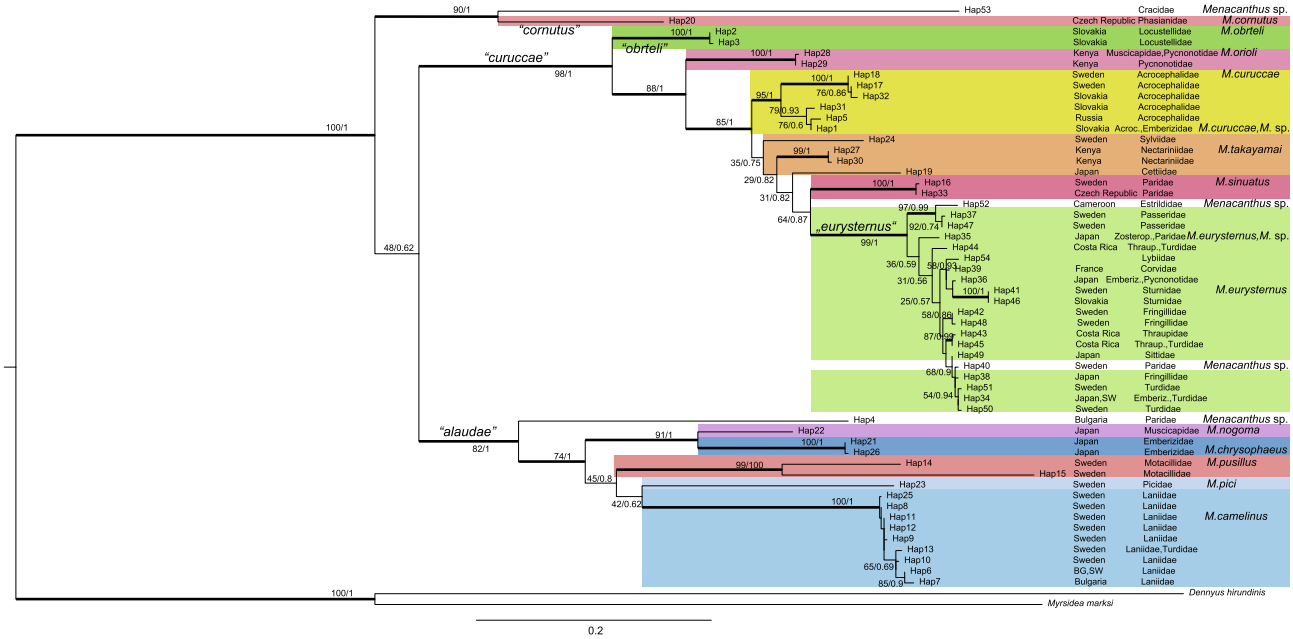


Fig. 3. Maximum Likelihood tree topology of *Menacanthus* based on concatenated sequence data of nuclear elongation factor 1-α and mitochondrial DNA cytochrome oxidase subunit I genes. Maximum Likelihood bootstrap values for 1,000 replicates and Bayesian Inference posterior probabilities are provided. Clades highlighted in bold indicate bootstrap values >70% and posterior probabilities >0.95. The tree was rooted with *Dennyus hirundinis* and *Myrsidea marksi*. Colours (shades of grey) mark morphological determinations of the species. Acroc, Acrocephalidae; BG, Bulgaria; Emberiz, Emberizidae; Hap, haplotype; SW, Sweden; Thraup, Thraupidae; Zosterop, Zosteropidae.

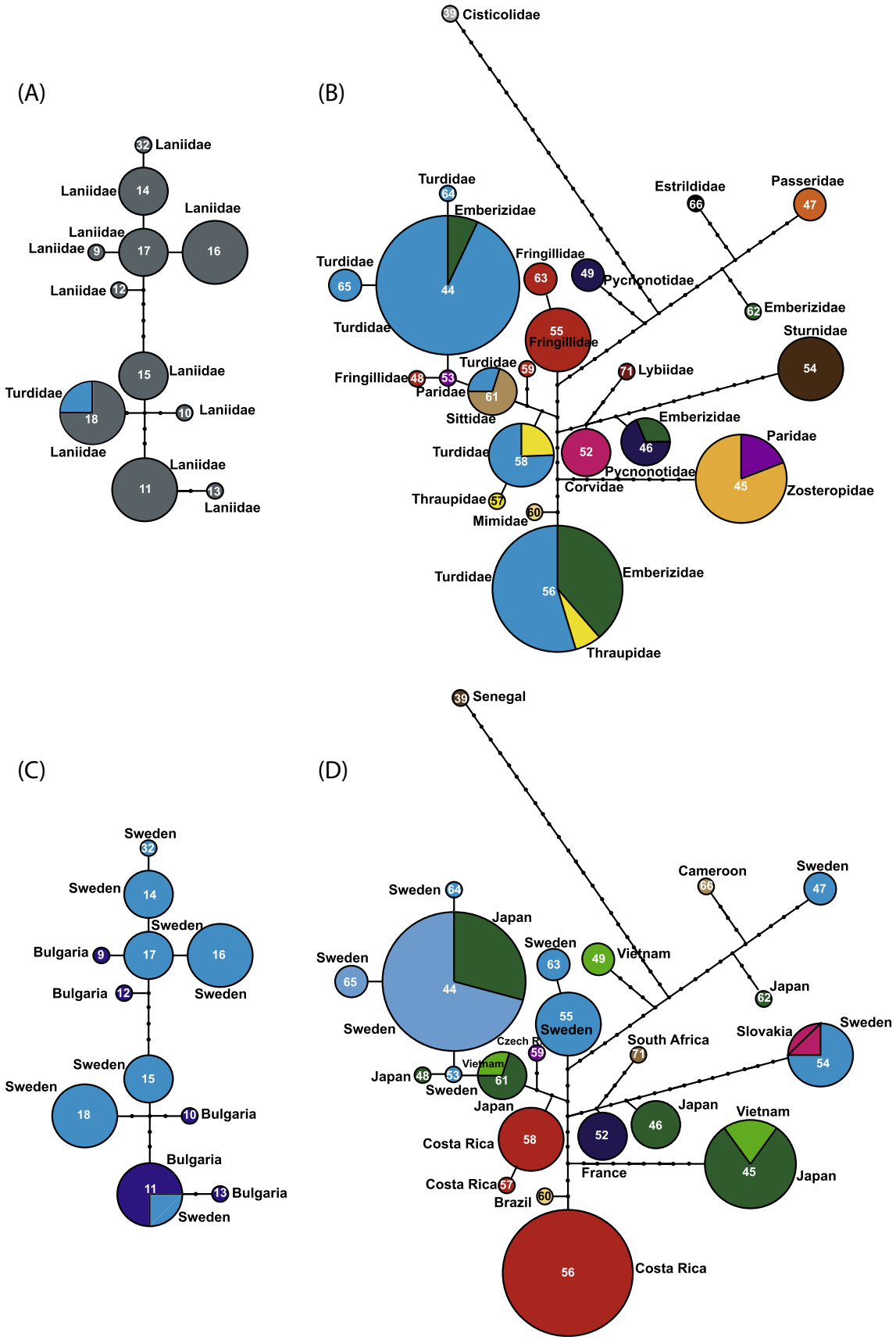


Fig. 4. *Menacanthus* cytochrome oxidase subunit I haplotype networks of "*camelinus*" and "*eurysternus*" clades generated with TCS software. Colours of the haplotypes refer to (A, B) host families or (C, D) the geographic origins of the specimens. Circle sizes reflect the numbers of specimens and show the haplotype number. Mutational steps between haplotypes are shown as dots.

distribution, the sequence diversity of this global "*eurysternus*" lineage was (in terms of the number of mutation steps between haplotypes) only moderately higher than that of the "*camelinus*" lineage.

In contrast to the rather straightforward "*camelinus*" clade, the widespread "*eurysternus*" clade showed a complex structure. It comprised lice from 13 passeriform host families. In approximately two-thirds of the host records, the lice from one host family formed unique haplotypes, while the remaining haplotypes were shared between two host families. Haplotypes did not cluster into specific lineages that would reflect evolutionary relationships between the hosts (Fig. 4B). For instance, haplotypes from turdid and emberizid birds were dispersed throughout the whole network. The genetic pattern was rather influenced by the geographic origin of the sequenced specimens (Fig. 4D). Except for one haplotype that was shared by lice from two distant parts of the Palearctic (Sweden and Japan), all haplotypes contained lice exclusively from one or two countries located on the same subcontinent. In concordance with this fact, the results of NCPA analysis performed for the "*eurysternus*" clade showed several instances of geography-determined evolutionary events (Fig. 5). In several cases allopatric fragmentation was identified (levels 2-20, 3-10, 4-4, 5-2). In addition, restricted gene flow with isolation by distance was suggested for levels 1-5, 3-3, 4-2 and 4-3. No pattern was found for the highest nesting level (6), probably due to the fact that the two lower categories (5-1 and 5-2) both contained samples from all biogeographic areas.

4. Discussion

In this study we analysed genetic relationships and variability in 14 species of a globally distributed ectoparasite genus, and showed that it contains species with strikingly different levels of host spectra and geographic distributions. Phylogenetic reconstructions based on mitochondrial COI and nuclear EF-1α sequences were used to infer inter-specific relationships. COI haplotype networks were then used to describe the patterns of intraspecific structure in the two most sampled species, which revealed striking differences between the two species.

Phylogenetic analyses mostly provided well-supported lineages with minor topological differences between genes. The differences between single gene analyses were partially caused by slightly different taxon sampling (due to amplification failure in some samples fixed in denatured alcohol) and by the lower genetic diversity of the nuclear EF-1α gene providing less information. These differences, however, did not affect the general picture of relationships between *Menacanthus* spp., which formed two major clusters termed "*alaudae*" and "*curuccae*" (Figs. 1 and 3, Supplementary Fig. S1).

The species with the widest host spectrum, *M. eurysternus*, was positioned within the "*curuccae*" clade, together with a series of host-specific or stenoxenous species clades (*Menacanthus orioli*, *M. curuccae*, *M. takayamai* group, *M. sinuatus* and unidentified *Menacanthus* sp. from Costa Rica and South Africa, Fig. 1). The terminal position of the euryxenous *M. eurysternus* within this host-specific

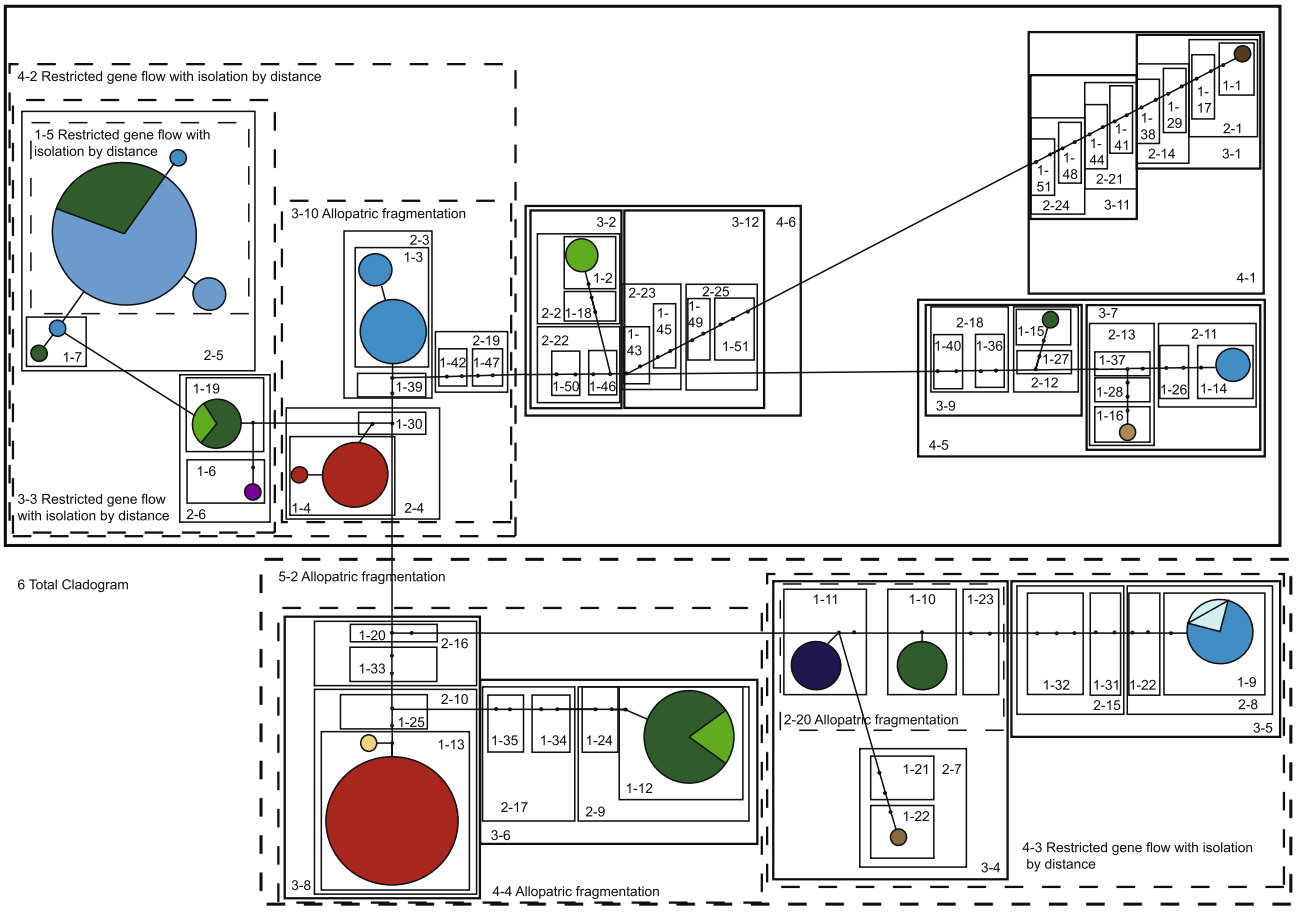


Fig. 5. Nested clade diagram obtained from *Menacanthus* cytochrome oxidase subunit I haplotypes of the "*eurysternus*" clade. Neighbouring haplotypes are connected by a single mutation; dots represent missing haplotypes along mutational pathways. The colours (shades of grey) of the haplotypes refer to the geographic origins of the specimens and are the same as in Fig. 4D. Nested clade levels are indicated by the numbers within particular nested clades. Dashed lines highlight nesting categories where population events were recognised using the nested clade phylogeographic analysis.

clade (Fig. 2) suggests that its ancestor underwent a life strategy reversal from a lineage of host specialists to a host generalist. An alternative interpretation would involve the independent evolution of host specialisation in each species clade from a generalist ancestor, which is less parsimonious (five changes rather than one). Despite containing many host-specific lineages, the *Menacanthus* tree topology does not reflect the evolutionary tree of their passerine bird hosts in any clear manner, not even for the clades containing related stenoxenous lineages (e.g. *M. curuccae* to *M. sinuatus* on Figs. 1 and 2). The evolution of the group probably did not progress through a co-speciation process known in other louse groups that infect less diverse ranges of hosts (e.g. Hafner et al., 1994; Clayton et al., 2003; Page et al., 2004; Hughes et al., 2007). Instead, host switching between different bird host families must have occurred.

The evolutionary patterns of *Menacanthus* lice sampled across multiple bird hosts presented here contribute new data that contradicts the traditional hypotheses that (i) parasites tend to evolve from host generalists into host specialists (Eichler, 1941), and (ii) parasites tend to co-speciate with their hosts (Fahrenholz, 1913; Eichler, 1948; Hafner and Nadler, 1988). Patterns revealing multi-host parasite species have often been considered artefacts caused by incomplete sampling (Dowling et al., 2003; Taylor and Purvis, 2003; Brooks et al., 2004) or cryptic speciation (Eichler, 1941). In contrast, we have shown that *Menacanthus* lice create complex patterns with post-speciation colonisation of new hosts (i.e. host switching), and that they tend toward switching from host specialists to host generalists in some lineages (*M. eurysternus* and possibly *M. obrteli*, Fig. 2). Similar results have recently been arrived at in other ectoparasitic insects such as fleas and ischnoceran body and wing lice (Poulin, 2006; Johnson et al., 2009, 2011).

Resolving inter-specific relationships in the phylogenetic analysis allowed us to explore genealogical differences between individual species, which revealed interesting facts about the ecology and evolution of host specialist and generalist parasites. The patterns revealed in the mtDNA haplotype networks of the "*camelinus*" and "*eurysternus*" species lineages nicely demonstrate the differences in life strategies between two species within a single parasite genus (Fig. 4).

The "*eurysternus*" network represents a host-generalist parasite that was recovered from 13 families of passeriform birds captured on four continents (Fig. 4B, D), which confirms the results of the taxonomic-morphological revision performed by Price (1975), and consequently adopted by Price et al. (2003) that have long been considered controversial by some authors (for example Fedorenko, 1983).

The "*camelinus*" network (Fig. 4A, C) represents a host-specific species where most specimens parasitise one host, *L. collurio*, and only one louse was found on an atypical host species, *T. merula* (Fig. 4A). That particular louse shared its COI haplotype with three other specimens from *Lanius* from the same locality in Sweden. We assume that it represents a straggler after an accidental host switch, rather than having established an independent long-term population on this host; otherwise, we would expect to recover *M. camelinus* from *T. merula* more frequently. The occurrence of other species of *Menacanthus* on atypical hosts (Supplementary Table S1) suggests that such accidental host switching can occur and may do so more often than expected.

Geographical patterns differed markedly between the network analyses of the two species (Fig. 4). The distribution of "*camelinus*" COI haplotypes was limited to Europe and thus they did not create clusters according to the geographic origin of their hosts (Sweden and Bulgaria, Fig. 4C). The geographic distribution may be connected to the migration patterns of the host, as all European populations of *L. collurio* migrate on a narrow front through Libya and Egypt during autumn migration and share relatively small wintering grounds in southern and eastern Africa (Harris and Franklin, 2000). Even if the populations are widely separated spatially during the breeding season, there may thus be ample opportunities for homogenisation of the louse populations on the hosts on their wintering grounds. A similar scenario has previously been suggested for cuckoo lice (Brooke and Nakamura, 1998) and shorebirds (Gustafsson and Olsson, 2012).

By contrast, a geography-dependent structure is apparent in the "*eurysternus*" network. Population structure is emerging in several parts of the network with many COI haplotypes specific to certain areas or localities but only rarely specific to particular host species (Fig. 4B, D). The overlapping distributions and habitat preferences of the hosts of *M. eurysternus* seem to be the most important factors maintaining genetic connectivity within geographic areas, as demonstrated by the six COI haplotypes (Nos. 44, 45, 46, 56, 58, 61), each being found on two to three unrelated families of birds (Fig. 4B and Supplementary Table S1). The best example is the Costa Rican haplotype, with 10 lice found on turdid, thraupid and emberizid birds caught in two nearby localities. Similar results have previously been arrived at for another widespread amblyceran genus, *Myrsidea*, where the sympatry of hosts may provide an opportunity for host switching between a turdid host and an ovenbird (Bueter et al., 2009). The sympatry and syntopy of host species was also found to be an important factor in the evolution of ischnoceran toucan lice of the genus *Austrophilopterus* (Weckstein, 2004). In addition, the "*eurysternus*" network contains both migrant and non-migrant hosts, as well as migrant hosts that follow very different migration routes.

The importance of geography rather than host specificity in driving the local genetic structure of *M. eurysternus* is also suggested by the results of the NCPA (Fig. 5). The analysis found several instances of statistically significant association between the COI haplotypes and their geographic distribution. The use of NCPA as a tool to analyse phylogeographic patterns has been challenged and model-based methods have been proposed as a replacement (Beaumont et al., 2010). However, in the present case, we think that the use of NCPA is valid. Inferring specific historical migrations or demographic events is beyond the scope of this study and would require more densely sampled sequence data or more genetic loci in order to draw any conclusions. Instead, we used NCPA to simply demonstrate the effect of geography on the distribution of "*eurysternus*" haplotypes. The results of the analysis imply that even if the haplotypes are closely related, many of them are unique to certain geographic units and/or must have dispersed across long distances.

The wide geographic distribution and emerging population structure in "*eurysternus*" populations provide opportunities for a future allopatric speciation and the evolution of new taxa. On the contrary, the narrow distribution of "*camelinus*" populations connected by the migration of their single bird host (*Lanius*) provides very little room for evolutionary changes other than host switching, which is a largely random and unpredictable process, probably with little success. Unless the parasite finds an unoccupied niche, which sometimes happens in depleted communities such as in island species (Whiteman et al., 2004) or in cases where the original parasite became extinct (Rozsa, 1993), straggling on atypical hosts is connected with high mortality rates and competition with established parasites. It is likely that there was probably an accidental host switch of the *camelinus* haplotype onto *Turdus merula* (COI Hap18 in Figs. 1 and 4A), which we consider a straggler rather than a representative of a new population. However, accidental host switching is probably not rare. Examining the diversity of hosts seen in the less densely sampled *Menacanthus* lineages revealed several other cases of stragglers on atypical hosts.

In the EF-1α phylogeny, *M. stramineus* from domestic chickens clustered together with *Menacanthus* sp. from two other host

orders and created a sister lineage to the rest of the *Menacanthus* samples (Supplementary Fig. S1). *Menacanthus stramineus* is presently known only from phasianid birds (Price et al., 2003), but our results indicate that this parasite may occasionally occur on hosts from other orders (Piciformes and Passeriformes). The sequences of *Menacanthus* recovered from the domestic chicken (EF-1α Hap19 and Hap20 in Supplementary Fig. S1) and from *Turdus* and *Dendrocopos* (EF-1α Hap16) were not identical, and their genetic distances are comparable with inter-specific levels seen elsewhere in the tree.

Several scenarios may explain such an unexpected distribution of the haplotypes. *Menacanthus stramineus* may comprise several lineages with complex population histories, potentially involving several species (represented here by the differentiated EF-1α haplotypes). A broader sampling of the domestic chicken could thus reveal the existence of EF-1α Hap16 (Supplementary Fig. S1) on this host as well, and the samples from the thrush and the woodpecker may be either genuine, but atypical, host associations, or serendipitous collections of rare stragglers. The overlapping microhabitats of the birds have probably played a major role in the establishment of naturally occurring populations of the same louse species on two or more distantly related hosts.

Similar host switching patterns were described by Clayton (1990) and Johnson et al. (2011) for ischnoceran lice, and by Bueter et al. (2009) for the amblyceran genus *Myrsidea*. Amblycerans (including *Menacanthus*) are in general more mobile than ischnocerans (Price et al., 2003), and under the conditions of poultry farming, where a food supply is also accessible to wild birds, opportunities for parasites to come into contact with new hosts may be common. Such host switching events from captive birds to distantly related birds have previously been reported for the poultry louse *Menopon gallinae* and *M. stramineus* on captive *Columba livia* (Dranzoa et al., 1999; Musa et al., 2011) and wild house sparrows, *Passer domesticus* (Hoyle, 1938). While this scenario is plausible for the EF-1α Hap16 (Supplementary Fig. S1) *Menacanthus* taken from *T. merula*, it seems less likely to be valid for that taken from *Dendrocopos major*, which is less likely to be feeding on seeds on the ground.

Alternatively, the host switch between domestic fowl and the two other bird groups may be older, with EF-1α Hap16 (Supplementary Fig. S1) representing a previously unknown species of *Menacanthus* that parasitises piciform and passeriform hosts. However, unless the passeriform louse population is very localised, we would also expect to recover this lineage at other localities where piciforms and passeriforms were sampled. A denser sampling would provide more data to resolve whether *M. stramineus* is a parasite with a complex population structure and capable of straggling to atypical hosts or whether a host switching event occurred in the past.

Whether these atypical host associations are well established or the result of straggling or contamination is presently unknown, as the atypical host populations have only been sampled once. However, while all three host species were sampled at the same locality, collection took place at different time periods: *G. gallus* samples were collected in May 2005, *T. merula* samples in January 2006 and *D. major* samples in February 2006. Chewing lice are not able to survive periods longer than a few days without their host (Mullen and Durden, 2002; Price et al., 2003), thus the contamination of birds by non-specific parasites can be excluded as a mode of transfer between unrelated hosts. However, only further sampling can establish whether there are continuous populations of *M. stramineus* on the atypical hosts.

The division of *Menacanthus* spp. into two major clades, here named "*curuccae*" and "*alaudae*", almost precisely follows the morphological division of *Menacanthus* spp. from passeriform birds according to Price (1977). The major division in his key is couplet 5, which separates the species here included in "*alaudae*" (*M. alaudae*, *M. camelinus*, *Menacanthus chrysophaeus*, *Menacanthus nogoma* and *Menacanthus pusillus*) from those included in "*curuccae*" (*M. curuccae, M. eurysternus*, *M. orioli*, *M. sinuatus* and *M. takayamai*). Within these two major clades ("*curuccae*" and "*alaudae*"), morphologically identified species typically created monophyletic lineages occurring on only one or two host families (Fig. 2). A notable exception is *M. eurysternus*, which has been discussed separately above. Price's (1977) key only includes the species on passeriforms and thus does not include *Menacathus pici*. This species is here placed as sister to "*alaudae*" in the COI phylogeny (Fig. 1), but nested within "*alaudae*" in the EF-1α and concatenated phylogenies (Fig. 3 and Supplementary Fig. S1). This placement in phylogenies is consistent with the morphology, as *M. pici* has the same number of lateroanterior metanotal setae, shape of the female subgenital plate, and shape of the male genitalia as those of the "*alaudae*" clade (Price and Emerson, 1975). An extended morphological revision, based on that of Price (1977), is thus likely to confirm the placement of *M. pici* in our phylogenies.

Menacanthus obrteli Balat, 1981, was described after the construction of Price's (1977) key, and was therefore not included in it, however material from hosts closely related to the type host of *M. obrteli* (*L. luscinioides*) was included under *M. takayamai*. Palma et al. (1998) considered *M. obrteli* indistinguishable from *M. takayamai*, and formally synonymised the two, while Mey (2003) recognised it as a subspecies of *M. takayamai*. Sychra et al. (2008) re-examined the type material of *M. obrteli*, as well as fresh material including males, and resurrected *M. obrteli* from synonymy. Both genes analysed here show that *M. obrteli* is well separated from the paraphyletic *M. takayamai*, and placed as a sister group (group "*obrteli*") to the rest of group "*curuccae*" (Figs. 1–3 and Supplementary Fig. S1).

Menacanthus obrteli has previously been recorded only from *L. luscinioides* (Sychra et al., 2008), however in our COI data set (see Fig. 1) material from this host is identical to specimens collected from *T. merula* and *L. collurio*. In addition, it forms a monophyletic lineage together with *Menacanthus* specimens from turdid and parid hosts (COI Hap 5, 6, 26). The fact that all specimens were collected in one area (central Europe) and on distantly related host taxa requires further explanation. Similar habitat preferences (as in the case of *M. stramineus*), shared nest holes (Johnson et al., 2002b; Weckstein, 2004), phoresy (Keirans, 1975; Harbison et al., 2009) or the overlapping migration routes and wintering ranges of hosts (e.g. Gustafsson and Olsson, 2012) are inapplicable, or insufficient, to completely explain the high level of louse dispersal between the hosts of *M. obrteli*. The hosts of *M. obrteli* do not share habitats or wintering routes and phoresy on vectors is not common in Amblycera (Price et al., 2003). Additional data collected from more host individuals and localities are needed to explore the level of host specificity in *M. obrteli* and the mechanism of dispersal between distantly related hosts.

The morphological homogeneity of *M. takayamai* samples may also be questioned, and the material determined to belong to this species on morphological grounds may be a cryptic assemblage of species. In all three data sets (Figs. 1 and 3, Supplementary Fig. S1), *M. takayamai* formed several lineages that are paraphyletic with respect to *M. eurysternus* and *M. sinuatus*, as well as other species in the EF-1α data set. The paraphyly of *M. takayamai* may have been caused by uneven sampling, as every lineage consisted of only a few individuals, each from one host family and each from a different continent or subcontinent (Fig. 1). As support is weak for these clades, the species as presently circumscribed morphologically is probably not a valid monophyletic taxon. Additional sampling focused on this taxon would provide more information to prove or disprove the polyphyly of *M. takayamai* and its dissolution to several species.

On a higher taxonomic level, it should be noted that several previously erected genera are here monophyletic and may warrant acceptance at least as subgenera. An important couplet in the key of Price (1977) is couplet 5, which divides most of the *Menacanthus* spp. on passerines into two groups; these groups are here referred to as the "*curuccae*" and "*alaudae*" groups. The latter of these groups contains *M. camelinus*, which is the type species of *Lanicanthus* Zlotorzycka, 1965. The type species of *Menacanthus*, *Menacanthus robustus* (Kellogg, 1896), was not included in the present study, but was placed by Price (1977) in the other large group, which corresponds to our "*curuccae*". The two groups can be differentiated by the number of lateroanterior setae of the pronotum, the margins of the female subgenital plate, the shape of the male genitalia (Price, 1977), and, supposedly, by the relative size of the facial hooks (Zlotorzycka, 1965). Further taxonomic considerations will be the subject of a future study.

In this study we demonstrated the importance of geography in forming population structure in multi-host parasites and discussed ecological factors facilitating host switches and maintenance of gene flow between unrelated host taxa. The differences in host specificity in *Menacanthus* spp. lineages were only partially congruent with the ecology of their hosts. *Menacanthus eurysternus* is typically found on hosts that allow for inter-specific transmission such as colonial nesters, cavity nesters and birds that form mixed-species feeding flocks, either during the breeding season or during the wintering season (Clayton, 1990; Price et al., 2003). However, there is no common biological pattern apparent for all hosts of this extremely euryxenous louse. The ecological proximity of hosts has been suggested to explain the transmission of lice through active dispersal to a new host after escaping preening (Johnson et al., 2011). Similar mechanisms might possibly facilitate the dispersal of *Menacanthus* lice between phylogenetically unrelated hosts.

Moreover, some intrinsic features of *M. eurysternus* may predispose it to maintain a wider host spectrum. *Menacanthus eurysternus* is an agile louse capable of moving quickly across the skin of its host (Price et al., 2003), and it can leave its host and survive for up to a few days without it (Mullen and Durden, 2002; Price et al., 2003). Finally, haematophagy may also play a role through interaction with endosymbiotic bacteria (Ries, 1931), providing a competitive advantage to some *Menacanthus* spp. or lineages. Additional sampling and experimental work may provide clues to help us distinguish between alternative mechanisms allowing louse dispersal and survival on new hosts.

Acknowledgements

We would like to thank all coworkers who provided their louse samples or helped in the field at locations where we examined the birds. Our special thanks goes to Mihaela Ilieva (Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences), Oleg Tolstenkov (Centre of Parasitology of A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences), Costica Adam ("Grigore Antipa" National Museum of Natural History, Romania), Zoltan Vas (Hungarian Natural History Museum, Budapest), Michel Valim (Museum of Zoology, University of São Paulo, Brasil), Martin Hromada (University of Presov, Slovakia), Wanyoike Wamiti (National Museums of Kenya, Nairobi). Funding was provided by the Grant Agency of the Academy of Sciences of the Czech Republic (Grant No. IAA601690901). Formal animal ethics approval number 215 99/2014-MZe-17214 was given by the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic for the work with wild birds. Care and maintenance of animals were in accordance with government/institution guidelines.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2014.09.001.

References

Agarwal, G.P., Saxena, A.K., Chandra, S., 1983. Haematophagous behaviour of *Menacanthus eurysternus* (Mallophaga, Amblycera). Angew. Parasitol. 24, 55–59.
Banks, J.C., Paterson, A.M., 2005. Multi-host parasite species in cophylogenetic studies. Int. J. Parasitol. 35, 741–746.
Bansal, N., Ahmad, A., Arya, G., Khan, V., Saxena, A.K., 2012. *Menacanthus palmai*, a new species of chewing louse (Menoponidae: Amblycera: Phthiraptera) from the *Coturnix coromandelica*. J. Parasit. Dis. 37, 276–280.
Beaumont, M.A., Nielsen, R., Robert, C., Hey, J., Gaggiotti, O., Knowles, L., Estoup, A., Panchal, M., Corander, J., Hickerson, M., Sisson, S.A., Fagundes, N., Chikhi, L., Beerli, P., Vitalis, R., Cornuet, J.M., Huelsenbeck, J., Foll, M., Yang, Z.H., Rousset, F., Balding, D., Excoffier, L., 2010. In defence of model-based inference in phylogeography REPLY. Mol. Ecol. 19, 436–446.
Boyd, E.M., 1951. A survey of parasitism of the Starling *Sturnus vulgaris* L. in North America. J. Parasitol. 31, 56–84.
Brooke, M.de L., Nakamura, H., 1998. The acquisition of host-specific feather lice by common cuckoos (*Cuculus canorus*). J. Zool. 244, 167–173.
Brooks, D.R., Dowling, A.P.G., van Veller, M.G.P., Hoberg, E.P., 2004. Ending a decade of deception: a valiant failure, a not-so-valiant failure and a success story. Cladistics 20, 32–46.
Bueter, C., Weckstein, J., Johnson, K.P., Bates, J.M., Gordon, C.E., 2009. Comparative phylogenetic histories of two louse genera found on Catharus thrushes and other birds. J. Parasitol. 95, 295–307.
Chandra, S., Agarwal, G.P., Singh, S.P.N., Saxena, A.K., 1990. Seasonal changes in a population of *Menacanthus eurysternus* (Mallophaga, Amblycera) on the Common Myna *Acridotheres tristis*. Int. J. Parasitol. 20, 1063–1065.
Cicchino, A.C., 2003. *Menacanthus bonariensis* new species (Phthiraptera: Menoponidae), parasitic on the White-bellied Sparrow, *Zonotrichia capensis hypoleuca* (Todd, 1915) (Aves: Passeriformes: Fringillidae) in Buenos Aires Province, Argentina. Zootaxa 358, 1–11.
Clay, T., Meinertzhagen, R., 1943. The relationship between Mallophaga and hippoboscid flies. Parasitology 35, 11–16.
Clayton, D.H., 1990. Host specificity of *Strigiphilus* owl lice (Ischnocera: Philopteridae), with the description of new species and host associations. J. Med. Entomol. 27, 257–265.
Clayton, D.H., Drown, D.M., 2001. Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). J. Parasitol. 87, 1291–1300.
Clayton, D.H., Johnson, K.P., 2003. Linking coevolutionary history to ecological process: doves and lice. Evol. Int. J. Org. Evol. 57, 2335–2341.
Clayton, D.H., Bush, S.E., Goates, B.M., Johnson, K.P., 2003. Host defense reinforces host–parasite cospeciation. Proc. Natl. Acad. Sci. Biol. 100, 15694–15699.
Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659.
Danforth, B.N., Ji, S., 1998. Elongation factor-1 alpha occurs as two copies in bees: implications for phylogenetic analysis of EF-1 alpha sequences in insects. Mol. Biol. Evol. 15, 225–235.
Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.
Demanche, C., Berthelemy, M., Petit, T., Polack, B., Wakefield, A.E., Dei-Cas, E., Guillot, J., 2001. Phylogeny of *Pneumocystis carinii* from 18 primate species confirms host specificity and suggests coevolution. J. Clin. Microbiol. 39, 2126–2133.
Dowling, A.P.G., van Veller, M.G.P., Hoberg, E.P., Brooks, D.R., 2003. A priori and a posteriori methods in comparative evolutionary studies of host–parasite associations. Cladistics 19, 240–253.
Dranzoa, C., Ocaido, M., Katete, P., 1999. The ecto-, gastro-intestinal and haemoparasites of live pigeons (*Columba livia*) in Kampala, Uganda. Avian Pathol. 28, 119–124.
du Toit, N., van Vuuren, B.J., Matthee, S., Matthee, C.A., 2013. Biogeography and host-related factors trump parasite life history: limited congruence among the genetic structures of specific ectoparasitic lice and their rodent hosts. Mol. Ecol. 22, 5185–5204.
Eichler, W., 1941. Wirtspezifität und stammesgeschichtliche Gleichläufigkeit (Fahrenholzsche Regel) bei Parasiten im allgemein und bei Mallophagen im besonderen. Zool. Anz. 132, 254–262.
Eichler, W., 1942. Die Entfaltungsregel und andere Gesetzmässigkeiten in den parasitogenetischen Beziehungen der Mallophagen und anderer ständiger Parasiten zu ihren Wirten. Zool. Anz. 137, 77–83.
Eichler, W., 1948. Some rules in ectoparasitism. Ann. Mag. Nat. Hist. 1, 588–598.
Fahrenholz, H., 1913. Ectoparasiten unde Abstammungslehre. Zool. Anz. 41, 371–374.
Fedorenko, I.A., 1983. Chewing Lice – Menoponidae. Fauna of Ukraine, vol. 22. Naukova Dumka, Kiev (Issue 5, Part 1, in Ukrainian).
Foster, M.S., 1969. Synchronized life cycles in orange-crowned warbler and its mallophagan parasites. Ecology 50, 315–323.
Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52, 696–704.

Guindon, S., Lethiec, F., Duroux, P., Gascuel, O., 2005. PHYML Online – a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res. 33, 557–559.
Gustafsson, D.R., Olsson, U., 2012. Flyway homogenisation or differentiation? Insights from the phylogeny of the sandpiper (Charadriiformes: Scolopacidae: Calidrinae) wing louse genus *Lunaceps* (Phthiraptera: Ischnocera). Int. J. Parasitol. 42, 93–102.
Hafner, M.S., Nadler, S.A., 1988. Phylogenetic trees support the coevolution of parasites and their hosts. Nature 332, 258–259.
Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spradling, T.A., Demastes, J.W., Nadler, S.A., 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science 265, 1087–1090.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.
Harbison, C.W., Jacobsen, M.V., Clayton, D.H., 2009. A hitchhiker's guide to parasite transmission: the phoretic behaviour of feather lice. Int. J. Parasitol. 39, 569–575.
Harris, T., Franklin, K., 2000. Shrikes and Bush-shrikes, Including Wood-shrikes, Helmet-shrikes, Flycatcher-shrikes, Philentomas. Batises and Wattle-eyes, Christopher Helm, London.
Hopkins, G.H.E., 1946. Stray notes on Mallophaga – VII. Ann. Mag. Nat. Hist. 11 (13), 170–183.
Hoyle, W.L., 1938. Transmission of poultry parasites by birds with special reference to the English or house sparrow and chickens. Trans. Kans. Acad. Sci. 41, 379–384.
Huelsenbeck, J.P., Bollback, J.P., 2001. Empirical and hierarchical Bayesian estimation of ancestral states. Syst. Biol. 50, 351–366.
Hughes, J., Kennedy, M., Johnson, K.P., Palma, R.L., Page, R.D.M., 2007. Multiple cophylogenetic analyses reveal frequent cospeciation between pelecaniform birds and *Pectinopygus* lice. Syst. Biol. 56, 232–251.
Johnson, K.P., Adams, R.J., Clayton, D.H., 2002a. The phylogeny of the louse genus *Brueelia* does not reflect host phylogeny. Biol. J. Linnean Soc. 77, 233–247.
Johnson, K.P., Williams, B.L., Drown, D.M., Adams, R.J., Clayton, D.H., 2002b. The population genetics of host specificity: genetic differentiation in dove lice (Insecta: Phthiraptera). Mol. Ecol. 11, 25–38.
Johnson, K.P., Cruickshank, R.H., Adams, R.J., Smith, V.S., Page, R.D., Clayton, D.H., 2003. Dramatically elevated rate of mitochondrial substitution in lice (Insecta: Phthiraptera). Mol. Phylogenet. Evol. 26, 231–242.
Johnson, K.P., Malenke, J.R., Clayton, D.H., 2009. Competition promotes the evolution of host generalists in obligate parasites. Proc. R. Soc. B 276, 3921–3926.
Johnson, K.P., Weckstein, J.D., Bush, S.E., Clayton, D.H., 2011. The evolution of host specificity in dove body lice. Parasitology 138, 1730–1736.
Jousson, O., Bartoli, P., Pawlowski, J., 2000. Cryptic speciation among intestinal parasites (Trematoda: Digenea) infecting sympatric host fishes (Sparidae). J. Evol. Biol. 13, 778–785.
Keirans, J.E., 1975. A review of the phoretic relationship between Mallophaga (Phthiraptera: Insecta) and Hippoboscidae (Diptera: Insecta). J. Med. Entomol. 12, 71–76.
Kounek, F., Sychra, O., Čapek, M., Lipková, A., Literák, I., 2011. Chewing lice of the genus *Myrsidea* (Phthiraptera: Menoponidae) from the Cardinalidae, Emberizidae, Fringillidae and Thraupidae (Aves: Passeriformes) from Costa Rica, with descriptions of four new species. Zootaxa, 1–16.
Krištofík, J., 2000. Synonymical notes to the *Menacanthus* species (Phthiraptera, Menoponidae) living on Passeriformes (Aves). Acta Parasitol. 45, 57–58.
Mey, E., 2003. Verzeichnis der Tierläuse (Phthiraptera) Deutschlands. In: Klausnitzer, B. (Ed.), Entomofauna Germanica. Entomologische Nachrichten und Berichte, Dresden, pp. 72–129.
Mullen, G.R., Durden, L.A., 2002. Medical and Veterinary Entomology. Academic Press, London.
Musa, S., Afroz, S.D., Khanum, H., 2011. Occurrence of ecto and endo parasites of pigeon (*Columba livia* Linn.). U. J. Zool. Rajshahi U. 30, 73–75.
Najer, T., Sychra, O., Kounek, F., Papoušek, I., Nguyen, M.H., 2014. Chewing lice Phthiraptera: Amblycera and Ischnocera) from wild birds in southern Vietnam, with descriptions of two new species. Zootaxa 3755, 419–433.
Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24, 581–583.
Page, R.D.M., Hafner, M.S., 1996. Molecular phylogenies and host–parasite cospeciation: gophers and lice as a model system. In: Harvey, P.H., Leigh Brown, A.J., Maynard Smith, J., Nee, S. (Eds.), New Uses for New Phylogenies. Oxford University Press, Oxford, pp. 255–270.
Page, R.D.M., Cruickshank, R.H., Dickens, M., Furness, R.W., Kennedy, M., Palma, R.L., Smith, V.S., 2004. Phylogeny of "*Philoceanus* complex" seabird lice (Phthiraptera: Ischnocera) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 30, 633–652.
Palma, R.L., Price, R.D., 2005. *Menacanthus rhipidurae*, a new species of chewing louse (Insecta: Phthiraptera: Menoponidae) from South Island fantails, *Rhipidura fuliginosa fuliginosa* (Aves: Passeriformes: Dicruridae). New Zeal. J. Zool. 32, 111–115.
Palma, R.L., Price, R.D., Hellenthal, R.A., 1998. New synonymies and host records for lice of the genus *Menacanthus* (Phthiraptera: Menoponidae) from the Passeriformes (Aves). J. R. Soc. N. Z. Zool. 28, 309–320.
Panchal, M., 2007. The automation of nested clade phylogeographic analysis. Bioinformatics 23, 509–510.
Pilgrim, R.L.C., Palma, R.L., 1982. A List of the Chewing Lice (Insecta: Mallophaga) from Birds in New Zealand Ornithological Society of New Zealand. National Museum of New Zealand, Wellington, New Zealand.
Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Posada, D., Crandall, K.A., Templeton, A.R., 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol. Ecol. 9, 487–488.
Poulin, R., 2006. Variation in infection parameters among populations within parasite species: intrinsic properties versus local factors. Int. J. Parasitol. 36, 877–885.
Price, R.D., 1975. *Menacanthus eurysternus* complex (Mallophaga, Menoponidae) of Passeriformes and Piciformes (Aves). Ann. Entomol. Soc. Am. 68, 617–622.
Price, R.D., 1977. *Menacanthus* (Mallophaga Menoponidae) of Passeriformes (Aves). J. Med. Entomol. 14, 207–220.
Price, R.D., Beer, J.R., 1965. A review of the *Colpocephalum* of the Corvidae with the description of a new species. Proc. Entomol. Soc. Wash. 67, 7–14.
Price, R.D., Emerson, K.C., 1975. *Menacanthus* (Mallophaga, Menoponidae) of Piciformes (Aves). Ann. Entomol. Soc. Am. 68, 779–785.
Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P., Clayton, D.H., 2003. The Chewing Lice. World Checklist and Biological Overview. Illinois Natural History Survey Special Publication, Champaign, Illinois.
Reed, D.L., Smith, V.S., Hammond, S.L., Rogers, A.R., Clayton, D.H., 2004. Genetic analysis of lice supports direct contact between modern and archaic humans. PLoS Biol. 2, 1972–1983.
Rheinwald, G., 2007. The position of *Trochiliphagus* Carriker within the Ricinidae (Insecta: Phthiraptera). Bonn. Zool. Beitr. 55, 37–46.
Ries, E., 1931. Die Symbiose der Läuse und Federlinge. Z. Morphol. Ökol. Tiere 20, 233–367.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Rozsa, L., 1993. Speciation patterns of ectoparasites and 'straggling' lice. Int. J. Parasitol. 23, 859–864.
Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W., Hebert, P.D.N., 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). Proc. Natl. Acad. Sci. U.S.A. 103, 3657–3662.
Srivastava, R., Kumar, S., Gupta, N., Singh, S.K., Saxena, A.K., 2003. Path coefficient analysis of correlation between breeding cycles of the common Myna *Acridotheres tristis* (Passeriformes: Sturnidae) and its phthirapteran ectoparasites. Folia Parasitol. 50, 315–316.
Štefka, J., Hypša, V., 2008. Host specificity and genealogy of the louse *Polyplax serrata* on field mice, *Apodemus* species: a case of parasite duplication or colonisation? Int. J. Parasitol. 38, 731–741.
Štefka, J., Hypša, V., Scholz, T., 2009. Interplay of host specificity and biogeography in the population structure of a cosmopolitan endoparasite: microsatellite study of *Ligula intestinalis* (Cestoda). Mol. Ecol. 18, 1187–1206.
Štefka, J., Hoeck, P.E., Keller, L.F., Smith, V.S., 2011. A hitchhikers guide to the Galapagos: co-phylogeography of Galapagos mockingbirds and their parasites. BMC Evol. Biol. 11, 284.
Sychra, O., Najer, T., Kounek, F., Nguyen, M.H., Tolstenkov, O.O., 2014. *Myrsidea claytoni* (Phthiraptera: Menoponidae) from *Cymbirhynchus macrorhynchos* (Passeriformes: Eurylaimidae), an interesting case of natural host switching. J. Parasitol. 100, 280–283.
Sychra, O., Sychrová, V., Literák, I., 2008. Identity of *Menacanthus obrteli* Balat (Phthiraptera: Menoponidae) from the Savi's warbler (Passeriformes: Sylviidae). Biologia 63, 686–688.
Taylor, J., Purvis, A., 2003. Have mammals and their chewing lice diversified in parallel? In: Page, R. (Ed.), Tangled Trees: Phylogeny, Cospeciation, and Coevolution. University Chicago Press, Chicago, pp. 240–261.
Templeton, A.R., 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. Mol. Ecol. 13, 789–809.
Templeton, A.R., Boerwinkle, E., Sing, C.F., 1987. A cladistic-analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping.1. Basic theory and an analysis of alcohol-dehydrogenase activity in *Drosophila*. Genetics 117, 343–351.
Templeton, A.R., Sing, C.F., 1993. A cladistic-analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. 4. Nested analyses with cladogram uncertainty and recombination. Genetics 134, 659–669.
Zlotorzycka, J., 1965. Mallophaga parasitizing Passeriformes and Pici. IV. Menacanthinae, Ricinidae, Degeeriellinae. Acta Parasitol. Pol. 13, 41–69.
Weckstein, J.D., 2004. Biogeography explains cophylogenetic patterns in toucan chewing lice. Syst. Biol. 53, 154–164.
Whiteman, N.K., Santiago-Alarcon, D., Johnson, K.P., Parker, P.G., 2004. Differences in straggling rates between two genera of dove lice (Insecta: Phthiraptera) reinforce population genetic and cophylogenetic patterns. Int. J. Parasitol. 34, 1113–1119.

Manuscript No. 4:
Mácová, A., Hoblíková, A., Hypša, V., Stanko, M., Martinů, J., Kvičerová, J. 2018. Mysteries of host switching: Diversification and host specificity in rodent-coccidia associations. Molecular Phylogenetics and Evolution, 127: 179–189.

Molecular Phylogenetics and Evolution 127 (2018) 179–189

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Mysteries of host switching: Diversification and host specificity in rodent-coccidia associations

Anna Mácová[a,b], Aneta Hoblíková[a], Václav Hypša[a,b], Michal Stanko[c], Jana Martinů[a,b], Jana Kvičerová[a,b,*]

[a] Department of Parasitology, Faculty of Science, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic
[b] Biology Centre, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic
[c] Institute of Parasitology and Institute of Zoology, Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovakia

ARTICLE INFO

Keywords:
Host switching
Host specificity
Coevolution
Ecological fitting
Parasite
Rodent

ABSTRACT

Recent studies show that host switching is much more frequent than originally believed and constitutes an important driver in evolution of host-parasite associations. However, its frequency and ecological mechanisms at the population level have been rarely investigated. We address this issue by analyzing phylogeny and population genetics of an extensive sample, from a broad geographic area, for commonly occurring parasites of the genus *Eimeria* within the abundant rodent genera *Apodemus*, *Microtus* and *Myodes*, using two molecular markers. At the most basal level, we demonstrate polyphyletic arrangement, i.e. multiple origin, of the rodent-specific clusters within the *Eimeria* phylogeny, and strong genetic/phylogenetic structure within these lineages determined at least partially by specificities to different host groups. However, a novel and the most important observation is a repeated occurrence of host switches among closely related genetic lineages which may become rapidly fixed. Within the studied model, this phenomenon applies particularly to the switches between the eimerians from *Apodemus flavicollis/Apodemus sylvaticus* and *Apodemus agrarius* groups. We show that genetic differentiation and isolation between *A. flavicollis/A. sylvaticus* and *A. agrarius* faunas is a secondary recent event and does not reflect host-parasite coevolutionary history. Rather, it provides an example of rapid ecology-based differentiation in the parasite population.

1. Introduction

The long-held view of host-parasite coevolution as being a process mainly determined by co-speciation events has dramatically changed in recent years, mainly due to the frequent incongruencies detected between host and parasite phylogenies (Paterson and Banks, 2001; Meinilä et al., 2004; Ricklefs et al., 2004; Hoberg and Brooks, 2008; Agosta et al., 2010; Araujo et al., 2015). This change has led to a recognition that parasites are not just passive companions of their hosts but rather organisms with their own biology and many host-independent traits. Consequently, well-established questions in this field, such as how parasites maintain their host spectra, how generalists become specialists, and *vice versa*, or what are the mechanisms, preconditions and frequency of host switching, are now seen in an entirely new light (Agosta et al., 2010).

Since genealogy constraint (manifested as a nearly strict co-speciation history) has been rejected as a predominant driver of the parasite speciation and distribution among the hosts, alternative hypotheses have had to be developed. Ecological fitting, a well-established hypothesis in the general ecological framework (Agosta and Klemens, 2008), has recently been adopted as a putative mechanism for new host colonization by a parasite (Agosta et al., 2010; Araujo et al., 2015; Messenger et al., 2015). Since parasites are long recognized as organisms strongly adapted to the environment provided by the specific host(s), their capability to survive in different environment should in theory be very limited. Frequent host switches observed in many parasite groups and often followed by speciation events thus pose an interesting question. The ecological fitting theory predicts that in some cases the adaptations evolved in particular environment (the host in the parasitological framework) and may allow the organism to survive under different conditions and successfully colonize the new environment (the new host). Malcicka et al. (2015) suggest that invasive species of parasites provide typical examples of such events. Since the ecological fitting mechanism is likely to work at the ecological level, i.e. on the short-term scale, it should result in a considerable variability in host spectra ranges among various parasite lineages/populations. Such

* Corresponding author at: Department of Parasitology, Faculty of Science, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic.
E-mail addresses: anniex.m@seznam.cz (A. Mácová), anetka027@seznam.cz (A. Hoblíková), vacatko@paru.cas.cz (V. Hypša), stankom@saske.sk (M. Stanko), martinu@paru.cas.cz (J. Martinů), janaq@centrum.cz (J. Kvičerová).

https://doi.org/10.1016/j.ympev.2018.05.009
Received 10 July 2017; Received in revised form 9 May 2018; Accepted 9 May 2018
Available online 16 May 2018
1055-7903/

a complex picture has indeed been detected in several host-parasite systems studied at the population level, e.g. *Pneumocystis*-primates, *Austrogoniodes*-penguins, *Polyplax-Apodemus*, or *Ligula*-fish hosts (Demanche et al., 2001; Banks et al., 2006; Štefka and Hypša, 2008; Štefka et al., 2009). These differencies in host spectra (i.e. the number and taxonomy of the host species) between the parasite species/populations could, in turn, affect the genetic structure of the species/populations. According to Nadler's hypothesis (Nadler, 1995), in multihost parasites, additional hosts increase the opportunities for dispersal, and thus reduce the parasites' population structure. Recently, Falk and Perkins (2013) supported this prediction with an empirical population study of two pinworm species, parasitizing in various reptiles in the Caribbean. Considering this recent development, it is clear that the elucidation of host switches/colonization in parasite populations is a key factor in understanding population genetics and evolution of parasites. Several such studies have been published on various parasitic associations, however, the majority of them were focused on the plants-phytophages, perhaps due to their better accessibility (Agosta, 2006; Habermannová et al., 2013; Nylin et al., 2014).

Here we propose the parasites of the genus *Eimeria*, associated with the rodent genus *Apodemus*, as a suitable model for such a coevolutionary study. Both counterparts, the hosts and the parasites, are well-studied organisms and thus provide a reliable background for the analyses. Mice of the genus *Apodemus* are the most common rodents in the Palaearctic region. The geographic distribution and habitats of some species (e.g. *A. flavicollis* and *A. sylvaticus*) overlap, so they live in sympatry, competing for food resources (Michaux et al., 2005; Sakka et al., 2010). They have been recorded from a variety of habitats, both natural and urban (Nowak, 1999; Anděra and Beneš, 2002; Wilson and Reeder, 2005), and have served as model species in several genetic/evolutionary studies (e.g. Nieberding et al., 2004, 2005; Meyer-Lucht and Sommer, 2005; Štefka and Hypša, 2008; Sakka et al., 2010; Demanche et al., 2015). Coccidia of the genus *Eimeria* are frequent parasites of this rodent genus (Lewis and Ball, 1983; Levine and Ivens, 1990; Higgs and Nowell, 2000). So far, morphological and molecular studies have indicated a complex relationship between *Eimeria* and their hosts, with some *Eimeria* species being able to infect several species of *Apodemus*, while others have so far been described only from a single host species (Lewis and Ball, 1983; Higgs and Nowell, 1991; Hůrková et al., 2005; Kvičerová and Hypša, 2013).

In our previous phylogenetic work, we showed that *Eimeria* samples collected from the genus *Apodemus* branched at different positions across the phylogenetic tree (Kvičerová and Hypša, 2013). This indicates that during eimerian evolution, the colonization of *Apodemus* by *Eimeria* occurred multiple times. Such a situation provides good opportunity to investigate host switches and evolution of host specificity at population level within a complex system. In this study we thus use an extensive sampling to analyze the polyphyletic distribution of *Eimeria* within the genus *Apodemus* (Muridae) from the genealogical and population genetics perspective. To provide a suitable ecological background, we further add the eimerian parasites collected from two additional and abundant rodent genera, *Myodes* and *Microtus* (Arvicolinae). We show that within this host spectrum, colonizations and complete host switches have occurred several times within the recent, i.e. species/population time-scale. We also determine several independent cases of recent host switches from a broader host spectrum (represented by several species or even genera) to a strictly specific association with *A. agrarius*, as possible examples of the ecology-based differentiation caused by immigration of a new host species.

This new insight into the evolution of host specificity within and among parasite populations may have important implications from both theoretical and practical perspective. From the theoretical point of view, it indicates that to achieve a reliable coevolutionary reconstruction, the studies and methodologies should take into account much broader spectrum of possibilities. For example, it demonstrates that there is no general probability of host switching vs. duplication, which could be established for a parasite species based merely on its biological features. Similarly, within the applied fields, such as epidemiology or disease control, the complex patterns observed in this study indicate that to establish a proper epidemiological models and control scheme may in many cases require a more detailed study based on extensive sample at population level. Particularly, possible occurrence of different genetic lineages and/or cryptic species of the pathogen, with different epidemiological characteristics, has to be examined.

Considering these circumstances, we specifically address in this study the following questions: (1) do the *Apodemus*-associated eimerians, scattered across the *Eimeria* tree, present consistently *Apodemus*-specific branches or just individual random infections? (2) considering close phylogenetic relationships and sympatric occurrence of the three *Apodemus* species, together with other rodent genera, do the *Eimeria* display any degree of host specificity? (3) if yes, how is such specificity reflected in their population genetic structure?

2. Material and methods

2.1. Sampling

Field studies were carried out in the course of 2006–2014, under official permits provided by the Czech Republic/European Union or collaborating institutions (Permit Numbers KUJCK 11134/2010 OZZL/2/Ou and 27873/ENV/11); the protocol was approved by the Committee on the Ethics of Animal Experiments of the University of South Bohemia and by the Ministry of the Environment of the Czech Republic (Permit Numbers 13841-11 and 22395/2014-MZE-17214). A list of localities and collected species is provided in Tables S1 and S2. Rodents were trapped using the classic wooden snap traps for mice. The traps were deployed in the late evening, left in the field overnight and picked up in the early morning. The faecal samples were collected directly from the gut of each individual animal. Faeces from each individual animal were kept in 4% potassium dichromate ($K_2Cr_2O_7$) solution. Host tissues (a small piece of ear or tail) were preserved in absolute ethanol for molecular determination of the host species.

2.2. Coprological examination and oocyst morphology

The presence of parasites in collected faeces was examined microscopically by flotation in Sheather's sucrose solution with a density of 1.30 (Duszynski and Wilber, 1997; Zajac and Conboy, 2006). Determination of the coccidian species/morphotypes was based on the morphology and morphometry of the sporulated oocysts, according to guidelines published by Duszynski and Wilber (1997) and Berto et al. (2014). An Olympus BX53 light microscope equipped with DP-73-1-51 high resolution image cooled digital camera Olympus and Olympus cellSens Standard 1.13 imaging software were used.

2.3. DNA extraction, PCR amplification of selected genes, sequencing

Eimerian DNA from positive faecal samples was isolated with FastDNA® SPIN for Soil Kit (MP Biomedicals, LLC, Santa Ana, California, USA) according to the manufacturer's instructions. For amplification, a mitochondrial gene for cytochrome *c* oxidase subunit I (COI) and a gene for the small subunit (SSU) of 18S rRNA, were selected. Both of these genes provide some methodological advantages but also suffer from certain shortcomings. While the COI sequences are known as good markers for intraspecific and interspecific studies, they are poorly represented for eimerians in the GenBank database. On the other hand, the 18S rRNA gene is the best represented eimerian gene in the GenBank, allowing for taxonomically broad analyses, but within *Eimeria* its variability is relatively low. A combination of these two genes thus provides an optimal means for extracting the available phylogenetic information on *Eimeria*.

Specific primers for amplification of ~800 bp of eimerian COI and

~1 400 bp of eimerian 18S rDNA were adopted from Schwarz et al. (2009) and Kvičerová et al. (2008), respectively. HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany) was used for all PCR reactions. PCR products were enzymatically purified and directly sequenced; five independent PCR products were sequenced for each sample. Consensi of the sequences were used for the subsequent analyses. Sequencing was carried out by Macrogen, Inc. (Amsterdam, the Netherlands) on an automatic 3730XL DNA analyzer.

2.4. Determination of the host species

Since some host species (namely *Apodemus flavicollis/A. sylvaticus*, *Microtus arvalis/M. agrestis*) have overlapping morphometries, and almost indiscernible juveniles and subadults, it was not able in several cases to determine them unequivocally to the species in the field. In such cases, we used the methods of molecular biology. Host DNA was extracted from the host tissue using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Species-specific primers were used for the PCR identification or verification of the host species, amplifying the mitochondrial DNA control region (in the case of *Apodemus* spp.; Bellinvia, 2004), or mitochondrial cytochrome *b* oxidase (in Arvicolinae; Jaarola and Searle, 2002). PCR products were enzymatically purified and directly sequenced by Macrogen, Inc. (Amsterdam, the Netherlands) on an automatic 3730XL DNA analyzer.

2.5. Sequence assembling, alignments, and phylogenetic analyses

The obtained sequences of *Eimeria* were identified by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), assembled using the Sequence Scanner v.1.0 (Applied Biosystems), EditSeq 5.05 and SeqMan 5.05 (DNASTAR, Inc., Madison, Wisconsin, USA) programs, and deposited in the NCBI GenBank database under the Accession numbers provided in Tables S2 and S3. The samples were tentatively assigned to the so far described *Eimeria* species based on their morphological examination (see Table S4) and similarity to the reference sequences available in the GenBank database. Alignments were created in BioEdit v.7.2.5 (Hall, 1999) and in MAFFT v.7 (Katoh et al., 2002; Katoh and Standley, 2013), and adjusted manually. 18S rDNA sequences were aligned in the nucleotide mode, COI sequences were aligned in the amino acid mode, then switched to nucleotide mode and used for the analyses. Using jModeltest (Posada, 2008, 2009), we selected GTR + Γ + I as the best fitting model to be used in the subsequent phylogenetic analyses. In addition, we performed Bayesian analysis of concatenated matrices under the above described model, and Bayesian analysis under the codon-based model for the COI matrix. Phylogenetic relationships were analyzed by Bayesian inference (BI) and maximum likelihood (ML) methods. BI was performed in MrBayes v.3.2 (Huelsenbeck and Ronquist, 2001) for 10 million generations; the trees were summarized after removing 25% burn-in. Average standard deviations of split frequencies were 0.033860 for the COI-derived tree, and 0.030280 for the 18S rDNA tree. ML was carried out in PHYML v.3.1 (Guindon and Gascuel, 2003), with bootstrap values calculated by 1000 replicates. Final trees were visualized and exported using TreeView v.1.6.6 program (Page, 1996).

Statistical significance of the proposed host specificities was formally tested for several lineages. More specifically, we tested *alorani* and offshoot of *apionodes* II for which we suggested secondarily established strict host specificity after switching to a new host, *A. agrarius* (see Results), and four additional lineages (*apionodes* I, *apionodes* II, *jerfinica*, and *uptoni*) for which the data within our sample indicate specificity either to *Apodemus* spp. or the subfamily Arvicolinae. In all cases, we used a statistical model to test whether the host species remains significant predictor of the parasite lineage even when possible effect of sampling localities is taken into account. The test was done using generalized linear mixed-effect models with binomial response in R platform (R Development Core Team, 2014) using the package lme4 (Bates et al., 2015), with host and locality as explanatory variables and the presence of parasite lineage as a response.

2.6. Population genetics analyses

Since we obtained COI sequences of different lengths, we used the following procedure to build an optimal set of haplotypes, i.e. the set of sufficient sequence lengths on one hand and reasonable taxonomical representation on the other. In the first step, we determined a maximum length limit under which all lineages delimited by the preceding phylogenetic analyses (Figs. 2–4) were represented by several sequences. We then removed all sequences shorter than the limit and trimmed the remaining sequences to the limit length. With this procedure, we obtained a matrix of 177 sequences 547 bp long. This matrix was used for the reconstruction of haplotype networks in programs TCS v.1.21 (Clement et al., 2000), PopART v.1.7 (http://popart.otago.ac.nz; Bandelt et al., 1999), and the calculation of diversity indices in DnaSP v.5 (Librado and Rozas, 2009). For the *Apodemus*-associated lineages, we estimated within-lineage differentiation due to host specificity by calculating GammaST, a measure of genetic distance between populations (Nei, 1982), among the samples from different hosts using the DnaSP program. For this comparison, we only considered the groups containing at least three samples collected from a single host species.

Finally, we used the BEAST v.1.8.2 program package (Bouckaert et al., 2014) to estimate the relative ages of the lineages. For the time calibration, we used a relative scale with the "age" of the root set arbitrary to 10. This procedure allowed for the results interpretation in both relative scale and a putative absolute scale. Within the relative framework, we could compare relative "ages" of all lineages to the obviously young *alorani* offshoot, without assuming specific ages. In the putative absolute framework, we used the estimate of *A. agrarius* age (4.5 mya; Sakka et al., 2010) as upper bound for the age of the *alorani* lineage. We based this putative calibration on the evolutionary scenario we derived from our phylogenetic analysis and the host specificities of the lineages (details in Results and Discussion). Briefly, we conclude that the *alorani* lineage originated by switching from *A. flavicollis/sylvaticus* to *A. agrarius*, after the latter host species spread into Europe from the Far East, and cannot therefore be older than *A. agrarius* itself. The analysis was done under the GTR + Γ + I model with the molecular clock set at the lognormal relaxed mode. By checking for convergence in the Tracer v.1.6.0 (http://beast.bio.ed.ac.uk), we eventually ran the analysis for 35 mil. generations. We then discarded 25% of the trees and created a consensus of the remaining samples. We prepared graphical representation of the tree in the FigTree program v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and applied the node relative ages from the BEAST results.

3. Results

3.1. Sampling

In total, 1 515 individuals of *Apodemus* spp., 200 of *Microtus* spp., and 364 of *Myodes glareolus* were collected within the period of 2006–2014 from an area covering 13 European countries and the west of Russia (Figs. 1, S1, S2a–c, Table S5). Of these samples, 680 (32.7%) were *Eimeria*-positive. Hosts of the positive samples were determined using both morphological and molecular methods. For the *Eimeria* samples from *Apodemus*, we obtained 165 sequences of the COI gene and 74 sequences of the SSU gene (see Table S2). For the *Eimeria* samples from Arvicolinae, we obtained 36 sequences of the COI gene and 37 sequences of the SSU gene (see Table S2). Furthermore, we obtained several *Eimeria* sequences from other small mammals (*Crocidura* sp., *Mus* sp., *Neomys* sp., and *Sorex araneus*). These sequences were included in the analyses to improve the sample background.

3.2. Phylogeny

The COI analyses yielded well-resolved trees with a strongly supported inner structure (Fig. 2). The same topologies were obtained under the GTR + Γ + I model and the codon-based model. The trees obtained via the SSU analyses were compatible regarding their main features with the COI trees, but they were less resolved (Fig. 3). Concatenation of both matrices yielded a tree similar to the topology based on 18S rDNA, however, the *kaunensis* lineage remained preserved there (Fig. S1). From the evolutionary point of view, the most notable feature of the trees was the taxa clustering being strongly influenced, but not entirely determined, by the taxonomic position of the host organisms. Eimerians collected from the two rodent groups investigated here, i.e. Muridae (represented by the genus *Apodemus*) and Arvicolinae (represented by the genera *Microtus* and *Myodes*), clustered in several well-formed and supported lineages. For the clarity of the following description and discussion, we delimited three dominant monophyletic lineages of these eimerians (Fig. 2). Since this delimitation was in close agreement with the established morphology-based taxa (species), we designated these lineages by the species names, i.e. *jerfinica, uptoni* and *apionodes*. In *apionodes* lineage, we designated individual sublineages as *apionodes* I–IV, as well as morphospecies *alorani* and *kaunensis*, paraphyletic in respect to *apionodes* (Fig. 2).

Of these lineages, two strictly *Apodemus*-specific lineages created monophyletic phylogenetically distinct groups, corresponding to *jerfinica* and *uptoni*. The rest of the lineages, encompassing the majority of the *Eimeria* samples, clustered as a monophyletic group with the posterior probability 1, and its sister group composed of three closely related sequences of *Eimeria* from *Mus* and *Heliophobius*. The host specificities of these lineages varied considerably. Interestingly, a tendency to a switch from Arvicolinae- to *Apodemus*-specificity could be seen within this cluster of lineages (Fig. 2). The most basal lineage *apionodes* I was entirely Arvicolinae-specific. Of the other lineages, the *kaunensis*, *apionodes* III, and *apionodes* IV were formed by a mix of samples from various host groups (i.e. Muridae, Arvicolinae, and a single sample from *Cavia porcellus*), with the more recent lineage (*apionodes* IV) prevailed by the *Apodemus* samples. Finally, two derived offshoots (*apionodes* II and *alorani*) were only *Apodemus*-specific, and the *alorani* lineage/species was even specific to a single *Apodemus* species, *A. agrarius* (Fig. 2, Table S2; for statistical test see Table S6).

This latest pattern strongly suggests a recent host switch from the *A. flavicollis/A. sylvaticus* (Af/As) to *A. agrarius* (Aa). In the tree derived via BEAST during calculations of relative ages, the arrangement of the lineages corresponded to that in the BI tree, except the switch between lineages *apionodes* II and *kaunensis* (Fig. 4). The COI-derived topology described above was compatible with the 18S rDNA based trees, except for six samples (designated AF 15_CZ7, AF 47_CZ9, AS20_IT63, AF 92D_DE47, AA B2A4_BG71, and AA 21655_SK36) showing conflicting positions; in all cases, these samples originated from mixed infections (as established by microscopical examination) and phylogenetic discrepancies were thus most likely due to amplification of different species/lineage for each genetic marker.

The sampling presented here covered a large part of Europe, from western France to eastern Bulgaria, and from southern Finland to southern Italy (Figs. 1, S2, S3a–c and Table S1). Within this area, the eimerian lineages varied slightly in their distribution, but showed considerable overlaps (Figs. 1 and S3a–c). Due to these many overlaps, all sampled areas were inhabited by multiple parasite lineages. Often the samples with the same host specificity, but of different phylogenetic position, were collected sympatrically, even at the same localities. An interesting geographic pattern was found for the eimerian lineages with the host switch from Af/As to Aa hosts. While in the less diversified lineage *apionodes* II the Aa samples were distributed inside the area of the Af/As samples, in the more diversified lineage *apionodes* IV the Af/As and its sister *alorani* Aa samples were collected from mutually disjunct localities (Fig. 5).

Apart from these lineages of the main interest (i.e. the *Apodemus*/Arvicolinae-associated samples), our data set also included samples from other rodent and non-rodent hosts. With one exception, all of these samples clustered outside the *Apodemus*/Arvicolinae-specific clusters. Some of them formed independent clusters based on their host characteristics, such as poultry-specific cluster, rabbit-specific cluster, or bird *Isospora*-specific cluster. However, few of the samples were unique by their origin (i.e. no other samples from the same host were available), and their phylogenetic clustering is thus difficult to interpret. The most peculiar case was *E. caviae*, the only sample which did not originate from the *Apodemus*/Arvicolinae hosts but clustered within one of the *Apodemus*-specific cluster (Fig. 2). Since the molecular data on eimerians from the same or closely related host are not available, we do not make at this point any evolutionary speculation and focus on the well-formed and supported *Apodemus*/Arvicolinae-specific clusters.

3.3. Population genetics

When collapsed into haplotypes, the set of the above mentioned 177 COI sequences (see chapter 2.6. in Material and Methods) yielded 45 unique haplotypes (Table S2) which split into two uncoupled networks and several isolated haplotypes when evaluated under the criteria of statistical parsimony as implemented in the TCS program (Fig. S4). The larger and substantially more complex network corresponded to the node A designated in the Fig. 4. The cohesion of this network, i.e. the low number of missing haplotypes, suggested that the sampling effectively covered relatively recent continuous diversification. This allowed for a reliable inference supporting the two independent switches to *A. agrarius* from the Af/As lineages (Figs. 2, 4 and 5). Their recent occurrence was further supported by a comparison of the relative ages of the lineages; e.g., the estimated relative age of the *alorani* lineage (3.5) was roughly one third of the whole tree depth (10) (Fig. 4). Apart from these topology-based arguments, a strong genetic barrier rising during the switch between Af/As on the one hand, and Aa on the other, was clearly reflected by the GammaST estimates within *jerfinica*, the only lineage with Aa samples considerably intertangled with Af/As. Of the three interspecific comparisons, the genetic differentiation expressed by GammaST is considerably lower between Af and As than differentiation between either of these species and Aa (Fig. 4).

4. Discussion

The phylogenetic trees and population networks show that *Eimeria* from several rodent genera form a remarkably complex system. The gross picture reveals three notable tendencies: (1) a strong genetic/phylogenetic structure in which well supported clusters are determined by specificities to different host groups, (2) polyphyletic arrangement, i.e. multiple origin, of the rodent-specific clusters within the *Eimeria* phylogeny, and (3) repeated host switches within the rodent-specific clusters (i.e. among closely related haplotypes) which may become rapidly fixed. The two former features are in line with several recent studies (e.g. Štefka and Hypša, 2008; Power et al., 2009; Štefka et al., 2009; Kvičerová and Hypša, 2013; Pineda-Catalan et al., 2013; Kvičerová et al., 2014; Ogedengbe et al., 2018) contradicting the traditional view of host specificity as a conserved and phylogenetically important parameter. The third feature is derived from the population-level analyses. In our opinion, it provides the most important contribution of the presented data to the conception of host-parasite coevolution.

Apart from these genetic features, it is interesting to note that compared to many other groups of parasites, the eimerian groups studied here display a surprising concordance between their taxonomy based on morphological features and the phylogenetic relationships inferred from molecular data. This is documented in the Table S4 that lists distinct sets of oocyst morphological features, corresponding to some of the previously described *Eimeria* species, which can be

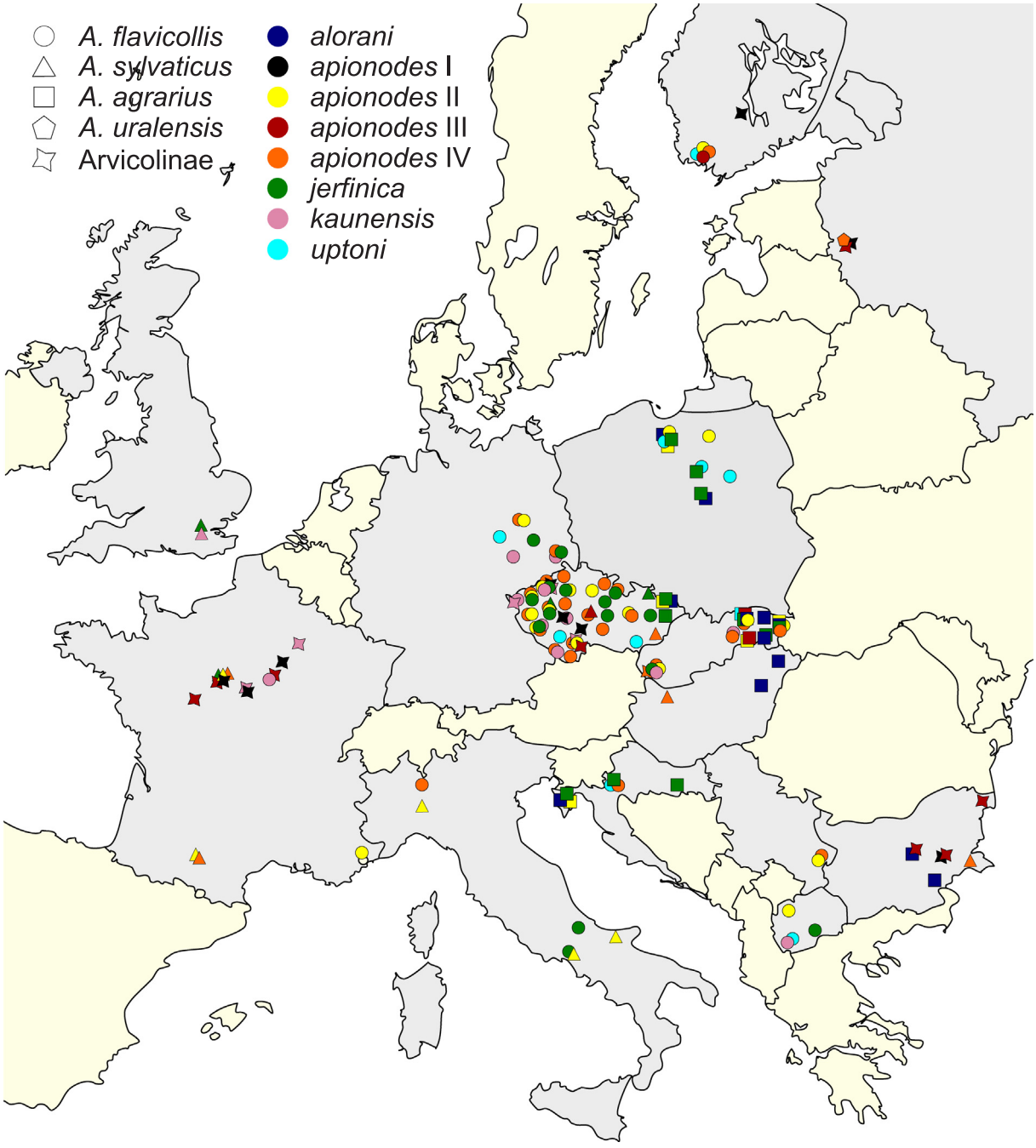


Fig. 1. Distribution of individual *Eimeria* lineages across the sampled localities. Symbols are attributed to the hosts, colours are attributed to the parasites.

attributed to the genetically-delimited lineages. However, apart from this general fit, the distribution of morphological traits across the tree also shows several peculiarities. For example, coccidia from the lineage corresponding to the *kaunensis* morphotype possess oocyst residuum (OR) but cluster within the group B, typical by its absence (Fig. 4). The other two rodent-specific *Eimeria* possessing OR (i.e. *E. cahirinensis* and *E. callospermophili*) cluster at a distant position within the tree (Fig. 2). This finding, rejecting the hypothesis of two distinct rodent *Eimeria* lineages based on the presence/absence of OR (Zhao and Duszynski, 2001a,b)), further demonstrates the effect of sampling effort, and hence the representativeness of the sampled material, on the phylogenetic/evolutionary inferences. Below, we discuss in more details the observed patterns and their possible consequences for the host-parasite coevolutionary concept.

4.1. Host specificity at the phylogenetic level

The non-monophyletic nature of the samples confirms and further extends our previous observation based solely on eleven samples of the *Apodemus*-associated *Eimeria* (Kvičerová and Hypša, 2013). However, due to the considerably larger number of samples (182 *Apodemus*-associated samples + 56 Arvicolinae-associated samples) a consistent picture of the parasites' distribution across their hosts and geographic ranges can now be drawn from the data. Thus, unlike our previous study, none of the samples presented here forms a single-sequence "orphan" lineage. All of the clusters containing eimerian parasites from *Apodemus* and/or Arvicolinae hosts form monophyletic groups composed of at least 8 sequences. This shows that each of the *Apodemus*-associated samples represents a specific branch, not just a random non-specific infection.

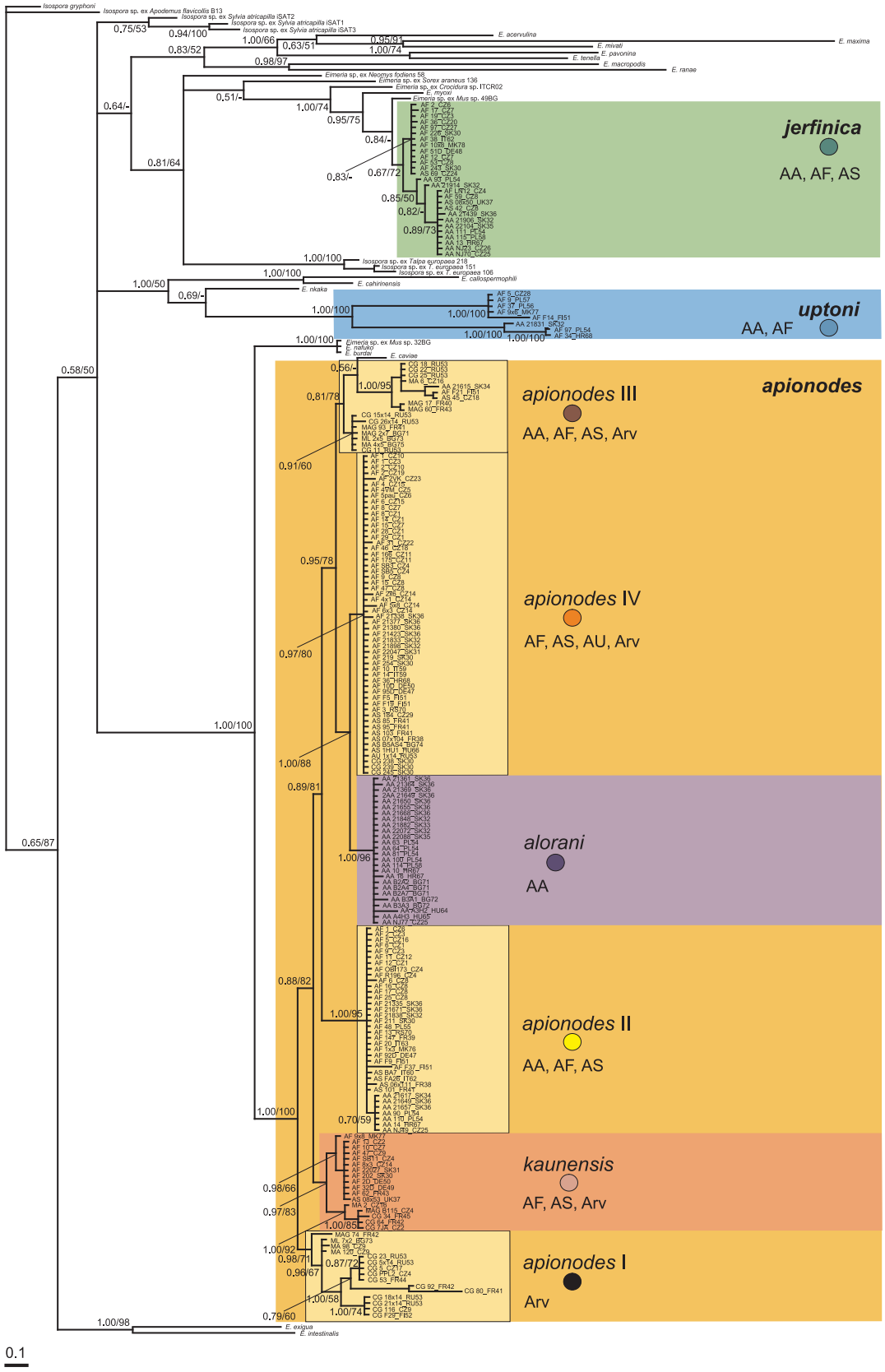


Fig. 2. Phylogenetic relationships of the eimerians inferred by the BI analysis of the COI sequences. Numbers at the nodes show posterior probabilities under the BI analysis/bootstrap values derived from ML analysis. Posterior probabilities and bootstrap supports lower than 0.50 or 50%, respectively, are marked with dash (–). Each original sample code consists of the abbreviation of the host species, specific code of the sample, country code, and the map reference. AA, *Apodemus agrarius*; AF, *Apodemus flavicollis*; AS, *Apodemus sylvaticus*; AU, *Apodemus uralensis*; Arv, Arvicolinae.

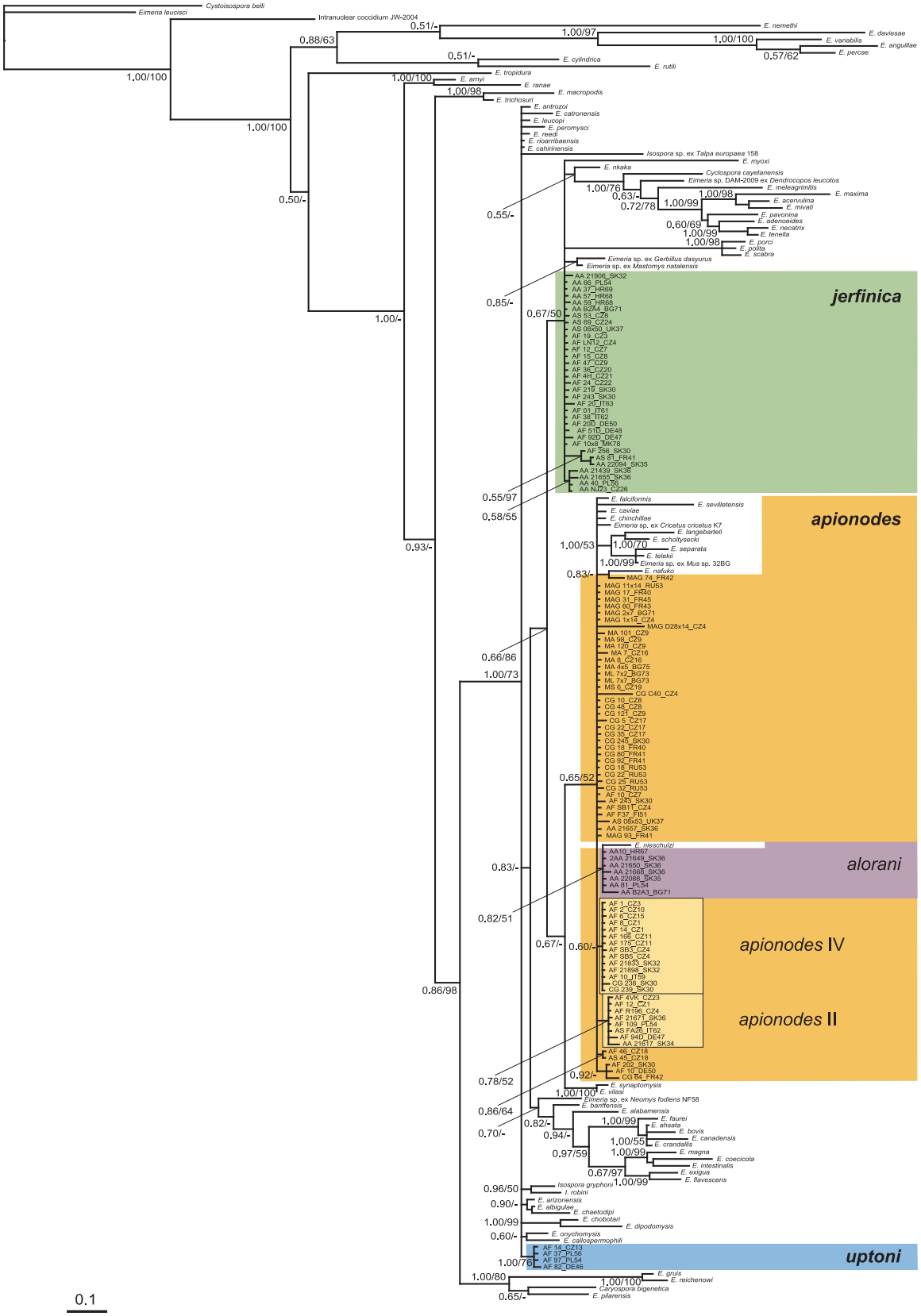


Fig. 3. Phylogenetic relationships of the eimerians inferred by the BI analysis of the 18S rDNA sequences. Numbers at the nodes show posterior probabilities under the BI analysis/bootstrap values derived from ML analysis. Posterior probabilities and bootstrap supports lower than 0.50 or 50%, respectively, are marked with dash (–). Each original sample code consists of the abbreviation of the host species, specific code of the sample, country code, and the map reference. AA, *Apodemus agrarius*; AF, *Apodemus flavicollis*; AS, *Apodemus sylvaticus*; AU, *Apodemus uralensis*; Arv, Arvicolinae.

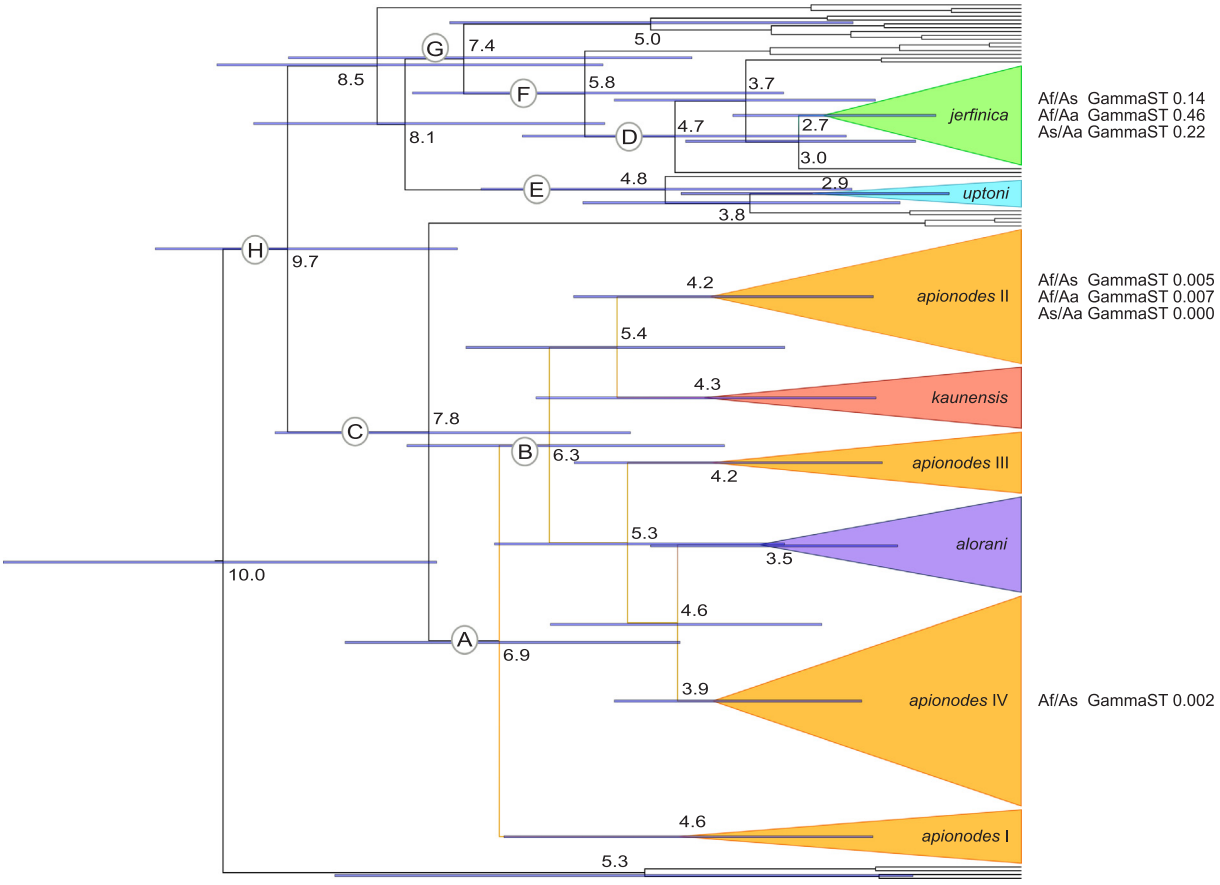


Fig. 4. Divergence times and genetic differentiations calculated by the BEAST package and DnaSP. The numbers at the nodes show relative "ages" of the lineages (see Material and Methods), the bars at the nodes represent posterior probabilities values and 95% credibility intervals.

Although the *Apodemus*/Arvicolinae-associated lineages branch in a non-monophyletic manner among the eimerians from other hosts, they all retain affinity to these rodent groups. An interesting example of the relationship between the host specificity and genetic structure is provided by the *Eimeria* associated with Af/As, and the Aa. Within the *apionodes* group, the Aa samples form two well-defined and strictly specific clusters, contrasting to the entirely intermixed sequences from Af and As. For these two clusters, the observed restriction to a single host proved statistically significant (i.e. not determined by the locality; see Table S6) for the *alorani* lineage composed of 26 samples, while it was nonsignificant for the less numerous offshoot of the *apionodes* II (n = 7). This arrangement, at least in the *alorani* lineage, is likely to reflect different evolutionary histories of the three *Apodemus* species. Two of them, Af and As, are closely related species of the subgenus *Sylvaemus* (Martin et al., 2000; Michaux et al., 2002) which separated around 4 million years ago (Michaux and Pasquier, 1974), and after Quaternary climatic oscillations recolonized the Europe from their southern refugia (Michaux et al., 2005). Currently, they co-occur in sympatry or even in syntopy throughout the majority of their European distribution (Michaux et al., 2005). In contrast, the distribution of phylogenetically distant Aa (subgenus *Apodemus*) overlaps with Af/As only in the eastern part of their geographical distribution (Suzuki et al., 2008). An analysis by Sakka et al. (2010) shows that populations of *A. agrarius* create a very complicated system with several main foci, the probable Quaternary refugia. China, Russian Far East, and Korea represent important centers of diversification for this species. Its eastern population covers southern parts of the Russian Far East, China, Korea, and Taiwan. The western population, isolated from the eastern one by several biogeographic barriers, is distributed across Kazakhstan, Kyrgyzstan, the Caucasus, and the Balkans, and reaches central Europe. Unlike the demographically stabilized eastern group, the western population shows clear signatures of a recent expansion (Sakka et al., 2010). Based on the dating, both Suzuki et al. (2008) and Sakka et al. (2010) suggest that colonization of the central Palaearct may have taken place around 175 000–190 000 years ago. The hypothesis, that only a single population lineage has penetrated into Europe, is supported by the genetic and molecular analyses (Filippucci et al., 2002; Suzuki et al., 2008).

It is reasonable to suppose that the Aa-specific lineages, e.g. the *alorani* branch, did not originate before the first waves of *A. agrarius* reached Europe. Therefore, considering the Sakka et al. (2010) dating of the *A. agrarius* origin in the Far East region (4.5 mya; see Material and Methods) and the delay of its expansion to Europe and hypothetical origin of the *alorani* group (shown in Fig. 4), the strictly *Apodemus*-specific branches described here seem of relatively recent origin in comparison to the suggested ages of the Af/As taxa (Michaux et al., 2005; Suzuki et al., 2008). The current patterns of phylogeny/specificity shown here thus seem to reflect recent evolutionary events (switches, adaptations), rather than stable long-term coevolution. These events resulted in origin of several lineages with different degree of host specificity, ranging from a single host species to several host genera. This makes the system of European *Eimeria* in rodents a promising model for investigating the diversification/speciation processes on the ecological scale.

4.2. Host switches and specificity at the population level

The pattern of genetic differentiation between Af/As and Aa samples is further strengthened by the situation in the *jerfinica* group. Here, although the samples from *A. flavicollis*, *A. sylvaticus* and *A. agrarius* are

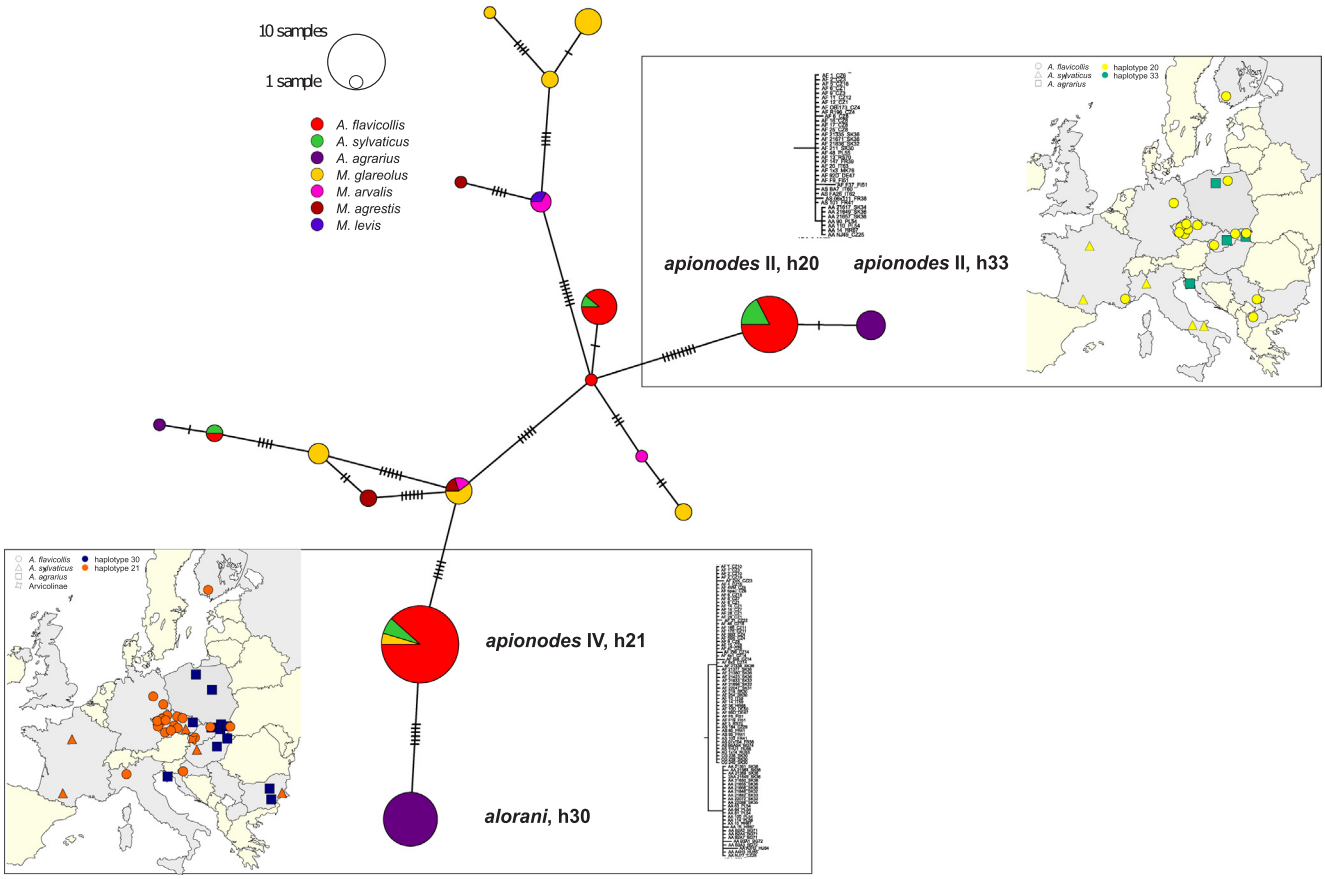


Fig. 5. Haplotype network of the lineage A (as defined in Fig. 4) constructed in PopART. Spatial relationship between the Af/As and Aa haplotypes are shown in the maps.

mixed, rather than forming distinct lineages, the differentiation test reveals barriers among the species. Of the three pairs of differentiation, the two involving *A. agrarius* (GammaST 0.46 for Af vs. Aa, and GammaST 0.22 for As vs. Aa) are considerably stronger than the Af/As differentiation (GammaST 0.14). Repeated genetic differentiation between the Af/As and Aa samples poses an interesting question about the underlying mechanism. No simple answer would fully explain the observed patterns. Since the Aa-specific samples clearly represent host switches, phylogenetic/genealogic constraint can not be responsible for this barrier. Neither can simple ecological parameters provide an explanation, as some of the Aa samples were obtained from the same localities as the Af and As samples, even during a single collection. In theory, a sampling bias, e.g. insufficient sampling effort, could result in an erroneous inference of seemingly host-specific haplotypes or lineages, such as the two Aa-specific offshoots. However, as shown in the Figs. 1 and 5, the Aa-specific samples were collected from a broad geographic range, shared with the Af/As samples. This provides evidence of long-maintained genetic separation of these groups in sympatry and shows that the Aa clusters are not artifacts of sampling just a local temporary subpopulation. It should be noted that the switch in specificity towards *A. agrarius* is not mere colonization, i.e. extension of the host spectrum, but a complete switch involving the entire abandoment of the original host taxa. In the *alorani* group, this process apparently gave rise to a morphologically distinguished lineage fully adapted to the new host. Thus, it seems likely that some more complex circumstances play a key role in this process, possibly, for example, differences in the hosts' physiologies and/or more subtle ecological differences. The patterns discussed above indicate that while the majority of coevolutionary studies address the question of host switches from the phylogenetic perspective, investigations of the early genetic differentiation may be the more pertinent approach. In our results, the well-resolved and supported part of the tree (*apionodes* + *alorani* + *kaunensis*) shows an interesting variation in degree of the host specificity among the lineages. While some of the lineages were found in several species of different families, others are specific to a single family or even a single species (Fig. 2; Table S6 with statistical tests). It is difficult with such unique events, even by applying rigorous parsimony rules, to establish direction of the evolutionary changes (i.e. narrowing the host spectrum by stronger specialization/adaptation vs. broadening the spectrum by colonization of new host species). However, several of the observed patterns may reflect a putative extension from the single-family specificity (e.g. the lineages *apionodes* I, *apionodes* II, *alorani*, *jerfinica*, and *uptoni*) to the mixture of two host families (e.g. lineages *apionodes* III, *apionodes* IV, and *kaunensis*), and can be perceived as possible instances of ecological fitting. In contrast, the sudden disruption between strict Af/As specificity and Aa specificity is more difficult to attribute to an established ecological mechanism.

These findings have also broader relevance within the established concepts of population structure and diversity in parasites. For example, the Nadler's hypothesis (Nadler, 1995) postulates direct relationship between the host range and genetic diversity, predicting that parasites (i.e. populations, species) with broader host spectrum display weaker population structure due to their better dispersal opportunity. Our results suggest that when analyzing such relationships, genetic diversity and host specificity should be evaluated within a common framework with genealogy and host switches dynamics. This can for example be illustrated on the lineages *apionodes* II and *apionodes* IV + *alorani* (Fig. 5). Within these groups, seemingly capable of dissemination via three or four host species living in sympatry, the parasites associated with *A. agrarius* do not contribute to the overall gene flow due to their strict host specificity. Similarly, such a monophyletic recent offshoot with narrower host specificity will likely

display lower genetic structure than the paraphyletic ancestral population with a broader host spectrum.

Acknowledgements

We thank Christopher Mark Steer for language correction of this manuscript. We are grateful to Ladislav Mošanský, Jana Fričová and Monika Onderová (Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia), Mária Kazimírová (Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia), Lucie Kordíková, Jakub Vlček, Jan Štefka, Roman Hrdlička, Michal Šorf, Michaela Matějková, Ema Hrouzková, Pavel Hrouzek and Tomáš Svozil (Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic), and Václav Mikeš (Museum of South Bohemia in České Budějovice, Czech Republic), who participated in the field studies or provided faecal samples. We also thank Petr Šmilauer for his guidance on the statistical test.

Funding

This work was supported by the Czech Science Foundation [grant number 14-07004S], and partially by the Bilateral Mobility Project (MAD) [grant number SAV-16-22], and the Slovak Research and Development Agency [grant number APVV-14-0274].

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.05.009.

References

Agosta, S.J., 2006. On ecological fitting, plant-insect associations, herbivore host shifts, and host plant selection. Oikos. Wiley Online Library 114, 556–565.
Agosta, S.J., Klemens, J.A., 2008. Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution. Ecol. Lett. 11, 1123–1134.
Agosta, S.J., Janz, N., Brooks, D.R., 2010. How specialists can be generalists: resolving the "parasite paradox" and implications for emerging infectious disease. Zoologia 27, 151–162.
Anděra, M., Beneš, B., 2002. Atlas rozšíření savců v České republice. Předběžná verze IV. Hlodavci (Rodentia) – část 2. Myšovití (Muridae), myšivkovití (Zapodidae). Národní museum; Praha, Czech Republic (in Czech).
Araujo, S.B.L., Braga, M.P., Brooks, D.R., Agosta, S.J., Hoberg, E.P., von Hartenthal, F.W., Boeger, W.A., 2015. Understanding host-switching by ecological fitting. PLoS One 10, 0139225.
Bandelt, H., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.
Banks, J.C., Palma, R.L., Paterson, A.M., 2006. Cophylogenetic relationships between penguins and their chewing lice. J. Evol. Biol. 19, 156–166.
Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.
Bellinvia, E., 2004. A phylogenetic study of the genus *Apodemus* by sequencing the mitochondrial DNA control region. J. Zool. Syst. Evol. Res. 42, 289–297.
Berto, B.P., McIntosh, D., Lopes, C.W.G., 2014. Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). Rev. Bras. Parasitol. Vet. 23, 1–15.
Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10, e1003537.
Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659.
Demanche, C., Berthelemy, M., Petit, T., Polack, B., Wakefield, A.E., Dei-Cas, E., Guillot, J., 2001. Phylogeny of *Pneumocystis carinii* from 18 primate species confirms host specificity and suggests coevolution. J. Clin. Microbiol. 39, 2126–2133.
Demanche, C., Deville, M., Michaux, J., Barriel, V., Pinçon, C., Aliouat-Denis, C.M., Pottier, M., Noël, C., Viscogliosi, E., Aliouat el, M., Dei-Cas, E., Morand, S., Guillot, J., 2015. What do *Pneumocystis* organisms tell us about the phylogeography of their hosts? The case of the woodmouse *Apodemus sylvaticus* in Continental Europe and western Mediterranean islands. PLoS One 10, e0120839.
Duszynski, D.W., Wilber, P.G., 1997. A guideline for the preparation of species descriptions in the Eimeriidae. J. Parasitol. 83, 333–336.
Falk, B.G., Perkins, S.L., 2013. Host specificity shapes population structure of pinworm parasites in Caribbean reptiles. Mol. Ecol. 22, 4576–4590.
Filippucci, M.G., Macholán, M., Michaux, J.R., 2002. Genetic variation and evolution in the genus *Apodemus* (Muridae: Rodentia). Biol. J. Linn. Soc. 75, 395–419.
Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenesis by maximum likelihood. Syst. Biol. 52, 696–704.
Habermannová, J., Bogusch, P., Straka, J., 2013. Flexible host choice and common host switches in the evolution of generalist and specialist cuckoo bees (Anthophila: Sphecodes). PLoS One 8, e64537.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.
Higgs, S., Nowell, F., 1991. A review of the species of *Eimeria* infecting hosts in the genus *Apodemus*. Syst. Parasitol. 20, 203–209.
Higgs, S., Nowell, F., 2000. Population biology of *Eimeria* (Protozoa: Apicomplexa) in *Apodemus sylvaticus*: a capture/recapture study. Parasitology 120, 355–363.
Hoberg, E.P., Brooks, D.R., 2008. Structuring the biosphere: episodic host-switching, geographic colonization, and diversification in complex host-parasite systems. J. Biogeogr. 35, 1533–1550.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Hůrková, L., Baker, M.A., Jirků, M., Modrý, D., 2005. Two new species of *Eimeria* Schneider 1875 (Apicomplexa: Eimeriidae) from the broad-toothed field mouse, *Apodemus mystacinus* Danford and Alston 1877 (Rodentia: Muridae) from Jordan. Parasitol. Res. 97, 33–40.
Jaarola, M., Searle, J.B., 2002. Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. Mol. Ecol. 11, 2613–2621.
Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl. Acids Res. 30, 3059–3066.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
Kvičerová, J., Hypša, V., 2013. Host-parasite incongruences in rodent *Eimeria* suggest significant role of adaptation rather than cophylogeny in maintenance of host specificity. PLoS One 8, e63601.
Kvičerová, J., Hypša, V., Dvořáková, N., Mikulíček, P., Jandzik, D., Gardner, M.G., Javanbakht, H., Tiar, G., Široký, P., 2014. *Hemolivia* and *Hepatozoon*: haemogregarines with tangled evolutionary relationships. Protist 165, 688–700.
Kvičerová, J., Pakandl, M., Hypša, V., 2008. Phylogenetic relationships among *Eimeria* spp. (Apicomplexa, Eimeriidae) infecting rabbits: evolutionary significance of biological and morphological features. Parasitology 135, 443–452.
Levine, N.D., Ivens, V., 1990. The Coccidian Parasites of Rodents. CRC Press, Boca Raton, FL, USA.
Lewis, D.C., Ball, S.J., 1983. Species of *Eimeria* of small wild rodents from the British Isles, with descriptions of two new species. Syst. Parasitol. 5, 259–270.
Librado, P., Rozas, J., 2009. DnaSP v. 5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Malcicka, M., Agosta, S.J., Harvey, J.A., 2015. Multi level ecological fitting: indirect life cycles are not a barrier to host switching and invasion. Glob. Chang. Biol. 21, 3210–3218.
Martin, Y., Gerlach, G., Schlötterer, C., Meyer, A., 2000. Molecular phylogeny of European muroid rodents based on complete cytochrome b sequences. Mol Phylogenet Evol 16, 37–47.
Meinilä, M., Kuusela, J., Ziętara, M.S., Lumme, J., 2004. Initial steps of speciation by geographic isolation and host switch in salmonid pathogen *Gyrodactylus salaris* (Monogenea: Gyrodactylidae). Int. J. Parasitol. 34, 515–526.
Messenger, L.A., Garcia, L., Vanhove, M., Huaranca, C., Bustamante, M., Torrico, M., Torrico, F., Miles, M.A., Llewellyn, M.S., 2015. Ecological host fitting of *Trypanosoma cruzi* TcI in Bolivia: mosaic population structure, hybridization and a role for humans in Andean parasite dispersal. Mol. Ecol. 24, 2406–2422.
Meyer-Lucht, Y., Sommer, S., 2005. MHC diversity and the association to nematode parasitism in the yellow-necked mouse (*Apodemus flavicollis*). Mol. Ecol. 14, 2233–2243.
Michaux, J.R., Chevret, P., Filippucci, M.G., Macholan, M., 2002. Phylogeny of the genus *Apodemus* with a special emphasis on the subgenus *Sylvaemus* using the nuclear IRBP gene and two mitochondrial markers: cytochrome b and 12S rRNA. Mol. Phylogenet. Evol. 23, 123–136.
Michaux, J.R., Libois, R., Filippucci, M.G., 2005. So close and so different: comparative phylogeography of two small mammal species, the yellow-necked fieldmouse (*Apodemus flavicollis*) and the woodmouse (*Apodemus sylvaticus*) in the western Palearctic region. Heredity 94, 52–63.
Michaux, J., Pasquier, L., 1974. Dynamique des populations de mulots (Rodentia, *Apodemus*) en Europe durant le Quaternaire. Premie`res données. Bull. Soc. Géol. France 7, 431–439 (in French).
Nadler, S.A., 1995. Microevolution and the genetic structure of parasite populations. J. Parasitol. 81, 395–403.
Nei, M., 1982. Evolution of human races at the gene level. In: Bonne-Tamir, B., Cohen, T., Goodman, R.M. (Eds.), Human Genetics. Part A. The Unfolding Genome. Alan R. Liss, New York, pp. 167–181.
Nieberding, C., Libois, R., Douady, C.J., Morand, S., Michaux, J.R., 2005. Phylogeography of a nematode (*Heligmosomoides polygurus*) in the western Palearctic region: persistence of northern cryptic populatios during ice ages? Mol. Ecol. 14, 765–779.
Nieberding, C., Morand, S., Libois, R., Michaux, J.R., 2004. A parasite reveals cryptic phylogeographic history of its host. Proc. Biol. Sci. 271, 2559–2568.
Nowak, R.M., 1999. Walkeŕs Mammals of the World, sixth ed. The Johns Hopkins University Press, Baltimore and London.
Nylin, S., Slove, J., Janz, N., 2014. Host plant utilization, host range oscillations and diversification in nymphalid butterflies: a phylogenetic investigation. Evolution 68, 105–124.
Ogedengbe, M.E., El-Sherry, S., Ogedengbe, J.D., Chapman, H.D., Barta, J.R., 2018. Phylogenies based on combined mitochondrial and nuclear sequences conflict with morphologically defined genera in the eimeriid coccidia (Apicomplexa). Int. J.

Parasitol 48, 59–69.
Page, R.D.M., 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. Comput. Applic. Biosci. 12, 357–358.
Paterson, A.M., Banks, J., 2001. Analytical approaches to measuring cospeciation of host and parasites: through a glass, darkly. Int. J. Parasitol. 31, 1012–1022.
Pineda-Catalan, O., Perkins, S.L., Peirce, M.A., Engstrand, R., Garcia-Davila, C., Pinedo-Vasquez, M., Aguirre, A.A., 2013. Revision of hemoproteid genera and description and redescription of two species of chelonian hemoproteid parasites. J. Parasitol. 99, 1089–1098.
Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Posada, D., 2009. Selection of models of DNA evolution with jModelTest. Methods Mol. Biol. 537, 93–112.
Power, M.L., Richter, C., Emery, S., Hufschmid, J., Gillings, M.R., 2009. *Eimeria trichosuri*: phylogenetic position of a marsupial coccidium, based on 18S rDNA sequences. Exp. Parasitol. 122, 165–168.
Ricklefs, R.E., Fallon, S.M., Bermingham, E., 2004. Evolutionary relationships, co-speciation, and host switching in avian malaria parasites. Syst. Biol. 53, 111–119.
Sakka, H., Quéré, J.P., Kartavtseva, I., Pavlenko, M., Chelomina, G., Atopkin, D., Bogdanov, A., Michaux, J., 2010. Comparative phylogeography of four *Apodemus* species (Mammalia: Rodentia) in the Asian Far East: evidence of Quaternary climatic changes in their genetic structure. Biol. J. Linn. Soc. 100, 797–821.
Schwarz, R.S., Jenkins, M.C., Klopp, S., Miska, K.B., 2009. Genomic analysis of *Eimeria* spp. populations in relation to performance levels of broiler chicken farms in Arkansas and North Carolina. J. Parasitol. 95, 871–880.
Štefka, J., Hypša, V., 2008. Host specificity and genealogy of the louse *Polyplax serrata* on field mice, *Apodemus* species: a case of parasite duplication or colonisation? Int. J. Parasitol. 38, 731–741.
Štefka, J., Hypša, V., Scholz, T., 2009. Interplay of host specificity and biogeography in the population structure of a cosmopolitan endoparasite: microsatellite study of *Ligula intestinalis* (Cestoda). Mol. Ecol. 18, 1187–1206.
Suzuki, H., Filippucci, M.G., Chelomina, G.N., Sato, J.J., Serizawa, K., Nevo, E., 2008. A biogeographic view of *Apodemus* in Asia and Europe inferred from nuclear and mitochondrial gene sequences. Biochem. Genet. 46, 329–346.
Wilson, D.E., Reeder, D.M., 2005. Mammal Species of the World: A Taxonomic and Geographic Reference, third ed., vol. 2. The Johns Hopkins University Press, Baltimore.
Zajac, A.M., Conboy, G.A., 2006. Veterinary Clinical Parasitology. Blackwell Publishing, Iowa, USA.
Zhao, X., Duszynski, D.W., 2001a. Molecular phylogenies suggest the oocyst residuum can be used to distinguish two independent lineages of *Eimeria* spp. in rodents. Parasitol. Res. 87, 638–643.
Zhao, X., Duszynski, D.W., 2001b. Phylogenetic relationships among rodent *Eimeria* species determined by plastid ORF470 and nuclear 18S rDNA sequences. Int. J. Parasitol. 31, 715–719.

Curriculum vitae

RNDr. Jana Martinů

University of South Bohemia in České Budějovice

Faculty of Science

Date of birth: May 15, 1984

Place of birth: Kadaň, Czech Republic

Education:

2003–2007: Bachelor's programme of study (Bc.) at Faculty of Science, University of South Bohemia in České Budějovice.

2007–2009: Master's degree study (Mgr.) at Faculty of Science, University of South Bohemia in České Budějovice, Department of Parasitology.

2009: State rigorosum examination (RNDr. title)

2009–present: Ph.D. programme of study at Faculty of Science, University of South Bohemia in České Budějovice, Department of Parasitology.

Employment:
2009-2014: Employed at Institute of Parasitology Biology Centre, CAS, v.v.i. in České Budějovice, Laboratory of Molecular Ecology and Evolution.
2009-present: Part-time employee at Faculty of Science, University of South Bohemia in České Budějovice.
2012-2014 & **2016-2018:** Maternity leave

International workshops and meetings:

2010: *ICP4 - The 4th International Conference on Phthiraptera*, Urgup, Turkey.

2011: *Workshop on Molecular Evolution, Europe*, Č. Krumlov, Czech Republic,

2014: *ICP5 - The 5th International Conference on Phthiraptera*, Park City, Utah, USA.

List of papers:

LITERÁK, I., KOCIANOVÁ, E., DUSBABEK, F., **MARTINŮ, J.**, PODZEMNÝ, P., SYCHRA, O. Winter infestation of wild birds by ticks and chiggers (Acari: Ixodidae, Trombiculidae) in the Czech Republic. *Parasitology Research*, 2007, vol. 101, no. 6, p. 1709-1711.

MARTINŮ, J., DUSBÁBEK, F., LITERÁK, I. A review of mites of the genus *Neharpyrhynchus* Fain (Acari : Harpirhynchidae) - Ectoparasites of wild birds, including description of four new species. *International Journal of Acarology*, 2008, vol. 1, no. 2, p. 197-209.

MARTINŮ, J., SYCHRA, O., LITERÁK, I., ČAPEK, M., GUSTAFSSON, D. L., ŠTEFKA, J. Host generalists and specialists emerging side by side: an analysis of evolutionary patterns in the

cosmopolitan chewing louse genus *Menacanthus*. *International Journal for Parasitology*, 2015, vol. 45, no. 1, p. 63–73.

MARTINŮ, J., ROUBOVÁ, V., NOVAKOVÁ, M., SMITH, VS., HYPŠA, V., ŠTEFKA, J. Characterisation of microsatellite loci in two species of lice, *Polyplax serrata* (Phthiraptera: Anoplura: Polyplacidae) and *Myrsidea nesomimi* (Phthiraptera: Amblycera: Menoponidae). *Folia Parasitologica*, 2015, vol. 62, p. 1–6.

MÁCOVÁ, A., HOBLÍKOVÁ, A., **MARTINŮ, J**., HYPŠA, V., STANKO M., KVIČEROVÁ, J. Mysteries of host switching: diversification and host specificity in rodent-coccidia associations. *Molecular Phylogenetics and Evolution*. 2018, vol. 8, p. 10008–10022.

MARTINŮ, J., HYPŠA, V., ŠTEFKA, J. Host specificity driving genetic structure and diversity in ectoparasite populations: co-evolutionary patterns in *Apodemus* mice and their lice. *Ecology and Evolution*. 2018, vol. 127, p. 179–189.