Charles University

Faculty of Science

Study programme: Zoology



Mgr. Tomáš Dvořák

Habitat requirements of Orthoptera – ecological, physiological and evolutionary factors

Biotopové preference rovnokřídlých – ekologické, fyziologické a evoluční faktory

Doctoral Thesis

Supervisor: doc. RNDr. Jakub Prokop, Ph. D.

Advisor: Mgr. Ondřej Balvín, Ph. D.

Prague, 2024

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu. AI technologie byla použita pouze k jazykovým korekturám.



Ve Znojmě, dne 14.12.2024 Mgr. Tomáš Dvořák

Acknowledgements

First and foremost, I would like to express my deepest gratitude to my supervisor, Jakub Prokop, whose guidance and support throughout the palaeontological aspects of this dissertation have been invaluable. His assistance in securing financial support for the ecological experiments was also essential and deeply appreciated.

I am also thankful to Ondřej Balvín, whose expertise was instrumental in incorporating the phylogenetic component of this thesis.

A significant portion of the work on the ecological part of this research would not have been possible without the contributions of my coauthors, Michal Knapp and Jiří Hadrava. Michal provided indispensable help with text corrections and the finalization of drafts, while Jiří's expertise in data analysis was crucial to this project's success.

I extend my gratitude to the members of the student entomological office, who provided a creative and supportive environment. Their camaraderie and encouragement not only facilitated the writing process but also offered a much-needed sense of relief during challenging moments.

Lastly, and most importantly, I would like to thank my family—my parents, my wife, and my daughter—for their unwavering support, patience, and love. Their belief in me has been the foundation of my perseverance throughout this journey.

The papers supporting the thesis was also financed with several grants, namely: Technology Agency of the Czech Republic (SS02030018–DivLand) and Grant Agency of the Czech Republic (18-03118S). Additionally, many thanks go to the Nature Conservation Agency of the Czech Republic for permitting experiments with protected grasshopper *Stenobothrus eurasius*.

Papers included in the thesis and author contribution statement:

Dvořák, T., Pecharová, M., Krzeminski, W., & Prokop, J. (2019). New archaeorthopteran insects from the Carboniferous of Poland: Insights into tangled taxonomy. Acta Palaeontologica Polonica, 64(4).

TD: *Writing-original draft, validation, conceptualization, investigation*

Dvořák, T., Pecharová, M., Leipner, A., Nel, A., & Prokop, J. (2021). New archaeorthopteran insects from the Pennsylvanian of Piesberg reveal unexpected mosaic of morphological traits and colouration pattern of the tegmina. Historical Biology, 33(12), 3375-3383.

TD: *Writing-original draft, validation, investigation, conceptualization*

Dvořák, T., Hadrava, J., & Knapp, M. (2022). The ecological niche and conservation value of Central European grassland orthopterans: A quantitative approach. Biological Conservation, 265, 109406.

TD: *Conceptualization, writing-original draft, formal analysis, validation, investigation, data curation, visualization, project administration, methodology*

Dvořák, T., & Knapp, M. (2023). Conserved temperature requirements but contrasting responses to humidity across oviposition preferences in temperate grasshoppers. Scientific Reports, 13(1), 21131.

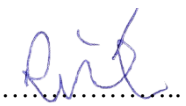
TD: *Conceptualization, writing-original draft, investigation, data curation, visualization, project administration, formal analysis, validation*

Dvořák, T., Krzemiński, W., & Prokop, J. (2023). New stem-dictyopteran insects from the Pennsylvanian deposits at Mazon Creek and Sosnowiec (Insecta: Polyneoptera). Historical Biology, 35(12), 2334-2339.

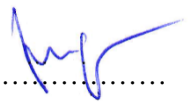
TD: *Writing-original draft, conceptualization, validation, investigation*

Dvořák, T., & Knapp, M. n. d. Species‘ mesoclimatic requierements affect body size, egg developmenttime and thermal sensitivity in temperate grasshoppers (manuscript).

TD: *Conceptualization, writing-original draft, formal analysis, validation, investigation, data curation, visualization, project administration*



Tomáš Dvořák



doc. RNDr. Jakup Prokop, Ph.D.

Abstract

Orthopterans play a vital role in grassland ecosystems in Central Europe. Species within this order inhabit a wide range of habitats, including microclimatic extremes such as rocky steppes, sand dunes, or conversely cold and wet areas and alpine meadows. Typically, each species exhibits a strong association with specific habitat characteristics. Thus, habitat microclimate and vegetation structure were postulated as the primary factors determining orthopteran occurrence in temperate grasslands.

Due to the strict yet diverse habitat preferences of individual species, orthopterans serve as popular bioindicators for studying the impacts of human activities and climatic change on grasslands. Moreover, they are valuable models for studying ecophysiological adaptations to diverse temperature and moisture conditions. However, there is a lack of proper identification and precise description of crucial habitat features driving habitat choice and subsequent ecophysiological and evolutionary background, which stands behind these preferences. Significant gaps exist, particularly concerning larval habitat requirements and oviposition site choice. To my knowledge, there is almost no comprehensive synthesis addressing orthopteran habitat requirements from community, ecophysiological and evolutionary perspectives.

In my thesis, I contribute to addressing mentioned issues. At first, I focus on the precise description of ecological niches for 38 orthopteran species inhabiting Central Europe and create a table of bioindication indices for various habitat parameters affecting Orthoptera communities. Additionally, I contribute to ecophysiological knowledge through two studies on the oviposition site choice in relation to microclimate and the temperature effects on embryonic development in 14 grasshopper species. Furthermore, I contribute to understanding the evolutionary history of orthopterans and their relatives with three studies describing several new fossils from the Carboniferous period.

Keywords: grasshoppers, grasslands, bioindication, ecophysiology, microclimate, vegetation structure, Archaeorthoptera

Abstrakt

Rovnokřídlí jsou význačnými obyvateli travnatých biotopů střední Evropy. Jednotliví zástupci obývají rozličné typy otevřených trávníků, včetně extrémních jako jsou skalní stepi, písčiny, nebo naopak chladné a vlhké louky či alpínské bezlesí. Nápadnou vlastností velké části druhů je silná vazba na specifický typ biotopu, především na jeho mikroklimatické podmínky a strukturu vegetace, jež jsou považovány za hlavní faktory stojící za biotopovými vazbami rovnokřídlých v travnatých biotopech mírného pásu.

Díky svým úzkým, avšak mezidruhově odlišným biotopovým vazbám, se rovnokřídlí stali oblíbenými indikátory vlivu hospodaření a klimatických změn na travnaté biotopy. Dále je jejich citlivost na mikroklima pasovala na význačné modelové organismy pro výzkum adaptace na rozličné teplotní a vlhkostní poměry stanoviště. Nicméně, stále přesně nevíme, jaké konkrétní vlastnosti biotopů stojí za jejich biotopovými preferencemi, a jaké je ekofyziologické a evoluční podhoubí, které za těmito vazbami stojí. Z tohoto hlediska víme málo především o preferovaných podmínkách pro kladení a o biotopových nárocích nymf. Obzvláště kritický je nedostatek studií, které by spojili tyto pohledy – identifikace biotopových preferencí a jejich ekofyziologické a evoluční příčiny – v jeden provázaný celek.

Ve své práci se snažím přispět k poznání biotopových preferencí rovnokřídlých z několika úhlů pohledu. V jedné studii jsem se zaměřil na přesnou identifikaci a kvantifikaci ekologických nik pro 38 rovnokřídlých obývajících střední Evropu. Výsledkem je tabulka bioindikačních indexů pro několik biotopových parametrů stojících za biotopovými vazbami rovnokřídlých. Z hlediska ekofyziologického jsem přispěl dvěma studiemi. První se zabývá identifikací preferovaných abiotických podmínek pro kladení vajíček, druhá se zabývá vlivem teploty na vývoj embryí u 14 druhů saranči. Nakonec jsem se věnoval i studiu evoluční historie orthopteroidních linií, jehož výsledkem jsou tři publikace přinášející popis několika nových fosilií karbonského stáří.

Klíčová slova: saranče, travnaté biotopy, bioindikace, ekofyziologie, mikroklima, struktura vegetace, Archaeorthoptera

Table of contents

1. Introduction **8**

2. Orthoptera and habitat **9**

2. 1 Orthoptera assemblages in Central Europe 9

2. 2 The role of microclimate and microhabitat 12

3. Thesis aims **15**

4. Habit requirements – community level **15**

4. 1 Orthoptera as bioindicators 17

5. Habitat requirements – ecophysiology **18**

5. 1 Oviposition preferences 19

5. 2 Embryonic development 21

5. 3 Nymphal development 22

5. 4 Adult thermal and moisture preference 23

6. Evolutionary history of orthopterans **25**

7. Gaps and future of studies on Orthoptera habitat requirements **28**

8. Conclusion **29**

9. References **31**

Study I **44**

Study II **45**

Study III **65**

Study IV **92**

Study V **103**

Study VI **107**

1. Introduction

Orthoptera is an insect order of intermediate size, comprising almost 30,000 described species, but the largest in Polyneoptera (Cigliano et al., 2024). It consists of two distinct suborders: Ensifera (katydids, crickets, cave crickets, etc.) and Caelifera (grasshoppers, ground hoppers, etc.). Throughout history these suborders were frequently assigned as two separated orders, and their monophyly was doubted repeatedly. However, recent phylogenetic studies confidently confirm Orthoptera as a monophyletic group with two suborders (Song et al., 2015). The order includes mainly medium to large species, with tiny myrmecophilous ant crickets from the Myrmecophilinae subfamily reaching only 2 mm as adults, being the smallest. Conversely, the largest ones, such as *Arachnacris* katydids and *Tropidacris* grasshoppers, can reach up to 15 cm in length and almost 30 cm in wingspan. The heaviest recognized species is the giant weta *Deinacrida heteracantha* from New Zealand, with females reaching up to 71 g (Whitman, 2008).

Representatives of orthoptera are characterized by excessively developed lateral lobes of the pronotum known as cryptopleuron, strong jumping hind legs, partially sclerotized tegmina, and the ability to produce sound – stridulation, mainly in a courtship context. Ensifera have the stridulation apparatus placed on the base of the tegmina. Caelifera usually stridulate by rubbing their hind legs against their forewings, more specifically against a modified radial vein. Complex courtship, including a variety of acoustic, chemical, and tactile stimuli, is an enigmatic and one of the most studied phenomena in orthopterans (Gwynne, 2008; Nattier et al., 2011). Regarding feeding strategies, Caelifera are almost exclusively phytophagous, with several detritophagous and bryophagous groups, mainly in Tetrigidae. However, occasional scavenging on dead specimens, frequently of the same species, has been observed. In Ensifera, a whole variety of feeding strategies, ranging from predators and scavengers to omnivores and strict herbivores, have been documented (Ingrisch & Köhler, 1998; Grimaldi & Engel, 2005; Kočárek et al., 2015). Orthopterans, especially grasshoppers, play a crucial role in ecosystems as they consume a significant amount of vegetation. On the other hand, they are also a vital part of the diet of other animals such as birds, small mammals, and reptiles. Many species hold cultural and economic significance. Some are well-known, such as the infamous locust swarms that have been detrimental to agriculture (Uvarov, 1966). Conversely, for many indigenous people, orthopterans serve as a considerable food source (Grimaldi & Engel, 2005; Bidau, 2014). Due to their importance, large size, and ease of breeding and handling, orthopterans have become valuable models for various biological disciplines, including physiology, behaviour, ecology, and evolution.

In recent years, there has been a rise in studies on the effects of climate change, habitat quality and management on Orthoptera populations, as well as the potential use of orthopterans as bioindicators of these phenomena (Fartmann et al., 2012; Penone et al., 2013; Poniatowski et al., 2020; Humbert et al.,

2021). However, for a complete understanding of an effect of environment on life histories of species and their bioindicative potential, it is essential to comprehend their habitat requirements. Despite a vast body of knowledge about Orthoptera, information on precisely measured habitat requirements, and especially the underlying factors, is largely missing, or is relatively well-known only for a few model species. Additionally, current distribution and habitat requirements of species are resulting from long coevolution of orthopterans and their habitat (Ingrisch & Köhler, 1998). Understanding these phenomena and their relations is of great importance for using orthopterans as reliable models for the study of mentioned habitat and climate changes and for a general understanding of biodiversity patterns.

2. Orthoptera and habitat

Orthoptera inhabit all continents except Antarctica and occupy a wide variety of habitats, ranging from tropical rainforests and deserts to various grasslands, boreal and alpine tundra. They can be found on trees, bushes, herbs, grasses, and the ground. Many species burrow below the ground, and some are known to inhabit special environments such as caves or ant colonies (Grimaldi & Engel, 2005; Bidau, 2014). Although orthopterans are generally considered a terrestrial order, some species can be considered semiaquatic, particularly from the Tetrigoidea superfamily. They are capable of actively diving underwater and feeding on water algae and plants. Additionally, many other species can swim or dive in response to danger (Amédégnato & Devriese, 2008; Derka & Fedor, 2010).

The centre of orthopteran diversity is located in the tropical parts of South America, Africa, Asia, and Oceania (Cigliano et al., 2024). Towards the poles, diversity decreases, with only a few species reaching beyond the polar circle (Haberski & Sikes, 2021). Nevertheless, some species can withstand harsh mountain climates. The highest-recorded orthopteran is the grasshopper *Hypernephia everesti*, described from Mount Everest at 5640 meters above sea level (Uvarov, 1922). In the European Alps, the highest occurrences for orthopterans are around 3000 meters above sea level (Zuna-Kratky et al., 2017).

2. 1 Orthoptera assemblages in Central Europe

Within the thesis I am focused primarily on temperate species inhabiting Czech Republic and surrounding countries i.e. Central Europe, as defined by the Encyclopaedia Britannica (Encyclopaedia Britannica, Inc, 2007), which includes the Czech Republic, Slovakia, Austria, Germany, Poland, Hungary, Slovenia, and Switzerland (Fig. 1). European fauna contains only about 1,114 out of approximately 30,000 described species (3%) (Cigliano et al., 2024) and only around 170 species inhabit Central Europe. This pattern of decreasing biodiversity towards the poles is typical for almost

all organisms (Brown, 2014). The effect can be further enhanced by periodic glaciations and the existence of mountain ranges hindering recolonization of Central Europe from Mediterranean refugia during interglacials (Hewitt, 2000; Svenning, 2003; Schmitt, 2007).



Fig. 1: Countries of Central Europe (in red) as defined by Encyclopaedia Britannica (Encyclopaedia Britannica, Inc, 2007).

For Central European Orthoptera assemblages, a characteristic is the meeting of species from several different faunal regions, whereas most species have a widespread Euro-Siberian or Palearctic range. Only a few species can be considered endemic to Central Europe, with most of them being endemic to the West Carpathian Mountain ranges, such as *Chorthippus tatrae*, *Isophya fatrensis*, *Isophya beybionkoi* or *Pseudopodisma nagyi* (Krištín et al., 2020; Cigliano et al., 2024).

Typical elements of steppe ecosystems, with the centre of diversity in Asian steppes, penetrate Central Europe from the southeast through Pannonia or, more rarely, directly from the east through Poland. The occurrences of these species are usually considerably fragmented and many of them reach their limits at the Pannonian border. For example, sand dune-inhabiting katydid *Montana montana* or *Dociostaurus brevicolis* grasshopper do not extend beyond the Pannonian region (Kočárek et al., 2015; Zuna-Kratky et al., 2017). Some steppe species have their north-western border in České středohoří Mountains in the Czech Republic, which is true for grasshoppers *Stenobothrus eurasius* and *Euchorthippus pulvinatus* (Holuša & Holuša, 2002; Kočárek et al., 2015). The suitable localities for

these species are usually very patchy and dependent on some form of management. The decline of steppe species, coupled with the abandonment of traditional agriculture and reforestation, resulting in a drastic change in habitat properties, is a well-known phenomenon (Báldi & Kisbenedek, 1999; Hochkirch et al., 2008; Fartmann et al., 2012; Habel et al., 2013).

Some species reach Central Europe from the Mediterranean. In recent years, with the rise in temperature, there has been a noticeable shift in the areas of many (sub)Mediterranean species to the north (Vlk, 2002; Kočárek & Holuša, 2006; Krištín et al., 2022; Hemala et al., 2023). Some of them were able to expand their areas on their own, as observed in *Ruspolia nitidula*, a mobile katydid that has expanded in recent years through Central Europe (Brodacki, 2015; Kaláb et al., 2021). Others were introduced by humans, mainly through railways, automobiles, or with exotic plants (Vlk et al., 2012; Krištín et al., 2022).

Specific fauna exists in high mountains, with two types of species distinguished. The first are generally cold-adapted species with alpino-arctic or bore-montane distribution, living in high European mountains and simultaneously in the boreal and tundra belt in the north (e.g., *Podisma pedestris*, *Bohemanella frigida* (Karjalainen, 2009; Zuna-Kratky et al., 2017)). The second kind of species can be considered truly mountainous, with substantial diversity usually arising from high topographic complexity enhancing speciation (Wallis et al., 2016; Rahbek et al., 2019; García-Rodríguez et al., 2021). The highest diversity in Central European Mountains is in the Alps, as they are the biggest and have a connection to the Mediterranean (Zuna-Kratky et al., 2017). The Carpathian Mountains have a less rich orthopteran fauna except for vigorously speciating *Isophya* spp. katydids (Krištín et al., 2020). In relatively low Hercynian Mountain ranges, specialized mountain species are practically missing, with the exception of the grasshopper *Miramella alpina*, which is abundant in the Alps and Carpathian Mountains, with a small population also in alpine grasslands of Hercynian Black Forest in Germany and Jeseníky Mountains and in moors in Šumava Mountains in the Czech Republic.

The Atlantic elements are hard to define within European orthopterans and only representative reaching Central Europe is the katydid *Leptophyes punctatissima*.

Regarding to occupied habitats, the vast majority of species occurring in Central Europe is associated with grasslands. Only several species occupy taller bushes or trees, and most of them still live in the lower vegetation on the forest edges, clearings, or glades in the larval stage (Ingrisch & Köhler, 1998). All of them belong to katydids (Ensifera); only one grasshopper species, *Anacridium aegyptium*, partially lives in taller bushes. Nevertheless, this species reaches central Europe only marginally, along the Adriatic coast of Slovenia (Gomboc & Šegula, 2014). The centre of diversity of grassland species is located in the warm Pannonian region, where different types of steppes, sand dunes, and marshlands

are inhabited. Locally high diversity can also be found in low mountain meadows, pastures, and forest edges adjoining Pannonia. High mountain alpine meadows support a few but highly specialized and rare species (Kočárek et al., 2015; Zuna-Kratky et al., 2017; Krištín et al., 2020). The remaining agricultural landscape is considerably impoverished and homogenized (Marini et al., 2010; Essl & Dirnböck, 2012; Chisté et al., 2016). Nevertheless, interesting biodiversity hotspots can be found in many types of post-industrial sites, which mimic high-quality xeric biotopes (Tropek et al., 2012; Bogusch et al., 2016; Heneberg et al., 2016).

2. 2 The role of microclimate and microhabitat

The role of climate and habitat on insects' life history, population dynamic, and evolution are one of the most classical topics in ecological and evolutionary studies (Bale et al., 2002; Parmesan, 2006; Deutsch et al., 2008). However, insects usually live on a much smaller scale than can be well assessed by commonly used macroclimatic variables such as air temperature, precipitation, insolation (Willmer, 1982; Duffy et al., 2015; Pincebourde & Woods, 2020), or general habitat characteristics such as land use and management (Essl & Dirnböck, 2012; Torma et al., 2014; Sutcliffe et al., 2015; Schirmel et al., 2019). Variables like local moisture or fine vegetation structure are rarely taken into account (Altmoos & Henle, 2010; Fartmann et al., 2012; Dvořák et al., 2022). Moreover, most studies that take into account the direct effect of local climate (microclimate) are usually performed in controlled laboratory conditions and detached from real field situations, including all potential interactions, spatial and temporal microclimatic diversity, and extremes (Uvarov, 1966; Willmer, 1982; Kreyling et al., 2014; Ma et al., 2015).

A very prominent feature of Central European grassland Orthoptera in the context of habitat requirements is usually a very specific preference for a certain type of grassland in individual species (Ingrisch & Köhler, 1998; Fartmann et al., 2012; Grzędzicka & Vahed, 2020; Dvořák et al., 2022; some examples in Figure 2). Therefore, for these were hypothesized that microclimate and vegetation structure play a main role in determining their habitat requirements (Joern, 1982; Ingrisch & Köhler, 1998; Weiss et al., 2013; Schirmel et al., 2019; Humbert et al., 2021). In particular, the most interesting in this context are species inhabiting extreme places, such as cold high alpine meadows with limited season length or wet owergrown wetlands, moors, etc. On the other side, many species thrive in extremely hot and dry habitats such as rocky steppes or almost bare sand dunes.



Fig. 2. Examples of grasshoppers and variety of their habitats in Central Europe (from top to the bottom: *Oedipoda germanica, Stenobothrus lineatus, Pseudochorthippus parallelus, Stethophyma grossum*).

Vegetation structure can affect orthopterans in two ways. It can affect animals directly, as different species prefer to dwell on different parts of vegetation (Ingrisch & Köhler, 1998; Hochkirch & Papen, 2001; Bauer & Kenyeres, 2006; Krištín et al., 2009) or eat different plants with different morphology (Gardiner et al., 2002; Franzke et al., 2010; Huang et al., 2016). Some species utilize stems and leaves of grasses and herbs for oviposition. In these species, the occurrence of suitable oviposition sites, and therefore specific vegetation cover, can be important (Ingrisch & Köhler, 1998).

However, the key principle of how vegetation structure shapes orthopterans' distribution is rather indirect through changing temperature and moisture characteristics (Fielding et al., 2001; Ahnesjö & Forsman, 2006; Weyer et al., 2012). Especially temperature can change at the scale of a few centimetres. The hottest part of grassland habitats tends to be the open ground surface with rapidly decreasing temperature together with increasing distance both under and above the ground. Even if the air temperature 1m above the ground is only 12°C, on exposed surfaces, it can reach up to 60°C (Stoutjesdijk & Barkman, 2014). In karst steppe, the ground between grass tussocks can heat up to 80°C with the air temperature 2m above the ground around 33°C (unpublished results). These facts clearly emphasize the importance of studies on microclimate and its effect on insects life histories, as these extreme temperatures are clearly outside general tolerable maxima. Too high temperatures in sun-exposed freshly mowed grasslands probably can account for low population abundance in some Great Britain grasshopper species (Gardiner & Hassall, 2009). Subadults and adult males of katydid *Decticus verrucivorus* clearly prefer a specific height and density of vegetation, but adult females tend to occur in places with more sparse vegetation to lay eggs, which need higher heat dosage associated with lower plant density (Cherrill & Brown, 1990a). On the other side, an interior of grass tussock can be a significantly colder, especially in the case of dense vegetation cover (Stoutjesdijk & Barkman, 2014).

The enough sum of temperature enabling the completion of the life cycle in a seasonal environment can be the main proximate explanation of the general warm microclimate choice in Central European Orthoptera (Sibly & Monk, 1987; Cherrill & Brown, 1990b). This has been shown to be true at least for egg development time (Van Wingerden et al., 1991), where embryos of more xerothermic species are more sensitive to low temperatures. Another, not mutually exclusive, explanations as eclosion failure at low temperatures (Whitman, 1986), or higher susceptibility to predation and parasitisation in larvae were also proposed (Grant et al., 1993). Though, some adaptation ability between populations of the same species occupying habitats with different microclimates was observed (Monk, 1985; Forsberg, 2004).

Results of the study (Dvořák et al., 2022) revealed microhabitat moisture as the most important variable delimiting grassland Orthoptera occurrence and communities' composition, surpassing the effect of vegetation cover, height, sun exposition, and mean air temperature. Surprisingly, the effect of moisture on Orthoptera habitat choices has been studied very rarely before (Powell et al., 2007; Weking et al.,

2016). It is generally known that different species prefer habitats with different moisture properties, but a robust proximate explanation of this relationship is still lacking (Ingrisch & Köhler, 1998; Gavlas et al., 2007). The scattered information suggests that the effect of moisture works in multiple ways. Evaporation cooling indirectly lowers temperatures in wet grasslands compared to dry ones (Ripley & Saugier, 1978), which on the one side lowers potentially lethal upper maxima (Uvarov, 1966), but lowers the general heat dosage, which hinders more thermophilic species. Ingrisch (1983) found that egg tolerance to desiccation differs between species. In congruence with expectation, species living in wet conditions have lower tolerance to desiccation, but even the most xerophilic species well tolerate high moisture. It was also found out that even slight moisture changes within one season can affect species compositions in Hungarian grasslands (Kenyeres et al., 2009).

Microclimatic and microhabitat conditions definitely play a crucial role in determining habitat requirements of Central European Orthoptera and studies searching for an unifying explanation on the effect of individual habitat parameters on different life stages and species of orthopterans are needed.

3. Thesis aims

The main goal of the thesis is to review and contribute to our understanding of Orthoptera habitat requirements in Central Europe. After three introducing chapters, the fourth chapter deals with a community-level approach and describes which habitat features shape Orthoptera assemblages and how these features are affected by habitat management and climatic change (**Study 1**). The fifth chapter is dedicated to searching for proximate ecological, physiological, and behavioural adaptations throughout the whole life cycle that can potentially explain the observed reactions to different habitat parameters in different species (**Studies 2 and 3**). The sixth chapter uses an evolutionary and paleontological approach to review the history and development of Central European orthopteran assemblages coupled with the evolution of grasslands. Additionally, I delve even further into the past and contribute to our knowledge about the early evolution of Orthoptera and its relatives (**Studies 4, 5, and 6**). In the seventh chapter I try to interconnect these approaches and discuss potential future ways of research on orthoptera habitat requirements.

4. Habit requirements – community level

Substantial portion of work was invested in an understanding an effect of general landscape patterns on Orthoptera communities' composition. In general, species diversity is positively enhanced with habitat diversity, landscape heterogenity and size of suitable habitat patches (Báldi & Kisbenedek, 1999; Schouten et al., 2007; Essl & Dirnböck, 2012; Sutcliffe et al., 2015; Weking et al., 2016), although some

studies didn't find a path size so important (Torma et al., 2014). These findings are logical, as heterogenous and large habitat can host sustainable populations of many species with different habitat requirements. Connectivity of habitat patches can play an important role in highly fragmented landscape, but its effect is much smaller in generally habitat rich landscape, where individual patches can be small and relatively isolated (Theuerkauf & Rouys, 2006; Poniatowski et al., 2016; Löffler & Fartmann, 2017).

Habitat characteristics and habitat connectivity ensuring functional metapopulation structure can be heavily shaped by weather and long-term changes in climate characteristics. Low precipitation and high spring and summer temperatures generally lead to higher densities in temperate grasshoppers, but warm winter can have a negative effect (Edwards, 1964; Powell et al., 2007; Poniatowski & Fartmann, 2011; Jonas et al., 2015). On the other side, when really extreme hot and dry summer occurs, vegetation wither early and grasshopper populations can be partially affected by food shortage (Branson, 2008; Fartmann et al., 2022). Climate change have a mixed effect on Orthoptera diversity in temperate climate. On the one side, there is a space for immigration of more thermophilous species from lower lattitudes and expansion of lowland species to a mountain. Nevertheless, low habitat quality and poor connectivity can often hinder a successful adaptation and migration to more favourable conditions in highly specialised and sedentary species (Löffler et al., 2019; Poniatowski et al., 2020; Prinster et al., 2020). Moreover, there is a substantial danger for alpine species, which have frequently a limited space to climb higher and local populations can went extinct in lower mountain ranges (Zuna-Kratky et al., 2017; Koot et al., 2022; Illich & Zuna-Kratky, 2022).

Practically all habitats in Central Europe are heavily affected and shaped by human activities (Hejcman et al., 2013), thus land use and management have a direct effect on Orthoptera populations (Löffler et al., 2019). Probably the major threat is a transformation of grasslands into arable fields, dense monocultural forests and constructions and thus rapid decline of suitable habitats and populations fragmentation (Isselstein et al., 2005; Williams et al., 2005; Carbutt et al., 2017). In remaining grasslands, changes in management create another pressure on Orthoptera communities (Marini et al., 2009, 2010; Löffler et al., 2019). Increased fertilisation decreases Orthoptera populations, mainly indirectly through inducing higher and denser vegetation leading to cooler microclimate, which affect especially more xerothermophilous species (Chisté et al., 2016; Humbert et al., 2021). Intensive mowing directly kills occurring grasshoppers and drastically change microclimatic properties (Gardiner & Hassall, 2009; Cizek et al., 2012). Grazing is considered as traditional management of grassland. Nevertheless, modern intensive grazing can have a mixed effect (Gardiner, 2018). Most beneficial is usually for xerothermic species, but species needing some ungrazed patches or species with eggs vulnerable to cattle disturbation can be affected negatively (Rada et al., 2014; Fonderflick et al., 2014; Chisté et al., 2016).

A special emphasis is placed on steppes, heathlands, sandy areas and other dry grasslands, which host the richest orthoptera communities. These habitats have partially natural character in Central Europe, but their quality and spread has been greatly enhanced with human traditional agriculture practices. Nowadays, they are prone to abandonment, overgrowing by bushes and trees, or fertilisation (Smits et al., 2008; Fartmann et al., 2012).

There is not much knowledge about an impact of human management on a few central European species associated with forest.

There are several approaches attempting to mitigate a decline of grassland grasshoppers in Central Europe. One of the examples can be uncut grass strips in meadows, which serve as refugees during mowing and enabling recolonisation of mown meadow (Badenhausser & Cordeau, 2012; Badenhausser et al., 2015). A good practice is maintaining a habitat mosaic with different management types and time regimes (Braschler et al., 2009; Schirmel et al., 2010). In latest years, increasing evidence for high conservation potential of post-mining sites and brownfields, which often mimic a high quality xerothermic and sparsely vegetated habitats, have been recognised (Tropek et al., 2012; Bogusch et al., 2016; Eckert et al., 2017). Controlled burning is also considered beneficial, especially in steppe remains, as it removes old vegetation and form more open grasslands, although overwintering eggs of some species laying above ground can be damaged (Chambers & Samways, 1998; Joubert et al., 2016). Formerly high quality steppe habitats can also profit form rewilding i.e. introducing of large (semi)wild grazers which can restore heterogenous and open vegetation (Fuhlendorf et al., 2018; Garrido et al., 2019).

Important note is, that practically all mentioned studies are performed on adults and we know very little about habitat preferences of nymphs and eggs and its reaction to management at the community level.

4. 1 Orthoptera as bioindicators

As indicated above, grasslands in Central Europe are under heavy pressure and decline in habitat quality and subsequent biodiversity is apparent. Thus, there is an effort for restoration and management changes to boost grassland biodiversity (van Swaay, 2002; Smits et al., 2008; Marini et al., 2009; Carbutt et al., 2017). An effective assessment of habitat condition and results of restoration measurements can be done through bioindicators. Due to narrow habitat requirements, rapid reaction to habitat changes and easy detection, orthopterans were often proposed as ideal bioindicators of grassland quality (McGeoch, 1998; Gerlach et al., 2013).

Despite a noticeable portion of work on grasshoppers bioindication, there is still a critical lack of exact description of habitat preferences, niche widths and importance of environmental parameters potentially standing behind habitat requirements of individual species. Usually only raw dividing into habitat

specialist/generalist, or general affinity to a specific environmental parameter is assessed (Bazelet & Samways, 2011; Essl & Dirnböck, 2012; Fartmann et al., 2012; Chisté et al., 2016; Weking et al., 2016). There are only a few works trying to more elaborately describe habitat preferences and set some bioindication indices (Ingrisch & Köhler, 1998). One older work assessed niches for vegetation type, local moisture and temperature, but only artificial numbers without proper analysis were used (Oschmann, 1991). Chisté et al. (2016) computed niches for land-use intensity, but underlaying factors causing observed patterns were not elaborated. Some studies defined indicator species for specific environmental parameters, but without assessing environmental optima or niche width (Bazelet & Samways, 2011; Weking et al., 2016).

Contribution:

We conducted the study (**Study 1**), in which we tried to exactly measure ecological niches of 38 Central European orthopterans. We visited 476 sites with various habitat types and Orthopteran communities and recorded Orthoptera diversity and five environmental parameters (local moisture, vegetation cover, vegetation height, sun irradiance and seasonal temperature).

We overcame mentioned shortcoming of current studies on Orthoptera bioindication potential by using the Maxent approach (Phillips et al., 2006; Elith et al., 2011). This enables us to construct occurrence probability curves for each species and environmental parameter separately. From these curves, environmental optimum and niche width for each parameter can be easily estimated. We also used jackknife of regularised training gain method to separate an individual effect of heavily correlated variables. In conclusion, local moisture shown to be the most important environmental variable leading orthopterans habitat requirements, yet rarely studied in the past (more in introduction). Resulting table of bioindication indices can be a useful for future conservational and ecological studies.

5. Habitat requirements – ecophysiology

The reviewed tendency to occupy very specific habitats with distinct microclimatic parameters emphasizes the importance of microclimate for orthopterans and suggests the existence of adaptations and trade-offs that underlie these strict, yet interspecifically very diverse preferences. This topic is not only interesting from an ecophysiological point of view but also has a direct connection to conservation studies, as we need to understand exactly why and through which mechanisms human activities and climate change shape Orthoptera populations.

The Orthopteran are capable of withstanding impressive amounts of temperature and moisture stress (Chapman et al., 2013), and there are various ways to confront unfavourable conditions. One of the simplest methods is to merely escape them. Even a subtle change in body position can have a significant

impact. For instance, grasshoppers exposed to high temperatures adopt a stilting posture, stretching their legs and raising their bodies above the ground. In this scenario, while the ground temperature may be a deadly 56°C, just 6mm above the ground, a tolerable 40°C is present (Willmer, 1982). Moreover, different life stages exhibit varying abilities to actively seek optimal conditions: from completely immobile eggs, through nymphs with limited mobility, to mobile (sometimes even flying) adults (Ingrisch & Köhler, 1998). Another aspect, that is the focus of this thesis, are ecophysiological adaptations. Within the constraints of abiotic limitations that enable multicellular life (Chapman et al., 2013), there is a significant scope for individual adaptations in how temperature and moisture affect development, reproductive effort, or the ability to escape predators and avoid diseases. Naturally, all these factors are heavily interconnected, constrained, and trade-offs are involved (Gillooly & Dodson, 2000; Berner & Blanckenhorn, 2006; Kingsolver & Huey, 2008; Marshall et al., 2018; Church et al., 2019). Moreover, temperature and moisture requirements and tolerance to extremes naturally change during the life cycle, with eggs generally exhibiting the highest cold and drought resistance (Shu-Guang & Kang, 2004; Pang et al., 2014; Srygley, 2021).

In conclusion, there are numerous ecophysiological factors, often constrained and changing throughout an individual's lifetime, that can be responsible for the observed habitat preferences of specific species. A significant amount of work on this topic has been conducted by Ingrisch & Köehler (1998), whose book is the best comprehensive study on the ecology of European orthopterans known to date. However, our understanding is still largely limited to a few model species, there is a lack of synthesis of different approaches and knowledge about some life stages remains fragmented. In this chapter, I review the known information about the effects of microclimate and subsequent adaptations throughout all life stages in Central European Orthoptera, and I describe my contribution to entangling these issues.

5. 1 Oviposition preferences

Life begins with an egg, and the act of oviposition determines the performance of future offspring (Refsnider & Janzen, 2010). There is a vast amount of literature addressing the effects of biotic factors, such as optimal food source selection and predation/parasitization avoidance, on oviposition site choice (Mitchell, 1975; Jaenike, 1978; Bernays & Graham, 1988; Almohamad et al., 2007; Morse, 2017). Conversely, there is only sparse information available about the effects of abiotic conditions such as local moisture and temperature (Stauffer & Whitman, 2007; Eilers et al., 2013; de Farias-Martins et al., 2017).

Two sets of hypotheses regarding oviposition site choice in relation to temperature and moisture can be identified for insects and orthopterans. Some authors have postulated that the choice of microclimate for oviposition should be consistent with adult habitat preferences; for example, species inhabiting dry

and warm habitats should lay their eggs in dry and warm conditions (Schnebel & Grossfield, 1986; Esbjerg & Lauritzen, 2010). A second hypothesis postulates a general tendency to lay eggs in moist conditions to avoid desiccation (Howard & Harrison, 1984; Brust & House, 1990; de Farias-Martins et al., 2017). Substantial testing of these hypotheses has been performed on Orthopteran models. These studies often compare two species or populations with contrasting habitat requirements (Choudhuri, 1958; Stauffer & Whitman, 2007; Herrmann et al., 2010; Fielding, 2011) and their results are mostly in congruence with the hypothesis stating correlation of oviposition site choice microclimate and adult habitat preference. However, there has been a lack of larger comparative studies that utilize more species, thus providing a more robust test of these hypotheses. Only one general study, which used multiple species, has been conducted on Drosophilas, and it was focused on a huge latitudinal gradient rather than microclimate (Schnebel & Grossfield, 1986).

As mentioned in previous chapters, the microclimate near the ground level can drastically change within just a few centimetres. Therefore, simply burying eggs a few centimetres underground can greatly reduce thermal and drought stress. Conversely, in colder habitats, placing eggs above the ground and exposing them to sunlight may be more beneficial (Ingrisch & Köhler, 1998; Stoutjesdijk & Barkman, 2014). Thus, differences in a vertical distribution of eggs oviposition can be a crucial behavioural adaptation of individual species, partially explaining their occupation of different habitats. More specifically, it is hypothesized that species living in hot and dry conditions should lay eggs deeper in the ground than oligothermic species, which should prefer shallow oviposition, or oviposit on a vegetation above the ground. However, there is very little information available regarding the validity of this hypothesis and the inter- or intraspecific plasticity of oviposition behaviour in temperate orthopterans (Ingrisch & Köhler, 1998; Stauffer & Whitman, 2007; Fielding, 2011).

Contribution:

In our study (**Study 2**), we investigated 14 species of temperate grasshoppers inhabiting grasslands with a wide variety of abiotic conditions, ranging from rocky steppes to marshes and alpine tundra, to test the validity of general oviposition site choice hypotheses and explore patterns of plasticity in oviposition depth among these species. We found that individual species differ in their moisture preference for oviposition, but there is no direct link between the preferred moisture of adults and oviposition sites. The effect was also not significant for temperature. In general, almost all species prefer a moist substrate, supporting the hypothesis of desiccation avoidance, which go against the current consensus about oviposition site choice in orthopterans. Regarding oviposition depth, in congruence with the hypothesis, we observed a significant increase in oviposition depth in species inhabiting more xerothermic habitats. This trend was also evident at the intraspecific level, where oothecae placed in warmer and drier conditions tended to be buried deeper.

Thus, while an oviposition site choice may explain some aspects of habitat choices, there is no direct link between general habitat preferences and preferences for oviposition. Instead, individual species exhibit an individualized approach, probably relative to the habitat context.

5. 2 Embryonic development

Most Central European Orthopteran species overwinter as eggs and undergo some form of diapause. Thus, embryonic development is divided into pre- and postdiapause stages. Grasshoppers generally overwinter once as eggs, with some exceptions in species inhabiting extreme alpine habitats (Ingrisch & Köhler, 1998). In katydids, there is higher variation with many species overwintering twice (representants of the genus *Saga* even more times) and in some species intrapopulation plasticity in overwintering numbers was discovered (Ingrisch, 1984, 1986a; b). Some prediapause development is needed to reach the diapausing stage, and a sufficient accumulation of temperature is required. For katydids with a variable overwintering count, a high accumulation of temperature preceding diapause can lead to hatching after the first overwintering. If the temperature accumulation is lower, they overwinter twice (Ingrisch, 1986a). In grasshopper *Chorthippus brunneus*, which obligatorily overwinters once, a pattern of delaying hatching in eggs laid late in autumn can still be observed (Cherrill & Begon, 1991). Day length can have a similar effect on some katydid species. Eggs laid during long days (at the beginning of summer) hatch the next year, whereas eggs laid during shorter days later in the season overwinter twice in *Roeseliana roeselii* katydid (Ingrisch, 1984). Sometimes, the overwintering regime can be plastic across populations from different climates (Ingrisch & Köhler, 1998).

Developmental rates are directly affected by ambient temperature as an egg is an immobile life stage, it is unable to actively thermoregulate or search for optimal temperatures, and it is fully reliant on the climatic conditions of the oviposition site, as mentioned in the previous chapter. The right timing of hatching, directly affected by accumulated temperature, is crucial for subsequent development, and many factors such as synchronizing with food resources, suitable climate, or avoiding predators and parasites, can play a role. There is also a hypothesis that xerothermophilous species are limited by season length and embryonic development time can be a main constraint on their occurrence (Sibly & Monk, 1987; Danks, 2004; Hodkinson, 2005). Eggs of Central European species can develop at fairly high continuous temperatures, up to 38°C, including species living in relatively cold and wet habitats. The lower developmental threshold for grasshoppers is around or slightly below 15°C, but this varies between species (Bruckhaus, 1990; Van Wingerden et al., 1991). Developing embryos generally survive below-zero temperatures, with supercooling capacity usually the highest in diapausing eggs during winter. After overwintering, cold tolerance rapidly declines (Shu-Guang & Kang, 2004; Pang et al., 2014).

The effect of moisture on egg development has been far less studied. Embryos need to absorb water after oviposition for continued development (Moriarty, 1970). High moisture content is generally preferred by all Central European grasshopper species studied from different habitat backgrounds. Nevertheless, in hygrophilous species, development is retarded at lower moisture level. In general, lower moisture prolongs postdiapause development in all species included in the study of Ingrisch (1983), or decreases hatching ratio after the second overwintering in species with a two-year life cycle (Ingrisch, 1986c). Also, spring rains can often initiate hatching (Ingrisch & Köhler, 1998).

However, we cannot rely on temperature alone when studying developmental rates and time, as they are directly related to size as one of the most important physiological constrain (Brown et al., 2004). The entangled relationship of temperature, size, and development time is one of the major determinants of insects life histories (Kingsolver & Huey, 2008). Theory predicts larger embryos in species or populations inhabiting harsher and colder environments with limited season length, as larger hatchlings are more resilient and mature relatively faster (Fox & Czesak, 2000; Marshall et al., 2018; Di Martino & Liow, 2021). However, this can be physiologically constrained, as larger embryos usually take longer to hatch (Gillooly & Dodson, 2000; Bueno & López-Urrutia, 2012).

Contribution:

I conducted a study on postdiapausal embryonic development in 14 grasshopper species from various habitat backgrounds (**Study 3**). The main goal was to assess whether species adapted to different microclimates demonstrate different development times at a constant temperature or have different sensitivities to temperature changes. Additionally, I also examined how these developmental parameters are coupled with relative/absolute hatchling and adult weight.

I found that species occupying habitats with warmer microclimate tend to develop for longer at a constant incubation temperature and are more sensitive to temperature changes, with their development being more prolonged at low temperatures. These findings are consistent with the general hypothesis expecting swifter development in colder climate to mitigate shorter seasons (Monk, 1985; Van Wingerden et al., 1991; Berner & Blanckenhorn, 2006). Additionally, consistent with hypothesis, species inhabiting hotter habitats have smaller adults, but there was no effect on relative hatchling size. This work provides scarce information about the validity of general hypotheses regarding adaptations of embryonic development time and coupled size traits at a microclimatic scale (as they are usually tested on large altitudinal or latitudinal gradients).

5. 3 Nymphal development

When developed embryos successfully hatch, another challenging life journey begins. Nymphs are more susceptible to harsh conditions than adults due to limited mobility and smaller size, which decreases

their buffering capacity (Chapman et al., 2013; Keosentse et al., 2021). The conditions to which nymphs are exposed further affect adult performance (Bochdanovits & De Jong, 2003; Christiansen-Jucht et al., 2015; Vasudeva, 2023). Some authors consider nymphs to be a crucial life stage, and assume that mortality during nymphal development can be the main determinant of adult population density. Increased predation in cold and wet conditions or failed eclosion has been proposed as the main explanatory factors (Cherrill & Brown, 1990b; Grant et al., 1993).

Nevertheless, little is known about Central European orthopterans and the effect of abiotic conditions on their nymphal development (Ingrisch & Köhler, 1998). Laboratory studies suggest a general high temperature demand, especially in grasshoppers (Ingrisch, 1978; Whitman, 1986; Lactin & Johnson, 1996; Willott & Hassall, 1998; Ren et al., 2016). A few field studies have found considerable developmental plasticity according to macro- and microclimatic conditions, where populations inhabiting colder and wetter habitats tend to develop faster, as they are more limited by season length under such conditions (Monk, 1985; Buckley et al., 2015). Note the similarity with a plasticity found in the embryonic development.

The effect of insolation and thermoregulatory ability is elaborated in the next chapter, as it is not known for nymphs of Central European species (Ingrisch & Köhler, 1998).

Research regarding the effect of moisture is almost absent. A rare study found that temperate orthopterans generally prefer dry air, and even nymphs of hydrophilic species generally thrive in rather dry conditions (Ingrisch, 1978; Ingrisch & Köhler, 1998).

In conclusion, the larval stage and its sensitivity to site conditions remain largely unexplored for Central European species. Some knowledge exists about notoriously well-known model organisms such as *Schistocerca gregaria* and *Locusta migratoria* (Hamilton, 1950; Uvarov, 1966; Maeno et al., 2021), both inhabiting tropical or subtropical regions and facing different challenges during development than Central European species. Future research studying how larval development is affected by microhabitat temperature and moisture and whether abiotic conditions are able to delimit species habitat preferences in this life stage is needed.

5. 4 Adult thermal and moisture preference

Adults have the most relaxed habitat requirements as they are the most resilient and mobile stage. However, suitable conditions for courtship, mating, egg maturation, and oviposition are still needed (Chapman et al., 2013; Hutchings, 2021). Particularly limiting can be the oviposition period, as a reproductive potential can be greatly constrained by the length of the season (Cherrill & Brown, 1990b; Willott & Hassall, 1998; Berger et al., 2008).

As mentioned, orthopterans are generally a thermophilous order, and some species belong to the most heat-tolerant species among insects. The general highest long-term liveable temperature for ectotherms is around 47°C (Hoffmann et al., 2013). Temperatures over 40°C are commonly preferred in some locusts such as *Schistocerca gregaria* or *Locusta migratoria* (Uvarov, 1966; Maeno et al., 2021). Central European species commonly prosper in temperatures around 35°C (Ingrisch & Köhler, 1998). The highest known upper thermal limit was found for the Australian desert ant *Melophorus bagoti* at 56.7°C (Christian & Morton, 1992), but various orthopteran species can withstand only slightly lower temperatures around 55°C (Uvarov, 1966).

Cold tolerance of non-diapausing adults is not well understood. In general, temperatures a few degrees below zero are tolerated for a short time (Uvarov, 1966). In a recent experiment, 14 species of temperate grasshoppers from various habitats survived -3°C for one hour without further consequences (unpublished results).

Insolation is a crucial requirement in Central European orthopterans, especially for grasshoppers thriving in high temperatures of 30-35°C, whereas such air temperatures are very limited in temperate climate. Sun basking is thus an important behavioural adaptation enabling them to gain enough heat for proper functioning (Begon, 1983; Whitman, 1987; Willott, 1997; Harris et al., 2015). Grasshoppers are adept at maintaining optimal body temperature during the day and are able to use a variety of thermoregulatory postures to achieve optimal heat accumulation (Whitman, 1987). The ability to increase body temperature above ambient varies between species. A study by (Willott, 1997) suggests an interesting link between occupied habitat and the ability to thermoregulate at various temperatures. *Omocestus viridulus*, which inhabits relatively cold places, was able to effectively raise its body temperature at low ambient temperatures (15-20°C), but at high temperatures exceeding 30°C, its body temperature remained high above ambient, posing a risk of overheating. On the other hand, relatively thermophilous species like *Stenobothrus lineatus* and *Myrmeleotettix maculatus* were unable to effectively raise their body temperature at low ambient temperatures, but were much better at avoiding overheating at around 35°C. Further studies on a larger set of species coupled with detailed measurements of microclimate could significantly enhance our understanding of thermoregulation ability and its contribution to habitat choice, providing more insight than simple laboratory examinations of upper thermal limits.

One specific hypothesis explains the high temperature demand as adaptation which can help against pathogens, whose generally hate high temperatures (Inglis et al., 1996; Springate & Thomas, 2005; Kistner & Belovsky, 2017).

6. Evolutionary history of orthopterans

To understand the current patterns in species distribution and their habitat bonding, we need to delve into the past, where current biomes and Orthoptera assemblages have formed. Most Central European species rely on grasslands, and therefore, their history is closely linked to the formation of this biome. Current grasslands in Central Europe have several origins: 1. Mountain grasslands above the tree line, maintained by the cold climate inhibiting tree growth, occur in high mountain ranges such as the Alps, higher parts of the Carpathians, and very fragmentarily in the highest parts of Hercynian Mountains. 2. Steppe grasslands, with their centrum in central Asia and fragmentarily spreading out in the eastern parts of Central Europe, are maintained by the dry environment and partially by grazing. 3. Additionally, there are small fragments of natural grassland-like (often ephemeral) vegetation on rock edges, river floodplains, etc. 4. However, the largest part of grasslands has a very recent origin, emerging from human activities, which expanded grasslands at the expense of forests after the last glacial period (Hejcman et al., 2013; Dixon et al., 2014; Feurdean et al., 2015; Kuneš et al., 2015).

The origin of temperate grassland as a biome is dated around the middle Miocene, approximately 15 million years ago, and it was initiated with climate drying and cooling (Jacobs et al., 1999). This event is coupled with the radiation of Acrididae, especially the subfamily Gomphocerinae, which are currently the dominant grasshopper group in temperate Eurasian grasslands (Song et al., 2015; Sevastianov et al., 2023).

However, the origin of Orthoptera dates much further back in the past (Song et al., 2015). The first confidently assigned orthopteran fossil belongs to *Oedischia williamsoni* from the Upper Carboniferous period in France (Béthoux & Nel, 2002). In this fossil, prominent "jumping" hind legs very similar to extant orthopterans are apparent. Nevertheless, ancient orthopterans are captured in a cloud of fossils, which definitely belong to Polyneoptera but are hardly assignable to any recent insect order. Usually, only wing fragments are preserved, and the whole taxonomy is based on forewing venation, which brings another issue with correct vein interpretation (Béthoux & Nel, 2002; Gorokhov, 2005; Béthoux, 2007) (For recent, most recognized wing venation interpretation, see Figure 3a). These taxa were formerly assigned to the wastebasket so called Protorthoptera (Carpenter, 1966; Moore & Carpenter, 1992). Opinions about species and natural taxonomic groups within Protorthoptera have frequently changed; for example Kukalová-Peck & Brauckmann (1992) considered some representatives close to "hemipteroid" lines. Some authors often do not respect taxonomic rules and arbitrarily assign these species to extant orders or creates new artificial groups (Rasnitsyn & Quicke, 2002; Béthoux & Nel, 2002). As a recent attempt to sort out the majority of "Protorthopterans," the new superorder Archaeorthoptera was created (Béthoux & Nel, 2002). The apomorphias of Archaeorthoptera are exclusively based on forewing venation patterns, especially entangled vein topology and fusions in the medio-cubital area. These include the basal fusion of CuA with M forming the common M + CuA vein.

After some time, CuA separates from M and runs alone, to finally fuse with the anterior branch of the cubitus posterior (CuPa) forming the CuA + CuPa (or CuPaα) common vein (Figure 3b). As a subordinate taxon, the superorder Panorthoptera, including Orthoptera, Titanoptera, Caloneurodea, or the "gerarid" line, was also created. The main apomorphy is the branching of CuPa to CuPa α and CuPaβ followed by fusion of CuPaα with CuA (Béthoux & Nel, 2002, 2005; Béthoux et al., 2012). However, the validity of the postulated taxonomic hierarchy and mentioned apomorphies must be thoroughly evaluated, and there is a high need for intensive research on fossils related to "orthopteroid" lines to finally resolve the exact origin of orthopterans and their relation to the other Polyneopteran insects.

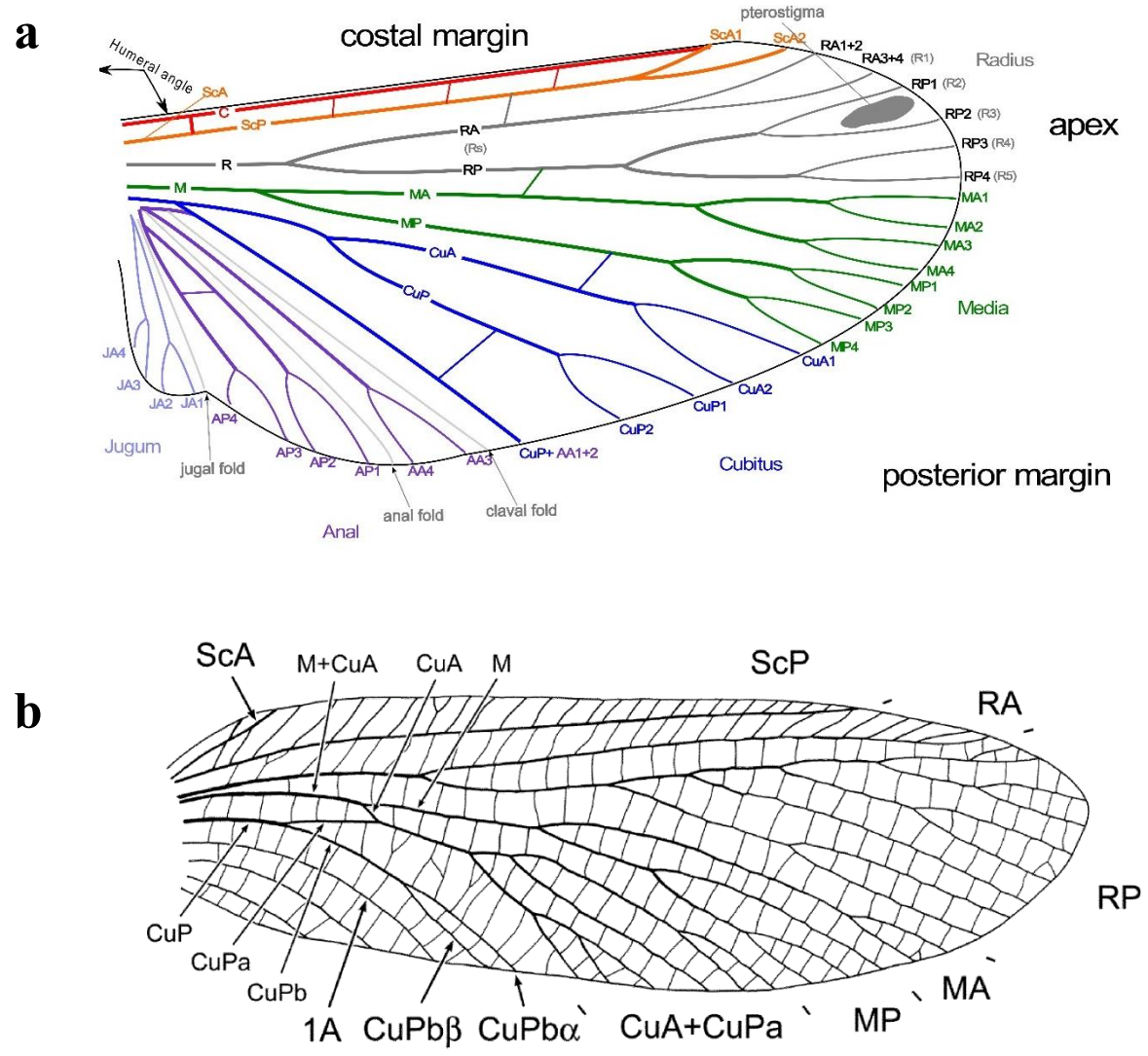


Fig. 3: Insects wing venation. **a)** general pattern, **b)** pattern typical for Archaeorthoptera.

Contribution:

As a contribution to our knowledge of the Upper Carboniferous insect fauna and the origin of Polyneopteran orders, we published three papers describing five new genera and seven new Polyneopteran species (mainly archaeorthopterans) from the Late Carboniferous (Pennsylvanian).

The first study (**Study 4**) presents a detailed description of two new archaeorthopteran genera and three new species from the Pennsylvanian of the Upper Silesian Coal Basin in Poland. These new taxa provide new insights into the wing venation disparity of this remarkable and insufficiently studied insect group. *Omaliella polonica* sp. nov. is based on a well-preserved forewing, including the wing base, which allows a thorough discussion and comparison with other archaeorthopterans. Surprisingly, it is the first complete wing for this group of related genera (*Omaliella*, *Omalia*, *Coselia*, and *Paleomastax*). *Owadpteron dareki* gen. et sp. nov. has an unusual arrangement of cubital veins. The marked resemblance of the venation of *Owadpteron* to that of some members of the gerarid line, such as *Nacekomia*, supports its placement within the family Geraridae (stem-group Orthoptera). Finally, the venation of *Parapalaeomastax dariuszi* gen. et sp. nov. strikingly resembles that of the genus *Palaeomastax*, differing only in the distally branched media. The discovery of these three new archaeorthopterans from the Upper Silesian Coal Basin fits well with that of closely related taxa known from other deposits in Euramerica, such as Mazon Creek Lagerstätte, Avion in the Pas-de-Calais Basin, and others. Furthermore, a new re-examination of the earliest archaeorthopteran from the Upper Silesian Coal Basin confirms the doubtful assignment of this fragmentary fossil to Archaeorthoptera or even to Pterygota.

The second study (**Study 5**) described another new archaeorthopterans *Contracladus impar* gen. nov. et sp. nov. (uncertain assignment), *Paraspiculum brauckmannii* gen. nov. et sp. nov. (Caloneurodea), and *Piesbergopterum punctatum* gen. nov. et sp. nov. (Cnemidolestodea) from the Moscovian of Piesberg quarry near Osnabrück (Lower Saxony, Germany). All new taxa display unique patterns of their forewing venations. The mosaic of characters found in *C. impar* with a particular lack of a free vein CuA demonstrates the limits of the current systematics for Archaeorthoptera. The evidence for a supposedly disruptive pattern of coloration is demonstrated on the forewing membrane of P. *punctatum*, which shows numerous spots in the distal part of the fore wing. Those are inside the cells delimited by the cross veins. The exceptionally preserved fossils found in Piesberg document fine structural details and complete our knowledge of the early history of this diverse group of polyneopteran insects.

The third and last study (**Study 6**) does not deal with Archaeorthoptera but representatives of the stem group relatives of Dictyoptera, comprising the common Palaeozoic and Mesozoic roachoids, a group well known for its problematic taxonomy. Here, two new taxa are described based on forewing venation well displaying convexity or concavity of the veins due to preservation in spherosideritic concretions. A new paoliid, *Stephanopsis testai* sp. nov. (Blattinopsidae), from the Moscovian deposits at Mazon Creek (Illinois, U.S.A.), supplements *Stephanopsis mirandus* found at the same locality, which mainly differs

in the branching patterns of MP and anal veins. A new stem dictyopteran, *Sosnowiecia dareki* gen. nov. et sp. nov. (?Mesorthopteridae), from Langsettian deposits at Sosnowiec-Klimontów (Lower Silesia, Poland) is problematic in terms of taxonomy as it has a mosaic of characters characteristic of Paoliida, Eoblattida, and Grylloblattida but retains affinities with roachoid insects, such as *Miroblatta* sp. Hence, these two new species increase the diversity of Carboniferous polyneopteran insects but reveal the problem of depending only on alar characters in taxonomy.

7. Gaps and future of studies on Orthoptera habitat requirements

One of the biggest current problems in understanding the ecological requirements of orthopterans and how these shape populations in relation to habitat and climate changes are significant gaps in some aspects of orthopteran life history knowledge and the lack of synthesis of different approaches. As described in Chapter 5, we know a relatively lot about the effects of temperature and moisture on egg development and on adults' requirements. However, work on oviposition site choice and the microclimatic requirements of low-mobile nymphs is scarce and there is still a lack of a consistent general explanation of the requirements of these stages. While one might expect a similar effect of microclimate on nymphs and adults, there are already hints that nymphs may prefer different microclimate and vegetation structure (Cherrill & Brown, 1990b; Ingrisch & Köhler, 1998; Wünsch et al., 2012). Studies that take into account the entire life cycle and different challenges that appear during life are almost missing (Monk, 1985; Grant et al., 1993; Willott & Hassall, 1998) and only a few model species have been studied. It would be very interesting to conduct complex studies across a whole life cycle on species inhabiting extremely hot and dry or conversely wet and cold habitats, where some adaptations and subsequent trade-offs should be easily observed. Another useful approach would be to focus on one specific life stadium and specific problem, but incorporate a larger number of species, to enable trends examination, as conclusions based on the comparison of a few species can be misleading (Dvořák & Knapp, 2023).

Studies on a community level have brought interesting insights into the general habitat, climatic, and management characteristics shaping Orthoptera populations. Nevertheless, local moisture has been surprisingly rarely studied (Powell et al., 2007; Weking et al., 2016), although it has been shown to be the main predictor shaping the occurrences of central European orthopterans (Dvořák et al., 2022). Another challenge is represented by often correlated explanatory variables, which is usually resolved by omitting the presumably less relevant one or fusing them into one representative variable. However, if two highly correlated types of predictors cannot be fused or omitted (e.g., they are both in the focus of the study), interpretation of such results can be very challenging (Weking et al., 2016). An ecological niche approach developed within the thesis (Dvořák et al., 2022) seems to be a promising tool to address these issues, but research on more species and more environmental parameters is still needed. Another

interesting topic could be studying the interaction effect of environmental variables or variability in habitat requirements between different populations of one species. For example, if populations inhabiting colder macroclimate prefer hotter microclimates and vice versa.

When studying ecological adaptation, it is essential to consider the evolutionary history and inherited traits of the species being examined. Understanding the interplay between evolutionary inheritance and ecological adaptability is crucial for fully comprehending observed habitat preferences. Expanding knowledge of grasshopper evolution and phylogenetic relationships, while integrating these insights into ecological studies, will help unravel the complex factors underlying observed habitat choices (Song et al., 2015; Hawlitschek et al., 2022; Sevastianov et al., 2023).

In conclusion, current knowledge on all points of view (community, ecophysiological and evolutional) on habitat choice of central European orthopterans is still very patchy. Moreover, to my knowledge, there is no comprehensive work where all community, ecophysiological and evolutionary approaches are merged together. There is a high need for studies where the detailed microclimate of specific habitats is measured in its whole complexity during the whole season and is coupled with close observation of behaviour, development, morphology and population dynamics of studied species. Common garden experiments with the transfer of species with different habitat requirements or laboratory experiments in cages closely simulating natural habitats, but enabling manipulation with climate, can also be very beneficial (Weiss et al., 2013; Schirmel et al., 2019). However, without such "whole life" studies, we cannot fully understand how orthopterans in Central European grasslands live, how they will react to management and climate changes, and how we can effectively protect them.

8. Conclusion

Temperate Orthoptera communities, spreading from hot rocky steeps to cold alpine meadows, provide a fascinating model for studying how insects cope with highly variable, often extreme, microclimatic conditions and how they will be affected by climatic changes and anthropogenic pressure.

In my thesis, I demonstrate that moisture is the most important determinant of habitat choice for most Orthoptera species in Central Europe, although this factor is rarely examined in other studies. Additionally, I developed ecological niche indices for five habitat parameters that determine the occurrence of most species. I offer new insight into oviposition site selection concerning the microclimatic properties of these sites. No link was found between adult habitat preferences and oviposition preferences; instead, most species showed a preference for generally moist sites, regardless of their occupied habitat. I also show that species from hotter environments have significantly longer embryonic development times and smaller adult sizes. Seven newly described fossil species from the

Upper Carboniferous reveal new, interesting fore-wing vein patterns, offering insights into the tangled relationships of early orthopteroids and related insects.

While I have partially addressed gaps in our understanding of Orthoptera habitat requirements and their evolutionary history, many new questions have emerged. More research is needed on the habitat requirements of different life stages, the ecological niches of unstudied species, the co-evolution of species and their habitats, and most importantly, connecting these often-scattered pieces of information together. Only by doing so can we fully understand how Orthoptera in Central European grasslands live, how they will react to management and climate changes, and how we can effectively protect them.

9. References

Ahnesjö J & Forsman A (2006). Differential Habitat Selection by Pygmy Grasshopper Color Morphs; Interactive Effects of Temperature and Predator Avoidance. *Evolutionary Ecology* 20:235–257.

Almohamad R, Verheggen FJ, Francis F & Haubruge E (2007). Predatory hoverflies select their oviposition site according to aphid host plant and aphid species. *Entomologia Experimentalis et Applicata* 125:13–21.

Altmoos M & Henle K (2010). Relevance of multiple spatial scales in habitat models: A case study with amphibians and grasshoppers. *Acta Oecologica* 36:548–560.

Amédégnato C & Devriese H (2008). Global diversity of true and pygmy grasshoppers (Acridomorpha, Orthoptera) in freshwater. *Hydrobiologia* 595:535–543.

Badenhausser I & Cordeau S (2012). Sown grass strip—A stable habitat for grasshoppers (Orthoptera: Acrididae) in dynamic agricultural landscapes. *Agriculture, Ecosystems & Environment* 159:105–111.

Badenhausser I, Gross N, Cordeau S, Bruneteau L & Vandier M (2015). Enhancing grasshopper (Orthoptera: Acrididae) communities in sown margin strips: the role of plant diversity and identity. *Arthropod-Plant Interactions* 9:333–346.

Báldi A & Kisbenedek T (1999). Orthopterans in small steppe patches: an investigation for the best-fit model of the species-area curve and evidences for their non-random distribution in the patches. *Acta Oecologica* 20:125–132.

Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM, Brown VK, Butterfield J, Buse A, Coulson JC, Farrar J, Good JEG, Harrington R, Hartley S, Jones TH, Lindroth RL, Press MC, Symrnioudis I, Watt AD & Whittaker JB (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology* 8:1–16.

Bauer N & Kenyeres Z (2006). Habitat preference studies of some species of the genus *Isophya* Brunner von Wattenwyl, 1878 (Orthoptera: Phaneropteridae) in the western part of the Carpathian Basin. *Journal of Orthoptera Research* 15:175–185.

Bazelet CS & Samways MJ (2011). Identifying grasshopper bioindicators for habitat quality assessment of ecological networks. *Ecological Indicators* 11:1259–1269.

Begon M (1983). Grasshopper populations and weather: the effects of insolation on *Chorthippus brunneus*. *Ecological Entomology* 8:361–370.

Berger D, Walters R & Gotthard K (2008). What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly. *Functional Ecology* 22:523–529.

Bernays E & Graham M (1988). On the Evolution of Host Specificity in Phytophagous Arthropods. *Ecology* 69:886–892.

Berner D & Blanckenhorn WU (2006). Grasshopper ontogeny in relation to time constraints: adaptive divergence and stasis. *Journal of Animal Ecology* 75:130–139.

Béthoux O (2007). Archaeorthoptera wing venation nomenclature: A reply to Gorokhov. *Paleontological Journal* 41:338–340.

Béthoux O, Gu J & Ren D (2012). A new Upper Carboniferous stem-orthopteran (Insecta) from Ningxia (China). *Insect Science* 19:153–158.

Béthoux O & Nel A (2002). Venation pattern and revision of Orthoptera sensu nov. and sister groups. Phylogeny of Palaeozoic and Mesozoic Orthoptera sensu nov. *Zootaxa* 96:1-88.

Béthoux O & Nel A (2005). Some palaeozoic 'protorthoptera' are 'ancestral' orthopteroids: Major wing braces as clues to a new split among the 'protorthoptera' (Insecta). *Journal of Systematic Palaeontology* 2:285–309.

Bidau CJ (2014). Patterns in Orthoptera biodiversity. I. Adaptations in ecological and evolutionary contexts. *Journal of Insect Biodiversity* 2:1.

Blanckenhorn WU (2004). Bergmann and Converse Bergmann Latitudinal Clines in Arthropods: Two Ends of a Continuum*? Integrative and Comparative Biology* 44:413–424.

Bochdanovits Z & De Jong G (2003). Temperature dependent larval resource allocation shaping adult body size in *Drosophila melanogaster*. *Journal of Evolutionary Biology* 16:1159–1167.

Bogusch P, Macek J, Janšta P, Kubík Š, Řezáč M, Holý K, Malenovský I, Baňař P, Mikát M, Astapenková A & Heneberg P (2016). Industrial and post-industrial habitats serve as critical refugia for pioneer species of newly identified arthropod assemblages associated with reed galls. *Biodiversity and Conservation* 25:827–863.

Branson DH (2008). Influence of a Large Late Summer Precipitation Event on Food Limitation and Grasshopper Population Dynamics in a Northern Great Plains Grassland. *Environmental Entomology* 37:686–695.

Braschler B, Marini L, Thommen GH & Baur B (2009). Effects of small-scale grassland fragmentation and frequent mowing on population density and species diversity of orthopterans: a long-term study. *Ecological Entomology* 34:321–329.

Brodacki M (2015). First records of the Large Cone-head Bush-cricket *Ruspolia nitidula* (Scopoli, 1786) (Orthoptera, Tettigoniidae) in Poland. *Naturalia* 3:123–126.

Brown JH (2014). Why are there so many species in the tropics? *Journal of Biogeography* 41:8–22.

Brown JH, Gillooly JF, Allen AP, Savage VM & West GB (2004). Toward a metabolic theory of ecology. *Ecology* 85:1771–1789.

Bruckhaus A (1990). Bedeutung der Temperatur für die Biotopbindung einiger einheimischer Feldheuschreckenarten. *Articulata* 5:43–57.

Brust GE & House GJ (1990). Influence of soil texture, soil moisture, organic cover, and weeds on oviposition preference of southern corn rootworm (Coleoptera: Chrysomelidae). *Environmental Entomology* 19:966–971.

Buckley LB, Nufio CR, Kirk EM & Kingsolver JG (2015). Elevational differences in developmental plasticity determine phenological responses of grasshoppers to recent climate warming. *Proceedings of the Royal Society B: Biological Sciences* 282:20150441.

Bueno J & López-Urrutia Á (2012). The Offspring-Development-Time/Offspring-Number Trade-Off. *The American Naturalist* 179:196–203.

Carbutt C, Henwood WD & Gilfedder LA (2017). Global plight of native temperate grasslands: going, going, gone? *Biodiversity and Conservation* 26:2911–2932.

Carpenter FM (1966). The Lowe Permian Insects from Kansas. Part II. The orders Protorthoptera and Orthoptera. *Psyche*:46–88.

Chambers BQ & Samways MJ (1998). Grasshopper response to a 40-year experimental burning and mowing regime, with recommendations for invertebrate conservation management. *Biodiversity and Conservation* 7:985–1012.

Chapman RF, Simpson SJ & Douglas AE (2013). The insects: structure and function. Cambridge University Press, New York.

Cherrill A & Begon M (1991). Oviposition Date and Pattern of Embryogenesis in the Grasshopper *Chorthippus brunneus* (Orthoptera, Acrididae). *Holarctic Ecology* 14:225–233.

Cherrill AJ & Brown VK (1990a). The Habitat Requirements of Adults of the Wart-bitter (*Decticus verrucivorus*) Orthoptera: Tettigoniidae in Southern England. *Biological Conservation* 53:145–157.

Cherrill AJ & Brown VK (1990b). The life cycle and distribution of the wart-biter *Decticus verrucivorus* (L.) (Orthoptera: Tettigoniidae) in a chalk Grassland in Southern England. *Biological Conservation* 53:125–143.

Chisté MN, Mody K, Gossner MM, Simons NK, Köhler G, Weisser WW & Blüthgen N (2016). Losers, winners, and opportunists: How grassland land-use intensity affects orthopteran communities. *Ecosphere* 7.

Choudhuri JCB (1958). Experimental Studies on the Choice of Oviposition Sites by Two Species of Chorthippus (Orthoptera: Acrididae). *The Journal of Animal Ecology* 27:201-216.

Christian KA & Morton SR (1992). Extreme Thermophilia in a Central Australian Ant, Melophorus bagoti. *Physiological Zoology* 65:885–905.

Christiansen-Jucht CD, Parham PE, Saddler A, Koella JC & Basáñez M-G (2015). Larval and adult environmental temperatures influence the adult reproductive traits of Anopheles gambiae s.s. *Parasites & Vectors* 8:456.

Church SH, Donoughe S, de Medeiros BAS & Extavour CG (2019). Insect egg size and shape evolve with ecology but not developmental rate. *Nature* 571:58–62.

Cigliano MM, Braun H, Eades DC & Otte D (2024). Ortohoptera Species File.

Cizek O, Zamecnik J, Tropek R, Kocarek P & Konvicka M (2012). Diversification of mowing regime increases arthropods diversity in species-poor cultural hay meadows. *Journal of Insect Conservation* 16:215–226.

Danks HV (2004). Seasonal Adaptations in Arctic Insects. *Integrative and Comparative Biology* 44:85–94.

Derka T & Fedor P (2010). *Hydrolutos breweri* sp. n., a new aquatic Lutosini species (Orthoptera: Anostostomatidae) from Churí-tepui (Chimantá Massif, Venezuela). *Zootaxa* 2653:51.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC & Martin PR (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences* 105:6668–6672.

Di Martino E & Liow L (2021). Larger offspring associated with lower temperatures across species of *Microporella*, a widespread colonial invertebrate. *Marine Ecology Progress Series* 662:1–13.

Dixon AP, Faber-Langendoen D, Josse C, Morrison J & Loucks CJ (2014). Distribution mapping of world grassland types. *Journal of Biogeography* 41:2003–2019.

Duffy GA, Coetzee BW, Janion-Scheepers C & Chown SL (2015). Microclimate-based macrophysiology: implications for insects in a warming world. *Current Opinion in Insect Science* 11:84–89.

Dvořák T, Hadrava J & Knapp M (2022). The ecological niche and conservation value of Central European grassland orthopterans: A quantitative approach. *Biological Conservation* 265:109406.

Dvořák T & Knapp M (2023). Conserved temperature requirements but contrasting responses to humidity across oviposition preferences in temperate grasshoppers. *Scientific Reports* 13:21131.

Eckert S, Möller M & Buchholz S (2017). Grasshopper diversity of urban wastelands is primarily boosted by habitat factors. *Insect Conservation and Diversity* 10:248–257.

Edwards RL (1964). Some Ecological Factors Affecting the Grasshopper Populations of Western Canada. *The Canadian Entomologist* 96:307–320.

Eilers S, Pettersson LB & Öckinger E (2013). Micro-climate determines oviposition site selection and abundance in the butterfly *Pyrgus armoricanus* at its northern range margin: Micro-climate and oviposition. *Ecological Entomology* 38:183–192.

Elith J, Phillips SJ, Hastie T, Dudík M, Chee YE & Yates CJ (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions* 17:43–57.

Encyclopaedia Britannica, Inc. (2007). The New Encyclopaedia Britannica. Encyclopaedia Britannica, Chicago.

Esbjerg P & Lauritzen AJ (2010). Oviposition response of the Turnip moth to soil moisture. *Acta Agriculturae Scandinavica, Section B - Plant Soil Science* 60:89–94.

Essl F & Dirnböck T (2012). What determines Orthoptera species distribution and richness in temperate semi-natural dry grassland remnants? *Biodiversity and Conservation* 21:2525–2537.

de Farias-Martins F, Sperber CF, Albeny-Simões D, Breaux JA, Fianco M & Szinwelski N (2017). Forest litter crickets prefer higher substrate moisture for oviposition: Evidence from field and lab experiments. *PLOS ONE* 12:1–16.

Fartmann T, Brüggeshemke J, Poniatowski D & Löffler F (2022). Summer drought affects abundance of grassland grasshoppers differently along an elevation gradient. *Ecological Entomology* 47:778–790.

Fartmann T, Krämer B, Stelzner F & Poniatowski D (2012). Orthoptera as ecological indicators for succession in steppe grassland. *Ecological Indicators* 20:337–344.

Feurdean A, Marinova E, Nielsen AB, Liakka J, Veres D, Hutchinson SM, Braun M, Timar-Gabor A, Astalos C, Mosburgger V & Hickler T (2015). Origin of the forest steppe and exceptional grassland diversity in Transylvania (central-eastern Europe). *Journal of Biogeography* 42:951–963.

Fielding DJ (2011). Oviposition Site Selection by the Grasshoppers *Melanoplus borealis* and *M. sanguinipes* (Orthoptera: Acrididae). *Journal of Orthoptera Research* 20:75–80.

Fielding DJ, Brusven MA, Shafii B & Price WJ (2001). Spatial heterogeneity of low-density populations of *Melanoplus sanguinipes* (Orthoptera: Acrididae) associated with grazing and vegetation treatments. *The Canadian Entomologist* 133:843–855.

Fonderflick J, Besnard A, Beuret A, Dalmais M & Schatz B (2014). The impact of grazing management on Orthoptera abundance varies over the season in Mediterranean steppe-like grassland. *Acta Oecologica* 60:7–16.

Forsberg B (2004). Microhabitat, phenology and diversity of Orthoptera in a seminatural pasture. *Thesi*s.

Fox CW & Czesak ME (2000). Evolutionary Ecology of Progeny Size in Arthropods. *Annual Review of Entomology* 45:341–369.

Franzke A, Unsicker SB, Specht J, Köhler G & Weisser WW (2010). Being a generalist herbivore in a diverse world: how do diets from different grasslands influence food plant selection and fitness of the grasshopper *Chorthippus parallelus* ? *Ecological Entomology* 35:126–138.

García-Rodríguez A, Martínez PA, Oliveira BF, Velasco JA, Pyron RA & Costa GC (2021). Amphibian Speciation Rates Support a General Role of Mountains as Biodiversity Pumps. *The American Naturalist* 198:E68–E79.

Gardiner T (2018). Grazing and Orthoptera: a review. *Journal of Orthoptera Research* 27:3–11.

Gardiner T & Hassall M (2009). Does microclimate affect grasshopper populations after cutting of hay in improved grassland? *Journal of Insect Conservation* 13:97–102.

Gardiner T, Pye M, Field R & Hill J (2002). The influence of sward height and vegetation composition in determining the habitat preferences of three *Chorthippus* species (Orthoptera: Acrididae) in Chelmsford, Essex, UK. *Journal of Orthoptera Research* 11:207–213.

Gavlas V, Bednár J & Krištín A (2007). A comparative study on orthopteroid assemblages along a moisture gradient in the Western Carpathians. *Biologia* 62:95–102.

Gerlach J, Samways M & Pryke J (2013). Terrestrial invertebrates as bioindicators: an overview of available taxonomic groups. *Journal of Insect Conservation* 17:831–850.

Gillooly JF & Dodson SI (2000). The relationship of neonate mass and incubation temperature to embryonic development time in a range of animal taxa. *Journal of Zoology* 251:369–375.

Gomboc S & Šegula B (2014). Pojoče kobilice Slovenije = Singing Orthoptera of Slovenia. EGEA, Zavod za naravo/Institution for nature, Ljubljana.

Gorokhov AV (2005). Review of Triassic Orthoptera with descriptions of new and little known taxa: Part 1. *Paleontological Journal* 39:178–186.

Grant A, Hassall M & Willott SJ (1993). An Alternative Theory of Grasshopper Life Cycles. *Oikos* 66:263–268.

Grimaldi DA & Engel MS (2005). Evolution of the insects. Cambridge university press, Cambridge [etc.].

Grzędzicka E & Vahed K (2020). Habitat requirements of the endangered heath bush-cricket *Gampsocleis glabra* (Orthoptera, Tettigoniidae) in an isolated population. *Journal of Insect Conservation* 24:935–945.

Gwynne DT (2008). Sexual Conflict over Nuptial Gifts in Insects. *Annual Review of Entomology* 53:83–101.

Habel JC, Dengler J, Janišová M, Török P, Wellstein C & Wiezik M (2013). European grassland ecosystems: threatened hotspots of biodiversity. *Biodiversity and Conservation* 22:2131–2138.

Haberski A & Sikes D (2021). Orthoptera of Alaska: A photographic key, new records, and synonymy of *Melanoplus gordonae*. Canadian Journal of Arthropod Identification 44.

Hamilton A (1950). Further studies on the rekation of humidit and temperature to the development of two species of African locusts – Locusta migratoria migratoides (R. & F.) and Schistocerca grefaria (Forsk.). *Transactions of the Royal Entomological Society of London* 101:1–58.

Harris RMB, McQuillan P & Hughes L (2015). The effectiveness of common thermo-regulatory behaviours in a cool temperate grasshopper. *Journal of Thermal Biology* 52:75–83.

Hejcman M, Hejcmanová P, Pavlů V & Beneš J (2013). Origin and history of grasslands in Central Europe – a review. *Grass and Forage Science* 68:345–363.

Hemala V, Svetlík J & Nuhlíčková S (2023). First record of the Common Crevice-cricket *Gryllomorpha dalmatina* (Ocskay, 1832) (Orthoptera: Gryllidae) in Slovakia: evidence of a viable population. *BioInvasions Records* 12:555–562.

Heneberg P, Hesoun P & Skuhrovec J (2016). Succession of arthropods on xerothermophilous habitats formed by sand quarrying: Epigeic beetles (Coleoptera) and orthopteroids (Orthoptera, Dermaptera and Blattodea). *Ecological Engineering* 95:340–356.

Herrmann DL, Ko AE, Bhatt S, Jannot JE & Juliano SA (2010). Geographic Variation in Size and Oviposition Depths of *Romalea microptera* (Orthoptera: Acrididae) Is Associated With Different Soil Conditions. *Annals of the Entomological Society of America* 103:227–235.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

Hochkirch A, Gärtner A-C & Brandt T (2008). Effects of forest-dune ecotone management on the endangered heath grasshopper, *Chorthippus vagans* (Orthoptera: Acrididae). *Bulletin of Entomological Research* 98:449–456.

Hochkirch A & Papen M (2001). Behaviour-related Microhabitat Utilization in *Chorthippus apricarius* (LINNÉ, 1758) and *Chorthippus biguttulus* (LINNÉ, 1758). *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* 13:343–346.

Hodkinson ID (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews* 80:489.

Hoffmann AA, Chown SL & Clusella-Trullas S (2013). Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology* 27:934–949.

Holuša J & Holuša O (2002). Occurrence of the grasshopper *Stenobothrus eurasius bohemicus* (Caelifera: Acrididae) in the Czech Republic. *Articulata* 17:89–93.

Howard DJ & Harrison RG (1984). Habitat Segregation in Ground Crickets: Experimental Studies of Adult Survival, Reproductive Success, and Oviposition Preference. *Ecology* 65:61–68.

Huang X, Wu H, McNeill MR, Qin X, Ma J, Tu X, Cao G, Wang G, Nong X & Zhang Z (2016). Quantitative analysis of diet structure by real-time PCR, reveals different feeding patterns by two dominant grasshopper species. *Scientific Reports* 6:32166.

Humbert J-Y, Delley S & Arlettaz R (2021). Grassland intensification dramatically impacts grasshoppers: Experimental evidence for direct and indirect effects of fertilisation and irrigation. *Agriculture, Ecosystems & Environment* 314:107412.

Hutchings JA (2021). A Primer of Life Histories: Ecology, Evolution, and Application. Oxford University Press.

Illich I & Zuna-Kratky T (2022). Population dynamics of an alpine grasshopper (Orthoptera) community over 30 years and the effects of climate warming and grazing. *Journal of Insect Conservation* 26:435–451.

Inglis GD, Johnson DL & Goettel MS (1996). Effects of Temperature and Thermoregulation on Mycosis by *Beauveria bassianain* Grasshoppers. *Biological Control* 7:131–139.

Ingrisch S (1978). Laboratory and Field Investigations on the Postembryonic Development of Some Mddle European Bush-crickets (Orthoptera: Tettigoniidlae) With Special Reference to the Influence of Temperature and Moisture. *Zoologischer Anzeiger* 200:309–320.

Ingrisch S (1983). Zum Einfluss der Feuchte auf die Schlupfrate und Entwicklungsdauer der Eier mitteleuropäischer Feldheuschrecken (Orthoptera, Acrididae). *Deutsche Entomologische Zeitschrift* 30:1–15.

Ingrisch S (1984). The influence of environmental factors on dormancy and duration of egg development in Metrioptera roeseli (Orthoptera: Tettigoniidae). *Oecologia* 61:254–258.

Ingrisch S (1986a). The plurennial life cycles of the European Tettigoniidae (Insecta: Orthoptera), 1. The effect of temperature on embryonic develepoment and hatching. *Oecologia* 70:606–616.

Ingrisch S (1986b). The plurennial life cycles of the European Tettigoniidae (Insecta: Orthoptera): 2. The effect of photoperiod on the induction of an initial diapause. *Oecologia* 70:617–623.

Ingrisch S (1986c). The plurennial life cycles of the European Tettigoniidae (Insecta: Orthoptera): 3. The effect of drought and the variable duration of the initial diapause. *Oecologia* 70:624–630.

Ingrisch S & Köhler G (1998). Die Heuschrecken mitteleuropas. Die Neue Brehm-Bücherei, Magdeburg.

Isselstein J, Jeangros B & Pavlu V (2005). Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe – A review. *Agronomy Research* 3:139–151.

Jacobs BF, Kingston JD & Jacobs LL (1999). The Origin of Grass-Dominated Ecosystems. *Annals of the Missouri Botanical Garden* 86:590.

Jaenike J (1978). On Optimal Oviposition Behaviour in Phytophagous Insect. *Theoretical Population Biology*:350–356.

Joern A (1982). Vegetation Structure and Microhabitat Selection in Grasshoppers (Orthoptera, Acrididae). *The Southwestern Naturalist* 27:197.

Jonas JL, Wolesensky W & Joern A (2015). Weather Affects Grasshopper Population Dynamics in Continental Grassland Over Annual and Decadal Periods. *Rangeland Ecology & Management* 68:29–39.

Joubert L, Pryke JS & Samways MJ (2016). Positive effects of burning and cattle grazing on grasshopper diversity. *Insect Conservation and Diversity* 9:290–301.

Kaláb O, Pyszko P & Kočárek P (2021). Estimation of the Recent Expansion Rate of Ruspolia nitidula (Orthoptera) on a Regional and Landscape Scale. *Insects* 12:639.

Karjalainen S (2009). Suomen heinäsirkat ja hepokatit. Tammi, Helsinki.

Kenyeres Z, Bauer N & Kisbenedek T (2009). differences in structural changes of orthopteran (Insecta: Orthoptera) assemblages during a vegetation period. *Ekología* 28:22–42.

Keosentse O, Mutamiswa R, Du Plessis H & Nyamukondiwa C (2021). Developmental stage variation in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) low temperature tolerance: implications for overwintering. *Austral Entomology* 60:400–410.

Kingsolver JG & Huey RB (2008). Size, temperature, and fitness: three rules. *Evolutionary Ecology Research*:251–268.

Kistner EJ & Belovsky GE (2017). Abiotic effects on the clear-winged grasshopper (Orthoptera: Acridae) and its fungal pathogen *Entomophaga grylli* (Entomophthorales: Entomophthoraceae) in an intermountain bunch-grass prairie. *The Canadian Entomologist* 149:315–325.

Kočárek P & Holuša J (2006). Recent expansion of bush-cricket Phaneroptera falcata (Orthoptera: Tettigoniidae) in northern Moravia and Silesia (Czech Republic). *Environmental Changes*:207-211.

Kočárek P, Holuša J, Vlk R & Marhoul P (2015). Rovnokřídlí České republiky. Academia, Praha.

Koot EM, Morgan-Richards M & Trewick SA (2022). Climate change and alpine-adapted insects: modelling environmental envelopes of a grasshopper radiation. *Royal Society Open Science* 9:211596.

Kreyling J, Jentsch A & Beier C (2014). Beyond realism in climate change experiments: gradient approaches identify thresholds and tipping points. *Ecology Letters* 17:125.

Krištín A, Čapka J, Zlámal N & Demeš P (2022). First records of the alien bush-crickets *Rhacocleis annulata* Fieber, 1853 and *Yersinella raymondii* (Yersin, 1860) (Orthoptera) in Slovakia. *BioInvasions Records* 11:383–389.

Krištín A, Jarčuška B & Kaňuch P (2020). An annotated checklist of crickets, grasshoppers and their allies (Orthoptera) in Slovakia. *Zootaxa* 4869.

Krištín A, Ka P, Fabriciusová V & Balla M (2009). On distribution and ecology of the bush-cricket *Polysarcus denticauda* (Orthoptera, Tettigoniidae) in Slovakia. *Polish Journal of Entomology* 78:185–191.

Kukalová-Peck J & Brauckmann C (1992). Most Paleozoic Protorthoptera are ancestral hemipteroids: major wing braces as clues to a new phylogeny of Neoptera (Insecta). *Canadian Journal of Zoology* 70:2452–2473.

Kuneš P, Svobodová-Svitavská H, Kolář J, Hajnalová M, Abraham V, Macek M, Tkáč P & Szabó P (2015). The origin of grasslands in the temperate forest zone of east-central Europe: long-term legacy of climate and human impact. *Quaternary Science Reviews* 116:15–27.

Lactin DJ & Johnson DL (1996). Behavioural optimization of body temperature by nymphal grasshoppers (Melanoplus sanguinipes, Orthoptera: Acrididae) in temperature gradients established using incandescent bulbs. *Journal of Thermal Biology* 21:231–238.

Löffler F & Fartmann T (2017). Effects of landscape and habitat quality on Orthoptera assemblages of pre-alpine calcareous grasslands. *Agriculture, Ecosystems & Environment* 248:71–81.

Löffler F, Poniatowski D & Fartmann T (2019). Orthoptera community shifts in response to land-use and climate change – Lessons from a long-term study across different grassland habitats. *Biological Conservation* 236:315–323.

Ma G, Rudolf VHW & Ma C (2015). Extreme temperature events alter demographic rates, relative fitness, and community structure. *Global Change Biology* 21:1794–1808.

Maeno KO, Piou C, Kearney MR, Ould Ely S, Ould Mohamed S, Jaavar MEH & Ould Babah Ebbe MA (2021). A general model of the thermal constraints on the world's most destructive locust, *Schistocerca gregaria*. *Ecological Applications* 31:e02310.

Marini L, Bommarco R, Fontana P & Battisti A (2010). Disentangling effects of habitat diversity and area on orthopteran species with contrasting mobility. *Biological Conservation* 143:2164–2171.

Marini L, Fontana P, Battisti A & Gaston KJ (2009). Response of orthopteran diversity to abandonment of semi-natural meadows. *Agriculture, Ecosystems & Environment* 132:232–236.

Marshall DJ, Pettersen AK & Cameron H (2018). A global synthesis of offspring size variation, its eco-evolutionary causes and consequences. *Functional Ecology*:1436–1446.

McGeoch MA (1998). The selection, testing and application of terrestrial insects as bioindicators. *Biological Reviews* 73:181–201.

Mitchell R (1975). The Evolution of Oviposition Tactics in the Bean Weevil, *Callosobruchus maculatus* (F.). *Ecology* 56:696–702.

Monk KA (1985). Effect of Habitat on the Life History Strategies of Some British Grasshoppers. *The Journal of Animal Ecology* 54:163.

Moore RC & Carpenter FM (1992). Arthropoda 4: Bd. 4: Superclass Hexapoda. Verlag nicht ermittelbar, Ort nicht ermittelbar.

Moriarty F (1970). The significance of water absorption by the developing eggs of five British Acrididae (Saltatoria). *Comparative Biochemistry and Physiology* 34:657–669.

Morse DH (2017). Where should I lay my eggs? Oviposition choices of a shelter-building moth and the shifting danger of being parasitized. *Entomologia Experimentalis et Applicata* 165:1–8.

Nattier R, Robillard T, Amedegnato C, Couloux A, Cruaud C & Desutter-Grandcolas L (2011). Evolution of acoustic communication in the Gomphocerinae (Orthoptera: Caelifera: Acrididae). *Zoologica Scripta* 40:479–497.

Oschmann M (1991). Zur Klassifizierung der ökologischen Ansprüche von Schaben (Blattodea) und Heuschrecken (Orthoptera). *Faunistische Abhandlungen Staatliches Museum für Tierkunde in Dresden* 18:51–57.

Pang B-P, Li N & Zhou X-R (2014). Supercooling Capacity and Cold Hardiness of Band-Winged Grasshopper eggs (Orthoptera: Acrididae). *Journal of Insect Science* 14.

Parmesan C (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics* 37:637–669.

Penone C, Le Viol I, Pellissier V, Julien J, Bas Y & Kerbiriou C (2013). Use of Large-Scale Acoustic Monitoring to Assess Anthropogenic Pressures on Orthoptera Communities. *Conservation Biology* 27:979–987.

Phillips SJ, Anderson RP & Schapire RE (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190:231–259.

Pincebourde S & Woods HA (2020). There is plenty of room at the bottom: microclimates drive insect vulnerability to climate change. *Current Opinion in Insect Science* 41:63–70.

Poniatowski D, Beckmann C, Löffler F, Münsch T, Helbing F, Samways MJ & Fartmann T (2020). Relative impacts of land-use and climate change on grasshopper range shifts have changed over time. *Global Ecology and Biogeography* 29:2190–2202.

Poniatowski D & Fartmann T (2011). Weather-driven changes in population density determine wing dimorphism in a bush-cricket species. *Agriculture, Ecosystems & Environment* 145:5–9.

Poniatowski D, Löffler F, Stuhldreher G, Borchard F, Krämer B & Fartmann T (2016). Functional connectivity as an indicator for patch occupancy in grassland specialists. *Ecological Indicators* 67:735–742.

Powell LR, Berg AA, Johnson DL & Warland JS (2007). Relationships of pest grasshopper populations in Alberta, Canada to soil moisture and climate variables. *Agricultural and Forest Meteorology* 144:73–84.

Prinster AJ, Resasco J & Nufio CR (2020). Weather variation affects the dispersal of grasshoppers beyond their elevational ranges. *Ecology and Evolution* 10:14411–14422.

Rada S, Mazalová M, Šipoš J & Kuras T (2014). Impacts of mowing, grazing and edge effect on Orthoptera of submontane grasslands: Perspectives for biodiversity protection. *Polish Journal of Ecology* 62:123–138.

Rahbek C, Borregaard MK, Antonelli A, Colwell RK, Holt BG, Nogues-Bravo D, Rasmussen CMØ, Richardson K, Rosing MT, Whittaker RJ & Fjeldså J (2019). Building mountain biodiversity: Geological and evolutionary processes. *Science* 365:1114–1119.

Rasnitsyn AP & Quicke DLJ (2002). History of Insects. Kluwer Academic Publishers, Dordrecht.

Refsnider JM & Janzen FJ (2010). Putting Eggs in One Basket: Ecological and Evolutionary Hypotheses for Variation in Oviposition-Site Choice. *Annual Review of Ecology, Evolution, and Systematics* 41:39–57.

Ren J-L, Tu X-B, Ge J, Zhao L & Zhang Z-H (2016). Influence of temperature on the development, reproduction, and life table of *Calliptamus italicus* (L.) (Orthoptera: Acridoidea). *Journal of Asia-Pacific Entomology* 19:203–207.

Ripley EA & Saugier B (1978). Biophysics of a Natural Grassland: Evaporation. *The Journal of Applied Ecology* 15:459.

Schirmel J, Blindow I & Fartmann T (2010). The importance of habitat mosaics for Orthoptera (Caelifera and Ensifera) in dry heathlands. *European Journal of Entomology* 107:129–132.

Schirmel J, Gerlach R & Buhk C (2019). Disentangling the role of management, vegetation structure, and plant quality for Orthoptera in lowland meadows. *Insect Science* 26:366–378.

Schmitt T (2007). Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Frontiers in Zoology* 4:11.

Schnebel EM & Grossfield J (1986). Oviposition Temperature Range in Four *Drosophila* Species Triads from Different Ecological Backgrounds. *American Midland Naturalist* 116:25.

Schouten MA, Verweij PA, Barendregt A, Kleukers RJM & De Ruiter PC (2007). Nested assemblages of Orthoptera species in the Netherlands: the importance of habitat features and life-history traits. *Journal of Biogeography* 34:1938–1946.

Sevastianov N, Neretina T & Vedenina V (2023). Evolution of calling songs in the grasshopper subfamily Gomphocerinae (Orthoptera, Acrididae). *Zoologica Scripta* 52:154–175.

Shu-Guang H & Kang L (2004). Supercooling capacity and cold hardiness of the eggs of the grasshopper *Chorthippus fallax* (Orthoptera: Acrididae). *European Journal of Entomology* 101:231–236.

Sibly R & Monk K (1987). A Theory of Grasshopper Life Cycles. *Oikos* 48:186–194.

Smits NAC, Willems JH & Bobbink R (2008). Long-term after-effects of fertilisation on the restoration of calcareous grasslands. *Applied Vegetation Science* 11:279–286.

Song H, Amédégnato C, Cigliano MM, Desutter-Grandcolas L, Heads SW, Huang Y, Otte D & Whiting MF (2015). 300 million years of diversification: elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. *Cladistics* 31:621–651.

Springate S & Thomas MB (2005). Thermal biology of the meadow grasshopper, *Chorthippus parallelus*, and the implications for resistance to disease. *Ecological Entomology* 30:724–732.

Srygley RB (2021). Elevational and Latitudinal Changes in Cold Tolerance of Nymph and Adult Mormon Crickets *Anabrus simplex* (Orthoptera: Tettigoniidae). *Environmental Entomology* 50:699–705.

Stauffer TW & Whitman DW (2007). Divergent oviposition behaviors in a desert *vs* a marsh grasshopper. *Journal of Orthoptera Research* 16:103–114.

Stoutjesdijk P & Barkman JJ (2014). Microclimate, vegetation and fauna. KNNV Publ, s.l.

Sutcliffe LME, Batáry P, Becker T, Orci KM & Leuschner C (2015). Both local and landscape factors determine plant and Orthoptera diversity in the semi-natural grasslands of Transylvania, Romania. *Biodiversity and Conservation* 24:229–245.

Svenning J-C (2003). Deterministic Plio-Pleistocene extinctions in the European cool-temperate tree flora. *Ecology Letters* 6:646–653.

van Swaay CAM (2002). The importance of calcareous grasslands for butterflies in Europe. *Biological Conservation* 104:315–318.

Theuerkauf J & Rouys S (2006). Do Orthoptera Need Human Land use in Central Europe? The Role of Habitat Patch Size and Linear Corridors in the Białowieża Forest, Poland. *Biodiversity and Conservation* 15:1497–1508.

Torma A, Gallé R & Bozsó M (2014). Effects of habitat and landscape characteristics on the arthropod assemblages (Araneae, Orthoptera, Heteroptera) of sand grassland remnants in Southern Hungary. *Agriculture, Ecosystems & Environment* 196:42–50.

Tropek R, Kadlec T, Hejda M, Kocarek P, Skuhrovec J, Malenovsky I, Vodka S, Spitzer L, Banar P & Konvicka M (2012). Technical reclamations are wasting the conservation potential of post-mining sites. A case study of black coal spoil dumps. *Ecological Engineering* 43:13–18.

Uvarov BP (1922). An interesting new grasshopper from Mount Everest. *Annals and Magazine of Natural History* 9:551–553.

Uvarov B (1966). Grasshoppers and Locusts: A handbook of general acridology. Cambridge University Press, Cambridge.

Van Wingerden WKRE, Musters JCM & Maaskamp FIM (1991). The influence of temperature on the duration of egg development in West European grasshoppers (Orthoptera: Acrididae). *Oecologia* 87:417–423.

Vasudeva R (2023). Experimental evidence for stronger impacts of larval but not adult rearing temperature on female fertility and lifespan in a seed beetle. *Evolutionary Ecology* 37:545–567.

Vlk R (2002). First record of bush-cricket *Phaneroptera nana* (Orthoptera: Tettigoniidae) in the Czech Republic. *Articulata* 17:101–102.

Vlk R, Balvín O, Krištín A, Marhoul P & Hrúz V (2012). Distribution of the Southern Oak Bush-cricket *Meconema meridionale* (Orthoptera, Tettigoniidae) in the Czech Republic and Slovakia. *Folia Oecologica* 39:155–165.

Wallis GP, Waters JM, Upton P & Craw D (2016). Transverse Alpine Speciation Driven by Glaciation. *Trends in Ecology & Evolution* 31:916–926.

Weiss N, Zucchi H & Hochkirch A (2013). The effects of grassland management and aspect on Orthoptera diversity and abundance: site conditions are as important as management. *Biodiversity and Conservation* 22:2167–2178.

Weking S, Kämpf I, Mathar W & Hölzel N (2016). Effects of land use and landscape patterns on Orthoptera communities in the Western Siberian forest steppe. *Biodiversity and Conservation* 25:2341–2359.

Weyer J, Weinberger J & Hochkirch A (2012). Mobility and microhabitat utilization in a flightless wetland grasshopper, *Chorthippus montanus* (Charpentier, 1825). *Journal of Insect Conservation* 16:379–390.

Whitman DW (1986). Developmental Thermal Requirements for the Grasshopper *Taeniopoda eques* (Orthoptera: Aeriididae). *Annals of the Entomological Society of America* 79:711–714.

Whitman DW (1987). Thermoregulation and daily activity patterns in a black desert grasshopper, Taeniopoda eques. *Animal Behaviour* 35:1814–1826.

Whitman DW (2008). The significance of body size in the Orthoptera: a review. *Journal of Orthoptera Research* 17:117–134.

Williams NSG, McDonnell MJ & Seager EJ (2005). Factors influencing the loss of an endangered ecosystem in an urbanising landscape: a case study of native grasslands from Melbourne, Australia. *Landscape and Urban Planning* 71:35–49.

Willmer PG (1982). Microclimate and the Environmental Physiology of Insect. *Advances in Insect Physiology*:1–57.

Willott SJ (1997). Thermoregulation in four species of British grasshoppers (Orthoptera: Acrididae). *Functional Ecology* 11:705–713.

Willott SJ & Hassall M (1998). Life-history responses of British grasshoppers (Orthoptera: Acrididae) to temperature change: Grasshopper life histories. *Functional Ecology* 12:232–241.

Wonglersak R, Fenberg PB, Langdon PG, Brooks SJ & Price BW (2020). Temperature-body size responses in insects: a case study of British Odonata. *Ecological Entomology* 45:795–805.

Wünsch Y, Schirmel J & Fartmann T (2012). Conservation management of coastal dunes for Orthoptera has to consider oviposition and nymphal preferences. *Journal of Insect Conservation* 16:501–510.

Zuna-Kratky T, Landmann A & Illich IP (2017). Die Heuschrecken Österreichs. Oberösterreichisches Landesmuseum Linz, Linz.

Study I

Dvořák, T., Hadrava, J., & Knapp, M. (2022). The ecological niche and conservation value of Central European grassland orthopterans: A quantitative approach. *Biological Conservation*, 265, 109406.

Biological Conservation 265 (2022) 109406

Contents lists available at ScienceDirect

Biological Conservation

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

Full-length article

The ecological niche and conservation value of Central European grassland orthopterans: A quantitative approach

Tomáš Dvořák [a,b,*], Jiří Hadrava [a,c], Michal Knapp [b]

[a] Department of Zoology, Faculty of Science, Charles University, Viničná 7, Prague 2 128 44, Czech Republic
[b] Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Prague - Suchdol 165 00, Czech Republic
[c] Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Branišovská 31, 370 05 České Budějovice, Czech Republic

ARTICLE INFO

Keywords:
Anthropogenic degradation
Endangered species
Habitat management
Microclimate
Microhabitat
Orthoptera

ABSTRACT

The ongoing biodiversity decline across taxonomic groups, including insects, is tightly related to land-use changes and habitat degradation. To assess habitat quality, proper bioindicators that allow efficient monitoring of temporal changes in habitat properties and help to understand the role of anthropogenic activities in shaping habitat characteristics, are needed. Orthopterans have been previously proposed as promising bioindicators for grassland ecosystems; however, comprehensive quantitative information on their habitat preferences is lacking, which hinders full utilization of their bioindication potential. In this study, we surveyed orthopteran assemblages and quantified local environmental conditions at 476 grassland sites in Central Europe. To model orthopteran habitat preferences, the Maxent approach was employed, which allowed us to detect continuous species responses to particular habitat characteristics (moisture, vegetation cover, vegetation height, average seasonal temperature, and direct radiation). We calculated ecological niche indices describing niche widths, species optima, and the relative importance of particular environmental variables for a total of 38 analysed species. Evaluation of final Maxent models showed that their predictive power was high for the great majority of investigated species. Red-listed species had significantly narrower niches compared to unlisted orthopterans. In addition, application of the developed quantitative ecological niche indices for ecological and conservation studies was demonstrated on an independent, previously published dataset. The development of quantitative ecological niche indices coupled with the existing time- and cost-effective survey methods, also allowing adoption of citizen science approaches, makes orthopterans an optimal model insect group for assessment of grassland condition and conservation.

1. Introduction

Recent studies have revealed an ongoing decrease in abundance for a wide range of insect species across the globe (Hochkirch, 2016; Hallmann et al., 2017; Goulson, 2019) and local extinctions have been reported even for previously common species in recent decades (Van Dyck et al., 2009). Well-documented drivers of insect decline are habitat loss and fragmentation (land-use changes resulting from increasing urbanisation, agriculture, and forestry), agricultural and forestry intensification, spread of invasive species, and climate change (Habel et al., 2019). Negative impacts often result from synergistic effects of several factors, among which, however, habitat loss and degradation nearly always play an essential role (Chisté et al., 2016; Habel and Schmitt, 2018; Habel et al., 2019). Thus, efficient ways to conserve biodiversity depend on habitat maintenance, creation, and restoration (Lundholm and Richardson, 2010; Perring et al., 2015). This requires rapid and reliable habitat quality assessments, using efficient tools, such as well-established bioindicator groups (Gerlach et al., 2013).

Orthopterans have been proposed as suitable ecological and environmental bioindicators in grassland ecosystems (McGeoch, 1998; Gerlach et al., 2013). Their large body size and conspicuous behaviour (Narisu et al., 1999) makes them easy to record and most individuals can be identified to the species level during field surveys (Gardiner et al., 2005; Kočárek et al., 2015). Furthermore, most orthopterans have species-specific stridulation sounds (Riede, 1998; Roca and Proulx, 2016), which can be recorded by amateurs or using automatic recording devices. Stridulation records can be later identified by specialists, providing a huge potential of orthopterans for citizen science projects

* Corresponding author at: Department of Zoology, Faculty of Science, Charles University, Viničná 7, Prague 2 128 44, Czech Republic.
E-mail address: dvorato1@natur.cuni.cz (T. Dvořák).

https://doi.org/10.1016/j.biocon.2021.109406
Received 1 August 2021; Received in revised form 1 November 2021; Accepted 17 November 2021
Available online 3 December 2021
0006-3207/© 2021 Elsevier Ltd. All rights reserved.

(Penone et al., 2013). In addition, many orthopteran species have narrow habitat requirements and high degrees of philopatry (Ingrisch and Köhler, 1998), making orthopterans better bioindicators compared to more mobile and less specialised insect groups (Talaśová et al., 2018). The species composition of orthopteran assemblages is thought to be affected mainly by local vegetation structure and microclimate (Ingrisch and Köhler, 1998; Weiss et al., 2013; Humbert et al., 2021), which allows efficient investigation of how local habitat quality is affected by grassland management, succession, or even ongoing climate change (Marini et al., 2009; Fartmann et al., 2012; Fonderflick et al., 2014; Rada et al., 2014; Loeffler et al., 2019).

Temperate grasslands are one of the largest biomes on the Earth and host rich biodiversity across a wide range of taxa (White et al., 2000; Habel et al., 2013). Since the Neolithic period, the maintenance of a large portion of grasslands has depended on human activities such as grazing by domestic animals, mowing, and anthropogenic fires, which complement natural processes such as wildfires and grazing by wild animals, and help grasslands to expand at the expense of forests (Vera, 2000; Kuneš et al., 2015). However, in the last century, natural and semi-natural temperate grasslands have been under strong pressure worldwide (Henwood, 2010; Carbutt et al., 2017; Aguilera et al., 2020). Intensification of anthropogenic activities, such as grassland transformation into arable fields and human settlements (Isselstein et al., 2005; Williams et al., 2005), intensive grazing, mowing and fertilisation (Bilotta et al., 2007; Smits et al., 2008; Cizek et al., 2012), as well as the abandonment of traditional farming systems and ongoing climate change (Grime, 2000; van Swaay, 2002), have resulted in grassland degradation globally. Many grassland specialist species are nowadays limited to artificial habitats such as quarries, post-mining sites, and brownfields, where xeric and sparse grasslands mimic original high-quality habitats (Box, 1999; Tropek et al., 2012; Bogusch et al., 2016). Therefore, conservation efforts aiming to boost grassland biodiversity have become common, including extensive grazing by ungulates (Lagendijk et al., 2019; Chodkiewicz, 2020). However, long-term conservation of grassland ecosystems can only be ensured by efficient monitoring of their quality which, in turn, requires proper bioindicators (McGeoch, 1998; Gerlach et al., 2013).

Despite the potential of orthopterans as grassland bioindicators, studies aiming to exactly quantify the habitat requirements of this group are unfortunately lacking. Previous studies have mainly used multivariate analyses or linear models to reveal key habitat features driving orthopteran assemblages or the response of particular species to management actions (e.g., Bazelet and Samways, 2011; Essl and Dirnböck, 2012; Buri et al., 2013; Fonderflick et al., 2014; Kenyeres et al., 2014; Chisté et al., 2016; Weking et al., 2016). However, the computation of environmental optima, niche width, and disentangling the contribution and importance of the often correlated environmental variables is missing. Moreover, detailed knowledge on autecology of particular orthopteran species is often lacking, and thus previous studies have frequently only employed approximate ecological classifications, e.g., classifying species as habitat specialists versus generalists (Fartmann et al., 2012; Sutcliffe et al., 2015; Kenyeres et al., 2020). Such a reductionist approach inevitably leads to low accuracy outcomes (Clavel et al., 2011; Smith and Smith, 2015). All the above-mentioned knowledge gaps hinder the full use of orthopterans as efficient bioindicators.

This study aims to comprehensively quantify the habitat requirements and conservation value of a wide range of orthopteran species inhabiting various grassland habitats in Central Europe. To do this, we employ the Maxent approach (Phillips et al., 2006; Elith et al., 2011) that allows direct evaluation of addressed issues by calculating exact response curves for the environmental variables investigated. In particular, we aim to: 1) evaluate the performance of Maxent models for all investigated species; 2) disentangle the relative importance of environmental variables potentially affecting orthopteran assemblages; 3) create a set of ecological niche indices ready to use in future studies; 4) give an example of the indices' use by re-analysing data from Fartmann et al. (2012); and 5) test the relationship between threat status and computed niche widths for all investigated orthopteran species.

2. Materials and methods

2.1. Study sites

Field data sampling was performed from July to early September of 2019 and 2020 at 476 grassland sites across the Czech Republic. Orthopterans live relatively long as adult, and thus mature individuals of the vast majority of species occurred throughout the whole sampling period, which allowed us to limit sampling to a single visit per site. The distribution of our study sites is shown in Fig. 1 (see Supplementary Table S1 for a full list of study sites and relevant environmental data). The climatic conditions of our focal sites are predominantly oceanic (Cfb) or humid continental (Dfb), with some sites being subarctic (Dfc) and polar (ET) in the highest mountains according to the Köppen-Geiger classification (Beck et al., 2018). The elevation of the study sites varies from 170 m to 1600 m a. s. l. The primary aim was to cover grassland-like sites across all available environmental conditions occurring in the Czech Republic, including both natural and human-made habitats. Increased focus was set on xerothermic habitats, which have the richest orthopteran fauna with many highly specialised and endangered species. The sites chosen are a good representation of habitats available in the Czech Republic, but additional effort should definitely be given to alpine habitats, which do not occur in the Czech Republic (e.g., High Tatras or Northern Alps).

2.2. Orthopteran survey

Surveys were performed in warm and at least partly sunny days between 8:00 and 19:00. A standardised transect walk (10 min) was performed within each sampling site. Orthoptera sampling was restricted to an area with relatively homogenous environmental conditions (especially moisture, vegetation characteristics, and slope). To avoid edge effects, we placed transects at a minimum distance of 10 m from other habitats. During the transect walk, the observer slowly walked and constantly swept the grass and herbaceous vegetation. Simultaneously, stridulating males and jumping orthopterans were identified to the species level if possible. After the transect walk, swept individuals were immediately identified and counted. We followed the nomenclature by Cigliano et al. (2021).To avoid observer bias, all surveys were performed by a single observer (TD). The combination of multiple sampling methods enabled us to detect the widest possible range of species, as also previously shown for other arthropod taxa (Štrobl et al., 2019; Knapp et al., 2020). Due to the high diversity of investigated habitat types, species-specific detectability of orthopterans, and the use of multiple sampling methods (Riede, 1998; Gardiner et al., 2005), we decided to quantify abundance of particular species using only a rough classification consisting of three categories: 1) rare (3–10 specimens); 2) common (11–50 specimens); and 3) abundant (>ca. 50 specimens). Species present as singletons and doubletons were omitted from our dataset as they could represent only migrating individuals or marginal local populations. Only species which were recorded in at least six investigated sites were employed in the final analyses.

2.3. Environmental variables

After each transect walk, a backward walk following the same trajectory was performed, and environmental variables were recorded for the sampled area. The following environmental variables were estimated: 1) Vegetation cover – visually estimated coverage of alive and standing vegetation with the accuracy to 10%; 2) vegetation height – average height of the dominant vegetation layer measured with a tape measure, where the dominant layer is defined as the highest layer forming at least 75% of vegetation cover (estimated by side view against

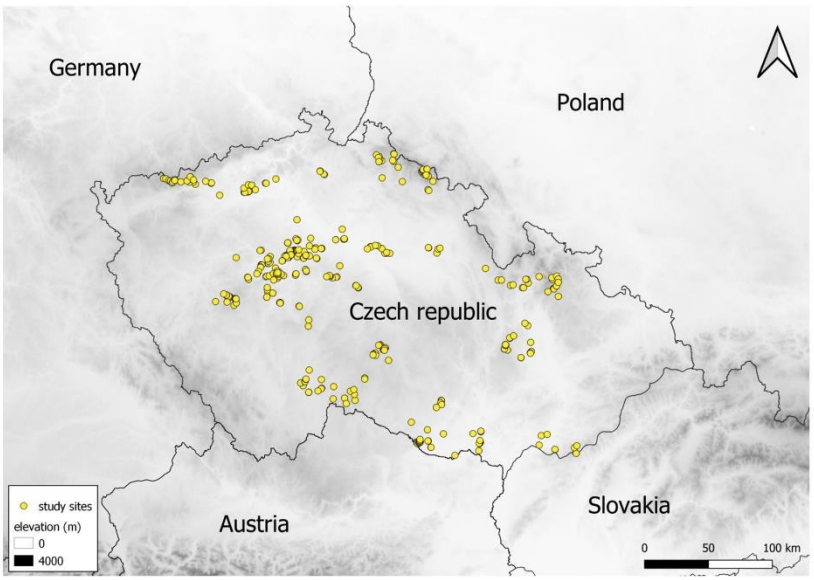


Fig. 1. Location of all the sites investigated in this study.

1 m distant white board). The following categories were used: low (<10 cm), low-medium (11–16 cm), medium (17–25 cm), medium-high (26–35 cm), high (36–60 cm), very high (>60 cm); 3) moisture – derived from Ellenberg's values of dominant plant species occurring in a 3 m wide buffer around the transect walk line (values adjusted for the Czech Republic were employed Chytrý et al., 2018). Plant species having at least 3% coverage were identified as dominant species. Typically, 10–15 plant species per site were used; however, in case of species-poor plant communities (e.g., forest clear-cuts on acidic soils, *Phragmites* or *Typha* dominated wetlands), lower numbers of dominant species were employed. Later, two more variables were additionally computed based on the GPS coordinates of particular sites; 4) direct radiation - as the seasonal sum of direct sun radiation computed using slope, aspect, satellite data on average monthly level of cloudiness (Wilson and Jetz, 2016), and field estimated shadiness of the given site (to 10%). Equations for computing direct radiation were derived from Harmon and Marks (1995); 5) average seasonal temperature – extracted from the Wordclim database (Fick and Hijmans, 2017; delimited to the growth season period, i.e., from March to November).

2.4. Data analysis

We used the software Maxent version 3.4.1 (Phillips et al., 2020) to reveal the relative importance of environmental variables and their optimum, minimum, and maximum values for each analysed species. Linear, quadratic, and hinge features were used for the model computation. Regularisation was set to 0.5, whereas default values were kept for other settings. Species data were converted into presence/absence data for the model. Vegetation height was transformed into a continuous variable using the standard value for each height category (i.e., 7, 13, 21, 30, 45, and 80 cm). For each species, the model run was repeated ten times, with one-tenth records randomly used as test data, and results of all runs were averaged. As background points, all survey sites were used (476 points). For each species, cloglog (complementary log-log) transformed presence probability curves were computed for each environmental variable. A cloglog transformation enables a comparison of species with contrasting abundances (Phillips et al., 2017). For niche width estimation, the 0.3 presence probability threshold was chosen, as this level is most consistent with our field data. A lower probability level will lead to unrealistically wide estimates of species niches. The optimum was chosen as the value which splits the area under the probability curve (and above the 0.3 probability threshold) in half. The relative importance of environmental variables was computed from a Jackknife of regularised training gain as:

$$I = \frac{G_i}{G_a} + \left(1 - \frac{G_{non-i}}{G_a}\right)$$

where I is the relative importance of a given variable, G_i is the model training gain with the given variable only, G_a is the training gain of full model with all variables, and $G_{non\,i}$ is the training gain without the given variable. The resulting relative importance (I) ranges from 0 to 2, where a value of 0 indicates that the given variable does not bring any useful information to the model. Conversely, a value of 2 indicates that the given variable alone fully contributes to the Maxent model. This approach can help to discriminate the net effects of correlated variables (Phillips et al., 2006; Wollan et al., 2008). Correlations between species' optima for particular environmental variables and their relative importance were computed and tested by a two-sided Pearson correlation test. The suitability of individual species for indication for a given environmental variable was measured as the niche width for a given variable related to the maximal available variable's range. The performance of particular models was evaluated by test AUC (Pearce and Ferrier, 2000; Wollan et al., 2008) and true skill statistics (Allouche et al., 2006).

To investigate the relationship between niche width and threat status of orthopteran species, the Spearman rank correlation coefficient was computed. When a species was not mentioned in the Red-list of the Czech Republic, we assigned a value of 0, near threatened species had a value of 1, endangered 2, and critically endangered 3.

2.5. Re-analysis of the existing dataset (Fartmann et al., 2012)

Finally, to demonstrate indices usefulness, we re-analysed the data available in the Appendix of Fartmann et al. (2012). In the original article, the authors analysed orthopterans species composition in five successional stages of steppe and examined the influence of the succession stage on specialist and generalist ratio. They also perform NMDS analysis to identify habitat properties driving orthopteran assemblages. Vegetation cover and height were shown to be the main drivers of community composition (they are also highly correlated). In addition, significant effect of solar radiation (sunshine duration and slope combination) was found in the original study. Here, we use computed niche widths for each environmental variable investigated in our study, instead of arbitrary discrete division to specialist and generalist, to analyse orthopteran community responses through steppe succession. Additionally, our indices also allow computing community-wide averages of optima for orthopterans occupying different steppe succession stages and evaluate relative importance (I) of investigated variables, and therefore bring more comprehensive information on orthopterans responses to steppe succession.

3. Results

3.1. Field data

In total, 50 orthopteran species were recorded in 442 out of the 476 visited grassland sites. This represents 51.5% of orthopteran species occurring in the Czech Republic (currently 97 species). However, if we consider only grassland inhabiting species and omit species which are close to extinction, our dataset covers about 80% of available species. Thirty-four sites without any orthopteran records were mostly heavily shaded grasslands within forests, had negligible vegetation cover, or represented overflooded shores of waterbodies. In unshaded grasslands with significant vegetation cover and non-extreme moisture, at least some orthopteran species were recorded in all cases. The average number of species recorded per site was 4.3 (excluding sites without any orthopteran species), while the maximum number of species recorded per site was nine. Detailed information on occurrence frequencies and abundances of recorded species are shown in Fig. 2. For 38 orthopteran species, sufficient data was collected to model their environmental requirements using Maxent software.

3.2. Predictive power of models

According to the Maxent test AUC, for seven orthopteran species the models achieved excellent performance (AUC > 0.9), for 29 species the models performed moderately well (AUC between 0.7 and 0.9), and only for two species did the models performed quite poorly (AUC < 0.7). True skill statistic (TSS) determined model performance for 2 species as excellent (TSS > 0.75), for 26 species as good (TSS between 0.4 and 0.75), and for ten species as poor (TSS < 0.4). In general, both performance metrics are well correlated ($\rho = 0.62$, $p < 0.0001$), but with great discrepancy in the two species *Stenobothrus eurasius* and *Stenobothrus stigmaticus*, where test AUC is high and TSS low. The full list of AUC, TSS, and species specificity values related to given environmental variables is available in Table 1.

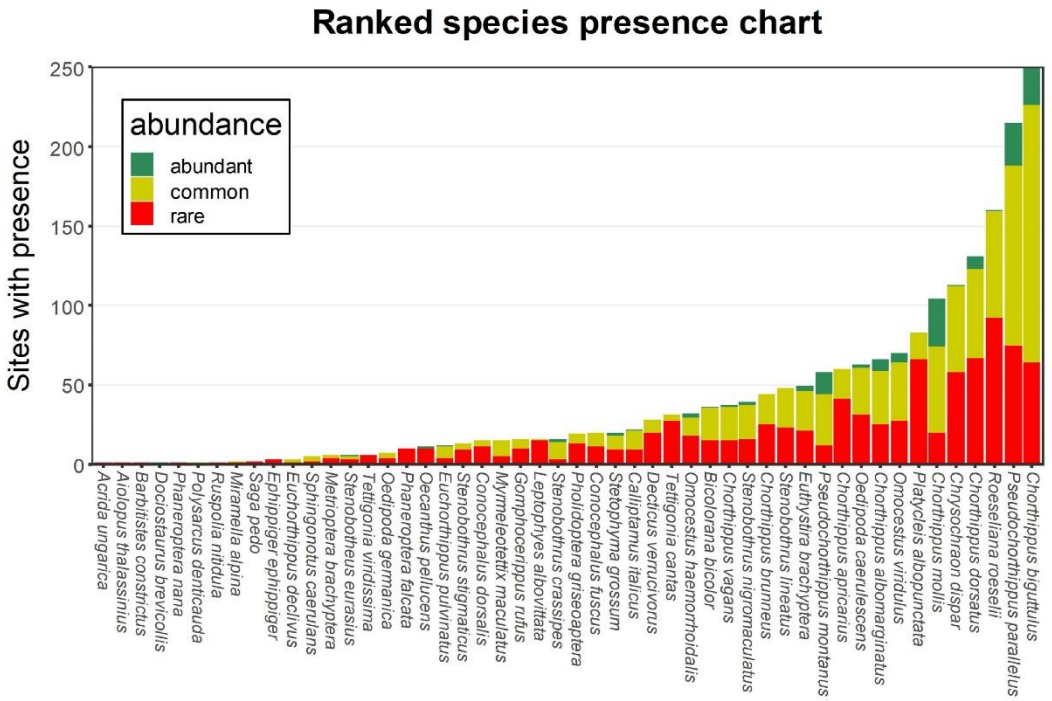


Fig. 2. Frequency distribution of species occurrences across all sites investigated in this study. Particular abundance categories defined in methods are displayed in different colours: abundant = green; common = yellow; rare = red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Summary of model performance (AUC and TSS) and niche width for all analysed species. For each environmental variable the occupied portion of potential available niche is shown (MOIST = moisture, COVER = vegetation cover, HEIGHT = vegetation height, RAD = direct radiation, TEMP = average seasonal temperature). Lower values indicate higher specificity for a given variable. The following abbreviations are used for red-listed species categories (status): CR = critically endangered; EN = endangered; and NT = near threatened.

Species	Status	AUC	TSS	MOIST	COVER	HEIGHT	RAD	TEMP
Bicolorana bicolor		0.89	0.64	0.18	0.33	0.32	0.44	0.27
Calliptamus italicus	NT	0.90	0.56	0.14	0.64	0.24	0.31	0.23
Conocephalus dorsalis		0.96	0.55	0.28	0.04	0.66	0.44	0.37
Conocephalus fuscus		0.93	0.80	0.60	0.26	0.65	0.27	0.27
Decticus verrucivorus		0.87	0.60	0.36	0.41	0.53	0.58	0.53
Euchorthippus pulvinatus	NT	0.83	0.33	0.26	0.84	0.42	0.33	0.31
Euthystira brachyptera		0.73	0.44	0.72	0.53	0.82	0.64	0.76
Gomphocerippus rufus		0.70	0.29	0.40	0.79	0.65	0.70	0.71
Chorthippus albomarginatus		0.81	0.49	0.46	0.41	0.61	0.31	0.82
Chorthippus apricarius		0.80	0.54	0.34	0.47	0.91	0.42	0.90
Chorthippus biguttulus		0.64	0.32	0.46	0.68	0.51	0.63	0.96
Chorthippus brunneus		0.82	0.61	0.38	0.60	0.44	0.68	1.00
Chorthippus dorsatus		0.73	0.43	0.65	0.41	0.83	0.51	0.59
Chorthippus mollis		0.82	0.61	0.25	0.69	0.33	0.43	0.23
Chorthippus vagans		0.89	0.71	0.26	0.55	0.23	0.69	0.40
Chrysochraon dispar		0.81	0.56	0.54	0.21	0.77	0.53	0.88
Leptophyes albovittata		0.71	0.30	1.00	0.96	1.00	0.63	0.26
Metrioptera brachyptera		0.79	0.29	0.56	0.10	0.94	0.61	0.61
Myrmeleotettix maculatus		0.90	0.52	0.28	0.66	0.11	0.82	0.38
Oecanthus pellucens		0.92	0.59	0.34	0.68	0.71	0.43	0.14
Oedipoda caerulescens		0.88	0.68	0.19	0.61	0.22	0.36	0.25
Oedipoda germanica	CR	0.97	0.55	0.11	0.33	0.13	0.43	0.16
Omocestus haemorrhoidalis		0.85	0.49	0.22	0.48	0.25	0.49	1.00
Omocestus viridulus		0.88	0.70	0.41	0.34	0.66	0.46	0.60
Phaneroptera falcata		0.74	0.04	0.91	0.47	0.81	0.66	0.71
Pholidoptera griseoaptera		0.85	0.45	0.46	0.24	0.78	0.46	1.00
Platycleis albopunctata		0.83	0.62	0.25	0.85	0.36	0.43	0.20
Pseudochorthippus montanus		0.88	0.72	0.48	0.17	0.80	0.40	0.40
Pseudochorthippus parallelus		0.68	0.35	0.54	0.43	0.87	0.53	1.00
Roeseliana roeselii		0.74	0.44	0.61	0.27	0.83	0.57	0.95
Stenobothrus crassipes	NT	0.86	0.58	0.14	0.43	0.29	0.52	0.30
Stenobothrus eurasius	EN	0.96	0.32	0.11	0.40	0.27	0.22	0.22
Stenobothrus lineatus		0.82	0.54	0.24	0.46	0.29	0.53	0.70
Stenobothrus nigromaculatus	NT	0.89	0.61	0.17	0.68	0.22	0.38	0.25
Stenobothrus stigmaticus	NT	0.93	0.37	0.35	0.51	0.22	0.27	0.47
Stetophyma grossum	NT	0.92	0.78	0.34	0.06	0.67	0.48	1.00
Tettigonia cantans		0.86	0.58	0.47	0.18	0.60	0.41	0.56
Tettigonia viridissima		0.70	0.04	0.75	0.28	0.80	0.62	0.74

3.3. Environmental variables and indices

The values of environmental optima, niche width, and relative importance (I) for each environmental variable and for each analysed species are given in Supplementary Table S2, and the occurrence probability curves are shown in Fig. 3. In general, species' optima for particular environmental variables showed quite strong significant intercorrelations, but their relative importance (I) for individual species was only weakly correlated (Table 2). The most important environmental variable determining orthopteran occurrence on grasslands was moisture, with an average index of relative importance $I = 0.70$. For other environmental variables, average indices' values were: $I = 0.50$ for vegetation cover, $I = 0.45$ for vegetation height, $I = 0.40$ for average seasonal temperature, and $I = 0.30$ for direct radiation.

3.4. Species conservation value

Eight out of 38 analysed species are included in the Red List of the Czech Republic (Farkač and Chobot, 2017). In general, red-listed species had narrower niches. A significant correlation between threat status and niche width was observed for moisture ($\rho = -0.56$, $p = 0.0003$), vegetation height ($\rho = -0.48$, $p = 0.0025$), direct radiation ($\rho = -0.46$, $p = 0.0038$), and average seasonal temperature ($\rho = -0.36$, $p = 0.025$), whereas niche width for vegetation cover was unrelated to species' threat status ($\rho = 0.04$, $p = 0.793$).

3.5. Example of using Maxent derived ecological niche indices

Fartmann et al. (2012) showed that the numbers of generalist orthopteran species did not change with the successional age of steppe grasslands, while specialist species declined in late-successional stages. Our analyses showed that the most important environmental variable determining orthopteran assemblages across all stages was moisture (Fig. 4), a variable not directly measured in the original study. The later successional stages were inhabited by more generalist orthopterans with higher moisture optima. Vegetation cover was shown to be the second most important predictor, which is consistent with the original findings of Fartmann et al. (2012). However, species inhabiting early stages were surprisingly more generalist, whereas in late-successional stages, orthopterans preferring denser vegetation were more common. Vegetation height had lower importance, although an overall preference for higher vegetation was apparent toward later succession stages. Direct radiation seemed to be of minor importance. This finding contrasts with the original results of Fartmann et al. (2012), where species inhabiting the intermediate stage were proposed to show preference for higher direct radiation.

It should be noted that the ordination plot published as Fig. 3 in the original study shows a clear preference of *Oedipoda caerulescens* and *Myrmeleotettix maculatus* for early-successional stages with sparse and short vegetation. Our ecological niche indices suggest that *O. caerulescens* is primarily affected by vegetation cover, whereas *M. maculatus* responds mainly to vegetation height. Indeed,

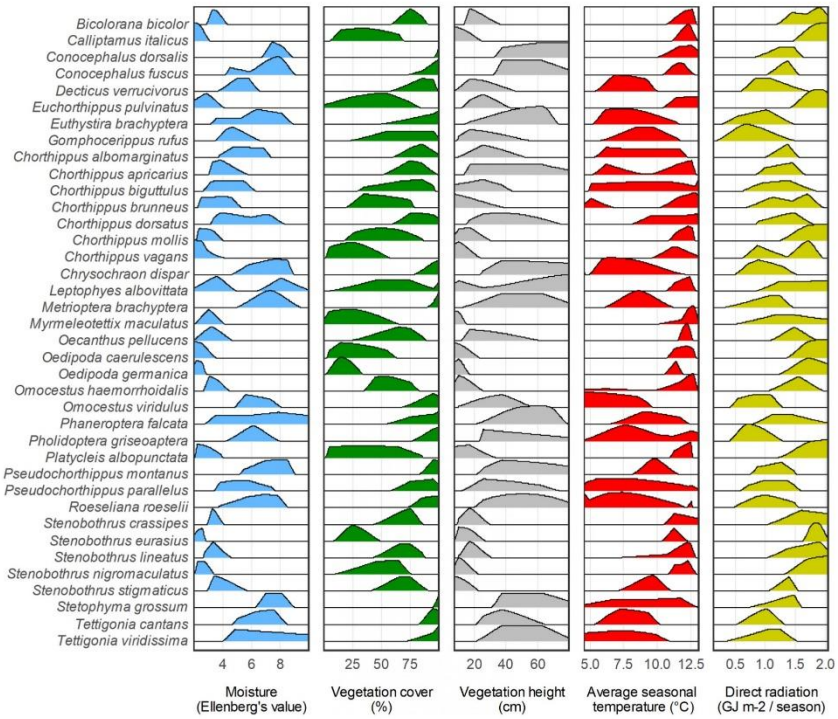


Fig. 3. Presence probability for all analysed species and measured environmental variable combinations. Moisture is shown in blue, vegetation cover in green, average seasonal temperature in red, vegetation height in grey, and direct radiation in yellow. Species are ordered alphabetically. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Correlations between environmental variables. Panel (A) shows pairwise Pearson correlation coefficients between species' optima. Panel (B) shows pairwise Pearson correlation coefficients between relative importance (I) of investigated variables for particular species. The following abbreviations representing environmental variables were used: MOIST = moisture; COVER = vegetation cover; HEIGHT = vegetation height; RAD = sum of seasonal direct solar radiation; TEMP = average seasonal temperature. Significant correlations are highlighted in bold, whereas asterisks indicate the levels of significance: *** ~ $p < 0.001$; ** ~ $p < 0.01$; * ~ $p < 0.05$.

A)

	COVER	HEIGHT	RAD	TEMP
MOIST	**0.91*** **	**0.89*** **	**−0.77*** **	**−0.68*** **
COVER		**0.80*** **	**−0.73*** **	**−0.67*** **
HEIGHT			**−0.60*** **	**−0.48** **
RAD				**0.85*** **

B)

	COVER	HEIGHT	RAD	TEMP
MOIST	0.22	**−0.35***	0.16	**−0.46** **
COVER		−0.08	−0.16	**−0.47** **
HEIGHT			−0.27	−0.28
RAD				−0.05

O. caerulescens can be found in dry ruderal habitats in Central Europe, where vegetation is sparse, but frequently relatively high.

4. Discussion

This study provides a comprehensive quantitative analysis of habitat preference of 38 Central European orthopteran species. Bioindication value of particular species was introduced with respect to five environmental variables (moisture, vegetation cover, vegetation height, direct radiation, and average seasonal temperature). The Maxent approach proved to be a very useful tool to determine the bioindication potential of particular species, as it produces presence probability curves, enabling a direct computation of species optima and niche width, which provide more accurate information than previous studies distinguishing only between good and bad bioindicators (Dufrene and Legendre, 1997; Weking et al., 2016). Computed niche widths correlate well with species Red List status. The application potential of quantitative ecological niche indices was illustrated by re-analysing a previously published dataset.

Microclimate and vegetation structure have been considered the most important determinants of the distribution of temperate grassland orthopterans (Ingrisch and Köhler, 1998; Poniatowski and Fartmann, 2008; Gardiner and Hassall, 2009; Humbert et al., 2021). Our results indicate that moisture is the most important predictor of orthopteran species occurrence, yet it is practically ignored in previous studies investigating the bioindication potential of orthopterans (e.g., Bazelet and Samways, 2011; Fartmann et al., 2012; Kenyeres et al., 2014).

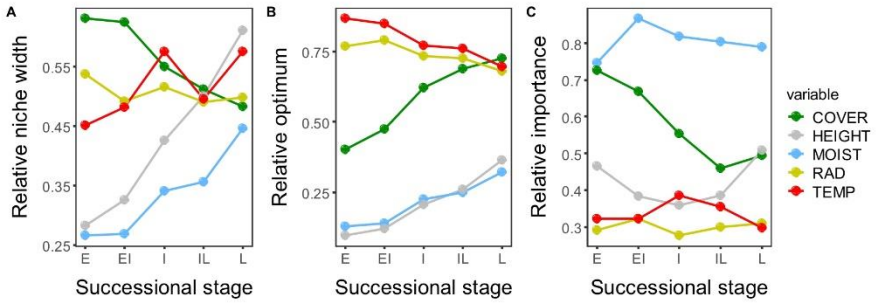


Fig. 4. Visualisation of re-analysed data from Fartmann et al. (2012). Particular panels show: A) average community-wide niche width; B) average community-wide optima; and C) average community-wide relative importance (*I*) of particular environmental variables for orthopteran assemblages occupying different steppe succession stages. Abbreviations of succession stages are as follows: E = early stage; EI = early-intermediate stage; I = intermediate stage; IL = intermediate-late stage; L = late stage. The following abbreviations for environmental variables are used: MOIST = moisture (blue); COVER = vegetation cover (green); TEMP = average seasonal temperature (red); HEIGHT = vegetation height (grey); and RAD = direct radiation (yellow). Source data can be found in the Appendix in Fartmann et al. (2012). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Vegetation cover and height followed moisture with respect to their relative importance across orthopteran species. Average seasonal temperature, which is closely related to altitude, was generally of lower importance. It should be noted that this pattern can be partially caused by the methodological limitation of our study. The climate database derived temperature data do not represent precise microclimatic conditions and a future study should complete recently available low-resolution temperature data by directly measured local temperature data. A necessary precondition is that thousands of dataloggers can be purchased at a reasonable price. However, our low-resolution temperature data were sufficient to identify that several investigated species, e.g., *Omocestus viridulus*, clearly preferred higher altitudes, i.e., lower seasonal sum of temperature, while they were generalist in other habitat parameters, even though orthopterans are generally considered to be thermophilic. For such mountain species, high summer temperatures can be a limiting factor (Willott, 1997). Surprisingly, although southern exposition (and thus high radiation) is considered to be important for many xerothermophilous species (Ingrisch and Köhler, 1998; Kočárek et al., 2015), this variable did not contribute much to species' distribution. Low moisture and sparser vegetation, which are often associated with southern exposition (Stoutjesdijk and Barkman, 2014), are of higher importance than exposition itself. It should be noted that, although species' optima of environmental variables were strongly intercorrelated (Table 2A), Maxent Jackknifes of regularised training gain indicated only a limited correlation of variable relative importance (*I*) across species (Table 2B). This suggests that particular orthopteran species responded to different environmental variables, and thus reducing correlated environmental variables into one representing variable, which is a common practice in ecological and conservation studies (Quinn and Keough, 2002), can be counterproductive.

The Maxent approach performed well for most of the analysed species. Orthopteran species for which model evaluation indicated weaker performance typically had wide niches and high prevalence, which is known to lower both AUC and TSS (Allouche et al., 2006). Despite suboptimal model performance evaluation, computed species niches are still reasonable in many cases, for example, *Chorthippus biguttulus*, *Pseudochorthippus parallelus*, and *Chorthippus dorsatus*. Ambiguous model evaluation results for *Stenobothrus eurasius* and *Stenobothrus stigmaticus* are probably caused by a low number of records for these species. They are frequently missing in potentially suitable sites, which results in low specificity that heavily affects TSS value (Allouche et al., 2006); however, due to strict habitat requirements, test AUC is still high (Smith, 2013). Only in few species (particularly *Leptophyes albovittata*, *Tettigonia viridissima*, and *Phaneroptera falcata*) is poor performance caused by a relatively low number of records coupled with wide niches. In these cases, resulting presence probability curves can be unreliable. It should be noted that Maxent only works with presence/absence data and thus does not allow incorporation of population sizes into models. This could decrease model precision; however, we tried to mitigate this issue with a high number of sites included in our study. In addition, if just one or two specimens were recorded per given site, the species was considered not to be regularly occurring there for the purpose of our analyses.

The time- and cost-effective survey method employed in this study enabled us to survey many sites and detect many orthopteran species, but it also has some limitations. A single visit per site performed during a period of peak orthopteran activity can result in underestimated distribution of some species. We probably missed species with early phenology, e.g., European field cricket *Gryllus campestris*, or katydids from the genera *Isophya* and *Poecilimon* (Kočárek et al., 2015). Despite combining several sampling methods, several species requiring specific monitoring techniques could be overlooked, such as small and non-stridulating geochortobionts like *Tetrix* spp. To further improve our understanding of orthopteran habitat preferences and accuracy of proposed ecological niche indices, additional sites could be included in the dataset, thus obtaining more records for less common species in particular. In addition, widening the geographical scope of our database beyond the Central Europe, e.g., to the pan-European level, will result in increased species coverage and wider applicability of derived ecological niche indices.

Computing precise species niches allows various ecological issues to be addressed. As an example, we re-examined the effect of steppe succession on orthopteran assemblages published by Fartmann et al. (2012). In the original study, species were arbitrarily divided into specialists and generalists, using a general (unquantified) knowledge of their habitat preferences. Our approach allowed the examination of quantitative specialisation level (represented by niche width) separately for each environmental variable, and we could also assess species' optima and variable importance (*I*). We also showed that Red-listed species have narrower niches than other species. This coincides with the common theory that specialised species are more threatened than generalists (Chichorro et al., 2019). Exceptionally, niche widths for vegetation cover were similar for species whether they were on the Red List or not. This could be caused by common species inhabiting managed meadows and pastures, where dense vegetation occurs and thus they seem to be dense vegetation specialist. In fact, the five most common species in our dataset can be typically found in conventional meadows, pastures and ruderalized road verges, but are almost absent in habitats with sparser vegetation (Ingrisch and Köhler, 1998). These dense vegetation

specialist species thus can work as bioindicators, and their absence rather than presence can indicate high conservation value habitats.

The ecological niche indices developed in this study can be a promising tool for evaluating various conservation measures. For example, rewilding, i.e., reintroduction of native large grazers to simulate the natural processes that maintain grassland vegetation, has become very popular recently (Fuhlendorf et al., 2018; Garrido et al., 2019). As orthopterans strongly respond to grazing regimes (Fonderflick et al., 2014; Gardiner, 2018) and grazing in general undoubtedly heavily affects vegetation structure and local microclimate, we suppose that they would be optimal bioindicators to follow changes caused by rewilding. Orthopterans have also shown their bioindication potential in studies investigating the conservation value of human-modified habitats such as quarries or urban grasslands (Tropek et al., 2012; Chisté et al., 2016). Only three out of 38 species analysed in the present study do not stridulate. Thus, Central European orthopterans are highly suitable for acoustic monitoring or even employment of automatic recording using various devices not requiring human presence (Roca and Proulx, 2016; McNeil and Grozinger, 2020). This allows employing orthopterans in rapid large-scale biodiversity screenings and extensive citizen science projects. For example, Penone et al. (2013) investigated the potential use of slow-moving vehicles equipped with a recording device to screen orthopteran assemblages around roads. The combination of accessible data collection, fine-scale distribution patterns, and the use of quantitative ecological niche indices developed in this study make orthopterans a highly suitable model group for a wide range of conservation studies.

5. Conclusions

The ongoing biodiversity decline and increasing anthropogenic pressure on grassland habitats across the globe require the development of appropriate bioindicators allowing a swift and cost-effective assessment of habitat quality and conservation measures effectiveness. In the present study, we deepened existing knowledge on habitat requirements of 38 orthopteran species occurring in Central Europe; for each investigated species we proposed quantitative ecological niche indices related to local moisture, vegetation cover, vegetation height, average seasonal temperature, and direct radiation. Our quantitative bioindication indices can serve as a corner stone for future ecological and conservation studies, as well as practical nature conservation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2021.109406.

CRediT authorship contribution statement

Tomáš Dvořák: Conceptualisation, Methodology, Formal analysis, Investigation, Writing – Original draft, Visualisation. **Jiří Hadrava:** Methodology, Formal analysis, Writing – Original draft. **Michal Knapp:** Review and editing, Formal analysis, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Many thanks go to Jakub Prokop (supervisor of T. Dvořák) for financial support of field works. This study was also supported by the Technology Agency of the Czech Republic (grant number SS02030018 – DivLand). We are grateful to Ezequiel González, Jan Douda, and Tiit Teder for insightful comments on the manuscript draft. Thank you to the editor and three anonymous reviewers for their valuable and constructive suggestions, and to Mark Sixsmith for language edits which improved the final version of the manuscript.

Data availability

The raw data used in the article will be available through Mendeley Data (Dvořák, 2021).

References

Aguilera, G., Roslin, T., Miller, K., Tamburini, G., Birkhofer, K., Caballero-Lopez, B., Lindström, S.A., Öckinger, E., Rundlöf, M., Rusch, A., Smith, H.G., Bommarco, R., 2020. Crop diversity benefits carabid and pollinator communities in landscapes with semi-natural habitats. J. Appl. Ecol. 57, 2170–2179.
Allouche, O., Tsoar, A., Kadmon, R., 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS): assessing the accuracy of distribution models. J. Appl. Ecol. 43, 1223–1232.
Bazelet, C.S., Samways, M.J., 2011. Identifying grasshopper bioindicators for habitat quality assessment of ecological networks. Ecol. Indic. 11, 1259–1269.
Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. Sci. Data 5.
Bilotta, G.S., Brazier, R.E., Haygarth, P.M., 2007. The impacts of grazing animals on the quality of soils, vegetation, and surface waters in intensively managed grasslands. Adv. Agron. 237–280.
Bogusch, P., Macek, J., Janšta, P., Kubík, Š., Řezáč, M., Holý, K., Malenovský, I., Bañař, P., Mikát, M., Astapenková, A., Heneberg, P., 2016. Industrial and post-industrial habitats serve as critical refugia for pioneer species of newly identified arthropod assemblages associated with reed galls. Biodivers. Conserv. 25, 827–863.
Box, J., 1999. Nature conservation and post-industrial landscapes. Ind. Archaeol. Rev. 21, 137–146.
Buri, P., Arlettaz, R., Humbert, J. Y., 2013. Delaying mowing and leaving uncut refuges boosts orthopterans in extensively managed meadows: evidence drawn from field-scale experimentation. Agric. Ecosyst. Environ. 181, 22–30.
Carbutt, C., Henwood, W.D., Gilfedder, L.A., 2017. Global plight of native temperate grasslands: going, going, gone? Biodivers. Conserv. 26, 2911–2932.
Chichorro, F., Juslén, A., Cardoso, P., 2019. A review of the relation between species traits and extinction risk. Biol. Conserv. 237, 220–229.
Chisté, M.N., Mody, K., Gossner, M.M., Simons, N.K., Köhler, G., Weisser, W.W., Blüthgen, N., 2016. Losers, winners, and opportunists: how grassland land-use intensity affects orthopteran communities. Ecosphere 7, 1–15.
Chodkiewicz, A., 2020. Advantages and disadvantages of polish primitive horse grazing on valuable nature areas – a review. Glob. Ecol. Conserv. 21, 1–11.
Chytrý, M., Tichý, L., Dřevojan, P., Sádlo, J., Zelený, D., et al., 2018. Ellenberg-type indicator values for the Czech flora. Preslia 90, 83–103.
Cigliano, M.M., Braun, H., Eades, D.C., Otte, D., 2021. Orthoptera species file. http://orthoptera.speciesfile.org/HomePage/Orthoptera/HomePage.aspx.
Cizek, O., Zamecnik, J., Tropek, R., Kocarek, P., Konvicka, M., 2012. Diversification of mowing regime increases arthropods diversity in species-poor cultural hay meadows. J. Insect Conserv. 16, 215–226.
Clavel, J., Julliard, R., Devictor, V., 2011. Worldwide decline of specialist species: toward a global functional homogenization? Front. Ecol. Environ. 9, 222–228.
Dufrene, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67, 345–366.
Dvořák, T., 2021. The ecological niche and conservation value of Central European grassland orthopterans: A quantitative approach. Mendeley Data V1. https://doi.org/10.17632/rxsgcnx547.1.
Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E., Yates, C.J., 2011. A statistical explanation of MaxEnt for ecologists: statistical explanation of MaxEnt. Divers. Distrib. 17, 43–57.
Essl, F., Dirnböck, T., 2012. What determines orthoptera species distribution and richness in temperate semi natural dry grassland remnants? Biodivers. Conserv. 21, 2525–2537.
Farkač, J., Chobot, K., 2017. Red list of threatened species of the Czech Republic. Příroda 36, 1–611.
Fartmann, T., Krämer, B., Stelzner, F., Poniatowski, D., 2012. Orthoptera as ecological indicators for succession in steppe grassland. Ecol. Indic. 20, 337–344.
Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37, 4302–4315.
Fonderflick, J., Besnard, A., Beuret, A., Dalmais, M., Schatz, B., 2014. The impact of grazing management on orthoptera abundance varies over the season in Mediterranean steppe like grassland. Acta Oecol. 60, 7–16.
Fuhlendorf, S.D., Davis, C.A., Elmore, R.D., Goodman, L.E., Hamilton, R.G., 2018. Perspectives on grassland conservation efforts: should we rewild to the past or conserve for the future? Philos. Trans. R. Soc., B 373, 1–10.
Gardiner, T., 2018. Orthoptera response to grazing. J. Orthop. Res. 27, 3–11.
Gardiner, T., Hassall, M., 2009. Does microclimate affect grasshopper populations after cutting of hay in improved grassland? J. Insect Conserv. 13, 97–102.
Gardiner, T., Hill, J., Chesmore, D., 2005. Review of the methods frequently used to estimate the abundance of orthoptera in grassland ecosystems. J. Insect Conserv. 9, 151–173.
Garrido, P., Mårell, A., Öckinger, E., Skarin, A., Jansson, A., Thulin, C., 2019. Experimental rewilding enhances grassland functional composition and pollinator habitat use. J. Appl. Ecol. 56, 946–955.

Gerlach, J., Samways, M., Pryke, J., 2013. Terrestrial invertebrates as bioindicators: an overview of available taxonomic groups. J. Insect Conserv. 17, 831–850.
Goulson, D., 2019. The insect apocalypse, and why it matters. Curr. Biol. 29, R967–R971.
Grime, J.P., 2000. The response of two contrasting limestone grasslands to simulated climate change. Science 289, 762–765.
Habel, J.C., Dengler, J., Janišová, M., Török, P., Wellstein, C., Wiezik, M., 2013. European grassland ecosystems: threatened hotspots of biodiversity. Biodivers. Conserv. 22, 2131–2138.
Habel, J.C., Samways, M.J., Schmitt, T., 2019. Mitigating the precipitous decline of terrestrial european insects: requirements for a new strategy. Biodivers. Conserv. 28, 1343–1360.
Habel, J.C., Schmitt, T., 2018. Vanishing of the common species: empty habitats and the role of genetic diversity. Biol. Conserv. 218, 211–216.
Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., de Kroon, H., 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLOS ONE 12.
Harmon, M.E., Marks, B.J., 1995. Programs to Estimate the Solar Radiation for Ecosystem Models.
Henwood, W.D., 2010. Toward a strategy for the conservation and protection of the world's temperate grassland. Great Plains Res. 20, 121–134.
Hochkirch, A., 2016. The insect crisis we can't ignore. Nature 539, 141–141.
Humbert, J. Y., Delley, S., Arlettaz, R., 2021. Grassland intensification dramatically impacts grasshoppers: experimental evidence for direct and indirect effects of fertilisation and irrigation. Agric. Ecosyst. Environ. 314, 107412.
Ingrisch, S., Köhler, G., 1998. Die Heuschrecken mitteleuropas. Die Neue Brehm Bücherei, Magdeburg.
Isselstein, J., Jeangros, B., Pavlu, V., 2005. Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe – a review. Agron. Res. 3, 139–151.
Kenyeres, Z., Bauer, N., Rácz, I.A., 2014. Local and global factors in organization of central-european orthopteran assemblages. Russ. J. Ecol. 45, 375–383.
Kenyeres, Z., Szabo, S., Gábor, T., Szinetár, C., 2020. Orthoptera assemblages as indicators for the restoration of sand grassland networks. Nort-Western J. Zool. 16, 7–14.
Knapp, M., Knappová, J., Jakubec, P., Vonička, P., Moravec, P., 2020. Incomplete species lists produced by pitfall trapping: how many carabid species and which functional traits are missing? Biol. Conserv. 245, 108545.
Kočárek, P., Holuša, J., Vlk, R., Marhoul, P., 2015. Rovnokřídlí České republiky. Academia, Praha.
Kuneš, P., Svobodová-Svitavská, H., Kolář, J., Hajnalová, M., Abraham, V., Macek, M., Tkáč, P., Szabó, P., 2015. The origin of grasslands in the temperate forest zone of east-Central Europe: long-term legacy of climate and human impact. Quat. Sci. Rev. 116, 15–27.
Lagendijk, D.D.G., Howison, R.A., Esselink, P., Smit, C., 2019. Grazing as a conservation management tool: responses of voles to grazer species and densities. Basic Appl. Ecol. 34, 36–45.
Loeffler, F., Poniatowski, D., Fartmann, T., 2019. Orthoptera community shifts in response to land-use and climate change - lessons from a long-term study across different grassland habitats. Biol. Conserv. 236, 315–323.
Lundholm, J.T., Richardson, P.J., 2010. MINI REVIEW: habitat analogues for reconciliation ecology in urban and industrial environments: habitat analogues. J. Appl. Ecol. 47, 966–975.
Marini, L., Fontana, P., Battisti, A., Gaston, K.J., 2009. Response of orthopteran diversity to abandonment of semi-natural meadows. Agric. Ecosyst. Environ. 132, 232–236.
McGeoch, M.A., 1998. The selection, testing and application of terrestrial insects as bioindicators. Biol. Rev. 73, 181–201.
McNeil, D.J., Grozinger, C.M., 2020. Singing in the suburbs: point count surveys efficiently reveal habitat associations for nocturnal orthoptera across an urban to-rural gradient. J. Insect Conserv. 24, 1031–1043.
Narisu, Lockwood J.A., Schell, S.P., 1999. A novel mark-recapture technique and its application to monitoring the direction and distance of local movements of rangeland grasshoppers (Orthoptera: Acrididae) in the context of pest management. J. Appl. Ecol. 36, 604–617.
Pearce, J., Ferrier, S., 2000. Evaluating the predictive performance of habitat models developed using logistic regression. Ecol. Model. 133, 225–245.
Penone, C., Le Viol, I., Pellissier, V., Julien, J., Bas, Y., Kerbiriou, Ch., 2013. Use of large-scale acoustic monitoring to assess anthropogenic pressures on orthoptera communities: insect monitoring with sound detection. Conserv. Biol. 27, 979–987.
Perring, M.P., Standish, R.J., Price, J.N., Craig, M.D., Erickson, T.E., Ruthrof, K.X., Whiteley, A.S., Valentine, L.E., Hobbs, R.J., 2015. Advances in restoration ecology: rising to the challenges of the coming decades. Ecosphere 6, 1–25.
Phillips, S.J., Anderson, R., Dudík, M., Schapire, R.E., Blair, M.E., et al., 2017. Opening the black box: an open-source release of Maxent. Ecography 40, 887–893.
Phillips, S.J, Dudík, M., Schapire, R.E., 2020. Maxent software for modeling species niches and distribution (Version 3.4.1.). . (Accessed 10 April 2020). Available from: http://biodiversityinformatics.amnh.org/open source/maxent/.
Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190, 231–259.
Poniatowski, D., Fartmann, T., 2008. The classification of insect communities: lessons from orthopteran assemblages of semi-dry calcareous grasslands in Central Germany. Eur. J. Entomol. 105, 659–671.
Quinn, G.P., Keough, M.J., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, New York.
Rada, S., Mazalová, M., Šipoš, J., Kuras, T., 2014. Impacts of mowing, grazing and edge effect on orthoptera of submontane grasslands: perspectives for biodiversity protection. Pol. J. Ecol. 62, 123–138.
Riede, K., 1998. Acoustic monitoring of orthoptera and its potential for conservation. J. Insect Conserv. 2, 217–223.
Roca, I.T., Proulx, R., 2016. Acoustic assessment of species richness and assembly rules in ensiferan communities from temperate ecosystems. Ecology 97, 116–123.
Smith, A.B., 2013. On evaluating species distribution models with random background sites in place of absences when test presences disproportionately sample suitable habitat. Divers. Distrib. 19, 867–872.
Smith, T.M., Smith, R.L., 2015. Elements of Ecology. Pearson Higher Education.
Smits, N.A.C., Willems, J.H., Bobbink, R., 2008. Long-term after-effects of fertilisation on the restoration of calcareous grasslands. Appl. Veg. Sci. 11, 279–286.
Stoutjesdijk, P., Barkman, J.J., 2014. Microclimate, Vegetation and Fauna. KNNV Publ, s. l.
Štrobl, M., Saska, P., Seidl, M., Kocian, M., Tajovský, K., Řezáč, M., Skuhrovec, J., Marhoul, P., Zbuzek, B., Jakubec, P., Knapp, M., Kadlec, T., 2019. Impact of an invasive tree on arthropod assemblages in woodlots isolated within an intensive agricultural landscape. Divers. Distrib. 25, 1800–1813.
Sutcliffe, L.M.E., Batáry, P., Becker, T., Orci, K.M., Leuschner, Ch., 2015. Both local and landscape factors determine plant and orthoptera diversity in the semi-natural grasslands of Transylvania, Romania. Biodivers. Conserv. 24, 229–245.
van Swaay, C.A.M., 2002. The importance of calcareous grasslands for butterflies in Europe. Biol. Conserv. 104, 315–318.
Talašová, A., Straka, J., Hadrava, J., Benda, D., Kocourek, F., Kazda, J., 2018. High degree of philopatry is required for mobile insects used as local indicators in biodiversity studies. Ecol. Indic. 94, 99–103.
Tropek, R., Kadlec, T., Hejda, M., Kocarek, P., Skuhrovec, J., Malenovsky, I., Vodka, S., Spitzer, L., Banar, P., Konvicka, M., 2012. Technical reclamations are wasting the conservation potential of post-mining sites. A case study of black coal spoil dumps. Ecol. Eng. 43, 13–18.
Van Dyck, H., Van Strien, A.J., Maes, D., Van Swaay, C.A.M., 2009. Declines in common, widespread butterflies in a landscape under intense human use. Conserv. Biol. 23, 957–965.
Vera, F.W.M., 2000. Grazing Ecology and Forest History. CABI Pub, Wallingford, Oxon; New York, NY.
Weiss, N., Zucchi, H., Hochkirch, A., 2013. The effects of grassland management and aspect on orthoptera diversity and abundance: site conditions are as important as management. Biodivers. Conserv. 22, 2167–2178.
Weking, S., Kämpf, I., Mathar, W., Hölzel, N., 2016. Effects of land use and landscape patterns on orthoptera communities in the Western siberian forest steppe. Biodivers. Conserv. 25, 2341–2359.
White, R.P., Murray, S., Rohweder, M., 2000. Pilot Analysis of Global Ecosystems: Grassland Ecosystems. World Resources Institute, Washington, DC.
Williams, N.S.G., McDonnell, M.J., Seager, E.J., 2005. Factors influencing the loss of an endangered ecosystem in an urbanising landscape: a case study of native grasslands from Melbourne, Australia. Landsc. Urban Plan. 71, 35–49.
Willott, S.J., 1997. Thermoregulation in four species of british grasshoppers (Orthoptera: Acrididae). Funct. Ecol. 11, 705–713.
Wilson, A.M., Jetz, W., 2016. Remotely sensed high resolution global cloud dynamics for predicting ecosystem and biodiversity distributions. PLoS Biol. 14, 1–20.
Wollan, A.K., Bakkestuen, V., Kauserud, H., Gulden, G., Halvorsen, R., 2008. Modelling and predicting fungal distribution patterns using herbarium data. J. Biogeogr. 35, 2298–2310.

Study II

Dvořák, T., & Knapp, M. (2023). Conserved temperature requirements but contrasting responses to humidity across oviposition preferences in temperate grasshoppers. *Scientific Reports*, 13(1), 21131.

scientific reports

Check for updates

OPEN

Conserved temperature requirements but contrasting responses to humidity across oviposition preferences in temperate grasshoppers

Tomáš Dvořák[1,2]✉ & Michal Knapp[2]

The right choice of an oviposition site is a crucial task for oviparous species without maternal care. In contrast to well investigated biotic factors, e.g., larval food preferences, parasitism, predation, and competition avoiding, abiotic factors affecting oviposition preferences in insects have been rarely investigated in comparative studies. To improve our current understanding of oviposition site selection in Orthoptera, we investigated the influence of substrate temperature and moisture on the oviposition behaviour of 14 temperate grasshopper species. Conspecific groups of adults were kept in arenas with simultaneous temperature and moisture gradients. For each ootheca produced during the experiment (n = 1192) we recorded its depth and local microclimatic conditions. Our results indicate that microclimatic oviposition preferences significantly differ among species, however, correlations between adult habitat preferences and microclimatic oviposition preferences were surprisingly weak. Even oligothermic species preferred substrate temperatures around 30 °C and some xerothermic species preferred higher humidity. The hypothesized tendency to place oothecae closer to the ground within grass tussocks under hot and dry conditions was confirmed. It is possible that species evaluate microclimatic conditions for oviposition in the context of occupied habitat, i.e., in a relative rather than absolute manner.

Searching for the optimal environment that allows for the successful development of eggs and subsequent offspring survival is one of the crucial issues faced by every oviparous ectotherm species without direct maternal care[1]. Most studies have focused on biotic factors associated with the optimal food availability for hatchlings or parasitism, predation, and competition avoiding[2–6]. Abiotic factors, such as moisture and temperature, have received significantly less attention[7–9] and comparative studies among species are largely missing. In addition, many studies are focused on reptile oviposition site selection[10–12], and there is only limited information on the most diverse and numerous group of ectotherms, insects[13,14]. With ongoing climate change and anthropogenic habitat degradation frequently affecting local moisture and temperature, understanding the role of microclimate on oviposition behaviour and preferences in insects has become an especially important research topic for ecologists and entomologists[15–17].

Current theory on oviposition site selection in insects can be classified into two main hypotheses: (1) the environmental matching hypothesis postulates that oviposition preferences are associated with the overall xerothermophility (an affinity for dry and warm sites) of a species[7,13,18], i.e., species inhabiting dry and warm habitats prefer to lay eggs in dry and warm microhabitats; (2) the insurance hypothesis postulates that all species prefer relatively humid substrate, independently of their habitat preferences, to avoid egg desiccation[9,19,20]. To test these hypotheses, multispecies comparative studies using standardized experimental designs are needed[13,21]. Unfortunately, the existing studies have mostly investigated a single species[9,18,20,22] or compared two species with contrasting habitat requirements[7,19,23]. For a few species, intraspecific differences between various populations have also been investigated[24,25]. Despite a potentially strong synergic effect, moisture and temperature have been

[1]Department of Zoology, Faculty of Science, Charles University, Viničná 7, Prague 2 128 00, Czech Republic. [2]Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Prague-Suchdol 165 00, Czech Republic. ✉email: Algalesie@seznam.cz

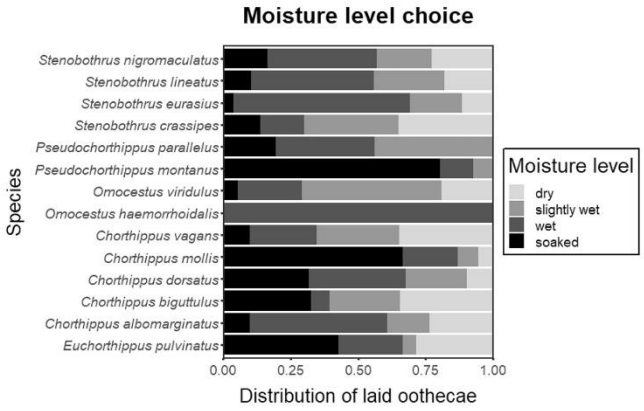


Figure 1. Species-specific distribution of oothecae in relation to humidity. Data for all species significantly differed from a random distribution (0.25:0.25:0.25:0.25).

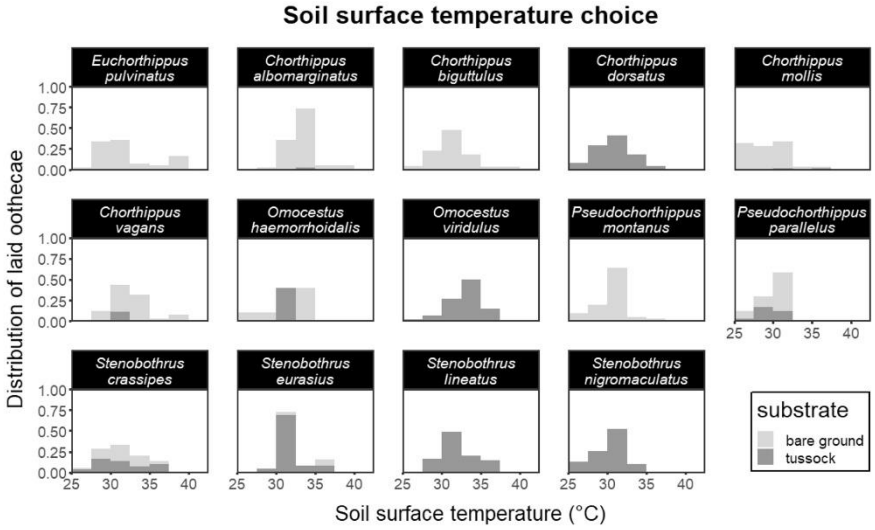


Figure 2. Species-specific distribution of oothecae in relation to temperature. Bare ground-laid and tussocks-laid oothecae are distinguished by colours (see the legend).

Vertical positions of oothecae ranged from 4 cm below ground to 5 cm above the soil surface (Table S1; Fig. S1). The deepest ootheca was buried by *Chorthippus vagans*, but the lowest mean depth was observed for *Euchorthippus pulvinatus* (2.37 ± 0.71 cm below ground). The species with the highest mean laying location was *Omocestus viridulus* with a mean of 1.34 ± 1.05 cm above the soil surface. There were significant differences in the vertical position of oothecae between the great majority of investigated species (bare ground-laid oothecae: $F = 20.3$, $df = 9$, $p < 0.001$; tussocks-laid oothecae: $F = 36.58$, $df = 10$, $p < 0.001$; Table S1).

Oviposition site selection hypotheses

Results showed that there was no significant relationship between species-specific oviposition preferences (preferred moisture level, substrate temperature, and partially the vertical position of oothecae) and the index of xerothermophility for both bare ground-laid and tussock-laid oothecae, with the exception of the vertical position of tussocks-laid oothecae (Table 2a). Highly xerothermophilic species placed their oothecae significantly

rarely investigated in parallel[26,27]. Experimental designs that combine independent gradients of both variables are needed to distinguish between independent and interactive effects of local temperature and substrate humidity.

Temperate grasshoppers from the subfamily Gomphocerinae represent an optimal model group for multi-species comparative studies. This subfamily contains a high number of morphologically uniform species with similar life histories[21] and, at the same time, particular species strongly differ in their habitat preferences[28–30]. The limited existing knowledge indicates that grasshoppers follow the environmental matching hypothesis rather than the insurance hypothesis, i.e., their microclimatic oviposition preferences are correlated with species habitat preferences[7,21,31,32]. However, systematic investigations are needed to provide a more nuanced and unambiguous conclusion.

Within a suitable microhabitat, ovipositing females can further adjust their laying behaviour to meet optimal conditions for egg development[8,33]. For example, species using soil and grass tussocks for egg laying can manipulate oviposition depth (according to the vertical distribution plasticity hypothesis), as moisture commonly increases and temperature decreases with increasing depth[21,24,34]. In particular, it has been hypothesized that xerothermophilic grasshopper species lay eggs deeper into the substrate to avoid exposure to extreme temperatures and drought, while species inhabiting cold and moist habitats prefer shallow oviposition or grass tussocks to increase developmental temperature or to escape egg flooding[7,21]. However, for orthopterans, there is still only very limited evidence on inter- and intra-specific variation in this trait, as well as the overall variability in condition-dependent oviposition depth[21,24].

This study investigated the oviposition preferences of 14 Central European Gomphocerinae grasshopper species using laboratory experiments that combined independent moisture and temperature gradients. The specific goals were to: (1) identify moisture, temperature, and depth preferences for egg-laying of all investigated species; (2) evaluate the oviposition preference hypotheses by comparing data from this study with adult habitat preferences investigated in our previous study (xerothermophility indices[30]); (3) assess whether grasshoppers modify their oviposition behaviour (laying depth) according to local microclimatic conditions.

Results

Oviposition preferences

A total of 1192 oothecae were produced by 280 females from the 14 investigated grasshopper species (Table 1). All species showed a non-random oviposition microhabitat selection with respect to moisture (Fig. 1), with *Stenobothrus nigromaculatus* being the closest to a random distribution of oothecae ($\chi^2 = 10.7$, df = 3, p = 0.013). The other extreme was represented by *Omocestus haemorrhoidalis*, which laid only 10 oothecae exclusively in the wet compartment. Three species preferred soaked substrate, six species preferred wet substrate and five species preferred slightly wet or dry substrate (Fig. 1; Table S1). Overall, across all species, wet substrate was the most preferred (contained 36% of all oothecae), followed by soaked (25%), slightly wet (22%) and dry substrate (17%). The strongest preference for moist substrate was recorded for *Pseudochorthippus montanus*, and the driest substrate was selected by *Chorthippus vagans* and *Stenobothrus crassipes*.

The highest average temperature for bare ground-laid oothecae was recorded for *Chorthippus albomarginatus* (33.2 °C), and the lowest for *Chorthippus mollis* (29.2 °C). Among tussocks-laid oothecae, the highest average temperature was observed for *Omocestus viridulus* (33.3 °C), and the lowest for *Pseudochorthippus parallelus* (29.6 °C). The preferred soil temperatures for oviposition are summarised in Table S1 and visualised in Fig. 2. Thermal preferences significantly differed between some species (bare ground-laid oothecae: F = 11.43, df = 9, p < 0.001; tussocks-laid oothecae: F = 14.47, df = 10, p < 0.001), however, there was also a large group of species with very similar thermal preferences (Table S1).

Species	Number of oothecae	Proportion of tussock-laid oothecae (%)	Index of xerothermophility
Euchorthippus pulvinatus	42	0	0.896
Chorthippus albomarginatus	51	2	0.336
Chorthippus biguttulus	160	0	0.518
Chorthippus dorsatus	145	100	0.375
Chorthippus mollis	54	4	0.900
Chorthippus vagans	92	12	0.960
Omocestus haemorrhoidalis	10	40	0.799
Omocestus viridulus	189	100	0.109
Pseudochorthipus montanus	41	0	0.021
Pseudochorthipus parallelus	41	32	0.291
Stenobothrus crassipes	80	51	0.780
Stenobothrus eurasius	26	88	1.000
Stenobothrus lineatus	183	100	0.777
Stenobothrus nigromaculatus	79	100	0.952

Table 1. List of investigated species and summary of their oviposition performance. Number of laid oothecae per species and proportion of tussock-laid oothecae are shown. In addition, the xerothermophility index value obtained from our previous study[30] is included for each species.

(a) Substrate type	Dependent variable	Chi-sq	d.f.	p-value
Bare ground	Moisture	0.007	1	0.934
	Temperature	0.658	1	0.417
	Vertical position	3.081	1	0.079
Tussocks	Moisture	1.883	1	0.170
	Temperature	0.773	1	0.395
	Vertical position	5.502	1	**0.019**
(b) Substrate type	**Dependent variable**	**Chi-sq**	**d.f.**	**p-value**
Bare ground	Moisture	1.063	1	0.303
	Temperature	0.455	1	0.500
	Vertical position	4.180	1	**0.041**
Tussocks	Moisture	0.379	1	0.538
	Temperature	0.476	1	0.491
	Vertical position	0.884	1	0.347

Table 2. Effects of species xerothermophility on oviposition preferences. Results of phylogenetic mixed-effects (a) and phylogenetically-uncoreccted (b) models investigating the relationship between the index of xerothermophility (independent variable) and species oviposition preferences for moisture, temperature, and ootheca vertical position (separate model was fitted for each response variable and substrate type). Bolded values indicate significant relationships. Significant values are in bold.

closer to the ground within grass tussock than less xerothermophilic species. Interestingly, the significance of this relationship was not confirmed for bare ground laying species despite the noticeable regression slope (Fig. 3). Phylogenetically-uncorrected models showed very similar results in the case of moisture and temperature. The only exception was the vertical position of oothecae, where the direction of the relationship was the same but the significance was switched between the bare ground and tussocks laying species (Table 2b). Our results for

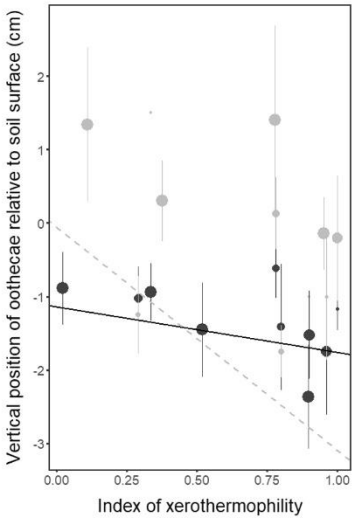


Figure 3. Relationship between vertical position of oothecae and species index of xerothermophility. Each dot represents mean vertical position of oothecae laid by one species (error bars represent ± SD). Bare ground-laid (dark grey) and tussocks-laid (light grey) oothecae are shown separately. Dot size represents species' affinity to the given substrate type. The full line represents the phylogenetically corrected significant relationship between the vertical position of oothecae and the index of xerothermophility for tussocks-laid oothecae ($p = 0.019$). The dashed line represents the same but only marginally significant relationship for bare ground-laid oothecae ($p = 0.079$). The zero at the y-axis refers to the soil surface. Thus, positive values are above the ground, negative bellow the ground.

moisture strongly support the insurance hypothesis, as wet substrate was, in general, the most preferred substrate and the fewest number of oothecae were placed in the dry compartment across all the investigated species (Fig. 1).

The vertical position of oothecae at the intraspecific level was significantly affected by moisture level and partially by temperature (Table 3a). Oothecae were placed shallower in bare ground or higher on tussocks with increasing local moisture (Figs. S2 and S3). Oothecae were placed significantly lower on tussocks and tend to be placed deeper in bare ground with increasing local temperature (Table 3a, Figs. 4 and S3). There was no significant interactive effects of local moisture and temperature on the vertical position of oothecae. Phylogenetically-uncorrected models performed practically identical compared to the corrected ones (Table 3b). Note that the above-described results represent overall patterns (across species) and patterns for particular species can be slightly different (for details see Figs. 4, S2 and S3).

Discussion

All the investigated orthopteran species showed a non-random choice of moisture level, surface temperature, and vertical position when ovipositing. Interestingly, there was no relationship between preferred moisture or temperature during oviposition and species xerothermophility based on adult habitat preferences[30]. Instead, most species preferred higher levels of moisture and a relatively narrow range of temperatures. Nevertheless, there was a significant relationship between species xerothermophility and vertical position of oothecae laid in tussocks. Furthermore, at the intraspecific level, oothecae were placed shallower in bare ground or higher on tussocks with increasing local moisture and partially also with increasing temperature. In general, our results indicate that orthopterans optimise oviposition depth and even xeric orthopteran species prefer substrates with higher moisture to protect oothecae from desiccation.

Moisture is the most relevant factor determining successful egg development in insects[14]. Many species need to absorb water before embryogenesis begins, and eggs have to continuously resist desiccation until hatching occurs[21]. Almost all the investigated species laid at least some eggs into each moisture level (compartment), which suggests a relatively relaxed moisture preferences across orthopterans. Even in the most drought preferring species, *Chorthippus vagans* and *Stenobothrus crassipes*, females only slightly preferred the dry substrate over slightly wet or wet substrates, and some oothecae were laid in soaked soil. In general, wet soil was preferred the most and dry soil the least. Our findings provide support for the insurance hypothesis, which predicts a general tendency to oviposit into substrates with high moisture levels independently of the habitat preferred by adults[9,20,35]. In contrast, we did not find evidence supporting the environmental matching hypothesis, which predicts a correlation between species habitat preferences (xerothermophility) and oviposition preferences[7,18]. It seems that species do not detect moisture in an absolute manner, but only relatively in the context of occupied habitat. For example, the xerothermophilic species *Chorthippus mollis* strongly preferred soaked substrate in the experiment, despite almost never coming into contact with soaked soil in nature. *Omocestus viridulus*, which usually occurs in humid habitats, preferred tussocks within only slightly wet soil in our experiment. This can be an adaptation to spring flooding, which is common in its preferred habitats[21,36].

The embryonic development rate is closely related to ambient temperature and the sum of degree days during a season can be a limiting factor for many temperate insects[32,37]. On the other hand, lethal temperature thresholds are usually very close to the thermal optima and a fine equilibrium between these two factors has to be reached[14,38]. Our data show that optima of all investigated species occurred between 29 and 34 °C, which fits well with previously published single-species data[21,25,31,39]. The relatively narrow range of preferred oviposition temperatures across species originating from variable habitats is quite surprising and seems to be quite conservative within the Gomphocerinae subfamily. In contrast to findings of Schnebel and Grossfield[13], we observed no tendency of xerothermophilic species to prefer higher surface temperature for oviposition. However, Schnebel & Grossfield (1986) investigated fruit fly species along a latitudinal gradient ranging from arctic to tropic regions,

(a) Substrate type	Independent variable	Chi-sq	d.f.	p
Bare ground	Moisture	18.02	3	**< 0.001**
	Temperature	3.26	1	0.071
	Moisture: temperature	2.6	3	0.459
Tussock	Moisture	27.61	3	**< 0.001**
	Temperature	6.09	1	**0.014**
	Moisture: temperature	6.25	3	0.100
(b) Substrate type	**Independent variable**	**Chi-sq**	**d.f.**	**p**
Bare ground	Moisture	18.48	3	**< 0.001**
	Temperature	3.33	1	0.068
	Moisture: temperature	2.74	3	0.434
Tussock	Moisture	27.50	3	**< 0.001**
	Temperature	6.32	1	**0.012**
	Moisture: temperature	6.24	3	0.101

Table 3. Effects of substrate moisture and surface temperature on vertical position of oothecae. Results are reported separately for the phylogenetic mixed-effect (a) and phylogenetically-uncorrected (b) models. Significant values are in bold.

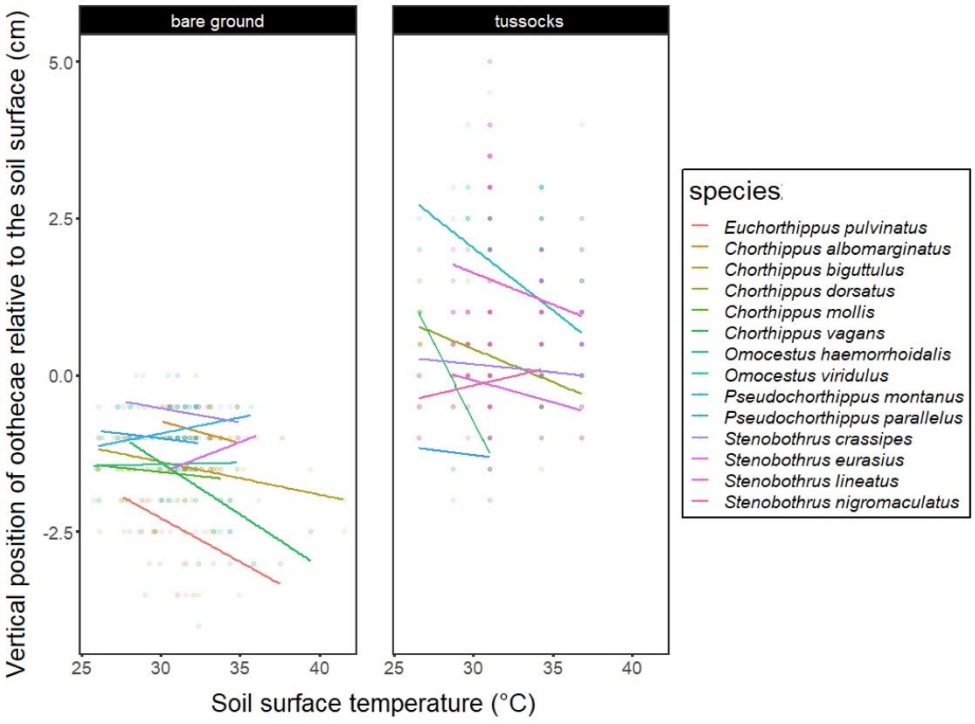


Figure 4. Effects of temperature on vertical position of oothecae. Separate regression lines are shown for each species. Each dot represents an individual ootheca (note that oothecae from different species placed to the same combination of conditions can overlap). The left graph shows bare ground-laid oothecae and the right shows tussocks-laid oothecae. The overall relationship (across species) between temperature and vertical position of oothecae was significant for tussock-laid oothecae ($p = 0.014$) and marginally significant for bare ground-laid oothecae ($p = 0.071$).

not species from a single region. Again, a possible explanation of our findings can be based on individual life strategies of different species. For example, despite its high xerothermophility, *Chorthippus mollis* preferred the lowest oviposition temperature out of all investigated species. This species has a relatively long embryonic development and the choice of relatively cold and moist microhabitats within hot and dry steppes, where it lives, can protect developing immobile embryos from potentially damaging temperatures at the start of summer when other species have already hatched[32,36]. The fact that we measured surface temperature can potentially represent a methodological issue. In nature, as well as in our experiment, soil temperature decreases with distance from the surface. However, existing information on the oviposition site selection process in orthopterans indicates that ovipositing females perform a batch of shallow probes with their ovipositor, so surface conditions (including temperature) are probably crucial for their decision making[21].

It has been hypothesized that xerothermophilic species should lay eggs deeper into the ground or lower within tussocks than oligothermic species to avoid damaging hot and dry conditions[7,21,40]. Our results partly supported this hypothesis, as tussocks-laid oothecae were placed closer to ground with increasing xerothermophility of species, and there was a similar, but non-significant tendency in bare ground-laid oothecae. In the case of phylogenetically-uncorrected models, significances were switched. This difference probably stems from accumulation of tussocks-laying species in the clade containing *Stenobothrus* and *Omocestus* genera (Fig. S4). In this case, results of phylogenetically-corrected models should be considered more relevant. Nevertheless, using more species, especially more hygro- and psychrophilic ones, in a future study will allow for a better evaluation of this pattern.

At the intraspecific level, we found a significant tendency to place oothecae deeper to the soil or closer to the ground within tussocks with decreasing moisture. A similar finding was previously reported for the grasshopper *Romalea microptera* in which females laid eggs shallower into the substrate when exposed to higher moisture levels[24]. Interestingly, the effect of temperature was surprisingly less pronounced and significant only for tussocks-laid oothecae. Phylogenetically-uncorrected models performed very similarly to corrected ones.

This indicates that species traits and ecology are more important than evolutionary history of species in the case of intraspecific analyses.

We are aware of some potential methodological issues emerging from our experimental design, specifically those arising from possible interactions between animals. As described in methods, we isolated the cages as much as possible without affecting the environmental gradients within each cage. To reduce potential interactions between ovipositing females, we employed relatively large cages to avoid extremely high population densities, which were similar to those used in other laboratory studies[32,41,42]. Finally, although only one cage was used per species, each individual oothecae represents our data points and laboratory observations suggests that the choice of oviposition sites was not influenced by other individuals. Ideally, future studies should replicate each species in several cages if methodologically possible. In addition, oviposition preferences can vary between geographically distant populations within species and this phenomenon would also deserve a future attention.

Only a limited number of studies have investigated the effects of microclimate on oviposition behaviour compared to the wider range of published works that explore the effects of biotic factors, e.g., optimal food, parasitism, or competition avoidance[5,6]. Abiotic factors are directly modified by ongoing climate change and anthropogenic habitat alterations, which increases the relevance of such studies for nature conservation[16,43]. Moreover, eggs are an immobile life stage unable to escape from suboptimal conditions and frequently the most vulnerable to extreme temperatures[32,44]. This highlights the importance of studies investigating microhabitat oviposition preferences in insects. Especially valuable are studies employing a comparative framework, i.e., investigating a high number of species using a standardized experimental setting. Unfortunately, such studies are rare with the exception of a historical study of *Drosophila* flies[13]. However, our understanding of oviposition site selection cannot be complete without further studies investigating survival of eggs (oothecae) placed in various microhabitats and the related fitness consequences for emerging offspring. This represents a priority topic for future research focused on abiotic factors or interaction of abiotic and biotic factors affecting oviposition behaviour in insects.

Materials and methods

Specimen collection

Grasshoppers used for the laboratory experiment were collected between June 15th and July 15th, 2018, when most of Central European grasshopper species reach adulthood. All collecting sites were located within the Czech Republic. More specifically, xerothermophilic species were obtained from karst areas of Český kras and steppe habitats in České středohoří, hygro- and psychrophilic *Omocestus viridulus* were collected from Brdy Mountains, and the rest of the species were collected within the capital city of Prague. All sampled sites represented typical habitats for each species[36]. Grasshoppers were visually checked, and only freshly emerged adults (with a soft cuticle and deflated abdomen) or subadults were chosen for the laboratory experiment. This approach reduced a potential age effect on oviposition preferences[21]. Finally, a set of 14 grasshopper species with contrasting habitat requirements was included in the laboratory experiment (see Supplementary Table S2 for additional details on their biology and sampling sites).

Experimental design

The laboratory experiment was performed in large cages ($100 \times 50 \times 30$ cm) made of 4 mm thick sheets of polycarbonate (bottom) and plastic mesh (walls). The bottom was divided lengthwise into four 100×12.5 **cm** sized compartments, and each compartment was filled 5 cm thick substrate layer consisting of 1:1 peat and sand mixture. During the testing stage we repeatedly measured water content of substrate samples originating from various areas of large cages (measured gravimetrically). Our substrate ensured even distribution of moisture within each compartment as confirmed by a pilot experiment (testing stage). Based on various watering regimes tested during the testing phase we developed the appropriate final experimental setting. Compartments (moisture treatments) within a cage differed in their moisture levels as follows: (1) dry (approximately 5% moisture), (2) slightly wet (20%), (3) wet (35%) or (4) soaked (50%). The dry compartment did not get any extra water during the experiment. The slightly wet compartment was watered every other day with 200 ml, which resulted in transient wetness of the upper soil layer. The wet compartment was watered with 400 ml every other day to maintain permanent wetness across the whole soil profile. The soaked compartment received 600 ml every other day to reach fully saturated soil. A line of three 53 W halogen bulbs arranged 10 cm above the soil level was used to create a temperature gradient perpendicular to moisture gradient (Fig. 1). The function describing the relation of soil-surface temperature and the distance from the heat source was computed based on measurements performed across the cage length in 5 cm intervals, separately for each moisture compartment. Maximal surface temperature (42 °C) was reached directly under the heat source in the dry compartment. In the other compartments, maximal temperature gradually decreased with increasing moisture level (32 °C in the soaked compartment). Moreover, there was no other light source in the breeding room and cages were visually isolated from each other.

Grasshopper species differ in their preferred oviposition substrate, some species preferring bare ground, others place eggs (oothecae) into grass roots or grass tussocks, and some species use both strategies[21]. Therefore, four grass tussocks (ca. 10×10 cm) were planted in each compartment on both sides of the heat source at a distance of 12 and 35 cm (see the cage setup in Fig. 5).

Twenty females and 15 males per species were released in each cage and each species had its own cage (i.e., 14 cages were used in total). Similar population densities were used in various laboratory studies on Central European gomphocerinae grasshoppers[32,41,42]. However, natural population densities are probably lower. Cages were maintained until the death of the last female, typically for two or three months. Grasshoppers were provided with food in the form of a mixture of grasses placed in water-filled plastic tubes. Several tubes were dispersed across each cage and grasses were changed every three days or when depleted. Cages were kept at a L16 : D8 photoperiod and ambient temperature fluctuating between 26 ± 2 °C during the light period and 17 ± 2 °C during

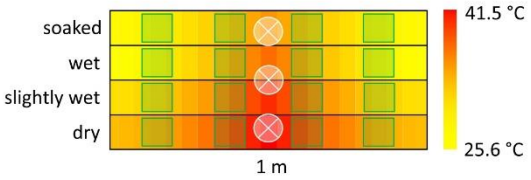


Figure 5. Scheme of our experimental cages (view from above). Background colours indicate local surface temperature, green squares represent grass tussocks, and crossed circles indicate positions of 53W halogen bulbs (placed 10 cm above the surface). Substrate humidity decreased from top to bottom. The bar on the right side shows the soil surface temperature recorded during the experiment.

the dark period. Ambient humidity ranged 40–60%. All investigated species are strictly day-active[21] and it is expected that oviposition took place solely during the daytime.

After the death of the last female, each cage was carefully inspected for oothecae. For each ootheca, the substrate (bare ground vs. tussock), the compartment moisture level (dry, slightly wet, wet, soaked), the vertical position relative to the soil surface (the depth in the soil, or the height above the ground in the case of an oviposition on grasses), and the distance from the heat source (to calculate temperature) were recorded. The vertical position of ootheca was measured as the position of its centre with a precision of ± 0.5 cm.

To minimize possible effects of neighbouring cages on experimental animals we tried to isolate the cages from each other as much as possible (at least one meter apart) and keep the same gradients within each cage. We also eliminated all outside sources of light. To reduce potential interactions between ovipositing females, we used relatively large cages (100 × 50 × 30 cm), reducing population densities. In addition, ovipositing females were observed ca. 10 h during the initial stage of the experiment and for several hours during following weeks and no interactions between ovipositing females were detected. Furthermore, we did not recorded any strange pattern in spatial distribution of oothecae (e.g., small-scale nesting or even distribution across the cage), indicating only negligible interactions between ovipositing females.

Data analysis

Phylogenetic data

To address phylogenetic relationships between investigated species, we constructed a phylogenetic tree following methods and data of the most recently published phylogeny[45] and added an additional COI sequence of *Stenobothrus crassipes*, which was obtained from the BOLD database ([46]; BOLD: AAD9833). The final phylogenetic tree is included in supplementary materials (Fig. S4). Four genes were used: two mitochondrial—Cytochrome C oxidase subunit I (COI, 575 bp) and Cytochrome B (cytB, 579 bp) and two nuclear—Internal transcribed spacers 1 and 2 (ITS1, 395 bp and ITS2, 293 bp). However, note that for particular species not all the above mentioned gene sequences are available (for details on other species than *Stenobothrus crassipes* see the original study[45]). Acquired sequences were aligned and trimmed in Aliview 1.28 software[47]. The Bayesian inference method (MCMC) was used to create phylogenetic trees and we performed a BEAST analysis (BEAST 2.7.4[48]). Evolutionary models were fitted using BEAUti 2.7.4 software[48]. A fixed clock model was used to estimate divergence time. Analysis was set to 20 million generations run and sampling step 2000 generations. Finally, the TreeAnnotator 2.7.4 software[48] (with 10% burn-in) was used to compute consensus tree and estimate the posterior probabilities (PP).

Moisture and temperature indices

To describe species oviposition preferences, we assessed the most preferred level (mode) and mean of moisture (dry, slightly wet, wet or soaked), the mean laying depth (vertical position according to the soil surface), and the mean preferred soil surface temperature. To test the existence of specific preferences for moisture, chi-squared tests were used for each species separately. Our null hypothesis assumed the random distribution among moisture compartments, i.e., 25% probability for each compartment. Significant differences between species oviposition temperature and vertical position were tested with a One-way ANOVA. Only species producing more than 10 oothecae per substrate type were included in our analyses.

To investigate the relationship between the index of xerothermophility (developed in our previous study; Table 1; for details see[30]) and species-specific microclimatic preferences, several phylogenetic linear mixed-effect models were fitted. Separate models were used for investigated response variables (moisture, temperature, vertical position of oothecae) and substrate type (bare ground, tussocks), i.e., in total six models were fitted. Index of xerothermophility was used as fixed effect and species identity as random effect in all models. To account for phylogenetic relations, we used "Almer" extension for "lme4" package[49] and the newly constructed phylogenetic tree (see above). For analyses of the vertical position of oothecae, the contribution of each species had to be weighted by the ratio of bare ground-laid or tussock-laid oothecae as records of rare oothecae in non-preferred substrate type could represent problematic cases. To investigate the importance of phylogenetic corrections, we also fitted phylogenetically-uncorrected version of all models (GLMMs, using "lmer" function).

Finally, we investigated the effects of microclimatic conditions on the intraspecific vertical position of oothecae using phylogenetic linear mixed-effect models in which the vertical position of ootheca was the response variable. Moisture, temperature, and their interaction were used as fixed effects. Species identity was included

as a random effect. Two separate models were fitted for bare ground-laid and tussock-laid oothecae. We also fitted phylogenetically-uncorrected versions of models for comparison. All analyses were performed in R 3.5.2[50].

Data availability

All data generated or analysed during this study are included in its supplementary information files.

Received: 15 November 2023; Accepted: 18 November 2023
Published online: 30 November 2023

References

1. Refsnider, J. M. & Janzen, F. J. Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annu. Rev. Ecol. Evol. Syst.* **41**, 39–57 (2010).
2. Mitchell, R. The evolution of oviposition tactics in the Bean Weevil, *Callosobruchus maculatus* (F.). *Ecology* **56**, 696–702 (1975).
3. Jaenike, J. On optimal oviposition behaviour in phytophagous insect. *Theor. Popul. Biol.* **14**, 350–356 (1978).
4. Bernays, E. & Graham, M. On the evolution of host specificity in phytophagous arthropods. *Ecology* **69**, 886–892 (1988).
5. Almohamad, R., Verheggen, F. J., Francis, F. & Haubruge, E. Predatory hoverflies select their oviposition site according to aphid host plant and aphid species. *Entomol. Exp. Appl.* **125**, 13–21 (2007).
6. Morse, D. H. Where should I lay my eggs? Oviposition choices of a shelter-building moth and the shifting danger of being parasitized. *Entomol. Exp. Appl.* **165**, 1–8 (2017).
7. Stauffer, T. W. & Whitman, D. W. Divergent oviposition behaviors in a desert *vs* a marsh grasshopper. *J. Orthoptera Res.* **16**, 103–114 (2007).
8. Eilers, S., Pettersson, L. B. & Öckinger, E. Micro-climate determines oviposition site selection and abundance in the butterfly *Pyrgus armoricanus* at its northern range margin: Micro-climate and oviposition. *Ecol. Entomol.* **38**, 183–192 (2013).
9. de Farias-Martins, F. *et al.* Forest litter crickets prefer higher substrate moisture for oviposition: Evidence from field and lab experiments. *PLoS ONE* **12**, 1–16 (2017).
10. Reedy, A. M., Zaragoza, D. & Warner, D. A. Maternally chosen nest sites positively affect multiple components of offspring fitness in a lizard. *Behav. Ecol.* **24**, 39–46 (2013).
11. Mainwaring, M. C. *et al.* Climate change and nesting behaviour in vertebrates: a review of the ecological threats and potential for adaptive responses: Climate change and nesting behaviour. *Biol. Rev.* **92**, 1991–2002 (2017).
12. Doody, J. S. *et al.* Plasticity in nest site choice behavior in response to hydric conditions in a reptile. *Sci. Rep.* **10**, 16048 (2020).
13. Schnebel, E. M. & Grossfield, J. Oviposition temperature range in four Drosophila species triads from different ecological backgrounds. *Am. Midl. Nat.* **116**, 25 (1986).
14. Chapman, R. F., Simpson, S. J. & Douglas, A. E. *The Insects: Structure and Function* (Cambridge University Press, 2013).
15. Gardiner, T. & Hassall, M. Does microclimate affect grasshopper populations after cutting of hay in improved grassland?. *J. Insect Conserv.* **13**, 97–102 (2009).
16. Loeffler, F., Poniatowski, D. & Fartmann, T. Orthoptera community shifts in response to land-use and climate change—Lessons from a long-term study across different grassland habitats. *Biol. Conserv.* **236**, 315–323 (2019).
17. Bladon, A. J. *et al.* How butterflies keep their cool: Physical and ecological traits influence thermoregulatory ability and population trends. *J. Anim. Ecol.* **89**, 2440–2450 (2020).
18. Esbjerg, P. & Lauritzen, A. J. Oviposition response of the Turnip moth to soil moisture. *Acta Agric. Scand. Sect. B Plant Soil Sci.* **60**, 89–94 (2010).
19. Howard, D. J. & Harrison, R. G. Habitat segregation in ground crickets: Experimental studies of adult survival, reproductive success, and oviposition preference. *Ecology* **65**, 61–68 (1984).
20. Brust, G. E. & House, G. J. Influence of soil texture, soil moisture, organic cover, and weeds on oviposition preference of southern corn rootworm (Coleoptera: Chrysomelidae). *Environ. Entomol.* **19**, 966–971 (1990).
21. Ingrisch, S. & Köhler, G. *Die Heuschrecken mitteleuropas* (Die Neue Brehm-Bücherei, 1998).
22. Fisher, J. R. Location of egg pods of Aulocara elliotti (Orthoptera: Acrididae) in a field of crested wheatgrass in Montana. *J. Kans. Entomol. Soc.* **65**, 416–420 (1992).
23. Ward, A. L. & Rogers, D. J. Oviposition response of scarabaeids: Does 'mother knows best' about rainfall variability and soil moisture?. *Physiol. Entomol.* **32**, 357–366 (2007).
24. Herrmann, D. L., Ko, A. E., Bhatt, S., Jannot, J. E. & Juliano, S. A. Geographic variation in size and oviposition depths of *Romalea microptera* (Orthoptera: Acrididae) is associated with different soil conditions. *Ann. Entomol. Soc. Am.* **103**, 227–235 (2010).
25. Fielding, D. J. Oviposition site selection by the grasshoppers *Melanoplus borealis* and *M. sanguinipes* (Orthoptera: Acrididae). *J. Orthoptera Res.* **20**, 75–80 (2011).
26. Willis, J. C. *et al.* The importance of temperature and moisture to the egg-laying behaviour of a pest slug, *Decoceras reticulatum*. *Ann. Appl. Biol.* **153**, 105–115 (2008).
27. Lepage, M. P., Bourgeois, G., Brodeur, J. & Boivin, G. Effect of soil temperature and moisture on survival of eggs and first-instar larvae of *Delia radicum*. *Environ. Entomol.* **41**, 159–165 (2012).
28. Kenyeres, Z., Bauer, N. & Rácz, I. A. Local and global factors in organization of Central-European orthopteran assemblages. *Russ. J. Ecol.* **45**, 375–383 (2014).
29. Jonas, J. L., Wolesensky, W. & Joern, A. Weather affects grasshopper population dynamics in continental grassland over annual and decadal periods. *Rangel. Ecol. Manag.* **68**, 29–39 (2015).
30. Dvořák, T., Hadrava, J. & Knapp, M. The ecological niche and conservation value of Central European grassland orthopterans: A quantitative approach. *Biol. Conserv.* **265**, 109406 (2022).
31. Choudhuri, J. C. B. Experimental studies on the choice of oviposition sites by two species of Chorthippus (Orthoptera: Acrididae). *J. Anim. Ecol.* **27**, 201 (1958).
32. Van Wingerden, W. K. R. E., Musters, J. C. M. & Maaskamp, F. I. M. The influence of temperature on the duration of egg development in West European grasshoppers (Orthoptera: Acrididae). *Oecologia* **87**, 417–423 (1991).
33. Čelik, T. Oviposition preferences of a threatened butterfly *Leptidea morsei* (Lepidoptera: Pieridae) at the western border of its range. *J. Insect Conserv.* **17**, 865–876 (2013).
34. Stoutjesdijk, P. & Barkman, J. J. *Microclimate, Vegetation and Fauna* (KNNV Publ, 2014).
35. Marrone, P. G. & Stinner, R. E. Effects of soil moisture and texture on oviposition preference of the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Coccinellidae). *Environ. Entomol.* **12**, 426–428 (1983).
36. Kočárek, P., Holuša, J., Vlk, R. & Marhoul, P. *Rovnokřídlí České republiky* (Academia, 2015).
37. Sibly, R. & Monk, K. A theory of grasshopper life cycles. *Oikos* **48**, 186–194 (1987).
38. Feder, M. E., Blair, N. & Figueras, H. Oviposition site selection: Unresponsiveness of Drosophila to cues of potential thermal stress. *Anim. Behav.* **53**, 585–588 (1997).
39. Fielding, D. J. Developmental time of *Melanoplus sanguinipes* (Orthoptera: Acrididae) at high latitudes. *Environ. Entomol.* **33**, 1513–1522 (2004).

40. Gustin, R. D. Effect of two moisture and population levels on oviposition of the western corn Rootworm123. *Environ. Entomol.* **8**, 406–407 (1979).
41. San Martin y Gomez, G. & Van Dyck, H. Ecotypic differentiation between urban and rural populations of the grasshopper *Chorthippus brunneus* relative to climate and habitat fragmentation. *Oecologia* **169**, 125–133 (2012).
42. Köehler, G. Erfahrungern zur Haltung und Zucht von Gomophocerinae (Acrididae) für ökophysiologische Experimente. *Articulata* **36**, 113–148 (2021).
43. Fartmann, T., Brüggeshemke, J., Poniatowski, D. & Löffler, F. Summer drought affects abundance of grassland grasshoppers differently along an elevation gradient. *Ecol. Entomol.* **47**, 778–790 (2022).
44. Knapp, M. & Nedvěd, O. Gender and timing during ontogeny matter: Effects of a temporary high temperature on survival, body size and colouration in *Harmonia axyridis*. *PLoS ONE* **8**, e74984 (2013).
45. Sevastianov, N., Neretina, T. & Vedenina, V. Evolution of calling songs in the grasshopper subfamily Gomphocerinae (Orthoptera, Acrididae). *Zool. Scr.* **52**, 154–175 (2023).
46. Ratnasingham, S. & Hebert, P. D. N. BARCODING: bold: The Barcode of Life Data System (http://www.barcodinglife.org): BARCODING. *Mol. Ecol. Notes* **7**, 355–364 (2007).
47. Larsson, A. AliView: A fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* **30**, 3276–3278 (2014).
48. Bouckaert, R. *et al.* An advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **15**, e1006650 (2019).
49. Bolstad, G. H. *et al.* genetic constraints predict evolutionary divergence in Dalechampia blossoms. *Philos. Trans. R. Soc. B Biol. Sci.* **369**, 20130255 (2014).
50. R Core Team. *R: A Language and Environment for Statistical Computing* https://www.R-project.org/. (R Foundation for Statistical Computing, 2020).

Acknowledgements

We are grateful to the Nature Conservation Agency of the Czech Republic for permitting experiments with endangered species *Stenobothrus eurasius*. Many thanks go to Jakub Prokop for financial and technical support, Ondřej Balvín for help with the phylogenetic analysis, Ezequiel González for insightful comments and David N. Awde for language corrections. This study was also partly supported by the Technology Agency of the Czech Republic (grant number SS02030018–DivLand).

Author contributions

T.D. came with the initial idea, designed methodology, collected data, performed the most of analyses and wrote the initial draft. M.K. significantly contributed to the data analyses, came with additional ideas, greatly improved and finalised initial draft and was involved in language corrections. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-47789-z.

Correspondence and requests for materials should be addressed to T.D.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2023

Study III

Dvořák, T., & Knapp, M. n. d. Species‘ mesoclimatic requierements affect body size, egg developmenttime and thermal sensitivity in temperate grasshoppers (manuscript).

Species‘ mesoclimatic requirements affect body size, egg development time and thermal sensitivity in temperate grasshoppers

Tomáš DVOŘÁK *[, 1, 2], Michal KNAPP [1]

[1]*Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Prague – Suchdol, 165 00, Czech Republic*

[2] *Department of Zoology, Faculty of Science, Charles University, Viničná 7, Prague 2, 128 44, Czech Republic*

* Corresponding author:

Email: Algalesie@seznam.cz

ORCID ID:

TD: 0000-0002-9182-4333

MK: 0000-0003-4487-7317

Abstract:

Environmental temperature significantly affects the development rates, size traits and performance of ectothermic organisms. However, most studies dealing with these phenomena studied them on large climatic gradients. This study investigates how mesoclimatic preferences, represented by a xerothermophility index, influence embryonic development time, thermal sensitivity, adult body size, egg and hatchling size, across 14 species of Central European grasshoppers. Specimens from different mesoclimatic habitats but similar macroclimates were reared in controlled conditions, with embryonic development and hatchling performance assessed across four temperature regimes (15.89–31.99°C).

Results reveal that xerothermophilous species (adapted to hot, dry habitats) exhibited prolonged embryonic development and higher thermal sensitivity, with development slowing at lower temperatures. Interestingly, embryonic development time was independent of hatchling weight, suggesting mesoclimatic factors outweigh metabolic constraints. Residual egg weight, which reflects surplus of water, positively correlated with thermal sensitivity, indicating potential buffering against thermal extremes. Adult body size increased in species from colder mesoclimates, aligning with Bergmann's rule. Despite temperature effects on embryonic development, hatchling weight and hatchability remained unaffected, indicating robustness to thermal variation during early stages.

This study underscores the importance of mesoclimatic preferences in shaping size and developmental traits in ectotherms, revealing fine-scale climatic adaptations often overlooked in large-scale studies. The findings suggest further research is needed to disentangle the role of residual egg mass, species phenology, and other life history traits in thermal adaptation. These insights highlight the complex interplay between developmental biology, environmental constraints, and evolutionary pressures.

Introduction:

Extremely high and low temperatures can limit survival and thus distribution of all organisms on Earth. However, whole range of environmental temperatures strongly affects mainly ectothermic organisms, which are unable to maintain their body temperature independent of the ambient temperature (Hutchings, 2021). The performance of ectotherms is significantly affected also by non-extreme temperatures as ambient temperature influences the speed of all biochemical reactions in their bodies (Gillooly et al., 2002; Brown et al., 2004; Marshall et al., 2018). More specifically, temperature affects their overall performance via temperature-dependent movement activity, right hatching timing, immune system functioning, reproduction investment etc. (Dingle & Mousseau, 1994; Berger et al., 2008; Warkentin, 2011; Chapman et al., 2013). During juvenile stages ambient temperature determine development and growth rates, and their interplay with offspring size determine adult body size in species with determined growth, e.g., insects (Berner & Blanckenhorn, 2006a; Kingsolver & Huey, 2008; Marshall et al., 2018).

It is expected that environmental conditions, e.g., temperature, strongly affect species traits which are frequently subjected to various other selection pressures at the same time, for example body size. Larger females typically have higher fitness as they are able to produce more and/or larger offspring (Honěk, 1993; Pincheira-Donoso & Hunt, 2017; Eckerström-Liedholm et al., 2017; Church et al., 2019). Larger offspring are predicted to better cope with harsh environmental conditions, e.g., low temperature, as they are more resistant to diverse stressors (Fox & Czesak, 2000; Marshall et al., 2018; Di Martino & Liow, 2021). Larger adult body size at lower temperature can result also from physiological mechanisms, e.g., decoupling of growth and development rates, the latter being more sensitive to temperature (Temperature-Size Rule; (Kingsolver & Huey, 2008; Arendt, 2011; Hutchings, 2021)). However, empirical evidence shows that for many univoltine species adult body size rather decreases with reduced growth season duration, i.e., at lower average annual temperature (Blanckenhorn, 2004; Berner & Blanckenhorn, 2006a; Marshall et al., 2018; Wonglersak et al., 2020). Therefore, large relative offspring body size (compared to adult body size) can benefit ectotherms in environments with lower temperature and limited growth season length, as higher relative offspring body size can reduce required future biomass accumulation and resulting development time (Rollinson & Rowe, 2016; Marshall et al., 2018). To achieve optimal adult size as well as offspring size, development rates can be subjected to selection, and existing evidence confirm the existence of latitudinal/altitudinal compensation, i.e., higher development

rates at higher latitudes/altitudes, across ectotherm species (Yamahira & Conover, 2002; Berner & Blanckenhorn, 2006a; Hao et al., 2021).

To disentangle relationships between environmental temperature, adult body size, offspring size (both absolute and relative) and development rate, we need to put strong emphasis on the investigation of egg stage. Eggs are immobile, i.e., cannot move to more suitable microclimatic conditions, and frequently represent the developmental stage most sensitive to extreme temperatures (Bonebrake et al., 2010; Knapp & Nedvěd, 2013). Moreover, right timing of hatching, which is directly affected by egg development time, is crucial for a future destiny of given individual. Precise synchronisation with food resources, optimal weather conditions and periods of limited predator and parasites activity increase juvenile survival and adult performance in ectotherm animals (Dingle & Mousseau, 1994; Berger et al., 2008; Warkentin, 2011).

To date, great majority of studies investigating responses of adult body size, egg size, development rate and hatchling performance to environmental temperature in ectotherm animals at both intraspecific and interspecific levels employed large-scale latitudinal and altitudinal gradients. For example, embryonic development was prolonged in populations from lower latitude in *Chorthippus dubius* grasshoppers inhabiting Asian steppes (Hao et al., 2021). Northern populations of eastern mosquito fish *Gambusia holdbrooki* tends to have larger embryos and adults, when a latitudinal gradient along the eastern coast of Northern America was investigated (Riesch et al., 2018). Higher altitude Alpine populations of *Omocestus viridulus* grasshopper consisted of smaller adults and had shorter embryonic development under standardized conditions compered to populations inhabiting lowlands (Berner et al., 2004; Berner & Blanckenhorn, 2006). Interspecific studies are much less abundant. For example, (Leingärtner et al., 2014) showed that Alpine Lepidopteran communities have on average shorter embryonic development and larger adult size with increasing altitude. Meta-analytic studies confirmed the existence of latitudinal converse Bergmann's rule in univoltine species, whereas no systematic shift in body size was observed along altitudinal gradient (Horne et al., 2015, 2018). Surprisingly limited information exists on adaptations acting at a smaller spatial scale, e.g., effects of mesoclimatic conditions.

The aim of this study is to investigate the effect of species mesoclimatic requirements (represented by species xerothermophility index; for details see method section and (Dvořák et al., 2022)) on adult body size, egg and hatchling size, temperature-dependent egg development

time and egg hatching rate. We use a study system consisting of 14 grasshopper species from the subfamily Gomphocerinae originating from the same geographical area and exposed to the same macroclimatic conditions. We are interested in answering the following main questions: 1) How species mesoclimatic requirements affects egg development time and its thermal sensitivity (Q10); 2) How species mesoclimatic requirements affects adult body size and hatchling sizes; 3) How species mesoclimatic requirements affects egg performance across investigated temperatures (from ca. 16 to 32 °C) represented by hatchability and hatchling weight. In addition, we investigate how are the above-mentioned variables interrelated (using a structural modelling approach).

Methods:

Specimen collection:

In total, 14 grasshopper species with contrasting habitat requirements were included in the laboratory experiment. Grasshoppers utilized for the experiment were collected in the early summer 2018, period when most of Central European grasshopper species reach adulthood. Collecting sites were located within Czech Republic in Central Bohemia. Most of the species were obtained on various grassland sites within the capital city of Prague. Specimens of hygro- and psychrophilic *Omocestus viridulus* were collected in nearby Brdy highlands. Some xerothermophilous species were obtained from steppes of nearby karst area of Český kras and in České středohoří. Additional details about species biology and collecting sites are available in Supplementary table S1. All sampled sites represented typical habitats for given species in Central Europe (Kočárek et al., 2015). All sites have very similar macroclimate (Tolazs et al., 2007) and altitude (300 – 400 m a. s. l.), which enable us to focus on differences in local climate at mesohabitat scale. All collected individuals were checked and only freshly emerged adults (with a soft cuticle and deflated abdomen) or subadults were chosen for the laboratory experiment. This approach reduced potential effect of female age on eggs parameters (Ingrisch & Köhler, 1998).

Adult body size:

In addition to 20 females per species used for experiment (see below), 40 freshly killed mature females from the same locality and with abdomens obviously not filled with eggs were

weighted. We focused our attention to females as they are expected to be subjected to stronger selection pressures in orthopterans. Females are larger, their sexual maturation takes longer, are less mobile and, in general, investment in egg development is more energy demanding than sperm production (Ingrisch & Köhler, 1998; Hochkirch & Gröning, 2008; García-Navas et al., 2017)

Laboratory experiment:

The grasshoppers were reared in large cages (100 × 50 × 30 cm) with bottom made from polycarbonate sheet and walls from plastic mesh. Bottom was filled 5 cm thick layer of 1 : 1 mixture of peat and sand. Heat and light were provided with three 53W halogen bulbs. Gradient of moisture from dry to soaked was maintained to enable each species to choose the right condition for oviposition and eggs development. Grasshopper species differ in their preferred oviposition substrate, some species preferring bare ground, others place eggs (oothecae) into grass roots or grass tussocks, and some species can utilize both strategies (Ingrisch & Köhler, 1998; Dvořák & Knapp, 2023). Thus, grass tussocks were placed in cages to allow oviposition of these tussocks laying species. Twenty females and 15 males per species were released in the cage, each species having his own cage, i.e., 14 cages were used in total. Grasshoppers were provided with food in form of a mixture of grasses placed in water-filled small plastic tubes. Cages were kept at a L16 : D8 photoperiod and ambient temperature fluctuating between 26 ± 2 °C during the light period and 17 ± 2 °C during the dark period. Ambient humidity ranged 40 – 60 %. Additional details about the cage design and rearing conditions can be find in our previous study utilizing the same approach (Dvořák & Knapp, 2023).

When all females died, oothecae with eggs were carefully collected and stored in humid environment at 20°C until winter. Before overwintering (November), 20 randomly chosen oothecae per species were dissected and three eggs from each ootheca (totally 60 eggs per species) weighted and photographed for further measurements. Although egg weight is changing rapidly during early development, approximately six weeks after oviposition the weight stabilise and remain almost the same until the end of overwintering (Moriarty, 1970). Therefore, we considered the egg weight in this period as the most relevant. During winter (December – February) eggs were stored at 4°C and high humidity to allow diapause termination at the end of this period. After overwintering, eggs were placed in climatic

chambers set to 17, 22, 27 and 32°C (exact long-term average temperatures measured with calibrated dataloggers were 15.89, 22.62, 26.64 and 31.99°C).

Climatic chambers were checked daily and hatched nymphs were collected and immediately weighted. Time (days) from the beginning of the experiment (end of overwintering) was recorded. Twenty nymphs (when available) were weighted per temperature treatment per species.

Data analysis:

To address phylogenetic relations of investigated species, we used phylogenetic tree developed in our previous study (for details see Dvořák & Knapp, 2023). Four phylogenetically corrected linear regression models (separate model for each incubation temperatures, i.e., 15.89, 22.62, 26.64 and 31.99 °C) were used to test the relationship between species mesoclimatic requirements (xerothermophility; explanatory variable) and embryonic developmental time (response variable)). Another phylogenetically corrected model was constructed to test the relationship between mesoclimatic requirements (explanatory variable) and egg thermal sensitivity (Q10; response variable). The xerothermophility index (Table S1) was computed for each investigated species in our previous studies (for details see Dvořák et al., 2022 and Dvořák & Knapp, 2023). This index is based on evaluation of habitat characteristics of occupied sites (moisture, vegetation cover, vegetation height, sun exposure and local climate) processed using a distribution MaxEnt model. Thermal sensitivity (Q10) was computed as natural logarithm of embryonic development time change between 17.5 and 27.5 °C in days. Precise (datalogger-measured) temperatures were used for the construction of species-specific regression formulas. Thermal sensitivity (Q10) was already used for grasshoppers by (Van Wingerden et al., 1991) and similar approach is frequently used also in studies investigating performance of other ectotherm organisms (DeLiberto et al., 2022).

To explore effects of adult and offspring size on developmental rates and to find how are individual parameters interconnected, we constructed piecewise structural equation model based on variables transformed to phylogenetically independent contrasts (PIC), which helped us to address phylogenetical relations between species. The focal variables were: 1) species mesoclimatic requirements, 2) embryonic developmental time at 22.62 °C (this temperature was suitable for all investigated species, i.e., provided the highest hatchability across species),

3) thermal sensitivity; 4) female adult weight, 5) hatchling weight, 6) residual egg weight and 7) relative hatchling weight. Hatchling weight (freshly nymph weight) was preferred over egg weight as it is considered to be more representative (Bosch & Bout, 1998; Wallace et al., 2006). Residual egg weight represents the difference between egg weight and hatchling weight (computed as regression residuals). We suppose that this redundant egg mass consisted mainly of water. The relative hatchling weight was computed as difference between adult weight and hatchling weight (based on regression residuals). Offspring size is inherently related to adult size and if we want to find and explain potential interspecific plasticity in hatchling weight, we need to control for the overall species size in this way (Church et al., 2019). In the basic model, we tested relationships hypothesized in the introduction, specifically: species inhabiting more xerothermic habitats should have: 1) smaller adults, 2) longer embryonic development, 3) higher egg termal sensitivity and 4) relatively smaller hatchlings; 5) species with larger adults should have larger hatchlings and 6) large hatchlings should have longer embryonic development. In addition, we investigated following potentially interesting links, which can reveal underlying mechanisms, e.g., the role of residual egg weight. Then, insignificant links between variables were removed until the best fit (lowest AIC and best chi-sq) was achieved (links based on basal hypotheses were left untouched).

Finally, we tested effect of different rearing temperatures on offspring performance represented by hatching rate and hatchling weight. A one-way ANOVA was used to assess differences between incubation temperature treatments. Pairwise comparisons between temperature treatments were assessed using Tukey post-hoc tests. To investigate potential effect of mesoclimatic requirements on hatchability and hatchling weight, we also employed phylogenetically corrected linear models for each temperature treatment separately.

All analyses were performed in Rstudio 3.5.3 (RStudio Team, 2023)

Results:

In total, we obtained 1192 oothecas (~ 7816 eggs, and 2993 live hatchlings) across 14 investigated species. The most oothecae were laid by *Omocestus viridulus* (189 oothecae ~ 1359 eggs). The least number was laid by *Omocestus haemorrhoidalis* (10 oothecas ~ 46 eggs). Median number of laid oothecae across species was 67 (211 eggs, respectively). Detailed information on reproductive performance of each particular species is summarized in Table S2.

Thermal sensitivity of embryonic development

Thermal sensitivity of embryonic development significantly increased together with increasing species xerothermophility index in Central European grasshopper species ($y = 3.755 + 0.746$, $df = 12$, $p = 0.033$; Figure 1). At the same time, higher species xerothermophility was positively linked to slower embryonic development at all investigated temperatures (Table 1; Figure 2). Embryonic development time in relation to incubation temperature for each species is visualised in Figure S1.

Table 1: Results of phylogenetically corrected linear models assessing an effect of species' xerothermophility on embryonic development time for four temperature setting. All models are significant.

Rearing temperature (°C)	**Regression formula**	**df**	**p**
15.82	$y = 4.078 + 0.261x$	10	0.0475
22.62	$y = 2.877+ 0.361x$	12	0.0055
26.64	$y = 2.325 + 0.350x$	12	0.0076
31.99	$y = 1.943 + 0.397x$	12	0.0074

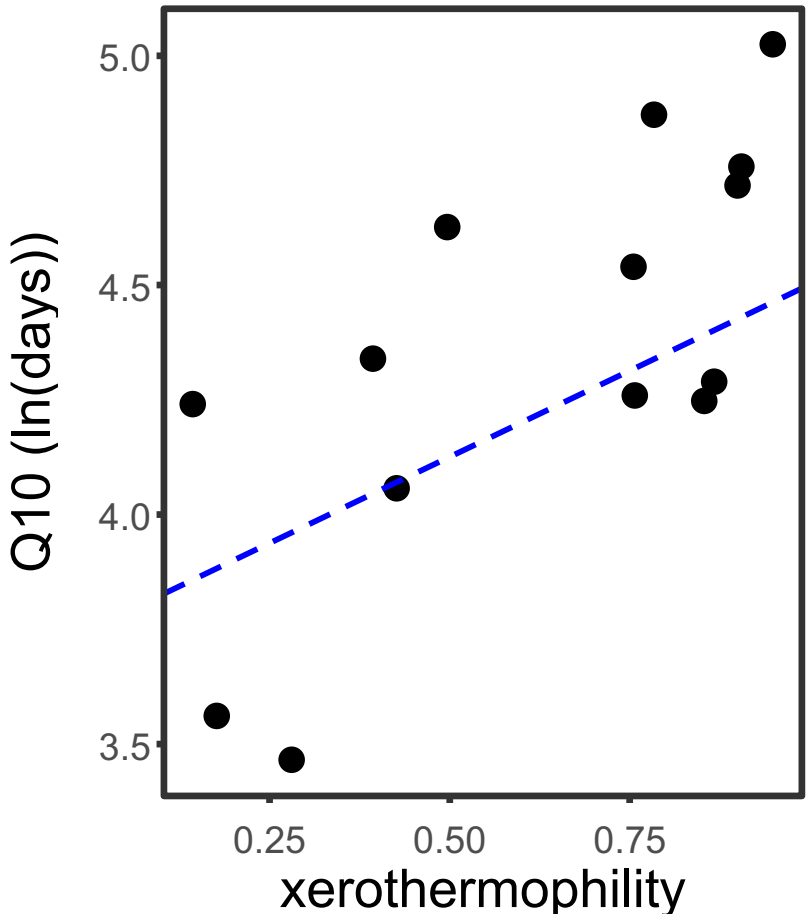


Figure 1: Effect of species xerothermophility on thermal sensitivity (Q10) of embryonic developmental time of Central European grasshoppers.

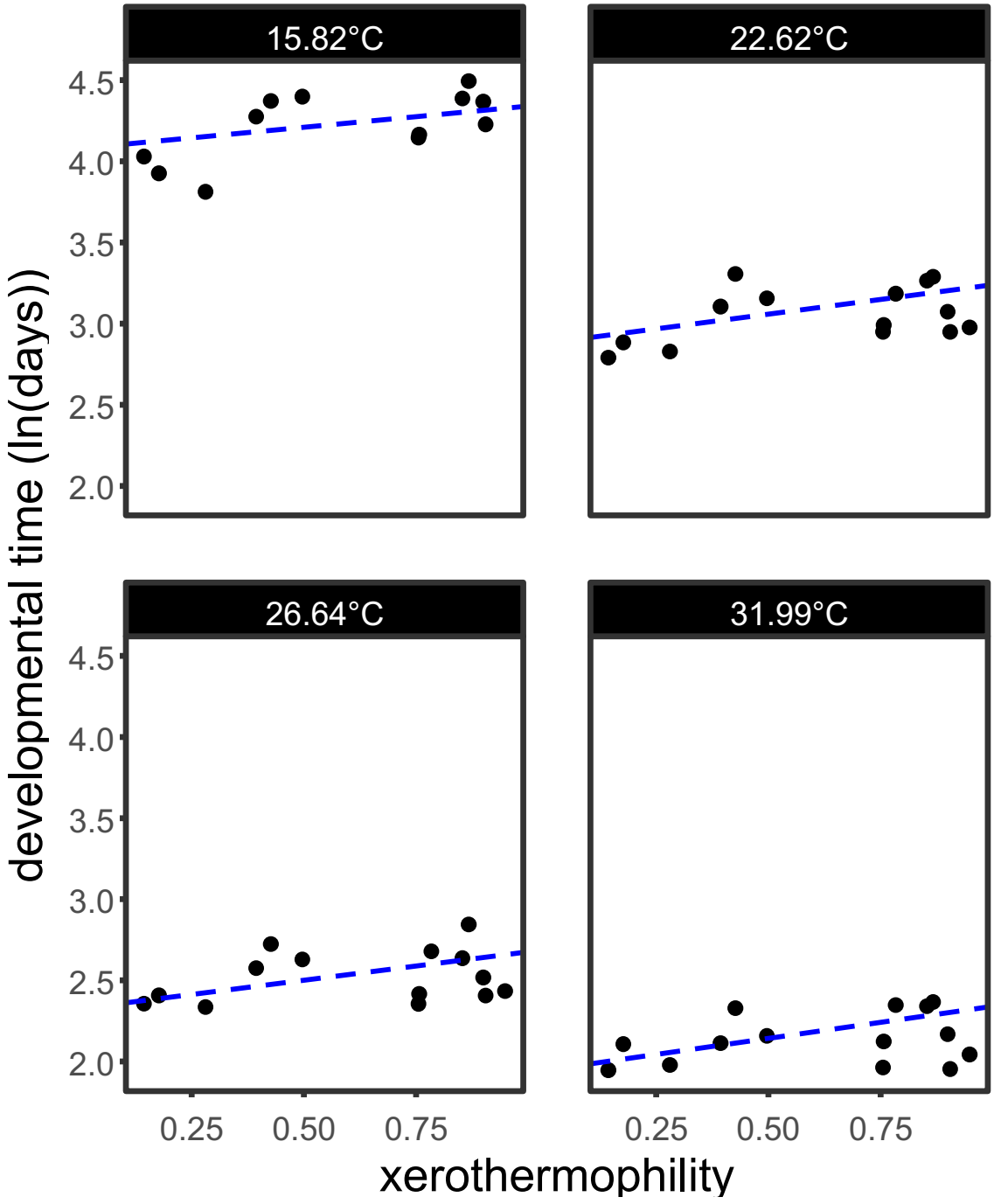


Figure 2: Effect of species xerothermophility on embryonic developmental time of Central European grasshoppers at different incubation temperatures. All relationships are significant (see Table 1).

Relationships between species mesoclimatic requirements, adult size and offspring traits

Species xerothermophility significantly negatively affected adult body size that was strongly positively related to offspring size (hatchling weight). In addition, species xerothermophility significantly positively affected embryonic development time (as also shown in detail in the previous paragraph). As our final structural equation model do not include significant relationship between nymphal weight and embryonic developmental time, we can conclude that more xerothermophilic species have smaller nymphs because of adult size constrains (Figure 3). Moreover, species xerothermophility significantly positively affected species thermal sensitivity (Q10) that significantly positively affected residual egg weight. However, there was no direct effect of species xerothermophility on residual egg weight (Figure 3).

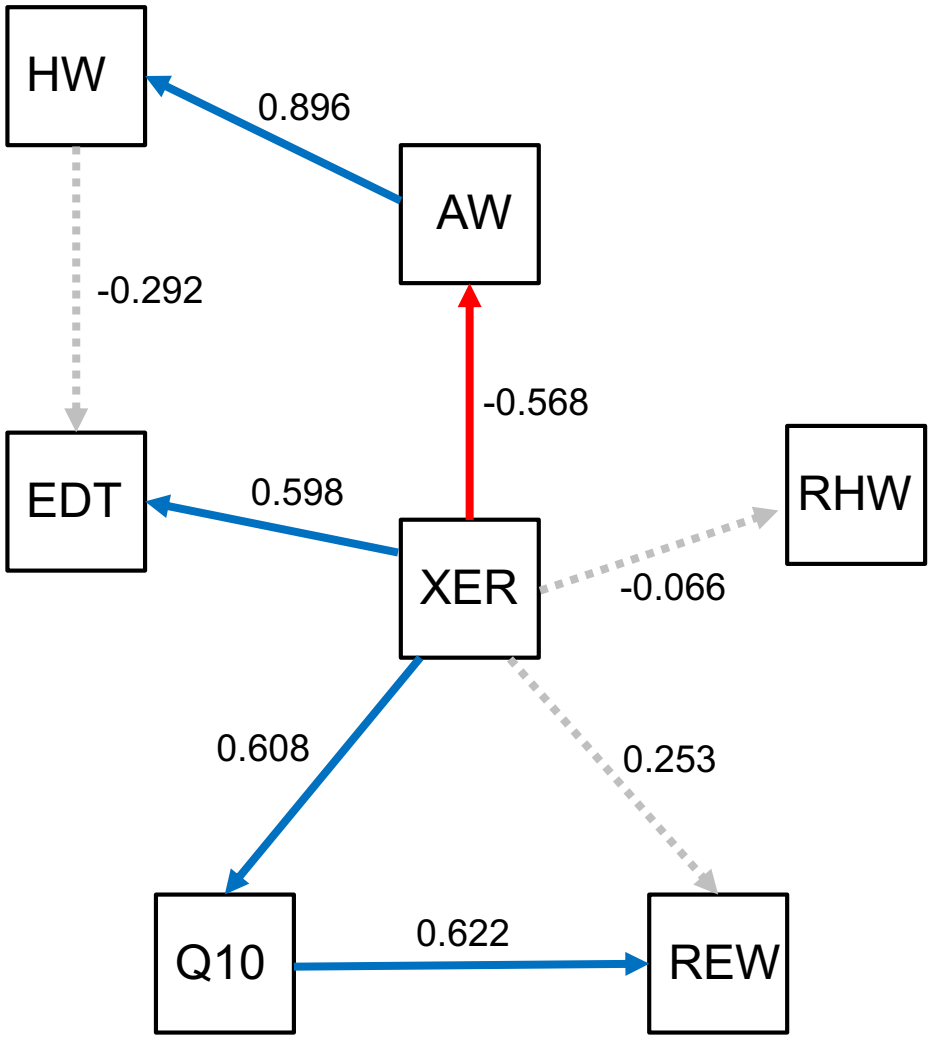


Figure 3: Piecewise structural equation model showing relationships and interactions between embryonic characteristics, adult size and mesoclimatic requirements (using phylogenetically independent contrasts). Arrows show direction of relationship. Blue colour

represents significantly positive relationships, red colour represents significantly negative relationships and dashed grey lines represent non-significant relationships. Numbers represent regression coefficient values (positive values indicate positive relationships). Abbreviations used for investigated variables are as follow: XER – species xerothermophility (based on the xerothermophility index), EDT – embryonic developmental time at 22.62°C, Q10 – temperature sensitivity, AW – adult weight, HW – hatchling weight, RHW – relative hatchling weight, REW – residual egg weight.

Hatchling performance

Average hatching rate across species was approximately 42%. The highest hatching rate was recorded in *Pseudochorthippus parallelus* (77%) and the lowest hatching rate was recorded in *Stenobothrus lineatus* (6%). Detailed results are shown in Table S2. Hatching rate was significantly higher at 22.62°C compared to some other temperatures (One-way ANOVA: df = 3, F = 4.424, p = 0.009). Tukey post-hoc tests showed that hatching rate at 22.62°C was significantly higher compared to 15.82°C (p = 0.019) and 26.64°C (p = 0.015), whereas, there was no significant difference between 22.62°C and 31.99°C (p = 0.155; Figure 4a). There was no significant effect of temperature on hatchling weight across investigated species (One-way ANOVA: df = 3, F = 0.049, p = 0.985; Figure 4b). Additionally, there was no significant effect of species xerothermophility on hatching rate nor hatchling weight across all temperature treatments (in all linear models p > 0.05).

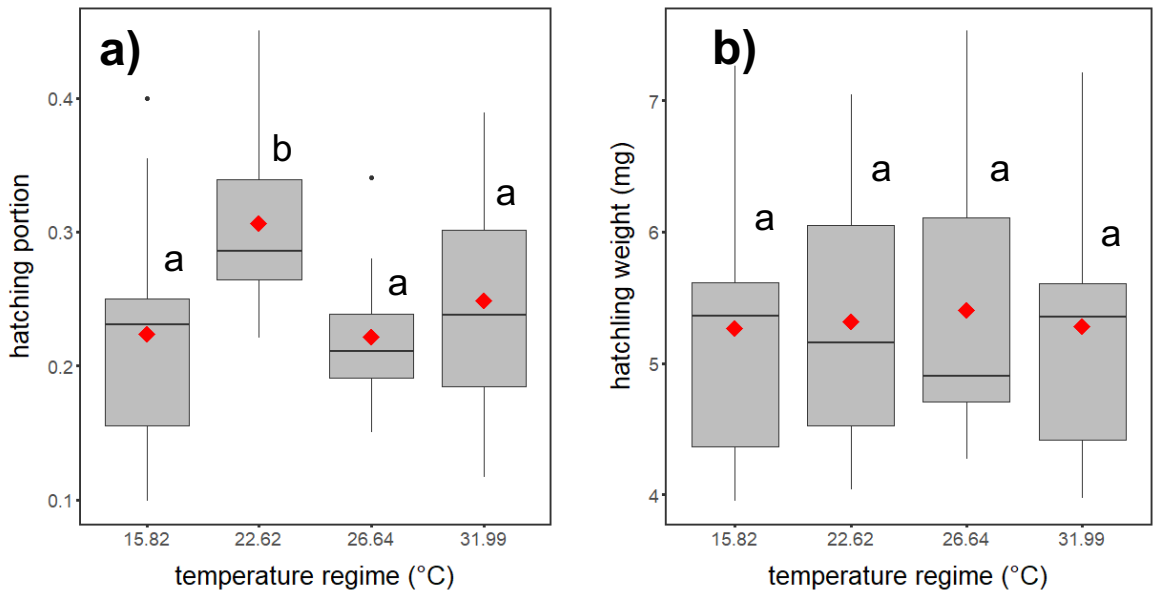


Figure 4: Effect of temperature on hatching rate portion a), and hatching weight b) Letters show differences between temperature regimes according to Tukey post-hoc tests. Coloured parts of boxplots indicate first and third quartile boundaries and internal horizonal line the median value. Red rhombus marks the average value.

Discussion:

An effect of environmental temperature on developmental rates and coupled size traits in ectotherms, both on intra- and interspecific scope, is routinely investigated along large-scale altitudinal or latitudinal gradients. Surprisingly, there is very limited information about the effect of mesoclimatic conditions on above mentioned traits. In our study we shown that in species occupying the same region with similar macroclimatic conditions their embryonic developmental time, temperature sensitivity and various size parameters are significantly affected by their mesoclimatic requirements.

In this study we found significant prolongation of embryonic developmental time in species preferring hot and dry microhabitat across relevant incubation temperatures. Existing evidence on intraspecific plasticity in embryonic developmental time related to climate confirms shorter embryonic development in mountain populations of various ectotherms (Berner et al. 2004; Roff 1980; Dingle and Mosseau 1994; Srygley 2020) or populations from higher latitudes (Hao et al., 2021), which is analogous to our findings for local mesoclimatic effects and interspecific scope. In addition, more xerothermophilous species from our study had higher thermal sensitivity (Q10) and thus their embryonic development time prolonged with decreasing temperature. Anecdotical older studies received comparable results. Wingerden (1991) found higher Q10 in xerothermophilic grasshoppers, however, he only used arbitrary sorting of species without real quantification of species mesoclimatic requirements and only few xerothermophilosu species were used in this study. Relatively slower development at lower temperature was discovered in lowland populations in the intrapecific study focused on *Rana temproraria* frogs from different altitudes (Beattie, 1987)

However, developmental rate patterns can be affected by confounding factors like target body size or weight gain of given species or population. Surprisingly, embryonic developmental time and its thermal sensitivity were not affected by species hatchling weight in our study, which is in contrast with expectations resulting from metabolic theory of ecology

(Brown et al., 2004). One possible limitation of our study is quite low variability in body size, especially hatchling weight, of chosen species. To confirm that the xerothermophility effect is really much stronger than the size effect, a future study based on larger number of species including those with extremely small or large hatchlings is need. However, our dataset include species with strongly varying in residual egg weight. It is quite common pattern in ectotherm animals that egg weight is not tightly linked with hatchling weight (Bosch & Bout, 1998; Wallace et al., 2006). We found out that residual egg weight was significantly positively affected by species thermal sensitivity, i.e, species strongly changing their embryonic developmental rate with temperature tends to have a more surplus egg weight. Despite species thermal sensitivity is strongly linked to their mesoclimatic requirements (xerothermophility), we did not observed direct effect of species xerothermophility on residual egg weight. This is surprising as it is expected that eggs of species more sensitive to microclimatic extremes need larger volume for better buffering capacity (Atkinson and Begon 1987; Sota and Mogi 1992; Hesran et al. 2019). Another factor that could obscure relationship between residual egg weight and species mesoclimatic requirements can be different species phenology. Eggs of species laying earlier in the season have to overcome more dry and warm periods (heatwaves) prior to overwintering than eggs of late laying species and thus need a larger buffering capacity, i.e., residual egg weight. This hypothesis needs further testing and especially a detailed information about grasshopper phenology. Additionally, measurement of egg's weight at the time of oviposition and direct examination of water contents needed for better evaluation of buffering hypothesis in grasshoppers.

Mesoclimatic preferences appeared to affect not only embryonic and hatchlings parameters, but also adult size. Our study system follows Bergmann's rule as adult size is significantly increasing in species inhabiting colder mesoclimate. This finding is less typical for insect, where inverse Bergmann's rule is more common (Colombo and Remis 2015; Marshall 2018), especially in those with univoltine life cycle and living in seasonal climate (Dingel and Mosseau 1994; Berner and Blackenhorn 2006). Adult size can be subjected to diverse physiological, ecological and evolutionary selection pressures and constraints and further investigation of other potentially contributing factors can help to uncover how big portion of adult size is caused by mesoclimatic adaptation. For example, despite our selected species were relatively closely related, they possess very different alar states form low mobile brachypterous species to fully macropterous and very agile ones. Trade-off between mobility

and fecundity (and resulting size differences) is well known phenomenon and can definitely play a role (Gibbs et al., 2010).

Suboptimal temperature during embryonic development can have further effect on larvae and adult fitness and one can expect that species with various mesoclimatic requirements will respond differently on these temperatures (Potter et al., 2011; Zhang et al., 2014; Quan et al., 2023). However, we didn't found any effect of incubation temperature on hatchling weight, including no effect of xerothermophility, which can be a sign of unaffected hatchling fitness. Similar finding are presented for fish (Karjalainen et al. 2015) or leafhoppers (Chuche & Thiéry, 2012). In moth *Manduca sexta* there were differences in hatchling weight according to incubation temperature, but the effect disappeared during larval development and no effect on subsequent fitness was found (Potter et al. 2011).

Our results are partially in concordance with general theory which states the highest hatching success at optimal temperature and decreasing to the both temperature extremes (e.g. Kime et al. 2006; Keena 2006; Garcia 2011; Chapman et al. 2013). However, we didn't find decreasing hatching success in the highest temperature regime 31.99°C. Probably, because we didn't get close enough to the upper thermal limit of embryos in our species studied, as grasshoppers are generally very heat tolerant (Uvarov, 1966). Interestingly, in the study of Wingerden (1991), the most of grasshopper species shown decreased hatching success at 32°C. But it can be consequence of adaptation on the more oceanic climate (as he studied populations from norther Germany and Netherland) and subsequent trade-off between better utilisation of lower temperatures versus survivability in the high temperature as discussed above.

Studying mesoclimatic preferences proved to be a potent way to partially explain embryonic developmental time and its intricated interplay with some size traits in temperate grasshoppers. In previous study we shown that mesoclimatic preferences can also affect oviposition preferences (Dvořák & Knapp, 2023). Nevertheless, there are still huge gaps in our knowledge, particularly potential interplay with macroclimatic parameters or plasticity within species areals. We also definitely have to examine more species to confirm found trends and to develop more complex models with more parameters enabling better understanding of how exactly mesoclimatic preferences affect life histories of grasshoppers.

References:

Arendt JD (2011) SIZE-FECUNDITY RELATIONSHIPS, GROWTH TRAJECTORIES, AND THE TEMPERATURE-SIZE RULE FOR ECTOTHERMS: SIZE-FECUNDITY RELATIONSHIPS AND THE TEMPERATURE-SIZE RULE. Evolution 65:43–51.

Beattie RC (1987) The reproductive biology of Common frog (*Rana temporaria*) populations from different altitudes in northern England. Journal of Zoology 211:387–398.

Berger D, Walters R & Gotthard K (2008) What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly. Functional Ecology 22:523–529.

Berner D & Blanckenhorn WU (2006) Grasshopper ontogeny in relation to time constraints: adaptive divergence and stasis. Journal of Animal Ecology 75:130–139.

Berner D, Körner C & Blanckenhorn WU (2004) Grasshopper populations across 2000 m of altitude: is there life history adaptation? Ecography 27:733–740.

Blanckenhorn WU (2004) Bergmann and Converse Bergmann Latitudinal Clines in Arthropods: Two Ends of a Continuum? Integrative and Comparative Biology 44:413–424.

Bonebrake TC, Boggs CL, McNally JM, Ranganathan J & Ehrlich PR (2010) Oviposition behavior and offspring performance in herbivorous insects: consequences of climatic and habitat heterogeneity. Oikos 119:927–934.

Bosch HAJ & Bout RG (1998) Relationships between Maternal Size, Egg Size, Clutch Size, and Hatchling Size in European Lacertid Lizards. Journal of Herpetology 32:410.

Brown JH, Gillooly JF, Allen AP, Savage VM & West GB (2004) TOWARD A METABOLIC THEORY OF ECOLOGY. Ecology 85:1771–1789.

Chapman RF, Simpson SJ & Douglas AE (2013) The insects: structure and function. Cambridge University Press, New York.

Church SH, Donoughe S, de Medeiros BAS & Extavour CG (2019) Insect egg size and shape evolve with ecology but not developmental rate. Nature 571:58–62.

DeLiberto AN, Drown MK, Ehrlich MA, Oleksiak MF & Crawford DL (2022) Feeling the heat: variation in thermal sensitivity within and among populations. Journal of Experimental Biology 225:jeb244831.

Di Martino E & Liow L (2021) Larger offspring associated with lower temperatures across species of Microporella, a widespread colonial invertebrate. Marine Ecology Progress Series 662:1–13.

Dingle H & Mousseau TA (1994) Geographic variation in embryonic development time and stage of diapause in a grasshopper. Oecologia 97:179–185.

Dvořák T, Hadrava J & Knapp M (2022) The ecological niche and conservation value of Central European grassland orthopterans: A quantitative approach. Biological Conservation 265:109406.

Dvořák T & Knapp M (2023) Conserved temperature requirements but contrasting responses to humidity across oviposition preferences in temperate grasshoppers. Scientific Reports 13:21131.

Eckerström-Liedholm S, Sowersby W, Gonzalez-Voyer A & Rogell B (2017) Time-limited environments affect the evolution of egg–body size allometry. Evolution 71:1900–1910.

Fox CW & Czesak ME (2000) Evolutionary Ecology of Progeny Size in Arthropods. Annual Review of Entomology 45:341–369.

García-Navas V, Noguerales V, Cordero PJ & Ortego J (2017) Ecological drivers of body size evolution and sexual size dimorphism in short-horned grasshoppers (Orthoptera: Acrididae). Journal of Evolutionary Biology 30:1592–1608.

Gibbs M, Breuker CJ, Hesketh H, Hails RS & Van Dyck H (2010) Maternal effects, flight versus fecundity trade-offs, and offspring immune defence in the Speckled Wood butterfly, Pararge aegeria. BMC Evolutionary Biology 10:345.

Gillooly JamesF, Charnov EL, West GB, Savage VM & Brown JH (2002) Effects of size and temperature on developmental time. Nature 417:70–73.

Hao S, Liu C, Ma C, Guo W & Kang L (2021) Embryonic Development of Grasshopper Populations Along Latitudinal Gradients Reveal Differential Thermoaccumulation for Adaptation to Climate Warming. Frontiers in Ecology and Evolution 9:736456.

Hochkirch A & Gröning J (2008) Sexual size dimorphism in Orthoptera (*sens. str* .) — a review. Journal of Orthoptera Research 17:189–196.

Honěk A (1993) Intraspecific Variation in Body Size and Fecundity in Insects: A General Relationship. Oikos 66:483.

Horne CR, Hirst AndrewG & Atkinson D (2015) Temperature-size responses match latitudinal-size clines in arthropods, revealing critical differences between aquatic and terrestrial species (B Enquist, Ed. by). Ecology Letters 18:327–335.

Horne CR, Hirst AG & Atkinson D (2018) Insect temperature–body size trends common to laboratory, latitudinal and seasonal gradients are not found across altitudes (JR Bell, Ed. by). Functional Ecology 32:948–957.

Hutchings JA (2021) A Primer of Life Histories: Ecology, Evolution, and Application. Oxford University Press.

Ingrisch S & Köhler G (1998) Die Heuschrecken mitteleuropas. Die Neue Brehm-Bücherei, Magdeburg.

Kingsolver JG & Huey RB (2008) Size, temperature, and fitness: three rules. Evolutionary Ecology Research:251–268.

Knapp M & Nedvěd O (2013) Gender and Timing during Ontogeny Matter: Effects of a Temporary High Temperature on Survival, Body Size and Colouration in Harmonia axyridis (SA White, Ed. by). PLoS ONE 8:e74984.

Kočárek P, Holuša J, Vlk R & Marhoul P (2015) Rovnokřídlí České republiky. Academia, Praha.

Leingärtner A, Krauss J & Steffan-Dewenter I (2014) Species richness and trait composition of butterfly assemblages change along an altitudinal gradient. Oecologia 175:613–623.

Marshall DJ, Pettersen AK & Cameron H (2018) A global synthesis of offspring size variation, its eco-evolutionary causes and consequences. Functional Ecology:1436–1446.

Moriarty F (1970) The significance of water absorption by the developing eggs of five British Acrididae (Saltatoria). Comparative Biochemistry and Physiology 34:657–669.

Pincheira-Donoso D & Hunt J (2017) Fecundity selection theory: concepts and evidence: Fecundity selection. Biological Reviews 92:341–356.

Potter KA, Davidowitz G & Woods HA (2011) Cross-stage consequences of egg temperature in the insect Manduca sexta. Functional Ecology 25:548–556.

Quan Y, Mason CE, He K, Wang Z & Wei H (2023) Impact of heat waves on egg survival and biological performance across life stages in the Asian corn borer. Entomologia Experimentalis et Applicata 171:129–137.

Riesch R, Martin RA, Diamond SE, Jourdan J, Plath M & Brian Langerhans R (2018) Thermal regime drives a latitudinal gradient in morphology and life history in a livebearing fish. Biological Journal of the Linnean Society 125:126–141.

Rollinson N & Rowe L (2016) The positive correlation between maternal size and offspring size: fitting pieces of a life-history puzzle. Biological Reviews 91:1134–1148.

RStudio Team (2023) RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Tolazs R, Míková T, Valeriánová A & Voženílek V (2007) Climate Atlas of Czechia. Tolasz, Radim a kol. Praha-Olomouc: Český hydrometeorologický ústav, Univerzita Palackého v Olomouci.

Uvarov B (1966) Grasshoppers and Locusts: A handbook of general acridology. Cambridge University Press, Cambridge.

Van Wingerden WKRE, Musters JCM & Maaskamp FIM (1991) The influence of temperature on the duration of egg development in West European grasshoppers (Orthoptera: Acrididae). Oecologia 87:417–423.

Wallace BP, Sotherland PR, Tomillo PS, Bouchard SS, Reina RD, Spotila JR & Paladino FV (2006) Egg components, egg size, and hatchling size in leatherback turtles. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 145:524–532.

Warkentin KM (2011) Environmentally Cued Hatching across Taxa: Embryos Respond to Risk and Opportunity. Integrative and Comparative Biology 51:14–25.

Wonglersak R, Fenberg PB, Langdon PG, Brooks SJ & Price BW (2020) Temperature-body size responses in insects: a case study of British Odonata. Ecological Entomology 45:795–805.

Yamahira K & Conover DO (2002) INTRA- VS. INTERSPECIFIC LATITUDINAL VARIATION IN GROWTH: ADAPTATION TO TEMPERATURE OR SEASONALITY? Ecology 83:1252–1262.

Zhang S, Cao Z, Wang Q, Zhang F & Liu T-X (2014) Exposing eggs to high temperatures affects the development, survival and reproduction of Harmonia axyridis. Journal of Thermal Biology 39:40–44.

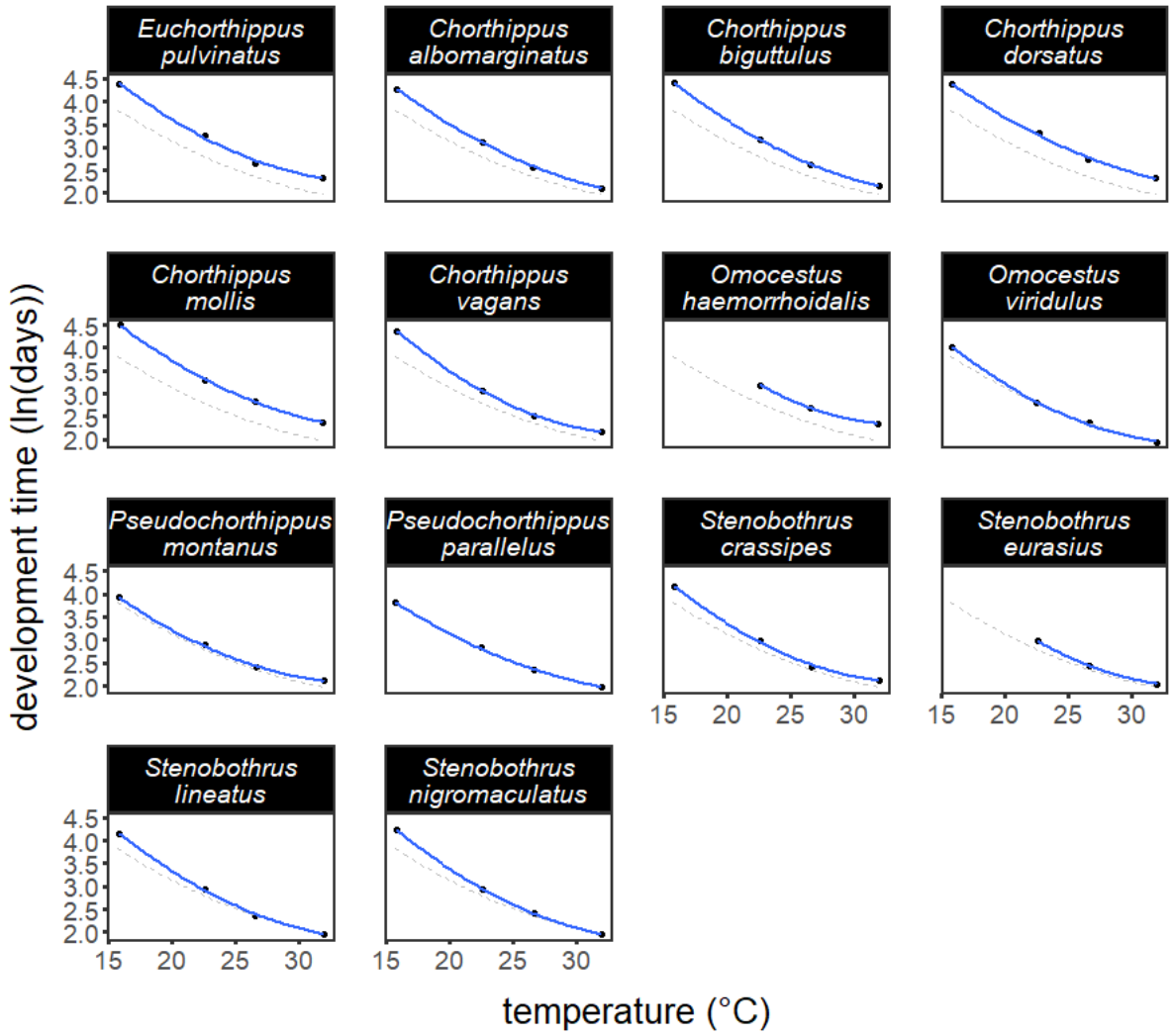


Figure S1: Relationship of natural log transformed development time on temperature in species studied. In each subplot, line representing *Pseudochorthippus parallelus* is added as reference. Note the noticeable differences in intercepts (representing embryonic developmental time at constant temperatures) and partially in slopes (representing temperature sensitivity, Q10) between species.

Table S1: Detail description of species habitat, distribution in Central Europe. Xerothermophility index and photo is also included.

Species	Photo	Xerothermophility index	Habitat description	Collection site	Distribution in Central Europe
Euchorthippus pulvinatus		0.896	Steppe with relatively high but sparse vegetation	*Stipa* spp. dominated steppes in Raná hill in České středohoří, Czech Republic	Rare, Czech Republic is the northwestern limit of its range
Chorthippus albomarginatus		0.336	Moderately wet to wet meadows and pastures, prefers lower vegetation	meadows near Šeberov, Prague, Czech Republic	Abundant from lowlands to lower mountains
Chorthippus biguttulus		0.518	Moderately wet to dry meadows, pastures, steppes; avoids too high and dense vegetation	meadows near Šeberov, Prague, Czech Republic	Very abundant from lowlands to lower mountains

Chorthippus dorsatus		0.375	From slightly dry to wet meadows, tolerates ruderalised high vegetation	meadows near Šeberov, Prague, Czech Republic	Abundant from lowlands to lower mountains
Chorthippus mollis		0.900	Steppes, rocky steppes, dry meadows and pastures with shorter and sparse vegetation	steppes and dry pastures near Radotín, Prague, Czech republic	Quite rare, can be locally abundant in lowlands
Chorthippus vagans		0.960	Dry forest clearings and edges, forest steppe, rocky steppe, prefers minimal vegetation cover	forest clearings in Kunratice, Prague, Czech Republic	Quite rare, occurs from lowlands to hills

Omocestus haemorrhoidalis		0.799	Dry meadows and pastures, heaths, sandy areas; occupies moderately high and not too dense vegetation	Heaths in Troja, Prague, Czech Republic	Locally abundant, from lowlands to lower mountains
Omocestus viridulus		0.109	From moderately wet to wet meadows, alpine grasslands, peat bogs; prefers higher altitudes	Wet meadows near Voznice, Brdy mountains, Czech Republic	Locally abundant, mostly in mountains
Pseudochorthippus montanus		0.021	Wet meadows, sedges	wet meadows near Háje, Prague, Czech Republic	Locally abundant from lowlands to lower mountains

Pseudochorthippus parallelus		0.291	Broad ecological niche, from dry to wet grasslands, only avoid extremely sparse and extremely dry vegetation	meadows near Šeberov, Prague, Czech Republic	Very abundant from lowlands to mountains
Stenobothrus crassipes		0.780	Moderately dry steppes and meadows without too high and dense vegetation	steppes and dry pastures near Radotín, Prague, Czech Republic	Rare, more abundant only in lowlands
Stenobothrus eurasius		1	Extremely dry and exposed rocky steppes with very sparse vegetation cover	Rocky steppes in Oblík hill in České středohoří, Czech Republic	Very rare, Czech Republic is the northwestern limit of its range

Stenobothrus lineatus		0.777	Dry meadows and meadow steppes with not too high vegetation	steppes and dry pastures near Radotín, Prague, Czech republic	Locally abundant from lowlands to lower mountains
Stenobothrus nigromaculatus		0.952	Diverse steppes, needs sparse and short vegetation cover	steppes and dry pastures near Radotín, Prague, Czech Republic	Rare, more abundant only in lowlands

Table S2: Summary of reproductional output of species studied. Twenty females per species were used.

species	Number of laid oothecae	Average number of eggs per ootheca	Estimated total number of laid eggs	Total number of hatched eggs	Egg hatchability (%)
Euchorthippus pulvinatus	42	7.1	300	98	33
Chorthippus albomarginatus	51	6.7	341	226	66
Chorthippus biguttulus	160	7.5	1197	396	33
Chorthippus dorsatus	145	7.4	1070	317	30
Chorthippus mollis	54	8.1	435	271	62
Chorthippus vagans	92	7.7	710	246	35
Omocestus haemorrhoidalis	10	6.2	62	36	58
Omocestus viridulus	189	7.2	1359	411	30
Pseudochorthippus montanus	41	6.0	248	145	58
Pseudochorthippus parallelus	41	6.3	260	200	77
Stenobothrus crassipes	80	3.2	258	100	39
Stenobothrus eurasius	26	4.6	120	45	37
Stenobothrus lineatus	183	5.6	1019	62	6
Stenobothrus nigromaculatus	79	5.5	436	110	25

Study IV

Dvořák, T., Pecharová, M., Krzeminski, W., & Prokop, J. (2019). New archaeorthopteran insects from the Carboniferous of Poland: Insights into tangled taxonomy. *Acta Palaeontologica Polonica*, 64(4).

New archaeorthopteran insects from the Carboniferous of Poland: Insights into tangled taxonomy

TOMÁŠ DVOŘÁK, MARTINA PECHAROVÁ, WIESŁAW KRZEMIŃSKI, and JAKUB PROKOP



Dvořák, T., Pecharová, M., Krzemiński, W., and Prokop, J. 2019. New archaeorthopteran insects from the Carboniferous of Poland: Insights into tangled taxonomy. *Acta Palaeontologica Polonica* 64 (4): 787–796.

Archaeorthoptera is a high rank insect taxon comprising Orthoptera as well as the extinct orders Titanoptera and Caloneurodea, and several other late Paleozoic groups formerly assigned to polyphyletic Protorthoptera. Synapomorphies defining Archaeorthoptera and some fossil subordinate taxa are exclusively based on wing venation. This study presents a detailed description of two new archaeorthopteran genera and three new species from the Pennsylvanian of the Upper Silesian Coal Basin in Poland. These new taxa provide new insights into the wing venation disparity of this remarkable and insufficiently studied insect group. *Omaliella polonica* sp. nov. is based on a well preserved forewing, including the wing base, which allows a thorough discussion and comparison with other archaeorthopterans. Surprisingly, it is the first complete wing for this group of related genera (*Omaliella*, *Omalia*, *Coselia* and *Paleomastax*). *Owadpteron dareki* gen. et sp. nov. has an unusual arrangement of cubital veins. The marked resemblance of the venation of *Owadpteron* to that of some members of the gerarid line, such as *Nacekomia*, supports its placement within the family Geraridae (stem-group Orthoptera). Finally, the venation of *Parapalaeomastax dariuszi* gen. et sp. nov. strikingly resembles that of the genus *Palaeomastax*, differing only in the distally branched media. Discovery of these three new archaeorthopterans from the Upper Silesian Coal Basin fits well with that of closely related taxa known from other deposits in Euramerica, such as Mazon Creek Lagerstätte, Avion in Pas-de-Calais Basin and others. Furthermore, a new re-examination of the earliest archaeorthopteran from the Upper Silesian Coal Basin confirms doubtful assignment of this fragmentary fossil to Archaeorthoptera or even to Pterygota.

Key words: Insecta, Archaeorthoptera, Polyneoptera, wing venation, Pennsylvanian, Poland, Upper Silesia.

Tomáš Dvořák [algalesie@seznam.cz], Martina Pecharová [pecharom@natur.cuni.cz] and Jakub Prokop [jprokop@natur.cuni.cz] Department of Zoology, Faculty of Science, Charles University, Viničná 7, CZ-128 00, Praha 2, Czech Republic.
Wiesław Krzemiński [wieslawk4@gmail.com] Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, ul. Sławkowska 17, 31-016, Kraków, Poland.

Received 5 March 2019, accepted 13 May 2019, available online 9 October 2019.

Copyright © 2019 T. Dvořák et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License (for details please see http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

The super-order Archaeorthoptera was established on the basis of its wing venation, with main apomorphies in the form of the complex fusions of the veins in medio-cubital area (Béthoux and Nel 2002, 2005). It consists of some taxa previously assigned to the polyphyletic group Protorthoptera listed by Carpenter (1992) and taxa assigned to the orders Orthoptera, Titanoptera, and Caloneurodea. However, the interrelationships within different groups of Archaeorthoptera remain poorly clarified. First, we can clearly separate the large group Panorthoptera (Béthoux and Nel 2002). While the assignments of some taxa to this group are still under debate, Panorthoptera consists of three main orders: Orthoptera, Caloneurodea, and Titanoptera, plus the gerarid line (Béthoux et al. 2012). The crucial diagnostic character of this group is the basal bifurcation of the CuPa vein into CuPaα and CuPaβ (Béthoux and Nel 2002). Aristov (2014) considered the placement of Geraridae among Gryllones and pointed out the resemblance of the venation to the family Cheliphlebiidae. However, the same author is not following the widely accepted concept of Archaeorthoptera and Panorthoptera (Béthoux and Nel 2002). Other established groups of Archaeorthoptera are Protophasmida (which is considered to be the most basal clade), Cnemidolestodea and Lobeattida (Béthoux 2005; Béthoux et al. 2012). However, there are many taxa that clearly belong to Archaeorthoptera, but can not be assigned to any of these high rank groups (Béthoux and Nel 2005; Béthoux 2008). Even the monophyly and validity of the

above mentioned taxa is still under debate as well as the homology and nomenclature of the veins of Archaeorthoptera, unlike the situation in other groups of Pterygota (see Rasnitsyn 2007, Desutter-Grandcolas et al. 2017; Li et al. 2018). This complicates the fact that some recent studies of certain authors avoid to use binomen for species in comparative discussion and follow the ICZN rules only formally in supplementary parts of their articles (e.g., Béthoux 2008; Béthoux and Schneider 2010). This practice is confusing and makes future cross generic comparison with studies accepting traditional Linnaean hierarchical classification of organisms more difficult.

Herein, we provide descriptions of two new fossil genera and three new species based on the pattern of their forewing venation. The first new species is based on an exceptionally well preserved holotype, which reveals the fine structural details of this genus. At the same time, this complete fossil demonstrates problems with previously described species based on very fragmentary wings on which sometimes even the crucial characters of higher taxa are missing. All specimens used in this study came from two Pennsylvanian deposits: Leszczyny-Czerwionka nearby Knurów and Sosnowiec-Klimontów, both situated in Upper Silesia, Poland. Moreover, we provide re-evaluation to the earliest archaeorthopteran known from the Upper Silesian Coal Basin.

Institutional abbreviations.—FM, The Field Museum of Natural History, Chicago, USA; MGL, Musée Géologique de Lille, Lille, France; MNHN, Muséum national d'Histoire naturelle, Paris, France; MP ISEA, Natural History Museum of the Institute of Systematics and Evolution of Animals, Polish Academy of Science, Cracow, Poland; NHM, The Natural History Museum, London, UK; RBINS, Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

Other abbreviations.—The wing venation concept and nomenclature follows that of Béthoux and Nel (2002) as established for Archaeorthoptera. 1A/2A, first/second anal vein; CuA/P, cubital anterior/posterior; CuPa/b, anterior/posterior branch of CuP; CuPaα/β cubital anterior/posterior branch of CuPa; M, stem of media; MA/P, medial anterior/posterior; RA/P, radial anterior/posterior; ScA/P, subcostal anterior/posterior.

Nomenclatural acts.—This published work and the nomenclatural acts it contains, have been registered in ZooBank: urn:lsid:zoobank.org:pub:758133BE-4660-40AF-A939-580B2F1D2E2B

Material and methods

The Carboniferous insects from localities in the Upper Silesian Coal Basin have been studied for more than a century (e.g., Roemer 1883; Handlirsch 1906). Most of the discoveries were linked with the intensive mining of coal in the past and only a few sites are currently accessible as spoil–heaps. One of them situated in the Polish part of the basin is Sosnowiec-Klimontów with material originally excavated from the Porąbka-Klimontów coal mine (e.g., Krawczyński et al. 1997). Fossils are preserved in sphero-sideritic concretions with 3D relief and include various groups of arthropods, molluscs, vertebrates and most commonly plant remains (Krawczyński et al. 2001, Stworzewicz et al. 2009, Pacyna and Zdebská 2010). The entomofauna consists predominantly of paoliids, palaeodictyopteran nymphs and their exuvia, followed by sparsely recorded other groups like Archaeognatha and Archaeorthoptera (Prokop et al. 2012, 2014, 2017, 2019).

The second locality Leszczyny-Czerwionka is situated west of Katowice and is especially well known for its fossil flora with a large accumulation of drifted long logs mainly attributable to Cordaites (Gradziński et al. 1982).

The fossils were studied in a dry state or under a film of ethyl alcohol using Leica MZ12.5 and Nikon SMZ 745 stereomicroscopes. Line drawings of the venation of both specimens were made directly with the aid of a camera lucida. Photographs were taken using a Canon D550 digital camera with EF 50mm and MP-65mm lenses. Original photographs were processed using the image-editing software Adobe Photoshop CS.

Systematic palaeontology

Super-order Archaeorthoptera Béthoux and Nel, 2002

Genus *Omaliella* Béthoux and Nel, 2005

Type species: *Omaliella ramosa* Béthoux and Nel, 2005, Pas-de-Calais Basin, France, Duckmantian (Westphalien B), Pennsylvanian.

Omaliella polonica sp. nov.

Fig. 1.

ZooBank LCID: urn:lsid:zoobank.org:pub:D0E08357-DADB-42EA-9E91-A75C0A4DE748

Etymology: From Latin *Polonia*, Poland.

Holotype: MP ISEA I–F/MP/8/1676/17 (imprint of forewing).

Type locality: Leszczyny-Czerwionka nearby Knurów, Rybnik, and Gliwice in Upper Silesia; found on the slag heaps that dates back to 19th century (Gradziński et al. 1982).

Type horizon: Carboniferous, Pensylvannian, Duckmantian (Westphalian B), Coal-bearing Mudstone Series, Orzesze Beds (Gradziński et al. 1982).

Diagnosis.—Based on fore-wing venation: width of costal area about midwing similar to subcostal area, ScP ending on anterior wing margin two thirds along wing length; RP partially connected with MA1 ending only with a terminal twig, anterior branch of RP distally fused with simple RA; CuA+CuPa extensively developed and posteriorly pectinate with 11 primary branches, basal branch distinctly undulated, CuPb simple.

Measurements.—Wing length 64.5 mm, maximum width 22.3 mm (about the level of division MA and MP).

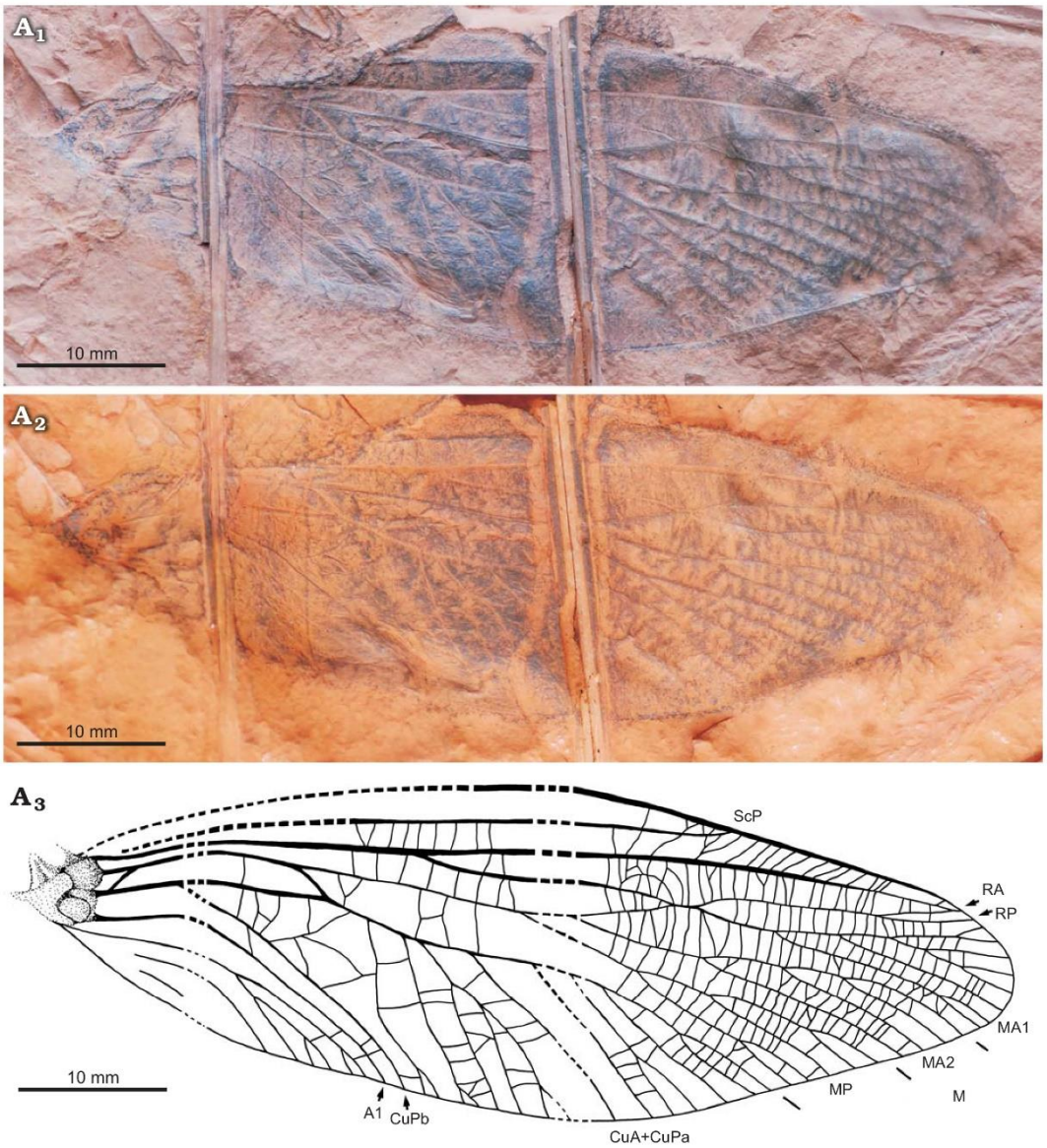


Fig. 1. Forewing venation of archaeorthopteran insect *Omaliella polonica* sp. nov., holotype (MP ISEA I–F/MP/8/1676/17), Carboniferous, Pensylvannian, Duckmantian (Westphalian B), Leszczyny-Czerwionka nearby the Knurów, Upper Silesia, Poland. Photograph in dry state (A_1), under a film of ethanol (A_2), and explanatory drawing (A_3). Abbreviations: A1, first anal vein; CuA, cubital anterior; CuPa/b, anterior/posterior branch of cubital posterior; M, media; MA/P, media anterior/posterior; RA/P, radius anterior/posterior; ScP, subcosta posterior.

Description.—Based on fore-wing venation. Nearly complete wing broadest approximately in the middle with originally hyaline membrane and numerous transverse or slightly oblique cross veins; ScA unknown because the basal anterior part of wing is not preserved; proximal part of ScP incomplete due to preservation; ScP reaches anterior wing margin in two thirds along the length of the wing, without discernible anterior branches; space between ScP and costal

margin of wing quite narrow, about the same width as space between ScP and RA in the middle of the wing; R strongly convex, division of RA and RP 22 mm from wing base, RA simple, running parallel with ScP, terminally fused with anterior branch of RP very close to anterior wing margin; RP slightly concave and almost simple, partially connected to MA for 1.9 mm, ending with short terminal twig; stem of M shortly behind the wing base connected to CuA for 11.9 mm, stem of M concave divided into MA and MP in about mid-wing; MA deeply separated into MA1 and MA2 shortly behind the division with MP; MA1 shortly connected to RP; MA1 and MA2 both pectinate ending with 4 main posterior branches; third branch of MA1 secondarily pectinate anteriorly ending with three short branches; MP ending with three main posterior branches, first and third branch with secondary twigs; division of CuA and CuP very close to wing base, CuA+CuPa pectinate with 8 main posterior branches; area between CuPa and CuPb with irregular network of veinlets; CuPb and A1 both simple and closely parallel to one another.

Remarks.—The present fossil can be assigned to the genus *Omaliella* based on the following combination of diagnostic forewing characters: RP long before it fuses with MA1, RP fused with MA1, CuA+CuPa posteriorly pectinate ending with about main 11 terminal branches. However, the type species of the genus *Omaliella* is based on the poorly preserved fossil of *O. ramosa* Béthoux and Nel, 2005, which does not have even the main apomorphies of the Archaeorthoptera. Therefore, it is necessary to discuss the assignment of *Omaliella polonica* sp. nov. and convincingly support the placement of *Omaliella* in the Archaeorthoptera.

We can assign *Omaliella polonica* sp. nov. to Archaeorthoptera sensu Béthoux and Nel, 2002 on the basis of the following apomorphies: basal division of CuP into CuPa and CuPb, fusion of M and CuA and fusion of distal part of CuA with CuPa. We can exclude its placement in clade Cnemidolestodea due to absence of anteriorly pectinate CuA+CuPa and the presence of a well-developed MP independent of CuA+CuPa (Béthoux 2005). Based on the non-differentiated vein CuPa (branches CuPaα and CuPaβ) the attribution to the clade Panorthoptera is also excluded (Béthoux and Nel 2002). We can also exclude the placement among lobeattid insects because of the more distal bifurcation of R with respect to the end of AA1 on posterior wing margin and quite wide space between RA and RP (Béthoux 2008). The apparently non-developed AP area also prompt us to exclude the assignment of *O. polonica* sp. nov. to Protophasmida neither (Béthoux 2003). Hence, *O. polonica* sp. nov. cannot be placed in any of the above mentioned higher clades of Archaeorthoptera.

Currently, there is no phylogenetic analysis of Archaeorthoptera that we could simply follow and therefore we avoid creating a new high level taxon. The placement of *O. polonica* sp. nov. is based on shared characters that could be indicative of the relationships of the closest taxa. Another problem is fragmentary preservation of some previously described taxa of *Omaliella*, which make it impossible to compare all the potentially important characters. Generally, we focused on the middle part of the forewing, which is usually well preserved in all the mentioned genera. So, we will compare and discuss these all significant characters of these relevant genera.

In the venation of *O. polonica* sp. nov. there is a prominent connection between RP and MA1 shared by for instance Geraridae (stem group of Panorthoptera) (Béthoux and Nel 2003) and some species of lobeattids (Béthoux 2005, 2008; Béthoux et al. 2012). It also resembles the situation in *Gerarus* Scudder, 1885, especially *Gerarus fisheri* (Brongniart, 1885) (MNHN-LP-R.51139), as in both RP and MA1 are partially fused (instead of the much commoner well separated MA), RP, MA, and MP have fewer branches and the region of CuA+CuPa is quite well developed. But *Gerarus fisheri* displays marked differences in venation as RA branched, connection between RP and MA is present only in some specimens, branching pattern of CuA+CuPa differs and furthermore CuPb with branches. Short fusion of RP and MA1 is also shared with *Miamia* Dana, 1864, of which the species *M. maimai* Béthoux, Gu, Yue, and Ren, 2012, is assigned to the lobeattids (Béthoux et al. 2012). But in this species there is only a weakly developed CuA+CuPa region and the pattern in the area CuA+M also differs.

Some other lobeattids have fussion of RP and MA instead of RA and MA1. *Sinopteron* with *Sinopteron huangheense* Prokop and Ren, 2007 has a posteriorly branched CuA+CuPa vein, and veins RP and MA are shortly connected by a short cross vein (Prokop and Ren 2007). *Chenxiella* with *Chenxiella liuae* shares also posteriorly branched CuA+CuPa vein, but MA is shortly connected to RP (Liu et al. 2009). However, the latter genus differs from *O. polonica* sp. nov. by a more basal division of RA and RP and richly branched RP. *Longzhua* with *Longzhua loculata* Gu, Béthoux, and Ren, 2011 has a short fusion of RP and MA and well developed CuA+CuPa region (Gu et al. 2011). On the other hand, R is branched more basally, RP is again richly branched and the forewing is distinctly smaller than that of *Omaliella polonica* sp. nov.

However, *Omaliella polonica* sp. nov. shares most traits with the following unassigned genera: *Omalia* Van Beneden and Coemans, 1867, *Coselia* Bolton, 1922, *Paleomastax*, and *Omalliela*. The main common difference is the connection of RP with MA instead MA1. Another difference in *Omalia macroptera* (RBINS a7687) is the non-regular branching pattern of CuA+CuPa. *Omalia* sp. (specimen MGL 4217) has a CuA+CuPa with a regular pattern of 5–6 main posterior branches. In this specimen we can also see a regular network of veinlets between CuA+CuPa and CuPb, which differs from that in *O. polonica* sp. nov. in which the irregular network of veinlets and most basal posterior branch of CuA+CuPa undulate. They are unfortunately not preserved in this specimen.

Due to incomplete preservation of the type specimens of *Coselia* and *Paleomastax* we do not know if MA is connected to RP. However, the general pattern of wing vena-

tion corresponds to that of *Omalia* and *Omaliella polonica* sp. nov. The preserved part of the wing of *Coselia palmiformes* (NHM I.15893) has a different branching pattern of CuA+CuPa and regular network of veinlets between CuA+CuPa. *Palaeomastax carbonis* Handlirsch, 1904 (RBINS a7700) has a similar pattern of regular posteriorly branching of veins CuA+CuPa, but the number of branches is unknown due to poor preservation.

The venation of *Omalliela polonica* sp. nov. shares numerous characteristics in the venation with the type species *Omalliela ramosa* Béthoux and Nel, 2005 known from Westphalian B of Pas-de-Calais Basin in France (Béthoux and Nel 2005). The vein RP is connected with MA1, area CuA+CuPa broadly developed and posteriorly pectinate with 10 main branches, unlike 11 branches found in *O. polonica* sp. nov., and simple CuPb. Unfortunately, we do not have any information on the branching patterns of R and M, but MP seems to be only weakly branched, as in *O. polonica*. Unfortunately, the basal part of forewing in *O. ramosa* is unknown. But the general course of the main veins in the type species strongly resembles that in *O. polonica* sp. nov., with the exception of the wider costal area, more basal connection of RP and MA1 and slightly different branching pattern of CuA+CuPa. On the basis of shared characters in their venations and general correspondence, we assign our new species to the genus *Omalliela* as *O. polonica*.

Stratigraphic and geographic range.—S Poland: Upper Silesia, Leszczyny-Czerwionka nearby Knurów; Pensylvannian, Duckmantian (Westphalian B).

Order Panorthoptera Crampton, 1928 (sensu Béthoux and Nel, 2002)

Family ?Geraridae Scudder, 1885

Genus *Owadpteron* nov.

ZooBank LCID: urn:lsid:zoobank.org:pub:38710148-8A61-4200-B0FC-42E0A6FAD893

Type species: *Owadpteron dareki* sp. nov.; by monotypy, see below.

Etymology: From Polish *owad*, insect and Greek *pteron*, wing; neuter in gender.

Diagnosis.—As for the type species by monotypy.

Owadpteron dareki sp. nov.

Fig. 2.

ZooBank LCID: urn:lsid:zoobank.org:pub:3BEC8E31-32AD-4601-8799-256FD9102B23

Etymology: In honour of our colleague and collector of Carboniferous fossils Dariusz Wojciechowski (Darek diminutive of Dariusz).

Holotype: MP ISEA I–F/MP/1488/26a,b/08 (print and counterimprint of forewing).

Type locality: Sosnowiec-Klimontów, originally Porąbka-Klimontów Mine, Upper Silesian Coal Basin, Poland (Krawczyński et al. 1997, 2001).

Type horizon: Langsettian (Westphalian A), Pensylvannian, Mudstone series (Załęże beds), Coal-bearing Mudstone Series (Pacyna and Zdebska 2012).

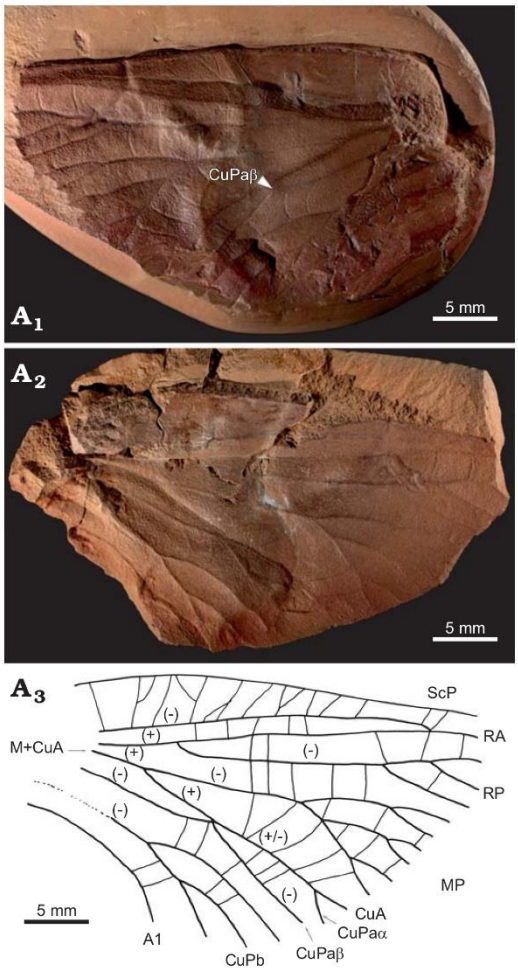


Fig. 2. Forewing venation of archaeorthopteran insect *Owadpteron dareki* sp. nov., holotype (MP ISEA I–F/MP/1488/26a,b/08), Carboniferous, Pensylvannian, Langsettian (Westphalian A), Sosnowiec-Klimontów, Upper Silesian Coal Basin, Poland. Photograph of imprint (A_1) and counterimprint (A_2), and explanatory drawing (A_3). Abbreviations: (+), convex vein; (-), concave vein; A1, first anal vein; CuA, cubital anterior; CuPa/b, anterior/posterior branch of cubital posterior; CuPaα/β, cubital anterior/posterior branch of CuPa; M, media; MP, media posterior; RA/P, radius anterior/posterior; ScP, subcosta posterior.

Diagnosis.—Based on fore-wing venation: broad costal area, RP ending with at least three branches, long stem of M+CuA; MP pectinate, each branch with secondary level branching; area between MP and CuA+CuPaα very broad at widest part and comparable to costal area; point connection of free part of CuA and CuPa; CuPa divides into branch CuPaα continuously attached to CuA and well separated

from branch CuPaβ; CuPb anteriorly branched and ending in at least three branches.

Measurements.—Forewing fragment length 36.4 mm long, estimated forewing length 55 mm, minimum width at widest part 21.7 mm (about the level of contact between CuA and CuPa).

Description.—Based on fore-wing venation. Broad wing incompletely preserved and lacking basal part and apex. Wing membrane originally rather thick with two types of cross veins present. Regular transverse cross veins broadly spaced between main veins occur together with a dense network of fine veinlets. ScA unknown; ScP ending on RA in distal part of wing; broad costal space between anterior margin of wing and ScP markedly narrowing posteriorly, subcostal field regularly narrow; R divides into RA and RP slightly behind the division of M and CuA, RA nearly straight and probably simple, RP distally pectinate ending with at least three branches; common stem of M+CuA present, free part of MP anteriorly richly branched ending with three main branches all secondarily bifurcated, broad space between veins MP and CuA with several straight cross veins, in widest part as wide as costal space; convex CuA diverging from stem of M+CuA proximally from division of RA and RP, one terminal twig of CuA apparent; concave CuPa divides into branch CuPaα continuously attached to CuA and well separated from branch CuPaβ; CuPb anteriorly branched and ending with three branches; A1 simple.

Remarks.—It is not easy to classify this wing due to incomplete preservation and especially the missing wing base. Presence of prominent convex veinal stem located posterior of the apparent strongly convex R, which is further divided into a weakly concave MP and convex CuA, which we consider to be the common stem of M+CuA. The presence of M+CuA is considered as one of the key apomorphies of the Archaeorhoptera (Béthoux and Nel 2002). Another important Archaeorthoptera apomorphy is the basal division of CuP into the branches CuPa and CuPb, which is also present on this wing. However, the free part of CuA does not fuse with CuPa and there is only one point connection between these two veins. This raises the question: was this situation an ancestral state for Archaeorthoptera before the complete fusion of these two veins occurred?

Due to the more distal bifurcation of R with respect to the end of AA1 on posterior wing margin and the presence wide space between branches of RA and RP, the placement to lobeattid taxa is unlikely (Béthoux 2008). The wing venation similarly lacks the typical character of Cnemidolestodea, in which there is an anteriorly pectinate CuA+CuPa (Béthoux 2005). We have no information on the area of AP, which is well developed in Protophasmida (Bethoux 2003), and the general venation in the preserved part differs considerably. So it cannot be placed in Protophasmida. There is also a weak correspondence of the venation with that of the genera *Omalia, Omaliella, Palomastax* and *Coselia*. Potential attribution to the clade Panorthoptera is discussed bellow.

However, the pattern in the venation of our wing resembles that in the monotypic *Ampeliptera*, with *A. limburgica* described from Namurian A in The Netherlands (Pruvost 1927). On both wings there is a dense network of veinlets, posteriorly pectinate RP and anteriorly pectinate MP with bifurcated branches. Even the short fusion of the veins CuA and CuPa is present in both taxa. However, this fusion in *Ampeliptera* is a bit longer. Branched CuPb is also present in both species. Main differences are a narrower space between anterior margin of wing and ScP, and wider space between RA and RP in *Ampeliptera*. The branching pattern of MP is also slightly different. But the missing distal part of wing poses a problem, we do not know the exact branching pattern. Same problem concerns the branching of CuA, but there are presumably fewer branches in *Owadpteron*. Systematic position of *Ampeliptera limburgica* has been debated for long time. Béthoux and Nel (2002), after examining of the holotype, placed *A. limburgica* into the Archaeorthoptera and considered it to be the oldest known member of this clade at that time. This supports our idea about the antiquity of *Owadpteron* based on an incompletely fused CuA and CuPa.

The venation does resemble that of *Nacekomia rossae* described from the Pennsylvanian strata at Mazon Creek, Ilinois (Richardson 1956), which is considered to be a member of the family Geraridae (Kukalová-Peck and Brauckmann 1992; Béthoux and Nel 2002). Both taxa share the same organisation of the cubital area, with CuA ending in only a few branches and a similar shaped CuPb with two anterior branches. The vein CuPa is fused with a free CuA over a short distance in *Nacekomia* (see Fig. 3), whereas in *Owadpteron* it is a point fusion. Nevertheless, this course of the CuPa vein is generally rare in Archaeorthoptera, so we can consider this character to be relatively stable in both taxa. Another resemblance between the venation of both taxa is the wide costal field and sparse but quite regular network of simple thin cross veins. On the other hand, there are some differences, such as ScP ends on RA instead of on the anterior margin of the wing in *N. rossae* and there is a more distal division of the veins RA and RP. The apparent resemblance of the venation to that of *Nacekomia* and the presence of several differences, lead us to assign our fossil to a new genus placed presumably in Geraridae (Panorthoptera). However, the exact taxonomic placement of *N. rossae* is under discussion because of the indistinct differentiation of CuPa into CuPaα and CuPaβ, which is a key apomorphy of Panorthoptera comprised of Geraridae (Béthoux and Nel 2002). Our re-examination of the type of *N. rossae* confirmed that the branches of CuPaα and CuPaβ are markedly concave, which justifies its placement in Panorthoptera (see Fig. 3). The bifurcation of CuPa is not clearly discernible in *Owadpteron*. However, the markedly different polarity of CuA from convex to neutral at its point of contact with CuPa support our notion that CuA continues



Fig. 3. Forewing venation of archaeorthopteran insect *Nacekomia rossae* Richardson, 1956, holotype (FM PE791), Pensylvannian, Moscovian (Westphalian D), Mazon Creek Lagerstätte, Ilinois, USA.

as an anterior branch of CuPaα, while CuPaβ diverges separately. An interpretation that would confirm the placement of *Owadpteron* in Panorthoptera and most probably close to *Nacekomia* (Geraridae). Nevertheless, we need a more complete specimen of *Owadpteron* in order to clarify its taxonomic status.

Stratigraphic and geographic range.—S Poland: Upper Silesia, Sosnowiec-Klimontów; Pensylvannian, Langsettian (Westphalian A).

Genus *Parapalaeomastax* nov.

ZooBank LCID: urn:lsid:zoobank.org:pub:13BC1408-34E6-4553-8714-B7D64A718552

Type species: *Parapalaeomastax dariuszi* sp. nov.; by monotypy, see below.

Etymology: Named after its similarities in venation to the extinct *Palaeomastax* Handlirsch, 1904; feminine in gender.

Diagnosis.—As for the type species by monotypy.

Parapalaeomastax dariuszi sp. nov.

Fig. 4.

ZooBank LCID: urn:lsid:zoobank.org:pub:08E34AA1-C66D-4FAF-A4C1-49F61D87A63B

Etymology: In honour of our colleague and collector of Carboniferous fossils Dariusz Wojciechowski.

Holotype: MP ISEA I−F/MP/1540/20/09 (imprint of forewing).

Type locality: Sosnowiec-Klimontów, originally Porąbka-Klimontów Mine, Upper Silesian Coal Basin, Poland (Krawczyński et al. 1997, 2001).

Type horizon: Pensylvannian, Langsettian (Westphalian A), Coal-bearing Mudstone Series, Mudstone series (Załęże beds) (Pacyna and Zdebska 2012).

Diagnosis.—Vein M distally divided into MA and MP, MA shortly braced to RP. Free part of M before bifurcation four times or less longer than width of costal field where CuA+M divides.

Measurements.—Forewing fragment length 34.4 mm long, estimated forewing length 55 mm, maximum width in widest preserved part 14 mm (estimated maximum width is probably only slightly larger).

Description.—Based on fore-wing venation**.** Wing generally incompletely preserved, cubital area partly distorted, apex missing. Wing membrane hyaline, numerous transverse or slightly oblique crossveins regularly arranged. ScA unknown, ScP ends on costal margin in distal third of wing. Costal area broad with regular pattern of numerous oblique veinlets. R almost straight, bifurcated 6.18 mm distal from divergence of M and CuA. RA probably simple. RP posteriorly pectinate, ending with at least three terminal branches. Short stout brace present between RP and MA. Long basal fusion between M+CuA. M diverging from the common stem well before the middle of the wing. M divided into MA and MP beyond the middle of the wing. Short brace between MA and RP 2.3 mm from M bifurcation. MA and MP run parallel to one another over a long distance. Distal bifurcation of both M veins present close to posterior margin of wing. CuA fused with CuPa shortly after its emergence from the M+CuA vein. CuPb probably simple. Details of cubital area indistinct, but posterior pectination of CuA+CuPa ending with at least 4 primary branches, the first of which is secondarily bifurcated. Anal area formed by at least two anal veins, second anal vein distally bifurcated.

Remarks.—This forewing corresponds to that of Archaeorthoptera sensu Béthoux and Nel, 2002 because of the following synapomorphies: CuP differentiated into two branches (CuPa and CuPb), M and CuA fused over long distance and distal part of CuA fused with CuPa. Despite a poorly preserved cubital area, there is no sign of a branched CuPa vein, so it is unlikely that it can be placed in Panorthoptera. Wide space between RA and RP lead us to exclude this fossil from Lobeattida. Due to posteriorly pectinate CuA+CuPa vein, we can also exclude the placement within Cnemidolestodea

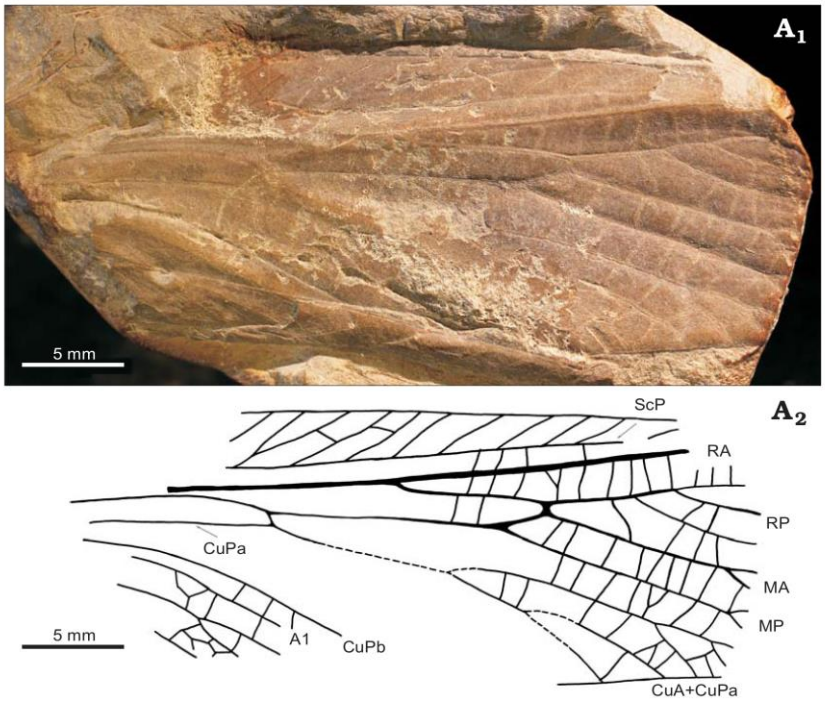


Fig. 4. Forewing venation of archaeorthopteran insect *Parapalaeomastax dariuszi* gen. et sp. nov., holotype (MP ISEA ISEA I–F/MP/1540/20/09), Carboniferous, Pensylvannian, Langsettian (Westphalian A), Sosnowiec-Klimontów, Upper Silesian Coal Basin, Poland. Photograph (A_1) and explanatory drawing (A_2). Abbreviations: A1, first anal vein; CuA, cubital anterior; CuPa/b, anterior/posterior branch of cubital posterior; MA/P, media anterior/posterior; RA/P, radius anterior/posterior; ScP, subcosta posterior.

(Béthoux 2005). AP area is indistinct, but is probably not well developed, and therefore our fossil cannot be placed in Protophasmida. Nevertheless, the general pattern of the venation of this fossil resembles that of the group of genera consisting of *Coselia*, *Omaliella*, *Paralongzhua*, *Omalia*, and especially *Palaeomastax* (Béthoux and Nel 2005; Prokop et al. 2015).

Parapalaeomastax gen. nov. differs from *Omalia* with *Omalia macroptera*, especially by its more distal division of MA and MP and connection of MA by only a short brace to RP, instead of a partial fusion (Béthoux and Nel 2005). There is also a more complicated pattern of cross veins in the costal area in *O. macroptera* and the same is true for *Omalia sp.* (specimen MGL 4217), where in addition, the bifurcation of MP is closer to the division of MA and MP (Béthoux and Nel 2005). *Coselia* mainly differs from *Parapalaeomastax* in having a markedly wider costal area with a prominent meshwork of cross veins, but the type species of *C. palmiformis* is based only on the basal part of a wing. In *Omaliella* with *Omaliella ramosa* the branching pattern of M is different and vein RP is connected to the anterior branch MA1. The comparison of *Parapalaeomastax* with early Permian *Paralongzhua* based on *Paralongzhua elongata* from Lodève (Hérault, France) reveals it has a much narrower costal area, the division of MA and MP located more basally and MA is deeply bifurcated (Prokop et al. 2015). *Palaeomastax* Handlirsch, 1904 based on *Palaeomastax carbonis* from Frameries in Belgium has the same general pattern of venation, with the exception of a long and simple M unlike our fossil with deeply branched M and MA shortly braced to R (Béthoux and Nel 2005). But the fossil of *P. carbonis* lacks distal part of wing and even the distal end of the preserved part is damaged. It is quite plausible, that a bifurcation of M and a connection of MA with RP can occur immediately distal to the preserved area. However, we have to stick to the facts. So, the long simple M is a crucial diagnostic character of the genus *Palaeomastax* and therefore provides sufficient differentiation of *Parapalaeomastax*, but we need to search for more complete fossils of *Palaeomastax* in order to accurately compare their wing apices.

Stratigraphic and geographic range.—S Poland: Upper Silesia, Sosnowiec-Klimontów; Pensylvannian, Langsettian (Westphalian A).

Re-evaluation of the earliest archaeorthopteran from the Upper Silesian Coal Basin

Prokop et al. (2005) reported the anterobasal part of wing attributable to Archaeorthoptera from the lowermost Namurian A/E1 (ca. 324 Ma) of Ostrava Formation in Upper Silesian Coal Basin (Czech Republic). Due to a poor and fragmentary preservation of this fossil the authors were unable to provide any formal attribution to a precise group or closer comparison at that time. However, this fossil potentially represents an important calibration point as it is at the same time the oldest Pterygota. Wolfe et al. (2016) regarded this fragmentary fossil as insufficiently characterized for dating. Our re-examination of this fossil specimen (No. B13711) reveals several obstacles as supposed longitudinal veins are interrupted and distinctly stronger in distal part, branching pattern of the main veins near the supposed wing base seems to be very unusual without clearly discernable veins ScP and R (including different polarity), and thus support uncertainties of the assignment. Therefore, we considered the placement of this fossil among Archaeorthoptera or even Pterygota as doubtful. Our new observation reveals that it could rather belong to another group of organisms (presumably a fragment of fish fin).

Conclusions

The Carboniferous insects from the Upper Silesian Coal Basin have been well studied for more than a century by the classic authors Ferdinand von Roemer, Anton Handlirsch and Pierre Pruvost. Recently discovered localities in the Polish part of the Basin at Czerwionka and particularly Sosnowiec with exceptionally well preserved specimens in sideritic nodules reveal a diverse entomofauna with a high potential for future studies.

This study focused on members of Archaeorthoptera and includes descriptions of two new genera and three new species based on fore-wing venation. A remarkably well preserved forewing of *Omaliella polonica* sp. nov. reveals structural details of the venation, which are unknown for the type species *Omaliella ramosa* due to its fragmentary preservation. In particular, this species allows us to study the venation near the wing base and apex for the first time.

Owadpteron dareki gen. et sp. nov. has an unusual trait, CuPaα is partially fused with CuA. Our hypothesis is supported by the examination of *Nacekomia rossae*, which has a similar venation, with the free part of CuPaα distinctly emerging from CuA. This pattern supports the assignment of *Owadpteron* to the Geraridae and Panorthoptera. However, the bifurcation of CuPa is an essential apomorphy of Panorthoptera and its absence in *O. dareki* would greatly weaken our taxonomic placement, or challenge the significance of this character. *Parapaleomastax dariuszi* gen. et sp. nov. is poorly preserved, but important apomorphies are visible and allow the specific assignment within Archaeorthoptera. The comparison with the genus *Palaeomastax* is complicated by the lack of a preserved wing apex with the crucial diagnostic characters.

All three new species of Archaeorthoptera differ greatly morphologically and in their wing venation, which could help in a future phylogenetic analysis of this stunning group.

Acknowledgements

We cordially thank Dariusz Wojciechowski (Katowice, Poland) for his effort and help with the fieldwork at Sosnowiec. The authors are grateful to Paul Mayer (The Field Museum, Chicago, USA), who kindly provided access to the collection under his care. We thank to André Nel (Muséum national d'Histoire naturelle, Paris, France) and anonymous reviewer for their insightful comments to early version of the manuscript. Authors are grateful Anthony F.G. Dixon (University of East Anglia, Norwich, United Kingdom) for improving the English and Tomáš Přikryl (Institute of Geology of the Czech Academy of Sciences, Praha) for valuable discussion on taphonomy. JP and MP were supported by a research project of the GAČR (No. 18-03118S).

References

Aristov, D.S. 2014. Classification of the order Cnemidolestida (Insecta: Perlidea) with descriptions of new taxa. *Far Eastern Entomologist* 277: 1–46.

Béthoux, O. 2003. *Protophasma dumasii*, a link between Orthoptera and the dictyopterid orders? *Journal of Orthoptera Research* 12: 57–62.

Béthoux, O. 2005. Cnemidolestodea (Insecta): an ancient order reinstated. *Journal of Systematic Palaeontology* 3: 403–408.

Béthoux, O. 2006. Revision of *Cacurgus* Handlirsch, 1911, a basal Pennsylvanian Archaeorthoptera (Insecta: Neoptera). *Bulletin of the Peabody Museum of Natural History* 47: 29–35.

Béthoux, O. 2008. Revision and phylogenetic affinities of the lobeattid species *bronsoni* Dana, 1864 and *silvatica* Laurentiaux & Laurentiaux-Vieira, 1980 (Pennsylvanian, Archaeorthoptera). *Arthropods Systematics & Phylogeny* 66: 145–163.

Béthoux, O. and Nel, A. 2002. Venation pattern and revision of Orthoptera sensu nov. and sister groups. Phylogeny of Palaeozoic and Mesozoic Orthoptera sensu nov. *Zootaxa* 96: 1–88.

Béthoux, O. and Nel, A. 2003. Wing venation morphology and variability of *Gerarus fischeri* (Brongniart, 1885) sensu Burnham (Panorthoptera; Upper Carboniferous, Commentry, France), with inferences on flight performance. *Organisms Diversity and Evolution* 3: 173–183.

Béthoux, O. and Nel, A. 2005. Some Palaeozoic Protorthoptera are ancestral Orthopteroids: major wing braces as clues to a new split among the Protorthoptera (Insecta). *Journal of Systematic Palaeontology* 2: 285–309.

Béthoux, O. and Schneider, J.W. 2010. Description of a hind wing of a new basal Archaeorthoptera (Mazon Creek, IL.; Pennsylvanian). *Alavesia* 3: 81–85.

Béthoux, O., Gu, J.-J., and Ren, D. 2012. A new Upper Carboniferous stem-orthopteran (Insecta) from Ningxia (China). *Insect Science* 19: 153–158.

Béthoux, O., Gu, J.-J., Yue, Y., and Ren, D. 2012. *Miamia maimai* n. sp., a new Pennsylvanian stem-orthopteran insect, and a case study on the application of cladotypic nomenclature. *Fossil Record* 15: 103–113.

Brauckmann, C. 1984. Weitere neue Insekten (Paleodictyoptera: Pro-

toorthoptera) aus Namurium B von HagenVorhalle. *Jahresberichte des Naturwissenschaftlichen Vereins in Wuppertal* 37: 108–115.

Carpenter, F.M. 1992. Superclass Hexapoda. *In*: R.C. Moore and R.L. Kaesler (eds.), *Treatise on Invertebrate Paleontology, Part R, Arthropoda 4, 3/4*, 1–655. The Geological Society of America, Boulder and the University of Kansas, Lawerence.

Desutter-Grandcolas, L., Jacquelin, L., Hugel, S., Boistel, R., Garrouste, R., Henrotay, M., Warren, B.H., Chintauan-Marquier, I.C., Nel, P., Grandcolas, P., and Nel, A. 2017. 3-D imaging reveals four extraordinary cases of convergent evolution of acoustic communication in crickets and allies (Insecta). *Scientific Reports* 7: 7099. [published online, https://doi.org/10.1038/s41598-017-06840-6].

Gradziński, R., Doktor, M., and Brzyski B. 1982. Accumulation of drifted logs and other large plant debris in a Carboniferous fluvial channel at Czerwionka, Upper Silesia. *Acta Geologica Polonica* 32: 69–81.

Gu, J.-J., Béthoux, O., and Ren, D. 2011. *Longzhua loculata* n. gen. n. sp., one of the most completely documented Pennsylvanian Archaeorthoptera (Insecta; Ningxia, China). *Journal of Palaeontology* 85: 303–314.

Handlirsch, A. 1906–1908. *Die fossilen Insekten und die Phylogenie der rezenten Formen. Ein Handbuch für Paläontologen und Zoologen*. 1430 pp. V.W. Engelman, Leipzig.

Krawczyński, W., Filipiak, P., and Gwoździewicz, M. 1997. Zespoł skamieniałości z karbońskich sferosyderitów (westfal A) NE części Górno–śląskiego Zagłębia Węglowego. *Przegląd Geologiczny* 45: 1271–1274.

Krawczyński,W., Filipiak, P., and Wojciechowski, D. 2001. Owady z warstw załęskich (westfal A) NE części Górnośląskiego Zagłębia Węglowego. *In*: *XXI Terenowa Szkoła Geologów Uniwersytetu Śląskiego*, 25–28. Uniwersytet Śląski, Sosnowiec.

Kukalová-Peck, J. and Brauckmann, C. 1992. Most Paleozoic Protorthoptera are ancestral hemipteroids: major wing braces as clues to a new phylogeny of Neoptera (Insecta). *Canadian Journal of Zoology* 70: 2452–2473.

Li, X.R., Zheng, Y.H., Wang, C.C., and Wang, Z.Q. 2018. Old method not old-fashioned: parallelism between wing venation and wing-pad tracheation of cockroaches and a revision of terminology. *Zoomorphology* 137: 519–533.

Liu, Yu., Ren, D., and Prokop, J. 2009. Discovery of a new Namurian archaeorthopterid from Ningxia, China (Insecta: Archaeorthoptera). *Zootaxa* 2032: 63–68.

Pacyna, G. and Zdebska, D. 2010. Upper Carboniferous seed fern (Pteridospermophyta) pollen organs from Silesia (Poland) and related evolution considerations. *Monographiae Botanicae* 100: 1–81.

Prokop, J. and Ren, D. 2007. New significant fossil insects from the Upper Carboniferous of Ningxia in northern China (Palaeodictyoptera, Archaeoptera). *European Journal of Entomology* 104: 267–275.

Prokop, J., Krzemińska, E., Krzemiński, W., Rosová, K., Pecharová, M., Nel, A., and Engel, M.S. 2019. Ecomorphological diversification of the Late Palaeozoic Palaeodictyopterida reveals different larval strategies and amphibious lifestyle in adults. *Royal Society Open Science* 6: 190460. [published online, https://doi.org/10.1098/rsos.190460]

Prokop, J., Krzemiński, W., Krzemińska, E., and Wojciechowski, D. 2012. Paoliida, a putative stem-group of winged insects: Morphology of new taxa from the Upper Carboniferous of Poland. *Acta Palaeontologica Polonica* 57: 161–173.

Prokop, J., Krzemiński, W., Krzemińska, E., Hörnschemeyer, T., Ilger, J.M., Brauckmann C., Grandcolas, P., and Nel, A. 2014. Late Palaeozoic Paoliida is the sister group of Dictyoptera (Insecta: Neoptera). *Journal of Systematic Palaeontology* 12: 601–622.

Prokop, J., Nel, A., and Hoch, I. 2005. Discovery of the oldest known Pterygota in the Lower Carboniferous of the Upper Silesian Basin in the Czech Republic (Insecta: Archaeorthoptera). *Geobios* 38: 383–387.

Prokop, J., Pecharová, M., Nel, A., Hörnschemeyer, T., Krzemińska, E., Krzemiński, W., and Engel, M.S. 2017. Paleozoic nymphal wing pads support dual model of insect wing origins. *Current Biology* 27: 263–269.

Prokop, J., Szwedo, J., Lapeyrie, J., Garrouste, R., and Nel, A. 2015. New middle Permian insects from Salagou Formation of the Lodève Basin in southern France (Insecta: Pterygota). *Annales de la Société Entomologique de France* 51: 14–51.

Pruvost, P. 1927. Sur une aile d'insecte trouvée au sondage de Gulpen. *Jaarvestag van voor het Nederlandsche Mijngebied te Heerlen* 1926: 76–77.

Rasnitsyn, A.P. 2007. On the discussion of the wing venation of (Archae) Orthoptera (Insecta). *Paleontological Journal* 41: 341–344.

Richardson, E.S. Jr. 1956. Pennsylvanian invertebrates of the Mazon Creek area, Illinois. Insects. *Fieldiana, Geology* 12: 15–56.

Roemer, F. 1883. Über einen im Schieferthon der zwischen Königshütte und Laurahütte gelegenen Alfredgrube, 10 m im Liegenden des Carolinen-flözes, gefundenen Insectenflügel. *Jahresbericht der Schlesischen Gesell-schaft für Vaterländische Kultur* 62: 226.

Stworzewicz, E., Szulc, J., and Pokryszko, B.M. 2009. Late Paleozoic continental gastropods from Poland: Systematic, evolutionary and paleoecological approach. *Journal of Paleontology* 83: 938–945.

Wolfe, J.M., Daley, A.C., Legg, D.A., and Edgecombe, G.D. 2016. Fossil calibrations for the arthropod tree of life. *Earth Science Reviews* 160: 43–110.

Study V

Dvořák, T., Pecharová, M., Leipner, A., Nel, A., & Prokop, J. (2021). New archaeorthopteran insects from the Pennsylvanian of Piesberg reveal unexpected mosaic of morphological traits and colouration pattern of the tegmina. *Historical Biology*, 33(12), 3375-3383.

HISTORICAL BIOLOGY
https://doi.org/10.1080/08912963.2020.1867127

Taylor & Francis
Taylor & Francis Group

Check for updates

New archaeorthopteran insects from the Pennsylvanian of Piesberg reveal unexpected mosaic of morphological traits and colouration pattern of the tegmina

Tomáš Dvořák[a], Martina Pecharová[a], Angelika Leipner[b], André Nel[c] and Jakub Prokop[a]

[a]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic; [b]Museum am Schölerberg, Osnabrück, Germany; [c]Institut Systématique Evolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université Des Antilles, Paris, France

ABSTRACT

New archaeorthopterans *Contracladus impar* gen. nov. et sp. nov. (uncertain assignement), *Paraspiculum brauckmannii* gen. nov. et sp. nov. (Caloneurodea) and *Piesbergopterum punctatum* gen. nov. et sp. nov. (Cnemidolestodea) are described from the Moscovian of Piesberg quarry near Osnabrück (Lower Saxony, Germany). All new taxa display unique patterns of their forewing venations. The mosaic of characters found in *C. impar* with a particular lack of a free vein CuA demonstrates the limits of the current systematics for Archaeorthoptera. The evidence for a supposedly disruptive pattern of colouration is demonstrated on the forewing membrane of *P. punctatum*, which shows numerous spots in the distal part of the wing. Those are inside the cells delimited by the crossveins. The exceptionally preserved fossils found in Piesberg document fine structural details and complete our knowledge on the early history of this diverse group of polyneopteran insects.

ARTICLE HISTORY
Received 23 November 2020
Accepted 17 December 2020

KEYWORDS
Insecta; Polyneoptera; Archaeorthoptera; wing venation; Carboniferous; subvariscan foreland basin

Introduction

The superorder Archaeorthoptera is one of the major polyneopteran insect groups with remarkable morphological diversity and species abundance in ecosystems recorded since the Late Carboniferous. The systematics is mainly based on the forewing venation, but certain fossils document the structure of other body parts in great detail (see e.g. Burnham 1983; Béthoux and Nel 2002). The representatives of Archaeorthoptera are also among the earliest recorded winged insects (Pterygota) known from the Namurian and Langsettian strata in Europe (e.g., Pruvost (1927); Brauckmann et al. 1996; Dvořák et al. 2019). Together with other polyneopteran groups as mainly Dictyoptera with its stem Paoliida, and Grylloblattida they belong to most common neopterous insects found in Pennsylvanian outcrops (e.g., Carpenter 1992; Brauckmann and Herd 2006; Prokop et al. 2012, 2014).

Herein, we provide descriptions of three new genera and species of Archaeorthoptera based on their patterns of forewing venation. One of these species, *Piesbergopterum punctatum* gen. nov. et sp. nov., shows an exceptional state of preservation with clear evidence of original pattern of colouration of the wing membrane.

Materials and methods

All examined specimens in this study come from the Osnabrück Formation (Pennsylvanian, latest Moscovian, lower Westphalian D/Asturian age). The fossils are found in the roof shales of the coal seams. The most basal visible coal seam 'Zweibänke' marks the base of the Westphalian D/Asturian of the Piesberg quarry near Osnabrück (Lower Saxony, Germany). The following coal seams are currently recognisable: "Zweibänke, 'Bänkchen', 'Dreibänke', 'Mittel', 'Johannisstein' and 'Schmitzchen'. For more detailed descriptions of the geology section and stratigraphy of the Piesberg see e.g. Josten et al. (1984), David (1990), Schultka (2000), Brauckmann and Herd (2002), Jones and Glover (2005) and Köwing and Rabitz (2005). Recently, Leipner and Chellouche (2019) reported a newly discovered lacustrine deposit, situated approximately 7 m above the coal seam 'Mittel'. The herein described insect fossils come from the roof shales of coal seams 'Zweibänke' and 'Dreibänke' as well as from the new lake deposit. The Piesberg quarry is one of few well documented Moscovian insect localities in Europe. The recorded entomofauna displays a remarkable diversity of palaeopterous and neopterous insects found in several horizons (e.g., Brauckmann and Herd 2002, 2006; Brauckmann et al. 2009). Alongside mainly preserved wings of insect adults, there are also larvae and their exuvia reported from Piesberg (Haug et al. 2013; Hörnig et al. 2014; Kiesmüller et al. 2019). The adult blattoid insects so-called 'roachoids' dominates with approximately 60% of all insect remains (Wrede et al. 2019).

This Pennsylvanian locality is one of few sites in Europe, which is currently well accessible and intensively sampled by the amateur palaeontologist Michael Sowiak and AL. The type material with Pal numbers is housed in the Museum am Schölerberg (Osnabrück, Germany). The specimen with prefix F in the accession number indicates the original collection of Mr. Michael Sowiak (Glandorf, Germany), which is temporarily housed in his collection and finally deposited via the Natural Science Association of Osnabrück (Naturwissenschaftlicher Verein Osnabrück) in the Museum am Schölerberg, coll. Sowiak.

The specimens were examined in a dry state and under a film of ethyl alcohol using Zeiss SteREO Discovery.V20 and Nikon SMZ 745 T stereomicroscopes. Line drawings were made using Zeiss SteREO Discovery.V20 with camera lucida attachment. Photographs of fossil specimens in a dry state or under a film of ethyl alcohol were taken with a Canon EOS 70D digital camera equipped with MP-E 65 mm and EF-S 60 mm macrolenses. To reduce reflectance on some pictures a polarising filter was used. Original photographs were processed using the

CONTACT Jakub Prokop jprokop@natur.cuni.cz Department of Zoology, Charles University, Vinicna 7, 12800 Prague 2, Czech Republic
© 2021 Informa UK Limited, trading as Taylor & Francis Group

Published online 21 Jan 2021

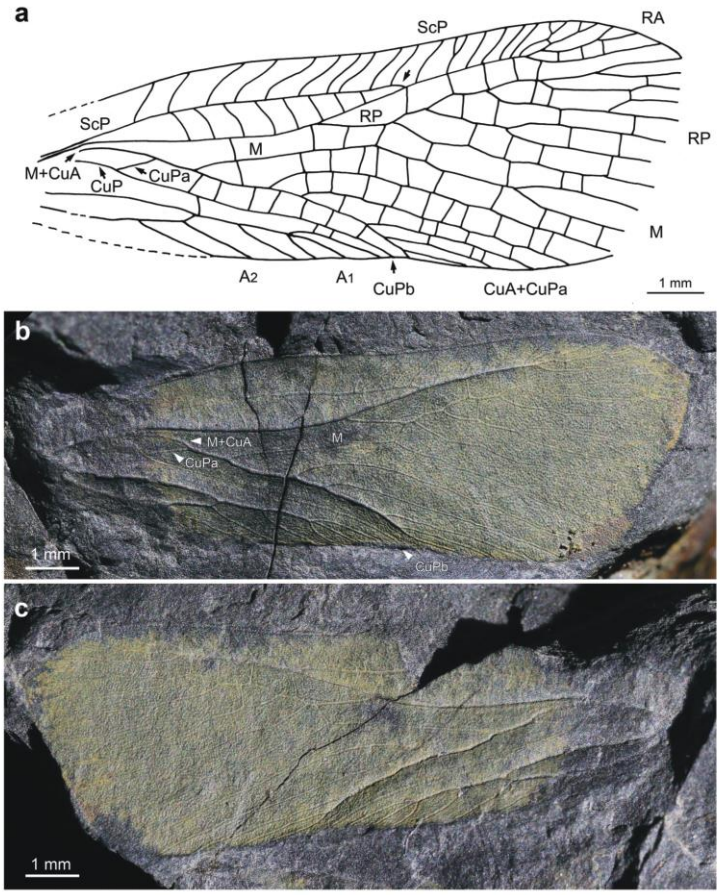


Figure 1. *Contracladus impar* gen. nov. et sp. nov., holotype specimen Pal 1609 (Museum am Schölerberg, Osnabrück), Pennsylvanian: Westphalian D/Asturian, Piesberg Quarry near Osnabrück, Germany. (a), drawing of forewing venation; (b), photograph of right forewing – part; (c), photograph of right forewing – counterpart.

image-editing software Adobe Photoshop CS4, and some were processed by the stacking software Zerene Stacker.

The wing venation nomenclature generally follows the scheme of Béthoux and Nel (2002), but there is an alternative concept of the wing venation for orthopteroid insects (see Rasnitsyn 2007). Wing venation abbreviations: A1/A2 – anal veins; CuPa/CuPb – anterior/posterior branch of cubitus posterior; CuPaα/CuPaβ – anterior/posterior branch of CuPa; CuA/CuP – cubitus anterior/posterior; M – stem of media; MA/MP – media anterior/posterior; RA/RP – radius anterior/posterior; ScA/ScP – subcosta anterior/posterior.

Results

Superorder Archaeorthoptera Béthoux and Nel, 2002
Order uncertain Genus *Contracladus* gen. nov.

http://www.zoobank.org/urn:lsid:zoobank.org:act:54261B72-4C47-4C9C-893F-1D21436D5D37

Type species. *Contracladus impar* sp. nov.

Etymology. Composite name after the Contra- and cladus as ending found in many genera of Cnemidolestodea, pointing out a few convergent characters in their venations.

Diagnosis

Forewing characters only. CuPa ending into M+ CuA; no CuPaβ; CuA+CuPa anteriorly pectinate; M not ending in CuA+CuPa.

Contracladus impar sp. nov. (Figure 1)

http://www.zoobank.org/urn:lsid:zoobank.org:act:940C8BA5-0C46-45F3-8E08-461DC6B26D93

Holotype. Pal 1609 (Part and counterpart), Museum am Schölerberg, Osnabrück: nearly complete right forewing with perfectly preserved venation lacking small part in the apex.

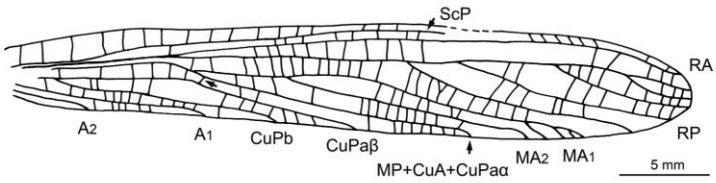


Figure 2. *Paraspiculum brauckmanni* gen. et sp. nov., holotype specimen F 564 (Museum am Schölerberg, Osnabrück – Sowiak coll.), drawing of forewing venation.

Type locality. Piesberg Quarry near Osnabrück, Lower Saxony, Germany.

Type strata. Claystone layer above the Dreibänke coal seam, Osnabrück Formation, Late Carboniferous (Pennsylvanian: Westphalian D/Asturian).

Etymology. After very odd pattern of archaeorthopteran venation.

Description

Based on right forewing. Preserved wing length 13 mm, maximum wing width 4.3 mm. Anterior and posterior wing margins slightly curved outwards at ca. mid length; numerous simple crossveins; entire surface of wing membrane densely covered with microstructures as microtrichia; ScP parallel to costal margin, ending into RA at ca. 3/5 of wing length; numerous simple sigmoidal crossveins in costal area; R divided into RA and RP just basal mid-wing; RA ending with three short posterior branches basal to wing apex; RP with six posterior branches regularly disposed, some being secondarily bifurcated; CuPa ending into M+ CuA; relatively concave M separating from common stem of M+ CuA+CuPa at ca. 1/4 of wing length, shortly connected by a crossvein to RP at about mid-wing, ending with two apical branches; CuA+CuPa anteriorly pectinated with five simple relatively concave branches reaching posterior wing margin; Branches of RP, M, and Cu regularly parallel and connected by simple, straight, more or less oblique, crossveins; concave CuPb simple, slightly undulated; two main anal veins connected by a crossvein at about 1/4 of wing length; A1 reaching posterior wing margin with four branches and A2 distally branched.

Discussion

Contracladus gen. nov. can be accurately attributed to the Archaeorthoptera because it has a forked concave CuP, with anterior branch ending into M+ CuA and vein M+ CuA basally fused with R. The absence of the second branch CuPaβ of CuPa excludes affinities with the Panorthoptera sensu Béthoux and Nel (2002). It has a very peculiar anteriorly pectinate vein CuA+CuPa, which is rarely found among the Orthoptera but also present in the Paoliida (Prokop et al. 2014b). Similar pectinations are found in the Cnemidolestodea sensu Béthoux (2005), but with the important difference in the first branch being itself forked several times. Interestingly, in the case of *Aviocladus* Prokop et al., 2014 the first branch of CuA+CuPa could be also simple, but unfortunately the posterior part of wing is incompletely preserved in the holotype (Prokop et al. 2014). Another difference to the members of Cnemidolestodea is the vein CuPa ending into M+ CuA in *Contracladus impar* gen. et sp. nov., while it ends into CuA in the Cnemidolestodea. Another difference with the Cnemidolestodea is the vein M not ending in CuA+CuPa.

The venation pattern of *Contracladus* gen. nov. is very particular as it lacks the synapomorphy of Archaeorthoptera as a free part of CuA emerging from M+ CuA (Béthoux and Nel 2002). Further examples of such problematic taxa, which lack free CuA and in which CuPa is connected to M+ CuA has been demonstrated in *Bruaylogus magnificus* Coty, Háva, Prokop, Roques, and Nel, 2014 (Coty et al. 2014; Gu et al. 2017). *Bruaylogus* is a Panorthoptera as it has a CuPaβ; and the vein CuPaα ends in M+ CuA at the point of separation of M with CuA, unlike *Contracladus* gen. nov. The corresponding pattern of veins in the basal part of the wing can also be found in *Nugonioneura* Tillyard, 1937 with *N. problematica* Tillyard, 1937 known from the Lower Permian of Elmo in Kansas, U.S. A. (Tillyard 1937; Aristov 2020, p. 6, Figs 3, 4). This fossil also shares lack of a free part of CuA emerging from M+ CuA according to the interpretation of Béthoux and Nel (2002), but showing less developed branching pattern of RP and CuA and less veinlets between ScP and C, in contrast to *Contracladus* gen. nov.

The main question is whether the CuPaβ was convergently developed in Caloneurodea and Panorthoptera. In this case,

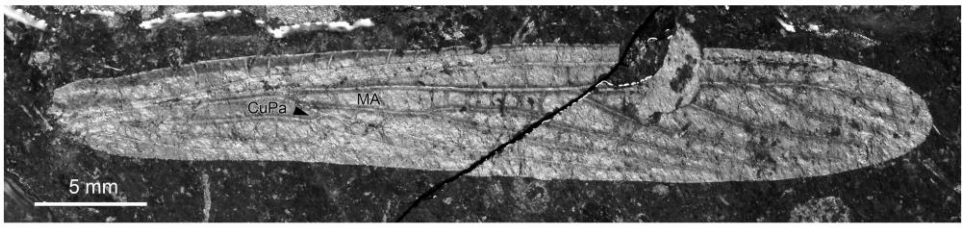


Figure 3. *Paraspiculum brauckmanni* gen. et sp. nov., holotype specimen F 564 (Museum am Schölerberg, Osnabrück – Sowiak coll.), photograph of forewing taken under a film of ethyl alcohol, Pennsylvanian: Westphalian D/Asturian, Piesberg Quarry near Osnabrück, Germany.

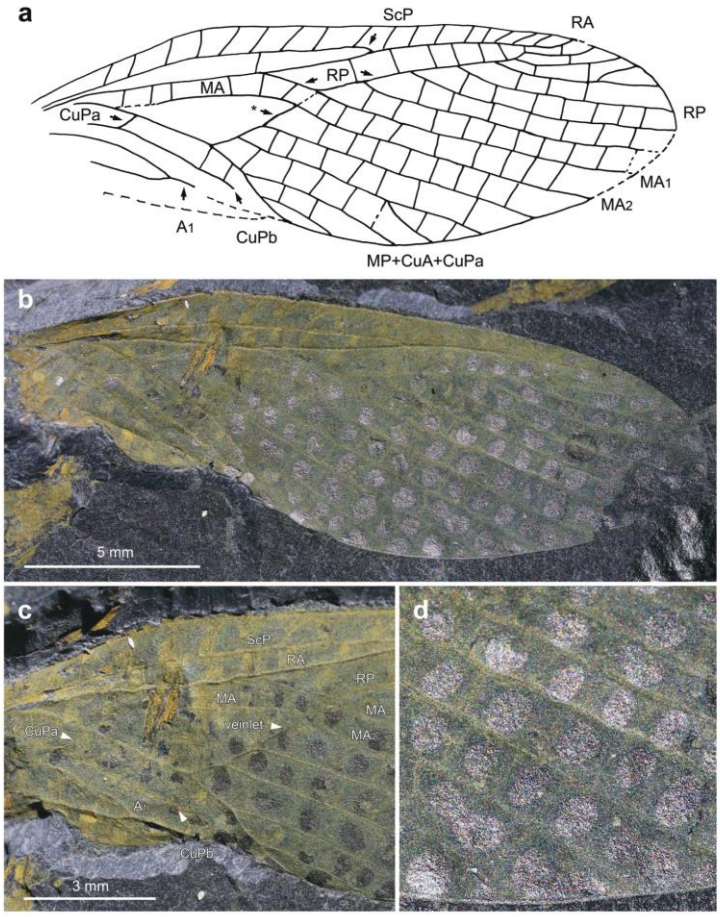


Figure 4. *Piesbergopterum punctatum* gen. et sp. nov., holotype specimen Pal 1389 (Museum am Schölerberg, Osnabrück), Pennsylvanian: Westphalian D/Asturian, Piesberg Quarry near Osnabrück, Germany. (a), drawing of forewing venation; (b), right forewing – part; (c), detail of branching pattern on forewing basal part; (d), detail of coloration pattern along the posterior wing margin.

Contracladus gen. nov. could fall in stem Caloneurodea. Alternatively, if we consider that the character 'presence of CuPaβ' is not homoplastic, *Contracladus* gen. nov. would fall in a different group from the Caloneurodea. Indeed both hypotheses are plausible, because a more or less basal fusion of CuPa with M+ CuA is possible, and the presence vs absence of CuPaβ is also possible.

As a conclusion, *Contracladus* gen. nov. can only be considered as an Archaeorthoptera of uncertain affinities. A new phylogenetic analysis will be necessary to clarify its position, together with those of several other Palaeozoic taxa in this superorder.

Along with the systematic placement, this fossil also diplays exceptional preservation of wing membrane, which was originally covered by dense surface microstructures, probably microtrichia, which are scarcely documented on Palaeozoic wings (see Prokop et al. 2016).

Clade Panorthoptera Crampton, 1928
Order Caloneurodea Handlirsch, 1937 Family uncertain

Remark. The subdivision of the Caloneurodea into different families, needs to be revised on the basis of a phylogenetic analysis. The monophyly of these families is not demonstrated.

Genus *Paraspiculum* gen. nov.

http://www.zoobank.org/urn:lsid:zoobank.org:act:1B83A1F1-2CDB-4292-857C-061AC3C29765

Type species. *Paraspiculum brauckmanni* gen. et sp. nov.

Etymology. Para – meaning similar, and the genus name *Spiculum*.

Diagnosis
Based on forewing venation. RP with several main branches; base of RP close to that of M+ CuA; first branch of RP emerging distally from mid-wing; MA1 not making a strong curve, with only short apical offshots distant from first branch of RP; MA2 simple; very long MP+CuA+CuPaα closely parallel to posterior margin of wing with short crossveins in-between; no small branches of CuPaβ near posterior wing margin

Paraspiculum brauckmanni gen. et sp. nov. (Figures 2, 3)

http://www.zoobank.org/urn:lsid:zoobank.org:act:7300BD87-864E-4780-A159-E5EF49992C40

Holotype. F 564 (Part and counterpart), originally from Sowiak coll.: nearly complete forewing lacking extreme wing base. No clear corrugation of veins discernible, therefore it´s not possible to clarify if a right or left wing is present.

Diagnosis. As for the genus. Also RP with five main branches.

Type locality. Piesberg Quarry near Osnabrück, Lower Saxony, Germany.

Type strata. Shale layer 3 of the new freshwater lake sediment, Osnabrück Formation, Late Carboniferous (Pennsylvanian: Westphalian D/Asturian).

Etymology. Patronymic; named after Carsten Brauckmann (Clausthal-Zellerfeld, Germany) to honour his merits to the Piesberg insect research.

Description
Based on forewing venation. Wing very narrow with 6.5:1 length: width ratio. Main veins generally only weakly branched and mostly parallel with numerous transversal crossveins. ScP ending to costal wing margin about three quarters of wing length. Costal area very broad near wing base (more than one third of wing width) and gradually narrowing towards wing apex. Stem of R divided into RA and RP about one quarter of wing length. RA simple and straight, only proximal part slightly curved to posterior wing margin. RP posteriorly pectinated, with five branches in apical part of wing; first branch of RP well distal to mid-length; all branches simple or with only terminal twig. Common stem of M+ CuA separating from R at level of base of RP; M+ CuA divided into MA and MP+CuA just distal to base of RP. MA deeply branched into two branches (MA1 and MA2) with several short apical veinlets emerging near posterior wing margin. Area between MA1 and MA2 narrow. Short free part of CuPaα diverging from CuPaβ in proximal third of wing and further ending into MP+CuA. MP+CuA+CuPaα and CuPaβ both simple and running parallel towards posterior wing margin; very long MP+CuA+CuPaα closely parallel to posterior margin of wing with short crossveins in-between; CuPb simple, diverging from CuPa close to wing base. Two simple anal veins preserved.

Discussion
This fossil can be attributed to the order Caloneurodea because of the following diagnostic characters (Béthoux et al. 2004): MA with two terminal branches; fusion of CuPaα with MP+CuA as a short anterior branch CuPaα between MP+CuA and CuPa (synapomorphy of the Panorthoptera); convex vein MP+CuA+CuPaα and concave vein CuPaβ running close, parallel and straight; MP running fused with CuA+CuPaα; anal area reduced to two veins (Béthoux and Nel 2002).

This fossil has the following diagnostic characters of the genus *Lusitaneura* Loureiro et al., 2010: ScP reaching costal margin in second third of wing length; three posterior branches of RP; MA with two branches with anterior branch not making a strong curve, and posterior branch with small secondary twigs emerging from it; no vein distally emerging from MP+CuA+CuPaα; no small branches of CuPaβ near posterior wing margin. Nevertheless, it differs from *Lusitaneura covensis* Loureiro et al., 2010 in the broader subcostal area and the longer MP+CuA+CuPaα, closely parallel to the posterior margin of wing (Loureiro et al. 2010).

The venation of this wing is most similar to that of the paratype of *Spiculum mendicum* Brauckman and Herd 2006, originally placed in the Hapalopteridae as Archaeorthoptera of uncertain position but later transferred in the Caloneurodea by Gu et al. (2017). However, the holotype of *Spiculum mendicum* differs from its paratype and *Paraspiculum brauckmanni* sp. nov. in the base of RP distinctly distal to separation of MA from MP+CuA (at same level in *Paraspiculum* gen. nov.), branched MA2 vein (simple in *Paraspiculum* gen. nov.), MA1 with long branches (very short in *Paraspiculum* gen. nov.). The paratype specimen of *Spiculum mendicum* differs from *Paraspiculum brauckmanni* sp. nov. in the very long free MP+CuA basal to its fusion with CuPaα. It also differs from *Paraspiculum* gen. nov. in RP with six main branches instead of five and first branch of RP emerging at about the mid-wing, instead of more distal postion in *Paraspiculum brauckmanni* sp. nov. These differences can be considered at most of specific significance. Note that Brauckmann and Herd (2006, p. 40–41) considered the paratype *Spiculum mendicum* as a metathoracic wing without strong argument. It would be very unusual that a hind wing would have such a reduced anal area, even if the Caloneurodea have narrow hind wings.

Among the other caloneurodean genera, *Nanogramma* Béthoux, Nel and Lapeyrie, 2004 has a short wing with reduced venation (two branches of RP). *Anomalogramma* Carpenter, 1943 has a shorter ScP, ending at mid-length and only two branches of RP. *Caloneura dawsoni* Brongniart, 1885 has remarkably broader wings, a longer ScP, and MA branched distally from mid-wing, unlike *Paraspiculum* gen. nov. *Gelasopteron gracile* Carpenter, 1976 has a wing narrower than that of *Paraspiculum* gen. nov., a very long MP+CuA+CuPaα, and with a simple RP (Carpenter 1976). *Aviogramma* Prokop et al., 2014 has a noticeably shorter ScP extending only slightly behind mid-wing and a fork of MA in a more distal position (Prokop et al. 2014). Short ScP can be seen also in *Anomalogramma parvum* Carpenter, 1943 (Carpenter 1943).

Gigagramma Béthoux, Nel and Lapeyrie, 2004 is a noticeably larger wing than *Paraspiculum* gen. nov. and has more branches of RP with the first branching proximally from mid-wing instead of distally as in *Paraspiculum* gen. nov. (Béthoux et al. 2004). *Gigagramma carpenteri* Béthoux, Nel and Lapeyrie, 2004 has also a free MP emerging from common stem MP+CuA+CuPaα. Available fossil wings of *Sthenarocera pachytyloides* Brongniart, 1885 are fragmentary, but with more than five RP branches, a ScP nearly reaching wing apex, and a wing broader than that of *Paraspiculum* gen. nov. *Sinaspidoneura* Huang, Fu, Lian and Nel, 2020 differs from *Paraspiculum* gen. nov. in the ScP reaching wing apex and the presence of only one anal vein (Huang et al. 2020). *Eohymen maculipennis* Martynov, 1937 has also a very long ScP unlike *Paraspiculum* gen. nov., but also a convex vein anterior to ScP that is unknown in the Caloneurodea, rendering its position in this clade somewhat uncertain (Rasnitsyn and Aristov in Aristov et al. 2013). *Isadistica* Rasnitsyn and Aristov, 2013 is characterised by a ScP ending into RA and very long MP+CuA+CuPaα and CuPaβ (Rasnitsyn and Aristov in Aristov et al. 2013). *Pruvostiella* Handlirsch, 1922 differs from *Paraspiculum* gen. nov. in the

distance between fork of MA and first branch of RP distinctly shorter than in *P. brauckmanni* sp. nov., the base of RP far from base of MA, and A2 reaching the posterior margin of wing distal to the division of R into RA and RP, while in *P. brauckmanni* sp. nov. A2 is short and ends on posterior wing margin close to wing base. *Apsidoneura* Carpenter, 1943 and *Homaloptila* Handlirsch, 1919 have a strong anterior curve of MA1 and MP+CuA+CuPaα is running very close to CuPaβ, making this species remarkably different from *P. brauckmanni* sp. nov. (Carpenter 1943). *Plesiogramma medialis* Carpenter, 1943 has a simple MA and wings much broader than *P. brauckmanni*. *Paleuthygramma* Martynov, 1930 and *Euthygramma* Martynov, 1928 have very long anal area, extending to three quarters of wing length (Béthoux et al. 2004). *Plesiogramma* Carpenter, 1943 has a very long ScP reaching the wing apex

The venation of *Paraspiculum* gen. nov. also resembles that of *Hapaloptera* Handlirsch, 1906 (holotype *H. gracilis*) especially in the pattern of the veins ScP, R and M (Carpenter 1965), but the putative absence of bifurcation of CuPa basal of its fusion with CuA would exclude *Hapaloptera* from the Panorthoptera (Béthoux and Nel 2002). *Hapaloptera* needs to be revised. *Ligogramma* Béthoux Nel and Lapeyrie, 2004 has the base of RP situated at midwing (Béthoux et al. 2004; Beckemeyer 2009).

Order Cnemidolestodea Handlirsch, 1937
Genus *Piesbergopterum* gen. nov.

http://www.zoobank.org/urn:lsid:zoobank.org:act:1765BBBD-157C-4952-8B20-76380FAFB811

Type species. *Piesbergopterum punctatum* sp. nov.

Etymology. Composite name after the locality Piesberg- and '*pterum*' for wing.

Diagnosis
Forewing characters. Area between ScP and costal margin markedly broader than area between ScP and RA; stem of RP short; base of RP situated far basal from fusion of ScP with RA; RP posteriorly pectinated ending with six simple branches; MA without posterior branch in a broad area between it and MP+CuA+CuPa; anterior branch of MP+CuA+CuPa with only two posterior branches and a short veinlet between it and MA; posterior branch of MP+CuA +CuPa with three anterior branches; anterior branch of MA touching RP; numerous simple crossveins nearly regularly spaced in distal part of wing.

Piesbergopterum punctatum sp. nov. (Figures 4)

http://www.zoobank.org/urn:lsid:zoobank.org:act:FC0D9DBD-E9FE-4298-BA92-466956825186

Holotype. Pal 1389 (Part and counterpart), Museum am Schölerberg, Osnabrück: right forewing with distorted basal part, wing membrane with distinct pattern of colouration.

Type locality. Piesberg Quarry near Osnabrück, Lower Saxony, Germany.

Type strata. Claystone layer above the Zweibänke coal seam, Osnabrück Formation, Late Carboniferous (Pennsylvanian: Westphalian D/Asturian).

Etymology. After the spotted pattern of wing colouration.

Diagnosis
As for the genus by monotypy. Pattern of colouration developed mainly in distal part of wing in form of numerous light circular spots inside cells.

Description
Based on right forewing venation. Estimated total wing length about 21 mm, maximum width at midwing 7 mm; prominent bending of costal margin in about basal third; wing membrane probably originally darkly coloured with a pattern of hyaline spots inside cells separated by crossveins; concave ScP slightly curved, running parallel with costal margin, ending to RA behind midwing; costal area broader than subcostal area with numerous simple and oblique crossveins; stem of R diverging from M+ CuA near base of wing; division of RA and RP proximal of midwing, 3 mm basal to connection of ScP on RA; strongly convex RA simple ending on costal margin well basal to wing apex; numerous oblique crossveins present in space between RA and costal wing margin; concave RP posteriorly pectinate ending with six simple branches covering whole distal part of wing; neutral vein MA diverging from M + CuA and further running parallel to stem of R/RP; MA deeply forked into two simple branches, anterior one MA1 shortly connected to RP and posterior one MA2 nearly straight; concave vein CuPa ending into MP+CuA shortly behind divergence of MA from MP+CuA. MP+CuA+CuPa emitting a posterior branch subdivided into three anterior branches; and an anterior branch parallel to MA, emitting two posterior branches, and distally ending into MA, thus defining a large area between it and MA crossed by irregular veinlets; a terminal twig on second posterior branch of anterior branch of MP+CuA+CuP; area between branches of RP, MA and MP+CuA +CuPa regularly connected by simple and oblique crossveins; concave CuP basally dividing into CuPa and CuPb, CuPb running parallel to MP+CuA+CuPa towards posterior wing margin; anal area incomplete with first convex anal vein A1 running parallel to CuP and CuPb; second anal vein A2 distally connected to A1.

Discussion
Piesbergopterum gen. nov. can be clearly attributed to the Archaeorthoptera as it shares the main diagnostic character of this superorder, namely the basal fusion of CuA with M and subsequent connection with the anterior branch of CuP as CuPa (Béthoux and Nel 2002). Furthermore, it displays characters typical of the order Cnemidolestodea, such as ScP terminating on RA, CuPa ending into MP+CuA, MP+CuA+CuPa emitting an anterior branch parallel to MA, with a specialised veinlet between it and MA, thus defining a large area between it and MA crossed by irregular veinlets (Béthoux 2005; Gu et al. 2014, Figs. 1–2).

Aristov (2014), using a different diagnosis and wing venation nomenclature for the Cnemidolestodea, proposed a key to families. *Piesbergopterum* gen. nov. falls in the family Cnemidolestidae Handlirsch, 1906 (sensu Aristov 2014, p. 10) that would comprise also the Ischnoneuridae Handlirsch, 1906, Aetophlebidae Handlirsch, 1906, Narkemidae Handlirsch, 1911, and Narkeminidae Pinto and Ornellas, 1991; because of the character 'MP weak, ending on CuA or MA, or absent'. Indeed, in *Piesbergopterum* gen. nov., MP is clearly basally fused CuA. Béthoux (2005) and Gu et al. (2014, Fig 2 A1, C1) supposed that the vein MP can be reduced to a weak veinlet between MA and CuA+CuP.

The family Cnemidolestidae comprises the following genera, after Aristov (2014) and Gu et al. (2014): *Aetophlebia* Scudder, 1885, *Amphiboliacridites* Langiaux et Parriat, 1974, *Anarkemina* Aristov, 2014, *Argentinonarkemina* Martins-Neto, Gallego and Brauckmann, 2007, *Bouleites* Lameere, 1917, *Carbonokata*

Aristov, 2013, *Cnemidolestes* Handlirsch, 1906, *Evenkiophlebia* Aristov, 2013, *Irajanarkemina* Martins-Neto, Gallego et Brauckmann, 2007, *Ischnoneura* Brongniart, 1893, *Longzhua* Gu, Béthoux, and Ren, 2011, *Narkema* Handlirsch, 1911, *Narkemina* Martynov, 1930, *Narkeminopsis* Whalley, 1979, *Narkeminuta* Aristov, 2013, *Narkemulla* Aristov, 2013, *Paranarkemina* Pinto et Ornellas, 1980, *Protodiamphipnoa* Brongniart, 1885, *Tshunoptera* Aristov, 2013, *Velizphlebia* Martins-Neto, Gallego et Brauckmann, 2007, *Xixia* Gu, Béthoux, and Ren, 2014,

The limits and definitions of these genera remain obscure in the absence of a phylogenetic analysis and global revision of the family.

The pattern of forewing venation of *Piesbergopterum* gen. nov. strongly resembles that of *Narkeminopsis* currently known by three species as *N. eddi* Whalley, 1979 (Carboniferous of Radstock in UK), *N. inversa* Aristov, 2013 (Carboniferous Chunya in Russia), and *N. sowiaki* Brauckmann and Herd, 2006 (Piesberg locality) (Whalley 1979; Béthoux and Nel 2005; Brauckmann and Herd 2006; Aristov 2013). But *Piesbergopterum* differs from *Narkeminopsis* in much broader area between MA and MP+CuA +CuPa (Whalley 1979, Figure 3; Brauckmann and Herd 2006, fig. 9; Béthoux and Nel 2005, fig. 20; Aristov 2013, Fig 1b). *Narkeminopsis eddi* and *N. sowiaki* have a pattern of colouration with prominent dark transversal or oblique stripes. *Aetophlebia, Irajanarkemina, Argentinonarkemina*, and *Longzhua* have a longer stem of RP and a narrower area between MA and MP+CuA+CuPa than in *Piesbergopterum* gen. nov. (Scudder 1890, p. 301–302, pl. 17, fig. 9; Martins-Neto et al. 2007, Figs 2, 7; Gu et al. 2011, Fig 3). *Paranarkemina* has a MP+CuA+CuPa completely separated from MA and a very long stem of RP (Martins-Neto et al. 2007.

Narkemina kata Aristov, 2013 differs from *Piesbergopterum* gen. nov. in the longer and narrower area between MA and MP+CuA +CuPa, more numerous branches of anterior branch of MP+CuA +CuPa, and rudimentary apical veinlet between MP+CuA+CuPa and MA (Aristov 2013, Fig 1a). *Narkemina angustata* Martynov, 1931 would better resemble *Piesbergopterum* gen. nov. in the broad area between MA and MP+CuA+CuPa and a better defined apical veinlet between MP+CuA+CuPa and MA but this veinlet is quite longer than in *Piesbergopterum* gen. nov. and the shape of the branches of the posterior branch of MP+CuA+CuPa is different (Martynov 1931). *Narkemina angustiformis* Sharov, 1961 has also an elongate apical veinlet between MP+CuA+CuPa and MA (Sharov 1961, fig. 94). *Narkemina genuina* Sharov, 1961 is based on a wing apex showing very few characters (Sharov 1961, figs. 95–96).

Aristov (2013, p. 275, Fig 1a–e) characterised the genus *Narkemulla* as follows: distance between base of RP and apex of ScP smaller than wing width (a character present in many other genera, e.g. *Narkeminopsis*). Base of RP located distal to wing middle (basal in *Piesbergopterum* gen. nov.); interradial space wide (an uncertain character, as in *Narkeminopsis* it is as wide as in *Narkemulla*). First bifurcations of MA and MP+CuA+CuPa located proximal to base of RP (also present in many other genera). MP simple or with short fork. MA starting branching proximal to base of RP (not present in *Piesbergopterum* gen. nov.). Anterior branch of MP+CuA+CuPa with three main bifurcations. Apex of A1 fused with A2. *Protodiamphipnoa* has a narrow area between MA and MP+CuA+CuP and a very long stem of RP (Béthoux and Nel 2005, fig. 15).

In *Evenkiophlebia*, Sc is fused with R immediately distal to base of RP, and MA is not touching Rs, unlike in *Piesbergopterum* gen. nov. (Aristov 2013, Fig 1f). In *Carbonokata*, RP has a long basal stem, a small posterior branch of MA in area between MA and MP +CuA+CuP, and a long veinlet at apex of MP+CUA+CuPa, unlike *Piesbergopterum* gen. nov. (Aristov 2013, Fig 1g). *Tshunoptera* has also a very long stem of RP (Aristov 2013, Fig 1h). *Bouleites* and *Cnemidolestes* have a very long stem of RP, and ca. six posterior branches of anterior branch of MP+CuA+CuPa (Béthoux and Nel 2005, figs. 18, 21). *Narkeminuta* and *Anarkemina* have no fusion between RP and MA and a MP separated from CuA+CuPa, which would put some doubt on their attributions to the Cnemidolestidae (Aristov 2013, Fig 1i; 2014, Figs 1–2). *Velizphlebia* has also a MA separated from RP and a posterior branch of MA looking like a MP (Martins-Neto et al. 2007, Figs 3–5). *Amphiboliacridites* has a rather broad area between MA and MP+CuA+CuPa and a pattern of spotted colouration as in *Piesbergopterum* gen. nov. but a long veinlet at apex of MP+CuA+CuPa and a very long stem of RP (Langiaux and Parriat 1974, p. 72). *Ischnoneura* has a rather well-defined posterior branch of MA in an opened area between MA and MP+CuA+CuPa, plus no contact between MA and RP (Béthoux and Nel 2005, fig. 19).

The species of *Narkema* (*N. taeniatum* Handlirsch 1911, *N. alternatum*; Cockerell 1924) have a very long stem of RP and a very long stem of anterior branch of MP+CuA+CuPa (Handlirsch 1911, fig. 28; Cockerell 1924, 1927).

Xixia (*X. huban* Gu et al. 2014, Xiaheyan in China) shares numerous characters with *Piesbergopterum* gen. nov., viz. costal area broader than subcostal; RP with numerous posterior branches; presence of specialised veinlet between MP+CuA+CuPa and posterior branch of MA (Gu et al. 2014). However, this veinlet is quite short in *Piesbergopterum punctatum* gen. nov. et sp. nov. while it is long and more or less sigmoidal in *Xixia*; also the posterior branch of MP+CuA+CuPa is regularly anteriorly pectinate in *Piesbergopterum* gen. nov., while its branches are quite irregular in *Xixia*.

The pattern of wing colouration is quite common among Cnemidolestodea. The spotted pattern of *Piesbergopterum* gen. nov., is also present in *Amphiboliacridites, Xixia huban, Narkemina angustiformis* Sharov, 1961 and weakly indicated in *Ischnoneura oustaleti* Brongniart, 1885 or *Protodiamphipnoa gaudryi* Brongniart, 1885. Other typical pattern of colouration is the transverse bands found in *Narkeminopsis eddi, Cnemidolestes woodwardi* Brongniart, 1893 or *Narkema taeniatum* Handlirsch, 1911. The spotted pattern can be found also in other Archaeorthoptera as in caloneurodean *Caloneura dawsoni* Brongniart, 1885 and partially in *Cacurgus spilopterus* Handlirsch, 1911 belonging to Archaeorthoptera inc. sed. These colour patterns have probably a disruptive camouflage function and can be found more frequently in Mesozoic and Cenozoic insects (e.g., Gao et al. 2020). The representatives of Cnemidolestodea are considered to be predacious due to massive and thorny first pair of legs (Handlirsch 1937; Hamilton 2011). And although a disruptive camouflage is considered to be protection against predators, it actually can serve in similar way as a confusion of a potential prey. Using various types of camouflage in this way is frequent e.g., in recent mantises or predaceous katydids (Cuthill et al. 2005).

Conclusions

The fossils from Piesberg quarry represent an exceptional source of data on diversity and morphological disparity of Pennsylvanian (Moscovian) insects. All three newly described taxa demonstrate unique combinations of characters in forewing venation pattern among Archaeorthoptera.

Contracladus gen. nov., established for *Contracladus impar* sp. nov. (type species), exhibits an unusual mosaic of characters with forked concave CuP, anterior branch ending into M+ CuA and vein M+ CuA basally fused with R as typical pattern for Archaeorthoptera, but it lacks the free vein CuA. The latter character is usually considered as synapomorphy for this group (see

Béthoux and Nel 2002). Therefore, it can be considered as an Archaeorthoptera of uncertain affinities pointing out the limits of current systematics.

Paraspiculum gen. nov. is established for *Paraspiculum brauckmanni* sp. nov. (type species), bearing a typical pattern of venation for Caloneurodea. It is the most similar to that of paratype of *Spiculum mendicum* Brauckman and Herd 2006, which is also surprisingly different from the holotype. The paratype of *S. mendicum* differs from *Paraspiculum brauckmanni* sp. nov. in total number of branches RP and the emergence of the first branch. Hence, the disparity of elongated wings of Caloneurodeans was unusual.

Piesbergopterum gen. nov. is established for *Piesbergopterum punctatum* sp. nov. (type species), showing a typical pattern of venation for Cnemidolestodea. Its venation strongly resembles that of *Narkeminopsis*, but with a less developed area between MA and MP+CuA+CuPa (Whalley 1979). In addition, *Piesbergopterum punctatum* sp. nov. shows spotted pattern of original colouration on tegmina, which had probably a disruptive function.

In conclusion, the polyneopteran insects reported from Piesberg and also other Moscovian localities like Avion and Commentary (both in France) or Mazon Creek (Illinois, U.S.A.), were incredibly diverse and abundant. Nevertheless, in contrast to the latter two localities, the insect fossils from Piesberg are almost entirely preserved as isolated wings or wing pads (only 1–3% of the insect remains are more or less complete specimens, see Wrede et al. 2019), which indicate decay and disarticulation of exoskeleton followed by short post-mortem transport and drowning (Duncan et al. 2003). Above the taphonomy significance, all three new species of Archaeorthoptera extend the disparity of morphological plans in the forewing venation which may contribute greatly to the analysis of phylogenetic relationships in the future.

Acknowledgements

The authors thank Alexander Rasnitsyn and two anonymous reviewers for their insightful comments and suggestions to the early version of this manuscript. We cordially thank Michael Sowiak (Glandorf, Germany) for his effort with the fieldwork at Piesberg quarry and willingness to provide specimens access for scientific evaluation. We also thank the GP Papenburg Company for their permission and technical support for palaeontological field work in their quarry. JP and MP gratefully acknowledge research support from the Grant Agency of the Czech Republic (No. 18-03118S).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Grantová Agentura České Republiky [18-03118S].

ORCID

Jakub Prokop http://orcid.org/0000-0001-6996-7832

References

Aristov DS. 2013. New and little-known Eoblattida (Insecta) from the Paleozoic of Russia. Paleont J. 47:272–282. doi:10.1134/S0031030113030039

Aristov DS. 2014. Classification of the order Cnemidolestida (Insecta: perlidea) with descriptions of new taxa. Far East Entomol. 277:1–46.

Aristov DS. 2020. Revision of the genera *Heteroptilon, Nugonioneura* and *Opistocladus* from the Lower Permian of USA (Insecta: cnemidolestida: Tillyardembiidae, Nugonioneuridae and Parmapteridae). Far East Entomol. 401:1–9.

Aristov DS, Bashkuev AS, Golubev VK, Gorochov AV, Karasev EV, Kopylov DS, Ponomarenko AG, Rasnitsyn AP, Rasnitsyn DA, Sinitshenkova ND, et al. 2013. Fossil insects of the Middle and Upper Permian of European Russia. Paleont J. 47:641–832. doi:10.1134/S0031030113070010

Beckemeyer RJ. 2009. *Ligogramma wichita*, a new species of Caloneurodea (Polyneoptera: orthopterida) from the Lower Permian Wellington Formation of Noble County, Oklahoma. J Kansas Entomol Soc. 82 (4):300–304. https://www.jstor.org/stable/20621965

Béthoux O. 2005. Cnemidolestodea (Insecta): an ancient order reinstated. J Syst Palaeont. 3:403–408. doi:10.1017/S147720190500163X

Béthoux O, Nel A. 2002. Venation pattern and revision of Orthoptera sensu nov. and sister groups. Phylogeny of Palaeozoic and Mesozoic Orthoptera sensu nov. Zootaxa. 96:1–88. doi:10.11646/zootaxa.96.1.1

Béthoux O, Nel A. 2005. Some Palaeozoic 'Protorthoptera' are 'ancestral' orthopteroids: major wing braces as clues to a new split among the 'Protorthoptera. J Syst Palaeont. 2:285–309. doi:10.1017/S1477201904001488

Béthoux O, Nel A, Lapeyrie J. 2004. The extinct order Caloneurodea (Insecta: pterygota: panorthoptera): wing venation, systematics and phylogenetic relationships. Ann Zool. 54:287–300.

Brauckmann C, Braukmann B, Gröning E. 1996. The stratigraphical position of the oldest known Pterygota (Insecta, Carboniferous, Namurian). Ann Soc Géol Belg. 117:47–56.

Brauckmann C, Herd KJ. 2002. Insekten-Funde aus dem Westfalium D (Ober-Karbon) des Piesberges bei Osnabrück (Deutschland). Teil 1: palaeoptera. Osnabrück Naturwiss Mitt. 28:27–69.

Brauckmann C, Herd KJ. 2006. Insekten-Funde aus dem Westfalium D (Ober-Karbon) des Piesberges bei Osnabruck (Deutschland). Teil 2: neoptera. Osnabrück Naturwiss Mitt. 30/31:19–65.

Brauckmann C, Herd KJ, Leipner A. 2009. Insekten-Funde aus dem Westfalium D (Ober-Karbon) des Piesberges bei Osnabrück (Deutschland). Nachtrag 1: palaeodictyopteroida. Osnabrück Naturwiss Mitt. 35::1–30.

Brongniart C. 1885. Les Insectes fossiles des terrains primaires. Coup d'oeil rapide sur la faune entomologique des terrains paléozoïques. Bull Soc Amis Sci Natur Rouen. 21:50–68.

Brongniart C. 1893. Recherches pour servir à l'histoire des insectes fossiles des temps primaires précédées d'une étude sur la nervation des ailes des insectes. Bull Soc Industr Minéral St Etienne. 3,7:4:1–491.

Burnham L. 1983. Studies on the Upper Carboniferous insects: 1. The Geraridae (order Prothortoptera). Psyche. 90:1–57. doi:10.1155/1983/42898

Carpenter FM. 1943. The Lower Permian insects of Kansas. Part 9. The orders Neuroptera, Raphidiodea, Caloneurodea and Prothortoptera (Prosbnisidae), with additional Protodonata and Megasecoptera. Proc Am Acad Arts Sci. 75:55–84.

Carpenter FM. 1965. Studies on North American Carboniferous insects. Part 4. Genera *Metropator, Eubleptus, Hapaloptera* and *Hadentomum*. Psyche. 72:175–178. doi:10.1155/1965/56936

Carpenter FM. 1976. The Lower Permian insects of Kansas. Part 12. Protorthoptera (continued), Neuroptera, additional Palaeodictyoptera, and families of uncertain position. Psyche. 83:336–376. doi:10.1155/1976/932123

Carpenter FM. 1992. Superclass Hexapoda. In: Moore RC, Kaesler RL, editors. Treatise on Invertebrate Paleontology (R), Arthropoda 4, 3/4. Boulder: The Geological Society of America and the University of Kansas; p. xxii+655.

Cockerell TDA. 1924. Fossil insects. Entomol News. 35:28–30.

Cockerell TDA. 1927. The Carboniferous insects of Maryland. Ann Mag Nat Hist. 9(19):385–416.

Coty D, Háva J, Prokop J, Roques P, Nel A. 2014. New archaeorthopteran insects from the Late Carboniferous of the Nord and Pas-de-Calais basins in northern France (Insecta: cnemidolestodea, Panorthoptera). Zootaxa. 3878:462–470. doi:10.11646/zootaxa.3878.5.4

Crampton GC. 1928. The grouping of insect orders and their lines of descent. Entomologist. 61:82–85.

Cuthill IC, Stevens M, Sheppard J, Maddocks T, Párranga AC, Troscianko TS. 2005. Disruptive coloration and background pattern matching. Nature. 434:72–74. doi:10.1038/nature03312

David F. 1990. Sedimentologie und Beckenanalyse im Westfal C und D des nordwestdeutschen Oberkarbons. DGMK-Bericht. 384(3):1–267.

Duncan IJ, Titchener F, Briggs DEG. 2003. Decay and disarticulation of the cockroach: implications for preservation of the blattoids of Writhlington (Upper Carboniferous), UK. Palaios. 18:256–265. doi:10.1669/0883-1351-(2003)018<0256:dadotc>2.0.CO;2

Dvořák T, Pecharová M, Krzemiński W, Prokop J. 2019. New archaeorthopteran insects from the Carboniferous of Poland: insights into tangled taxonomy. Acta Palaeont Pol. 64:787–796. doi:10.4202/app.00614.2019

Gao T, Shih C, Ren D. 2020. Behaviors and interactions of Insects in ecosystems of mid-mesozoic northearsten China. Ann Rev Entomol. 66:1–18. doi:10.1146/annurev-ento-072720-095043

Gu J-J, Béthoux O, Ren D. 2011. *Longzhua loculata* n. gen. n. sp., one of the most completely documented Pennsylvanian Archaeorthoptera (Insecta; Ningxia, China). J Paleont. 85:303–314. doi:10.1666/10-085.1

Gu J-J, Béthoux O, Ren D. 2014. A new cnemidolestodean stem-orthopteran insect from the Late Carboniferous of China. Acta Palaeont Pol. 59:689–696. doi:10.4202/app.2011.0204

Gu J-J, Béthoux O, Ren D. 2017. A new, rare and distinctive species of Panorthoptera (Insecta, Archaeorthoptera) from the Upper Carboniferous of Xiaheyan (Ningxia, China). Fossil Rec. 20:253–257. doi:10.5194/fr-20-253-2017

Hamilton KGA. 2011. Cnemidolestodea: an ancient insect order resurrected. Biodiversity. 4:22–24. doi:10.1080/14888386.2003.9712686

Handlirsch A. 1906. Die fossilen Insekten und die Phylogenie der rezenten Formen. Ein Handbuch für Paläontologen und Zoologen. Leipzig: Wilhelm Engelman; p. 1430.

Handlirsch A. 1911. New Paleozoic insects from the vicinity of Mazon creek, Illinois. Am J Sci. 31(4):297–326 + 353–377. doi:10.2475/ajs.s4-31.185.353.

Handlirsch A. 1919. Revision der paläozoischen Insekten. Denks K Akad Wissen, Math-Naturwissen Kl. 96:511–592.

Handlirsch A. 1922. Animalia Insecta Palaeozoica. In: Diener C, editor. Fossilium Catalogus. Vol. 16. Berlin: Junk W. (Publ.); p. 230.

Handlirsch A. 1937. Neue Untersuchungen über die fossilen Insekten. Ann Naturhist Mus Wien. 48:1–140.

Haug JT, Leipner A, Wappler T, Haug C. 2013. Palaeozoic insect nymphs: new finds from the Piesberg quarry (Upper Carboniferous, Germany). Bull Geosci. 88:779–791.

Hörnig MK, Haug C, Herd KJ, Haug JT. 2014. New insights into dictyopteran early development: smallest Palaeozoic roachoid nymph found so far. Palaeodiversity. 7:159–165.

Huang D, Fu Y, Lian X, Nel A. 2020. *Sinaspidoneura magnifica* nov. gen., nov. sp., first Chinese Caloneurodea (Insecta: archaeorthoptera). Geobios. 63:33–37. doi:10.1016/j.geobios.2020.10.003

Jones NS, Glover BW. 2005. Fluvial sandbody architecture, cyclicity and sequence stratigraphical setting – implications for hydrocarbon reservoirs: the Westphalian C and D of the Osnabrück Ibbenbüren area, northwest Germany. Occ Publ Ser Yorkshire Geol Soc. 7:57–74.

Josten KH, Köwing K, Rabitz A. 1984. Oberkarbon. In: Klassen H, editor. Geologie des Osnabrücker Berglandes. Osnabrück: Naturwissenschaftliches Museum Osnabrück; p. 7–77.

Kiesmüller C, Hörnig MK, Leipner A, Haug C, Haug JT. 2019. Palaeozoic palaeodictyopteran insect nymphs with prominent ovipositors from a new locality. Bull Geosci. 94:23–40.

Köwing K, Rabitz A. 2005. Osnabrücker Karbon. Cour Forschungsinst Senckenberg. 254:255–270.

Langiaux J, Parriat H. 1974. Faune entomologique du bassin de Blanzy-Montceau. "La Physiophile". Soc Et Sci Nat Hist Montceau-les-Mines. 81:62–74.

Leipner A, Chellouche P. 2019. A newly discovered lake deposit of Pennsylvanian age (upper Moscovian, Wesphalian D) from the Piesberg Quarry, Osnabrück, Northwestern Germany [abstract]. In: hartenfels S, Herbig HG, Amler MRW, Aretz M editors. 19th Int Congr Carb Perm, Cologne, July 29 – august 2, 2019. Kölner Forum Geol Paläont. 23:197–198.

Loureiro JP, Correia P, Nel A, Pinto de Jesus A. 2010. *Lusitaneura covensis* n. gen., n. sp., first Caloneurodea from the Carboniferous of Portugal (Insecta: pterygota: panorthoptera). Ann Soc Entomol Fr (N S). 46:242–246. doi:10.1080/00379271.2010.10697664

Martins-Neto RG, Gallego OF, Brauckmann C, Cruz JL. 2007. A review of the South American Palaeozoic entomofauna. Part I: the Ischnoneuroidea and Cacurgoidea, with description of new taxa. Afr Invert. 48:87–101.

Martynov AV. 1930. On the Paleozoic insects of the Kuznetsk Basin. Izvet Glavn- Geol Upravl. 49:73–100. [In Russian].

Martynov AV. 1931. New fossil insects from Tikhie Gory. 2. Neoptera (excluding Miomoptera). Trudy Geol Muz Akad Nauk SSSR. 8:149–212. [In Russian].

Martynov AV. 1937. Permian fossil insects from Kargala and their relationships. Trudy Paleont Inst Akad Nauk SSSR. 7:8–92. [in Russian].

Prokop J, Krzeminski W, Krzeminska E, Hörnschemeyer T, Ilger JM, Brauckmann C, Grandcolas P, Nel A. 2014b. Late Palaeozoic Paoliida is the sister group of Dictyoptera (Insecta: neoptera). J Syst Palaeont. 12:601–622. doi:10.1080/14772019.2013.823468

Prokop J, Krzemiński W, Krzemińska E, Wojtechowski D. 2012. Paoliida, a putative stem-group of winged insects: morphology of new taxa from the Upper Carboniferous of Poland. Acta Palaeont Polonica. 57:161–173. doi:10.4202/app.2010.0064

Prokop J, Pecharová M, Ren D. 2016. Hidden surface microstructures on Carboniferous insect *Brodioptera sinensis* (Megasecoptera) enlighten functional morphology and sensorial perception. Sci Rep. 6:28316. doi:10.1038/srep28316

Prokop J, Roques P, Nel A. 2014. New non-holometabolous insects from Pennsylvanian of Avion locality in Pas-de-Calais, France (Insecta: 'Exopterygota'). Alcheringa. 38:155–169. doi:10.1080/03115518.2014.848620

Pruvost P. 1927. Sur une aile d'insecte trouvée au sondage de Gulpen. Jaarvest Nederland Mijngeb Heerlen. 1926:76–77.

Rasnitsyn AP. 2007. On the discussion of the wing venation of (Archae) Orthoptera (Insecta). Paleont J. 41:341–344.

Schultka S. 2000. Zur Palökologie der Euproopiden im Nordwestdeutschen Oberkarbon. Mitt Mus Natur Be Geowissen Reihe. 3:87–98.

Scudder SH. 1890. The fossil insects of North America (with notes on some European species). 1. The Pretertiary insects. Rep US Geol Surv Terr. 13:1–453.

Sharov AG. 1961. Order Plecoptera. In: Rohdendorf BB, Becker-Migdisova EE, Martynova OM, Sharov AG, editors. Paleozoiskie nasekomye Kuznetskogo basseina [Paleozoic insects of the Kuznetsk basin.], Trudy Paleontol Inst Akad Nauk SSSR. 85:166–175.

Tillyard RJ. 1937. Kansas Permian insects. 17. The order Megasecoptera and additions to the Palaeodictyoptera, Odonata, Protoperlaria, Copeognatha and Neuroptera. Am J Sci. 33(5):81–110.

Whalley PES. 1979. New species of Protorthoptera and Protodonata (Insecta) from the Upper Carboniferous of Britain, with a comment on the origin of wings. Bull Br Mus Nat Hist Geol. 32:85–90.

Wrede V, Drozdzewski G, Juch D, Leipner A, Sowiak M. 2019. Field Trip A2: the Pennsylvanian of the Ruhr Basin and Osnabrück region, western Germany – facies, stratigraphy and tectonics of a paralic foreland basin of the Variscides. In: herbig HG, Aretz M, Amler MRW, Hartenfels S., editors. Field Guide of the 19th Int Congr Carb Perm, July 29 – august 2; Cologne. Kölner Forum Geol Paläont. 24:43–80.

Study VI

Dvořák, T., Krzemiński, W., & Prokop, J. (2023). New stem-dictyopteran insects from the Pennsylvanian deposits at Mazon Creek and Sosnowiec (Insecta: Polyneoptera). *Historical Biology*, 35(12), 2334-2339.

Historical Biology
An International Journal of Paleobiology



Taylor & Francis
Taylor & Francis Group

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ghbi20

New stem-dictyopteran insects from the Pennsylvanian deposits at Mazon Creek and Sosnowiec (Insecta: Polyneoptera)

Tomáš Dvořák, Wiesław Krzemiński & Jakub Prokop

To cite this article: Tomáš Dvořák, Wiesław Krzemiński & Jakub Prokop (2023) New stem-dictyopteran insects from the Pennsylvanian deposits at Mazon Creek and Sosnowiec (Insecta: Polyneoptera), Historical Biology, 35:12, 2334-2339, DOI: 10.1080/08912963.2022.2140423

To link to this article: https://doi.org/10.1080/08912963.2022.2140423

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

Published online: 07 Nov 2022.

Submit your article to this journal

Article views: 891

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ghbi20

HISTORICAL BIOLOGY
2023, VOL. 35, NO. 12, 2334–2339
https://doi.org/10.1080/08912963.2022.2140423

Taylor & Francis
Taylor & Francis Group

OPEN ACCESS Check for updates

New stem-dictyopteran insects from the Pennsylvanian deposits at Mazon Creek and Sosnowiec (Insecta: Polyneoptera)

Tomáš Dvořák[a], Wiesław Krzemiński[b] and Jakub Prokop[a]

[a]Department of Zoology, Faculty of Science, Charles University, Praha 2, Czech Republic; [b]Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland

ABSTRACT

Polyneopteran clade of insects is very diverse and has a remarkable fossil history starting in the Late Palaeozoic. Representatives of Dictyoptera comprise roaches, termites and mantids and their stem group relatives like the common Palaeozoic and Mesozoic roachoids, a group well known for its problematic taxonomy. In this study, two new taxa are described based on forewing venation well displaying convexity or concavity of the veins due to preservation in spherosideritic concretions. A new paoliid, *Stephanopsis testai* sp. nov. (Blattinopsidae), from Moscovian deposits at Mazon Creek (Illinois, U.S.A.), supplements *Stephanopsis mirandus* found at the same locality, which mainly differs in the branching patterns of MP and anal veins. A new stem dictyopteran, *Sosnowiecia dareki* gen. nov. et sp. nov. (?Mesorthopteridae), from Langsettian deposits at Sosnowiec-Klimontów (Lower Silesia, Poland) is problematic in terms of taxonomy as it has a mosaic of characters characteristic of Paoliida, Eoblattida and Grylloblattida, but retains affinities with roachoid insects, such as *Miroblatta* sp. Hence, these two new species increase the diversity of Carboniferous polyneopteran insects, but reveals the problem of depending only on alar characters in taxonomy.

LSID urn:lsid:zoobank.org:pub:49299826-0F1E-4892-8D88-82FBDCC192BF

ARTICLE HISTORY
Received 11 July 2022
Revised 21 October 2022
Accepted 22 October 2022

KEYWORDS
Stem Dictyoptera; Paoliida; Blattinopsidae; roachoids; wing venation; Carboniferous

Introduction

Dictyoptera is a well-supported clade of polynepteran insects comprising crown groups of Mantodea (mantids) and Blattodea (cockroaches and termites) with long evolutionary histories and stem group relatives in the Late Palaeozoic and Mesozoic strata (Legendre et al. 2015; Wipfler et al. 2019). These include particularly well-known roachoids, the females of which had very long ovipositors (Grimaldi and Engel 2005; Hörnig et al. 2018). Although these insects were very abundant and diverse in Late Palaeozoic ecosystems, their systematics is unsatisfactory mainly due to large intraspecific variability in wing venation, which is frequently used as characters for their classification. On the other hand, the alar characters of the stem group of Dictyoptera, such as Paoliida, are not so variable at the intraspecific level and therefore more suitable for taxonomy (Prokop et al. 2014). Paoliida currently consists of three families, Paoliidae, Blattinopsidae and Anthracoptilidae, but the position of some others remains uncertain (Guan et la. 2016).

In this paper two new Pennsylvanian taxa of stem-Dictyoptera are designated: *Stephanopsis testai* sp. nov. (Blattinopsidae) from Mazon Creek in Illinois, U.S.A and *Sosnowiecia dareki* gen. et sp. nov. (?Mesorthopteridae) from Sosnowiec-Klimontów in Lower Silesia, Poland. In addition, problems with the classification of Palaeozoic polyneopteran insects are discussed.

Materials and methods

Materials examined in this study came from spherosideritic concretions/nodules from two Pennsylvanian deposits; Mazon Creek Lagerstätte in Illinois, U.S.A. (Moscovian, Carbondale Formation) and Sosnowiec-Klimontów in Lower Silesia, Poland (Langsettian, Mudstone series – Załęże beds, Coal-bearing Mudstone). While Mazon Creek is a world-famous site with a diverse entomofauna (Carpenter 1997; Kukalová-Peck 1997), insects from Sosnowiec are only reported in a few publications (Krawczyncski et al. 1997; Prokop et al. 2012, 2014, 2017, 2022; Dvořák et al. 2019).

The specimens were observed in a dry state using a Nikon SMZ 745T stereomicroscope. Line drawings of venation were made using a Zeiss SteREO Discovery.V20 with camera lucida attachment. Habitus drawing of *S. dareki* gen. nov. et sp. nov. was done by a scientific illustrator, Zuzana Čadová (Liberec, Czech Republic). Photographs of fossil specimens in a dry state were taken with a Canon EOS 550D digital camera equipped with either a MP-E 65 mm or EF-S 60 mm macro-lens. Original photographs were processed using the image-editing software Adobe Photoshop CS and some were processed using the stacking software Helicon Focus.

Both type specimens are housed in the institutional collections of The Field Museum of Natural History (Chicago, U.S.A.) and the Natural History Museum of the Institute of Systematics and Evolution of Animals PAS (Cracow, Poland).

The nomenclature of the wing venation generally follows the scheme of Brodsky (1994) and Schubnel et al. (1961). Abbreviation of veins: A1/A2 – anal veins; CuA/CuP – cubitus anterior/posterior; M – stem of media; MP – media posterior; PCu – postcubital vein; RA/RP – radius anterior/posterior; ScA/ScP – subcosta anterior/posterior.

CONTACT Jakub Prokop jprokop@natur.cuni.cz Department of Zoology, Faculty of Science, Charles University, Viničná 7, CZ-128 00, Praha 2, Czech Republic

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.

Published online 07 Nov 2022

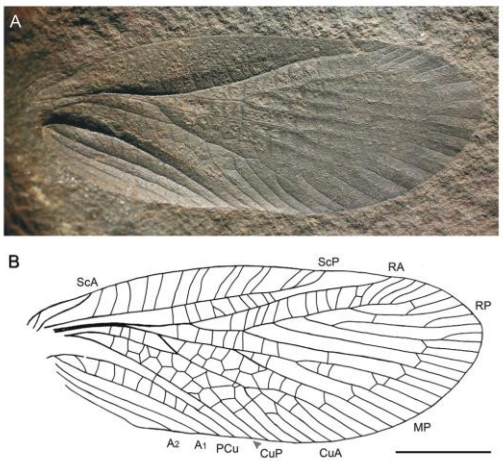


Figure 1. *Stephanopsis testai* sp. nov., holotype TVT 4023 (originally in Thomas V. Testa coll., currently deposited in FMNH), Moscovian: Middle Pennsylvanian, Mazon Creek, Illinois U.S.A. (A) photograph of forewing venation; (B) line drawing of venation of forewing. Scale bars = 5 mm.

Results

Order Paoliida Handlirsch 1906
Family Blattinopsidae Bolton, 1925
Genus *Stephanopsis* Kukalová, 1958

Stephanopsis testai sp. nov. (Figure 1)
ZooBank LSID: urn:lsid:zoobank.org:act:ECF9934E-1F55-46E1-9FA7-A59DADFD6796

Etymology

Patronymic; named after Thomas V. Testa in honour of his fieldwork at Mazon Creek.

Diagnosis

Area between C and ScP twice as wide as the area between ScP and RA. First branches of RP and MP at the same level of about half of the length of the wing. RP posteriorly pectinated with five primary branches. Two rows of cells between CuA and CuP below the arculus. PCu vein well developed. Anal veins simple.

Description

Based on forewing venation. Wing very well preserved with numerous cross veins and noticeable reticular pattern with two rows of large irregular cells in cubital and partially in medial area. Wing length about 23.9 mm, and maximum width 9 mm. Noticeable short weakly sigmoidal ScA ending at the costal margin 3.7 mm from the wing base. ScP with numerous anterior and slightly oblique veinlets, ending at the costal margin two-thirds along the length of the wing. Area between C and ScP wide (1.8 mm), about twice the width at the widest part of the area between ScP and RA. RA apparently convex, slightly sigmoidal and simple. RP emerging from RA 8.7 mm from the base of the wing, regularly posteriorly pectinated with five main branches and several sigmoidal anterior terminal shoots that reach the apical margin of the wing. MP with three branches, with the first at the same level as the first branch of RP, around half of the length of the wing. CuA with five primary posterior branches. Branches of RP, MP and CuA run parallel and form a regular pattern distally with veinlets between adjoining branches about 3 mm in length. Strong oblique cross vein mp-cua (arculus) present between MP and CuA. CuP simple and distally nearly straight. Convex PCu running parallel to CuP (*sensu* Schubnel et al. 2020). Three simple anal veins discernible.

Type material

Holotype TVT 4023 (originally in Thomas V. Testa coll., currently deposited in FMNH), positive (B) and negative imprint (A) of nearly complete forewing preserved in siderite nodule.

Type locality

Mazon Creek, Peabody Coal Pit 11, Grundy County, Illinois, U.S.A.

Type strata

Francis Creek Shale Member, Carbondale Formation, Will-Kankakee-Grundy Co., Illinois, U.S.A.; Moscovian (315.2–307.0 Ma), Middle Pennsylvanian, Carboniferous (Shabica and Hay 1997).

Discussion

This fossil wing clearly belongs to Blattinopsidae (Paoliida) because ScP ends on the costal margin with exception in *Avionblattinopsis* Quispe et al., 2021, stem of R sigmoidal, RP pectinated with high number of posterior branches and slightly, but not extensively extends the area between MP and CuA, which differentiates Blattinopsidae from Paoliidae (Prokop et al. 2014; Quispe et al. 2021). There is also a superficial resemblance in the venation with some Archaeorthoptera, but this group differs in a common stem for M + CuA and the fusion of CuA and CuP(a), which are missing in this fossil (Béthoux and Nel 2002).

The general pattern of venation *S. testai* sp. nov. resembles some other blattinopsid like species of the genus *Glaphyrophlebia* Handlirsch, 1906, but *Glaphyrophlebia* has a regular parallel pattern of R, M and CuA branches with many supplementary veins. For example, in *Glaphyrophlebia wettinensis* Fritsch, 1899 the first branch of RP and MP is at the same level and RP is posteriorly pectinated and has a similar number of branches (Hörnschmeyer and Stapf 2001). But there is a marked difference between *S. testai* sp. nov. and *Glaphyrophlebia* in that it lacks the typical vein-bow and has a distinctly narrower space between veins ScP and RA (Hörnschemeyer and Stapf 2001; Aristov et al. 2021; Aristov and Rasnitsyn 2022a).

The present forewing can be clearly assigned to the genus *Stephanopsis* Kukalová, 1958 as the width of the costal area (between C and ScP) is much wider than the area between ScP and RA (Hörnschemeyer and Stapf 2001). *Stephanopsis testai* sp. nov. clearly differs from *Stephanopsis incerta* Laurentiaux, 1950 and *Stephanopsis elegans* Schlechtendal in Handlirsch 1906 by the position of the first branch of RP being about half way along the wing, whereas in the latter species, the first branch of RP is about a third of the way along the length of the wing and more anterior to the first branch of MP. Moreover, *S. elegans* has a much longer ScP reaching far beyond two-thirds of the way along the length of the wing and in *S. incerta* there is a much finer reticular pattern between veins CuA and CuP. But as the preserved fossil of *S. incerta* is probably a hindwing (Kukalová 1958), an exact comparison is difficult. The venations of *S. testai* sp. nov. and *S. mirandus* (Richardson, 1956) are similar and they are described from the same locality. The vein

RA has a similar sigmoidal shape, first branch of RP and MP occur at the same level of about half way along the length of the wing and the general branching pattern of RP is similar (Richardson 1956). However, MP has the same number of branches in both species, but their branching pattern is different in respect of topology. The most remarkable difference is the posterior branching of the anal veins in *S. mirandus*, whereas in *S. testai* sp. nov. they are clearly all simple. Hörnschmeyer and Stapf (2001) also consider the venation of *Klebsiella extincta* (Meunier, 1908) to be similar to that of the genus *Stephanopsis* (Meunier 1908). As the holotype of *K. extincta* was recently reviewed by Quispe et al. (2021), we compared both these species. The general pattern of veins is comparable, especially the primary branching of the main veins. However, a better developed medial area with six terminal branches instead of three as in *S. testai* sp. nov. and a less developed cubital area clearly distinguish these two species. Also, the regular pattern of terminal veinlets is absent in *K. extincta*.

Superorder Dictyoptera
Family ?Mesorthopteridae
Genus *Sosnowiecia* gen. nov.
ZooBank LSID: urn:lsid:zoobank.org:act:1EEACE1C-BDE9-4332-83A7-9D255F5C1858

Type species. *Sosnowiecia dareki* sp. nov.

Diagnosis
Based on forewing characters: RA and RP branch near the middle of the wing behind the first and second branches of M. Long stem of Cu. CuA posteriorly pectinated with four convex branches. Area between MP and CuA very narrow with one row of cells. Arculus (cross vein mp-cua) weakly developed. CuP terminally bifurcated. Area between CuA and CuP slightly enlarged with numerous irregular cells.

Etymology
Named after the locality, Sosnowiec (Lower Silesia, Poland), where the specimen was found; gender feminine.

Sosnowiecia dareki sp. nov. (Figures 2, 3)
ZooBank LSID: urn:lsid:zoobank.org:act:8151F945-C84D-41FA-BB68-E259C6178014

Etymology
Named after Darek (diminutive of Polish first name Dariusz) the collector of this fossil; gender feminine.

Diagnosis
By monotypy, that of the genus.

Description
Body preserved with arched mesonotum and both mesothoracic wings flexed and slightly open, partially preserved metathorax hidden under the anal areas of wings and four slender segments of abdomen (proximal part of abdomen hidden as well). Forewing incompletely preserved with about one-third of the distal part missing. Regular net of transverse veinlets or irregular cells, the so-called archedictyon, present. ScP slightly oblique, running parallel to stem of R and ending in distal third of the length of the wing. Costal area between anterior wing margin and ScP with a coarse network and two rows of cells. Costal area 1.4 mm wide without apparent widening near wing base; costal area about one and half times wider than the widest part of the area between ScP and RA. RA/RP bifurcation relatively distant, 13.9 mm from base of wing, which is approximately between one-third and half of the length of the wing. RA and RP simple in preserved basal parts of the wing. Stem of M/MP clearly concave and anteriorly pectinated with four visible branches. First division of MP is 3.3 mm from the bifurcation of RA and RP. Straight or oblique simple cross veins in area

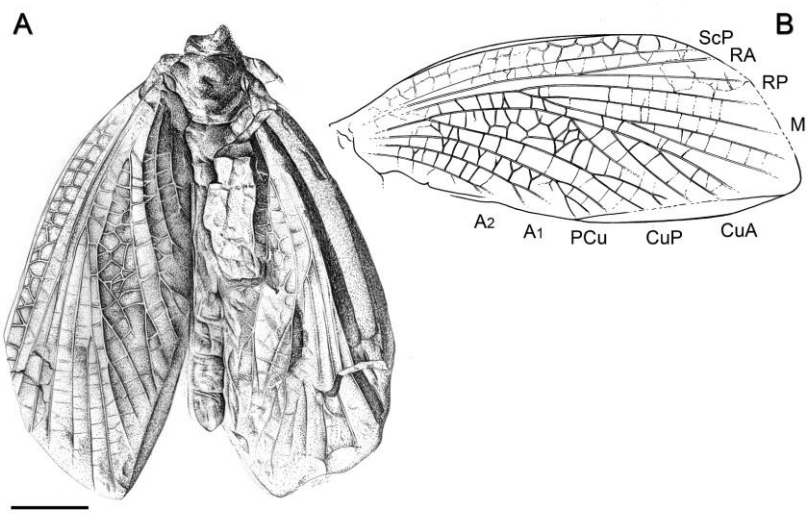


Figure 2. *Sosnowiecia dareki* gen. et sp. nov., holotype MP ISEA I–F/MP/1488/22-08 (Natural History Museum of the Institute of Systematics and Evolution of Animals PAS, Cracow coll.), Langsettian (Westphalian A): Pensylvannian, Sosnowiec-Klimontów, originally Porąbka-Klimontów Mine, Poland. (A) line drawing of habitus, (B) line drawing of forewing venation. Both line drawings were done by Zuzana Čadová. Scale bars = 5 mm.

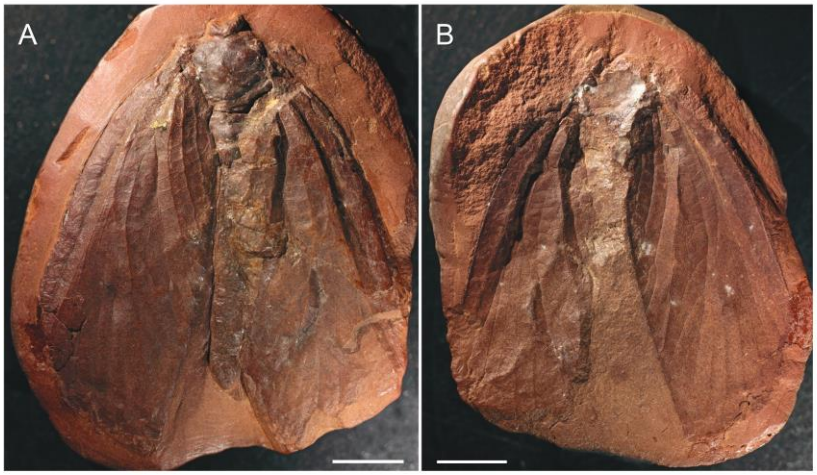


Figure 3. *Sosnowiecia dareki* gen. et sp. nov., holotype MP ISEA I–F/MP/1488/22-08 (Natural History Museum of the Institute of Systematics and Evolution of Animals PAS, Cracow coll.), Langsettian (Westphalian A): Pensylvannian, Sosnowiec-Klimontów, originally Porąbka-Klimontów Mine, Poland. (A) photograph of habitus – imprint, (B) photograph of habitus – counter-imprint. Scale bars = 5 mm.

between branches of MP. Stem of Cu relatively long with the division into CuA and CuP about 4.6 mm from wing base. Space between CuA and CuP widely open with irregular network of cells. Strongly convex CuA posteriorly pectinated and ending with four simple branches on posterior wing margin. First division of CuA nearly level with division of RA and RP. CuP concave with distal branches and anterior branch ending with short twig. Convex PCu distally bifurcated and posterior branch ending with a short twig on posterior margin of wing. Two anal veins ending with terminal twigs. Numerous simple or Y shaped cross veins between branches of CuP, PCu and anal veins.

Type material

Holotype MP ISEA I–F/MP/1488/22-08 (print and counter imprint of thorax with mesothoracic wings and partially preserved abdomen in sideritic concretion).

Type locality

Sosnowiec-Klimontów, originally Porąbka-Klimontów Mine, Upper Silesian Coal Basin, Poland (Krawczyński et al. 1997; Prokop et al. 2012).

Type strata

Langsettian (Westphalian A), Pensylvannian, Mudstone series (Załęże beds), Coal-bearing Mudstone Series (Pacyna and Zdebska 2002).

Discussion

The pattern of forewing venation of this fossil corresponds to that of polyneopteran insects and clearly differs from that of Archaeorthoptera in the absence of a basal division of CuP, absence of distal fusion of anterior branch of CuP with CuA and also lack of basal fusion of veins M with CuA (Béthoux and Nel 2002). There is a clear resemblance to 'blattoid' lines Dictyoptera (Blattodea + Mantodea) and especially Paollida, but not an exact match. The most remarkable characters shared with Paoliida are a long common stem of Cu in front of the bifurcation into CuA and CuP, M running closely parallel to R near base of wing and relatively broad area with irregular network of cross veins between CuA and CuP. On the other hand, a short weakly developed convex arculus between M/MP and CuA is not very distinct and there are no convex anterior branches of CuA, which are typical for roachoid-like or paoliid insects (e.g. Zhang et al. 2013; Prokop et al. 2014; Correia et al. 2019). Other typical features of Paoliidae are oblique anterior veinlets of ScP or a broad area between MP and CuA, which are not present in *S. dareki* gen. et sp. nov. (Prokop et al. 2014), but are present in some Blattinopsidae and also some Palaeozoic roachoids, like *Miroblatta costalis* Laurentiaux-Vieira & Laurentiaux, 1987 (see Béthoux et al. 2011). However, the position of these insects remains uncertain, but they most probably belong to the stem group of Dictyoptera (Correia et al. 2019).

Possible affinities to Eoblattida, including Gryloblattida *sensu* Storozhenko (1997), are also apparent, but the concept of this rich group is still questionable as the type species, *Eoblatta robusta* (Brongniart, 1893), seems to belong to Archaeorthoptera (Schubnel et al. 2020). General arrangement of the main veins in the mid wing area, for example, resemble 'eoblattid' *Permula lebachensis* Schlechtendal, 1913, with the first branch of M close to that of the first branch of R and CuA posteriorly pectinated (Aristov 2015). However, in *S. dareki* the width of the costal area between ScP and C clearly narrows distally and there is a different pattern of cross veins. In contrast, sigmoidal CuA and the costal field narrows towards wing base and the wing tip differs in *P. lebachensis* Schlechtendal, 1913. Moreover, a large part of the wing is missing and thus an exact comparison is impossible. There is a most definite resemblance to *Paridelia pusilla* Sharov, 1961 (see Aristov 2015). The R division in both taxa is located beyond the first third of the length of the wing and behind the first branch of M. Both veins RA and RP run simple for a long distance. They also share posteriorly pectinated CuA with four branches and anterior branches of ScP forming a cellular meshwork instead of simple oblique veinlets. But

P. pussila differs from *S. dareki* in the more basal placement of the first CuA branch in relation to the first branch of M, CuP lacks a distal bifurcation and the branching pattern of M is different. Taxonomic placement of *P. pussila* has been changed several times. Initially it was placed in the family Ideliidae (Sharov 1961) and subsequently in Kortshakoliidae, which are both part of Grylloblattida (Storozhenko 1997, 1998). Later, Aristov (2015) transferred *Paridelia* to the family Mesorthopteridae within Eoblattida. However, due to uncertain position of Eoblattida, the placement of the family Mesorthopteridae must be re-examined. Another problem complicating a correct taxonomic placement arises from the uncertain relationships between extinct Grylloblattida and presumably crown group Grylloblattodea with Grylloblattidae (Storozhenko 2002; Cui et al. 2015). A mosaic of characters (particularly resembling Paoliida, Dictyoptera and Grylloblattida) and uncertain, probably very basal position (within Polyneoptera) of the most similar species *P. pussila*, lead us to consider *S. dareki* as a representative of a stem group of Dictyoptera with affinity to blattoids, but without an exact placement in currently described orders. This statement is congruent with the recent analysis supporting a relatively close phylogenetic relationship of extant Grylloblattodea and Dictyoptera (Wipfler et al. 2019).

Conclusion

In this study one new genus and two new species of polyneopteran insects are described based on the venation of their forewings. *Stephanopsis testai sp.* nov. is based on well-preserved venation, which clearly placed it in Blattinopsidae (Paollida). *Sosnowiecia dareki* gen. nov. et sp. nov. is based on an incomplete forewing which has a combination of characters that prevents an accurate placement, but affinities with blattoids are clearly apparent. The situation is further complicated by the uncertain status of Grylloblattida, the stem group of Dictyoptera (like roachoids) and especially Eoblattida. In order to determine the Palaeozoic evolutionary history of Polyneoptera, a comprehensive phylogenetic study and clarification of the status of these high ranked taxa is needed.

Acknowledgments

We are indebted to the scientific illustrator Zuzana Čadová for the magnificent line drawing of *Sosnowiecia dareki*. We cordially thank Darek Wojciechowski for his fieldwork at Sosnowiec. The authors are grateful to Paul Mayer (The Field Museum, Chicago, U.S.A.), who kindly provided access to the collection under his care. The authors are grateful to Anthony F. G. Dixon (University of East Anglia, Norwich, United Kingdom) for improving the English and an anonymous referee for suggestions for improving an earlier version of the manuscript. TD & JP were supported by the Grant Agency of the Czech Republic (No. 18-03118S).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Grant Agency of the Czech Republic [18-03118S]; Czech Science Foundation.

ORCID

Jakub Prokop http://orcid.org/0000-0001-6996-7832

References

Aristov DS. 2015. Classification of the order Eoblattida (Insecta: Blattidea) with description of new taxa. Far Eastern Entomologist. 301:1–56.

Aristov DS, Rasnitsyn AP. 2022a. New Blattinopsidae (Insecta: Blattinopsida) from the Middle Permian of European Russia. Paleontological Journal. 56 (2):187–193. doi:10.1134/S0031030122020034.

Aristov DS, Rasnitsyn AP. 2022b. New species of the genus *Glaphyrophlebia* (Insecta, Blattinopsida: Blattinopsidae) from the Upper Carboniferous of Ukraine. Far Eastern Entomologist. 450:9–11. doi:10.25221/fee.450.2.

Aristov DS, Rasnitsyn AP, Naugolnykh SV. 2021. New Blattinopsidae (Insecta: Blattinopsida) in the Permian of the Pechora Basin (Komi Republic, Russia) in the context of landscape and vegetation evolution. Paleontological Journal. 55(6):641–649. doi:10.1134/S0031030121060022.

Béthoux O, Nel A. 2002. Venation pattern and revision of Orthoptera *sensu nov.* and sister groups. Phylogeny of Palaeozoic and Mesozoic Orthoptera Sensu Nov. Zootaxa. 96:1–88. doi:10.11646/zootaxa.96.1.1

Béthoux O, Schneider JW, Klass KD. 2011. Redescription of the holotype of *Phyloblatta gaudryi* (Agnus, 1903) (Pennsylvanian; Commentry, France), an exceptionally well-preserved stem-dictyopteran. Geodiversitas. 33 (4):625–635. doi:10.5252/g2011n4a4.

Brodsky AK. 1994. The Evolution of Insect Flight. Oxford (New York, Tokyo): Oxford University Press.

Brongniart C. 1893. Recherches pour servir à l'histoire des insectes fossiles des temps primaires précédées d'une étude sur la nervation des ailes des insectes. Bulletin de la Société d'Industrie Minérale de Saint-Etienne. 7(4):1–491.

Carpenter FM. 1997. Insecta. Chapter 14A. In: Shabica CW, Hay AA, editors. Richardson's guide to the fossil fauna of Mazon creek. Chicago, Ilinois: Northeastern Illinois University; p. 184–193.

Correia P, Schubnel T, Nel A. 2019. What is the roachoid genus *Eneriblatta* (Dictyoptera: Phyloblattidae) from the Carboniferous of Portugal. Historical Biology. 33(6):777–782. doi:10.1080/08912963.2019.1661407.

Cui YY, Béthoux O, Klass KD, Ren D. 2015. The Jurassic Bajanzhargalanidae (Insecta: Grylloblattida?): new genera and species, and data on postabdominal morphology. Arthropod Structure & Development. 44(6 Pt B):688–716. doi:10.1016/j.asd.2015.04.008.

Cui YY, Béthoux O, Ren D. 2011. Intraindividual variability in Sinonamuropteridae forewing venation (Grylloblattida; Late Carboniferous): taxonomic and nomenclatural implications. Systematic Entomology. 36(1):44–56. doi:10.1111/j.1365-3113.2010.00545.x.

Dvořák T, Pecharová M, Krzemiński W, Prokop J. 2019. New archaeorthopteran insects from the Carboniferous of Poland: insights into tangled taxonomy. Acta Palaeontologica Polonica. 64:787–796. doi:10.4202/app.00614.2019

Fritsch A. 1899. Beschreibung einiger neuen fossilen Insekten in Schleferton des Steinkohlengebirge v. In: Beyschlag F, Fritsch KW, editors. Das Jüngere Steinkohlengebirge und das Rothliegende in der Provinz Sachsen und den Angrenzenden Gebieten. Abhandlungen der Königlich Preußischen-Geologischen Landesanstalt Berlin: Im Vertrieb der S. Schropp'schen Hof-Landkartenhandlung (J.H. Neumann). p. 1–263. Wettin.

Grimaldi DA, Engel MS. 2005. Evolution of the insects. Cambridge: Cambridge University Press.

Guan Z, Prokop J, Roques P, Lapeyrie J, Nel A. 2016. Revision of the enigmatic insect family Anthracoptilidae enlightens the evolution of Palaeozoic stem-dictyopterans. Acta Palaeontologica Polonica. 61:71–87.

Handlirsch A. 1906. Die fossilen Insekten und die Phylogenie der rezenten Formen. In: Ein Handbuch für Paläontologen und Zoologen. Leipzig: Wilhelm Engelman; p. 1430.

Hörnig MK, Haug C, Schneider JW, Haug JT. 2018. Evolution of reproductive strategies in dictyopteran insects—clues from ovipositor morphology of extinct roachoids. Acta Palaeontologica Polonica. 63:1–24. doi:10.4202/app.00324.2016.

Hörnschemeyer T, Stapf H. 2001. Review of Blattinopsidae (Prothortoptera) with description of new species from the Lower Permian of Niedermoschel (Germany). Neue Jahrbuch für Geologie und Paläontologie, Abhandlungen. 221(1):81–109. doi:10.1127/njgpa/221/2001/81.

Krawczyński W, Filipiak P, Gwoździewicz M. 1997. Zespoł skamie–niałości z karbońskich sferosyderitów (westfal A) NE części Górno–śląskiego Zagłębia Węglowego. Przeglad Geologiczny. 45:1271–1274.

Kukalová J. 1958. Remarks to the family Blattinopsidae Bolton, 1925 (Insecta, Protorthoptera). Vestnik Ustredniho Ustavu Geologickeho. 33:129–131.

Kukalová-Peck J. 1997. Mazon Creek insect fossils: the origin of insect wings and clues about the origin of insect metamorphosis. Chapter 14B. In: Shabica CW, Hay AA, editors. Richardson's Guide to the Fossil Fauna of Mazon Creek. Chicago, Illinois: Northeastern Illinois University; p. 194–207.

Laurentiaux D. 1950. Les insectes des bassins houillers du Gard et de la Loire. Annales de Paléontologie. 36:63–84.

Laurentiaux-Vieira F, Laurentiaux D. 1987. Un remarquable Archimylacridae du Westphalien inférieur belge. Ancienneté du dimorphisme sexuel des blattes. Annales de la Société Géologique du Nord. 106:37–47.
Legendre F, Nel A, Svenson GJ, Robillard T, Pellens R, Grandcolas P. 2015. Phylogeny of Dictyoptera: dating the origin of cockroaches, praying mantises and termites with molecular data and controlled fossil evidence. PLos ONE. 10(7):1–27. doi:10.1371/journal.pone.0130127.
Meunier F. 1908. Quatrième note sur de nouveaux insectes du Stéphanien de Commentry. Bulletin du Muséum National d'Histoire Naturelle. 14:244–249.
Pacyna G, Zdebska D 2002. Upper Carboniferous plant macrofossils from sideritic concretions in Sosnowiec (Upper Silesian Coal Basin) and Mazon Creek (Illinois, USA). In: Lipiarski I (ed.), Proceedings XXV Symposium Geology of Coal–Bearing Strata of Poland, 123–127. University of Mining and Metallurgy, Cracow.
Prokop J, Krzemińska E, Wojtechowski D, Wojciechowski D. 2012. Paoliida, a putative stem-group of winged insects: morphology of new taxa from the Upper Carboniferous of Poland. Acta Palaeontologica Polonica. 57 (1):161–173. doi:10.4202/app.2010.0064.
Prokop J, Krzeminski W, Krzeminska E, Hörnschemeyer T, Ilger J-M, Brauckmann C, Grandcolas P, Nel A. 2014. Late Palaeozoic Paoliida is the sister group of Dictyoptera (Insecta: Neoptera). Journal of Systematic Palaeontology. 12(5):601–622. doi:10.1080/14772019.2013.823468.
Prokop J, Pecharová M, Nel A, Hörnschemeyer T, Krzemińska E, Krzemiński W, Engel MS. 2017. Paleozoic nymphal wing pads support dual model of insect wing origins. Current Biology. 27(2):263–269. doi:10.1016/j.cub.2016.11.021.
Prokop J, Rosová K, Krzemińska E, Krzemiński W, Nel A, Engel MS. 2022. Abdominal serial homologues of wings in Paleozoic insects. Current Biology. 32(15):3414–3422. doi:10.1016/j.cub.2022.06.024.
Quispe L, Roques P, Garrouste R, Nel A. 2021. Carboniferous Blattinopsidae: revision of *Klebsiella* and new genus and species from Avion (Insecta, Paoliida). Historical Biology. 34(3):383–389. doi:10.1080/08912963.2021.1916817.
Richardson ES Jr. 1956. Pennsylvanian invertebrates of the Mazon Creek area, Illinois. Insects. Fieldiana Geology. 12:15–56.
Schlechtendal DHR. 1913. Untersuchung ueber die karbonischen Insekten und Spinnen von Wettin unter Berucksichtigung berwandter Faunen (Revision der Originale von Germar, Giebel und Goldenberg). Nova Acta Akademia Leopoldina-Carolinae. 98(1):1–186.
Schubnel T, Desutter-Grandcolas L, Legendre F, Prokop J, Mazurier A, Garrouste R, Grandcolas P, Nel A. 2019. To be or not to be: postcubital vein in insects revealed by microtomography. Systematic Entomology. 45 (2):327–336. doi:10.1111/syen.12399.
Schubnel T, Roberts D, Roques P, Garrouste R, Desutter-Grandcolas L, Nel A. 2020. Moscovian fossils shed light on the enigmatic polyneopteran families Cacurgidae and Eoblattidae (Insecta: 'Eoblattida. Archaeorthoptera). Journal of Systematic Palaeontology. 18(6):499–511. doi:10.1080/14772019.2019.1627595.
Shabica CW, Hay AW. 1997. Richardson's guide to the fossil fauna of Mazon Creek. Northeastern Illinois Studies. 1–307.
Sharov AG. 1961. Order Paraplecoptera. In: Rohdendorf BB, Becker-Migdisova EE, Martynova OM, Sharov AG. Paleozoiskie nasekomye Kuznetskogo basseina [Paleozoic insects of the Kuznetsk basin.] Trudy Paleontologicheskogo Instituta Akademii nauk SSSR. (Vol: 85, pp. 164–224).
Storozhenko SY. 1997. Classification of order Grylloblattida, with description of new taxa. Far Eastern Entomologist. 42:1–20.
Storozhenko SY. 1998. Sistematika, filogeniya i evolyutsiya grilloblattidovykh nasekomykh (Insecta: Grylloblattida) [Systematics, phylogeny and evolution of the grylloblattids (Insecta: grylloblattida).] Dal'nauka. Dordrecht (Boston, London): Kluwer Academic Publishers; p. 517.
Storozhenko SYu. 2002. Order Grylloblattida Walker, 1914 (= Notoptera Crampton, 1915, = Grylloblattodea Brues et Melander, 1932, + Protorthoptera Handlirsch, 1906, = Paraplecoptera Martynov, 1925, + Protoperlaria Tillyard, 1928). In: Rasnitsyn AP, and Quicke DLI, editors. History of insects. Dordrecht, Boston, London: Kluwer Academic Publishers; pp. 278–284.
Wipfler B, Letsch H, Frandsen PB, Kapli P, Mayer C, Bartel D, Buckley TR, Donath A, Edgerly-Rooks JS, Fujita M, et al. 2019. Evolutionary history of Polyneoptera and its implications for our understanding of early winged insects. Proceedings of the National Academy of Sciences. 116:3024–3029. doi:10.1073/pnas.18177941.
Zhang Z, Schneider JW, Hong Y. 2013. The most ancient roach (Blattodea): a new genus and species from the earliest Late Carboniferous (Namurian) of China, with a discussion of the phylomorphogeny of early blattids. Journal of Systematic Palaeontology. 11(1):27–40. doi:10.1080/14772019.2011.634443.