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

Selected factors affecting species and genetic diversity of oribatid mites (Acari: Oribatida) in Central Europe

Ph.D. Thesis

Mgr. Petra Kokořová

Supervisor:
RNDr. Josef Starý, CSc.
Institute of Soil Biology and Biogeochemistry, Biology Centre CAS

Supervisor – specialist
PaedDr. Martina Žurovcová, Ph.D.
Institute of Entomology, Biology Centre CAS

České Budějovice
2022

This thesis should be cited as:

Kokořová P. (2022): Selected factors affecting species and genetic diversity of oribatid mites (Acari: Oribatida) in Central Europe. Ph.D. Thesis Series, No. 15; University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic. 89 pp.

Annotation:

The first part of the thesis focused on the impact of natural disturbances and subsequent management practices (natural regeneration vs. clear cutting) on the oribatid mites in the montane Norway spruce forest in Šumava National Park (Czech Republic). Our results showed that changes caused by logging after a natural disturbance could be evident even more than 15 years later, at least in oribatid mite communities. The results of a phylogeographic study of two closely related species are presented in the second part. Except for revealing distinct phylogeographic patterns in the populations of these species across Europe, one new species was discovered, and its morphological and genetic description is provided.

Declaration:

I hereby declare that I am the author of this thesis and that I have used only those sources and literature detailed in the list of references.

Petra Kokořová

České Budějovice

16.9. 2022

Financial support:

The work presented in this thesis originated from a partnership between the Faculty of Science, University of South Bohemia in České Budějovice, Institute of Soil Biology and Biogeochemistry, and Institute of Entomology, Biology Centre of the Czech Academy of Sciences, České Budějovice. The Grant Agency of the Czech Republic provided financial support for two grant projects: No. P504/12/1218 and No 14-09231S. Investigation in Slovak caves was supported by Slovak Research and Developmental Agency, grant project: APV-17-0477.

Acknowledgments:

I want to thank my supervisor, Josef Starý, for his patience and support at this place. I really appreciate his help with sometimes tricky determination of oribatid mites into species. I am grateful to Martina Žurovcová and all the people from the Laboratory of Entomopathogenic Nematodes (Institute of Entomology, Biology Centre CAS), who allowed me to use their lab and equipment and taught me how to extract and amplify DNA. Likewise, I want to thank RNDr. Karel Tajovský, CSc. (Institute of Soil Biology and Biogeochemistry, Biology Centre CAS) and RNDr. Peter Ľuptáčik, Ph.D. (P.J. Šafárik University in Košice, Slovakia) for their help with the sampling in Czech and Slovak caves.

Furthermore, I would like to thank my family and friends for all the support they provided to me during my studies and for convincing me and helping me to finish this work.

List of scientific publications and the author´s contribution:

Paper I

Kokořová P., Starý J. (2017): Communities of oribatid mites (Acari: Oribatida) of naturally regenerating and salvage-logged montane spruce forests of Šumava Mountains. Biologia 72(4):445-451. (IF = 1.532)

PK participated in the soil sampling and microarthropods extractions, determined oribatid mites, statistically analyzed all data, wrote the manuscript, and completed the revisions. (80%)

Paper II

Kokořová P., Žurovcová M., Ľuptáčik P., Starý J. (2021): Distinct phylogeographic patterns in populations of two oribatid mite specie from the genus *Pantelozetes* (Acari, Oribatida, Thyrsomidae) in Central Europe. Experimental and Applied Acarology 83:493-511. (IF = 2.265)

PK designed the study, conducted the DNA extractions and amplifications, did the subsequent statistical analysis of the obtained data, wrote the manuscript, and completed the revisions. (70%)

Paper III

Starý J., **Kokořová P.**, Ľuptáčik P., Žurovcová M. A new species of the genus *Pantelozetes* (Acari, Oribatida, Thyrisomidae) from the caves of Eastern Slovakia. (manuscript)

PK performed the DNA extractions and amplifications, analyzed obtained molecular data and wrote the corresponding part of the manuscript. (40%)

The senior author and supervisor of the listed publications acknowledges the contribution of PK as stated above.

RNDr. Josef Starý, CSc.

Table of Contents:

1. Introduction

1.1. World of oribatid mites

Mites (Acari), with more than 45,000 species described, are one of the oldest, most abundant, and most diverse arthropod group (Selden 2017). For a long time, there has been an agreement that Acari comprise of two separate and well-defined superorders: **Parasitiformes** (with four orders: Opilioacarida, Holothyrida, Ixodida, and Mesostigmata) and **Acariformes**, the second being considered as the largest and most biologically diverse chelicerate superorder with more than 32 000 described species. Specimens that are classified as Acariformes are further divided into Trombidiformes (suborders Sphaerolichida and Prostigmata) and Sarcoptiformes (suborders Endeostigmata and Oribatida) (Krantz and Walter 2009).

Oribatida, which are in this study's spotlight, represent the largest suborder of Acariformes with almost 12 000 species described to date (Subías, online version 2020). However, the estimations of the real numbers reach up to 120 000 species (Walter and Proctor 2013). They are an evolutionarily ancient group as their first accepted fossil records are documented in Early Devonian sediments that date back at least 380 million years (Norton et al. 1988). According to molecular dating analyses, their origin might be even older, around 570 million years ago (Schaefer et al. 2010).

Oribatid mites are cosmopolitan and predominantly soil-dwelling microarthropods (in size from 0.15 to 2.5 millimeters) that can be found in almost every terrestrial habitat from tropics to poles, generally in high diversity as well as density. Some of the species adapted to arboreal or even aquatic lifestyles.

In the upper soil layers, the population densities of Oribatida reach from a few hundred individuals per square meter in arid or intensively used agricultural soils up to hundreds of thousands of individuals in acidic soils of temperate and boreal forests with a well-developed organic horizon (Maraun and Scheu 2000). Very often, especially in the northern forest ecosystems,

where the abundance of macrofauna is low, oribatids overtake its functions and represent the dominant group of mesofauna. It is already well recognized that in case of environmental changes, soil fauna has the potential to modify terrestrial ecosystems (i.e., soil characteristics and then plant composition) due to their incorporation into soil processes that help to sustain soil stability and fertility (Harte et al. 1996).

Recently, the interest in the reaction of oribatid mite populations to the changes in environmental conditions has been increasing because of their essential role in detrital food webs. They are involved in nutrient cycling, decomposition, and the distribution of organic matter within soils. Many species can selectively accumulate mineral substances (including heavy metals and alkaloids) in their bodies and cuticula and spread them through the ecosystem (Raspotnig et al. 2011; Skubała and Zaleski 2012). Moreover, they interact with the microbial community and act as the stimulators of fungal and bacterial growth by grazing on them or by transporting them through the soil profiles (Luxton 1972). It is clear that the role of oribatid mites in ecosystem processes is important (Wallwork 1983; Norton 1994), although the details are still poorly understood (Potapov et al. 2022).

The knowledge of their trophic ecology is limited and given their high densities in soils, it remains a question of how they can coexist without extensive trophic niche differentiation (Schneider et al. 2005). Multiple laboratory experiments showed that oribatid mites consume a wide range of fungi and bacteria, decomposing organic material, lichens and algae, and may even act as predators of soil Nematoda (Luxton 1972; Behan-Pelletier 1999; Coleman et al. 2004; Schneider et al. 2005). The term "choosy generalists," which tend to prefer one type of food resource, but in case of its insufficiency, they skip to another, used by Schneider and Maraun (2005), summarises best their feeding habits. According to most recent studies, oribatid species belong to at least four trophic guilds, i.e., lichen feeders, primary decomposers, secondary decomposers, and predators/scavengers with the classification into these trophic guilds above the species level being very often not reliable (Potapov et al. 2022).

In general, oribatid mites usually exhibit K-strategist life traits, as they are slowly reproducing with long life cycles. Typically, one female produces only 20 - 30 eggs over her life (Norton 1994). The generation time can vary even in one species depending on the environmental conditions, especially the temperature being the most crucial control factor - low temperatures prolonging the generation time (Coleman et al. 2004). For the species of temperate to boreal regions, the life cycle length typically varies from a couple of months up to two years, but in some cases, it can reach four or five years (Ciancialo and Norton 2006). For example, small species like *Oppiella nova* undergoes whole postembryonic development in just three weeks, and its lifespan is only 50 days, while bigger *Platynothrus peltifer* evolves 150 – 200 days and its lifespan is about 400 days (Norton 1994).

Within oribatid mites, more than 10% of described species are known to reproduce exclusively via parthenogenesis (Maraun et al. 2004), which is the greatest known concentration of this type of reproduction in the Acari. These groups of parthenogens are usually species-rich (whole genera and families, see Figure 1) and likely radiated while being parthenogenetic despite the predictions about parthenogenetic lineages being "dead ends" for various reasons in evolution (Maraun et al. 2004; more details in chapter 1.3.2.).

Overall, oribatid mites are not just passive inhabitants of soil ecosystems. Rather, they are effective nexuses, important indicators of changes in their environment and they represent a vast reservoir of biodiversity.

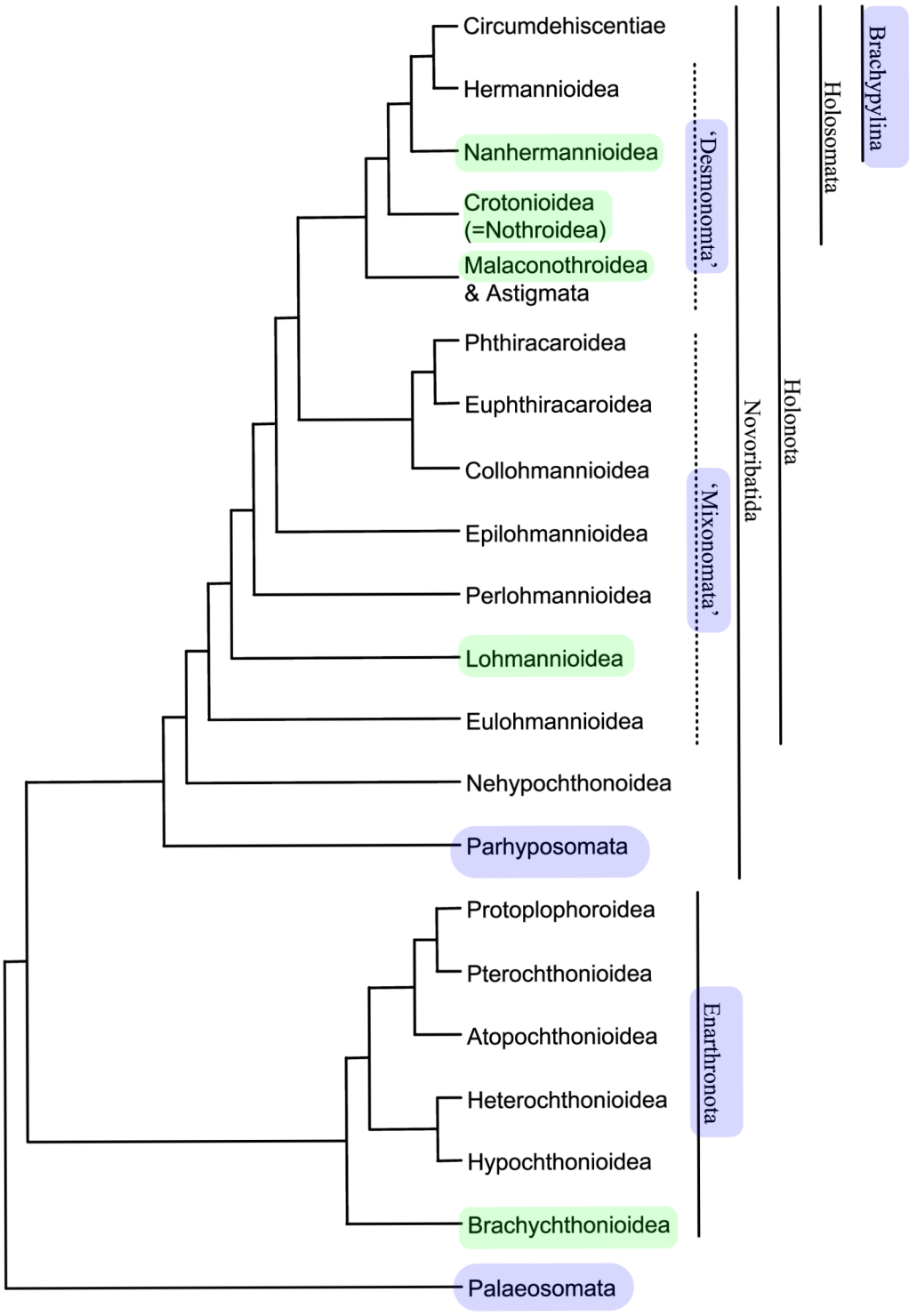

Figure 1: Cladogram of the phylogenetic relationships of oribatid mites based on morphological characters as depicted in Maraun et al. (2004). Six major groups established by Grandjean (1969) are highlighted with blue and highlighted with green are superfamilies with parthenogenetic lineages.

1.1.2. Classification of oribatid mites

Conventionally, based on morphological data and following the hypothesis presented by Grandjean (1969), oribatids are divided into six major groups: (1) the primitive sack-like **Palaeosomata** (4 families with less than 50 species) with a limited degree of sclerotization, found worldwide in all types of habitats, characteristic for dry soils, (2) the armoured **Enarthronota** abundant especially in dry soils or mosses with a high concentration of parthenogenetic species, their fossils are known from the Devonian era, (3) the small group **Parhyposomata** (only three families with 18 species) resembling immature oribatids, (4) the paraphyletic **Mixomonata** also known as true box mites occurring in soils rich in an organic litter, they often burrow in wood litter or live in the tree bark, (5) species-rich group **Desmonomata** (more than 7 500 species described), with a high concentration of parthenogenetic species and (6) **Brachypylina**, the so-called "higher" Oribatida – the most diverse and derived lineage.

A complete phylogenetic system of oribatid mites does not exist yet, as they are highly diverse, and the taxon sampling is usually limited per one study. Many authors focused their studies on the phylogenetic relationship between lower taxonomical groups of Oribatida (Salomone et al. 2002; Heethoff et al. 2007; Schäffer et al. 2010a; Kreipe et al. 2015; Pachl et al. 2017). A hypothetical phylogeny of oribatid mites based on morphology, as extracted from Maraun et al. (2004), is depicted in Figure 1.

1.2. Oribatid mites and changes in the environment

On a local scale, oribatid mites could be pretty efficient bioindicators for evaluating the impact of disturbances and environmental changes on the ecosystems as they occur (especially in temperate zone habitats) in high species numbers and densities. Most oribatid mites are connected to the upper soil layers with organic matter, where the conditions can shift rapidly. Oribatids are sensitive to fluctuations, especially in abiotic factors, as they are unable to escape unfavourable conditions due to their limited long-distance dispersal abilities (Ojala and Huhta 2001). Hence the recolonization after large-scale or repeated disturbances may be slow, and the changes in their populations can be evident even after decades (Lindberg and Bengtsson 2006).

The main abiotic factors regulating oribatid mite populations in the soil are moisture and temperature. Generally, with increasing temperature and decreasing moisture, the diversity and density of oribatids is declining. Adult oribatid mites have developed many defence mechanisms that should protect them from drying up, most importantly, sclerotized cuticle and a strong layer of cerotegument on the body surface (Starý 1990). However, less sclerotized juveniles are more susceptible to shifts in moisture.

Moreover, the effect of drought on mesofauna communities might be indirect, as it might negatively affect the availability of food resources – a decrease in biomass and diversity of fungi and other microorganisms (Taylor and Wolters 2005). The previous shows that the interaction of Oribatida with other soil organisms is complex and very often connected to the shifts in abiotic conditions (Maraun et al. 2020).

1.2.1. Montane spruce forests and their management

Without human intervention, nearly all mountain regions in Central Europe would be covered with mixed forest types dominated by European beech (*Fagus sylvatica*) and silver fir (*Abies alba*), with the top parts crossing to sub-alpine forests dominated by Norway spruce (*Picea abies*) (Svoboda et

al. 2012). Since the bronze age and especially during the last few centuries, when the human population grew rapidly, the natural forest ecosystems (not only in Europe) had to step aside to agricultural lands or to coniferous monocultures meant to provide wood for the industry. Only a small part of montane spruce forests nowadays is considered in a relatively natural state.

Natural disturbances (i.e., fire, windstorms, and insect outbreaks) are key driving factors of forest ecosystem dynamics worldwide. They strongly impact forest structure and composition, and their scale and effects differ according to the forest type. For maintaining the structure and functioning of natural montane spruce forests in Central Europe, the large-scale and high-severity disturbance events, particularly windstorms followed up by insect outbreaks, seem to be essential (Svoboda et al. 2012). These events preserve biological diversity by creating a mosaic of differently aged forests with different types of abiotic and biotic conditions. For efficient forest management, it is crucial to understand the effects of disturbance regimes that will likely intensify with the ongoing climate change (Seidl et al. 2017).

The Bohemian Forest, a complexly forested mountain region where the first part of this thesis was conducted, lies on the borders of the Czech Republic, Germany, and Austria. The most valuable localities with natural or semi-natural Norway spruce forests are included in the National Parks both in the Czech Republic and Germany. During the 1990s, a series of strong windstorms destroyed a vast area of the natural spruce forests along the Czech and German border in both National Parks. Subsequently, massive bark beetle (*Ips typographus*) outbreaks devastated most mature trees in the locality. And additionally, in January 2007, another strong windstorm destroyed the rest of the surviving spruce trees.

Two utterly opposing management practices were used in the area in the Czech Republic to protect the rest of the healthy forests from bark beetle. While parts of the affected forest areas in the core zones of the National Park Šumava were left to regenerate naturally without human intervention, others were clearcut logged, the wood and branches completely removed, and then new seedlings were planted. Forest management practices have a

considerable impact not only on the overground biota of the managed area but also on less visible parts of the system, in particular the soil layer.

After a natural disturbance, the affected forest ecosystem is already altered (i.e., change of the microclimatic conditions, disrupted soil, and changes in the organic matter content and nutrient fluxes) and with subsequent logging of dead trees, this disruption is intensifying (Lindenmayer and Noss 2006). The most extreme changes are in temperature and moisture. The temperature is elevated on the large forest openings, and its daily fluctuations above the soil surface are more significant than in the mature or naturally regenerating forest (Hais and Kučera 2008). Soil moisture is decreasing due to the higher temperatures and due to the higher outflow caused by soil compaction and missing herb layer after logging. On the contrary, the microclimatic conditions in the non-intervention decaying spruce forest naturally regenerating after the disturbance are very similar to those in the mature forest (Hais and Kučera 2008). Decaying wood and standing dead trees help to hold soil moisture and temperature and provide many shaded places and sheltered microhabitats, protecting microbiota and soil fauna from desiccation (Jabin et al. 2004).

The changes described above dramatically impact the soil biota and the processes in which they participate. Immediately after the disturbance, the abundance of Oribatid mites declines rapidly, and some sensitive species could completely disappear (Siepel 1996). The most dominant species quickly become eurytopic and parthenogenetic species (i.e., *Tectocepheus velatus, Platynothrus peltifer,* or *Oppiella nova*) as they are more tolerant to stress conditions (Behan-Pelletier 1999). Clearcut salvage-logging is an extreme type of ecosystem disturbance, especially its size and the type of used mechanization decides how big the impact on the soil fauna would be (Siepel 1996).

The two different management practices used in the National Park Šumava after the windstorm and bark beetle outbreak offered a unique opportunity to survey the impact of different post-disturbance regimes on the structure of oribatid mite communities in the long term. Overall, oribatid mites have the

little capacity (low metabolic rates, slow development, low fecundity, and low dispersal ability) to respond fast to environmental alternations (Behan-Pelletier 1999). Not many studies were performed to evaluate the development of the oribatid mite community in recovering non-intervention spruce forests. However, this knowledge might help simplify the decision about which management practices apply in the protected areas to be less harmful to soil biota.

1.3. Review of molecular markers used in the research of oribatid mites

The rapid development of molecular biology techniques over the last three decades supported resolving diverse biological issues, including population genetics, phylogenetic reconstructions, and taxonomy. Various types of molecular markers are currently used for the DNA-based research of mites on different levels (Zhao et al. 2020). However, most of the published studies were aimed at parasitic and medically or economically important mites, the soil-dwelling species are still often neglected, and their research is expanding slowly (Dabert 2006; Zhao et al. 2020). A proper selection of suitable DNA markers should be made before starting any new ones. It is important to point out that using the same set of markers as in already published studies brings many advantages, most notably an opportunity to combine newly obtained data with sequences reported in publicly available databases. The form of obtained DNA information depends on the chosen DNA fragment, its evolution and mutation rates, and the organism's life traits that might simplify the fixation of mutation that brings an advantage for the organism (dos Santos and Tixier 2017). Typically, fast-evolving genes are usually suitable for studying relationships between recently diverged taxa (at species or population level), while highly conserved genes are useful in assessing deeper phylogenetic nodes (dos Santos and Tixier 2017).

Salomone et al. (1996) published the very first study of oribatid mites where the amplification of a fragment of nowadays popular mitochondrial cytochrome c oxidase I (COI – commonly used for DNA barcoding, i.e., species identification) gene was used to clarify the confusion in the determination of two *Steganacarus* species based on their missing morphological differences (Bernini and Avanzati 1988). The choice of COI was not accidental. Back then, mitochondrial DNA was already widely used for studying evolutionary relationships of related species in various animal groups, and a proper set of universal arthropod primers for the COI gene was already designed (Simon et al. 1994). Since then, more than 60 scientific papers dealing with oribatid's DNA on different taxonomic levels have been published. According to what

the authors try to clarify, it is possible to categorize the papers into three main topics: (i) the most extensive portion is dedicated to phylogenetic reconstructions of Oribatida on different taxonomic levels, (ii) investigation of the origin of parthenogenetic species, and (iii) search for a molecular marker suitable for species identification.

1.3.1. Phylogenetic and phylogeographic studies

The investigation of phylogeny tries to map evolutionary history and relationships on different taxonomic levels, which for some groups of invertebrates resulted in deep modifications in the classification (dos Santos and Tixier 2017). In order to describe the entire evolution of a taxonomic group, a combination of different genes providing different information might be required, as fast evolving genes are not able to resolve deep nodes because of multiple substitutions and contrary, highly conserved genes do not work for resolving of the relationships between recently diverged taxa (dos Santos and Tixier (2017).

Several different molecular markers, mitochondrial as well as nuclear, were used in the phylogenetic reconstructions of Oribatid mites as the authors tried to figure out which marker would answer their questions best. Most papers used the combination of mitochondrial COI and a nuclear gene.

The most extensive insight into the phylogeny of Oribatida was done by Maraun et al. (2004; phylogenetic reconstruction based on the D3 region of the 28S rDNA gene), Dabert et al. (2010; a combination of COI and 18S rDNA) and Pachl et al. (2017; a combination of 18S rDNA and 28S rDNA), who included species of all six major taxa defined by Grandjean (1969) (Chapter 1.1.2. and Figure 1). Despite the necessarily limited taxon sampling, these studies provided the first glimpse into the DNA-based phylogeny that roughly follows the one based on morphology. The obtained phylogenetic trees constructed from the sequences of nuclear genes provided similar resolution of the relationship between the major taxa, and therefore, these genes seem to be suitable for phylogenetic reconstructions of deep nodes in oribatid

mites. However, further investigation based on more extensive sampling is required.

Moreover, Dabert et al. (2010) tried to reconstruct phylogenetic relationships within Acariformes. The study confirmed the monophyly of this superorder and recognized two orders within Acariformes: Sarcoptiformes (Endeostigmata + Oribatida + Astigmata) and Trombidiformes (=Prostigmata).

A greater part of the studies focused on lower taxonomic levels and tried to reconstruct the relationship at the family or genus level. Again, a combination of COI and a nuclear gene was used frequently. For instance: Schäffer et al. (2010a) analyzed the genus *Scutovertex* with the use of mitochondrial COI gene and two nuclear (28S rDNA and a coding gene for elongation factor 1-α (*ef 1-α*)) genes in combination with a morphological approach. Both genetic and morphological data revealed paraphyly in this genus and suggested the existence of several cryptic species. Pfingstl et al. (2018 and 2019) used COI and 18S rDNA combined with a morphological approach for phylogeny and description of intertidal mites. Lienhard and Krisper (2021) described five new distinct species of the genus *Caleremaeus* based on the COI and *ef 1-α* genes, and also, minor morphological differences were found.

Phylogeographic investigation tries to clarify the origin of geographical structuring of genetic variation within and among closely related species across the landscape, combining biogeography, population genetics and phylogenetic analysis (Emerson et al. 2011). Schäffer et al. (2010b) investigated the evolutionary and demographic history of *Scutovertex minutus* and *Scutovertes sculptus* with the use of the COI gene and revealed distinct phylogeographic patterns in these closely related species. While populations of *S. minutus* formed a clear geographic pattern, no phylogeographic structure was detected in *S. sculptus*. Rosenberger et al. (2013) tried to track the impact of climatic oscillations during the Pleistocene that affected the genetic and species diversity and the distribution of European flora and fauna on the populations of widespread species *Steganacarus magnus* with the use of COI in addition with the nuclear gene *ef 1-α*. Genetic diversity in the COI gene was

very high (32%), and the analysis of *ef 1-α* (a subset of 26 individuals, mean p-distances between populations 5.9%) supported the existence of distinct genetic lineages within Europe. However, the detected value of COI diversity might indicate the presence of cryptic species within *S. magnus* populations.

To sum it up, the COI gene provided good resolution between the populations, which makes COI a good marker for phylogeographic studies of populations or closely related species. In contrast, the nuclear genes sometimes fail to distinguish between closely related species but are suitable for resolving deep nodes in the phylogeny. In all the studies, the importance of combining different approaches (i.e., genetic, morphological, ecological) is highlighted and it is necessary to redescribe the systematics of highly diverse taxa such as oribatid mites.

1.3.2. Parthenogenetic oribatid mites

One of the unsolved mysteries in evolutionary biology is the dominance of sexual reproduction in the animal kingdom despite its many evolutionary costs - such as dilution of the genome, production of males, mate searching, and mating (Bell 1982; Brandeis 2018). The consensus that sex and gene recombination are beneficial for the long-term persistence of species was established. However, asexual species seem to have a number of advantages when compared to sexual ones, especially the twofold benefit of not producing males and not diluting their genome (Williams 1975; Tagg et al. 2005). In theory, parthenogenetic species should outcompete sexual ones in most environments as they show faster colonization and population growth in new habitats (Bell 1982; Lindberg and Bengtsson 2006; Schön 2007), yet only 1% of Metazoa reproduces parthenogenetically (Bell 1982).

Parthenogenesis is defined as the development of offspring from unfertilized eggs (Bell 1982). Thelytoky species (like parthenogenetic Oribatida), the "true" parthenogens, are comprised exclusively of females who develop from unfertilized eggs.

In general, all the theories that try to explain why sex dominates in most Metazoa uniformly predict the extinction of the parthenogenetic species in the long term (Williams 1975; Bell 1982; Barton and Charlesworth 1998; West et al. 1999; Birky et al. 2005). For species' long-term survival, sexual reproduction seems essential thanks to an accelerated rate of adaptive evolution to changing environmental conditions and prevention of accumulation of deleterious mutations by gene recombination.

Despite the predictions about parthenogenetic lineages being "dead ends" in evolution, three very old (millions of years) clusters of parthenogenetic species do exist, suggesting that they have radiated into discrete genetic and morphological entities without sexual reproduction (Barraclough et al. 2003). These monophyletic groups are Bdelloidea – the class of Rotifera (including 363 species, Welch and Meselson 2000), Darwinulidae – the family of Ostracoda (with 36 species, Martens et al. 2003), and several species-rich clades of oribatid mites (Maraun et al. 2004).

In fact, more than 10% of described Oribatida species are known to reproduce exclusively via parthenogenesis (Maraun et al. 2004). These clades of parthenogens are usually species-rich and presumably radiated while being parthenogenetic. Some of them belong to Desmonomata (families Nanhemanniidae 56 spp., Malaconothridae 104 spp., Trhypochthoniidae 68 spp., Camisiidae 92 spp. and the genus *Nothrus* 54 spp.), while the others belong to Enarthronota (family Brachychthoniidae 102sp.) and Mixonomata (family Lohmanniidae 156sp.) (Maraun et al. 2004), see Figure 1 for visualization.

Maraun et al. (2003) used comparisons of sequence variability of the D3 region of closely related parthenogenetic species to demonstrate that these species did not recently split from a sexual lineage and radiated asexually. Further phylogenetic analyses of the D3 region confirmed ancient asexual radiation into distinct genetic and morphologic species in the rest of the above-mentioned taxa (Maraun et al. 2004). Laumann et al. (2007) combined four nuclear genes (18S and 28S rDNA and coding genes for heat shock protein 82 (*hsp82*) and *ef 1-α*) to prove that three known morphotypes of the

parthenogenetic genus *Tectocepheus* are distinct species that evolved in the absence of sexual reproduction.

Interestingly, one case of revolution of sex from parthenogenetic ancestors, which is unique in the animal kingdom, was described in the family Crotoniidae that frequently colonize trees, which suggests that sexual reproduction is crucial under certain environmental conditions (combination of 18S rDNA, *hsp82* and *ef 1-α;* Domes et al. 2007). Pachl et al. (2020) used 18S rDNA to investigate the diversification of Acariformes and found that the primary mode of reproduction during the evolution was sexual. However, species-rich parthenogenetic taxa evolved at least four times independently in Oribatida and confirmed the results of all other previous studies suggesting that these clusters diversified without sexual reproduction.

1.3.3. Which molecular marker for species identification?

The correct identification of species is fundamental for the research of biodiversity, biogeography, ecology, or genetics. For a long, the classification and delimitation of oribatid mites were mainly based on the morphological characteristics of each species. This field could be very challenging as they are a diverse group of microscopic arthropods with many morphologically similar species. Moreover, even for small-scale studies, many individuals are usually extracted from the soil. Therefore, identification at the species level is often expert- and time-limited (Zhao et al. 2020). The previous is valid for adult oribatids, yet juvenile identification is often impossible. This all signals a need for a new approach to taxon recognition.

Hebert et al. (2003) proposed using DNA sequences as genetic "barcodes" (the analogy to the Universal Product Codes that uniquely identify commercial products) for species identification. As the best candidate for the DNA barcode, they chose a part of a protein-coding gene, cytochrome c oxidase I, as the universal primers for this gene are very robust, and COI appears to possess a greater range of phylogenetic signal than any other mitochondrial gene. It allows discrimination of not only closely allied species but also

phylogeographic groups within a single species. The intraspecific divergences in this gene are ordinarily much lower among individuals of one species than interspecific divergences between closely related species. The divergence over 3% in the nucleotide sequences, often termed as “barcoding gap”, was originally taken as an indication of a distinct species' existence (Hebert et al. 2004a).

A web platform with an online database, The Barcode of Life Data Systems (BOLD), was created to collect, manage, and share DNA barcode data. To date, there are only 754 species with barcodes from an order Sarcoptiformes recorded in BOLD, which is only about 5% of all described species. A huge oribatid mite sampling was performed in Canada. They employed DNA barcoding to begin the assessment of mite diversity across the country; today, approximately 60-70% of 230 recognized oribatid mite families are currently supported by barcode clusters (Beaulieu et al. 2019).

Latter studies revealed that the standard COI barcoding gene shows in general more variability between the populations of flightless or less mobile widespread taxa (Papadopoulou et al. 2009), which was already documented also for the populations of soil-living microarthropods (Schäffer et al. 2010a; Rosenberger et al. 2013; Saltzwedel et al. 2016, 2017; Parimuchová et al. 2017). In oribatid mites, the intraspecific COI variances vary noticeably among species - i.e., up to 2% - *Platynothrus peltifer* (see Heethoff et al. 2007); 4% - *Scutovertex minutus* (see Schäffer et al. 2010b); 1.24 – 1.78% - *Carinozetes bermudensis* (see Pfingstl et al. 2014); up to 0.6% - cryptic species complex of *Cymbaeremaeus*, however, the number of analyzed individuals was low (see Schäffer et al. 2019); up to 4.2% - cryptic species complex of *Caleremaeus* (see Lienhard and Krisper 2021).

Later, other thresholds above which the COI gene indicates distinct species were suggested, i.e., 10x the mean intraspecific divergence (Hebert et al. 2004b) or it can be calculated independently for each empirical dataset (for instance Automatic Barcode Gap Discovery (ABGD) available online) (Puillandre et al. 2012). Usage of the COI as a reliable species marker is controversial and requires further investigation, as it was already

demonstrated that the accuracy of species delimitation is influenced by the quality of the reference database, the geographic extent of sampling, and the intensity of intraspecific sampling (Chapple and Ritchie 2013).

Recently, employing the D3 region of the nuclear 28S rDNA gene as a species marker for oribatids was proposed (Lehmitz and Decker 2017). Lehmitz and Decker (2017) examined sequences from 89 species, observed intraspecific variability of the D3 fragment was low, ranging from 0 to 0.5%, which is promising (average interspecific variability was 2.7%). However, only 93% of the analyzed species were identified successfully, as the D3 fragment fails to separate between some closely related taxa. The same issue was reported in multiple other studies (Maraun et al. 2003; Kreipe et al. 2015; Schäffer et al. 2019; Schäffer and Koblmüller 2020). Studies based on the ribosomal 18S rDNA region indicate that this gene separates oribatid mite species well (Dabert et al. 2010; Pachl et al. 2017; Schäffer and Koblmüller 2020). Yet, any proper investigation of the intraspecific variation of 18S in Oribatida was not presented until now. Overall, this issue is still not fully resolved. The suitable species marker in combination with metabarcoding, that does not focus on one specific organism but aims to determine complete species composition within a sample and enables time- and cost-effective assessment of diversity by allowing the analysis of bulk samples of specimens or soil (Young and Hebert 2022), might help with large scale studies.

2. Aims of the thesis

The thesis revolves around different ecological and genetic aspects affecting the populations of oribatid mites in Central Europe.

The main aims were set as follows:

1) Exploring the oribatid mite communities and their diversity in Norway spruce forests of the Šumava Mountains affected by windstorms, bark beetle outbreaks and subsequent management practices.

2) Selection of DNA markers suitable for studying genetic variability in the populations of oribatid mites.

3) Analysis of genetic variability in the geographically distant populations of selected related species.

3. Summary of results and discussion

3.1. Outline of the research

This thesis is based on three papers. In the Paper I, the effects of natural disturbances (windstorms and bark beetle outbreak) and subsequent management on the oribatid mites in the montane spruce forest in the National Park are described in terms of their abundance, diversity, species composition and functional traits. The most valuable parts of the affected area were left to regenerate naturally, while the rest was clear-cut, the wood was removed, and new spruce seedlings were planted. We expected that the oribatid mite community at the naturally regenerating sites would be in better condition (higher abundance and diversity) and similar to the communities from control sites in mature spruce forest, which was confirmed in our study.

The time-consuming and often difficult determination of oribatid mites to species level under the microscope brought us to the thought of exploring the possibilities of identification based on DNA markers. A review of the papers that used molecular markers (summarized in chapter 1.2. of this thesis) pointed out that the selection of a suitable species marker for oribatids is still not fully resolved and much more data are needed. On the other hand, a DNA marker for phylogeographic studies of populations was already well established and we decided to focus our following research on this topic. Two species from one genus with completely different ecology, one widespread and the other known mostly from caves, were selected. Their genetic variability and phytogeography in Central Europe were studied in the Paper II.

And finally, in the Paper III a new species discovered during the analysis of the data obtained for the Paper II, is described in terms of its morphology and genetics.

3.2. Research Paper I

Communities of oribatid mites (Acari: Oribatida) of naturally regenerating and salvage-logged montane spruce forests of Šumava Mountains.

During the 1990s series of windstorms followed by a massive bark beetle outbreak devastated a vast part of the Norway spruce forests in the Šumava National Park. Although most of the affected forest was part of the protected area, two utterly different management strategies were applied. The most valuable parts (so-called "core" zones of the NP) were left to regenerate naturally without human intervention, while the rest of the forest was salvage-logged, the dead wood was removed, and new spruce seedlings were planted.

The study was part of more extensive research, where other soil faunas and soil conditions were observed, conducted on the selected sampling sites near Březník Mt. in one of the core zones of the Šumava NP. Our aim was to explore the effect of applied management on the structure of the oribatid mite communities and to compare it with the community from the mature spruce forest. As the soil samples were taken more than 15 years after the disturbance, the communities should have enough time to regenerate. The analysis of the impact of applied management on oribatid mite communities was significant. We found the communities on the naturally regenerating sites in better condition than those on salvage-logged ones, with the species composition and density similar to the control site. The abundances observed on the salvage-logged sites reached only numbers normally found in the forest opening or meadows with often occurrence of heliophilous and xerophilous species. Conversely, no significant differences were discovered in the occurrence of sexual vs. parthenogenetic species and in the trophic structure of the community. Our results showed that the changes caused by logging in the montane spruce forest ecosystems could be pronounced for an extended time period, at least for oribatid mites.

3.3. Research Paper II

Distinct phylogeographic patterns in populations of two oribatid mite species from the genus *Pantelozetes* (Acari, Oribatida, Thyrisomidae) in Central Europe.

In this study, two molecular markers (mitochondrial COI, nuclear D3 region) were used to track the phylogenetic relationships between populations of two related oribatid mite species, one documented mainly from caves in Central Europe and the other widespread in soils across the Holarctic region.

Despite our expectation to find phylogeographic structure in the populations of both species, only troglobiotic *P.cavaticus* formed a clear pattern with two genetically distant, geographically separated lineages – Czech and Slovak. The molecular clock dating set the parting of these lineages into the Late Pliocene during which climatic and biotic changes (cooling, spread of grasslands) occurred in Europe and may have forced the ancestors of current *P.cavaticus* to escape the changing surface conditions to cave ecosystems that offer more stable climatic conditions. Despite this ancient separation, the individuals from both lineages remained morphologically consistent. Moreover, the analysis of the COI sequences led to a morphological reinspection of one cave population because of its high interspecific genetic distance from the rest of the populations (more than 20%) and revealed the existence of a new species.

In contrast, individuals from the populations of *P.paolii* were divided into three separate genetic lineages not corresponding with the geographical distance of the sampling sites. Individuals from different lineages even coexisted at some sites. This points to this species's effective long-distance dispersal ability, possibly in the feather of birds. Again, no evident morphological differences were found.

In this study, we confirmed that COI is a good DNA marker for the phylogeographic research of oribatid mite populations as its intraspecific

variation is high. On the other hand, the nuclear D3 fragment is too conserved to distinguish between populations or even closely related species.

3.4. Research Paper III

A new species of the genus *Pantelozetes* (Acari, Oribatida, Thyrisomidae) from the caves of Eastern Slovakia.

In the last chapter of this thesis, a novel species is described in terms of its morphology and genetics. The analysis of the COI sequences obtained for the study of *Pantelozetes cavaticus* revealed in one population a significant imbalance in the distribution of genetic variability, while between the other populations the variability was around 2 to 5%, in this case the variability was more than 20% which is the level of COI nucleotide variability usually observed between distinct species. The specimens from this population were re-examined under the stereomicroscope and some noticeable morphological differences were detected – especially tridactylous tarsi (vs. monodactylous tarsi), the punctulation between the anterior part of notogaster (vs. smooth), the absence of solenidion on tarsi, and the rounded posterior part of notogaster (vs. bluntly pointed). The species was named *Pantelozes kunsti* and its delimitation from the other three common European species of the genus *Pantelozetes* was verified by further analysis of their COI sequences. This study shows that COI can be used as good supplementary evidence for species delimitation if another independent (morphologic) characteristics are involved.

Moreover, we provided a revised identification key to the known species of the genus *Pantelozetes* from Europe. This paper was submitted to the journal Systematic and Applied Acarology and is now under revision.

3.5. Conclusions and future perspectives

The effects of forest management after natural disturbance on the communities of oribatid mites were examined in the initial stage of the presented project. The management selection in the commercial spruce forest attacked by bark beetle is clear – clear-cutting and logging to prevent further spreading of bark beetles. However, these techniques also negatively impact the soil fauna and its perspective regeneration. Protection of soil fauna in the forest ecosystems of National Parks requires an entirely different and more sensitive approach.

In terrestrial ecosystems, the soil contains up to 90% of species diversity and biomass (Anderson 2009), yet the knowledge of soil fauna as indicators of ecosystem disturbance is still fragmentary. Understanding what is happening in the soil after a disturbance is essential for assessing the impact and possibly applying reasonable practices to ease the regeneration of the affected ecosystem. Changes in the soil might considerably alter the restoration of the aboveground plant layer. The disturbed spruce forests in the Šumava National Park and the different management practices that were applied offered valuable insight into the development of soil fauna.

Our research confirmed that management practices alter the composition of oribatid mite communities after large-scale disturbances even more than 15 years later. Siepel (1996) showed that changes caused by logging could persist over decades. Results clearly favor the natural regeneration of the spruce forest ecosystem, at least from the perspective of oribatid mites. The communities on naturally regenerating sites were recovering well and resembled the communities from healthy forest ecosystem in terms of species composition and abundance, which was in agreement with the results of other studies (Starý and Matějka 2008; Farská et al. 2014). On the other hand, the communities from salvage-logged sites were more similar to a meadow community with an increased concentration of heliophilous species and low abundance. Nevertheless, many questions remain to be addressed for a deeper understanding of shifts in oribatid mite communities and their interaction with other parts of soil and aboveground ecosystems.

In addition to the traditional approach (total abundance, diversity indices and species composition), we also considered functional traits (reproduction mode, food preferences), which might be a good indicator of community change and allow us to identify general patterns (Vandewalle et al. 2010). However, the success of analyses based on functional traits largely depends on the definition of these functional groups (Pik et al. 1999). Hevia et al. (2017) identified body size and diet as the two most common invertebrate traits showing significant relationships with land management. There is still a severe limitation for the broader use of functional traits in Oribatida, as many characteristics even of widespread species are missing or differ in the available literature. More research in this field could bring valuable information.

The overall shortage of acarologists, lack of identification keys and time-consuming determination of Oribatida to species level might be partially solved with the concept of taxonomic sufficiency (TS; Ellis 1985). TS is based on selecting coarser taxonomic resolution (i.e., families or genera instead of species) that is still able to recognize ecological patterns in the community. TS is well established for some terrestrial invertebrate taxa (Pik et al. 1999; Groc et al. 2010), but it is not frequently applied to oribatid mites. Correctly chosen aggregated taxonomic levels are essential in this concept to avoid losing the information. The results of Minor et al. (2017) suggest that the best taxonomic level for Oribatida for maintaining ecological information and to ease of identification appears to be suborders and superfamilies in case of Brachypylina. As TS is still not well established in the research of Oribatida, we decided to explore the possibilities of DNA markers for species identification. However, this question seems to be very complicated and requires much deeper investigation. It also overreached possibilities (time and financial) of this thesis, as mitochondrial COI, traditionally used for species identification in other taxa, displays high intraspecific variability in Oribatida.

Using of COI might represent a good way for conducting quick, preliminary studies of poorly known taxonomic groups or geographic regions with the initial barcoding study providing a framework for more detailed taxonomic

approach (Chapple and Ritchie 2013) especially in combination with quickly evolving metabarcoding techniques.

Therefore, the second part of the project was focused on the genetic variability of isolated populations of oribatid mites, for which the COI gene was already well established as a suitable molecular marker. Our research confirmed it and revealed that even closely related species can display completely opposite phylogeographic patterns. While cave-dwelling *Pantelozetes cavaticus* created a clear pattern with populations from different karst areas being strictly separated from each other, soil living *Pantelozetes paolii* showed very effective long-dispersal abilities with individuals from sampling sites hundreds of kilometres apart sharing the identical haplotypes. Long-term geographic and genetic isolation of the populations of one species could provoke an evolution of new species different not only on the molecular level but also morphologically. The discovery of new species from the genus *Pantelozetes* in one of the studied caves seems to support this theory. However, these two studies provided only a first glimpse into the genetic diversity and evolutionary history of the examined species. Many other things remain to be discovered and described.

4. References:

Anderson J.M. (2009): Why should we care about soil fauna? Pesquisa Agropecuária Brasileira 44:835–842.

Barraclough T.G., Birky C.W. Jr., Burt A. (2003): Diversifications in sexual and asexual organisms. Evolution 57:2166–2172.

Barton N.H., Charlesworth B. (1998): Why sex and recombination? Science 281:1986-1990.

Beaulieu F., Knee W., Nowell V., Schwarzfeld M., Lindo Z., Behan-Pelletier V.M., Lumley L., Young M.R., Smith I., Proctor H.C., Mironov S.V., Galloway T.D., Walter D.E., Lindquist E.E. (2019): Acari of Canada. In: Langor D.W.,Sheffield C.S. (Eds): The Biota of Canada – A Biodiversity Assessment. Part 1: The terrestrial Arthropods. ZooKeys 819:77–168.

Behan-Pelletier V.M. (1999): Oribatid mite biodiversity in agroecosystems: role for bioindication. Agriculture, Ecosystems and Environment 74:411–423.

Bell G. (1982): The Masterpiece of Nature: The Evolution and Genetics of Sexuality. University of California Press, California. 635pp.

Bernini F., Avazenti A.M. (1988): Notulae oribatologicae XLVII. Intraspecific variability in *Tropacarus*: The example of *Steganacarus (Tropacarus) pulcherrimus* (Berlese, 1887) junior synonym of *S. (T.) carinatus* (Koch, 1841) (Acarida, Oribatida). Plant Ecology and Diversity 14(3):107-114).

Birky C.W. Jr., Wolf C., Maughan H., Hebertson L.,Henry E. (2005): Speciation and selection without sex. Hydrobiologia 546:29–45.

Brandeis M. (2018): New-age ideas about age-old sex: Separating meiosis from mating could solve a century-old conundrum. Biological Reviews 93(2):801–810.

Chapple D.G., Ritchie P.A. (2013): A retrospective approach to testing DNA barcoding method. PLOS ONE 8(11):e77882.

Cianciolo J.M., Norton R.A. (2006): The ecological distribution of reproductive mode in oribatid mites, as related to biological complexity. Experimental and Applied Acarology 40(1):1–25.

Coleman D.C., Crossley D.A. jr., Hendrix P.F. (2004): Fundamentals of Soil Ecology. Academic Press Inc., second edition, San Diego. 404pp.

Dabert M. (2006): DNA markers in the phylogenetics of the Acari. Biological Letters 43(2):97-107.

Dabert M., Witaliński W., Kaźmierski A., Olszanowski Z. (2010): Molecular phylogeny of acariform mites (Acari, Arachnida): Strong conflict between phylogenetic signal and long-branch attraction artifacts. Molecular Phylogenetics and Evolution 56(1):222-241).

Domes K., Norton R.A., Maraun M., Scheu S. (2007): Reevolution of sexuality breaks Dollo's law. PNAS 104(17):7139-7144.

Ellis D. (1985): Taxonomic sufficiency in pollution assessment. Marine Pollution Bulletin 16:442–461.

Emerson B.C., Cicconardi F., Fanciulli P.P., Shaw P.J.A. (2011): Phylogeny, phylogeography, phylobetadiversity and the molecular analysis of biological communities. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 366(1576):2391-2402.

Farská J., Prejzková K., Starý J., Rusek J. (2014): Soil microarthropods in non-intervention montane spruce forest regenerating after bark-beetle outbreak. Ecological Research 29: 1087 – 1096. **Grandjean F.** (1969): Considérations sur le classement des oribates leur division en 6 groupes majores. Acarologia 11(1):127-153.

Groc S., Delabie J.H.C., Longino J.T., Orivel J., Majer J.D., Vasconcelos H.L., Dejean A. (2010): A new method based on taxonomic sufficiency to simplify studies on Neotropical ant assemblages. Biological Conservation 143:2832–2839.

Hais M., Kučera T. (2008): Surface temperature change of spruce forest as a result of bark beetle attack: Remote sensing and GIS approach. European Journal of Forest Research 127:327-336.

Harte J., Rawa A., Price V. (1996): Effects of manipulated soil microclimate on mesofaunal biomass and diversity. Soil Biology and Biochemistry 28(3):313–322.

Hebert P.N.D., Cywinska A., Ball S.L., deWaard J.R. (2003): Biological identifications through DNA barcodes. Proceedings, Biological sciences 270(1512):313-321.

Hebert P.D.N., Penton E.H., Burns J.M., Janzen D.H., Hallwachs W. (2004a): Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. PNAS 101(41):14812–14817.

Hebert P.N.D., Stoeckle M.Y., Zemlak T.S., Francis C.M. (2004b): Identification of birds through DNA barcodes. PLOS Biology 2:1657-1663.

Heethoff M., Domes K., Laumann M., Maraun M, Norton R.A., Scheu S. (2007): High genetic divergences indicate ancient separation of parthenogenetic lineages of the oribatid mite *Platynothrus peltifer* (Acari, Oribatida). Journal of Evolutionary Biology 20(1):392–402.

Jabin M., Mohr D., Kappes H., Topp W. (2004): Influence of deadwood on density of soil macroarthropods in a managed oak-beech forest. Forest Ecology and Management 194:61-69.

Krantz G.W., Walter D.E. (eds., 2009): The manual of acarology. Third edition, Texas Tech University Press, 807pp.

Kreipe V., Corral-Hernández E., Sheu S., Schaefer I., Maraun M. (2015): Phylogeny and species delineation in European species of the genus *Steganacarus* (Acari, Oribatida) using mitochondrial and nuclear markers. Experimental Applied Acarology 66:173 – 186.

Laumann M., Norton R.A., Weigmann G., Scheu S., Maraun M., Heethoff M. (2007): Speciation in the parthenogenetic oribatid mite genus Tectocepheus (Acari, Oribatida) as indicated by molecular phylogeny. Pedobiologia 51 (2), pp. 111 – 122.

Lienhard A., Krisper G. (2021): Hidden biodiversity in microarthropods (Acari, Oribatida, Eremaeoidea, *Caleremaeus*). Scientific reports 11:23123.

Lindberg N., Bengtsson J. (2006): Recovery of forest soil fauna diversity and composition after repeated summer droughts. Oikos 114(3):494–506.

Lindenmayer D.B., Noss R.F. (2006): Salvage logging, ecosystem processes, and biodiversity conservation. Conservation Biology 20(4):949-958.

Lehmitz R., Decker P. (2017): The nuclear 28S gene fragment D3 as species marker in oribatid mites (Acari, Oribatida) from German peatlands. Experimental and Applied Acarology 71(3):259–276

Luxton M. (1972): Studies on the oribatid mites of a Danish beech wood soil, I. Nutritional biology. Pedobiologia 12(6):434–463.

Maraun M., Scheu S. (2000): The structure of oribatid mite communities (Acari, Oribatida): patterns, mechanisms and implications for future research. Ecography 23(3):374–383.

Maraun M., Salamon J.A., Schneider K., Schneider K., Schaefer M., Scheu S. (2003): Oribatid mite and collembolan diverzity, density and community structure in a moder beech forest (*Fagus sylvatica*): effects of mechanical perturbations. Soil Biology and Biochemistry 35(10):1387–1394.

Maraun M., Heethoff M., Schneider K., Scheu S., Weigmann G., Cianciolo J., Thomas R.H., Norton R.A. (2004): Molecular phylogeny of oribatid mites (Oribatida, Acari): evidence for multiple radiations of parthenogenetic lineages. Experimental and Applied Acarology 33(3):183–201.

Maraun M., Augustin D., Pollierer M.M., Scheu S. (2020): Variation in trophic niches of oribatid mites in temperate forest ecosystems as indicated by neutral lipid fatty acid patterns. Experimental and Applied Acarology 81:103-115.

Martens K., Rossetti G., Horne D. J. (2003): How ancient are ancient asexuals? Proceedings of the Royal Society of London B 270:723-729.

Minor M.A., Ermilov S.G., Tiunov A.V. (2017): Taxonomic resolution and functional traits in the analysis of tropical oribatid mite assemblages. Experimental and Applied Acarology 73:365-381.

Norton R.A., Bonamo P.M., Grierson J.D., Shears W.A. (1988): Oribatid mite fossils from a terrestrial Devonian deposit near Gilboa. New York Journal of Paleontology 62, pp.259-269.

Norton R.A. (1994): Evolutionary aspects of oribatid mite life histories and consequences for the origin of Astigmata. In: Houck M.A. (ed.): Mites: Ecological and Evolutionary Analyses of Life History Patterns. Chapman and Hall, New York, pp. 99–135.

Ojala R., Huhta V. (2001): Dispersal of microarthropods in forest soil. Pedobiologia 45:443–450.

Pachl P., Lindl A.C., Krause A., Scheu S., Schaefer I., Maraun M. (2017): The tropics as an ancient cradle of oribatid mite diversity. Acarologia 57(2):309–322.

Pachl P., Uusitalo M., Scheu S., Schaefer I., Maraun M. (2020): Repeated convergent evolution of parthenogenesis in Acariformes (Acari). Ecology and Evolution 11(1):321-337.

Papadopoulou A., Anastasiou I., Keskin B., Vogler A.P. (2009): Comparative phylogeography of tenebrionid beetles in the Aegean archipelago: the effect of dispersal ability and habitat preference. Molecular Ecology 18(11):2503–2517.

Parimuchová A., Kováč Ľ., Žurovcová M., Miklisová D., Paučulová L. (2017): A glacial relict in the Carpathian caves – population variability or a species complex? Arthropod Systematics and Phylogeny 75(3):351–362.

Pfingstl T., Baumann J., Lienhard A. (2014): The Caribbean enigma: the presence of unusual cryptic diversity in intertidal mites (Arachnida, Acari, Oribatida). Organisms Diversity and Evolution 19(4):609–623.

Pfingstl T., Lienhard A., Shimano S., Bin Yasin Z., Tan Shau-Hwai A., Jantarit S., Petcharad B. (2018): Systematics, genetics, and biogeography of intertidal mites (Acari, Oribatida) from the Andaman Sea and Strait of Malacca. Journal of Zoological Systematics and Evolutionary Research 57(1):91-112.

Pfingstl T., Lienhard A., Baumann J. (2019): New and cryptic species of intertidal mites (Acari, Oribatida) from the Western Caribbean – an integrative approach. International Journal of Acarology 45(1-2):10-25.

Pik A.J., Oliver I., Beattie A.J. (1999): Taxonomic sufficiency in ecological studies of terrestrial invertebrates. Australian Journal of Ecology 24:555–562.

Potapov A.M., Beaulieu F., Birkhofer K., Bluhm S.L., Degtyarev M.I. et al. (2022): Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. Biological Reviews 97(3):1057-1117.

Puillandre N., Lambert A., Brouillet S., Achaz G. (2012): ABGB, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology 21: 1864-1877.

Raspotnig G., Norton R.A., Heethoff M. (2011): Oribatid mites and skin alkaloids in poison frogs. Biology Letters 7(4):555–556.

Rosenberger M.J., Maraun M., Scheu S., Schaefer I. (2013): Pre– and post–glacial diversifications shape genetic complexity of soil-living microarthropod species. Pedobiologia 56(2):79–87.

Salomone N., Emerson B.C., Hewitt G.M., Bernini F. (2002): Phylogenetic relationships among the Canary Island Steganacaridae (Acari, Oribatida) inferred from mitochondrial DNA sequence data. Molecular Ecology 11:79-89.

Saltzwedel von H., Scheu S., Schaefer I. (2016): Founder events and pre-glacial divergences shape the genetic structure of European Collembola species. BMC Evolutionary Biology 16:148.

Saltzwedel von H., Scheu S., Schaefer I. (2017): Genetic structure and distribution of *Parisotoma notabilis* (Collembola) in Europe: Cryptic diversity, split of lineages and colonization patterns. PLoS ONE 12(2):e0170909

Salomone N., Frati F., Bernini F. (1996): Investigation on the taxonomic status of *Steganacarus magnus* and *Steganacarus anomalus* (Acari: Oribatida) using mitochondrial DNA sequences. Experimental and Applied Acarology 20(11): 607-615.

dos Santos V.V., Tixier M.S. (2016): Which molecular markers for assessing which taxonomic level? The case study of the mite family Phytoseiidae (Acari: Mesostigmata). Cladistics 33(3):251-267.

Seidl R., Thom D., Kautz M., Martin-Benito D., Peltoniemi M., Vacchiano G., Wild J., Ascoli D., Petr M., Honkaniemi J., Lexer M.J., Trotsiuk V., Mairota P., Svoboda M., Fabrika M., Nagel T.A., Reyer C.P.O. (2017): Forest disturbances under climate change. Nature Climate Change 7(6):395–402.

Selden P.A. (2017): Arachnids. Reference Module in Life Sciences.

Schäffer S., Pfingstl T., Koblmüller S., Winkler K.A., Sturmbauer Ch., Krisper G. (2010a): Phylogenetic analysis of European *Scutovertex* mites (Acari, Oribatida, Scutoverticidae) reveals paraphyly and cryptic diversity: A molecular and morphological approach. Molecular Phylogenetics and Evolution 55(2):677-688.

Schäffer S., Koblmüller S., Pfingstl T., Sturmbauer Ch., Krisper G. (2010b): Contrasting mitochondrial DNA diversity estimates in Austrian *Scutovertex minutus* and *S. sculptus* (Acari, Oribatida, Brachypylina, Scutoverticidae). Pedobiologia 53(3):203-211.

Schäffer S., Kerschbaumer M., Koblmüller S. (2019): Multiple new species: Cryptic diversity in the widespread mite species *Cymberemaeus cymba* (Oribatida, Cymbaeremaeidae). Molecular Phylogenetics and Evolution 135:185–192.

Schäffer S., Koblmüller S. (2020): Unexpected diversity in the host-generalist oribatid mite *Paraleius leontonychus* (Oribatida, Scheloribatidae) phoretic on Palearctic bark beetles. PeerJ – Life and Environment 8:e9710.

Schaefer I., Norton R.A., Scheu S., Maraun M. (2010): Arthropod colonization of land - linking molecules and fossils in oribatid mites (Acari, Oribatida). Molecular Phylogenetics and Evolution 57(1):113-121.

Schneider K., Maraun M. (2005): Feeding preferences among dark pigmented fungal taxa ("Dematiacea") indicate limited trophic niche differentiation of oribatid mites (Oribatida, Acari). Pedobiologia 49(1):61–67.

Schneider K., Renker S., Maraun M. (2005): Oribatid mite (Acari, Oribatida) feeding on ectomycorrhizal fungi. Mycorrhiza 16(1):67–72.

Schön I. (2007): Did Pleistocene glaciations shape genetic patterns of European ostracods? A phylogeographic analysis of two species with asexual reproduction. Hydrobiologia 575:33-50.

Siepel H. (1996): The importance of unpredictable and short-term environmental extremes for biodiversity in oribatid mites. Biodiversity Letters 3(1):26–34.

Simon C., Frati F., Beckenbach A., Crespi B., Liu H., Flook P. (1994): Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. Annals of the Entomological Society of America 87(6):651-701.

Skubala P., Zaleski T. (2012): Heavy metal sensitivity and bioconcentration in oribatid mites (Acari, Oribatida). Gradient study in meadow ecosystems. Science of the Total Environment 414:364–372.

Starý J. (1990): Ekologie pancířníků (Acari: Oribatida) v sukcesní řadě půd. Kandidátská disertační práce, ÚPB ČSAV České Budějovice. 179pp.

Starý J., Matějka K. (2008): Pancířníci (Acari: Oribatida) vybraných lokalit horských lesů na Šumavě. An interim report from the project 2B06012 Management

biodiversity v Krkonoších a na Šumavě v roce 2007. http://www.infodatasys.cz/biodivkrsu/rep2007_Oribatida.pdf (1.9.2022).

Subías L.S. (2020): Listado sistemático, sinonímico y biogeográfico de los ácaros oribátidos (Acariformes: Oribatida) del mundo (excepto fósiles) (17ª actualización); 527pp. Online version (http://bba.bioucm.es/cont/docs/RO_1.pdf) accessed in July 2022.

Svoboda M., Janda P., Nagel T.A., Fraver S., Rejzek J., Bače R. (2012): Disturbance history of an old-growth sub-alpine *Picea abies* stand in the Bohemian Forest, Czech Republic. Journal of Vegetation Science 23(3): 86-97.

Tagg N., Doncaster C.P., Innes D.J. (2005): Resource competition between genetically varied and genetically uniform populations of *Daphnia pulex* (Leydig): does asexual reproduction confer a short-term ecological advantage? Biological Journal of the Linnean Society 85:111–123.

Taylor A.R., Wolters V. (2005): Responses of oribatid mite communities to summer drought: The influence of litter type and quality. Soil Biology and Biochemistry 37(11): 2117–2130.

Vandewalle M., de Bello F., Berg M.P. et al (2010): Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodiversity and Conservation 19:2921–2947.

Young M.R., Hebert P.D.N. (2022): Unearthing soil arthropod diversity through DNA metabarcoding. PeerJ – Life and Environment 10:e12845.

Walter D.E., Proctor H.E. (2013): Mites: Ecology, evolution and behaviour. Life at a microscale (second edition). Springer, Dordrecht. 494pp.

Wallwork J.A. (1983): Oribatids in forest ecosystems. Annual Review of Entomology 28:109-130.

Welch D.M., Meselson M. (2000): Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. Science 288(5469):1211-1215.

West S.A., Lively C.M., Read A.F. (1999): A pluralistic approach to sex and recombination. Journal of Evolutionary Biology 12(6):1003-1012.

Williams, G. C. (1975). Sex and evolution. Princeton University Press, London. Monographs on Population Biology, Volume 8. 210pp.

Zhao Y., Zhang W.Y., Wang R.L., Niu D.L. (2020): Divergent domains of 28S ribosomal RNA gene: DNA barcodes for molecular classification and identification of mites. Parasites & Vectors 13(251):1-12.

5. Attached publications

5.1. Paper I

DE GRUYTER

Biologia **72**/4: 445—451, 2017
Section Zoology
DOI: 10.1515/biolog-2017-0050

Communities of oribatid mites (Acari: Oribatida) of naturally regenerating and salvage-logged montane spruce forests of Šumava Mountains

Petra Kokořová[1,2] & Josef Starý[1]

[1]*Institute of Soil Biology, Biology Centre v.v.i, Academy of Sciences of the Czech Republic, Na Sádkách 7, CZ-37005, České Budějovice, Czech Republic; e-mail: petra.kokorova@post.cz, jstary@upb.cas.cz*
[2]*Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, Branišovská 1760, CZ-37005, České Budějovice, Czech Republic*

Abstract: The main aim of this study was to describe and compare communities of oribatid mites of selected areas in montane spruce forests near Březník in the Šumava National Park, where different management strategies were used after the spruce dieback caused by massive bark beetle gradation more than 15 years ago. Naturally regenerating and salvage-logged areas were compared. Significant differences were found in the oribatid mite community composition between differently managed areas. The oribatid mite community in naturally regenerating areas was in better shape and its abundance and species composition was similar to the community of a control area in a mature and healthy spruce forest.

Key words: oribatid mites; spruce forest; community; bark beetle gradation; forest management

Introduction

The national parks Šumava (Czech Republic) and the Bavarian Forest (Germany), which spread out across the Šumava Mts, represent one of the largest complexly forested areas in Central Europe. During whole its existence this area and especially its forests have been shaped by windstorms usually followed up by bark beetle (*Ips typographus* L., 1758) outbreaks of different range (Svoboda et al. 2012). Natural disturbances such as windstorms, insect outbreaks and fires are one of the key drivers of natural forests dynamics all over the world and therefore also in Central Europe (Svoboda et al. 2012). At the end of 1990s a large area of natural and semi-natural Norway spruce (*Picea abies*) forests on the top parts of the Šumava Mts suffered a series of windstorms followed by a significant bark beetle outbreak that killed almost all mature trees (Skuhravý 2002). If similar event happened in a commercial forest, the affected trees would be clear cut. However, disturbed ecosystems in natural parks need to be treated much more sensitively as natural processes are supposed to drive their development. In the Šumava National Park, two different management strategies were used. Part of the area located in the core zones of National Park was left to regenerate naturally, whereas the rest of the affected area was salvage-logged (dead trees were removed, branches and needles were left on site) (Jonášová & Prach 2004). Salvage-logging in such disturbed ecosystems can cause more damage than natural disturbance itself (Lindenmayer & Noss 2006). Besides destroying the seedlings waiting for their chance to grow and most of the vegetation cover, logging also seriously affects belowground parts of ecosystem (Marshall 2000). For example, in logged areas, soil and litter temperatures increase with higher day-time temperatures compared to naturally regenerating areas in which dead trees and remaining vegetation provide shadow and keep microclimatic conditions close to those in healthy forests (Hais & Kučera 2008; Griffin et al. 2011). Decaying wood is also important source of nutrients for the new generation of trees and soil organisms, especially in mountain ecosystems, and therefore its removal after dieback further depletes and damages ecosystems (Šantrůčková et al. 2010).

In our study we focused on one major group of soil microarthropods – oribatid mites (Acari: Oribatida), which are sensitive to moisture and temperature changes and play an important role in ecosystem functioning – particularly in organic matter decomposition and nutrient cycling (Niedbała 1980; Norton & Behan-Pelletier 2009). Oribatid mites are common in high densities in soils all over the world and particularly dominant in coniferous forests of temperate zones with well-developed organic horizons (Siira-Pietikäinen et al. 2008). Due to their slow reproduction and relatively long ontogenesis, oribatids are classified as K-strategists (Coleman et al. 2004), which means that they, together with their inability to escape unfavourable conditions due to limited long-distance dispersal (Ojala & Huhta 2001), react to changes in their environment slower than other mesofauna organisms and that their com-

Table 1. Exact GPS position and altitude of the study sites.

Site	N	E	Altitude (m a.s.l.)
S3	48°59′03.42″	13°25′26.08″	1,215
S5	48°58′42.65″	13°27′45.30″	1,283
S7	48°59′00.79″	13°25′54.62″	1,183
P2	48°59′06.78″	13°27′27.16″	1,214
P3	48°59′14.45″	13°26′10.67″	1,197
P5	48°58′39.04″	13°27′55.99″	1,288
M1	48°59′07.06″	13°25′35.44″	1,181
M4	48°59′19.08″	13°27′06.38″	1,185
M5	48°59′02.11″	13°26′54.81″	1,237
BSW	48°59′32.30″	13°49′01.92″	1,291

munities could be affected by disturbances even after decades.

The aim of our study was therefore to describe and compare oribatid mite communities of differently managed disturbed forests in terms of (1) density and diversity and (2) species composition. Considering the fact that the environmental conditions in naturally regenerating forest after bark beetle disturbance stay similar to those in undisturbed forest and that the soil is not damaged by logging, we hypothesized that the oribatid mite community in naturally regenerating forests will be in better condition than the community in salvage-logged forests with abundances and species composition close to those in mature, undisturbed forests.

Material and methods

The study area is located in the central part of the Šumava Mts (Czech Republic), close to Březník and Studená Mountain in the core zone of the Šumava National Park. This area was chosen because it already contains established study plots for the evaluation of vegetation cover (Jonášová & Prach 2008), soil characteristics (Krausová 2011; Pavlas 2014) and other soil fauna (Bryndová 2013; Velíšek 2014). The study sites are situated in acidophilic Norway spruce forests between 1,180 and 1,280 m a.s.l. (see Table 1 for exact position and altitude with GPS coordinates). The area is characterised by a cold and wet climate with a mean annual temperature of 4 °C and mean annual precipitation exceeding 1,500 mm (Jonášová & Prach 2008). The bedrock is dominated by gneiss partly combined with granodiorite covered with podsols and kambisols or gley soils in marshy forests. The forests of our study area are naturally dominated by Norway spruce (*Picea abies*) with addition of rowan (*Sorbus aucuparia*), European beech (*Fagus sylvatica*), silver fir (*Abies alba*) and hairy birch (*Betula pubescens*). The dominants of the herb layer are *Calamagrostis villosa*, *Deschampsia flexuosa*, and *Vaccinium myrtillus* (Jonášová & Prach 2008). The area suffered a massive bark beetle outbreak in the 1990s, followed by forest dieback. Different management strategies were used for regeneration.

We collected soil samples from nine study sites, of which three were located in a naturally regenerating forest (marked S – S3, S5, S7), three in a salvage-logged forest (marked P – P2, P3, P5) and three in a naturally regenerating marshy forest with moderate dieback of mature trees (marked M – M1, M4, M5). This design enabled us to compare oribatid mite communities' development in disturbed forests under different management strategies. As control site, we used a site located in the top part of Boubín Mountain in a mature healthy spruce forest (see Table 1 for GPS coordinates). Boubín is a part of the Šumava Lanscape Protected Area with weather and soil conditions similar to those in Březník, which is 25 kilometres to the east. For comparison, we used data from Starý & Matějka (2008) – i.e. for the site marked BSW, where 45 oribatid species were identified and the mean abundance was 101,000 ind. m^{-2}. This locality was sampled during spring and autumn 2007, using the same methodology as described in our paper.

Soil samples from our study sites (10 cm^2 surface area and 10 cm depth) were taken twice a year at the beginning of summer (June 2013 and 2014) and during autumn (October 2012 and 2013), when oribatid mite abundance is expected to be at its maximum. At each sampling time, we randomly took five soil samples from every site. Soil arthropods were extracted in a high-gradient extractor described by Marshall (1972) for five days with increasing temperatures. Specimen of Oribatida, Collembola, Gamasida and Tarsonemida were then counted in each sample. Oribatid mites were determined to species level (Kunst 1971; Balogh & Mahunka 1983; Weigmann 2006) and, when possible, juveniles were also determined, counted and added to respective adults.

Oribatid mite communities were described by mean abundance (A – individuals per square meter), species richness (R – number of species per the sample) and Shannon-Weaver's species diversity index (H') (Shannon & Weaver 1949). In addition, some life-history traits (reproductive mode, feeding guilds, biotope and moisture preferences) of determined species were used in this study to provide insight into community recovery and the role in ecosystem functioning after disturbance (Strenzke 1952; Schuster 1956; Hartenstein 1962; Kunst 1971; Luxton 1972; Giljarov & Krivoluckij 1975; Seniczak & Stefaniak 1978; Starý 1990; Schatz 1983; Weigmann 2006). These data were collected from multiple sources and are shown in Electronic supplementary file 1 (Table S4).

A preliminary nested ANOVA was performed at $P < 0.05$ to verify whether density and diversity indices differed between the soil samples collected at one study site. As we did not find significant differences, data from each site were pooled and treated together. Prior to analysis, data were log-transformed. Partial Principal Components analysis (PCA) with hierarchical design was used to evaluate the relationship between oribatid mite communities and forest management. A linear response model was selected for small scale gradients as recommended by Šmilauer & Lepš (2014). To compare the control site at Boubín with our sites, we used the data from Boubín as supplementary and then pro-

Table 2. Oribatid mite communities' characteristics.

Sampling site	S	R (mean ± SE)	A (ind. m^{-2})	H'
S3	43	8.85 ± 4.51	79,150	2.06
S5	35	6.35 ± 4.22	28,150	2.37
S7	45	10.05 ± 3.97	67,950	2.98
P2	41	7.40 ± 3.47	34,900	2.84
P3	39	5.60 ± 4.04	19,900	2.93
P5	39	5.70 ± 4.15	26,400	2.44
M1	42	6.85 ± 4.91	26,550	2.98
M4	33	5.90 ± 5.39	28,550	2.79
M5	47	8.05 ± 4.12	35,500	3.12

Explanations: S – total number of species found, R – species richness (mean ± SE), A – mean abundance (ind. m^{-2}), H' – Shannon-Weaver index of species diversity.

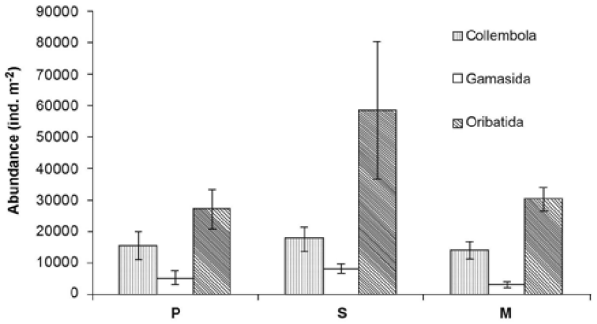

Fig. 1. Mean abundances ± SE of soil microarthropods at the sampling sites according to the type of used management. P – salvage-logged, S – naturally regenerating, M – marshy forest.

jected this site into the graph obtained by partial PCA of the influence of management on oribatid mite communities.

Partial Redundancy Analysis (RDA) with hierarchical design was used to analyse the influence of forest management on functional groups. We used the Monte Carlo permutation test (499 permutations) for evaluation of the methods. Analyses were carried out using Canoco (version 5) and Statistica 12.0.

Results

In total, we obtained 11,625 soil microarthropods during the extractions, of which 6,952 were oribatid mites (5,488 adults and 1,464 juveniles), 984 individuals of Gamasida, 868 individuals of Tarsonemida and 2,825 Collembolans. Oribatid mites were the most dominant group of soil microarthropods at all sampling sites, with a mean abundance of 58,417 ± 21,884 ind. m^{-2} (mean ± SE) at S (naturally regenerating) sites, 27,067 ± 6,142 ind. m^{-2} at P (salvage-logged) sites and 30,200 ± 3,835 ind. m^{-2} at M (marshy forest) sites (Fig. 1). We recorded 82 oribatid mite species from 31 families (see the list with numbers and ecological characteristics in the Electronic supplementary file 1, Tables S1–S4), of which 10 are rare species of the Šumava Mts (*Brachychthonius pius*, *Caenobelba montana*, *Liebstadia willmanni*, *Neoribates aurantiacus*, *Oppiella translamellata*, *Oppiella loksai*, *Parachipteria bella*, *Protoribates capucinus*, *Suctobelba reticulata* and *Suctobelbella perforata*).

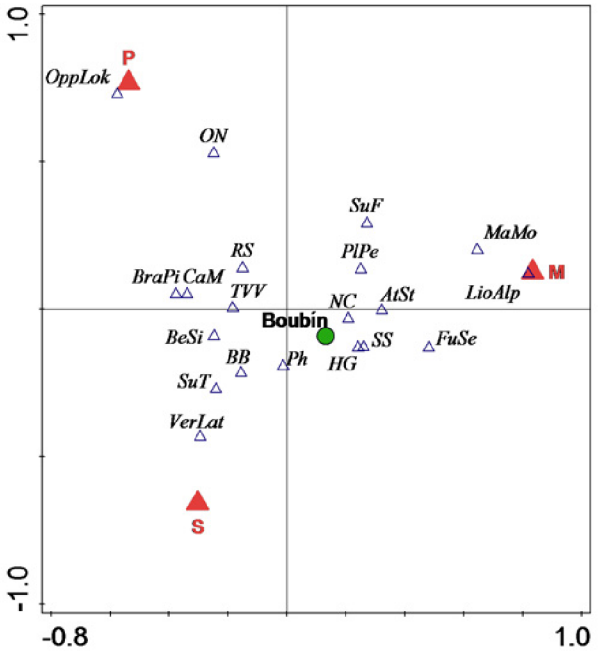

Fig. 2. Partial CCA ordination diagram, the effect of management on the oribatid mite species composition (20 best explained species are shown – abbreviations in Appendix 1) with the position of control site at Boubín.

Species richness of oribatid mites was very similar at salvage-logged and marshy forest sites and slightly higher at naturally regenerating forest sites, but without any significant differences. Species richness per soil sample was 8.42 ± 4.23 (mean ± SE) at S sites, 6.23 ± 3.87 at P sites and 6.93 ± 4.81 at M sites. The oribatid mite communities' characteristics of all nine sampling sites are shown in the Table 2.

At the sampling site P5 in salvage-logged forest, we found the xerophilous and heliophilous species *Oribatula exilis*, which was not found at any other site.

Principal Components Analysis (PCA) was used to illustrate the effect of forest management on the mesofauna community and on oribatid mite communities. Forest management had no significant effect on mesofauna abundance ($F = 4.9$; $P = 0.068$) but we found significant differences between oribatid mite communi-

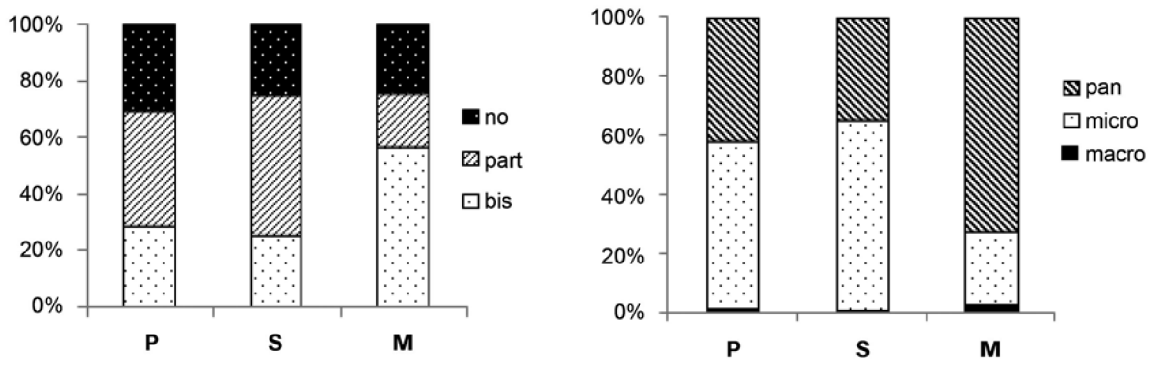

Fig. 3. The left graph shows the ratio of oribatid mite species according to their way of reproduction (no – unknown; part – parthenogenetic; bis – bisexual). The right graph shows the ratio of oribatid mite species according to the feeding groups (pan – panphytophages; micro – microphytophages; macro – macrophytophages).

ties at differently managed sites ($F = 1.7$; $P = 0.002$).

The main gradient, characterized by the first axis, which is most probably represented by increasing soil moisture, quite well separated oribatid mite species according to their moisture preferences (Fig. 2). The position of the control site at Boubín is projected into the results of the PCA in Fig. 2.

The communities differed significantly among all forest management strategies – most important was the significant difference between S (naturally regenerating) and P (salvage-logged) sites ($F = 1.4$; $P = 0.016$). The effect of used management explained 3.9% of the variability in the oribatid mite community.

Parthenogenetic species reached higher abundances at P and S sites, whereas bisexual species dominated at M sites (Fig. 3), but the results were not significant ($F = 2.4$; $P = 0.58$). We also found a higher occurrence of microphytophagous oribatid mites at P and S sites, while panphytophagous oribatid mites were more abundant at M sites (Fig. 3); these differences were only marginally significant ($F = 2.6$; $P = 0.052$).

In all sites, silvicolous and eurytopic species were most common, but we found heliophilous species in salvage-logged sites (P) in higher density than in naturally regenerating sites (they were not present in marshy forest). At marshy forest sites (M), tyrphophilous species were more common, but these differences were not significant ($F = 1.8$; $P = 0.102$). The distribution of species according to their moisture preferences was significant ($F = 2.9$; $P = 0.034$) and is shown in the ordination diagram at Fig. 4. The effect of management explained 10.4% of the variability in oribatid mite community.

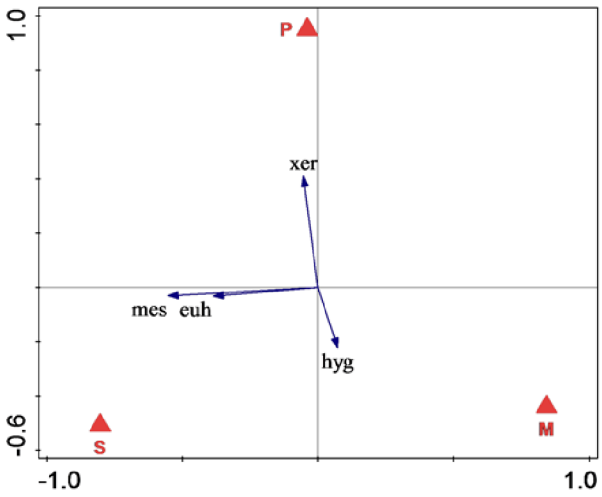

Fig. 4. Partial RDA analysis, the effect of management on the distribution of oribatid species according to their moisture preferences. xer – xerophilous, euh – euhydric, mes – mesophilous, hyg – hygrophilous.

Discussion

The results of this research suggest that forest management strategy after large-scale disturbances in mountain spruce forests significantly influences the composition of the oribatid mite community, even more than 15 years after the events. Changes in oribatid mite communities caused by logging can persist over many years (Siepel 1996), but long-term studies about this issue are scarce. It was not a surprise, that the abundances of oribatid mites outnumbered the other microarthropod groups on all sampling sites, since coniferous forests are habitats in which oribatid mites normally dominate (Siira-Pietikäinen et al. 2008). However, the observed abundances were relatively low, with values ranging between 20 and 30,000 ind. m^{-2} found at P (salvage-logged) sites, which corresponded much more to forest openings or meadows than to regenerating forests (Starý 1990; Luptáčik et al. 2012). Similar values were found for M (marshy forest) sites, probably because of higher moisture levels in such sites, which can limit reproduction of some species. By contrast, mean abundances ranging between 60 and 80,000 ind. m^{-2} found at S (naturally regenerating) sites were in agreement with values found in other studies (Starý & Matějka 2008; Farská et al. 2014b) and comparable to abundances in fully grown healthy forests (about 100–150,000 ind. m^{-2}; Starý & Matějka 2008).

The high variability in oribatid mite communities at all sites corresponded with the results of other studies which observed that such imbalance in the distribution of species is characteristic for areas affected by any type of disturbance (Caruso et al. 2007). Generally, in disturbed ecosystems, a high number of species with low abundance and just a few opportunistic species with wide ecological valence with very high abundance (for instance *Tectocepheus velatus velatus* or *Platynothrus peltifer*) can be found (Maraun et al. 2003; Murvanidze et al. 2013), which was also confirmed in our study – a maximum of six tolerant species dominated the sampling sites and the remaining species were sensitive species with low abundance. Divergences between sampling sites with different management strategies were mainly caused by the presence of subdominant and infrequent species. The dissimilarity between sites in marshy forests and dry forests (S and P) was not surprising, as these forest types have different microclimatic conditions The most crucial was higher moisture that did not fluctuate much during the year as well as smaller fluctuation of surface soil temperature by virtue of higher number of grown trees that survived the disturbance (Pavlas 2014). Better availability of nutrients and their equable distribution throughout the soil profile than at other sampling sites (Krausová 2011) is most probably the reason why more spruces survived the bark beetle outbreak. This could be one of reasons why the oribatid mite communities on these sites were relatively stable with domination of silvicolous and hygrophilous species, such as *Berniniella sigma*, *Liochthonius alpestris, Malaconothrus monodactylus, Nanhermannia coronata* or *Oppiella translamellata*.

Nevertheless, it would be advisable to include sites in salvage-logged marshy forest for specification of the results.

A much more considerable result of this research was the finding that the communities differed between salvage-logged and naturally regenerating sampling sites. Heliophilous and xerophilous species, such as *Achipteria coleoptrata, Damaeus gracilipes, Oppiella loksai* or *Oribatula exilis*, which prefer dry habitats without tree layer, were more abundant at salvage-logged sites (P). This corresponds with the fact that at these sites, lower moisture and higher soil temperature fluctuations were detected than at S sites (Pavlas 2014). On the other hand, at naturally regenerating sites (S), silvicolous and hygrophilous species, such as *Parachipteria bella* or *Verachthonius laticeps*, were more abundant. Surprisingly, the species *Brachychthonius pius* was very abundant in these sites, although species of the family Brachychthoniidae are very susceptible to disturbances and their recovery after such events is very slow (Maraun el al. 2003). This suggests that these sites are recovering relatively quickly and are in better ecological condition than the P sites.

The comparison of our research sites with the site at Boubín in a fully grown healthy spruce forest implied that the oribatid mite communities of our S and M sites developed and regenerated well and were similar to the community of a healthy forest. The mean abundance of oribatid mites at the Boubín site was 101,000 ind. m^{-2}, which was slightly higher than in other spruce forests in Central Europe (Starý & Matějka 2008); however, the abundances in our S sites, with microclimatic and moisture conditions similar to Boubín, were approaching these values. In a similar study, Farská et al. (2014b), who compared oribatid mite communities from naturally regenerating spruce forests with those in healthy forests, obtained similar results. On the contrary, the communities of the P sites developed differently, and even more than 15 years after the disturbance, species composition was very different from that of the control site at Boubín. The same trend (lower abundances and changes in species composition) was found in the oribatid mite communities at the High Tatras National Park in sites that were salvage-logged after windstorms (Lóšková et al. 2013).

The dominance of parthenogenetic oribatid mites at salvage-logged and naturally regenerating sites in our research corresponded with the results of the study by Farská et al. (2014b), which was also performed in a forest regenerating after bark beetle outbreak. The supremacy of bisexual species in marshy forest was caused mainly by high abundances of species *Hermannia gibba* and *Nanhermannia coronata*. Nevertheless, due to diverse information found in the literature, the role of reproduction in community and ecosystem recovery after disturbance is still unclear. Abundances of parthenogenetic oribatid species recovered quickly after summer droughts (Lindberg & Bengtsson 2006) and were quite high in intensively managed montane spruce forests (Farská et al. 2014a). This corresponds with Norton's (1994) statement that parthenogenesis is a useful strategy for colonizers who can reproduce quickly and effectively and start a new population from just a few individuals. On the other hand, the bisexual species were more successful in colonization of new habitats during laboratory experiments (Domes et al. 2007), which is consistent with the theory mentioned by Hamilton (1980), stating that sexual reproduction is superior to parthenogenesis, especially in unstable ecosystems after disturbances, because high genetic variability of the population allows faster adaptation to changing environmental conditions.

Recent investigations (Maraun et al. 2013; Mumladze et al. 2015) suggest that also the availability of resources could be the driving factor of the prevailing reproductive mode. Sexual taxa were favoured at the sites with resource shortage while parthenogenetic species dominated at sites with high quality litter material. These findings correspond with our results – parthenogenetic species were more abundant in naturally regenerating and salvage-logged sites, where higher input of nutrients from decaying wood into soil could be expected.

The equable distribution of microphytophagous and panphytophagous species on salvage-logged and naturally regenerating sites is an interesting finding and stands in conflict with the results of Farská el

al. (2014b), who found predominance of detritivorous macrophytophagous species in forests regenerating after bark beetle disturbance. Due to higher inputs of dead wood into soil organic matter, the same trend could be expected in our research in naturally regenerating forests. In contrast, a dominance of panphytophagous oribatids could be expected on salvage-logged sites as a consequence of soil disturbance by logging, considerably decreasing microbial biomass and activity (Dahlberg et al. 2001) and leading to major changes in composition of primary fungivorous oribatid mite communities.

However, for better specification and interpretation of the results, it would be suitable to partition the oribatid species into smaller classes according to their diets, but such information is not yet available for most of the species.

The distribution of oribatid mite species in our study sites according to their moisture preferences fully corresponded with the moisture and temperature soil conditions found in these sites by Pavlas (2014) and could serve as a control of previous results. It was obvious that sites located in salvage-logged forest differed from the other two site types in terms of species composition. The results of this study prove that the consequences of salvage-logging in montane spruce forests, at least for the oribatid mite community, were apparent more than 15 years after logging.

Acknowledgements

We would like to thank to Dr. P. Šmilauer, (University of South Bohemia, Faculty of Sciences, České Budějovice, Czech Republic) and Dr. M. Devetter (Institute of Soil Biology, Biology Centre, ASCR, v.v.i., České Budějovice, Czech Republic) for their assistance and help with using of PCA and RDA analysis. The research was supported by the Academy of Sciences of the Czech Republic, under Research Plan No. AV0Z606960521 and by grant projects of the Czech Science Foundation (projects No. P504/12/1218). We thank the Administration of the National Park Šumava for permissions to conduct the study in the nature reserves.

References

Balogh J. & Mahunka S. 1983. Primitive Oribatids of the Palaearctic Region. Series: The Soil Mites of the World. Akadémia Kiadó, Budapest, 372 pp. ISBN-10: 0444996540

Bryndová M. 2013. Vliv kalamitní těžby na populaci želvušek (Tardigrada) v horských smrčinách NP Šumava [Effect of salvage logging on soil tardigrade population in mountain spruce forest of the Šumava Nation Park after 16 years from treatment]. Unpublished Mgr. thesis, Faculty of Science, University of South Bohemia, 43 pp.

Caruso T., Pigino G., Bernini F., Bargagli R. & Migliorini M. 2007. The Berger-Parker index as an effective tool for monitoring the biodiversity of disturbed soils: a case study on Mediterranean oribatid (Acari: Oribatida) assemblages. Biodivers. Conserv. **16:** 3277–3285. DOI: 10.1007/s10531-006-9137-3

Coleman D.C., Crossley D.A. jr. & Hendrix P.F. 2004. Foundamentals of Soil Ecology. Academic Press Inc., 2nd edn, San Diego, 408 pp. ISBN: 9780121797263

Dahlberg A., Schimmel J., Taylor A.F.S. & Johannesson H. 2001. Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. Biol. Conserv. **100:** 151–161. DOI: 10.1016/S0006-3207(00)00230-5

Domes K., Scheu S. & Maraun M. 2007. Resources and sex: soil re-colonization by sexual and parthenogenetic oribatid species. Pedobiologia **51:** 1–11. DOI: 10.1016/j.pedobi.2006.11.001

Farská J., Prejzková K. & Rusek J. 2014a. Management intensity affects traits of soil microarthropod community in montane spruce forest. Appl. Soil Ecol. **75:** 71–79. DOI: 10.1016/j.apsoil.2013.11.003

Farská J., Prejzková K., Starý J. & Rusek J. 2014b. Soil microarthropods in non-intervention montane spruce forest regenerating after bark-beetle outbreak. Ecol. Res. **29:** 1087–1096. DOI: 10.1007/s11284-014-1197-3

Giljarov M.S. & Krivoluckij D.A. (eds). 1975. Opredelitel obitayushchikh v pochve kleshcheĭ. Sarcoptiformes. Nauka, Moscow, 492 pp.

Griffin J.M., Turner M.G. & Simard M. 2011. Nitrogen cycling following mountain pine beetle disturbance in lodgepole pine forests of Greater Yellowstone. Forest Ecol. Manag. **261:** 1077–1089. DOI: 10.1016/j.foreco.2010.12.031

Hais M. & Kučera T. 2008. Surface temperature change of spruce forest as a result of bark beetle attack: Remote sensing and GIS approach. Eur. J. For. Res. **127:** 327–336. DOI: 10.1007/s10342-008-0208-8

Hamilton W.D. 1980. Sex versus non-sex versus parasite. Oikos **35:** 282–290. DOI: 10.2307/3544435

Hartenstein R.C. 1962. Soil Oribatei. Feeding specifity among forest soil Oribatei (Acarina). Ann. Entomol. Soc. Am. **55:** 202–206. DOI: 10.1093/aesa/55.2.202

Jonášová M. & Prach K. 2004. Central-European mountain spruce (*Picea abies* (L.) Karst.) forests: regeneration of tree species after a bark-beetle outbreak. Ecol. Eng. **23:** 15–27. DOI: 10.1016/j.ecoleng.2004.06.010

Jonášová M. & Prach K. 2008. The influence of bark beetle outbreak vs. salvage logging onground layer vegetation in Central European mountain spruce forests. Biol. Conserv. **141:** 1525–1535. DOI: 10.1016/j.biocon.2008.03.013

Krausová M. 2011. Odhad dostupnosti živin v půdě asanovaných a přirozeně se vyvíjejících porostů po kůrovcové kalamitě na území NP Šumava [Estimate of available nutrients in forest soil after bark beetle outbreak of stands after salvage logging and of stands left without intervention in the Šumava National park]. Unpublished Bc. thesis, Faculty of Science, University of South Bohemia, 41 pp.

Kunst M. 1971. Nadkohorta pancířníci – Oribatei [Supercohort moss mites – Oribatei], pp. 531–580. In: Daniel M. & Černý V. (eds), Klíč zvířeny ČSSR, díl IV. Želvušky, jazyčnatky, klepítkatci: sekáči, pavouci, štírci, roztoči, Československá akademie věd, Praha, 603 pp.

Lindberg N. & Bengtsson J. 2006. Recovery of forest soil fauna diversity and composition after repeated summer droughts. Oikos **114:** 494–506. DOI: 10.1111/j.2006.0030-1299.14396.x

Lindenmayer D.B. & Noss R.F. 2006. Salvage logging, ecosystem processes, and biodiversity conservation. Conserv. Biol. **20:** 949–958. DOI: 10.1111/j.1523-1739.2006.00497.x

Lóšková J., Luptáčik P., Miklisová D. & Kováč L. 2013. The effect of clear-cutting and wildfire on soil Oribatida (Acari) in windthrown stands of the High Tatra Mountains (Slovakia). Eur. J. Soil Biol. **55:** 131–138. DOI: 10.1016/j.ejsobi.2013.01.001

Luptáčik P., Miklisová D. & Kováč L. 2012. Diversity and community structure of soil Oribatida (Acari) in an arable field with alluvial soils. Eur. J. Soil Biol. **50:** 97–105. DOI: 10.1016/j.ejsobi.2011.12.008

Luxton M. 1972. Studies on the oribatid mites of a Danish beech wood soil, I. Nutritional biology. Pedobiologia **12:** 434–463.

Maraun M., Fronczek S., Marian F., Sandmann D. & Scheu S. 2013. More sex at higher altitudes: Changes in the frequency of parthenogenesis in oribatid mites in tropical montane rain forests. Pedobiologia **56:** 185–195. DOI: 10.1016/j.pedobi.2013.07.001

Maraun M., Salamon J.A., Schneider K., Schaefer M. & Scheu S. 2003. Oribatid mite and collembolan diversity, density and

community structure in a moder beech forest (*Fagus sylvatica*): effects of mechanical perturbations. Soil Biol. Biochem. **35:** 1387–1394. DOI: 1016/S0038-0717(03)00218-9

Marshall V.G. 1972. Comparison of two methods of estimating efficiency of funnel extractors for soil microarthropods. Soil Biol. Biochem. **4:** 417–426. DOI: 10.1016/0038-0717(72)90056-9

Marshall V.G. 2000. Impact of forest harvesting on biological processes in northern forest soil. Forest Ecol. Manag. **133:** 43–60. DOI: 10.1016/S0378-1127(99)00297-2

Mumladze L., Murvanidze M., Maraun M. & Salakaia M. 2015. Oribatid mite communities along an elevational gradient in Sairme gorge (Caucasus). Exp. Appl. Acarol. **66:** 41–51. DOI: 10.1007/s10493-015-9893-4

Murvanidze M., Mumladze M., Arabuli T. & Kvavadze E. 2013. Oribatid mite colonization of sand and manganese tailing sites. Acarologia **53:** 203–215. DOI: 10.1051/acarologia/20132089

Niedbała W. 1980. Mechowce – roztocze ekosystémów ladowych [Oribatida – Mites of Terrestrial Ecosystems]. PWN, Warsava, 255 pp.

Norton R.A. 1994. Evolutionary aspects of oribatid mite life histories and consequences for the origin of Astigmata, pp. 99–135. In: Houck M.A. (ed.), Mites: Ecological and Evolutionary Analyses of Life-History Patterns, Chapman and Hall, New York, 357 pp. ISBN: 978-1-4615-2389-5

Norton R.A. & Behan-Pelletier V.M. 2009. Oribatida, pp. 430–564. In: Krantz G.W. & Walter D.E. (eds), A Manual of Acarology, 3rd ed. Texas Tech University Press, Lubbock, Texas, 807 pp. ISBN: 978-0-89672-620-8

Ojala R. & Huhta V. 2001. Dispersal of microarthropods in forest soil. Pedobiologia **45:** 443–450. DOI: 10.1078/0031-4056-00098

Pavlas J. 2014. Vliv disturbance lesa na teplotu a vlhkost půdy [Influence of the forest disturbances on the temperature and humidity of soil]. Unpublished Mgr. thesis, Faculty of Science, University of South Bohemia, 60 pp.

Seniczak S. & Stefaniak O. 1978. The microflora of the alimentary canal of *Oppia nitens* (Acarina, Oribatei). Pedobiologia **18:** 110–119.

Schuster R. 1956. Der Anteil der Oribatiden an den Zersetzungsvorgängen im Boden. Z. Morphol. Ökol. Tiere **45 (1):** 1–33. DOI: 10.1007/BF00699814

Shannon C.E. & Weaver W. 1949. The mathematical theory of communication. Illinois University Press, Urbana USA, 282 pp. ISBN: 0-252-72546-8

Siepel H. 1996. The importance of unpredictable and short-term environmental extremes for biodiversity in oribatid mites. Biodivers. Lett. **3:** 26–34. DOI: 10.2307/2999707

Siira-Pietikäinen A., Penttinen R. & Huhta V. 2008. Oribatid mites (Acari: Oribatida) in boreal forest floor and decaying wood. Pedobiologia **52:** 111–118. DOI: 10.1016/j.pedobi.2008.05.001

Skuhravý V. 2002. Lýkožrout smrkový v Bavorském lese a na Šumavě [Eight-toothed spruce bark beetle in the Bavarian forest and Šumava Mts]. Živa **5:** 220–222.

Starý J. 1990. Ekologie pancířníků (Acari: Oribatida) v sukcesní řadě půd [Ecology of oribatid mites (Acari: Oribatida) during succession in brown soils]. Unpublished PhD. thesis, Institute of Soil Biology, ASCR, České Budějovice, 179 pp.

Starý J. & Matějka K. 2008. Pancířníci (Acari: Oribatida) vybraných lokalit horských lesů na Šumavě. Průběžná zpráva z řešení projektu 2B06012 Management biodiversity v Krkonoších a na Šumavě v roce 2007 [Oribatid mites (Acari: Oribatida) of selected localities at montain forests on the Šumava Mts.. Report of the project 2B06012: Biodiversity management in the Krkonoše Mts and the Šumava Mts in 2007], http://www.infodatasys.cz/biodivkrsu/rep2007_Oribatida.pdf

Strenzke K. 1952. Untersuchungen über die Tiergemeinschaften des Bodens: Die Oribatiden und ihre Synusien in den Böden Norddeutschlands. Zoologica **37 (104),** 172 pp.

Svoboda M., Janda P., Nagel T.A., Fraver S., Rejzek J. & Bače R. 2012. Disturbance history of an old-growth sub-alpine *Picea abies* stand in the Bohemian forest, Czech Republic. J. Veg. Sci. **23:** 86–97. DOI: 10.1111/j.1654-1103.2011.01329.x

Šantrůčková H., Vrba J., Křenová Z., Svoboda M., Benčoková A., Edwards M., Fuchs R., Hais M., Hruška J., Kopáček J., Matějka K. & Rusek J. 2010. Co vyprávějí šumavské smrčiny [What can the spruce forests of Šumava Mts. narrate]. Správa NP a CHKO Šumava, Vimperk, 153 pp.

Šmilauer P. & Lepš J. 2014. Multivariate analysis of ecological data using Canoco 5. 2nd edn. Cambridge University Press, 362 pp. ISBN: 978-1-107-69440-8

Velíšek L. 2014. Společenstva mnohonožek a stonožek přirozeně se vyvíjejících a asanovaných horských smrčin Šumavy [Communities of millipedes and centipedes in the naturally disturbed and rehabilitated mountain spruce forests of the Šumava Mts]. Unpublished Mgr. thesis, Faculty of Science, University of South Bohemia, 71 pp.

Weigmann G. 2006. Die Tierwelt Deutschlands, Teil 76: Hornmilben (Oribatida). Goecke and Evers, Keltern, 520 pp. ISBN: 978-3-937783-18-5

Received November 1, 2016
Accepted January 21, 2017

Appendix 1. Oribatid mite species abbreviations.

Species	Abbreviation
Athropacarus striculus	AtSt
Belba bartosi	BB
Berniniella sigma	BeSi
Brachychthonius pius	BraPi
Caenobelba montana	CaM
Fuscozetes setosus	FuSe
Hermannia gibba	HG
Liochthonius alpestris	LioAlp
Malaconothrus monodactylus	MaMo
Nanhermannia coronata	NC
Oppiella loksai	OppLok
Oppiella nova	ON
Phtiracarus sp.	Ph
Platynothrus peltifer	PlPe
Rhinoppia subpectinata	RS
Steganacarus spinosus	SS
Suctobelba trigona	SuT
Suctobelbella falcata	SuF
Verachthonius laticeps	VerLat

5.2. Paper II

Experimental and Applied Acarology (2021) 83:493–511
https://doi.org/10.1007/s10493-021-00605-7

Distinct phylogeographic patterns in populations of two oribatid mite species from the genus *Pantelozetes* (Acari, Oribatida, Thyrisomidae) in Central Europe

Petra Kokořová[1,2,3] · **Martina Žurovcová**[3] · **Peter Ľuptáčik**[4] · **Josef Starý**[2]

Received: 4 November 2020 / Accepted: 2 March 2021 / Published online: 3 April 2021

Abstract

Oribatid mites are important decomposers of dead organic matter in soils across the world. Their origin dates back at least 380 Mya. Multiple severe climatic changes during Late Pliocene and Pleistocene shaped the migration patterns of these organisms and should be reflected in the genetic variability of their current populations. In this study, we examined the genetic diversity and phylogeographic structure as well as the evolutionary history of populations of two ecologically different oribatid mite species. *Pantelozetes cavaticus* is a troglophile oribatid mite known mainly from Central European caves, whereas *Pantelozetes paolii* is a common surface eurytopic species with Holarctic distribution. We used two molecular markers—mitochondrial cytochrome c oxidase subunit I (COI) and the nuclear D3 region of the 28S rDNA gene—to reveal phylogenetic relationships between contemporary populations. Whereas the D3 region showed minimal or no variability within populations, COI appeared to be a relevant marker for population studies. Phylogeographic analysis based on COI detected two lineages of *P. cavaticus* ('Czech' and 'Slovak'), which separated during the Late Pliocene (2.9 Mya) and revealed the existence of one new species. In contrast, three identified genetic lineages of *P. paolii* (radiation time 2.9 and 1.2 Mya, respectively) uncovered in this study were found to coexist in the distant sampling localities, suggesting a connection between populations even over long distances.

Keywords Oribatid mites · Genetic diversity · Phylogeography · COI · D3 region

✉ Petra Kokořová
petra.kokorova@post.cz

[1] Department of Ecosystem Biology, Faculty of Science, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic

[2] Biology Centre, Institute of Soil Biology, Czech Academy of Sciences, České Budějovice, Czech Republic

[3] Biology Centre, Institute of Entomology, Czech Academy of Sciences, České Budějovice, Czech Republic

[4] Faculty of Sciences, Institute of Biology and Ecology, Pavol Josef Šafárik University in Košice, Košice, Slovakia

Introduction

Oribatid mites (Acari: Oribatida) are mostly a soil-dwelling and very diverse group of microarthropods with more than 11,000 species described so far (Subías 2020). Their representatives can be found in almost every terrestrial habitat from tropics to poles, being the most abundant group in the first few centimeters of soil, with a density up to hundreds of thousands of individuals per m^2 in temperate forests with a thick humus layer (Maraun and Scheu 2000). They play an important functional role in soils as decomposers of dead organic matter and stimulators of fungal and bacterial growth (Luxton 1972).

Oribatid mites are among the oldest terrestrial animals, with earliest fossil record from Devonian sediments dated at 380 Mya (Norton et al. 1988) and molecular analyses dating their origin to at least 580 Mya (Schaefer et al. 2010). In Europe, climatic oscillations and consequential biome changes over the past 3 million years caused considerable shifts in distributional patterns of surface as well as soil-living animals (Hewitt 2004). During the cold periods, the warm-adapted fauna and flora either went extinct or were forced to look for more favourable habitats, i.e., refugia. However, active dispersal skills of the soil mesofauna, such as Oribatida or Collembola, are quite limited (Lehmitz et al. 2012) and more recent climatic and habitat changes during Pliocene and Pleistocene defined the migration patterns of the individual species, which should be still reflected in the genetic composition of their current populations. Such effects on the genetic diversity have been studied only scarcely in soil-dwelling arthropods (Beheregaray 2008; Rosenberger 2010), although present-day distributional patterns of intraspecific genetic diversity and estimations of its degree have been shown to provide important insights into the phylogeography and evolutionary history of species (Beebee and Row 2008).

Yosii (1956) pointed out that caves with constant microclimatic conditions in the northern temperate zone can serve as refugia, especially for small soil-dwelling species. Subterranean ecosystems are generally considered as habitats where species from different external ecological conditions can successfully cohabit during hostile surface conditions (Kováč et al. 2016).

The troglophile *Pantelozetes cavaticus* (Kunst) is a sexually reproducing oribatid mite that has been reported mainly from Central European caves, in eastern Czech Republic (Starý 2008), in the Western Carpathians and the Slovak Karst in Slovakia (Ľuptáčik and Miko 2003), in eastern Austria (Bruckner 1995), in southern Poland (Żbikowska-Zdun et al. 2009) and in some caves in Germany, Belgium and Hungary (Ľuptáčik 2004). The species does not possess any obvious troglobiomorphic adaptations (e.g., depigmentation, elongated antennae and legs), but it shows a strong affiliation to the cave environment. Only two findings have been reported from surface environments (Starý 2008; Żbikowska-Zdun et al. 2009). It is considered coprophilous (guanophilous) with frequent occurrence on rotten wood (Ľuptáčik and Miko 2003). The distribution pattern along with absent adaptations to cave life of *P. cavaticus* indicates that it could be a glacial relict, most likely a descendant of an old Pleistocene fauna, or even older Tertiary fauna.

Żbikowska-Zdun et al. (2009) examined populations of *P. cavaticus* from two isolated caves in Poland and found no significant morphological differences between them. Here we take an approach of investigating possible differences between populations at the molecular level. Deep genetic divergences in soil-dwelling arthropods at the population level have been previously reported and well documented in oribatid mites (Heethoff et al. 2007; Rosenberger 2010; Saltzwedel et al. 2014; Schäffer et al. 2019).

To evaluate whether the association of *P. cavaticus* with subterranean habitats caused a geographical isolation and consequently a reproductive isolation, we compared its genetic variability with that of populations of a closely related surface species *Pantelozetes paolii* (Oudemans). In contrast to *P. cavaticus*, *P. paolii* is a widespread and abundant eurytopic pan-phytophagous species with sexual reproduction and Holarctic distribution. *Pantelozetes paolii* is a type species of the genus *Pantelozetes*, which now comprises 22 species and one subspecies based on morphological characters (Subías 2020); phylogenetic relationships among these species are unknown.

In the present study, we used the combination of two DNA markers—the mitochondrial cytochrome c oxidase subunit I (COI, the barcoding region) and the nuclear D3 region of the 28S rDNA gene—as both markers were previously successfully used in other studies aimed at phylogeny and phylogeography of oribatid mites (COI: Rosenberger 2010; Schäffer et al. 2010, 2019; Pfingstl et al. 2019; D3: Lehmitz and Decker 2017; Pachl et al. 2017). Furthermore, Kreipe et al. (2015) suggested that the combination of these two genes should provide a reliable insight into the phylogeny and species radiation within oribatid mites.

Our objectives were (1) to investigate the genetic variability within and between the populations of the troglobiotic *P. cavaticus* and to determine whether the studied caves were reproductively isolated; (2) to compare the genetic variability of two congeneric oribatid mite species with a different ecology—*P. cavaticus* and *P. paolii*; and (3) to trace the evolutionary history of potential lineages of both species. Assuming that the active dispersal abilities of oribatid mites are limited, we expected similar patterns of genetic variability, with genetic isolation more pronounced in the troglobiotic species even over shorter geographical distances.

Materials and methods

Specimen sampling, DNA extraction and sequencing

Organic litter, decaying wood or bat guano samples for the extraction of *P. cavaticus* were taken from sediments in 10 caves between 2015 and 2017 (three caves in Czech Republic, seven in Slovakia; see Fig. 1), where the presence of this species was demonstrated in previous research (Ľuptáčik 2004; Starý 2008). Nová Amatérská Cave and Sloupsko-Šošůvské Caves are in the Moravian Karst, which is the largest karst area in Czech Republic based in the Middle Devonian limestone. These two cave systems are connected via a subterranean stream. A third cave sampled in Czech Republic, the Javoříčské Caves, is situated in a small outcrop of Devonian limestone in the Špraněk Hill. Five of the caves sampled in Slovakia are part of the Slovak Karst (the largest karst area in Central Europe composed of several layers of Mesozoic limestone and dolomite) and are relatively close to each other—500 m to 40 km. Belianska Cave is the largest cave in the Slovak part of the Tatra Mountains established in Mesozoic limestone. The last cave sampled in Slovakia, Andrejová Cave II, located in the Čierna Hora Mountains (part of the Slovak Ore Mountains), is a small joint cave in a limestone rock cliff.

For the common soil living *P. paolii*, soil samples to the depth of 5 cm were collected at 12 already established study sites of the Institute of Soil Biology (Biology Centre CAS, České Budějovice) between 2016 and 2017 (Czech Republic, Slovakia, and Poland; Fig. 1).

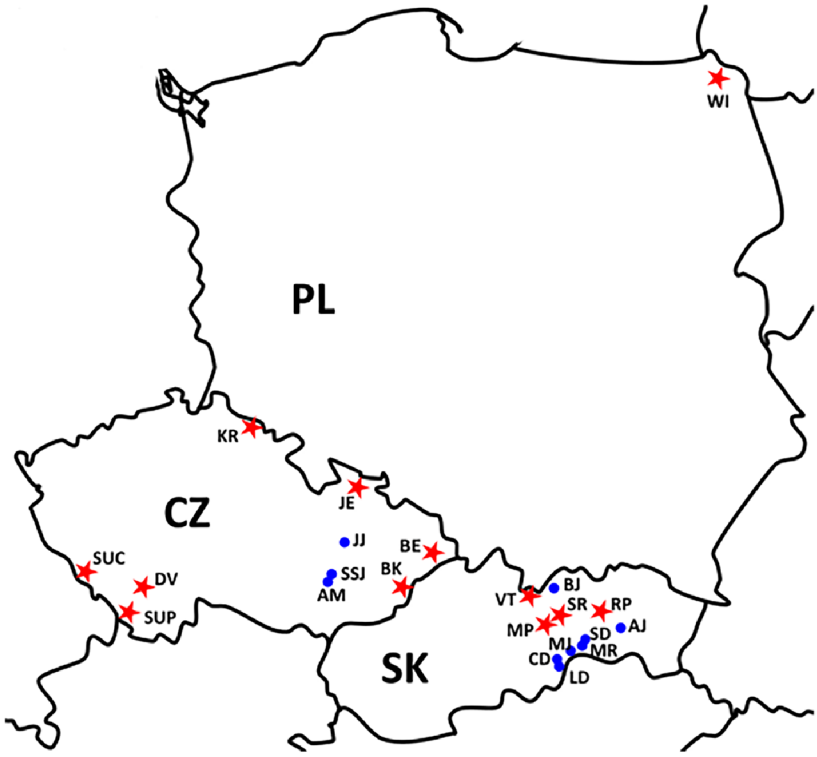

Fig. 1 Map of Czech Republic, Slovakia and Poland with the sampling localities; dots for *Pantelozetes cavaticus* and stars for *P. paolii* (for detailed information about each sampling locality see Table 1, Online Resource 1)

The name of the sampling locality and its abbreviation, number of collected specimens as well as the number of sequences obtained for each locality and GenBank reference numbers are provided for all records in Table 1. More detailed information about sampling localities (GPS, altitude, material collected, etc.) are given in Online Resource 1.

Soil arthropods were extracted in a high-gradient extractor by Marshall (1972) for 5 days with increasing temperatures. *Pantelozetes cavaticus* and *P. paolii* specimens were identified to species level under a light microscope after Weigmann (2006) and stored in 96% ethanol at −20 °C until preparation.

Genomic DNA was extracted from single whole individuals (20 specimens of *P. cavaticus* and 10 of *P. paolii* from every sampling locality if such a number was available—see Table 1 for details) using the Exgene Tissue SV mini kit following the manufacturer' protocol for insects with final elution in 50 µl instead of 200 µl (GeneAll® Biotechnology). A fragment of cytochrome c oxidase subunit I (COI) was amplified using the primers OriLCO (5′-TCAACAAATCATAAAGAYATYGG-3′; slightly modified primer COIarchI used in Heethoff et al. 2007), and standard HCO2198 (5′-TAAACT GGGTGACCAAAAAATCA-3′; Folmer et al. 1994). For the amplification of the D3 fragment of nuclear 28S rDNA gene, the forward primer D3A and the reverse primer D3B were used as described in Kreipe et al. (2015).

The polymerase chain reaction (PCR) contained 0.75 µl of each primer (0.5 pmol/µl), 1 µl of dNTPs (2.5 mM of each dNTP), 1.25 µl of 10×reaction buffer, 0.1 µl of TaKaRa Ex Taq® polymerase, 7.75 µl of PCR water and 2 µl (for COI) and 1 µl (for

Table 1 List of sampling locality names in Czech Republic (CZ), Slovakia (SK) and Poland (PL) with their abbreviations and specifications of obtained DNA sequences

Species	Locality name	Country	Abbreviation	No. of specimens collected/isolated	No. of seq COI/D3	Accession no COI	Accession no D3
Pantelozetes cavaticus	Javoříčské Caves	CZ—Špraněk Hill	JJ_CZ	176/20	20/10	MW034736-755	MW581985-994
	Nová Amatérská Cave	CZ—Moravian Karst	AM_CZ	42/20	20/10	MW034678-697	MW581965-974
	Sloupsko-Šošůvské Caves	CZ—Moravian Karst	SSJ_CZ	31/25	16/10	MW034817-832	MW581975-984
	Belianska Cave	SK—Belianske Tatry	BJ_SK	17/17	17/10	MW034698-714	MW582045-054
	Majkova Cave	SK—Slovak Karst	MJ_SK	27/21	21/10	MW034776-796	MW582005-014
	Marciho Cave	SK—Slovak Karst	MR_SK	50/20	20/10	MW034797-816	MW582015-024
	Snieżna diera Cave	SK—Slovak Karst	SD_SK	50/25	25/10	MW034833-857	MW581995-2004
	Čertova diera Cave	SK—Slovak Karst	CD_SK	122/21	21/10	MW034715-735	MW582025-034
	Liščia diera Cave	SK—Slovak Karst	LD_SK	48/20	20/10	MW034756-775	MW582035-044
Pantelozetes sp.	Andrejová Cave II	SK—Slovak Ore Mountains	AJ_SK	65/23	23/10	MW649874-896	MW581955-964
P. paolii	Dlouhá Ves	CZ	DV_CZ	25/15	3/5	MW193960-962	MW646033-037
	NP Šumava	CZ	SUP_CZ	25/15	6/5	MW193900;903;933-936	MW646087-091
	Šumava PLA	CZ	SUC_CZ	17/15	5/5	MW193901-902;930-932	MW646038-042
	Jeseníky PLA	CZ	JE_CZ	12/12	4/5	MW193926-929	MW646043-047
	NP Krkonoše	CZ	KR_CZ	15/15	9/5	MW193917-924	MW646048-052
	Beskydy PLA	CZ	BE_CZ	8/8	6/5	MW193911-916	MW646053-057
	Bílé Karpaty PLA	CZ	BK_CZ	9/9	7/5	MW193904-910	MW646058-062
	NP Muránska planina	SK	MP_SK	14/14	6/5	MW193954-959	MW646063-067
	Ružín water dam	SK	RP_SK	19/15	5/5	MW193949-953	MW646068-072
	NP Slovenský ráj	SK	SR_SK	21/15	4/5	MW193945-948	MW646073-077
	Tatranský NP	SK	VT_SK	9/9	3/5	MW193942-944	MW646078-082
	NP Wigry	PL	WI_PL	19/15	5/5	MW193937-941	MW646083-087

NP national park, *PLA* protected landscape area

D3), respectively, of template DNA. The PCR conditions consisted of initial 94 °C for 1 min; 40 cycles at 94 °C for 15 s, 47 °C for 40 s and 72 °C for 50 s; and final extension at 72 °C for 2 min. PCR products were visualized on 2% agarose gel and samples with positive products were enzymatically purified with a mixture of 0.5 µl Exonuclease I, 1 µl FastAP (ThermoFisher Scientific, USA) and 0.5 µl PCR water per sample (incubated at 37 °C for 30 min followed by 15 min at 80 °C) before direct sequencing. The purified PCR products were sequenced by GATC Biotech (Germany).

A surface-dwelling oribatid mite *Nothrus silvestris* (Nicolet) (Crotonioidea) was used as an outgroup taxon. Its COI and D3 sequences were taken over from Rosenberger (2010) and Lehmitz and Decker (2017), respectively (GenBank acc. nr. JF263835 and KY681356, respectively).

All sequences generated for this study were checked to be consistent with oribatid mite DNA via Blast searches (Altschul et al. 1997); no contaminations were discovered. All sequences generated for this study are publicly available from GenBank.

Data analysis

Sequences were manually edited: ambiguous positions were corrected by hand and unreadable short stretches were trimmed using the chromatograms (ca. 30 bp at the 5′ and 3′ ends) with BioEdit v.7 (Hall 1999). Both COI and D3 sequences together with the outgroup taxon *N. silvestris* were aligned with the MEGA X software (Kumar et al. 2018) by Muscle algorithm with default parameters. The final alignment of the COI fragment contained 203 sequences with the length of 630 bp for *P. cavaticus* and 63 sequences with the length of 626 bp for *P. paolii*, respectively. The final alignment of the D3 gene fragment contained 100 sequences (462 bp) for *P. cavaticus* and 60 sequences (456 bp) for *P. paolii*. Mitochondrial gene sequences were translated into amino acid sequences using the Invertebrate Mitochondrial Gene Code implemented in MEGA and as there were no stop codons and the alignments were gap-free, all of them were considered as true mitochondrial and not nuclear copies and were reverse-translated into nucleotide sequences for further analyses. All alignments are available from the authors upon request.

The number of synonymous and non-synonymous mutations in the COI alignment were calculated in MEGA, nucleotide (π) and haplotype diversity (Hd) were calculated in DnaSP v.5.10 (Librado and Rozas 2009). The number of protein haplotypes was determined using the online tool DNAcollapser in FaBox v.1.5 (Villesen 2007). The independent analysis of molecular variance (AMOVA) was performed only for *P. cavaticus* in ARLEQUIN v.3.5 (Excoffier et al. 2005), to investigate within- and between-populations structure based on uncorrected p-distances selecting Czech Republic and Slovakia as groups. Isolation by distance was tested by Mantel Test (10,000 permutations) implemented in ARLEQUIN.

To measure the effect of demographic changes on the DNA sequences of the populations, the neutrality tests Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) were performed in ARLEQUIN. Haplotype networks were constructed in PopART v.1.7 (Leigh and Bryant 2015) using Median Joining Network, separately for *P. cavaticus* and *P. paolii*, to determine and visualize the relationships and history between haplotypes.

The best-fitting model for phylogenetic analyses of COI alignments of both species was selected using the Bayesian Information Criterion (BIC) in the ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE v.1.6.12 (Nguyen et al. 2015). The model of sequence evolution was HKY + F + G4 for *P. cavaticus* and HKY + F + I for *P. paolii*, respectively. Phylogenetic trees of both species were calculated separately using Maximum

Likelihood (ML) and Bayesian Inference (BI) methods, implementing the models selected by ModelFinder. ML analyses were conducted in IQ-TREE with default parameter settings and 10,000 ultrafast bootstrap replicates (Hoang et al. 2018). BI was conducted in MrBayes v.3.2.7 (Ronquist et al. 2012) and BEAST v.2.6.0 (Bouckaert et al. 2019). The Bayesian Markov Chain Monte Carlo simulations in MrBayes were performed in two independent runs with four chains for each species separately, each for 10 million generations sampled every 1000th generation, a burn-in of 2500 was applied. To assess run convergence, the Tracer v.1.7.1 (Rambaut et al. 2018) was used.

Molecular divergence times of major lineages were estimated separately for *P. cavaticus* and *P. paolii*. Prior to this analysis, substitution saturation of the COI sequences was measured in DAMBE 6 software (Xia 2017) with the test by Xia et al. (2003)—the sequences experienced little substitution saturation. The relaxed clock log-normal analysis was performed in two runs for both species with BEAUti, BEAST and TreeAnnotator, all v.2.6.0 (Bouckaert et al. 2019). We set a fixed substitution rate of 0.0115, which corresponds to a standard arthropod mutation rate of COI, 2.3% sequence divergence per million years (Avise 1994; Brower 1994). The site model was HKY + F + G4 for *P. cavaticus* and HKY + F + I for *P. paolii*, respectively, and 'Coalescent Constant Population' was used as tree prior (as recommended for population-level studies by the authors of BEAST). The population size was set as log normal; all the other priors were estimated by the software. The convergence of the MCMC chain after 10,000,000 generations with every 1000th generation sampled and a burn-in of 1000 was confirmed using the Tracer.

Results

DNA extraction and subsequent PCR amplification of the COI gene was successful in 203 out of 212 *P. cavaticus* specimens and in 63 out of 157 *P. paolii* specimens analysed (Table 1); various sets of primers and PCR conditions were tested on the problematic individuals, albeit without success.

PCR amplification of the D3 region worked for all processed samples; however, it showed only a very low molecular variation between the two studied species (uncorrelated p-distances were 0.6%), and none within them. Only four positions of the 462 bp fragment varied in the 100 analysed individuals of *P. cavaticus* and no position of the 456 bp fragment varied in the 60 analysed individuals of *P. paolii*. Accordingly, the phylogenetic trees had no structure and sampling localities were mixed (ML phylogenetic tree of studied species together with an outgroup based on the D3 is shown in Online Resource 2a). Therefore, D3 sequences were not used for further analyses.

Phylogenetic analysis of COI sequences split the analysed species into three lineages and suggested the existence of a new species independent of *P. cavaticus* and *P. paolii*—hereafter it is referred to as *Pantelozetes* sp. The new species showed a closer relationship to *P. cavaticus*, values of uncorrected p-distances in the COI gene were 20.4% for *P. cavaticus* vs. *Pantelozetes* sp. and 22.7% between *P. paolii* and *Pantelozetes* sp. (Table 2a). D3 sequences failed to distinguish *Pantelozetes* sp. from *P. cavaticus*. *Pantelozetes* sp. was found only at the sampling locality Andrejová Cave II. Subsequent re-inspection under the light microscope revealed distinctive morphological features of the new species and its detailed description is currently in progress and will be published separately. Intraspecific p-distances were 0.2% and five haplotypes were detected in the 23 analysed *Pantelozetes* sp. COI sequences, which were not used for the reconstruction of phylogenetic trees and

Table 2 Intra- and interspecific distances (uncorrected p-distances, %) for the studied COI gene marker: (**a**) for all studied *Pantelozetes* species, (**b**) for *P. cavaticus* populations, and (**c**) for *P. paolii* populations, according to sampling locality and lineages

(a)

Species	*P. cavaticus*	*P. paolii*	*Pantelozetes* sp.
P. cavaticus	*2.4*		
P. paolii	21.7	*3.7*	
Pantelozetes sp.	20.4	22.7	*0.2*
Nothrus silvestris	22.6	23.4	25.9

(b)

Locality	AM_CZ	BJ_SK	CD_SK	JJ_CZ	LD_SK	MJ_SK	MR_SK	SSJ_CZ	SD_SK
AM_CZ	*0.5*								
BJ_SK	4.7	*0.6*					Lineage	CZ	SK
CD_SK	3.9	2.1	*0.8*				CZ	*1* *0.6*	
JJ_CZ	1.0	4.8	3.7	*0.04*			SK	4	*1* *1.3*
LD_SK	4.1	2.5	1.0	4.1	*0.1*				
MJ_SK	3.9	2.1	0.7	3.7	1.7	*0.03*			
MR_SK	4.1	2.5	1.2	4.1	0.7	1.7	*0.8*		
SSJ_CZ	0.4	4.6	3.7	0.7	3.9	3.7	3.9	*0.1*	
SD_SK	4.2	2.6	0.9	4.1	0.4	1.4	0.7	4.0	*0.2*

(c)

Locality	BE_CZ	BK_CZ	DV_CZ	JE_CZ	KR_CZ	RP_SK	SR_SK	MP_SK	SUC_CZ	SUP_CZ	VT_SK	WI_PL
BE_CZ	*4.3*											
BK_CZ	3.9	*3.4*							Lineage	1	2	3
DV_CZ	3.7	5.7	*0*						1	*0.1*		
JE_CZ	3.9	3.6	4.1	*5*					2	2.6	*0*	
KR_CZ	3.8	4.8	1.9	4	*3.3*				3	7	6.7	*0.1*
RP_SK	3.8	4	3.3	3.8	3.6	*4.6*						
SR_SK	3.6	3.9	3.5	3.8	3.7	3.7	*4.7*					
MP_SK	3.6	3.9	3.5	3.8	3.7	3.7	3.5	*4.2*				
SUC_CZ	3.6	4.2	2.8	3.9	3.3	3.6	3.5	3.5	*4.2*			
SUP_CZ	3.6	4.5	2.3	3.9	3.1	3.6	3.5	3.5	3.3	*3.7*		
VT_SK	4.8	4.8	2.5	4	3.3	3.7	4.6	4.6	4.2	3.9	*0*	
WI_PL	3.6	4.2	2.8	3.9	3.3	3.6	3.5	3.5	3.4	3.3	4.2	*4.2*

Intraspecific and intrapopulation distances are given in italics

haplotype network of the studied species. ML phylogenetic tree of all studied species (*P. cavaticus*, *P. paolii*, *Pantelozetes* sp.) with an outgroup *N. silvestris* is shown in the Online Resource 2b.

Pantelozetes cavaticus

In each of the phylogenetic trees *P. cavaticus* was monophyletic and separated with high support from the outgroup taxon *N. silvestris* (as well as from *P. paolii* and *Pantelozetes* sp.; Online Resource 2b). Phylogenetic reconstructions based on BI and ML methods of the COI nucleotide alignment revealed very similar topologies with slightly different resolution. Only the BI tree (created in BEAST) is shown in Fig. 2a (see Online Resource 3 for the other trees). COI haplotypes generally clustered according to sampling locations and separated with high support into two main lineages—'Czech' (1) and 'Slovak' (2) (Fig. 2a). Applying a standard invertebrate mitochondrial substitution rate of 2.3% per million years, 'Czech' and 'Slovak' lineages diverged in the Late Pliocene, 2.9 ± 1.6 Mya (Fig. 2a).

The COI nucleotide haplotype network also showed a strong cave-related structure once more with an obvious separation of Czech and Slovak caves (Fig. 3a). Individuals from nearby located sampling localities (caves) shared identical or closely related haplotypes. Haplotypes of the individuals from Belianska Cave (SK) and Javoříčské Caves (CZ) were clearly separated and not shared with any other individual from another caves. In total, 37 nucleotide haplotypes were sampled within the 180 individuals sequenced. The estimated haplotype diversity was relatively high within the populations and lineages (average values 0.502 and 0.802, respectively), whereas nucleotide diversity was considerably lower in both cases (on average 0.004 in populations and 0.009 in lineages) (Table 3). As for the amino acid haplotypes, 16 were identified, two (most abundant) shared among the individuals from Czech and Slovak caves, while the remaining 14 were detected only in Slovak caves (ML phylogenetic tree based on amino acid sequencies is shown in the Online resource 4a).

Genetic distances between the populations from discrete caves were moderately higher and ranged between 0.4 and 4.8% for the COI gene (Table 2b). Within-population genetic distances were generally low (Table 2b). Accordingly, as indicated by AMOVA, genetic variance was highest between groups (Czech and Slovak caves: 70.5%), markedly lower among populations within groups (21.1%) and lowest within populations (8.5%) (Table 4). A significant correlation between genetic and geographical distances of populations, isolation by distance, was revealed using Mantel test ($R^2 = 0.877$, $p = 0.004$). The neutrality analyses did not show any significant differences from zero within the populations ($p > 0.05$ for both D and F_S), except for two caves—Majkova Cave and Sniežná diera Cave—indicating a population expansion after a bottleneck event (Table 3).

Pantelozetes paolii

The phylogenetic trees based on BI and ML methods of the COI nucleotide alignment showed similar topologies and only the BI tree (MrBayes) is shown in Fig. 2b (ML tree is shown in Online Resource 5). *Pantelozetes paolii* was monophyletic and separated with high support from the outgroup taxon *N. silvestris* (as well as from *P. cavaticus* and *Pantelozetes* sp., Online Resource 2b) and the COI haplotypes clustered into three main lineages independent of the sampling site (Fig. 2b; for detailed information about the number and geographic origin of the individuals from each lineage see Online resource

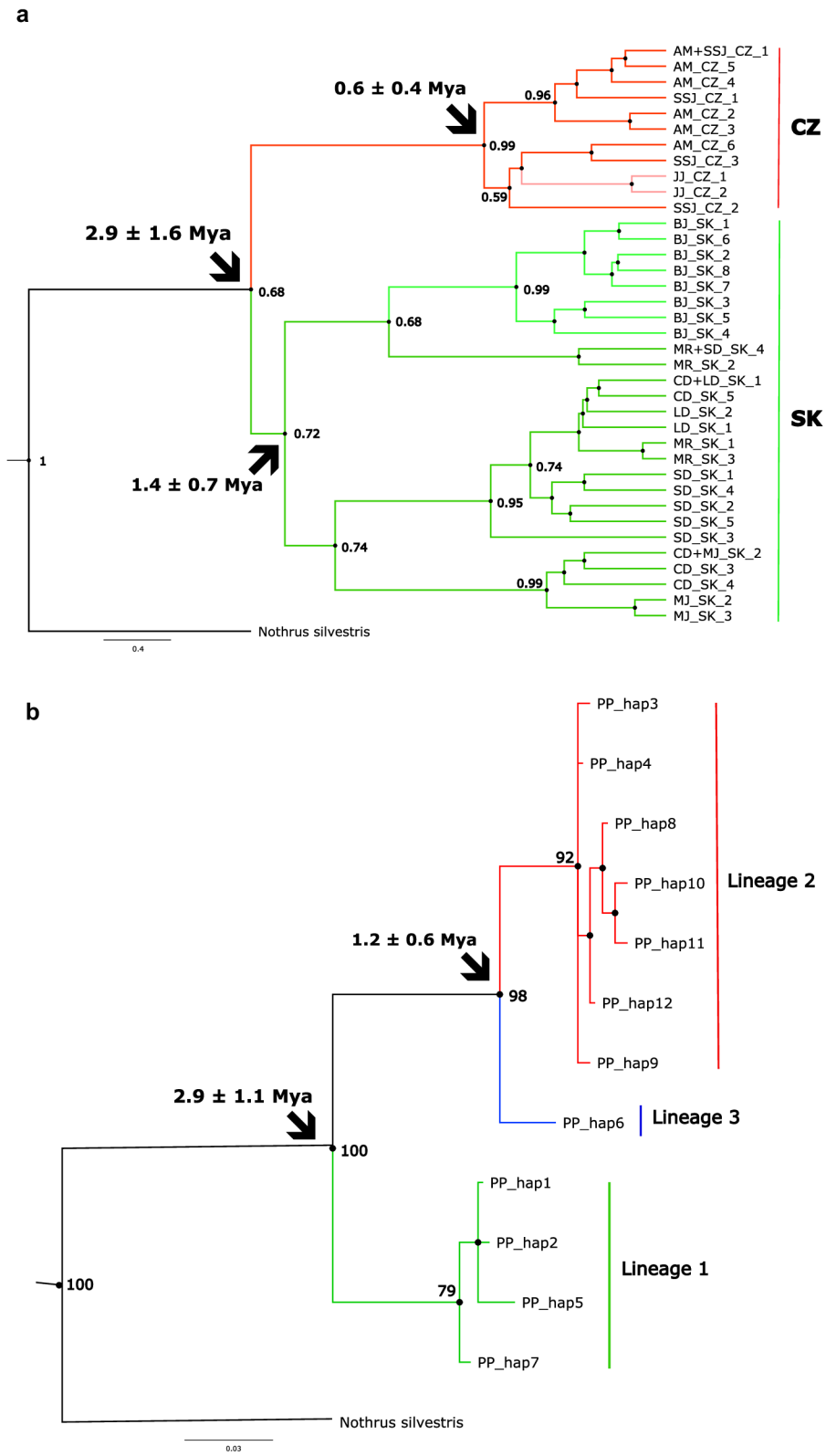

Fig. 2 Bayesian inference trees based on the COI gene showing the relatedness among individuals of (**a**) *Pantelozetes cavaticus* (calculated in BEAST) and (**b**) *P. paolii* (calculated in MrBayes). Different branch colors indicate genetic lineages identified within the species (see color version online). Numbers at the nodes represent posterior probabilities, bold numbers are median estimated divergence times ± 95% HPD (highest posterior density) calculated in BEAST

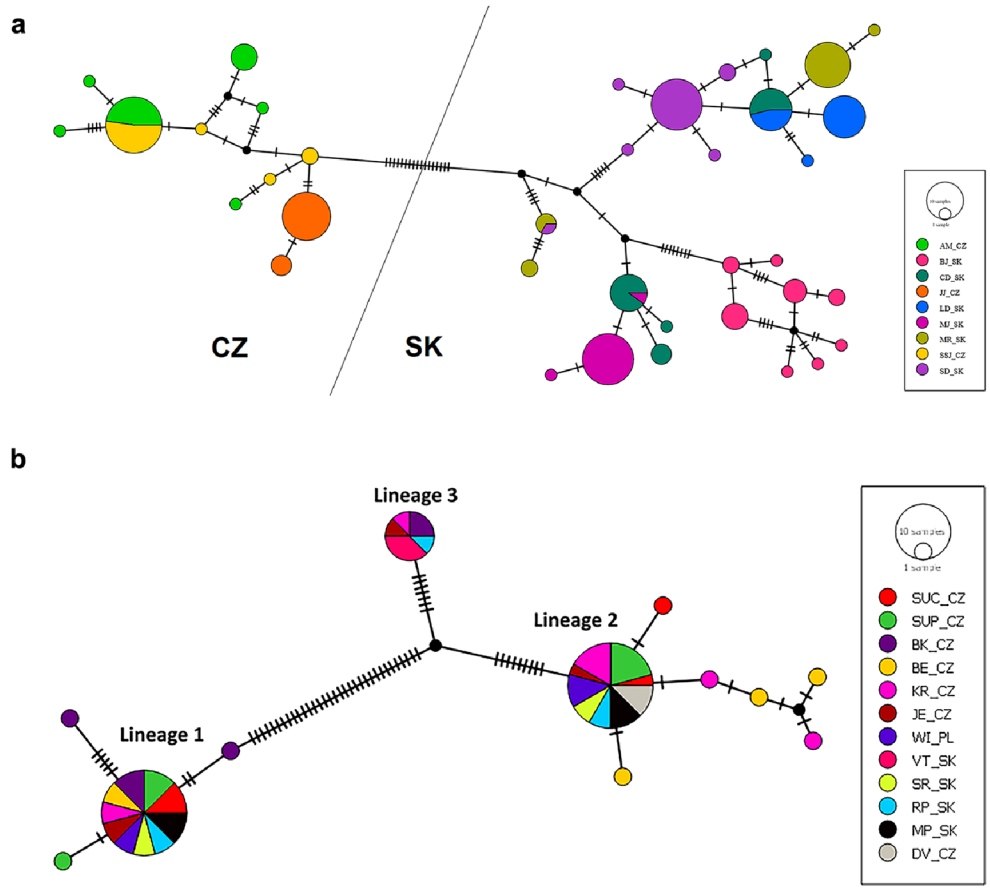

Fig. 3 Median Joining Haplotype Network of COI sequences of (**a**) *Pantelozetes cavaticus* and (**b**) *P. paolii* (see color version online). The size of the circles is proportional to the number of sequences per haplotype, bars on the lines represent number of mutation steps separating haplotypes

6). Applying a mitochondrial substitution rate of 2.3% per million years, the lineage 1 diverged from the rest 2.9 ± 1.1 Mya during the Late Pliocene, and lineages 2 and 3 split during the Pleistocene 1.2 ± 0.6 Mya (Fig. 2b).

The COI haplotype network showed a clear structure of three dominant haplotypes, i.e., three lineages shared among individuals from often very distant sampling localities (Fig. 3b). The network showed individuals from multiple sampling localities in all lineages, i.e., in the sites Ružín Water Dam (RP_SK), NP Krkonoše (KR_CZ) and Jeseníky PLA (JE_CZ) individuals with all three haplotypes coexisted, and in the rest of the sampling sites, a mix of two of the three main haplotypes was found. In total, 12 haplotypes were found in the 63 individuals sequenced. The most abundant haplotypes 1 and 2 were associated with some other haplotypes (represented only by one individual) by a mutational step. Contrary to *P. cavaticus*, the estimated haplotype diversity was relatively high within the populations (an average value 0.545) but much lower within the lineage (an average value 0.198), whereas nucleotide diversity was low in both cases (and markedly lower within the lineages) (Table 3). We identified six amino acid haplotypes, two abundant: first shared only between the individuals from lineage 1, second shared between individuals from lineages 2 and 3; the rest of the amino acid haplotypes were detected only in one individual on different sampling localities (ML phylogenetic tree is shown in Online Resource 3b).

Table 3 Basic molecular diversity parameters and neutrality tests for the studied *Pantelozetes* species, sampling localities (see Table 1 for code explanation) and lineages

	N	H	Hd	π	Tajima's D	Fu's Fs
P. cavaticus						
All individuals	180	37	0.933	0.024	0.738	0.806
AM_CZ	20	6	0.658	0.005	−0.736	1.058
JJ_CZ	20	2	0.268	0.001	−0.086	0.381
SSJ_CZ	16	4	0.442	0.002	0.227	−0.316
BJ_SK	17	8	0.878	0.006	−0.152	−0.663
CD_SK	21	5	0.714	0.008	2.083	4.030
LD_SK	20	3	0.511	0.001	−0.526	0.382
MJ_SK	21	3	0.186	0.001	−1.514*	−1.920*
MR_SK	20	4	0.437	0.008	0.738	5.656
SD_SK	25	6	0.427	0.002	−2.179*	−1.220
Lineage CZ	56	11	0.738	0.006	0.035	−0.086
Lineage SK	124	26	0.912	0.013	−0.097	−1.200
P. paolii						
All individuals	63	12	0.726	0.037	1.306	6.085
DV_CZ	3	1	0	0	0	
BE_CZ	6	4	0.800	0.044	2.078	4.966
BK_CZ	7	4	0.810	0.034	0.901	5.678
JE_CZ	4	3	0.833	0.049	1.315	4.649
KR_CZ	9	5	0.806	0.033	0.162	5.449
SUC_CZ	5	4	0.900	0.042	1.812	3.245
SUP_CZ	6	2	0.533	0.037	1.392	11.584
MP_SK	6	2	0.600	0.042	2.365	12.112
RP_SK	5	3	0.800	0.046	1.455	6.519
SR_SK	4	2	0.667	0.046	2.309	8.541
VT_SK	3	1	0	0	0	–
WI_PL	5	2	0.600	0.042	1.883	10.274
Lineage 1	25	4	0.230	0.001	−2.216*	−0.806*
Lineage 2	30	7	0.366	0.001	−1.582*	−3.484*
Lineage 3	8	1	0	0	0	–

N number of sequenced individuals, *H* number of observed haplotypes, *Hd* haplotype diversity, *π* nucleotide diversity

*Significant differences from zero, suggesting population expansion after a bottleneck event

Genetic distances detected between populations from discrete sampling localities were high and ranged between 1.9 and 5.7% for the COI gene. Intrapopulation genetic distances in the COI gene were high as well (3.3–5%), except for two populations where the value was zero (possibly the result of the small number of successfully sequenced individuals). After redefining the groups of individuals according to their assignment to the genetic lineage, the genetic distances among the lineages ranged between 2.6 and 7% and between 0 and 0.1% within the lineages, respectively (Table 2c).

Isolation by distance was rejected, Mantel test being not significant ($R^2 = 0.034$, $p = 0.14$). The neutrality analyses did not show any significant differences from zero within

Table 4 Result of AMOVA based on the uncorrected p-distances for *Pantelozetes cavaticus*

Source of variation	Between groups (CZ and SK)	Within groups	Within populations
Degrees of freedom	1	7	171
Sum of squares	764.87	393.28	189.53
Variance components	9.21 Va*	2.75 Vb*	1.11 Vc*
Variation %	70.46	21.05	8.49
Fixation indices	Fst 0.915*		

Fst F-statistics

*Indicate significant differences ($p < 0.05$)

the populations ($p > 0.05$ for both D and F_S) leading to the assumption that there is no evidence of size changes in the populations. However, after this analysis was carried out on every identified lineage (except lineage 3 with only one haplotype), values significantly different from zero were observed ($p > 0.05$ for both D and F_S) indicating that the lineages may have undergone a process of population expansion after a bottleneck event (Table 3).

Discussion

The results of our study provide the first insight into the genetic diversity and population structure of two oribatid mite species from the genus *Pantelozetes*. In agreement with our hypothesis, the cave-associated species, *P. cavaticus*, is represented by several phylogeographically subdivided populations that are reproductively well isolated between discrete karst areas, falling into two main genetic lineages ('Czech' and 'Slovak'). Conversely, the other investigated species, the common soil-living *P. paolii*, lacks any geography-related population structure, i.e., the three identified main genetic lineages coexist on individual sampling locations, suggesting good dispersal abilities of this species.

The intraspecific variation of the COI gene was quite high between the identified lineages of both species (ranging from 2.6 to 7%). The standard COI barcoding gene shows in general more variability between the populations of flightless or less mobile wide-spread taxa (Papadopoulou et al. 2009), which was already documented also for the populations of soil-living microarthropods (Heethoff et al. 2007; Schäffer et al. 2010; Rosenberger et al. 2013; Kreipe et al. 2015; Lehmitz and Decker 2017) and which corresponds with our results. The phylogenetic analysis of *P. cavaticus* COI sequences further revealed existence of new species *Pantelozetes* sp., which was clearly separate from *P. cavaticus* (20.4% interspecific distance). A morphological re-inspection of the samples from a small, isolated locality in Čierna Hora Mts. (Andrejová Cave II), where the genetic analysis indicated the presence of the new species, revealed individuals with minor yet distinctive morphological features.

The D3 region was considered as a possible species marker in several studies (Maraun et al. 2003; Laumann et al. 2007; Lehmitz and Decker 2017), although some have reported it to fail to separate closely related species (Lehmitz and Decker 2017; Schäffer et al. 2019). In our study we found only little variation in D3 between *P. cavaticus* and *P. paolii* (0.6%); furthermore, the locus also failed to distinguish the new species, with its sequences being

identical to those of *P. cavaticus*. It seems that the D3 fragment is too short and conserved to be reliably used as the sole species marker for oribatid mites.

Pantelozetes cavaticus

The phylogeographic analysis of *P. cavaticus* sampled in the mid-point of its distribution revealed deep genetic differences between the individual populations. Considering the limited active migration possibilities of the species and, more importantly, the discontinuous nature of the cave environment to which the species is bound, the geographic isolation was expected. Two main lineages of *P. cavaticus* were identified, 'Czech' and 'Slovak'. Both lineages had specific, non-synonymous substitutions in the COI gene and were never found to coexist in a single cave, indicating selection followed by a subsequent spread of the most competitive genotype. This is consistent with the results of several other studies that revealed high intraspecific genetic diversity in COI of widespread oribatid mite species (Rosenberger 2010; Schäffer et al. 2010, 2019; von Saltzwedel et al. 2014; Pfingstl et al. 2019).

Mutation rate of the mitochondrial COI gene is relatively high, which could lead to the formation of a significant inter-population diversity over a short evolutionary timescale (Hebert et al. 2003). Therefore, the presence of individuals from different caves that shared the same haplotypes (i.e., AM_CZ + SSJ_CZ; LD_SK + MJ_SK + CD_SK; SD_SK + MR_SK) points to a continual gene flow between these populations or to a recent colonization of one cave from the other. This could be easily explained by a short geographical distance between these caves and their potential connection via underground streams or crevices (e.g., Nová Amatérská Cave and Sloupsko-Šošůvské Caves are connected by a subterranean stream, and all the caves from Slovak Karst are close to each other).

In contrast, no shared haplotypes and presumably no gene flow between the populations from distant and not inter-connected karst areas indicate effective reproductive isolation of these populations from each other. A very similar trend of COI genetic variability increasing with geographical distance was detected by Parimuchová et al. (2017) in the populations of the troglophile collembolan *Protaphorura janosik* in Slovak caves.

Molecular divergence estimates based on *P. cavaticus* COI sequences indicated that the separation of 'Czech' and 'Slovak' lineages substantially predated Quaternary glaciations (Pleistocene) and happened during the Late Pliocene (2.9 Mya). This radiation event coincides with the climatic and biotic changes occurring in Europe during this epoch, i.e., global cooling, less precipitation, and the spread of grasslands (Retallack 2001). This might have forced the common ancestor of extant *P. cavaticus* lineages to escape from the changing surface conditions to cave ecosystems with more stable conditions. Despite this relatively ancient radiation, the lineages have remained morphologically consistent and no clear morphological differences were evident between individuals during the identification prior to the DNA extraction. The stable conditions of the subterranean environment may have contributed to the morphological consistency of the emerging genetic lineages. Homogenous habitat conditions were suggested to enforce stabilizing selection, which can maintain a constant phenotype across the range of the group, resulting in conserved morphologies on a diverse genetic background (Colborn et al. 2001; Pfingstl et al. 2019).

Nevertheless, long-term geographical and genetic isolation of the lineages could be expected to provoke, through the growing genetic distances, an evolution of new species differing even in their morphology. Indeed, our discovery of a new species in Andrejová Cave II, seems to support this theory. Based on the molecular divergence estimates,

Pantelozetes sp. separated from the ancestor of *P. cavaticus* 10.1 (± 1.8) Mya, during the Late Miocene.

Pantelozetes paolii

Pantelozetes paolii is a eurytopic and abundant species that can be found in a variety of surface habitats (with Holarctic distribution), indicating that this species can cope with a wide range of environmental conditions. Unfortunately, the number of specimens obtained during sample collection was limited and PCR amplification of the COI gene did not work very well in this species, therefore the number of successfully sequenced individuals from some sampling localities was low (i.e., only three individuals from DV_CZ and VT_SK).

We assumed that given its small size and limited active locomotion powers, the populations from distant areas would create a distinct phylogeographic pattern as this was already described in many other studies investigating the genetic structure of populations of small widespread soil arthropods (Schäffer et al. 2010; Rosenberger et al. 2013; Saltzwedel et al. 2016, 2017). Waters et al. (2013) suggested that a founder effect may play a major role in the colonization of new habitats, which results in a low genetic variance within the populations but a high one between them.

Contrary to this, in 63 sequenced individuals we found three main genetic lineages with deep genetic differences (genetic distances between the lineages ranged from 2.6 to 7%) cohabiting at distinct sampling localities. High haplotype diversity indicated ancient separation and independent evolution of these lineages. It was common that individuals from at least two lineages (and in a few cases even all three—in Ružín Water Dam, Jeseníky PLA and NP Krkonoše) coexisted at one locality (one haplotype was shared among the individuals from distinct localities). This points to an effective long-distance dispersal ability of this species.

Poor active dispersal, even on distances of only couple of centimeters, is characteristic for oribatid mites (Lehmitz et al. 2012). However, given that many species have huge geographical distribution ranges, efficient dispersal pathways must exist. This topic has not yet been completely resolved. Passive dispersal by wind, though documented, is species-specific and not very common over a longer distance in oribatid mites given their small size and susceptibility to dehydration (Lehmitz et al. 2011; Schuppenhauer et al. 2019). Transport by running water and on larger animals, especially on birds, is more common and well documented (Lebedeva and Krivolutsky 2003; Krivolutsky and Lebedeva 2004; Schuppenhauer et al. 2019). *Pantelozetes paolii* has been sporadically found in the feathers of waterfowl (Krivolutsky and Lebedeva 2004), which is probably the most likely dispersal manner of this species.

High haplotype diversity (Hd, on average 0.545 and 0.198 for populations and lineages, respectively) and relatively low nucleotide diversity (π, on average 0.035 and 0.001 for populations and lineages, respectively) of *P. paolii* obtained in this study is consistent with what has been described for some other species of oribatid mites. For example, Schäffer et al. (2010), in their analysis of the COI region of two *Scutovertex* species (sampled also in Central Europe), observed values of Hd between 0.818 and 0.989 and values of π between 0.018 and 0.051.

These differences in Hd and π can indicate recent population growth (Korstian et al. 2015), which is consistent with the results of the neutrality tests performed within the detected lineages of *P. paolii* in our study. It seems that the species experienced an event that caused a drastic reduction in its abundance (bottleneck effect) followed by a rapid

population growth. First of these events might have happened during the Late Pliocene (2.9 Mya per molecular divergence time estimates) when the climatic and habitat conditions were rapidly changing—similarly to the situation in *P. cavaticus* as described above. The second event of radiation (1.2 Mya) was probably caused by strong climatic oscillations during the Pleistocene Epoch (Hewitt 2004). Moreover, bottleneck events followed by demographic expansions have been consistently shown to leave a genetic mark in the current populations in the pattern of some haplotypes being broadly shared and others, less frequent, differing by only a few mutations (Bas 1995).

Initial morphological examination of specimen did not reveal substantial differences, however an in-depth morphometric study of individuals from different genetic lineages may reveal relevant morphological traits. Additional coupled genetic and morphological data from representative sampling sites across the *P. paolli* distribution range are necessary to clarify the relationships among its populations.

Conclusions

This study provides the first insight into the genetic diversity, population structure and evolutionary history of two ecologically different species from the genus *Pantelozetes.* We show that populations of the cave-dwelling *P. cavaticus* are effectively confined to the respective karst areas, whereas the populations of the surface-dwelling *P. paolii* from comparably distant localities are intermixed, demonstrated as three main lineages that coexist on multiple sampling sites, suggesting effective long-distance dispersal of the latter species. The estimated divergence of the genetic lineages of *P. cavaticus* and *P. paolii* of 2.9 Mya coincides with the onset of major climatic changes during Late Pliocene, when the European climate began to cool rapidly. In addition, we confirmed that COI is a good marker for studies of population structure, but found the D3 fragment too conserved to distinguish populations or even closely-related species. Finally, we report a new candidate species, *Pantelozetes* sp., from one of the sampled caves in Slovakia (Andrejová Cave II); its description will be published separately.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10493-021-00605-7.

Acknowledgements The research was supported by the Czech Academy of Sciences (under Research Plan No. AV0Z606960521), by two grant projects of the Czech Science Foundation (projects No. P504/12/1218 and No. 14-09231S) and one project of the Slovak Research and Development Agency (APVV-17-0477). The research in Czech caves was undertaken in the collaboration with the Cave Administration of Czech Republic. We want to thank to Dr. Koudelka (Head of the Javoříčské Caves) for cooperation and enabling the access to the Javoříčské Caves; Dr. Štefka (Head of the Moravian Karst PLA), Dr. Tůma and Dr. Kovařík for cooperation and help with the field sampling in Sloupsko-šošůvské Caves and Amatérská Cave. We thank also to the Slovak Caves Administration and to the Administrations of Šumava NP, Wigry NP, Tatranský NP and Slovenský kras NP for their permission to conduct the study in the national parks.

Funding This study was supported by the Academy of Sciences of the Czech Republic (under Research Plan No. AV0Z606960521), by two grant projects of the Czech Science Foundation (projects No. P504/12/1218 and No.14-09231S) and by one project of the Slovak Research and Development Agency (APVV-17-0477).

Data availability All the sequences obtained for this study are publicly available from the GenBank (accession numbers are listed in Table 1). The datasets (alignments) generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest The authors have no conflict of interest to declare that are relevant to the content of this article.

Ethics approval No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402

Avise JC (1994) Molecular markers, natural history and evolution. Chapman & Hall, New York

Bas C (1995) Ecological structures: expansion and replacement. Sci Mar 59:373–380

Beebee T, Row G (2008) An introduction to molecular ecology, 3rd edn. Oxford University Press, Oxford

Beheregaray LB (2008) Twenty years of phylogeography: the state of the field and the challenges of the southern hemisphere. Mol Ecol 17(17):3754–3774

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A et al (2019) BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. PLoS Comput Biol 15(4):e1006650

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. PNAS USA 91:6491–6495

Bruckner A (1995) Cave-dwelling oribatid mites (Acarina, Cryptostigmata) from East Austria. Verh Zool-Bot Ges Österr 132:81–107

Colborn J, Crabtree RE, Shaklee JB, Pfeiler E, Bowen BW (2001) The evolutionary enigma of bonefishes *(Albula*spp.): cryptic species and ancient separations in a globally distributed shorefish. Evolution 55(4):807–820

Excoffier LG, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

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. Mol Mar Biol Biotechnol 3(5):294–299

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147(2):915–925

Hall TA (1999) BioEdit: A User-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acid Symp Ser 41:95–98

Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. Proc R Soc B 270(1512):313–321

Heethoff M, Domes K, Laumann M, Maraun M, Norton RA, Scheu S (2007) High genetic divergences indicate ancient separation of parthenogenetic lineages of the oribatid mite *Platynothrus peltifer* (Acari, Oribatida). J Evol Biol 20:392–402

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Philos Trans R Soc Lond B Biol Sci 359(1442):183–195

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol 35(2):518–522

Kalyaanamoorthy S, Minh BQ, Wong TKW, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589

Korstian KM, Hale AM, Williams DA (2015) Genetic diversity, historic population size, and population structure in 2 North American tree bats. J Mamm 96(5):972–980

Kováč Ľ, Parimuchová A, Miklisová D (2016) Distributional patterns of cave Collembola (Hexapoda) in association with habitat conditions, geography and subterranean refugia in the Western Carpathians. Biol J Linn Soc 119(3):571–592

Kreipe V, Corral-Hernández E, Scheu S, Schaefer I, Maraum M (2015) Phylogeny and species delineation in European species of the genus *Steganacarus* (Acari, Oribatida) using mitochondrial and nuclear markers. Exp Appl Acarol 66(2):173–186

Krivolutsky DA, Lebedeva NV (2004) Oribatid mites (Oribatei, Acariformes) in bird feathers: non-passerines. Acta Zool Litu 14(1):26–45
Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549
Laumann M, Norton RA, Weigmann G, Scheu S, Maraun M, Heethoff M (2007) Speciation in the parthenogenetic oribatid mite genus *Tectocepheus* (Acari, Oribatida) as indicated by molecular phylogeny. Pedobiologia 51(2):111–122
Lebedeva NV, Krivolutsky DA (2003) Birds spread soil microarthropods to arctic islands. Dokl Biol Sci 391:329–332
Lehmitz R, Decker P (2017) The nuclear 28S gene fragment D3 as species marker in oribatid mites (Acari, Oribatida) from German peatlands. Exp Appl Acarol 71(3):259–276
Lehmitz R, Russel D, Hohberg K, Christian A (2011) Wind dispersal of oribatid mites as mode of migration. Pedobiologia 54(3):201–207
Lehmitz R, Russell D, Hohberg K, Christian A, Xylander WER (2012) Active dispersal of oribatid mites into young soils. Appl Soil Ecol 55:10–19
Leigh JW, Bryant D (2015) PopART: full-feature software for haplotype network construction. Methods Ecol Evol 6(9):1110–1116
Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452
Ľuptáčik P (2004) Príspevok k rozšíreniu *Gemmazetes cavaticus* (Kunst, 1962) (Acarina, Oribatida, Thyrisomidae) v Európe so zameraním na územie Slovenska. Book of Abstracts Zoologické dny. Brno, Brno
Ľuptáčik P, Miko L (2003) Oribatid mites (Acarina, Oribatida) of Slovak caves. Subterr Biol 1:25–29
Luxton M (1972) Studies on the oribatid mites of a Danish beech wood soil. I. Nutritional biology. Pedobiologia 12(3):434–463
Maraun M, Scheu S (2000) The structure of oribatid mite communities (Acari, Oribatida): patterns, mechanisms and implications for future research. Ecography 23:374–383
Maraun M, Heethoff M, Scheu S, Norton RA, Weigmann G, Thomas RH (2003) Radiation in sexual and parthenogenetic oribatid mites (Oribatida, Acari) as indicated by genetic divergence of closely related species. Exp Appl Acarol 29(3–4):265–277
Marshall VG (1972) Comparison of two methods of estimating efficiency of funnel extractors for soil microarthropods. Soil Biol Biochem 4:417–426
Nguyen LT, Schmidt HA, von Haeseler A, Bui QM (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32(4):268–274
Norton RA, Bonamo PM, Grierson JD, Shears WA (1988) Oribatid mite fossils from a terrestrial Devonian deposit near Gilboa, New York. J Paleontol 62:259–269
Ojala R, Huhta V (2001) Dispersal of microarthropods in forest soil. Pedobiologia 45:443–450
Pachl P, Lindl AC, Krause A, Scheu S, Schaefer I, Maraun M (2017) The tropics as an ancient cradle of oribatid mite diversity. Acarologia 57(2):309–322
Papadopoulou A, Anastasiou I, Keskin B, Vogler AP (2009) Comparative phylogeography of tenebrionid beetles in the Aegean archipelago: the effect of dispersal ability and habitat preference. Mol Ecol 18(11):2503–2517
Parimuchová A, Kováč Ľ, Žurovcová M, Miklisová D, Paučulová L (2017) A glacial relict in the Carpathian caves—population variability or a species complex? Arthropod Syst Phylo 75(3):351–362
Pfingstl T, Baumann J, Lienhard A (2019) The Caribbean enigma: the presence of unusual cryptic diversity in intertidal mites (Arachnida, Acari, Oribatida). Org Divers Evol 19(4):609–623
Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using tracer 1.7. Syst Biol 67(5):901–904
Retallack GJ (2001) Cenozoic expansion of grasslands and climatic cooling. J Geol 109(4):407–426
Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61(3):539–542
Rosenberger MJ (2010) Phylogeography in sexual and parthenogenetic European Oribatida. Dissertation, Göttingen Centre for Biodiversity and Ecology
Rosenberger MJ, Maraun M, Scheu S, Schaefer I (2013) Pre- and post-glacial diversifications shape genetic complexity of soil-living microarthropod species. Pedobiologia 56:79–87
Schaefer I, Norton RA, Scheu S, Maraun M (2010) Arthropod colonization of land-linking molecules and fossils in oribatid mites (Acari, Oribatida). Mol Phylogenet Evol 55:113–121

Schäffer S, Pfingstl T, Koblmüller S, Winkler KA, Sturmbauer Ch, Krisper G (2010) Phylogenetic analysis of European *Scutovertex* mites (Acari, Oribatida, Scutoverticidae) reveals paraphyly and cryptic diversity: a molecular genetics and morphological approach. Mol Phyl Evol 55:677–688

Schäffer S, Kerschbaumer M, Koblmüller S (2019) Multiple new species: cryptic diversity in the widespread mite species *Cymberemaeus cymba* (Oribatida, Cymbaeremaeidae). Mol Phylogenet Evol 135:185–192

Schuppenhauer MM, Lehmitz R, Xylander WER (2019) Slow-moving soil organisms on a water highway: aquatic dispersal and survival potential of Oribatida and Collembola in running water. Mov Ecol. https://doi.org/10.1186/s40462-019-0165-5

Skoracka A, Magalhães S, Rector BG, Kuczyński L (2015) Cryptic speciation in the Acari: a function of species lifestyles or our ability to separate species? Exp Appl Acarol 67(2):165–182

Starý J (2008) Diversity and distribution of oribatid mites (Acari: Oribatida) in caves of Czech Republic. Acta Carsol Slovaka 46(1):185–197

Subías LS (2020) Listado sistemático, sinonímico y biogeográfico de los Ácaros Oribátidos (Acariformes: Oribatida) del mundo (excepto fósiles), 15ª actualización. http://bba.bioucm.es/cont/docs/RO_1.pdf. Accessed June 2020

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. G3 (Bethesda) 123:585–595

Villesen P (2007) FaBox: an online toolbox for fasta sequences. Mol Ecol Notes 7(6):965–968

von Saltzwedel H, Mark M, Scheu S, Schaefer I (2014) Evidence for frozen niche variation in a cosmopolitan parthenogenetic soil mite species (Acari, Oribatida). PLoS ONE 9(11):e113268

von Saltzwedel H, Scheu S, Schaefer I (2016) Founder events and pre-glacial divergences shape the genetic structure of European Collembola species. BMC Evol Biol 16:148. https://doi.org/10.1186/s12862-016-0719-8

von Saltzwedel H, Scheu S, Schaefer I (2017) Genetic structure and distribution of *Parisotoma notabilis* (Collembola) in Europe: cryptic diversity, split of lineages and colonization patterns. PLoS ONE 12(2):e0170909

Waters JM, Fraser CI, Hewitt GM (2013) Founder takes all: density-dependent processes structure biodiversity. Trends Ecol Evol 28(2):78–85

Weigmann G (2006) Die Tierwelt Deutschlands, Teil 76: Hornmilben (Oribatida). Goecke and Evers, Keltern

Xia X (2017) DAMBE6: new tools for microbial genomics, phylogenetics and molecular evolution. J Hered 108:431–437

Xia X, Xie Z, Salemi M, Chen L, Wang Y (2003) An index of substitution saturation and its application. Mol Phylogenet Evol 26:1–7

Yosii R (1956) Monographie zur Höhlencollembolen Japans, vol 3. Biological Laboratory, Kyoto University, Kyoto, p 1

Żbikowska-Zdun K, Piksa K, Smaczyńska A (2009) Variation of selected morphological characters of the cave mite Oribella cavatica Kunst, 1962 (Acari, Oribatida). Biol Lett 46(2):123–127

5.3. Paper III

A new species of the genus *Pantelozetes* (Acari, Oribatida, Thyrisomidae) from the caves of Eastern Slovakia

JOSEF STARÝ[1*], PETRA KOKOŘOVÁ[1,2], PETER ĽUPTÁČIK[3] & MARTINA ŽUROVCOVÁ[4]

[1]*Biology Centre of the Czech Academy of Sciences, Institute of Soil Biology, České Budějovice, Czech Republic. E-mail: jstary@upb.cas.cz*
[2]*Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic. E-mail: petra.kokorova@post.cz*
[3]*Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Slovakia. E-mail: peter.luptacik@upjs.sk*
[4]*Biology Centre of the Czech Academy of Sciences, Institute of Entomology, České Budějovice, Czech Republic. E-mail: martina@entu.cas.cz*
[*]*Corresponding author*

Abstract

Pantelozetes kunsti **sp. nov.,** the new species, named is described based on the adult individuals obtained from the detritus of Andrejová cave II in Eastern Slovakia. The new species is morphologically very similar to *Pantelozetes cavaticus* (Kunst, 1962), from which it differs especially by tridactylous tarsi (versus monodactylous tarsi), the punctulation between the anterior part of costulae (vs. smooth surface), the absence of solenidion ω_2 on tarsi II, and the rounded posterior part of notogaster (vs. slightly bluntly pointed). The identification key to the known species of the genus *Pantelozetes* from Europe and a list of identified species of this genus from 11 localities in the Czech Republic and Slovakia are presented. In addition, the delimitation of *P. kunsti* **sp.nov.** from other common European species of the genus *Pantelozetes* was verified by analyses of the COI (DNA barcoding) sequences. The interspecific distances in COI varied between 19% to 22.1% and the newly described species showed closest phylogenetic relationship to *P. cavaticus*.

Key words: genetic diversity, COI, barcoding, troglophilous oribatid mites, morphology

Introduction

Phylogenetic analyses of oribatid mite DNA sequences of the genus *Pantelozetes* from the localities in the Czech Republic and Slovakia suggested the existence of a new species. *Pantelozetes kunsti* **sp. nov.** was identified as the mitochondrial COI sequences of the populations of *Pantelozetes cavaticus* were analysed. The sequences from one population (sampling site Andrejová cave II) showed much higher nucleotide variability when compared with other studied populations. The separation of the new species

was further supported by a subsequent morphological analysis of the specimens under the stereomicroscope.

Currently, the genus *Pantelozetes* comprises 22 species and one subspecies, which are distributed in the Holarctic, Oriental and Neotropical Regions (Subías 2004, updated 2021). The main morphological characters of this genus were summarized by Kunst (1971), Fujikawa (1979), Weigmann (2006), Bayartogtokh (2010) and Ermilov et al. (2015). The identification keys to the selected species of this genus were presented earlier by Fujikawa (1979), Rakhimbaeva (1995), Bayartogtokh (2003, 2010) and Weigmann (2006).

The main objective of this study was to describe and illustrate newfound *Pantelozetes kunsti* **sp.nov.** and to present an identification key to the European species of the genus. The additional objective of this paper was to compare divergences on the DNA level between the new species and other common *Pantelozetes* species.

Material and methods

Specimens. In total, 594 specimens from four species of the genus *Pantelozetes* were obtained at two localities in the Czech Republic and nine localities in Slovakia. Most of this material was used for DNA analyses, the smaller part was used for species identification. The sampling localities are given in "*Material examined"* section.

Observation and documentation. Soil arthropods were extracted from the soil and leaf litter samples in a modified high-gradient extractor (Marshall 1972). All mite specimens were preserved in 96% ethanol at -20 °C until further preparation. Oribatid mites were mounted and cleared in 80% lactic acid on temporary cavity slides for determination, measurement, and illustration. The determined material was preserved in the vials with 80 % ethanol with drops of glycerol. Observations, determination, measurements, and illustrations were performed with the use of a transmission light microscope "Leica DRM" equipped with a drawing attachment. The body length was measured in a lateral view from the tip of the rostrum to the posterior edge of the ventral plate. The notogastral width refers to the maximum width of the notogaster in dorsal aspect. The length of the body setae was measured in a lateral aspect. All measurements are given in micrometres. The leg setae formulas are given in parentheses according to the sequence trochanter - femur - genu - tibia - tarsus (famulus included). The formulas for leg solenidia are given in square brackets according to the sequence genu - tibia - tarsus.

Terminology. Morphological terminology used in this paper follows that of F. Grandjean: see Travé & Vachon (1975) for references, Norton (1977) for leg setal nomenclature, and Norton & Behan–Pelletier (2009) for overview.

Abbreviations. The following abbreviations were used (including text, figures and tables): *ro*, *le*, *in*, *bs*, *ex* = rostral, lamellar, interlamellar, bothridial and exobothridial setae, respectively; *cos* = costulae;

opisthonotal gland opening; *cr* = dorso-lateral cristae; *cp* = circumpedal carianae; *a*, *m*, *h* = subcapitular setae; *or* = adoral seta; *v*, *l*, *d*, *cm*, *acm*, *ul*, *sul*, *vt*, = palp setae; *ω* = palp and leg solenidion; *cha*, *chb* = cheliceral setae; Tg = Trägårdh's organ; Df = digital fixus; Dm = digitus mobilis; PdI = pedotectum I; *1a*, *1b*, *1c*, *2a*, *3a*, *3b*, *3c*, *4a*, *4b*, *4c* = epimeral setae; $g_{1\text{-}6}$, *ag*, $an_{1\text{-}2}$, $ad_{1\text{-}3}$ = genital, aggenital, anal and adanal setae, respectively; *iad* = adanal lyrifissure; Tr III, Tr IV = leg trochanter III and IV, respectively; σ, φ, *ω* = leg solenidia; ε = tarsus I famulus; *v*, *ev*, *bv*, *l*, *d*, *ft*, *tc*, *it*, *p*, *u*, *a*, *s*, *pv*, *pl* = leg setae.

DNA extraction and analysis. Total genomic DNA of *P. kunsti* **sp. nov.** was extracted from the whole single individuals (23 specimens) with the Exgene Tissue SV mini kit (GeneAll® Biotechnology) following the manufacturer´ protocol for insects with final elution in 50 µl instead of 200 µl. A fragment of cytochrome c oxidase subunit I (COI) was amplified by PCR and sequenced as described in Kokořová et al. (2021). COI sequences of other *Pantelozetes* species used for comparisons were obtained from Kokořová et al. (2021) (for GenBank accession numbers see Appendix 1). Three specimens of *P. alpestris* sampled in the High Tatras were additionally sequenced for this study. The newly obtained sequences are deposited in GenBank with accession numbers: *P. kunsti* **sp. nov.** MW649874-MW649896; *P. alpestris* MZ645101-MZ645103.

All sequences were assembled and then aligned with the MEGA X software (Kumar et al. 2018) by Muscle algorithm with default parameters. Pairwise genetic distances were calculated in MEGA X. The distance based Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021) analysis was performed with default settings (p-distances set as nucleotide substitution model) and ran on the ASAP website (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html). Only the partitions showing the lowest and second lowest asap-score were considered according to Puillandre et al. (2021). The hypothetical species composition generated by ASAP was compared with morphologically defined *Pantelozetes* species, including the newly described species *P. kunsti* **sp. nov.** The phylogenetic trees of studied species were calculated with Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The best fitting model for both methods was generated by Bayesian Information Criterion (BIC) in the ModelFinder (Kalyaanamoorthy et al. 2017) implemented in the IQ-TREE v.1.6.12 software (Nguyen et al. 2015). The ML analysis was conducted in IQ-TREE with default parameter settings and 10,000 ultrafast bootstrap replicates (Hoang et al. 2018). The BI analysis was performed in MrBayes v.3.2.7 (Ronquist et al. 2012) in two independent runs with four chains, each for 10 million generations sampled every 1,000th generation, a burn-in of 2,500 was applied. To assess run convergence, the Tracer v.1.7.1 (Rambaut et al. 2018) was used. A representative of other genus *Nothrus silvestris* (Crotonioidea) was used as an outgroup taxon, the COI sequence was obtained from Rosenberger et al. (2013) (GenBank accession no JF2638835).

Description

Family: Thyrisomidae Grandjean, 1954

Genus *Pantelozetes* Grandjean, 1953

Syn: *Montizetes* Kunst, 1971; *Oribellopsis* Kunst, 1971; *Gemmazetes* Fujikawa, 1979 (see Ermilov et al. 2015).

Type species: *Xenillus paolii* Oudemans, 1913

***Pantelozetes kunsti* sp. nov.**

Material examined

Eastern Slovakia, 26.VIII.2017, Andrejová Cave II, Čierna hora Mts., Pokryvy, latitude 48°48'03.45"N, longitude 21°09'48.62"E, altitude 485 m, sample of litter and detritus near cave entrance, (11 ex.), leg. P. Ľuptáčik.

Diagnosis

Rostrum rounded without dorsolateral teeth. Costulae longer than half of prodorsum length. Rostral and lamellar setae setiform, barbed. Interlamellar setae erect, barbed. Bothridial setae fusiform, lanceolate, barbed. Prodorsum without dorsolateral ridges. Central part of prodorsum between anterior part of costulae finely punctate. Notogastral setae barbed, similar in length. Cristae long. Two pairs of adoral setae. Sternal apodeme short. Epimeral and ano-genital setae thin, smooth. Leg tarsi with three claws.

Description

Measurements. Body length: 453 (holotype, female), 421–468 (ten paratypes, eight males and two females); notogaster width: 284 (holotype), 281–295 (ten paratypes). No distinct difference between males and females in body size.

Integument (Figs. 1A, B, C). Body color light brown to brown. Body surface, subcapitular mentum and genae, genital and anal plates punctate, which is visible only under high magnification (x 1000). Lateral sides of prodorsum (between acetabula and bothridia, and laterally to lamellae) tuberculate. Central part of prodorsum between anterior part of costulae finely punctate. Lateral side of prodorsum outside of anterior part of costula with fine reticulation.

Prodorsum (Figs. 1A, B). Rostrum rounded, without teeth. Costulae (*cos*) well developed, longer than half of prodorsum length, slightly S-shaped. Rostral (*ro*, 45-51) setae setiform barbed inserted laterodorsally. Lamellar (*le*, 63-71) setae setiform, barbed, inserted on the lamellar ends. Interlamellar setae (*in*, 32-38) shorter than rostral and lamellar ones, rod-like, erect, blunted and distinctly barbed.

smooth stalk, narrowly spindle-shaped, barbed head. Inter bothridial region smooth without distinct muscle sigillae. Dorsolateral ridges absent.

Notogaster (Figs. 1A, C). Anterior margin slightly convex medially. Posterior margin broadly rounded. One pair of distinct humeral tubercles present, punctate. Dorsophragma well visible. Ten pairs of notogastral setae, fine, slightly barbed, similar length (28-38). Seta p_1 (28) the shortest, setae *la*, h_3 and p_3 (38) the longest ones. Cristae (*cr*) long reaches the level of lyrifisure *im.* All lyrifissures (*ia, im, ip, ips, ih*) distinct, in usual location. Opisthonotal glandopenings (*gla*) located posterior to h_3.

Gnathosoma (Figs. 2E, F, G). Subcapitulum longer than wide (89-97 x 71-79). Subcapitular setae, setiform *a* 17-19), and *m* (19-21) shorter than *h* (23-26) slightly barbed. Two pairs of adoral setae (or_1, or_2 (5-7)) present thin, smooth. Palps (length 63-67) with setae formula 0-2-1-3-9(ω). Solenidion short (ω), pressed to the surface of palptarsi, blunted. Chelicerae, digitus fixus (90-99) with three teeth, digitus mobilis (24-28) with four teeth, with two barbed setiform cheliceral setae; *cha* (27-34) cambered, distinctly longer than curved *chb* (10-17). Trägårdh's organ (Tg 34-37) long, pointed.

Epimeral and lateral podosomal regions (Figs. 1B, C). Epimeres I and apodemes II separated medially by short sternal apodeme. Epimeres II, III, and IV and apodemes III and sejugal apodemes fused medially. Apodemes 2 and sejugal apodemes with tubercle-like or bridge-like structures. Epimeral setae formula: 3-1-3-3. All epimeral setae thin, setiform smooth. Lateral setae *1c, 3c* and *4c* (19-25) longer than medial ones *1a, 1b, 2a, 3a, 3b, 4a, 4b* (12-16). Pedotecta I distinct, punctate. Circumpedal carinae (*cp*) short directed to acetabula IV.

Anogenital region (Figs. 1B, C). Six pairs of genital (g_1-g_6, 12-15), one pair of aggenital (*ag*, 15-18), two pairs of anal (an_1, an_2, 16-17), and three pairs of adanal (ad_1, ad_2, ad_3, 19-22) setae setiform, thin and smooth Adanal lyrifissures (*iad*) distinct, located in adanal position parallel and near to anal aperture.

Legs (Figs. 2A-D). Tridactylous. Median claw thick, smooth, distinctly longer than lateral thin ones. Formulae of leg setation and solenidia: I (1-5-2-4-17) [1-2-2], II (1-5-3-4-15) [1-1-1], III (2-3-1-3-15) [1-1-0], IV (1-2-2-3-12) [0-1-0]. Famulus (ε) short, straight, blunted. Solenidia ω1 on tarsi I, ω on tarsi II and σ on genua II and III thickened, other solenidia thin setiform.

Type deposition

The holotype and 5 paratypes are deposited in the collection of the Museum of Natural History, Geneva, Switzerland; other 5 paratypes are deposited in the collection of the Biology Centre of the Czech Academy of Sciences, Institute of Soil Biology, České Budějovice, Czech Republic.

Etymology

The new species was named in honour of Prof. Dr. Miroslav Kunst (1923–1987), renowned Czech acarologist and zoologist, as well as the university teacher of the first author, for his significant contribution to our knowledge of the oribatid mite fauna especially in the caves of Central Europe.

Remarks

The new species is morphologically most similar to *P. cavaticus* (Kunst, 1962), differs from it by tridactylous tarsi (versus monodactylous tarsi), punctulation between anterior part of costulae, (vs. smooth surface), absence of solenidion ω_2 on tarsi II, and rounded posterior part of notogaster (vs. slightly blunty pointed); from *P. mongolicus* (Balogh & Mahunka, 1965) it differs by distinctly longer notogastal setae (vs. short), longer costulae (vs. shorter costulae), by absence of muscle sigillae in interbothridial region (vs. presence of three pairs of sigillae), lyrifissurae *iad* in adanal position (vs. apoanal position); from *P. arcticus* (Pankov, 1993) by absence of dorsolateral ridge on prodorsum (vs. presence), absence of muscle sigillae in interbothridial region (vs. presence of one pair of sigillae), presence of punctulation between anterior part of costulae (vs. smoth surface); from *P. alpestris* (Willmann, 1929) by distinctly longer costulae than half of prodorsum (vs. shorter costulae), absence of dorsolateral ridge, borthidial setae distinctly barbed head (vs. smooth head terminated by one long seta); from *P. forsslundi* (Moritz, 1965) by tridactylous tarsi (vs. monodactylous tarsi), presence of punctulation in between anterior part of costulae (versus smooth body surface in this region).

Key to European species of the genus *Pantelozetes*

1 - Prodorsum with lateralodorsal teeth on rostrum 2
- Prodorsum without laterodorsal teeth on rostrum 3

2 - Costulae connected in anterior part, muscle sigillae present in interbothridial region, at most three laterodorsal teeth on each side of rostrum ..... *P. berlesei* Fujikawa, 1979 - distribution: South - East Europe.
- Costulae never connected in anterior part, without muscle sigillae in interbothridia region, more than three teeth on each side of rostrum ..... *P. paolii* (Oudemans, 1913) - distribution: semicosmopolitan.

3 - Dorsolateral ridges on prodorsum present, costulles shorter reach half of prodorsum, insertions of lamellar setae in the middle part of prodorsum 4
- Dorsolateral ridges on prodorsum absent, costules distinctly longer than half of prodorsum, insertions of lamellar setae closer to the rostral margin 9

4 - Leg tarsi with three claws 5
- Leg tarsi with one claw 7

5 - Bothridial setae with smooth, fusiform head terminated by one long hair .....
..... *P. alpestris* (Willmann, 1929) - distribution: Holarctic Region

6 - Costulae absent, prodorsum with three pairs of muscle sigillae in interbothridial region .....
…….. *P. delamellatus* (Pérez-Iñigo, jr. 1990) - distribution: Spain.
- Costulae present on prodorsum, without muscle sigillae in interbothridial region .....
…….. *P. abulensis* (Pérez-Iñigo, 1984) - distribution: Spain.

7 - Costulae very short, do not reach insertions of lamellar setae ..... *P. grecus* (Mahunka et Mahunka-Papp, 2010) - distribution: Greece.
- Costulae longer reach insertions of lamellar setae 8

8 - Interlamellar setae smooth, two times longer than lamellar and rostral ones with sharp top .....
..... *P. etruscus* (Bernini, 1980) - distribution: Italy.
- Interlamellar setae barbed, thickened, and blunted, as long as lamellar and rostral ones, ...
..... *P. crosbyi* (Berlese, 1908) - distribution: Holarctic Region (see Ermilov 2016)

9 - Leg tarsii tridactylous, middle part of prodorsum between costulae with punctulation .....
..... *P. kunsti* **sp. nov.** - distribution: Slovakia.
- Leg tarsii monodactylous middle part of prodorsum between costulae smooth 10

10 - Bothridial setae with pectinate head ..... *P. clavigerus* (Mihelčič, 1958) - distribution: Austria.
- Bothridial setae without pectinate head 11

11 - Bothridial setae with distinct barbed head, notogastral setae strong and roughed, posterior part of notogaster rounded ..... *P. forsslundi* (Moritz, 1965) - distribution: Central and Western Europe.
- Bothridial setae with narrowly elongated head, notogastral setae thin and smooth, posterior part of notogaster slightly bluntly pointed ..... *P. cavaticus* (Kunst, 1962) - distribution: Palaearctic Region (Central, Western and Southern Europe, Mongolia, China) (see – Bayartogtokh 2010, Wang et al. 2000, Wen 1990).

Analyses of molecular data

The fragment of COI, standardly used for DNA barcoding of species, was successfully obtained from 23 specimens of *P. kunsti* **sp. nov.** and from three specimens of *P. alpestris*. Additional sequences of *P. cavaticus* and *P. paolii* for phylogenetic analyses were obtained from Kokořová et al. (2021). Five haplotypes were discovered in 23 sequences of *P. kunsti* **sp. nov.** The final alignment of four analysed *Pantelozetes* species consisted of sequences with 624-631 base pairs. Of the 242 variable sites, 210 were parsimony informative positions. No insertions, deletions, or stop codons were identified.

Phylogenetic reconstructions based on BI and ML methods revealed very similar topologies, therefore only BI tree is shown in Fig.3. The analyses yielded phylogenies with good to high statistical support for the monophyly of four studied *Pantelozetes* species.

The ASAP species delineation algorithm distinguished five (best asap-score) and four (second best asap-score) hypothetical *Pantelozetes* species. All five partitions coincided with the partitions derived from the ML and BI phylogeny, however one lineage of *P. paolii* was considered as a separate species (cluster A1 and A2 in Fig.3) by this algorithm. The four groups hypothesis (second best asap-score) corresponded with the morphologically defined and in the phylogenetic tree recognized *Pantelozetes* species including the newly proposed one, namely *P. paolii* (cluster A), *P. alpestris* (cluster B), *P. cavaticus* (cluster C) and *P. kunsti* **sp. nov.** (cluster D) (Fig.3).

The values of uncorrected p-distances within studied species ranged between 0.2% to 5.4% and the distances between species ranged between 19% to 22.1% (Table 2a-b). The newly proposed species *P.kunsti* **sp. nov.** showed closest relationship to *P. cavaticus*. Thus, a distinct barcoding gap was evident with inter-specific distances highly exceeding intra-specific ones (Fig.4).

Discussion on species delimitation

The analysis of DNA sequences can provide valuable information that can help resolve problematic arthropod species delimitation. In this study, we combined the morphological diagnostic characters with the COI dataset for the description of *P. kunsti* **sp. nov**. and its delimitation within the most common *Pantelozetes* species in Central Europe.

The analyses of the COI sequences recognized *P. kunsti* **sp. nov**. as a distinct monophyletic lineage within studied *Pantelozetes* species, which was likewise supported by relatively high genetic distances to other species (19-22.1%). These high species-level divergences in DNA barcoding region are well in line with the results previously reported in other oribatid mite species (Schäffer et al. 2010; Pfingstl et al. 2019; Schäffer et al. 2019; Kokořová et al. 2021) and suggests that this radiation is not of recent origin. The ASAP analysis also successfully delimited all studied morphologically defined species and suggested the existence of one extra species within *P. paolii* (the intraspecific divergences were 5.4%). Nevertheless, it was already well documented that DNA barcoding gene shows in general higher variability between the populations of small widespread microarthropods (Heethoff et al. 2007; Rosenberger et al. 2013; Kokořová et al. 2021), therefore we consider this partition to be just independent lineage of one species (for more details see Kokořová et al. 2021).

For oribatid mites, the DNA barcoding alone is considered of limited value for reliable determination of species boundaries (Kreipe et al. 2015), however it can be regarded as supplementary evidence, if other independent characteristics are involved in the analyses (White et al. 2014). Moreover, the main aim of the reconstruction of the phylogenetic trees was to use the obtained trees as tools to visualize possible species delimitations (DeSalle & Goldstein 2019), not to obtain accurate phylogenetic hypotheses which would require a broader spectrum of genetic markers as well as higher number of

List of identified *Pantelozetes* species

P. paolii

Material examined: Slovakia, 15.VIII.2017, Stolické vrchy Mts., SW slope of Stolica Mt., 5.5 km NE of Muráňská Zdychava, latitude 48°46'13,4"N, longitude 20°12'03,5"E, altitude 1348 m, spruce forest, sample of Norway spruce (*Picea abies*) litter, (22 ex.), leg. J. Starý; Slovakia, 2.VIII.2016, near Ružín dam, 4.8 km N of Opátka, latitude 48°49'54,2"N, longitude 21°04'24,7"E, altitude 401 m, beech forest (*Fagetum nudum*), sample of beech (*Fagus sylvatica*) litter, (33 ex.), leg. J. Starý; Slovakia, 5.VIII.2016, Biele vody, Slovenský ráj Mts., 0.6 km NE of Biele vody, latitude 48°52'31,0"N, longitude 20°24'21,3"E, altitude 877 m, beech forest with Norway spruce (*Picea abies*), sample of beech litter and mosses, (15 ex.), leg. J. Starý; Slovakia, 19.IX.2016, Tatra National Park, the High Tatra Mts., Tomanova valley, 8.8 km N of Podbánské, latitude 49°13'20,3"N, longitude 19°55'46,8"E, altitude 1361 m, spruce forest, with bilberry (*Vaccinium myrtillus*), sample of spruce litter, (16 ex.), leg. P. Čuchta.

P. cavaticus

Material examined: Czech Republic, 11.XI.2015, Moravian Karst Landscape Protected Area, Nová Amatérská Cave, White fountain, Ráztoka site, latitude 49°22'42.325"N, longitude 16°43'39.113"E, altitude 393 m., sample of decaying wood, (42 ex.), leg. K. Tajovský and P. Kokořová; Czech Republic, 21.X.2015, Javoříčské Caves, Giant Dome, latitude 49°40'13.400"N, longitude 16°54'50.300"E, altitude 452 m, sample of decaying wood, (176 ex.), leg. P. Kokořová and K. Tajovský; Slovakia, 18.V.2017, Slovenský kras National Park, Majkova Cave, Silická Plateau, latitude 48°32'44.489"N, longitude 20°32'49.320"E, altitude 494 m, sample of organic detritus near cave entrance, (20 ex.), leg. P. Ľuptáčik; Slovakia, 19.X.2017, Tatra National Park, Belianska Cave, Belianské Tatry Mts., latitude 49°13'43.888"N, longitude 20°18'40.461"E, altitude 890 m, Music-hall site, hand collection from stalactites near small cave lake, (120 ex.), leg. P. Ľuptáčik; Slovakia, 1.X.2015, Slovenský kras National Park, Čertova diera, Silická Plateau, latitude 48°28'57.810"N, longitude 20°27'44.500"E, altitude 339 m, Big Bat Hall, sample of bat's guano, (122 ex.), leg. P. Ľuptáčik.

***P. kunsti* sp. nov.**

Material examined: Slovakia, 26.VIII.2017, Čierna hora Mts., Andrejová Cave II., above Andrejová Rock, latitude 48°48'3.450"N, longitude 21°09'48.620"E, altitude 485 m, sample of litter and detritus near cave entrance, (43 ex.), leg. P. Ľuptáčik.

P. alpestris

Material examined: Slovakia, 4.VII.2016, Tatra National Park, the High Tatra Mts., Tomanova valley, latitude 49°12'46,7"N, longitude 19°54'46,7"E, altitude 1963 m, SW slope of Polská Tomanová Mt., alpine meadow with dominant highland rush (*Juncus trifidus*), with blueberry (*Vaccinium myrtillus*), (3

Acknowledgements

The authors thank to Ing. M. Koudelka, Head of Javoříčské Caves, for cooperation and access to Javoříčské Caves; to Dr. L. Štefka, Head of the Moravian Karst Protected Landscape Area, for cooperation and help in field sampling in Amatérská Cave. The research in the Czech caves was undertaken in collaboration with the Cave Administration of the Czech Republic. We also thank to the Slovak Caves Administration, and to the Slovak Karst National Park for the permission to conduct the study in the national parks and nature reserves. The research was supported by the Academy of Sciences of the Czech Republic, under Research Plan No. AV0Z606960521 and by two grant projects of the Czech Science Foundation (projects No. P504/12/1218 and No. 14-09231S) and by one project of the Slovak Research and Development Agency (APVV-17-0477).

References

Balogh, J. & Mahunka, S. (1965) Ergebnisse der zoologischen Forschungen von Dr. Z. Kaszab in der Mongolei 34. Acarina: Oribatei. *Annales Historico-Naturales Musei Nationalis Hungarici, Pars Zoologica,* 57, 452–465.

Bayartogtokh, B. (2003) Four species of oribatid mites (Acari: Oribatida) from Central and Southern Mongolia with notes on the genera *Montizetes* and *Zachvatkinibates*. *Mongolian Journal of Biological Sciences,* 1(1), 3–19.

Bayartogtokh, B. (2010) *Oribatid mites of Mongolia (Acari, Oribatida)*. Moscow, KMK, 372 pp.

Berlese, A. (1908) Elenco di generi e specie nuove di Acari. *Redia*, 5, 1–15.

Bernini, F. (1980) Notulae Oribatologicae XXIV. Gli Acari Oribatei di alcune piccole grotte del Senese. *Redia*, 63, 358–405.

DeSalle, R. & Goldstein, P.Z. (2019) Review and interpretation of trends in DNA barcoding. *Frontiers in Ecology and Evolution*, 7, 302.

Ermilov, S.G. (2016) Redescription of *Pantelozetes crosbyi* (Berlese, 1908) (Acari, Oribatida, Thyrisomidae). *Acarina,* 24(2), 167–173.

Ermilov, S.G., Savatulin, V.M. & Tolstikov, A.V. (2015) A new species of *Pantelozetes* (Acari, Oribatida, Thyrisomidae) from environs of lake Baikal (Russia). *Systematic and Applied Acarology*, 20(1), 51–60.

Fujikawa, T. (1979) Revision of the family Banksinomidae (Acari, Oribates). *Acarologia,* 20(3), 433–467.

Grandjean, F. (1953) Observations sur les Oribates (25^{e} série). *Bulletin du Muséum National d'Histoire Naturelle*, 25(2), 155–162.

Grandjean, F. (1954) Essai sur les Oribates (Acariens). *Bulletin de la Société Zoologique de France,* 78, 421–445.

Heethoff, M., Domes, K., Laumann, M., Maraun, M., Norton, R.A. & Scheu, S. (2007) High genetic divergences indicate ancient separation of parthenogenetic lineages of the oribatid mite *Platynothrus peltifer* (Acari, Oribatida). *Journal of Evolutionary Biology*, 20, 392–400.

Hoang, D.T., Chernomor, O., von Haeseler, A., Bui Quang, M., Le Sy Vinh (2018) UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology Evolution,* 35(2), 518–522.

Kalyaanamoorthy, S., Bui Quang, M., Wong, T.K.W., von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat Methods*, 14, 587–589.

Kokořová, P., Žurovcová, M., Ľuptáčik, P. & Starý, J. (2021) Distinct phylogeography patterns in populations of two oribatid mite species from the genus *Pantelozetes* (Acari, Oribatida, Thyrisomidae) in Central Europe. *Experimental and Applied Acarology,* 83(4), 493–511.

Kreipe, V., Corral-Hernández, E., Scheu, S., Schaefer, I., Maraum, M. (2015) Phylogeny and species delineation in European species of the genus Steganacarus (Acari, Oribatida) using mitochondrial and nuclear markers. *Experimental and Applied Acarology*, 66(2), 173–186.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution,* 35(6), 1547–1549.

Kunst, M. (1962) *Oribella cavatica* n. sp., eine neue Moosmilbe aus dem Guano der Fledermäuse (Acarina: Oribatei). *Acta Universitatis Carolinae, Biologica* (Supplementum), 1–6.

Kunst, M. (1971) Nadkohorta pancířníci – Oribatei. *In:* Daniel, M. & Černý, V. (Eds), *Klíč zvířeny ČSSR IV*, Academia, Praha, pp. 531–580.

Mahunka, S. & Mahunka-Papp, l. (2010) New and little known oribatid mites from the Carpathian Basin and the Balkan Peninsula (Acari: Oribatida). *Acta Zoologica Academiae Scientiarum Hungaricae*, 56(3), 211–234.

Marshall, G.V. (1972) Comparison of two methods of estimating efficiency of funnel extractors for soil microarthropods. *Soil Biology and Biochemistry*, 4, 417–426.

Mihelčič, F. (1958) Algunas descripciones de Oribátidos hallados en yacimientos húmedos. *Eos,* 34(1), 55–68.

Moritz, M. (1965) Neue Oribatiden (Acari) aus Deutschland I. *Oribella forsslundi* n. sp. und *Oppia nasuta* n. sp. *Zoologischer Anzeiger*, 175(4–6), 451–456.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Bui, Q.M. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, 32(4), 68–274.

Norton, R.A. (1977) A review of F. Grandjean's system of leg chaetotaxy in the Oribatei (Acari) and its application to the family Damaeidae. *In*: Dindal, D.L. (Editor), *Biology of oribatid mites*. SUNY College of Environmental Science and Forestry, Syracuse, pp. 33–61.

Norton, R.A. & Behan-Pelletier, V.M. (2009) Suborder Oribatida. Chapter 15. *In*: Krantz, G.W. & Walter, D.E. (Editors), *A Manual of Acarology*. Texas Tech University Press, Lubbock, pp. 430–

Oudemans, A.C. (1913) Acarologische Aanteekeningen XLVII. *Entomologische Berichten,* 23, 372–376.

Pérez-Iñigo, C. (1984) *Montizetes abulensis* n. sp., a new species of oribatid mite (Acari, Oribatei, Banksinomidae) from the Spanish Central Mountain Range. *Redia,* 67, 261–268.

Pérez-Iñigo, C. Jr. (1990) Contribucion al conocimiento de los Oribatidos (Acari, Oribatei) de la Provincia de Huesca, II. Zona Pirenaica. *Eos,* 66(2), 127–150.

Pfingstl, T., Baumann, J. & Lienhard, A. (2019) The Caribbean enigma: the presence of unusual cryptic diversity in intertidal mites (Arachnida, Acari, Oribatida). *Organisms Diversity & Evolution*, 19(4), 609–623.

Puillandre, N., Brouillet, S. & Achaz, G. (2021) ASAP: Assemble species by automatic partitioning. *Molecular Ecology Resources,* 21(2), 609–620.

Rakhimbaeva, A.K. (1995) New species Pantelozetes tianchinicus sp.n. Rakhimbaeva (Oribatei, Thyrisomidae Grandjean, 1953) and a key to the genus *Pantelozetes* Grand. *Selevinia*, 4, 13–16.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarisation in Bayesian phylogenetics using tracer 1.7. *Systematic Biology*, 67(5), 901–904.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MRBAYES 3.2: eficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology,* 61(3), 539–542.

Rosenberger, M.J., Maraun, M., Scheu, S., & Schaefer, I. (2013) Pre- and post-glacial diversifications shape genetic complexity of soil-living microarthropod species. *Pedobiologia*, 56, 79–87.

Ryabinin, N.A. & Pan'kov A.H. (1993) New species of mites of the family Banksinomidae from the Far East. Short communications (in Russian). *Zoologicheskii Zhurnal*, 72(4), 129–134.

Schäffer, S., Pfingstl, T., Koblmüller, S., Winkler, K.A., Sturmbauer, Ch. & Krisper, G. (2010) Phylogenetic analysis of European *Scutovertex* mites (Acari, Oribatida, Scutoverticidae) reveals paraphyly and cryptic diversity: A molecular genetics and morphological approach. *Molecular Phylogenetics and Evolution*, 55, 677–688.

Schäffer, S., Kerschbaumer, M. & Koblmüller, S. (2019) Multiple new species: Cryptic diversity in the widespread mite species *Cymberemaeus cymba* (Oribatida, Cymbaeremaeidae). *Molecular Phylogenetics and Evolution*, 135, 185–192.

Subías, L.S. (2004) Listado sistemático y sinonímico y biogeográfico de los ácaros oribátidos (Acariformes: Oribatida) del Mundo (except fósiles). *Graellsia,* 60 (número extraordinário), 3–305. Online version accessed in March 2021, 532 pp.; http://bba.bioucm.es/cont/docs/RO_1.pdf.

Travé, J. & Vachon, M. (1975) François Grandjean. 1882–1975 (Notice biographique et bibliographique). *Acarologia*, 17(1), 1–19.

Wang, H., Hu, S. & Yin S. (2000) The oribatid fauna and distribution different zones in China. *In*: Yin W.Y. (Editor), *Soil Animals of China*. Science Press, Beijing, pp. 251–265.

Weigmann, G. (2006) *Hornmilben (Oribatida)*. Die Tierwelt Deutschlands. Teil 76, Keltern, Goecke and Evers, 520 pp.

Wen, Z. (1990) Description of new and unrecorded oribatid mites from Jilin Province, China (Acari: Oribatida). *Journal of Northeast Normal University (*Supplementum*)*, 125–131.

White, B., Pilgrim, E., Boykin, L., Stein, E. & Mazor, R. (2014) Comparison of four species-delimitation methods applied to a DNA barcode data set of insect larvae for use in routine bioassessment. *Freshwater Science*, 33(1), 338–348.

Willmann, C. (1929) Neue Oribatiden II. *Zoologischer Anzeiger*, 80(1/2), 43–46.

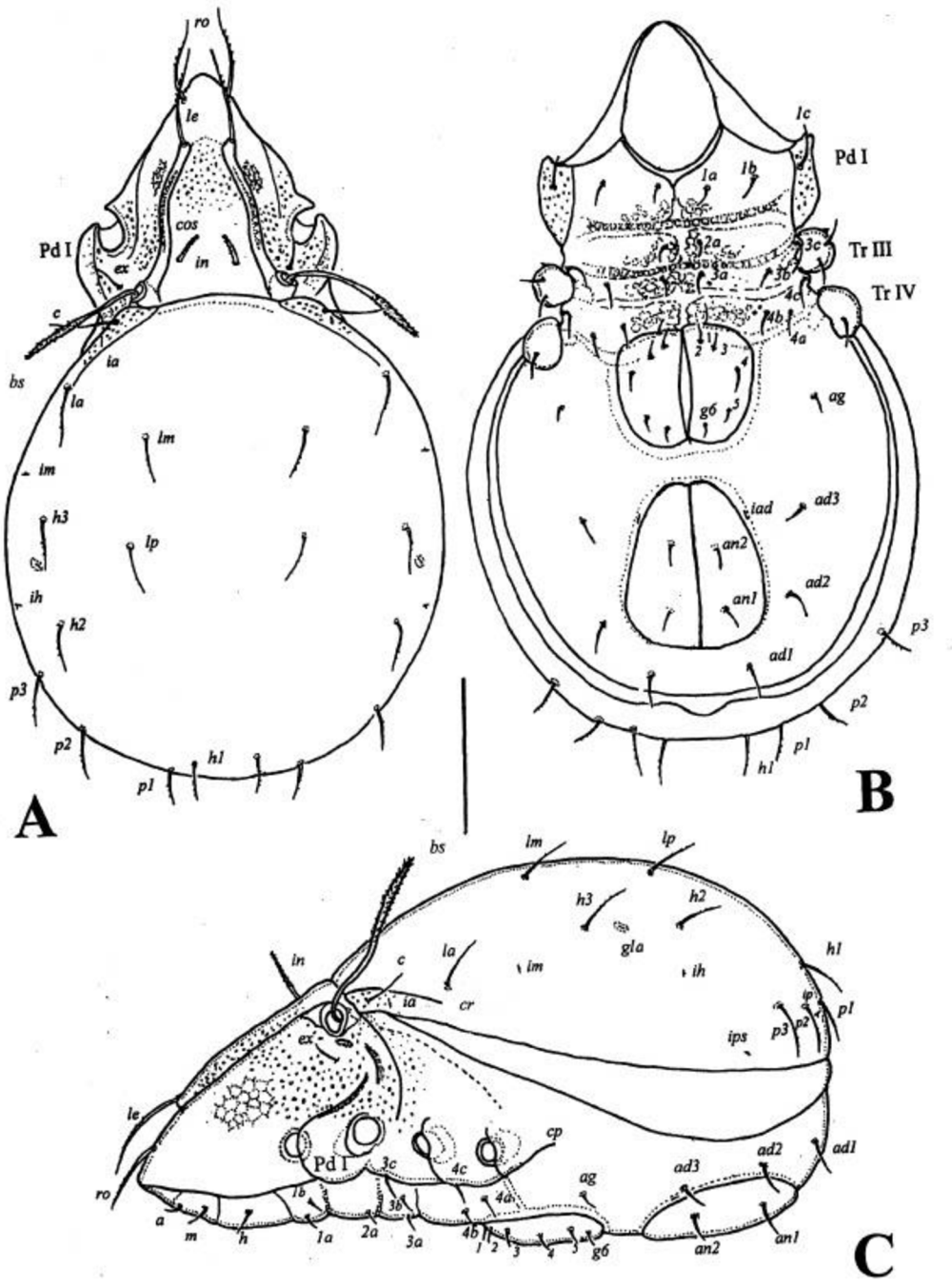

FIGURE 1. *Pantelozetes kunsti* **sp. nov.**, adult: A — dorsal view (legs not shown); B — ventral view (gnathosoma and legs except trochanters III, IV not shown); C — lateral view (legs not shown). Scale bar 100 μm.

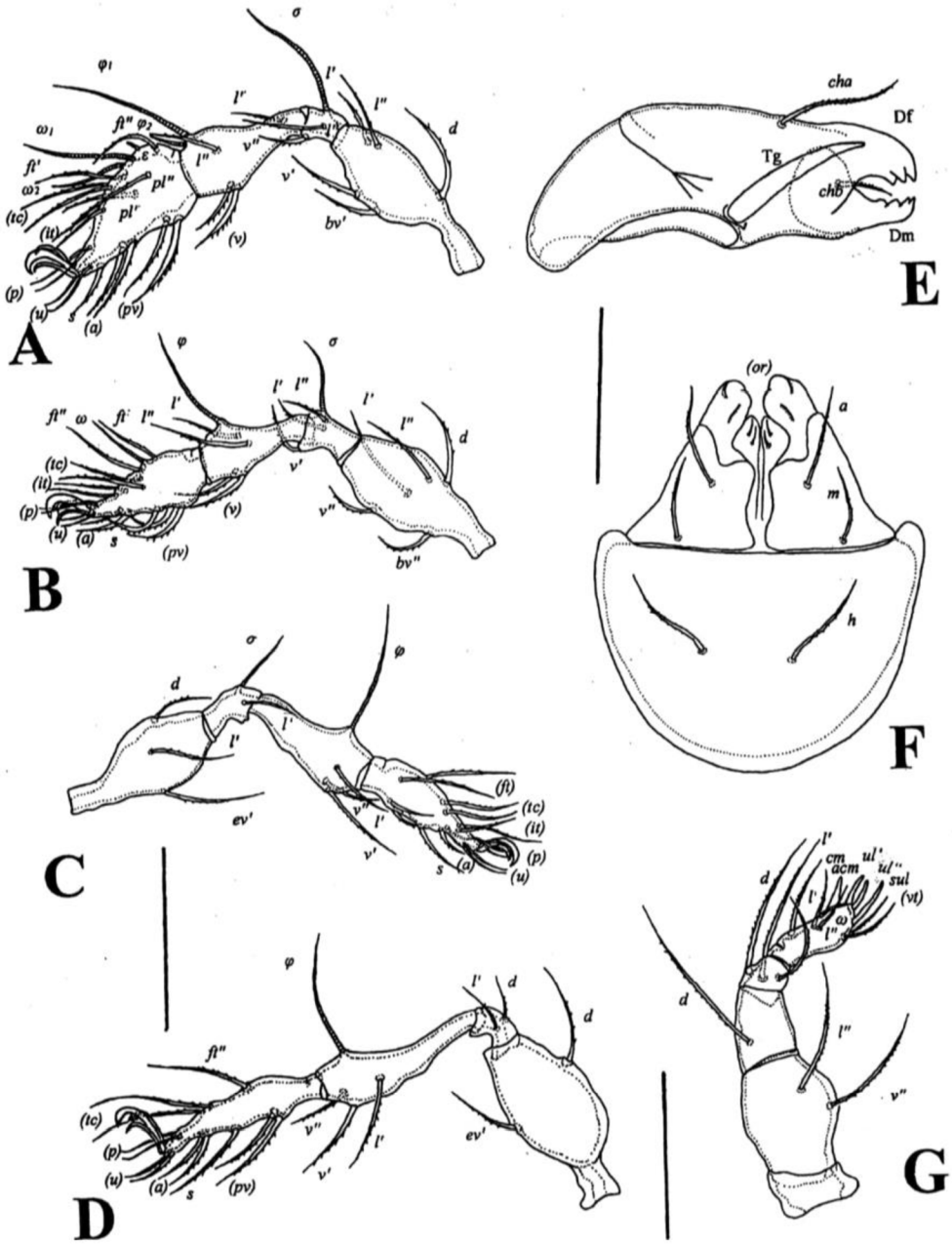

FIGURE 2. *Pantelozetes kunsti* **sp. nov.**, adult: A — left leg I antiaxial view (trochanter I not shown); B — left leg II antiaxial view (trochanter II not shown); C — left leg III antiaxial view (trochanter III not shown); D — right leg IV antiaxial view (trochanter IV not shown); E — chelicera, left, paraxial view; F — subcapitulum, ventral view; G — palp, right, antiaxial view. Scale bar 100 μm (A, B, C, D), 40 μm (E, F), 30 μm (G).

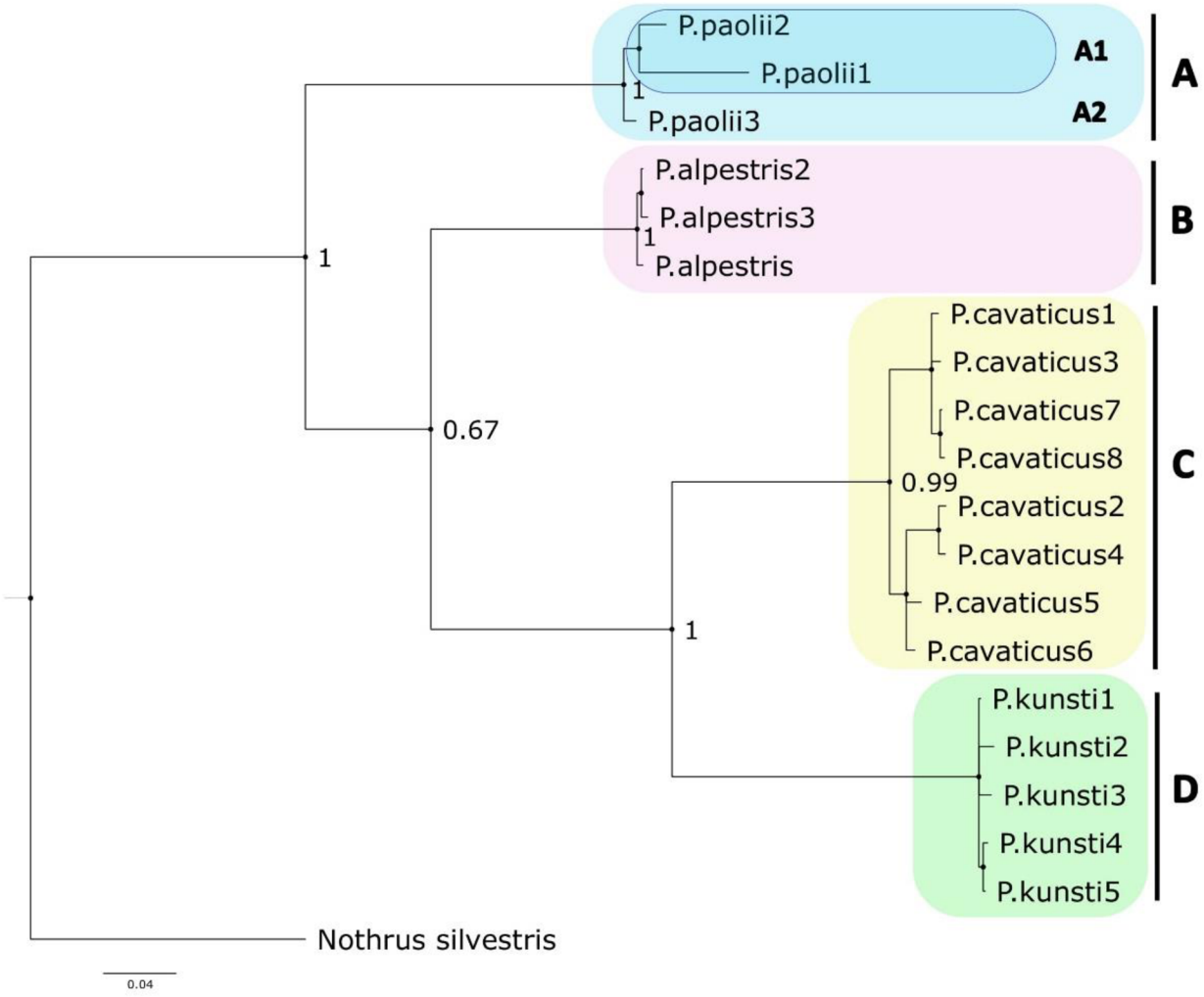

FIGURE 3. Bayesian inference tree based on COI gene of *Pantelozetes* species: (A-D) represents hypothetical species generated by ASAP, coloured clusters represent morphology-based species. Only posterior probability values ≥ 0.5 are shown. (For visualisation and interpretation of the colours in this figure, the reader is referred to the web version of this article).

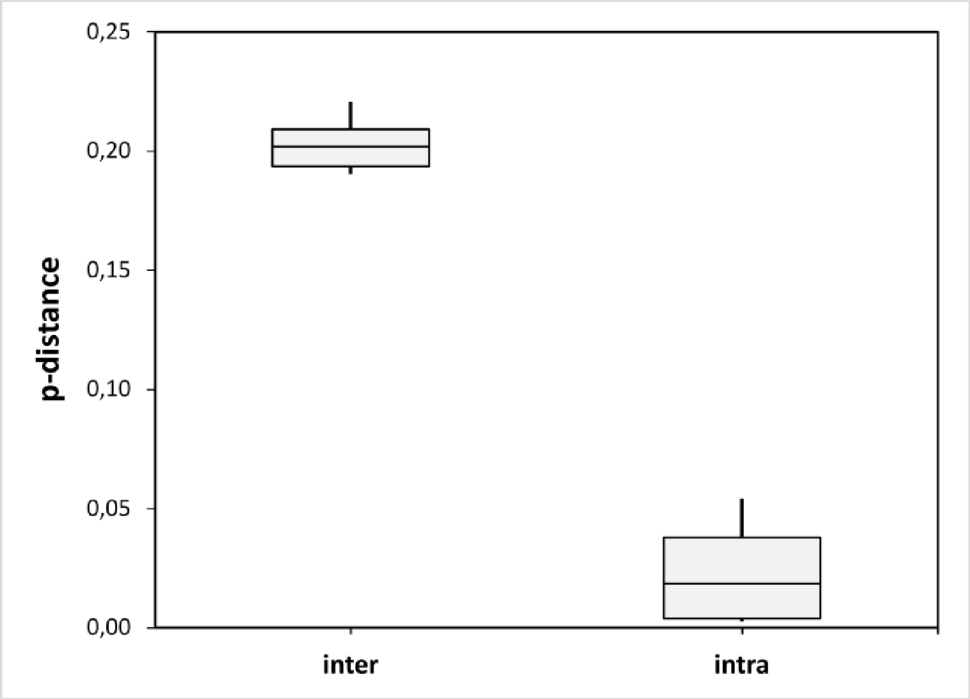

FIGURE 4. Intra- and interspecific distances (uncorrected p-distances) for the used COI marker for all studied *Pantelozetes* species.

TABLE 1. Leg setation and solenidia of adult *Pantelozetes kunsti* **sp. nov.**

Leg	*Tr*	*Fe*	*Ge*	*Ti*	*Ta*
I	*v'*	*d, (l), bv', v'*	*l'', v'',* σ	*(l),(v),* φ_1, φ_2	*(ft), (tc), (it), (p), (u), (a), s, (pv), (pl),* ε, ω_1, ω_2
II	*v'*	*d, (l), bv'', v''*	*(l), v',* σ	*(l), (v),* φ	*(ft), (tc), (it), (p), (u), (a), s, (pv),* ω
III	*l', v'*	*d, l', ev'*	*l',* σ	*l', (v),* φ	*(ft), (tc), (it), (p), (u), (a), s, (pv)*
IV	*v'*	*d, ev'*	*d, l'*	*l', (v)* φ	*ft'', (tc), (p), (u), (a), s, (pv)*

Note: Roman letters refer to normal setae, Greek letters refer to solenidia (except ε = famulus). Single apostrophe (') marks setae on the anterior and double apostrophe ('') setae on the posterior side of a given leg segment. Parentheses refer to a pair of setae.

TABLE 2. (a) Values of intraspecific distances (d; uncorrected p-distances) with standard error (SE). (b) Values of interspecific distances (lower left; uncorrected p-distances) with standard error (upper right).

(a)

	d	**SE**
P. paolii	0.054	*0.007*
P. alpestris	0.004	*0.002*
P. cavaticus	0.032	*0.005*
P. kunsti sp.nov.	0.002	*0.001*

(b)

	P. paolii	***P. alpestris***	***P. cavaticus***	***P. kunsti sp. nov.***
P. paolii		*0.015*	*0.016*	*0.016*
P. alpestris	0.191		*0.015*	*0.016*
P. cavaticus	0.221	0.203		*0.015*
P. kunsti sp.nov.	0.211	0.201	0.190	

Petra Kokořová
petra.kokorova@post.cz

Selected factors affecting species and genetic diversity of oribatid mites (Acari: Oribatida) in Central Europe
Ph.D. Thesis Series, 2022, No. 15

Printed in the Czech Republic by Typodesign
Edition of 8 copies

University of South Bohemia in České Budějovice
Faculty of Science
Branišovská 1760
CZ-37005 České Budějovice, Czech Republic
Phone: +420 387 776 201
www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz