Are there really cryptic species within the myrmecophilous butterfly species *Phengaris (Maculinea) teleius* and *P. (M.) nausithous* (Lepidoptera: Lycaenidae)? Analyses across Eurasian distribution ranges, confusing effects of the endosymbiotic bacterial parasite *Wolbachia*, and implications for *Phengaris (Maculinea)* conservation

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von Frau Sylvia Jaschinsky (geb. Ritter)

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Gutachter:

1. Prof. Dr. J. Thomas
2. Prof. Dr. J. Settele
3. Prof. Dr. R.F.A. Moritz

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Preface

The present dissertation was mainly produced from 2007 – 2011 during my employment as PhD student at the Helmholtz Centre for Environmental Research – UFZ in Halle/Saale (Department of Community Ecology), under the scientific supervision by Prof. Dr. Josef Settele and Dr. Walter Durka. Many thanks also to Dr. Stefan Michalski and Dr. Martin Wiemers, who participated with numerous hints and discussions for the peer-reviewed publication "*Wolbachia* infections mimic cryptic speciation in two parasitic butterfly species, *Phengaris teleius* and *P. nausithous* (Lepidoptera: Lycaenidae)" (Ritter *et al.* 2013). The research project was financially supported by the VolkswagenStiftung (http://www.volkswagenstiftung.de/) (Sylvia Ritter, Az 1/82 751). Furthermore, research has been conducted within the project CLIMIT (Climit Change Impacts on Insects and their Mitigation; http://www.climit-project.net/) funded by Deutsches Zentrum für Luft- und Raumfahrt-Bundesministerium für Bildung und Forschung (www.pt-dlr.de/) (Germany), the Natural Environment Research Council (http:// www.nerc.ac.uk/) and the Department of Environment, Food and Rural Affairs (http://www.defra.gov.uk/) (UK), the French National Research Agency (http://www. agence-nationale-recherche.fr/) (France), Formas (http://www.formas.se/) (Sweden), and Swedish Environmental Protection Agency (http://www.naturvardsverket. se/) (Sweden) through the FP6 BiodivERsA Eranet (http://www.biodiversa.org/) and was also funded within the EU funded project SCALES (http://www.scalesproject.net/) (FP7 grant agreement no. 226852).

1 Summary / Zusammenfassung

Summary

Objective of present dissertation is a phylogenetic and phylogeographic investigation by using COI gene sequences, nuclear microsatellites, and *Wolbachia*-screens to test for cryptic species and phylogeographic scenarios in strongly protected (EU habitats directive) butterfly species *Phengaris (Maculinea) teleius* and *P. (M.) nausithous* (Lepidoptera: Lycaenidae)*, based on a comprehensive sample across their Eurasian ranges.

All *Phengaris* species ("Large Blues") are myrmecophilous social parasites that spend most of their life in close relationship with ants. Their fascinating life-style stimulates an intense research in all areas of biology (evolutionary biology, behavioural biology/ecology, population ecology, population genetics, acoustics, etc.) for decades and many studies and research projects promote an advanced and broad understanding of the whole genus (this is true at least for those 4 species which are distributed also in Europe). The highly specialised life-style of all *Phengaris* species and their dependence on solely one or few host plant species and solely few host ant species of the genus *Myrmica* (Hymenoptera: Formicidae) entail also an augmented sensibility in the face of environmental changes which lead to an ongoing dissappearence of local populations in the whole of Europe. Main habitat of the often co-occuring study species *P. (M.) teleius* and *P. (M.) nausithous* are extensively grazed or mown *Sanguisorba officinalis* meadows. Due to a complete abandonment of agricultural usage, or converse, their intensification, the plant and ant communities change their quality and may therefore no longer be suitable for *Phengaris (Maculinea)*. Consequently, the survival of populations often depends on a species oriented management.

In a previous, genus-wide phylogenetic study, cryptic species have been hypothesised for *P. (M.) teleius* and *P. (M.) nausithous* based on divergent mtDNA sequences. This hypothesis was the starting point of present thesis. Since *Phengaris (Maculinea)* species are highly endangered, the existence of cryptic species would have drastic consequences for conservation and management: Which habitat requirements do the cryptic (sister) species have compared to their already known "morphospecies"? On which host ant species do they depend? Where is their distributional range? Which protective status needs to be assigned to the cryptic counterpart? How can populations be conserved, which management strategies have to be formulated? Etc.

Due to the high relevance for species protection, the hypothesis of cryptic species within *P. (M.) teleius* and *P. (M.) nausithous* was investigated most intensely from 2007 – 2011 (and with minor intensity from 2012 – 2016). Obtained results and drawn interpretations are the objectives of present dissertation, which in part had been published in an international peer-reviewed journal in 2013.

The analyses based on mitochondrial COI sequences have shown a deep mitochondrial split in both species, which is estimated to have happened between 0.65–1.97 million years ago. Deep

mitochondrial divergence within species may result from cryptic species existence, from phylogeographic isolation, or from endosymbiotic bacteria like *Wolbachia*. This phylogenetic split was not reflected in the nuclear microsatellite pattern, but was concordant with an infection with the bacterial endosymbiont *Wolbachia* in both species. Haplotypes previously attributed to cryptic species were part of the "*Wolbachia* influenced" clades.

Wolbachia is a bacterial, endosymbiotic parasite which manipulates host reproduction by different, yet poorly understood, mechanisms to maximize its own transmission into the next host generation. A *Wolbachia* infection can act as a reproductive barrier within a population between infected and uninfected individuals, which can be maintained over Millions of years. *Wolbachia* infection was detected for the first time in *P. (M.) teleius* und *P. (M.) nausithous* within the present analyses; and *Wolbachia* mechanisms "cytoplasmatic incompatibility" (CI) in *P. (M.) teleius* and "male-killing" or "feminization" in *P. (M.) nausithous* are suggested, but testing of these hypotheses require further and more detailed studies.

In both species the remaining phylogeographic structure was largely consistent between mitochondrial and nuclear genomes. In *P. (M.) teleius* several mitochondrial and nuclear groups were observed in East Asia while a single haplogroup and nuclear cluster prevailed across continental Eurasia. Neutrality tests suggested rapid demographic expansion into the latter area. On the contrary, *P. (M.) nausithous* had several mitochondrial and nuclear groups in Europe, suggesting a complex phylogeographic history in the western part of its range. Observed phylogeographic groups are regarded preliminary as conservation units/evolutionarily significant units (CUs/ESUs), but detailled ecological studies on host specificity, or cuticular hydrocarbon profiling within each genetic group are needed for a fundamental CU/ESU-assignment. The conclusion is that deep intraspecific divergences found in DNA barcode studies do not represent cryptic species but instead resulted by both, infection by *Wolbachia* and phylogeographic structure.

* Phylogenetic studies on genus- and section-level revealed the congeneric status of palaearctic *Maculinea* VAN EECKE 1915 with Asiatic *Phengaris* DOHERTY 1891 (Ugelvig *et al.* 2011, Als *et al.* 2004, Pech *et al.* 2004) wherefore *Maculinea* was synonymised with *Phengaris* by Fric *et al.* (2007). Until the International Commission on Zoological Nomenclature hasn´t ruled out the valid generic name (Balletto *et al.* 2010, Fric *et al.* 2010) I add the (sub-) genus name *Maculinea* in parentheses in present dissertation.

Zusammenfassung

Gegenstand der hier vorgestellten Arbeit ist eine phylogenetische und phylogeographische Untersuchung zur Existenz von kryptischen Arten bei den nach europäischer FFH-Richtlinie geschützten Schmetterlingsarten *Phengaris (Maculinea) teleius* und *Phengaris (Maculinea) nausithous* (Heller und Dunkler Wiesenknopfameisenbläuling) (Lepidoptera: Lycaenidae)* basierend auf umfangreichem Probenmaterial aus dem gesamten eurasischen Verbreitungsgebiet. Eine Besonderheit aller *Phengaris*-Arten ist die myrmekophile, sozialparasitische Lebensweise der Schmetterlingsraupen in Ameisennestern der Gattung *Myrmica*, welche seit Jahrzehnten intensive Forschung auf allen Gebieten der Biologie anregt (Evolutionsbiologie, Verhaltensbiologie und -ökologie, Populationsökologie und -genetik, Akustik etc.). Zahllose Untersuchungen und Forschungsprojekte in den letzten Jahrzehnten in vielen Teilen des europäischen Verbreitungsgebietes beförderten ein fortgeschrittenes und breit gefächertes grundlegendes Verständnis der gesamten Gattung (zumindest bei den auch in Europa verbreiteten Vertretern). Die hoch spezialisierte Lebensweise aller *Phengaris*-Arten und ihre jeweilige Abhängigkeit von nur einer oder wenigen Wirtspflanzenart(en) sowie wenigen Wirtsameisenarten bedingen eine hohe Empfindlichkeit gegenüber Veränderungen der Umwelt- und Habitatbedingungen, was seit Jahrzehnten zu einem fortdauernden Verschwinden von lokalen Populationen in ganz Europa führt. Haupthabitat der beiden betrachteten, oft gemeinsam vorkommenden Arten sind land-/weidewirtschaftlich extensiv genutzte *Sanguisorba officinalis*-Feuchtwiesengesellschaften, deren Fortbestehen v. a. aufgrund von Nutzungsaufgaben oder, als anderes Extrem, Nutzungsintensivierungen bedroht ist. Die Erhaltung der Habitate ist oftmals von pflegerischen Maßnahmen abhängig, die sich idealerweise anhand wissenschaftlich fundierter, naturschutzfachlicher Empfehlungen orientieren.

2004 ist in einer phylogenetischen Analyse auf die mögliche Existenz von kryptischen Arten bei *P. (M.) teleius* und *P. (M.) nausithous* hingewiesen worden (Als *et al.* 2004). Diese Hypothese ist Ausgangspunkt der vorliegenden Dissertation. Eine Bestätigung dieser Hypothese würde unmittelbare Konsequenzen für den Artenschutz und das Management nach sich ziehen: Welche Habitatansprüche haben die kryptischen (Schwester)arten verglichen mit den schon bekannten "Morphospezies"? Von welchen Wirtsameisenarten sind sie direkt abhängig? Wo erstreckt sich ihr Gesamtverbreitungsgebiet? Welcher Schutzstatus muss ihnen zugewiesen werden? Wie können Populationen erhalten werden? Welche Managementempfehlungen sind zu formulieren? Etc.

Aufgrund der hohen artenschutzfachlichen Relevanz wurde im Rahmen eines Drittmittelgeförderten Forschungsvorhabens von 2007 – 2011 der Frage nach der Existenz von kryptischen Arten sowie weiterführenden Fragen intensiv nachgegangen. Die erzielten Ergebnisse und Interpretationen sind Gegenstand vorliegender Dissertationsschrift. Ein Teil der Ergebnisse und Interpretationen ist 2013 im sehr komprimierten Format einer wissenschaftlichen Publikation veröffentlicht und der internationalen Fachgemeinde zugänglich gemacht worden. Für weitere Informationen und weiterführende Interpretationen, Diskussionen und Ausblicke bietet vorliegende Dissertation hinreichend Raum.

Hier präsentierte Analysen auf Basis mitochondrialer COI-Sequenzen und nuklearer Mikrosatelliten-Daten zeigten bei beiden Arten eine tiefe mitochondriale phylogenetische Aufspaltung auf,

welche vor ca. 0.65–1.97 Millionen Jahren begann. Die mitochondriale Aufspaltung fand keine Entsprechung bei dem analysierten Mikrosatelliten-Datensatz, jedoch war sie kongruent mit bei *P. (M.) teleius* und *P. (M.) nausithous* erstmalig detektierten *Wolbachia*-Infektionen. Die Haplotypen, welche zur Vermutung von kryptischen Arten bei *P. (M.) teleius* und *P. (M.) nausithous* führten, konnten in vorliegender Arbeit eindeutig den "*Wolbachia*-beeinflussten" phylogenetischen Zweigen zugewiesen werden.

Wolbachia ist ein bakterieller Endosymbiont, welcher als so genannter "Reproduktionsparasit" verschiedene, bisher wenig verstandene Mechanismen aufweist, um seine Chance, in die nachkommende Generation des sich sexuell reproduzierenden Wirtes übertragen zu werden, zu maximieren. Die *Wolbachia*-Infektion kann zur reproduktiven Abtrennung infizierter Individuen von nicht-infizierten Individuen führen und somit eine reproduktive Barriere innerhalb einer Population darstellen. Diese Barriere kann, wie mehrfach vermutet wurde, über Millionen von Jahren bestehen. Inwieweit solche Phänomene zur Artbildung beitragen, ist Gegenstand aktueller Forschung, jedoch nicht Gegenstand vorliegender Arbeit. Erste hier präsentierte Analysen lassen auf die *Wolbachia*-Mechanismen *„cytoplasmatische Inkompatibilität“* (CI, *P. (M.) teleius*) und *„male-killing“* oder *„feminization“* (*P. (M.) nausithous*) schließen, allerdings sind zur Verifizierung/Falsifizierung weiterführende Untersuchungen erforderlich.

Die phylogeographische Struktur ist zwischen dem mitochondrialen (mtDNA) und dem nuklearen Datensatz (Mikrosatelliten) bei beiden Arten weitestgehend konsistent. Bei *P. (M.) teleius* wurden in Ost-Asien mehrere mitochondriale und nukleare Gruppen identifiziert, wohingegen in Europa nur eine dieser Gruppen detektiert wurde. Neutralitäts-Tests deuten auf eine schnelle demographische Ausbreitung der Art aus dem asiatischen in den europäischen Teil des Verbreitungsgebietes hin. Bei *P. (M.) nausithous* zeigt sich eine gegenteilige phylogeographische Struktur mit mehreren mitochondrialen und nuklearen Gruppen in Europa, was auf eine komplexe phylogeographische Geschichte im westlichen Verbreitungsgebiet der Art schließen lässt. Die beobachteten phylogeographischen Gruppen werden vorläufig als *conservation units/evolutionarily significant units* = CUs/ESUs betrachtet. Für eine grundlegende Abgrenzung von ESUs sind jedoch detaillierte Untersuchungen zu u. a. Wirtspezifität oder den zugrunde liegenden chemischen Hydrocarbon-Oberflächenprofilen innerhalb jedes phylogeographischen Clusters notwendig.

Zusammenfassend kann gesagt werden, dass die tiefen phylogenetischen Aufspaltungen, die zur Hypothese der Existenz von kryptischen Arten bei beiden Wiesenknopfameisenbläulingsarten führten, das Ergebnis von *Wolbachia*-Infektionen sind, die bei beiden Arten möglicherweise seit Millionen von Jahren persistent bestehen. Alle weiteren phylogenetischen Aufspaltungen und populationsgenetischen Gruppierungen sind Ergebnis ihrer phylogeographischen, nacheiszeitlichen Geschichte.

* Phylogenetische Analysen auf Gattungs- und Sektionsebene zeigten auf, dass die paläarktische Gattung *Maculinea* VAN EECKE 1915 und die asiatische Gattung *Phengaris* DOHERTY 1891 lediglich eine einzige Gattung repräsentieren (Ugelvig *et al.* 2011, Als *et al.* 2004, Pech *et al.* 2004), mit der Konsequenz dass *Maculinea* mit *Phengaris* synonymisiert wurde (Fric *et al.* 2007). Solange die Internationale Kommission für Zoologische Nomenklatur nicht über den international gültigen Namen für die Gattung entschieden hat (Balletto *et al.* 2010, Fric *et al.* 2010) ergänze ich in vorliegender Arbeit den Untergattungsnamen *Maculinea* in Klammern.

2 Outline of present thesis

The present thesis extends contents of the peer-reviewed publication "*Wolbachia* infections mimic cryptic speciation in two parasitic butterfly species, *Phengaris teleius* and *P. nausithous* (Lepidoptera: Lycaenidae)" (Ritter *et al.* 2013) considering more and newer literature. Furthermore, it also gives room to introduce the main characteristics of the family Lycaenidae, to explain what cryptic species are, to shortly review effects of the endosymbiotic reproductive parasite *Wolbachia* on arthropods and butterflies, and to introduce the concept of and criteria for evolutionarily significant units (ESUs) as conservation unit below species level (chapter 3). Moreover, the present thesis gives a short overview on the substantial research body of the *Phengaris (Maculinea) – Myrmica* model system for evolutionary biology (chapter 4).

Genetic information from COI sequences of mitochondrial DNA and nuclear microsatellite markers were used to test hypotheses (chapter 5). Genetic information was extracted from a comprehensive sample set from broad Eurasian species distribution ranges during three collection trips in Mongolia (Juli 2007), Russia (Juli 2008), and China (Juni 2009). Further samples were provided by numerous helpful European *Phengaris (Maculinea)* specialists.

The following questions were answered (chapter 6 - 7):

- Are there two, or more, cryptic species hiding behind both "morphospecies", *Phengaris (Maculinea) teleius* BERGSTRAESSER 1779 and *P. (M.) nausithous* BERGSTRAESSER 1779?
- Or, alternatively, are distinct lineages the result of *Wolbachia* infections?
- Can the obtained phylogenetic and microsatellite pattern be explained by hypotheses for phylogeographic histories of the species?

Additionally, I combined available information on host ant specificity, and subspecific differentiations with mitochondrial and microsatellite genetic data for a preliminary demarcation of evolutionarily significant units (ESUs) as conservation units (CUs) below species level (chapter 7).

Finally, it is outlined which further questions emerge from the results, and which implications results bear for *Phengaris (Maculinea)* and insect species conservation (chapter 8).

References of all chapters were listed at the end of the thesis.

3 Introduction

3.1 Butterflies

Butterflies and moths (Lepidoptera) are one of the most diverse orders of insects on Earth. They are distributed widely across all terrestrial ecosystems, except Antarctica, and play an important role in pollination of flowering plants (Bloch *et al.* 2006). Furthermore, some species cause significant damage to crops and stored food, but also to natural forests (Bloem *et al.* 2005). The total number of all described Lepidoptera species in the world is estimated to be more than 157.000; the number of described butterfly species (Papilionidea) was estimated to be ca. 18.400 (van Nieukerken *et al.* 2011), which constitutes only 9% of all Lepidoptera species. Three families (Nymphalidae: N=6152, Lycaenidae: N=5201, and Hesperiidae: N=4113) comprise 84%, the Lycaenidae family alone comprises 28% of all "true" butterfly (Papilionoidea) species (van Nieukerken *et al.* 2011). Around two-thirds of them occur in the tropics and a lot of new species are described each year, mainly from there (Shields 1989). Further, many cryptic species have been detected and still awaits their detection (e. g., Bickford *et al.* 2007, Hebert *et al.* 2003).

The diversification of butterflies started presumably in Gondwana, around 110 - 100 mya, during the Mesozoic age (Heikkilä *et al.* 2012, Eliot 1973). Currently known lineages began to diversify at 65 mya, after the Cretaceous–Paleogene (K–Pg) extinction event (Heikkilä *et al.* 2012).

3.2 Myrmecophily in Lycaenidae

Lycaenidae show quite distinct characteristics within Papilionoidea because many of them are entomophagous feeders and disproportionaly rich in species where butterfly-larvae associate in diverse ways with ants (myrmecophily). About 75% of lycaenid butterfly species have a facultative or obligate ant-associated lifestyle, varying from coexistence to specific mutualistic, where a few species have evolved ant parasitism (e.g., Fiedler 2012, Pierce *et al.* 2002).

Obligate interactions show a high ant specificity, typically involving only a single species or genus of ants (Pierce *et al.* 2002). Lycaenidae have evolved highly adapted sets of myrmecophilous organs to manipulate ant behaviour in diverse ways by chemical and acoustical mimicry: e. g., to suppress ant aggression, to be guarded by ants against predators or parasites, to maintain mutualistic interactions by delivering nutritious secretions for ants, to release brood carrying behaviour, or to achieve queen ant status in the host ant colony hierarchy (Barbero *et al.* 2009a, Daniels *et al.* 2005, Pierce *et al.* 2002, Fiedler 1991).

The myrmecophilous lifestyle is assumed to be one source of the high diversity within the lycaenid family considering that the communication between butterflies and their host ants is connected with the evolution of specialized adaptations and life-cycles (Fiedler *et al.* 1996). Shifts

to a novel "ant environment" with different behavioural and chemical characteristics permits diversification in Lycaenidae, or even may facilitate subsequent radiation (Pierce *et al.* 2002). Eastwood *et al.* (2006) demonstrated the contribution of biogeography, host-plant and host-ant association to the variation in butterfly genetic diversity. Further research would be necessary to understand the role ant association has played in the evolution and diversification of Lycaenidae (Pierce *et al.* 2002). The ant parasitic Palaearctic small genus *Phengaris* belongs to the Glaucopsyche section of the Lycaenidae (Als *et al.* 2004). The fourth instar caterpillars live as social parasites within ant nests of their specific host ant species, either feeding predaciously on the ant brood, or being fed by the ant workers through trophallaxis (Fiedler 2012, Thomas and Settele 2004).

3.3 Cryptic species

Most of the currently known species had been delineated traditionally on morphological characters ("morphospecies") (Mayr 1996). Cryptic species, also called sibling species, are two or more discrete “good” biological, reproductively isolated species classified erroneously as a single nominal species because of the difficulties to distinguish them morphologically (Bickford *et al.* 2007, Mayr 1942). They often differ on behavioural, or ecological characters, and fulfil all criteria of the Biological Species Concept (BSC), as they “are groups of interbreeding natural populations that are reproductively isolated from other such groups”. Or, as Mayr revised later in his definition, individuals of a species maintain “biological properties (…) which prevent the interbreeding (fusion) of populations” to protect its gene pool. Individuals of a species share certain characteristics because they belong to a single reproductive entity, a biological species (Mayr 1996). Note, that reproductive isolation is a byproduct of the process of ongoing evolutionary divergence. Inefficiency of isolating mechanisms is a natural phenomenon in different degrees, as hybridisation between closely related sympatric species occurs occasionally. Nevertheless, the complete fusion of such species populations is prevented (at least among higher animals) (for details see, e.g. Mayr 1996).

Rapid PCR and DNA sequencing analysis provided a new and simple tool to taxonomists for the detection of cryptic species, as many cryptic species genetically differ in similar frequencies like morphologically distinct species do (Bickford *et al.* 2007, Mayr 1996). Enhanced genetic distances within "morphospecies" have uncovered cryptic species in most habitats and organisms, often concordant with subtle morphological differences recognized by a more closer look (e.g., Sañudo-Restrepo *et al.* 2013, Dincă *et al.* 2011a), or characterized by divergent ecological niches (e.g., McBride *et al.* 2009), or different geographical distribution ranges (e.g., Vodă *et al.* 2015, Sañudo-Restrepo *et al.* 2013).

The detection of cryptic complexes influences the understanding of dietary requirements from a "species" being a generalist to a species complex of several dietary specialists (Bickford *et al.* 2007). Furthermore, the existence of ecotypes or geographic host races in many species may lead, if persisting long enough, to speciation (Drés and Mallet 2002). This is similar in parasitic species with "host-generalists" being in fact cryptic species complexes of specialists, each of

them with only one or a few host species (e.g., Smith *et al.* 2006, Kankare *et al.* 2005, Schönrogge *et al.* 2002).

Thus, the hidden existence of cryptic species strongly affects current knowledge of biodiversity and, consequently, conservation efforts. In endangered undetected cryptic species complexes the extinction risk for each cryptic taxa will be much higher than it is evaluated for the nominate "morphospecies", due to their more specific requirements and their more limited distributions. Furthermore, application of management strategies developed for the "morphospecies" may be detrimental for underlying non-detected cryptic species. Additionally, habitat prioritization for conservation is often based on estimations of species richness and/or endemism which may be wrong without information on that (Bickford *et al.* 2007, Witt *et al.* 2006).

3.3.1 Cryptic species within parasites and myrmecophiles

Arthropods are expected to contain many cryptic species and are considered to be key targets for cryptic species investigations (Bickford *et al.* 2007). In butterflies it has been recently suggested that the existence of cryptic diversity in one of the best studied continets (Europe) is with 27.7% much higher than previously expected (Dincă *et al.* 2015).

For parasitic or myrmecophilous species it is expected that they have a higher evolutionary potential than free-living species (Huyse *et al.* 2005, Schönrogge *et al.* 2002). The frequency of founder events in parasites is enhanced due to their short generation times, seasonal changes in their prevalences (Huyse *et al.* 2005), or the colonization of new hosts (Malenke *et al.* 2008). Further, speciation processes can be accelerated by co-evolutionary arms races between hosts and parasites (e.g., Schmid-Hempel 2011, Kawecki 1998).

Myrmecophiles are organisms dependent on ants, at least in parts of their developmental stages. Further, myrmecophily is a common phenomenon in ecology, as it has evolved independently and convergently in many taxonomic groups. Most of them are insects, e.g., beetles, mites, butterflies, wasps, crickets, flies, or other arthropods, but also snails, and even snakes can have a myrmecophilous lifestyle (Kronauer and Pierce 2011, Hölldobler and Wilson 1990). Among insects presumably 100.000 myrmecophilous species exist (Elmes 1996). The modes of interactions between myrmecophiles and ants are mainly commensalic or mutualistic, but also parasitic relationships have evolved (Hölldobler and Wilson 1990). Diverse behavioural, morphological, and physiological adaptations make a myrmecophilous lifestyle possible (e.g., to be protected by ant attacks, to reward ants with nutritious supplements, or to communicate with ants). To manipulate ant social behaviour, myrmecophiles use the chemical and acoustical communication systems of ants (e.g., Sala *et al.* 2014, Thomas *et al.* 2005, Lenoir *et al.* 2001, Hölldobler and Wilson 1990).

Most commensalic or free-living mutualistic myrmecophiles are not highly specific regarding associated ant partner, as they are able to interact with ant species from several subfamilies (Pierce *et al.* 2002, Hölldobler and Wilson 1990). In contrast, ant nest inhabiting myrmecophiles are much more host specific (Hölldobler and Wilson 1990). It has been estimated that around 10.000 – 20.000 "morphospecies" of insects have evolved as social ant parasites. Most of them

are extremely rare compared to the abundance and distributions of their ant hosts (Thomas *et al.* 2005).

75% of all lycaenid butterfly species are myrmecophiles; perhaps 5% of them live as social parasites within ant nests (e.g., Thomas *et al.* 2005, Pierce 1995, Fiedler 1998). Myrmecophilous parasites are in general highly specific with one or a few host ant species. Thus, they experience strong selection on physiological or behavioural characters. Fundamental differences in nonvisual biological features may develop easily which may result in reproductive isolation and speciation without any visible changes in morphological features (Bickford *et al.* 2007, Schönrogge *et al.* 2002).

3.3.2 MtDNA analysis to discover cryptic species

The discovery of cryptic species increased exponentially in the last two decades, as application of rapid PCR- and DNA-sequencing methods became relatively inexpensive. An investigation of intraspecific genetic diversity is a powerful first step in recognizing cryptic units in many groups of animal taxa (including butterflies and moths) (e.g., Dincă *et al.* 2015, Silva-Brandão *et al.* 2009) and the progresses in their detection in the last decade revealed that cryptic species, e. g. the presence of phylogenetically distinct units within a morphologically defined taxon (Bickford *et al.* 2007), are much higher than previously expected (Dincă *et al.* 2015, Trontelj and Fiser 2009).

DNA barcoding using the mitochondrial gene Cytochrome c Oxidase I (COI) has become a standard method to assign unknown individuals to species, to assess biodiversity, and to discover new species including cryptic units within well-defined morphospecies, as recombination is absent/low and substitution rates are relatively fast (e.g., Saitoh *et al.* 2015, Dincă *et al.* 2015, Silva-Brandão *et al.* 2009, Hebert *et al.* 2004, Sperling 2003). For some of the cryptic units it has additionally been shown that they correspond well with a divergent ecological niche (e.g., McBride *et al.* 2009), with divergent karyotypes (e.g., Lukhtanov *et al.* 2015), with previously undetected morphological differences (e.g., Georgieva *et al.* 2013, Sañudo-Restrepo *et al.* 2013), with a not co-occuring chequered distribution (Vodă *et al.* 2015), or, in parasites, with different host species (e.g., Malenke *et al.* 2008).

However, the sole use of mtDNA sequences as a tool for species detection and delimitation can be problematic (e.g., Duplouy *et al.* 2010, Galtier *et al.* 2009, Rubinoff 2006). Patterns of deep divergence of mitochondrial DNA sequences within species may be due to historical processes like introgression between species (Forister *et al.* 2008), or phylogeographic isolation (Wiemers and Fiedler 2007). Further, the application of the mtDNA barcoding method alone can be problematic for closely related species which recently diverged by natural selection, when their differences are not yet reflected in mtDNA patterns. This can happen, since neutral processes operate more slowly than adaptive ones (e.g., Silva-Brandão *et al.* 2009, Forister *et al.* 2008). Finally, in invertebrates the assumption of neutral evolution of mtDNA may not be met due to the presence of endosymbiotic bacteria, like *Wolbachia* (e.g., Russell *et al.* 2012, Werren *et al.* 2008, Hurst and Jiggins 2005). Nevertheless, in spite of these problems mtDNA barcoding is an easy method to explore potential cryptic diversity and to elucidate where to look closer with

traditional time-consuming analysis like morphometrics, behavioural and/or physiological analysis. Thus, its application is widely supported to recover new or taxonomic problematic species (e.g., Dincă *et al.* 2015, Silva-Brandão *et al.* 2009).

3.4 *Wolbachia*

Microbial endoparasites of the genus *Wolbachia* are common intracellular bacteria in arthropod or nematod hosts. They belong to the order Rickettsiales (Werren *et al.* 2008, Stouthamer *et al.* 1999), and are divided into several supergroups (e.g., Bing *et al.* 2014, Baldo and Werren 2007). *Wolbachia* lives mainly within reproductive tissues (ovaries and testes) of their hosts and is vertically transmitted from mother to offspring through host eggs (e.g., Werren *et al.* 2008), but also horizontal transmission among and within species seems to be frequent, possibly through host-parasite or predator-prey interactions (e.g., Bing *et al.* 2014, Russell *et al.* 2009, Stahlhut *et al.* 2010, Jiggins *et al.* 2002, Vavre *et al.* 1999). To achieve high transmission rates into the next host generation, *Wolbachia* manipulates the reproductive system of its host in diverse ways, often causing a substantial host fitness decrease (e.g., Zug and Hammerstein 2015, Werren *et al.* 2008). Reproductive phenotypes *Wolbachia* induce in infected arthropod hosts are: feminization of genetic males (Hemiptera, Isopoda, Lepidoptera); induction of parthenogenesis resulting in development of unfertilized eggs into females (Hymenoptera, Thysanoptera, Acari); killing of male progeny during embryogenesis or early larval stages (Coleoptera, Diptera, Lepidoptera, orders of Pseudoscorpiones); and, as most frequent phenotypic effect, cytoplasmic incompatibility (CI) of sperm from *Wolbachia* infected males with eggs from females not harbouring any or the same *Wolbachia* type (or types) (Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, Isopoda, Acari). Only females transmit the bacterial infection with their eggs to the offspring generation. Infected females are compatible with both, uninfected males and males infected by the same *Wolbachia* strain. Uninfected females are compatible with uninfected males only, which results in strongly reduced opportunities to reproduce within a population compared to infected females. Thus, CI is functioning as a post-mating barrier to the formation of embryos even within a population (e.g., Werren *et al.* 2008, Stouthamer *et al.* 1999). Further, CI results in high proportions of embryonic lethality (Saridaki and Bourtzis 2010). Regardless of the specific above mentioned mechanisms by which host reproduction is manipulated, *Wolbachia* quickly enhances the proportion of infected females in a host population (e.g., Werren *et al.* 2008, Werren 1997).

Some *Wolbachia* strains are multi-potent: the same *Wolbachia* strain can cause a different phenotypic effect in another host, a trait not yet understood very well (e.g., Hornett *et al.* 2008, Jaenike 2007, Fujii *et al.* 2001). Underlying *Wolbachia*-caused defects in hosts occur during early embryogenesis in all induced phenotypes, but the exact mechanisms remain unclear yet, despite extensive investigations (Werren *et al.* 2008). Epigenetic changes induced by the inheritable "environmental" factor *Wolbachia* are revealed as one such mechanisms: The expression and splicing of genes which are involved in sex differentiation and development is influenced, if *Wolbachia* is exceeding a threshold in bacterial density (Sugimoto *et al.* 2010, Negri *et al.* 2009).

When a *Wolbachia* infection has no relevant detrimental fitness effects on its host (Hoffmann and Turelli 1988, Caspari and Watson 1959) and a host species/population has no *Wolbachia* suppressing or countering elements evolved (e.g., Mitsuhashi *et al.* 2011, Hornett *et al.* 2006, Charlat *et al.* 2005), the infection can spread rapidly through whole populations and species to persistent fixation (e.g., Zug and Hammerstein 2015, Kodandaramaiah *et al.* 2013, Narita *et al.* 2006, Dyson and Hurst 2004). This *Wolbachia*-sweep will be accompagnied by maternally inherited mitochondria of the initially infected female. As a consequence of the maternal inheritance of the infection, one particular mitochondrial haplotype (and its mutational derivatives) will hitchhike along with *Wolbachia* and replace all mtDNA haplotypes. This will change the mtDNA population structure drastically which will not longer reflect real evolutionary history (e.g., Charlat *et al.* 2009, Werren 1997, Turelli and Hoffmann 1991, Caspari and Watson 1959). Thus, an infected population (or the infected part of a population) is maintaining lower mtDNA diversity than uninfected ones. However, empirical studies have found that selfish genetic elements like *Wolbachia* are often maintained within populations at relatively low frequencies (Hatcher 2000, Hurst *et al.* 1999). Under which conditions *Wolbachia* persists at low frequencies, thus maintaining mitochondrial polymorphism, is less clear, as fixation frequency depends on various factors like reproductive fitness effects, population size and structure, infection and transmission frequency, reproductive system of the host, bacterial density and/or phage presence (e.g., Caspari and Watson 1959, Bordenstein *et al.* 2006, Egas *et al.* 2002, Jansen *et al.* 2008). Furthermore, fitness effects of *Wolbachia* on host individuals can be conditional on environmental factors (Mouton *et al.* 2006, Reynolds *et al.* 2003, Werren 1997).

It has been estimated that between 40% and 66% of the worlds insect species and the vast majority (around 80%!) of Lepidoptera species harbour *Wolbachia*, which make it to one of the most common and widespread intracellular bacterial genus on Earth (Ahmed *et al.* 2015, Zug and Hammerstein 2012, Hilgenboecker *et al.* 2008). It is not well understood yet, why host resistance to *Wolbachia* is found so rarely. Recent evidence suggests yet undetected positive effects of *Wolbachia* on arthropod host fitness and thus, behaving as mutualists (e.g., Narita *et al.* 2009). Indeed, there is no clear-cut distinction between both effects, reproductive parasitic and mutualistic, even at the same time, implying relatively easy transitions between both effects. Fitness benefits in arthropod species include increased fecundity and longevity, protection against pathogens, and supply with nutrients (Zug and Hammerstein 2015).

3.4.1 *Wolbachia* effects on mtDNA patterns

*Wolbachia*s influence on mtDNA patterns may seriously undermine the power of mtDNA barcoding for species detection. It can either mask species diversity due to mtDNA introgression, e.g. resulting from rare hybridization events between closely related species ("one barcode - two species" phenomenon) (e.g., Narita *et al.* 2006, Gompert *et al.* 2006, Hurst and Jiggins 2005). Or, in contrast, it can promote high mtDNA divergence due to long lasting post-zygotic reproductive isolation between infected and uninfected lineages (e.g., Kvie *et al.* 2013, Charlat *et al.* 2009, Lohman *et al.* 2008) or between populations infected by different *Wolbachia* strains ("two barcodes - one species" phenomenon) (e.g., Kodandaramaiah *et al.* 2013). Further, it may even cause or facilitate the formation of new host species (Sun *et al.* 2011, Telschow *et al.* 2005,

Bordenstein *et al.* 2001, Shoemaker *et al.* 1999). *Wolbachia* may also become lost because of inefficient transmission (Hurst and Werren 2001, Stouthamer *et al.* 1999, Stevens 1989) which may further complicate the interpretation of mtDNA patterns.

To assess whether an observed mtDNA haplotype pattern was the result of *Wolbachia* infection, additional analyses are needed including tests for the presence of *Wolbachia,* as well as the use of additional markers from the nuclear genome (e.g., Kvie *et al.* 2013, Smith *et al.* 2012, Dupius *et al.* 2012, Dasmahapatra *et al.* 2010, Gompert *et al.* 2006). Variation patterns at nuclear genes should not be affected by *Wolbachia* presence. This would lead to mito-nuclear discordance in case of a *Wolbachia* influenced mtDNA-pattern (e.g., Kodandaramaiah *et al.* 2013, Jiggins 2003).

3.4.2 *Wolbachia* effects on butterfly biology and *Phengaris (Maculinea)*

In six out of seven butterfly families *Wolbachia* infections of supergroup A or B have been revealed. In Riodinidae no infection with *Wolbachia* has been detected, which instead showed comparably high proportions of species infected with *Spiroplasma* spp., another reproductive endosymbiotic parasite in insects. Highest proportions of *Wolbachia* infection have been estimated in Pieridae (>40% of screened species are infected with *Wolbachia*); in Hesperidae, Nymphalidae and Lycaenidae proportions are also high (between 20% and 40%) (Russell *et al.* 2012, Salunke *et al.* 2012, Tagami and Miura 2004). Note, that these results are based on molecular surveys which often, due to small sample sizes, make infections with low prevalences hard to detect, resulting in systematic underestimations. To correct for such biases a recent beta-binomial modelling approach revealed that the majority of butterfly species (around 80%) are infected with *Wolbachia* (Ahmed *et al.* 2015).

Several butterfly species have been studied intensively regarding *Wolbachia* effects. To date, CI, male-killing, and feminization have been reported in butterflies; *Wolbachia* induced parthenogenesis has not been observed yet (Kageyama *et al.* 2012, Kodandaramaiah 2011, Werren *et al.* 2008).

Basic biological processes like sex ratio distortion, sex determination, or sperm-egg compatibility are related to *Wolbachia* (Kodandaramaiah 2011). Investigations on the nymphalid butterfly species *Hypolimnas bolina* delivered profound insights into effects of a male-killing *Wolbachia* strain (strain wBol1) on demography, morphology, or mating behaviour. Extreme female-biased sex-ratios (100 females per male) has been observed in a population with a *Wolbachia* infection persistent for >100 years. Furthermore, the ability of males to mate is >50 times higher and size of male spermatophores are much smaller in populations harbouring the long-lasting infection. The decreased male productivity per mate could explain its increased mating frequency observed, as well as the increased female promiscuity in such populations (Charlat *et al.* 2007, Charlat *et al.* 2005, Dyson and Hurst 2004). In some populations *H. bolina* has evolved resistence to male-killing which has recently spread to fixation (Mitsuhashi *et al.* 2011, Hornett *et al.* 2006, Charlat *et al.* 2005). Another interesting *Wolbachia* strategy observed in *H. bolina* populations is the immediate expression of a "backup" phenotype (CI) after inhibiting the primary

male killing phenotype by the host (Hornett *et al.* 2008). Genome-wide analyses of the male killing strain wBol1 reveal recent horizontal gene transfers from multiple sources (Duplouy *et al.* 2013).

In female biased populations of *Acraea encedon* and its close co-occuring sister *A. encedana* (Nymphalidae) an interesting reversal of sex roles has been observed. Both species harbour the same male killing *Wolbachia* strain, indicating either its inheritance from a common ancestor or its horizontal transfer between both host species (which may occur since sibling competition and egg-cannibalism is likely). Mating strategies of sexes are converse with sexual selection of males, instead of females, as usual in butterflies (Wickman 2009). Females exhibit a lekking behaviour, flying together in swarms nearby prominent landmarks and display diverse behaviours to attract males. Further, a near-perfect vertical transmission of *Wolbachia* and efficient male killing has been reported (Jiggins *et al.* 2002, Jiggins *et al.* 2000a, Jiggins *et al.* 2000b, Jiggins *et al.* 1998). Different populations are at different stages of the *Wolbachia* spread, as bacterial prevalences and population sex ratios vary considerably on spatial and temporal scales in both, *A. encedon* and *A. encedana*. Thus, the gradual spread of the male killer through populations toward fixation begins with a host response at behavioural level (full sex-role reversal) and lead finally, at demographic levels, toward population extinctions due to a lack of males as essential reproductive partner (Hassan and Idris 2013).

The effects of infections by multiple strains inducing different phenotypes (CI, feminization) have been studied in *Eurema mandarina* (family Pieridae), a species where a complete *Wolbachia*-caused feminization of genetic males into morphological, behavioural, and functional females has been reported for the first time in insects (Narita *et al.* 2007). Ongoing studies provided also discussions about mechanistic principles of male killing and feminization, revealing that *Wolbachia* interact with the sex determining system. Further, the suppression of the male phenotype occurs continuously over larval development, and not, as suggested previously, at a triggered switching point (Kageyama *et al.* 2012, Narita and Kageyama 2008, Hiroki *et al.* 2004). Feminization was recently reported also in *Eurema hecabe*, a close sister to *E. mandarina* (Narita *et al.* 2011), the only two butterfly species currently known to harbour a feminizing *Wolbachia* strain (Kageyama *et al.* 2012).

Apart from above mentioned examples for reproductive parasitism also mutualistic *Wolbachia* effects have been observed in butterflies (Kodandaramaiah 2011). In *Colias erate* a CI inducing *Wolbachia* strain showed a perfect vertical transmission, as well as infection frequencies up to 100% in several populations. Further, laboratory experiments revealed survival rates during larval stages were significantly higher in broods of infected females compared to brood of females cured by tetracycline antibiotics indicating that the *Wolbachia* infection has beneficial effects on the fitness of the butterfly host (Narita *et al.* 2009).

Species with a parasitic lifestyle like *Phengaris* are potential candidates for cryptic species existence (Huyse *et al.* 2005), and particularly prone to be horizontally infected by endoparasites like *Wolbachia* (Kageyama *et al.* 2012, Heath *et al.* 1999). In fact, recent screens for *Wolbachia* infections explored a presence of diverse strains from supergroup A and B within all *Phengaris (Maculinea)* species (Bereczki *et al.* 2015, Patricelli *et al.* 2013, Sielezniew *et al.* 2012).

3.5 Species conservation units

Species conservation efforts are restricted by limited resources and conflicting economic interests. Thus, *species conservation* actually and practically means *conservation and management of a limited number of populations of an endangered species*. The application of the Evolutionarily Significant Unit (ESU) concept, firstly published by Ryder 1986, as the most accepted conservation unit (CU) below species level, may help to select individual (meta-) populations for optimal habitat management actions to conserve a broad within-species diversity of the endangered species in focus. Further, the concept of Geminate Evolutionarily Unit (GEU) has been proposed as another Conservation Unit below species level (Bowen 1998).

3.5.1 Definitions and designations

To deliver an objective method to find and prioritize unambiguous units for protection below taxonomic levels, the concept of *Evolutionarily Significant Unit* (ESU) was developed (Ryder 1986). It suggests that conservational efforts should be addressed to ESUs as major intraspecific units instead of inconsistent subspecies: "To identify an ESU, information about natural population history, morphometrics, distribution range, as well as molecular and/or cytogenetic information is essential". Further, "the concordance between sets of data derived by different techniques" was recommended (Ryder 1986). Since the late 1980´s the ESU concept evolved over time, e.g. a hierarchical classification scheme have been proposed to detect those "populations that are most likely to be evolutionarily significant units". Populations with ESU status show a discontinuous genetic divergence pattern with a significant genetic distance to other populations and are geographically separated from those other populations. Within an ESU, genome assemblages are closely related and locally adapted to their environment (Dizon et al. 1992).

Moritz (1994) defined ESUs for conservation in relation to ongoing developments in molecular population genetics and phylogenetics and suggested a genetic criterion for the recognition of an ESU (which he defined as a historically isolated set of populations): "ESUs should be reciprocally monophyletic for mtDNA alleles and show *significant* divergence of allele frequencies at nuclear loci." Further, he defined *Management Units* (MUs) as "populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles." In fact, MUs are the real units at which population monitoring, demographic studies or management actions are practiced (Moritz 1994).

Bowen (1998) emphasized to focus also on the *future evolutionary potential of populations* in order to detect Conservation Units (CUs), and not on their evolutionary history alone. He defined *Geminate Evolutionary Units* (GEUs) for the conservation of populations as another category below species level which might play a role in future speciation processes. In the face of genetic homogeneity such populations may be identified by morphological, behavioral, and/or ecological divergence and will not show a reciprocal monophyletic pattern of genetic markers. To identify GEUs five criteria have been proposed: 1. isolated populations which likely remain isolated for an extended geological period; 2. behavioral barriers to gene flow, e.g. reproduce/mate at different times of the year or belonging to different drainages; 3. subpopulations invading a new habitat and thus, expanding the species niche; 4. polytypic species with high

morphological, ecological, or genetic diversity; 5. morphological differentiated species with homogeneity at neutral genetic markers.

Crandall and colleagues (2000) focussed on natural selection as evolutionary process, which ideally acts on functional/adaptive divergent populations and emphasized that maintaining evolutionary processes and the potential for evolutionary change in the future (adaptive diversity) should be the goal of management, rather than maintaining historical population structure and variants alone. Further, authors underlined that the conceptual framework of ESU designation which allows only 2 categories (ESU or not) is inadequate to reflect the continuous distribution of genetic diversity, habitat types, and selective pressures. To represent this continuum, eight categories of distinctiveness between populations with decreasing divergence are suggested to designate important conservation units. The genetic and ecological exchangeability for both, recent and historical time frames, is considered, each case linked with a recommended management action (Crandall *et al.* 2000).

Fraser and Bernatchez (2001) reviewed ESU concepts and its strengths and weaknesses and proposed the unified conceptual framework of *Adaptive Evolutionary Conservation* (AEC) to reconcile opposing views. "An universal definition of an ESU across all species may not be possible and designating ESUs should be done flexibly case-by-case with incorporating the positive attributes of each approach, either alone or in combination, depending on situational circumstances." Differing ESU approaches can be applied as tools in the AEC tool box and can operate in a complementary way. Authors supported the view that an ESU definition should ideally be based on a combination of ecological and genetic data, although under AEC, ESUs can be designated with molecular genetic markers alone.

De Guia and Saitoh (2007) introduced the concepts of *partial* and *full* ESUs: Identification of a *partial ESU* is based on a single set of data, neutral genetic variation or adaptive variation, whereas designation of a *full ESU* needs information of both. "Identification of partial ESUs should be regarded as a first step toward identifiying full ESUs" and authors stress the importance of adding information on further aspects of variation for reaching full ESU status (De Guia and Saitoh 2007).

More recently, ESUs have been considered as largest intraspecific conservation unit with high genetic *and* ecological distinctiveness "that have been historically isolated from each other and that likely have important adaptive differences among them" (Funk *et al.* 2012). Both, neutral and adaptive loci, should be used to delineate ESUs, rather than be considered as alternatives, because ESUs are shaped by both, neutral (e.g., historical isolation) and adaptive (e.g., divergent selection) processes. Further, authors promote high-throughput technologies like next-generation sequencing to gather data from the whole genome. "When possible, ecological, phenotypic, and environmental data should also be used to complement genomic data" (Funk *et al.* 2012).

3.5.2 Applications of ESU-concepts in butterflies and *Phengaris (Maculinea)*

A survey on published genetic data on 147 butterfly studies revealed that in >20% of the studies genotypic information were in conflict with nominal taxonomic boundaries due to incomplete lineage sorting, hybridization, or introgression. In 1/3 of the studies with conflicting results, endangered, threatened, or rare species were involved (Forister *et al.* 2008). Results demonstrated that population differentiation and adaptive evolution can proceed at a more rapid pace compared to molecular evolutional rates at a presumably neutral marker. Further, the authors critizised that a single locus approach to designate ESUs is "based upon the assumption that historically distinct populations have the greatest potential to contain distinct adaptive variation." Thus, also for butterfly species it is highly recommended that all population-level processes should be considered when attempting to identify units for conservation, not solely the presumed neutral dynamics that underlie mtDNA evolution (Forister *et al.* 2008).

In *P. (M.) alcon* molecular studies mainly based on mtDNA sequences did not reveal any evidence for a clade separation between both ecological forms "*alcon*" and "*rebeli*" (Ugelvig *et al.* 2011, Pecsenye *et al.* 2007, Als *et al.* 2004). This uniformity may be a result of a selective sweep of a single mtDNA haplotype hitchhiking a *Wolbachia* infection which may have spread through the species and ecotypes (Sielezniew *et al.* 2012; see also chapter 7.1.). Further, it has been shown that phylogenetic grouping as well as microsatellite clustering did not completely reflect different host ant dependent groups within *P. (M.) alcon*, implicating that applying of both genetic markers alone may not be sufficent enough for a suitable designation of ESUs in this species (Sielezniew *et al.* 2012; see also chapter 7.1.).

By reviewing *Phengaris (Maculinea) arion*s´ and *P. (M.) alcon*s´ host specificity (ant and food plant) and phenology in different European study sites again it was demonstrated that adaptive processes cannot be detected by current molecular analysis in *Phengaris (Maculinea)* species (Casacci *et al.* 2014). With consideration of their extinction history and extinction risks in Europe authors illustrated further, why other sources (ecological, behavioural, and physiological) than genetic are urgently needed and equally valid for an assessment of conservation units (Casacci *et al.* 2014). Moreover, they highly emphasize the importance of recognizing myrmecophilous insect parasites exploiting different host ant species as separate ESUs, because of their non-inter-exchangeability of differently adapted populations in periods of stress, or deprivation (Casacci *et al.* 2014, Thomas *et al.* 2009, Elmes *et al.* 2002, Crandall *et al.* 2000).

4 Study species

4.1 *Phengaris* as a model system for insect biology

Several butterfly species became model organisms in basic biology research areas, like ecology, evolutionary biology, behaviour or conservation biology (Dincă *et al.* 2015, Roe *et al.* 2010, Ehrlich 2003, Pierce *et al.* 2002). Further, they serve as sensitive indicators for monitoring biodiversity or climate change (Van Swaay *et al.* 2015a, Settele *et al.* 2008, Thomas *et al.* 2005).

Especially *Phengaris (Maculinea)* species have been studied quite extensively over the last decades, and much has been learned about their general biology and host specificity (e.g., Thomas *et al.* 2013, Patricelli *et al.* 2010, Sliwinska *et al.* 2006, Elmes *et al.* 2002, Schönrogge *et al.* 2000, Thomas *et al.* 1998, Elmes *et al.* 1991a), or population genetics, ecology and dynamics of the butterfly parasites, its *Myrmica* hosts (Hymenoptera: Formicidae), and its initial food plants (e.g., Sielezniew *et al.* 2015, Pecsenye *et al.* 2015, Solazzo *et al.* 2014, Kajzer-Bonk *et al.* 2016, Kajzer-Bonk *et al.* 2013, Batáry *et al.* 2009, Musche *et al.* 2008, Nash *et al.* 2008, Anton *et al.* 2007, Hovestadt *et al.* 2005, Nowicki *et al.* 2005a, Bereczki *et al.* 2005, Elmes *et al.* 2004). Behavioural biology was also studied including egg-laying-behaviour or particular parasite host interactions (e.g., Skorká *et al.* 2013, Solazzo *et al.* 2013, Patricelli *et al.* 2011, Musche *et al.* 2006, Nowicki *et al.* 2005b, Thomas 2002, Als *et al.* 2001, Thomas and Elmes 2001, Elmes *et al.* 1991b, Thomas 1984). Moreover, mechanisms to integrate into the ant colony have been studied intensively, particularly the mimicked cuticular chemical components behind the specific relationship of ant host and butterfly parasite (e.g., Solazzo *et al.* 2015, Thomas *et al.* 2013, Nash *et al.* 2008, Schlick-Steiner *et al.* 2004, Schönrogge *et al.* 2004, Elmes *et al.* 2002, Akino *et al.* 1999). More recently, mimicry patterns of host and parasite acoustics attracted scientific attention (Sala *et al.* 2014, Barbero *et al.* 2009a, Barbero *et al.* 2009b). Finally, there is a lively discussion about taxonomy, especially regarding the generic name (*Phengaris* or *Maculinea*?), or the species status of the ecotype "*rebeli*" of *P. (M.) alcon* (e.g., Kudrna and Fric 2013, Ugelvig *et al.* 2011, Balletto *et al.* 2010).

To date, *Phengaris (Maculinea)* is one of the best studied butterfly genera globally and has become a flagship taxon for insect conservation (Settele *et al.* 2011, Thomas *et al.* 2009). Further, the group serves as a model for species ecology, host parasite interactions, or co-evolution in myrmecophilous insects (Nash *et al.* 2008, Settele *et al.* 2005).

4.2 Ecology of *Phengaris (Maculinea)*

Compared to other Lepidoptera species the ecology of all studied *Phengaris* species is, due to its myrmecophilous socially parasitic life style, highly complex. Each species depends on one or a few host plant species (*Gentiana* spp., *Origanum* spp., *Thymus* spp., *Sanguisorba officinalis*), and one or more *Myrmica* host ant species (Thomas *et al.* 2013, Als *et al.* 2004, Thomas and

Settele 2004, Thomas *et al.* 1989). In Asia another myrmicine ant species, *Aphaenogaster japonica* FOREL 1911, has been observed as *Phengaris* host (Sibatani *et al.* 1994).

4.2.1 Life cycle of *Phengaris (Maculinea) teleius* and *P. (M.) nausithous*

The two often co-occurring, closely related species studied here, *Phengaris (Maculinea) teleius* BERGSTRAESSER 1779 and *P. (M.) nausithous* BERGSTRAESSER 1779 share *Sanguisorba officinalis* LINNAEUS 1753 (Rosaceae) as their only foodplant (Thomas 1984). Adults lay their eggs in the flower heads, where caterpillars feed on the flower buds and seeds. After the third moult caterpillars leave the flower heads, settle beneath the food plant until being discovered by trophobiotic *Myrmica* ants (Thomas and Settele 2004). In this stage caterpillars produce sounds resembling those produced by queen ants to attract foraging ant workers over wider distances (Sala *et al.* 2014). Due to additional chemical mimicry of *Myrmica* recognition hydrocarbon profiles, foraging *Myrmica* ants mistake caterpillars as their own brood and bring them, after a complex adoption ritual, into their colony (Thomas *et al.* 2010). Inside the colony caterpillars live as social parasites for up to two years (Witek *et al.* 2006), living on the ants´ brood and completing their life cycle. In some *Phengaris (Maculinea)* species caterpillars feed as predators on the ants´ brood (*P. (M.) teleius, P. (M.) arion*), while other species (ecotypes "*alcon"* and *"rebeli"* of *P. (M.) alcon*) live as "cuckoos" among the ant community, the latter being fed directly by nurse ants, even neglecting their own brood. For *P. (M.) nausithous* an ecologically intermediate status between predatory and cuckoo species is suggested (Thomas and Settele 2004), or rather the "cuckoo" strategy alone is assumed (Patricelli *et al.* 2010). Detailled behavioural studies on feeding strategies of *P. (M.) nausithous* are still lacking.

In the early summer of the consecutive year (or year after; Witek *et al.* 2006) pupation occurs inside the ant nest, and some weeks later the adult butterflies eclose. Adults are unable to produce the specific surface chemicals and therefore are in danger of being detected as foreigners by the ants. Thus, they must leave the colony immediately in order not be killed by the ants (e.g., Thomas and Settele 2004, Elmes and Thomas 1992).

4.2.2 Distribution and host ant species of *Phengaris (Maculinea) teleius* and *P. (M.) nausithous*

Both species have wide and overlapping distribution areas in temperate regions of the Palaearctic (Wynhoff 1998a). *P. (M.) teleius* is morphologically variable and a number of subspecies have been described from Asia of which only a few may be valid (Sibatani *et al.* 1994). In contrast, in *P. (M.) nausithous* most authors only recognize the nominate form. Recently, the subspecies *kijevensis* SHELJUZHKO 1928 has been recognized in Eastern Europe (Rakosy *et al.* 2010).

The primary (and nearly exclusive) host ant of *P. (M.) nausithous* over wide European ranges is *Myrmica rubra* LINNAEUS 1758 (Patricelli *et al.* 2010, Tartally and Varga 2005, Stankiewicz and Sielezniew 2002, Thomas *et al.* 1989, own unpublished data; summarized in Witek *et al.*

2014). *Myrmica scabrinodis* NYLANDER 1846 and *M. ruginodis* NYLANDER 1846 have also been observed as hosts of *P. (M.) nausithous,* often in regions where *Myrmica rubra* is absent (Romo *et al.* 2015, Witek *et al.* 2008, Tartally *et al.* 2008). Host ant specificity among *P. (M.) teleius* is much less pronounced and shows regional and local differences with *Myrmica scabrinodis, M. rubra, M. ruginodis, M. rugulosa* NYLANDER 1849*, M. gallienii* BONDROIT 1920, *M. salina* RUZSKY 1905*, M. specioides* BONDROIT 1918, and *M. vandeli* BONDROIT 1920, all used as host ant species, some of these even in the same *Phengaris* population in comparable frequencies (Witek et al. 2010, Witek *et al.* 2008, Pech *et al.* 2007, Stankiewicz and Sielezniew 2002; summarized in Witek *et al.* 2014). In a study area in Northern Mongolia *Myrmica kamtschatica* KUPYANSKAYA 1986*, M. angulinodis* RUZSKY 1905, and *M. forcipata* KARAVAIEV 1931 have been observed as hosts in similar frequencies, suggesting a low *Myrmica* host specificity also in the East Palaearctic (Woyciechowski *et al.* 2006). In Japan *Myrmica ruginodis* and *Aphaenogaster japonica* serve as hosts of *P. (M.) teleius* which is the only currently known non-*Myrmica* host ant species in *Phengaris (Maculinea)* (Sibatani *et al.* 1994).

4.2.3 *Phengaris (Maculinea)* strategies to integrate into the host ant colony

Chemical mimicry is one crucial mechanism to integrate into the host ant colony: Fourth instar caterpillars synthesize cuticular hydrocarbons resembling those of the *Myrmica* ants. After adoption caterpillars produce additional hydrocarbons ensuring a closer similarity to its primary regional *Myrmica* host (Thomas *et al.* 2013, Elmes *et al.* 2002, Akino *et al.* 1999). Another mechanism to trick the ants is acoustical mimicry: caterpillars and pupae produce sounds very similar to queen ant sounds in order to gain a similar high protection status by workers as queen ants have (Sala *et al.* 2014, Barbero *et al.* 2009a, Barbero *et al.* 2009b).

Investigations into the recognition of hydrocarbon profiles of five *Myrmica* species show that there are differences between species, as well as between distinct populations among species. These different cuticular chemical signatures can explain the geographic pattern and local specialisiations of host specificity among European *Phengaris (Maculinea)* species (Thomas *et al.* 2013, Nash *et al.* 2008, Schlick-Steiner *et al.* 2004, Elmes *et al.* 2002). Further, experimental analysis has shown that a succesful survival of cuckoo *Phengaris (Maculinea)* caterpillars within non-host *Myrmica* ant nests strongly depends on continiously good conditions without any food stress (Elmes *et al.* 2004). Thus, it is crucial for butterfly caterpillars to be fully integrated into the host society in environmental stressful situations with food shortages, at least in those *Phengaris (Maculinea)* species which live as cuckoos within ant nests, as it is also suggested for *P. (M.) nausithous* (Patricelli *et al.* 2010). Such a full integration can be achieved only with their specific primary *Myrmica* hosts (Elmes *et al.* 2004).

In order to be initially accepted by its specific host ant species and to maintain this acceptance, the primary mechanism used by the butterfly parasite seems to be chemical mimicry, but to enhance its colony member status inside the colony up to queen ant status, acoustic mimicry plays the major role (Thomas *et al.* 2010). Acoustic strategies and sounds differ according to context and depend on feeding behaviour of the *Phengaris (Maculinea)* species, larval devel-

opment, or life history traits (Sala *et al.* 2014). Which role combined effects of both mechanisms, chemical and acoustical mimicry, play in each interspecific stages (e.g., adoption) has to be explored in future (Settele *et al.* 2011).

The enhanced number of documented local host ant species and a successful regional (multi-) host use in *Phengaris (Maculinea)* suggest a certain kind of flexibility in host ant exploitation indicating their *potential* to shift to new main hosts, at least in evolutionary times (Filz and Schmitt 2015, Jansen *et al.* 2011, Schlick-Steiner *et al.* 2004). A host shift is expected to be more probable with a high chemical similarity between the new and old host ant species (Jansen *et al.* 2011).

4.3 General threats in *Phengaris (Maculinea)* species

The extremely high contemporary anthropogenic impact on the environment globally lead to habitat disturbances and losses and consequently to a "defaunation" (denotes species and population losses, as well as abundance declines) (Dirzo *et al.* 2014). Also in butterfly populations humans cause major losses reflecting the general destruction of humans' life-support systems (Ehrlich 2003) and for numerous butterfly species in Europe a decline in distribution has been assessed (Van Swaay *et al.* 2009).

The overlapping mosaic of host ant and host plant distributions in myrmecophilous Lycaenidae lead to small isolated populations (Pierce *et al.* 2002) and due to their highly complex life cycles they are exceptionally sensitive to environmental disturbances (Thomas *et al.* 2005, New 1993), or to climatic changes, as one of the greatest global threats to biodiversity (Settele *et al.* 2014, Settele *et al.* 2008). Over evolutionary times, population fragmentations may have promoted diversification in Lycaenidae, but with extensive environmental destructions in the current "Anthropocene" the risk of local extinctions is strongly enhanced (Dirzo *et al.* 2014, Settele and Spangenberg 2013, Pierce *et al.* 2002). Further, due to the high host specificity observed in ant parasites with only one or a few host ant species, the risk of co-extinction may be exceptionally high (Fiedler 2012, Settele and Kühn 2009, Koh *et al.* 2004). Habitat destruction and increasing fragmentation has been considered as the most important driver for global defaunation processes (Dirzo *et al.* 2014) and the main driver in butterfly declines is the change in agricultural land use (Van Swaay *et al.* 2015a). Without active habitat management actions butterflies cannot survive in modern European landscapes (Settele *et al.* 2009).

Also European *Phengaris (Maculinea)* populations suffer from local extinctions and increasing fragmentations in most of their ranges for decades, mainly because of changes in habitat suitability caused by changes of local farming practices in grasslands, the main habitat of the species. This lead to changes of *Myrmica* host ant communities within grassland habitats, no longer suitable for *Phengaris (Maculinea)* (Van Swaay *et al.* 2015a, Bubová *et al.* 2015, Filz *et al.* 2013, Munguira and Martin 1999). Another reason for the endangered/threatened conservation status lies in the small population sizes which make *Phengaris (Maculinea)* populations particularly prone to local extinctions (Nowicki *et al.* 2005a, Figurny-Puchalska *et al.* 2000). Finally, adult dispersal abilities are limited and long distance migrations through unsuitable areas are

rare (Nowicki *et al.* 2014) making natural re-colonizations of inhospitable habitats in far distant localities nearly impossible (Romo *et al.* 2015).

All European *Phengaris (Maculinea)* species are regarded as indicator species of high biodiversity in their declining grassland habitats (Thomas *et al.* 2005, Maes and van Dyck 2005). Furthermore, they are recorded in the Red List of the International Union for the Conservation of Nature with decreasing population trends in their habitats (IUCN, 2010). Three of them, namely *Phengaris (Maculinea) arion,* as well as the study species *P. (M.) teleius* and *P. (M.) nausithous,* are listed in annexes of the European Habitats Directive (Van Swaay *et al.* 2012, Van Helsdingen *et al.* 1996) which means that populations must be kept in a favourable state of preservation by European Union law (Settele and Kühn 2009).

4.4 Cryptic species within myrmecophilous social parasites *P. (M.) teleius* and *P. (M.) nausithous?*

Phengaris (Maculinea) species have been intensively studied over decades with respect to their myrmecophilous adaptations and specificities, or their characteristics in population ecology, which make them to an arthropod model system for social parasites of ants (Thomas *et al.* 2005). Different ecotypes, regional and geographic host races exist in *Phengaris (Maculinea)* species (e.g., Thomas *et al.* 2013, Sielezniew *et al.* 2012, Witek *et al.* 2010, Woyciecowski *et al.* 2006, Als *et al.* 2002), which may trigger speciation (e.g., Smith *et al.* 2006, Thomas and Settele 2004, Kankare *et al.* 2005, Schönrogge *et al.* 2002, Drés and Mallet 2002). Further, fragmentation of populations and rare long distance migrations (Nowicki *et al.* 2014), reflected in low gene flow between distant populations (Nowicki *et al.* 2005a, Hovestadt *et al.* 2005) may lead to further genetic isolation. Indeed, an ongoing co-evolutionary arms race between *P. (M.) alcon* and its *Myrmica* ant host is indicated (Nash *et al.* 2008). Thus, the evolution of genetically determined reproductive barriers is possible (Avise *et al.* 1987) which is a major criterion for the delineation of species by applying the Biological Species Concept (Mayr 1996, Mayr 1942).

Phylogenetic studies of mtDNA and nuclear sequences of different samples from distant Eurasian localities revealed that several of the *Phengaris (Maculinea)* species may represent different cryptic species, e.g. *P. (M.) teleius* and *P. (M.) nausithous* (Als *et al.* 2004, Ugelvig *et al.* 2011). Without accurate designation of a species and without general knowledge of its habitat requirements, the application of management strategies for the conservation of endangered species may remain a cost-intense game with uncertain success. Thus, due to their status as model organisms and their conservational need at least in Europe a precise assessment of cryptic diversity in *Phengaris (Maculinea)* species is required urgently (e.g., Dincă *et al.* 2015, Thomas *et al.* 2009).

5 Materials and methods

5.1 Sampling

Phengaris (Maculinea) teleius, including the subspecies *sinalcon* MURAYAMA 1992 (Murayama 1992), *obscurata* STAUDINGER 1892 (Staudinger 1892), *euphemia* STAUDINGER 1887 (Staudinger 1887), *hosonoi* TAKAHASHI 1973 (Takahashi 1973), *kazamoto* DRUCE 1875 (Druce 1875), *ogumae* MATSUMURA 1910 (Matsumura 1910), and *daisensis* MATSUMURA 1926 (Matsumura 1926), as well as *Phengaris (Maculinea) nausithous* were sampled throughout their distribution ranges from 44 and 36 populations, respectively (Fig. 1, Tab. 4). Note that *P. (M.) teleius* and *P. (M.) nausithous* co-occurred in 19 populations. Up to 10 individuals were sampled per species and location. Hand netted adults (N = 110) were killed with potassium cyanide and kept either in glassine envelopes or in 99.8% ethanol. Caterpillars (N = 149) taken from the food plant *Sanguisorba officinalis* were conserved in ethanol. Sex ratios were not assessed, as sample sizes per population were too low and more than half of the specimens were larvae. Collection permits were obtained from Struktur- und Genehmigungsdirektion Nord (Koblenz, Germany), Regierungspräsidium Leipzig (Germany), Regierung von Unterfranken (Würzburg, Germany), Thüringer Landesverwaltungsamt (Weimar, Germany), and specimen collectors' own collection permits, if required in respective countries.

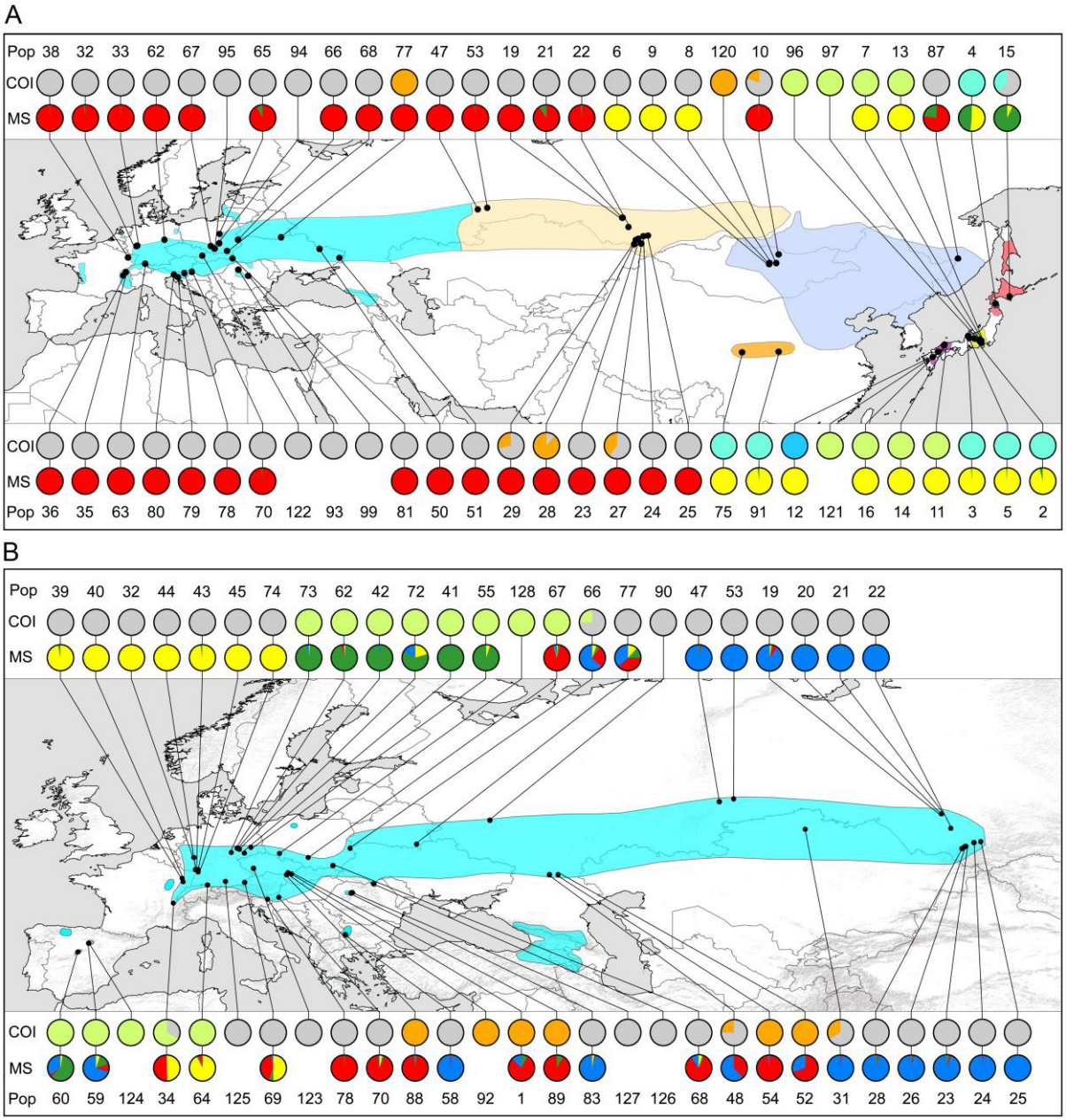

Figure 1: Distribution range, sampling sites and genetic structure at mitochondrial and nuclear genomes. COI = cytochrome oxidase I (for details see Fig. 2) and MS = microsatellites (for details see Fig. 4) for *Phengaris (Maculinea) teleius* (A) and *P. (M.) nausithous* (B). For details on populations (Pop), see Tab. 4. Distribution ranges are based on published records (Europe: Wynhoff 1998a, Kudrna *et al.* 2011; Asia: Lukhtanov and Lukhtanov 1994, Tshikolovets *et al.* 2002, Tshikolovets *et al.* 2009a, Tshikolovets *et al.* 2009b), and expert knowledge (Asia: personal communication Oleg Kosterin, Min Wang). Shading color corresponds to subspecific affiliations of *P. (M.) teleius* according to Tab. 4 (cyan: nominate species, peach: *P. (M.) t. obscurata*, blue: *P. (M.) t. euphemia*, orange: *P. (M.) t. sinalcon*, pink: *P. (M.) t. ogumae*, yellow: *P. (M.) t. kazamoto*, green: *P. (M.) t. hosonoi*, violet: *P. (M.) t. daisensis*).

5.2 DNA barcoding and tests for *Wolbachia* infection

Total genomic DNA was extracted using the QIAGEN Dneasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. Two fragments of the COI gene were amplified using the primer combinations: LCO – Nancy and Tonya – Hobbes (Ugelvig *et al.* 2011). PCR was performed in 20 ml reactions, containing 0.5 pmol of each primer, 200 mM dNTPs, PCR buffer, 1.875 mM MgCl2, and 0.8 units Fermentas Taq DNA polymerase (Fermentas, Leon-Rot, Germany). The thermocycler protocol was: denaturation at 95°C (2 min) followed by 37 cycles of 95°C (1 min), 47°C (1 min) and 72°C (1.5 min), and a subsequent final extension step at 72°C (10 min). PCR-products were directly cyclesequenced using the ABI BigDye Terminater v3.1 cycle sequencing Kit using the same primers. Products were sequenced on an Applied Biosystems 31306l Genetic Analyzer (Applied Biosystems, Foster City, USA). About 10% of the fragments were treated with a multiple tube approach and 20% of the fragments were sequenced in both directions which did not show any mismatches. Sequences were obtained for 147 samples of *P. (M.) teleius* and 112 samples of *P. (M.) nausithous*. GenBank accession numbers for all concatenated sequences are provided in Tab. 4. All individuals were tested for infection with *Wolbachia* performing two independent PCR screens for the *Wolbachia* surface protein (wsp) following the protocol of Zhou and colleagues (Zhou *et al.* 1998). PCR products were visualized on 1.5% agarose gels and scored for the presence of *Wolbachia* infections (Tab. 4). The wsp-genes of *Wolbachia* endosymbionts of 8 *P. (M.) teleius* and 3 *P. (M.) nausithous* were sequenced to determine allele and supergroup correspondence, using the *Wolbachia* wsp Database (Jolley *et al.* 2004) and BLAST. I did not screened for further endosymbiotic heritable bacterials due to their negligible prevalence in Lycaenidae (Russell *et al.* 2012).

5.3 MtDNA sequence analysis

COI fragments of 147 and 112 individuals of *P. (M.) teleius* and *P. (M.) nausithous*, respectively, were manually concatenated and aligned with BioEdit (Hall 1999). To avoid the inclusion of mitochondrial pseudogenes (Williams and Knowlton 2001), translated amino acid sequences were tested for substitutions and stop-codons using the program MEGA 5 (Tamura *et al.* 2011). In both *P. (M.) teleius* and *P. (M.) nausithous* ten non-synonymous substitutions were found leading to a change in the amino acid sequence. However, I did not regard these substitutions as indicative for a pseudogene because the mutations occurred in parts of the protein known for their high amino acid variability (Lunt *et al.* 1996, Kim *et al.* 2006), or because substitutions led to amino acids of similar characteristics. Additionally, all published COI sequences of *P. (M.) teleius* and *P. (M.) nausithous* available from GenBank as of 1 June 2013 (Tab. 4) were added. These also included all publicly available barcode sequences from the barcode of life database (BOLD; Ratnasingham and Hebert 2007). Note that the test for *Wolbachia* infection could not be performed for sequences retrieved from GenBank. Sequences of further *Phengaris* species (*P. (M.) arion* LINNAEUS 1758, *P. (M.) alcon* DENIS and SCHIFFERMÜLLER 1775, *P. albida* LEECH 1893, *P. atroguttata* OBERTHÜR 1876, *P. daitozana* WILEMAN 1908) were taken from GenBank (Tab. 4) as outgroup taxa. A haplotype analysis was carried out using TCS 1.21 (Clement *et al.* 2000). Prior to this analysis, parts of the alignment which were only availa-

ble for a minority of sequences (alignment positions 1–60, 649–766 and 1193–2210) were removed, and sequences were sorted according to the number of non-ambiguous sites in decreasing order. All short sequences (below 680 bp, i.e. all short barcode sequences) which were included in the first haplotype analysis were removed from further analysis due to the low level of overlap resulting in 157 sequences for *P. (M.) teleius* and 120 for *P. (M.) nausithous*. Gene evolution was visualized with a haplotype network using statistical parsimony as implemented in TCS 1.21 using default options. Phylogenetic trees were inferred applying two criteria, i.e. unweighted Maximum Parsimony (MP), and Maximum Likelihood (ML), using the consensus haplotype sequences of the complete alignment. MP analysis was conducted in MEGA 5 (Tamura *et al.* 2011) doing a heuristic search (Close Neighbor-Interchange algorithm). Initial trees were obtained by random addition of sequences (10 replicates). All codon positions were included and alignment gaps were treated as missing data. For ML inference, the Tamura-Nei model (Tamura and Nei 1993) with a gamma distribution for rate variation among sites (G = 0.084) was selected using jModelTest 0.1.1 (Posada 2008) as the best fitting evolutionary model. Tree searches were performed with PhyML version 3.0 (Guindon *et al.* 2010) using the SPR search option and a BIONJ starting tree. Branch support for MP- and ML-trees was estimated by bootstrapping the dataset 500 times. Average sequence divergence for COI was calculated as uncorrected pairwise p distances of all haplotype sequence pairs within and between clades using MEGA 5 (Tamura *et al.* 2011). Because fossil data of *Phengaris* are not available, and geological events cannot be linked with branching events in the phylogenetic trees, age estimates of splitting events were calculated by using three COI substitution rates reported for arthropods, i.e. 1.3% (Quek *et al.* 2004), 2.3% (Brower 1994), and 3.5% per million years (Papadopoulou *et al.* 2010). Nucleotide diversity p (Tajima 1989) was estimated. To test whether sequence diversity was concordant with expectations of neutral evolution Tajima's D (Tajima 1989) and Fu's F (Fu 1996) were computed, as implemented in Arlequin 3.5.1.2 (Excoffier and Lischer 2010). Deviations from neutral evolution may suggest recent demographic expansions or bottlenecks. For these analyses only nucleotide positions represented in every sample were included (*P. (M.) teleius* N= 845; *P. (M.) nausithous* N= 768). Furthermore, samples from divergent "*Wolbachia*" clades were excluded.

5.4 Nuclear microsatellite analysis

Samples were genotyped at eight nuclear microsatellite loci (Macu1, Macu3, Macu7, Macu8, Macu9, Macu11, Macu15, Macu16; Zeisset *et al.* 2005). Loci were amplified in three reactions with a multiplex PCR kit (QIAGEN) using fluorescent labelled primers and separated on an Applied Biosystems 31306l Genetic Analyzer (Applied Biosystems, Foster City, USA). Individuals for which fewer than four loci yielded interpretable results were excluded from the analysis resulting in a data set of 143 *P. (M.) teleius* and 109 *P. (M.) nausithous* genotypes (Tab. 5). To assess population structure of individual multilocus genotypes a Bayesian clustering method were used separately for each species using STRUCTURE 2.3 (Pritchard *et al.* 2000). For each K ranging from 1 to 10, 10 replicate runs with 100.000 steps after a burn-in period of 50.000 steps were performed. The admixture model without prior population information and with correlated allele frequencies were used. Most likely K values were estimated following

Evanno *et al.* (2005); see Fig. 7. The program CLUMPP 1.1.1 (Jakobsson and Rosenberg 2007) was used to estimate the mean cluster assignment across replicate runs. For the resulting main clusters gene diversity (He), allelic richness (Ar), private allelic richness (pAr), and shared allelic richness (sAr) were calculated using ADZE 1.0 (Szpiech *et al.* 2008). Differentiation among clusters was quantified as h, an estimator of Wright's FST (Weir and Cockerham 1984) and as standardized G'ST (Hedrick 2005; eq. 4b) calculated in Fstat 2.9.3.2. (Goudet 2001). Individuals with highly ambiguous cluster membership (inferred ancestry <0.8; *P. (M.) teleius*: N=4; *P. (M.) nausithous*: N= 24) were excluded from this analysis. The relationship between nuclear and mitochondrial genomes were assessed by correlating inter-individual genetic distances and testing the significance by a Mantel test with 1000 randomizations in R version 2.12.2 (R core team, 2011). For microsatellites, genetic distances were quantified as proportion of shared alleles calculated with MSA v. 3.0 (Dieringer and Schlötterer 2003). For COI sequences I used Maximum Composite Likelihood estimates with pairwise deletion of missing data and gamma distributed substitution rates among sites, calculated in MEGA 5 (Tamura *et al.* 2011).

6 Results

6.1 *Wolbachia* infection

In *P. (M.) teleius* 19 out of 147 (13%) individuals investigated were found to be infected with *Wolbachia*, while in *P. (M.) nausithous* 6 out of 112 (5.4%) were found (Tab. 4). The *Wolbachia* wsp genes had one allele each in *P. (M.) teleius* and *P. (M.) nausitous* (GenBank accession no. JX470438, JX470439). The sequence from *P. (M.) teleius* is identical with allele 431 found in Heteroptera from Japan (Kikuchi and Fukatsu 2003). The sequence from *P. (M.) nausithous* differs only slightly from three known alleles (264, 266, 436) detected in Lepidoptera and Hemiptera also originating from Japan (Kikuchi and Fukatsu 2003, Tagami and Miura 2004) and was submitted as new allele 639 to the *Wolbachia* wsp database. The two wsp alleles are very distinct (nucleotide p distance: 9.4%; protein p-distance: 14%), however, both are affiliated with *Wolbachia* supergroup B.

6.2 Phylogenetic inference in *P. (M.) teleius* and *P. (M.) nausithous*

The final mtDNA alignment contained 282 sequences (157 *P. (M.) teleius*, 120 *P. (M.) nausithous*, 5 outgroup) with a total length of 2253 bases of which 333 (14.8%) sites were variable and 210 were parsimony informative (9.3%). No indels were detected. In total 124 unique haplotypes were observed (Tab. 4), 72 in *P. (M.) teleius* and 52 in *P. (M.) nausithous*. Of these, 3 haplotypes (N50, N51, N52) were observed exclusively in barcode sequences, which were excluded from further analysis. However, these haplotypes only differed in single nucleotide positions from other haplotypes (N06, N42, and N49, respectively). The mtDNA haplotype network calculation resulted in independent networks for *P. (M.) teleius*, *P. (M.) nausithous*, and for each outgroup species. Further, within both study species a clade dominated by *Wolbachia*-infected individuals under the 95% parsimony limit (0.956=13 steps) resulted. A slightly relaxed parsimony limit (0.949=14 steps in *P. (M.) nausithous*; 0.942=15 steps in *P. (M.) teleius*) led to a connection between the "*Wolbachia*" and the respective remaining haplotypes (Fig. 2). Thus, in both species there is a majority phylogroup plus several long branching groups, one of which is characterised by *Wolbachia* infection. Phylogenetic inference using both ML and MP yielded essentially the same results, with both *P. (M.) teleius* and *P. (M.) nausithous* being monophyletic (Fig. 3, Fig. 5, Fig. 6). Together with *P. (M.) arion* the two species formed a clade clearly separated from other members of the genus. However, only in the parsimony analysis *P. (M.) teleius* and *P. (M.) nausithous* were supported as sister species (Fig. 6). In both species there is a basal "*Wolbachia*" clade sister to four (*P. (M.) teleius*) or two (*P. (M.) nausithous*) further haplogroups. In *P. (M.) teleius* the majority of haplotypes formed a star-like network with many single steps (haplogroup *P. (M.) teleius* I) (Fig. 2). This phylogroup was distributed throughout continental Eurasia except for one haplotype which occurred in the most northern Japanese population (Hokkaido; ssp. *ogumae*) (Fig. 1). Three additional longbranched clades were geographically confined to Eastern Asia: *P. (M.) teleius* II to Honshu (ssp. *kazamoto* and *daisensis*),

P. (M.) teleius III to Kyushu (ssp. *daisensis*) and *P. (M.) teleius* IV to China (ssp. *sinalcon*) and Japan (Hokkaido and Northern Honshu; ssp. *ogumae*, *kazamoto* and *hosonoi*). In the long branched *P. (M.) teleius* "*Wolbachia*" clade most individuals (94%; N= 15/16) were infected, significantly more than within the rest of *P. (M.) teleius* (2.8%; 4/141; X^2-test: $p<0.0001$, Fig. 2). This clade was geographically restricted to Belarus, the Russian Altai, and Mongolia (Fig. 1). The subspecies within *P. (M.) teleius* showed no clear correspondence to haplogroups since subspecies either consisted of several haplotypes (ssp. *kazamoto, daisensis* and *ogumae*), or haplogroups harboured several subspecies (*P. (M.) teleius* I, II and IV). In *P. (M.) nausithous* the majority haplogroup I was distributed through most of the species range. One additional clade was formed (*P. (M.) nausithous* II) by European haplotypes from Poland, Eastern Germany, Southern Germany, the Western Alps and Spain (Fig. 1, Fig. 2). In *P. (M.) nausithous* the "*Wolbachia*" clade harboured 56% infected individuals (N = 5/9) in contrast to the rest of *P. (M.) nausithous* (<1%; 1/110; X^2-test: $p<0.0001$, Fig. 2). Infected individuals originated from Eastern Europe and Western Asia (Fig. 1). Subspecies *kijevensis* (Rákosy *et al.* 2010) had haplotypes of two clades, *P. (M.) nausithous* "*Wolbachia*" and *P. (M.) nausithous* I.

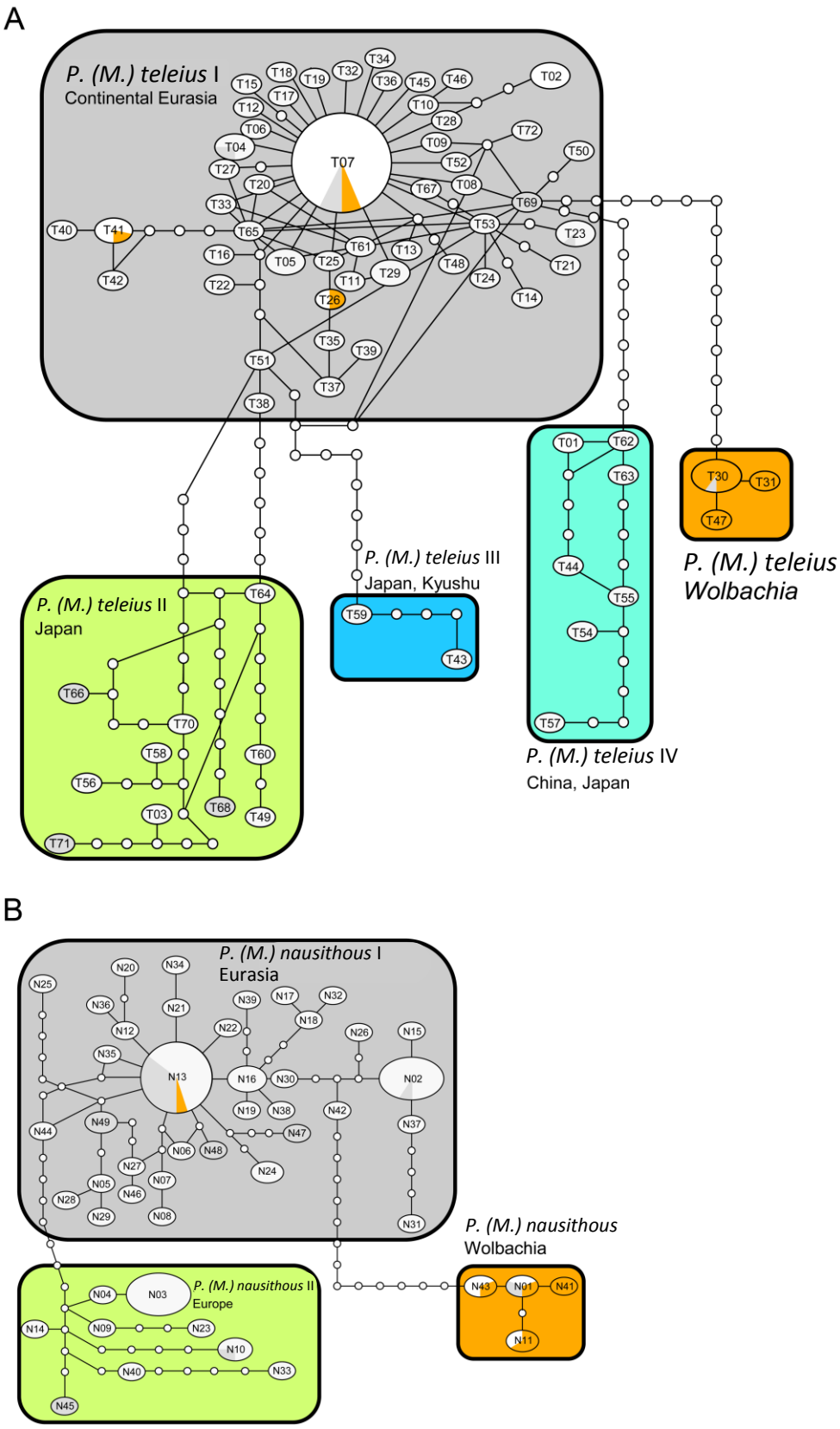

Figure 2: COI haplotype networks for *Phengaris (Maculinea) teleius* (A) and *P. (M.) nausithous* (B). Circle size is proportional to haplotypes frequency (Tab. 4). The proportion of individuals infected with *Wolbachia* is indicated by an orange-coloured pie chart. Note that in several haplotypes samples could not be tested for *Wolbachia* since the corresponding sequence was extracted from Genbank (grey shaded pie chart).

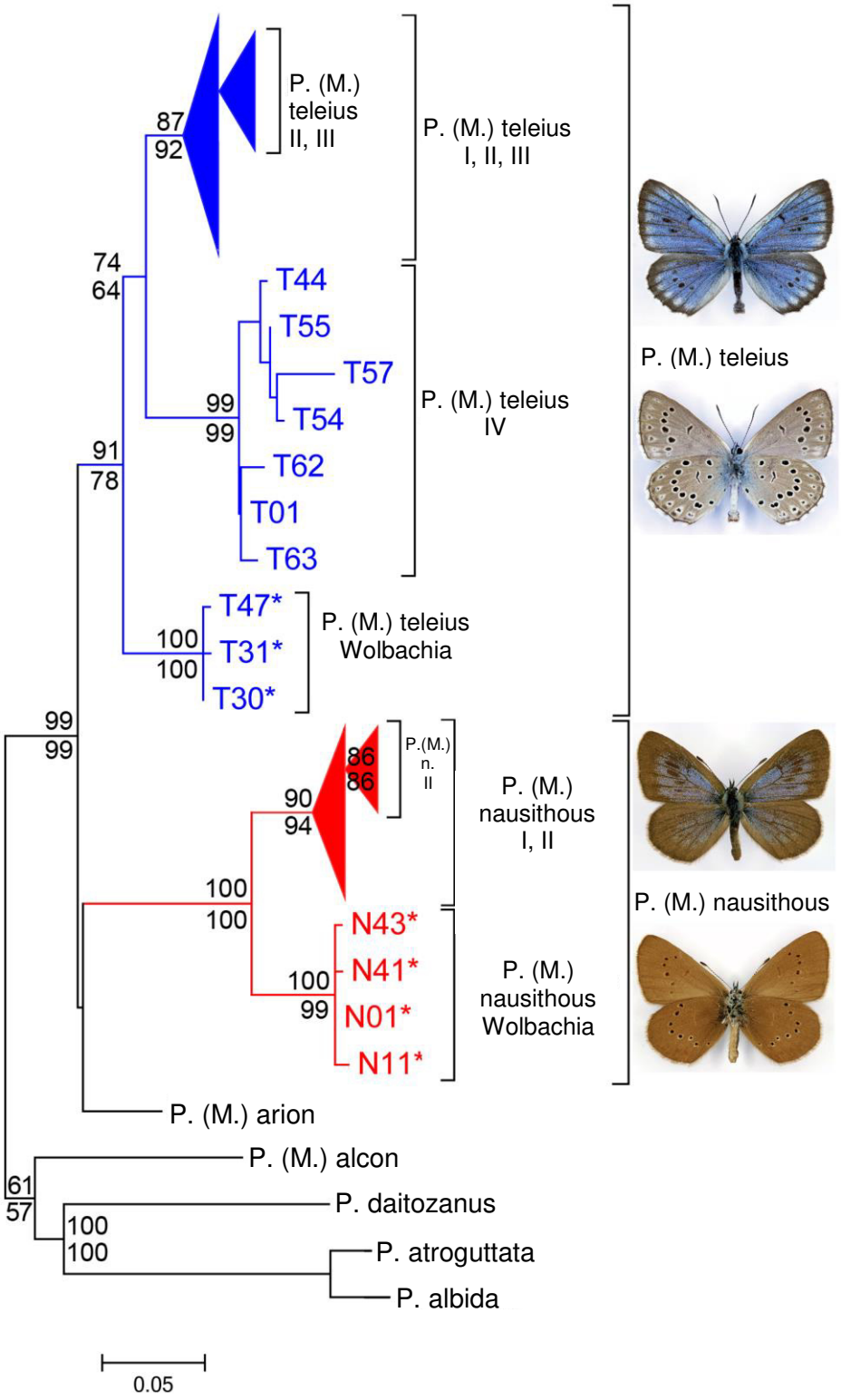

Figure 3: Phylogram for haplotypes of *Phengaris (Maculinea) teleius* and *P. (M.) nausithous* based on ML analysis for mitochondrial COI. Bootstrap values in percent (>50%) are given above branches (based on ML analysis) and below branches (MP analysis). Bootstrap values within subclades are not shown (see Fig. 5, 6). *haplotypes associated with *Wolbachia*.

6.3 Sequence divergence and nucleotide diversity

Average sequence divergence between *P. (M.) teleius* and *P. (M.) nausithous* was 4.19% ± 0.54%, placing the split between the species at the end of the Pliocene or beginning of the Pleistocene (Tab. 1). In both species the sequence divergence between haplotypes of the "*Wolbachia*" clades and all other haplotypes was similar and translated into estimated ages between 0.65 and 1.97 Million years. Nucleotide diversity was $\pi = 5.52$ (including *Wolbachia* infected individuals: 7.73) for *P. (M.) teleius* and $\pi = 5.41$ (6.82) for *P. (M.) nausithous* (Tab. 2). Neutrality tests for different geographic areas revealed contrasting results for the two species. *P. (M.) teleius* showed low π combined with significantly negative Tajima's D or Fu's F for continental Asia and Europe suggestive of rapid demographic expansion in that area, whereas samples from Japan showed high π and no deviation from neutrality. In *P. (M.) nausithous*, clade *P. nausithous* I showed significant deviation from neutrality suggesting demographic expansion in the eastern part of the range, whereas in the western part *P. nausithous* II conformed to a neutral model.

Table 1: Sequence divergence values and estimated node dates for prominent splits of recovered phylogenetic trees (Fig. 3).

Split	**Sequence divergence (%) between clades**	**MYA (Evolutionary rate of COI 1.3% per 1 Million years)**	**MYA (Evolutionary rate of COI 2.3% per 1 Million years)**	**MYA (Evolutionary rate of COI 3.5% per 1 Million years)**
P. (M.) teleius versus *P. (M.) nausithous*	4.19 ± 0.54	3.22	1.82	1.19
P. (M.) teleius I-IV versus *P. (M.) teleius* "Wolbachia"	2.56 ± 0.47	1.97	1.11	0.73
P. (M.) teleius IV versus *P. (M.) teleius* I-III	2.16 ± 0.37	1.66	0.94	0.62
P. (M.) teleius II versus *P. (M.) teleius* I+III+IV	1.39 ± 0.22	1.07	0.60	0.40
P. (M.) teleius III versus *P. (M.) teleius* I+II+IV	1.52 ± 0.26	1.17	0.66	0.43
P. (M.) nausithous I-II versus *P. (M.) nausithous* "Wolbachia"	2.28 ± 0.43	1.75	0.99	0.65
P. (M.) nausithous I versus *P. (M.) nausithous* II	1.42 ± 0.30	1.09	0.62	0.41

Table 2: Nucleotide diversity π, Tajima´s *D*, and Fu´s *F* estimates at mtDNA COI of different phylogenetic clusters and geographic zones. N = number of sequences, S = number of polymorphic sites

Sample pool	**N**	**S**	**π ± s.d.**	***Tajima´s D***	**P** value	***Fu´s F***	**P** value
P. (M.) teleius I+II+III+IV	141	88	5.52 ± 2.96	-2.07	**0.002**	-25.18	**0.000**
P. (M.) teleius I + partly IV (Continental Eurasia)	117	57	2.56 ± 1.53	-2.40	**0.000**	-26.83	**0.000**
P. (M.) teleius II+III+partly IV (Japan)	24	50	14.58 ± 7.54	0.35	0.694	-00.71	0.414
P. (M.) nausithous I+II	110	40	5.41 ± 2.91	-0.89	0.177	-20.01	**0.000**
P. (M.) nausithous I	76	33	2.21 ± 1.37	-2.13	**0.002**	-27.07	**0.000**
P. (M.) nausithous II	34	12	2.52 ± 1.55	-0.44	0.325	-01.03	0.330

6.4 Nuclear microsatellite analysis

In *P. (M.) teleius*, the STRUCTURE analyses revealed consistent outcomes with K = 2, separating two geographically coherent clusters (Fig. 4, Fig. 7). The "Main Cluster" was formed by all samples from Europe and extended to continental Asia. The second cluster "East Asia" was formed by all samples from Japan, China and Central Mongolia. A few individuals showed admixture in the border region of the two clusters (Fig. 1). In additional separate STRUCTURE analysis of the two clusters the East Asian cluster was again split into two groups separating Hokkaido from the rest. Within the "Main Cluster" no further substructure was found as a peak of $\Delta K = 12$ at K = 4 was very low compared to the other analyses (Fig. 7) and the resulting groups showed a high degree of admixture and no clear geographic pattern. The "Main Cluster" in *P. (M.) teleius* corresponded largely to haplogroup *P. teleius* I obtained in the COI analysis, while the different Japanese clades and the "Wolbachia" clade were not retrieved in the microsatellite analysis. In *P. (M.) nausithous* the STRUCTURE analysis revealed four clusters (Fig. 4, Fig. 7). Three clusters corresponded to areas in Europe (I, II, III) comprising western, central and eastern European populations, respectively. The fourth and largest cluster extended from Eastern Europe into Asia. Admixture was observed in contact zones of the clusters in specimens from France, Czech Republic, Belarus, Poland, SW Germany, and E Germany. Specimens from peripheral sites in Spain, Germany and Southern Russia also appeared admixed (Fig. 1). In STRUCTURE analyses at lower values of K, a strong East-West split was found. At K = 2, a western cluster comprising Europe I+II and an eastern cluster comprising Europe III+Asia was formed. At K = 3, Europe III was separated from Asia. Genetic differentiation between clusters was strong in both species (*P. (M.) teleius*: h = 0.265 (SE 0.063), G'ST =0.671; *P. (M.) nausithous*: h =0.143 (SE 0.02), G'ST = 0.497) although lower in *P. (M.) nausithous,* as expected from the larger number of clusters. Genetic variation within clusters is shown in Tab. 3.

In *P. (M.) teleius*, cluster East Asia I was the most genetically diverse, as indicated by higher values of He, Ar and private Ar. In *P. (M.) nausithous*, the four clusters showed similar levels of genetic variation. Genetic divergence was largely consistent between nuclear and mitochondrial genomes, but influenced by the inclusion of *Wolbachia* haplogroups which did not form similarly divergent microsatellite clusters. In *P. (M.) teleius* genetic distances of microsatellites and COI sequences were not correlated when all haplotypes were considered (r = 0.083, Mantel-p= 0.12), but became significantly positively correlated when *Wolbachia* haplotypes were removed (r = 0.405, Mantel-p= 0.001). For *P. (M.) nausithous* genetic distances were correlated both overall (r= 0.269, Mantel-p= 0.001) and without *Wolbachia* haplotypes (r = 0.177, Mantel-p= 0.001).

Table 3: Mean estimates of genetic diversity across 8 microsatellite loci in clusters identified in the STRUCTURE analysis. H_e expected heterozygosity, A mean number of alleles, Ar allelic richness based on 7 and 10 individuals, for *P. (M.) teleius* and *P. (M.) nausithous*, respectively.

Species / cluster	**N individuals**	**H_e (SD)**	**A**	**Ar (SE)**	**private Ar (SE)**	**% private alleles**
P. (M.) teleius						
Main Cluster	103	0.55 (0.26)	11.8	4.7 (1.0)	2.8 (1.0)	60%
East Asia I	26	0.67 (0.21)	9.0	5.4 (1.0)	3.3 (1.0)	61%
East Asia II	9	0.41 (0.31)	3.3	3.2 (0.7)	1.5 (0.7)	47%
P. (M.) nausithous						
Europe I	16	0.70 (0.23)	8.3	6.7 (1.5)	3.4 (1.4)	51%
Europe II	21	0.70 (0.24)	9.6	7.1 (1.0)	2.4 (0.6)	34%
Europe III	11	0.70 (0.23)	6.4	6.2 (1.3)	2.6 (1.0)	42%
Asia	37	0.71 (0.30)	13.9	7.8 (1.5)	3.5 (0.9)	45%

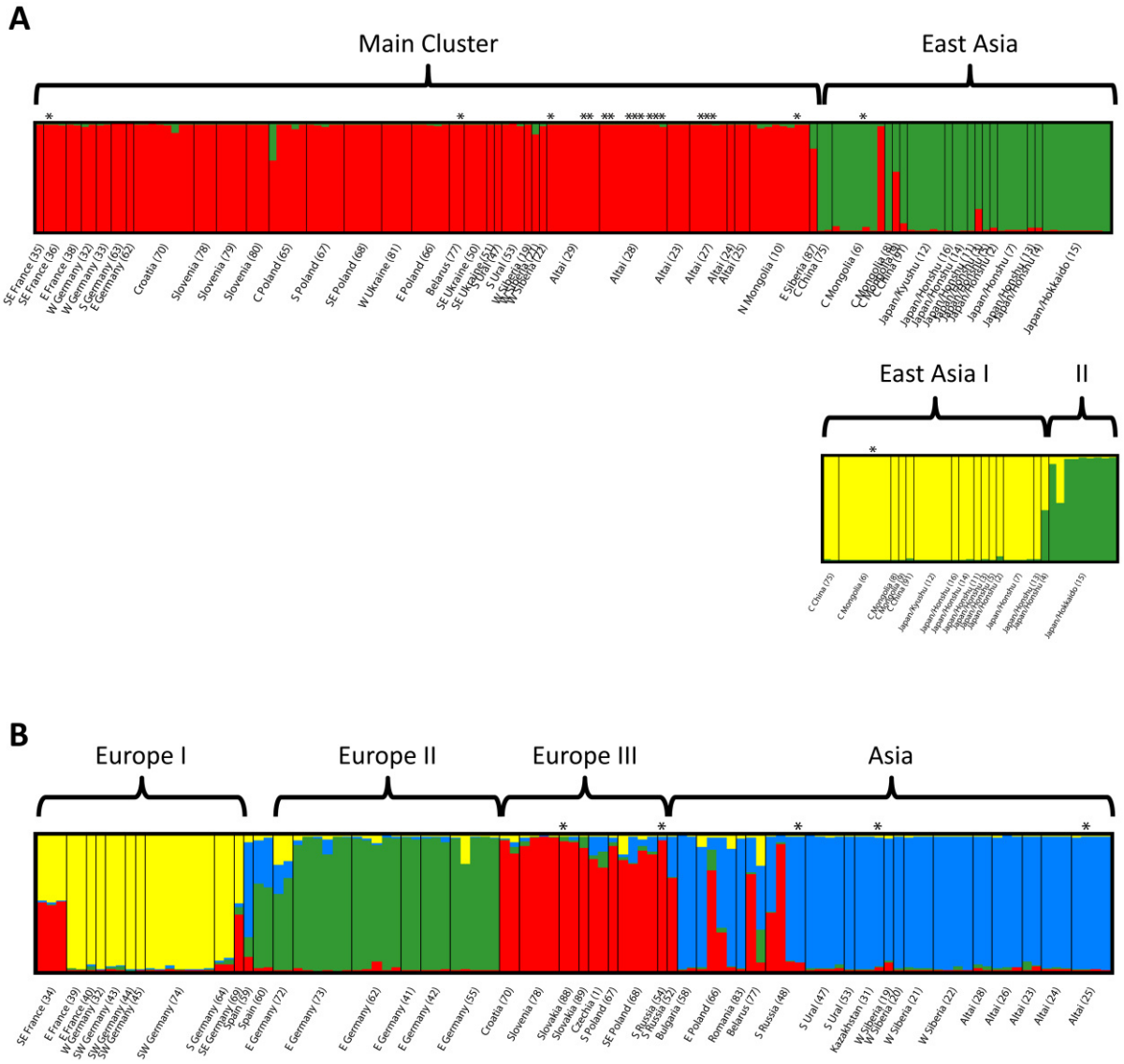

Figure 4: Results of STRUCTURE analysis of microsatellite genotypes for *Phengaris (Maculinea) teleius* (A) and *P. (M.) nausithous* (B). Small bars represent individuals and their cluster membership coefficients. For details see text, Fig. 1, and Fig. 7; for population details see Tab. 4. *individuals infected with *Wolbachia.*

6.5 Conservation units (CUs) in *Phengaris (Maculinea)*

6.5.1 Definition of CUs in *P. (M.) nausithous*

For a demarcation of CUs in *P. (M.) nausithous* I combined the mitochondrial and microsatellite genetic data with available information on host ant specificity, as suggested by Casacci *et al.* 2014. Solid host ant information is available from Western and Central European populations. In France (Thomas *et al.* 1989), Germany (own unpublished data), Poland (Patricelli *et al.* 2010, Witek *et al.* 2008, Stankiewicz and Sielezniew 2002, Thomas *et al.* 1989), Slovakia (Witek *et al.* 2008), Western Ukraine (Witek *et al.* 2008), and Hungary (Tartally and Varga 2005) *Myrmi-*

ca rubra has been found to be the only primary host ant species supporting *P. (M.) nausithous*. These *M. rubra*-dependent populations belong to COI-clades I, or II (Fig. 2B, Fig. 3), and to one of each microsatellite clusters (Europe I, II, III, or Asia; Fig. 4B). I regard Central European *M. rubra* adapted populations of the microsatellite clusters "Europe I" (East France, West Germany), "Europe II" (East Germany), and "Europe III" (Czechia, Slovakia, South Poland, Slovenia, Croatia) *preliminarily* as at least three *partial* ESUs according to each cluster (Fig. 1B, 4B) (Funk et al 2012, De Guia and Saitoh 2007). For populations in Eastern Europe and Western Siberia (microsatellite cluster "Asia") information about specific ant hosts is completely lacking and therefore I did not defined any preliminary CUs.

An East Polish *M. rubra*-dependent population (pop. no. 66) has been detected as part of the contact zone between microsatellite clusters "Asia" and "Europe III" (Fig. 1B, 4B) and therefore, I consider it to belong to a separate conservation unit (CU). Further contact zone populations I detected are located in Belarus (Fig. 1B; pop. no. 77), South Russia (pop. no. 48, and 52), and in the Alps (pop. no. 34, 64, and 69). Contact zone populations generally harbour an enhanced genetic richness and/or adaptiveness to climatic changes, as they harbour alleles survived in different Ice Age refugia. Thus, contact zone populations may harbour enhanced future evolutionary potential and should gain special conservation efforts (Bowen 1998).

Strongly isolated *M. scabrinodis*-adapted *P. (M.) nausithous* populations in Central Europe / Transylvania (*M. scabrinodis* is the only *Myrmica* species present in these populations) (Tartally *et al.* 2008) belong to microsatellite cluster "Asia". Further, these populations survive at low densities and are recognized to represent the subspecies *kijevensis* (Rákosy *et al.* 2010). Moreover, "steppic" habitat conditions characterize these populations as strongly differing from typical *P. (M.) nausithous* habitats in Central Europe (Rákosy *et al.* 2010, Tartally *et al.* 2008). Based on these characteristics I regard isolated "steppic" Transylvanian *P. (M.) nausithous* populations as important ESU (Casacci *et al.* 2014, Funk *et al.* 2012, Bowen 1998). The only genetic sample included in present analysis (pop. no. 83) belongs to COI clade I and to microsatellite cluster "Asia". Of course, more samples need to be analysed for a robust genetic membership. Additionally, also Bulgarian populations (pop. no. 58), characterized by the same genetic combination as Transylvanian samples (COI clade I, microsatellite cluster "Asia"), I regard as separate CU due to its high isolation from the main distribution range (Fig. 1B) (Funk *et al.* 2012, Bowen 1998).

In populations in Spain, which are completely isolated and far distantly located from the main distributional area of the species too (Fig.1B), in addition to *Myrmica rubra* also *Myrmica scabrinodis* has been identified as host ant species (Romo *et al.* 2015, Munguira and Martin 1999). Spanish populations included in present analyses belong to COI clade II and harbour alleles of all four microsatellite clusters (Fig 1B, 4B). Unfortunately, only three samples were available and only two samples delivered reliable microsatellite information. Nevertheless, due to their presumably long-term isolation from the main distributional range, and due to its importance as Ice Age refugia also Iberian *P. (M.) nausithous* populations should be regarded as one, or even more, separate ESUs, depending on its host ant specificity pattern (Casacci *et al.* 2014, Funk et al. 2012, Bowen 1998), which still has to be investigated.

6.5.2 Definition of CUs in *P. (M.) teleius*

Also in *P. (M.) teleius* information on host ant specificity is sparse compared to its total Eurasian distributional range. Generally, ant specificity is much less pronounced in *P. (M.) teleius*. Eleven regionally or locally differing *Myrmica* host ant species have been detected up to now: *Myrmica scabrinodis, M. rubra, M. ruginodis, M. rugulosa*, *M. gallienii*, *M. salina, M. specioides,* and *M. vandeli* in Central Europe (Witek *et al.* 2008, Stankiewitz and Sielezniew 2002, Elmes *et al.* 1998; summarized in Witek *et al.* 2014), and *M. kamtschatica, M. angulinodis,* and *M. forcipata* being detected as hosts of *P. (M.) teleius* in an open population in Central Asia (northern Mongolia) (Woyciechowski *et al.* 2006). Further, in Japan *Myrmica ruginodes* and *Aphaenogaster japonica*, the only currently known non-*Myrmica* host ant species in *Phengaris (Maculinea)*, harbour *P. (M.) teleius* (Sibatani *et al.* 1994). In many localities several *Myrmica* species were parasitized to similar frequencies, why it is not appropriate to distinguish a primary host (Witek *et al.* 2008, Woyciechowski *et al.* 2006, Stankiewitz and Sielezniew 2002). Due to this low specificity compared to other *Phengaris (Maculinea)* species I did not incorporate information on host ant adaptation in present CU designation.

Considering mitochondrial DNA patterns as major criteria in *P. (M.) teleius* I define CUs corresponding completely with mitochondrial haplogroups (Fig. 2A) (De Guia and Saitoh 2007, Fraser and Bernatchez 2001). Haplogroup "*P. (M.) teleius* I" comprises populations of Continental Eurasia (Fig. 1A), including subspecies *P. (M.) teleius teleius*, *P. (M.) teleius obscurata,* (which both also represent microsatellite "Main cluster"; Fig. 4A), and *P. (M.) teleius euphemia.* Each subspecies may represent a separate CU. Further, in Japan at least three CUs may be relevant. Two of them according to haplogroups "*P. (M.) teleius* II" (island Honshū; ssp. *kazamoto* and *daisensis*), and "*P. (M.) teleius* III" (island Kyushu; ssp. *daisensis*). They are geographically clearly separated from the main distributional range (Fig. 2A, Fig. 1A), which is another relevant criteria suggested for defining a CU. The third CU in Japan I consider corresponds not completely with haplogroup "*P. (M.) teleius* IV"; solely populations from Japanese islands Hokkaido and northern Honshū (ssp. *kazamoto, ogumae* and *hosonoi*) I assign to this CU. Chinese populations (ssp. *sinalcon*) which also belong to haplogroup "*P. (M.) teleius* IV" I consider as separate CU, as they are geographically isolated from the contiguous Eurasian distributional range and far distantly located from Hokkaido and Honshū (Funk et al. 2012, Bowen 1998; Fig. 1A).

7 Discussion

7.1 Phylogenetic inference and *Wolbachia* infection

The present phylogenetic analysis based on mtDNA sequences revealed that *P. (M.) teleius* and *P. (M.) nausithous* were clearly separated and formed well supported monophyletic clades. However, within both species highly distinct evolutionary lineages were found. These clades were not concordant with known subspecies nor did they represent spatially contiguous groups. Such an intraspecific phylogenetic pattern could be the result of either recent, secondary contact of formerly geographically separated populations of the species or it could be evidence for intrinsic reproductive barriers among sympatric cryptic species (Avise *et al.* 1987). Indeed, the observed average sequence divergence between haplotypes of these highly divergent clades and the rest of the species (2.28–2.56%) for *P. (M.) teleius* and *P. (M.) nausithous* resembled the divergence that has been reported between species (e.g., McBride *et al.* 2009). Similar levels of sequence divergence have already been found in *P. (M.) teleius* and *P. (M.) nausithous* and have led to the hypothesis of cryptic species existence (Als *et al.* 2004, Ugelvig *et al.* 2011). However, the divergent haplogroups in both species were strongly associated with *Wolbachia* infections ("*Wolbachia*" – clades) in contrast to the remaining haplotypes. A similar pattern has been already described within other butterfly species (e.g., Kodandaramaiah *et al.* 2013, Charlat *et al.* 2009, Lohman *et al.* 2008). In fact, the COI sequences which led to the hypotheses of cryptic species within *P. (M.) teleius* (Ugelvig *et al.* 2011: specimen Uk-08-J627) and within *P. (M.) nausithous* (Als *et al.* 2004: specimen ZD-99-S301) corresponded perfectly to haplotypes that were associated with *Wolbachia* in my new samples originating from the same regions. This suggests that these specimens were also likely to be infected with *Wolbachia*. This interpretation is corroborated by the fact that divergent haplotypes of infected and uninfected individuals co-occurred at several localities and that in both species the mtDNA *Wolbachia* clades were not reflected in the nuclear genome. Cryptic species should result in similar patterns across different genomes (e.g., Dasmahapatra *et al.* 2010). Thus, these inconsistencies between mitochondrial and nuclear data sets are evidence against cryptic species. Similar *Wolbachia*-influenced mito-nuclear discordant patterns has been described in other butterfly species leading to similar conclusions (e.g., Kodandaramaiah *et al.* 2013, Kvie *et al.* 2013). Further, I do not expect, that mtDNA haplogroups *P. (M.) teleius* II, III, and IV and *P. (M.) nausithous* II (Fig. 2) reflect cryptic species, as microsatellite pattern is not congruent with mtDNA pattern (Fig. 4, Fig. 1).

The results suggest that the *Wolbachia* infection took place between 0.7–2.0 mya and 0.6–1.7 mya in *P. (M.) teleius* and *P. (M.) nausithous*, respectively, and well after species diversification, which is estimated between 1.2 and 3.2 mya, a time span consistent with previous estimates using external calibration points for chronology estimation (Als *et al.* 2004). However, the infection persists only in a minority of individuals from few populations. Hence, mitochondrial sequences of infected and uninfected parts of the populations accumulated substantial divergence, resulting in well separated clades in the inferred phylogeny. A similar phylogenetic

pattern has been shown for other butterfly species (Kodandaramaiah *et al.* 2013, Kvie *et al.* 2013, Charlat *et al.* 2009, Nice *et al.* 2009, Narita *et al.* 2006) and can be explained by the "two barcodes - one species" effect a *Wolbachia* infection can have on mtDNA patterns (e.g., Kodandaramaiah *et al.* 2013).

In 44% of the specimens of the *P. (M.) nausithous* "*Wolbachia*" clade no infection was detected. These individuals might indeed lack an infection, which can happen when *Wolbachia* is not efficiently transmitted to the next generation (Hurst *et al.* 2001, Werren *et al.* 1997). Thus, a negative *Wolbachia* test in particular samples does not disprove *Wolbachia* infection as causal for mtDNA lineage divergence. Further, extreme low titer infections are sometimes hard to detect with a simple *wsp*-screen I performed here (Schneider *et al.* 2013). Moreover, 45% of DNA-samples included in my analysis had been extracted from butterfly legs instead from abdominal tissue or larvae. As *Wolbachia* is living mainly in reproductive germ tissues (e.g., Werren *et al.* 1997), it is generally recommended to preferably use fresh abdominal tissue to implement *Wolbachia* screens (e.g., Smith *et al.* 2012). In contrast, it has been shown that *Wolbachia* can be detected in both, reproductive *and* in somatic tissues in similar concentrations (Dobson *et al.* 1999). Recently, a 100% infection frequency had been detected in DNA extracts from butterfly legs in another *Phengaris (Maculinea)* species (Patricelli *et al.* 2013). However, I cannot exclude that the PCR-screening for *Wolbachia* might have produced false negatives, e.g. due to mutations in the primer binding sites.

Within 19 populations examined, *P. (M.) teleius* and *P. (M.) nausithous* co-occurred in the same locality. Three of these populations harbour a *Wolbachia* infection, either hosted by *P. (M.) teleius* (populations 28 and 77), or hosted by *P. (M.) nausithous* (population 25), but never hosted by both species at any locality. My observation suggests a lack of horizontal transmission between the two sister species, although potential routes for horizontal transmissions between both species exist. E.g., a *Wolbachia* infection may be transferred between caterpillars of both species by parasitoid individuals, e.g. of *Neotypus* ssp. or *Ichneumon* ssp. (Shaw *et al.* 2009), which may act as vector for *Wolbachia* infections (Jiggins *et al.* 2002, Vavre *et al.* 1999). Further, "accidental" cannibalism of *Wolbachia* infected eggs or caterpillars in times of high larval densities within flowerheads of the larval foodplant *S. officinalis* shared by both, *P. (M.) teleius* and *P. (M.) nausithous,* in populations in which they do co-occur, may happen. Such a scenario could be another possibility for horizontal *Wolbachia* transmission, as hypothesized earlier for another butterfly sister species pair *Acraea encedon/encedana* (Jiggins *et al.* 2000a).

It has been shown that different *Wolbachia* strains can have different effects on the fitness of their hosts, ranging from positive to detrimental (Sarakatsanou *et al.* 2011, Bordenstein and Werren 2000). In both species *Wolbachia* infected individuals were found across large parts of the distribution ranges from Belarus to Mongolia and from Slovakia to the South Ural Mountains, for *P. (M.) teleius* and *P. (M.) nausithous*, respectively. Because the *Wolbachia* infections were present within populations at low frequency in wide distributional areas, infected individuals might experience a positive fitness effect due to the presence of *Wolbachia* (Jansen *et al.* 2008). Transmission rates into the next host generation seem to be imperfect, since the infection did not sweep through whole populations (Jansen *et al.* 2008). Further, this effect could depend on certain environmental conditions, e.g. in *Leptopilina* wasps *Wolbachia* density was highest at

high temperatures (Mouton *et al.* 2006). Indeed, in *P. (M.) teleius* only populations inhabiting steppe habitats with relatively hot and dry conditions in summer harboured *Wolbachia* infected individuals. This observation could be an indication for a temperature-dependent survival- and vertical transmission success of *Wolbachia* within its *P. (M.) teleius* host with best transmission rates at high temperatures (Werren *et al.* 1997). Furthermore, in *P. (M.) teleius* two adults were *Wolbachia* infected, both of them were males which might be an indication for a CI strain in *P. (M.) teleius*. In *P. (M.) nausithous*, however, all four *Wolbachia* infected adults were females which might be an indication for a male-killing or feminization strain in that species (Werren *et al.* 2008).

Wolbachia supergroups A and B are currently the only ones known to occur in butterflies (Salunke *et al.* 2012). Both *Wolbachia* strains detected in my samples belong to supergroup B which is prevalent in butterflies. In a recent study (Bereczki *et al.* 2015) several strains of supergroup A and B have been detected in Central European populations of both, *P. (M.) teleius* and *P. (M.) nausithous*, with infection frequencies of 14.4% and 36.2%, respectively, without any geographical pattern. Since Bereczki and colleagues (2015) did not amplified the same DNA regions as I did (wsp), it is not clear, if B strains detected in samples from the Carpathians / Carpathian Basin are the same ones, as detected in my samples. At least, a high similarity of several COI sequences of *Wolbachia* infected *P. (M.) nausithous* samples from the Carpathians / Carpathian Basin with COI sequences from the high divergent *P. nausithous* "*Wolbachia*" clade suggests their membership to the same B strain I detected, but this needs further sequence analysis at minimum in both marker genes, wsp and 16S rRNA, or more comprehensive, a *Wolbachia* multilocus sequence typing (MLST) approach (Baldo *et al.* 2006).

The prevalence and infestation patterns highly differ among *Phengaris* species, as *P. (M.) arion* and *P. (M.) alcon* are *Wolbachia*-infected in 100%, each with a single strain from supergroup A and B, respectively (Patricelli *et al.* 2013, Sielezniew *et al.* 2012). In *P. (M.) arion* another A strain was found in the Carpathian Basin with identical 100% *Wolbachia* prevalence (Bereczki *et al.* 2015). These high prevalences in *P. (M.) arion* and *P. (M.) alcon* suggest perfect vertical *Wolbachia* transmissions from mother to offspring within both species. Further, the complete lack of mtDNA diversity in *P. (M.) alcon* indicate a recent selective sweep of *Wolbachia* through the species, accompanied by the spread of a single mitotype (Sielezniew *et al.* 2012). This spread may have deleted a potentially former existing mtDNA diversity and its informational content for phylogenetic / phylogeographic analysis (Smith *et al.* 2012). In fact, at nuclear genome level genetic diversity reflect ecological host races (Sielezniew *et al.* 2012). In contrast, the high genetic differentiation of Italian and Polish *P. (M.) arion* mtDNA did not suggest a selective sweep of *Wolbachia*, instead indicates that the infection took place before the species expanded from its glacial refugia and differentiated ecologically and morphologically (Patricelli *et al.* 2013).

7.2 Phylogeography of *P. (M.) teleius* and *P. (M.) nausithous*

In both species the samples that were not affected by *Wolbachia* showed considerable divergence in both, the mitochondrial and the nuclear genome. However, the two species showed

contrasting geographical patterns of differentiation and likely evolutionary scenarios. In *P. (M.) teleius* there was little mtDNA variation across the western part of its range (*P. (M.) teleius* I). However, in East Asia three divergent haplogroups were found (*P. (M.) teleius* II, III, and IV). Although not fully concordant with the mtDNA pattern, the nuclear microsatellite data also revealed a stronger sub-structuring in Eastern Asia (East Asia I + II). This pattern might be well explained by the following scenario: After speciation of *P. (M.) teleius* between 1.2 and 3.2 mya, which likely took place in Central or Eastern Asia (Sibatani *et al.* 1994, Fiedler 1998), lineages may have spread and diversified throughout Eurasia. However, climatic conditions in one of the last glacial phases could have eliminated the species from Europe and from most parts of continental Asia (Schmitt 2007, Hewitt 2000). In the Far East of continental Asia and in Japan the species may have found larger or more suitable refugial areas (Tsukada 1982, De Lattin 1967), concordant with the high genetic diversity there and represented by East Palaearctic mtDNA haplogroups presented here (Fig. 2A). This phylogeographic hypothesis is also corroborated by the fact that all described subspecies in *P. (M.) teleius* are restricted to the Eastern Palaearctic and mainly to Japan (Sibatani *et al.* 1994). Re-colonization of continental Asia and Europe may have started by founder individuals surviving in East Asia or Japan. The presence of isolated refugia in this area is likely given the complex topography and may be mirrored e.g. in the haplotypes T40, T41 and T42, or T02, forming distinct groups within *P. (M.) teleius* I (Fig. 2A) and geographically confined to the Hustai Mountains in Central Mongolia (pop. no. 6, 8, 9), or Hokkaido (pop. no. 15). Low Tajima's D and Fu's F values coupled with the low nucleotide diversity (p) values of East Asia corroborate such a recent range expansion. Similar east-to-west colonization routes of butterflies have been suggested for an Eastern clade of *Melitaea cinxia* coming from Far Eastern populations and migrating into Scandinavia (Wahlberg and Saccheri 2007), as well as for *Coenonympha hero* which seems to have had a glacial centre of survival in the Southern Ural Mountains and expanded from there westwards to Europe (Cassel and Tammaru 2003). However, although Eastern Asia clearly emerges as a centre of diversification within *P. (M.) teleius* in present analyses, the mtDNA phylogenetic clades and the microsatellite STRUCTURE groupings were not congruent with the morphologically defined subspecies. Similar patterns were found, e.g. in *Aglais urticae*, a nymphalid butterfly, and could be due to ecological differentiation occurring more rapidly than evolution at the mtDNA level (Vandewoestijne *et al.* 2004). *P. (M.) teleius* populations of different haplogroups had been isolated in their East-Asiatic glacial refugia at maximum between 1.07 - 1.66 million years (Tab. 1), as estimated sequence divergences reflect the maximum time populations spent isolated in a refugium (Hewitt 1999). In other animal species comparable maximum times of isolation had been estimated (Hewitt 1999).

In contrast to *P. (M.) teleius*, in *P. (M.) nausithous* the mitochondrial, as well as the nuclear data set showed a stronger structuring of genetic diversity in the western part of its distribution. However, the divergence of the geographically separated haplogroups *P. (M.) nausithous* I and II was not concordant with the microsatellite clusters Europe I, II and III and their geographic patterns. For *P. (M.) nausithous* a likely scenario is that after speciation in xero-montane Siberia (Sibatani *et al.* 1994, Fiedler 1998) it subsequently spread towards Western Asia and Europe where it diversified. Haplogroup *P. (M.) nausithous* II located in Central and Western Europe has slightly increased nucleotide diversity and non-significant Tajima's D and Fu's F values

which suggest that the species survived during Pleistocene Ice Ages within European glacial refugia. For *P. (M.) nausithous* haplogroup I recent range expansion into the Eastern parts of its distribution range is likely to have started from a limited set of individuals indicated by significantly low Tajima's D and Fu's F values of Eurasian samples. The microsatellite clusters are also in line with the survival of *P. (M.) nausithous* in several European refugia. Three major European glacial refugia for animal species have been identified on the Iberian, Italian and Balkan peninsulas (Schmitt 2007, Hewitt 2000, De Lattin 1967). Further, in micro-climatically buffered zones in small areas of Central and Eastern Europe, or even north of the Alps, several extra-Mediterranean cryptic refugia for a glacial survival of animal and plant species are evident (Schmitt and Varga 2012, Stewart and Lister 2001). Cluster Asia and cluster Europe III represent genetic groups that may survived in Europe in Mediterranean refugia on the Balkan peninsula, or in extra-Mediterranean refugia in the Carpathians / Carpathian Basin, the Balkans, or near the Bulgarian mountain system, as these regions have been detected as important European refugial areas for animal species survival (Schmitt and Varga 2012, Hewitt 2000). Recolonization of Northern Europe and Asia may have started from one of these glacial refugia. Vice versa, these genetic groups may also have survived in a Central Asiatic or Caspian / Caucasian refugia and Europe was postglacially re-populated by these genetic lineages starting from there (Schmitt and Varga 2012, Hewitt 2000, De Lattin 1967). Cluster Europe II may represent a refugium located on the Iberian Peninsula with postglacial re-colonizations from there into Central Europe (Hewitt 2000, De Lattin 1967). Cluster Europe I may represent a cryptic extra-Mediterranean refugia east, or west of the Alps (Schmitt and Varga 2012). However, admixed populations in contact zones of clusters Europe I and Europe III, admixed populations in Spain and the distribution of cluster Europe III both, east and west of the Alps, indicate a complex phylogeographic history in Western and Central Europe. The East European (Europe III) and Asian cluster are overlapping, as evidenced by several hybrid populations located in East Poland, Belarus, and South Russia (Hewitt 2000). In *P. (M.) nausithous* populations of different haplogroups had been isolated in Ice Age refugia 1.09 million years at maximum (Tab. 1) which is comparable to other animal species survived in European glacial refugia (Hewitt 1999).

7.3 Conservation units (CUs) in *Phengaris (Maculinea)*

An evaluation of evolutionarily significant units (ESUs) based on evidence from molecular data solely is highly critisized in *Phengaris* and other butterfly species (Casacci *et al.* 2014, Sielezniew *et al.* 2012, Funk *et al.* 2012). Highly supporting this view I defined several CUs in *P. (M.) nausithous* in Europe based on a combination of my genetic data with available information about host ant specificity. The main host ant species of *P. (M.) nausithous* over wide European ranges is *Myrmica rubra.* Results show that *M. rubra*-dependent *P. (M.) nausithous* populations were not consistently represented by a single genetic entity, but are represented by all four microsatellite clusters. The host *M. rubra* is a genetically highly differentiated species (Seppä and Walin 1995) and also their cuticular chemical profiles may be variable between far distant regions. This may have lead to diverse chemical signatures of their social parasites, like *P. (M.) nausithous*. Thus, recognition chemicals of caterpillars and ant larvae may not sufficiently match between far distant populations, as observed in different host ant races of *P. (M.)*

alcon, ecotype *"rebeli"* (e.g., Thomas *et al.* 2013, Elmes *et al.* 2002). Consequently, a survival for *P. (M.) nausithous* in a far distant *Myrmica rubra* population may not possible, at least with environmental / nutritional bad conditions (Elmes *et al.* 2004). A *robust* demarcation of *full* ESUs (De Guia and Saitoh 2007) within Western and Central European *M. rubra* hosted populations deserves further information, e.g. detailled physiological (chemo-taxonomic CHC profiling; Guillem *et al.* 2012) or, if chemo-taxonomy is not applicable, behavioural investigations (e.g., reciprocal cross-infectivity experiments) to reliably evaluate the ecological exchangeablity between far distant *P. (M.) nausithous* populations. Ecological exchangeablity is a main parameter in defining ESUs according to Crandall *et al.* 2000, and is essentially important to know prior to re-introduction efforts of this endangered species. Furthermore, *P. (M.) nausithous* microsatellite cluster "Asia" covers a huge geographical area (Fig. 1B) and of course, more than one CU should be regarded based on ecological informations about host ant specificity, which are still completely lacking from the Eastern distributional range. Also in isolated parts of the species range, like in Bulgaria, or on the Iberian peninsular extensive host ant investigations would put a clear delination of units for conservation forward. Moreover, *P. (M.) nausithous* populations, where multiple host ant species supports the parasite as found in Poland (Witek *et al.* 2008) should also be regarded as separate CUs due to the high potential to evolutionary significant host switches (Jansen *et al.* 2011, Crandall *et al.* 2000, Bowen 1998). Such populations should gain a high protection status and especially adapted management strategies should be applied to keep *Phengaris (Maculinea)* stable.

The definition of CUs in *P. (M.) teleius* is more complicated at current stages of knowledge as there are no ecological criteria available which are discriminitave enough for this purpose. Morphological subspecific differentiations are suboptimal neither (Ryder 1986), also due to lacking correspondence to mtDNA haplogroups or microsatellite clusters (Fig. 1A). Also in conspecific *P. (M.) arion* genetic data did not support subspecific devisions (Patricelli *et al.* 2013). Further, a major revision on subspecific differentiation of *P. (M.) teleius* has been suggested (Sibatani *et al.* 1994).

Beyond that, although I could not include any samples from the Caucasus *P. (M.) teleius* and *P. (M.) nausithous* populations in this Mountain range should be regarded as separate CU(s) also, as they are strongly isolated from the main distribution ranges of the species (Funk *et al.* 2012, Bowen 1998). Further, the Caucasus may have served as important glacial refugia for both species (Hewitt 1999, De Lattin 1967) which is another sufficient criteria to treat populations there as separate CUs (Funk *et al.* 2012). Of course, as long as no ecological, and genetic informations from a representative set of Caucasian populations are available a clear CU/ESU designation is impossible. Generally, regardless of morphological, ecological, or genetic characteristics, all *Phengaris*-populations isolated from the contiguous distributional Eurasian ranges should be treated as separate CUs, as geographically isolated populations bear a high evolutionary potential and thus, may diverge separately (Funk et al. 2012, Crandall et al. 2000, Bowen 1998).

In summary, due to limited information on host ant specificity and due to limited sample numbers in several parts of the distributional ranges differentiation of CUs presented herein remain preliminary! Main focus of my dissertation was covering the total Eurasian distribution range

which consequently resulted in a limited density of sampling points at a regional, or national scale. Thus, exact boundaries of defined CUs could not be determined. For delineation of boundaries and, furthermore, delineation of MUs (management units; Moritz 1994) phylogenetic and population genetic analysis on smaller scales are needed (e.g., Patricelli *et al.* 2013, Sielezniew *et al.* 2012). Nevertheless, with preliminary designations of CUs presented herein several regions of special conservation concern for *P. (M.) nausithous* and P. *(M.) teleius* have been depicted and represent a first step in designation of conservation units in both species. Further investigations are needed urgently to include information not only about ecological (host ant specificity), but also about physiological differentiations. An exact delineation and protection of several CUs in *P. (M.) teleius* within the East Palaearctic is essential, as this region is clearly emerging as divergence centre of the species. Furthermore, several Ice Age refugial areas are located in the East Palaearctic (Tsukada 1982, De Lattin 1967), which also served as centre of survival for *P. (M.) teleius* during last glacial maxima (see also chapter 7.2.).

8 Synthesis

Based on mtDNA barcoding, nuclear microsatellite analyses, and *Wolbachia* screening the hypothesis of cryptic species within *Phengaris (Maculinea) teleius* and *P. (M.) nausithous* is rejected. The major splits in the mtDNA phylogeny in both species can be explained by *Wolbachia* infections. Furthermore, geographic isolation during Pleistocene glaciations contributed to differentiation at mitochondrial and nuclear genomes. Thus, *Wolbachia* infections and complex phylogeographic histories shaped the observed genetic structure of both species, instead of cryptic species presence. Present investigation also has shown that DNA barcoding studies can deliver robust information on cryptic species in combination with tests for *Wolbachia* infections only, as well as with additional analysis on nuclear markers, especially in groups with high prevalences of *Wolbachia* infection (Gerth *et al.* 2011). Moreover, present study provides further insights into postglacial movement patterns of Palaearctic insect species: *P. (M.) teleius* showed increased genetic structuring in Eastern Asia whereas *P. (M.) nausithous* was more structured in Western Eurasia, likely indicating opposing refugial areas during past glacial maxima.

8.1 Options for further studies emerging from present results

8.1.1 *Wolbachia* in *Phengaris (Maculinea)*

Beside findings in *P. (M.) teleius* and *P. (M.) nausithous* presented herein, numerous further European populations of all *Phengaris (Maculinea)* species have been identified recently to be infected with *Wolbachia* (Bereczki *et al.* 2015, Patricelli *et al.* 2013, Sielezniew *et al.* 2012). Further, the central European geographic entity Carpathians / Carpathian Basin seem to be a "*Phengaris / Wolbachia*-hot spot" with multiple *Wolbachia* strains infecting all *Phengaris (Maculinea)* species present there (which are all *Phengaris (Maculinea)* species present in Europe) with different infection frequencies (Bereczki *et al.* 2015). As *Wolbachia* can have strong impacts on host ecology and evolution (Zug and Hammerstein 2014), intense basic studies are needed to characterize the different effects *Wolbachia* may have in this unique arthropod model system. Firstly, *Wolbachia* strains infecting *Phengaris (Maculinea)* species need to be designated by MLST genotyping (Salunke *et al.* 2012, Baldo *et al.* 2006). Further, VNTR molecular screening to differentiate *Wolbachia*-infections that share 100% identity at the *wsp*-locus, as well as *wsp* PCR-blotting to detect extreme low-titer infections should be carried out (Schneider *et al.* 2013). Closely related *Wolbachia* strains can have different phenotypic effects on closely related host species, as observed in *Acraea* butterflies (Jiggins *et al.* 2002). Thus, comprehensive analyses of adult stage sex ratios, egg hatch-rates, larval development rates, or infection frequences in combination with behavioral studies on e.g., mating behaviour should be carried out in different populations to characterize *Wolbachias´* phenotypic and fitness effects on selected *Phengaris* ssp. / *Wolbachia*-strain combinations (Jiggins *et al.* 2000a, Hurst *et al.* 1999). Further, demographic and metapopulation models have to be developed to assess effects of

Wolbachia on *Phengaris* (meta-) populations (Nice *et al.* 2009). Aside these approaches, laboratory rearing experiments to assess survival rates and development times during *Phengaris (Maculinea)* larval and pupal stages in comparison to individuals from uninfected and/or antibiotica cured populations will provide solid information on *Wolbachias´* fitness effects (Narita *et al.* 2009). Generally, overarching investigations of all aspects of the *Wolbachia*-infection (and, if appropriate, other reproductive parasitic endosymbionts) at large Eurasian scales and/or within a potential hot-spot of *Wolbachia-Phengaris* would be worthwile for the understanding of the whole model system.

8.1.2 Population genetic studies

P. (M.) nausithous shows complex phylogeographic patterns especially in contact zones and peripheral areas which deserve further analysis based on denser sampling. The four geographically separated microsatellite groups detected here were observed in a small area in Europe. Thus, more detailed studies are necessary on the extent and delineation of these European clusters and the affiliation of peripheral populations in the Alps and isolated populations, e.g. in Spain, Bulgaria, and Romania. Further, Caucasian populations need to be included into analysis, which unfortunately was not possible in the present study. In *P. (M.) teleius* more detailed analysis should be performed in East Asia with denser sampling in Japan, China, Russia (Transbaikalia), and Mongolia. Also *P. (M.) teleius* populations from the Caucasian mountains should be included in future studies.

Fine-scaled population genetic analyses are not only necessary for a better understanding of the phylogeographic history of the species, but also for a clear delineation of CU/ESU-boundaries, as conservation of the species´ evolutionary potential is maximized when protecting several ESUs, especially in the face of environmental changes (Funk *et al.* 2012). My sampling scheme was adapted to answering the main question about cryptic species existence. Thus, ESU-designations presented herein have a strong preliminary character. Comprehensive population genetic analysis on broad regional scales are needed, as already performed in other *Phengaris (Maculinea)* species in certain parts of Europe (Patricelli *et al.* 2013, Sielezniew *et al.* 2012, Bereczki *et al.* 2011). Furthermore, ecological information on host ant specificity in Eastern European and Asian populations is essential for a comprehensive designation of *full* ESUs in *Phengaris (Maculinea)* butterflies (Casacci *et al.* 2014, Funk *et al.* 2012, De Guia and Saitoh 2007)

8.1.3 Beyond that: Integration of laboratory cross-infection experiments and chemo-taxonomic analysis in *Phengaris (Maculinea)* ESU-designation

Phengaris (Maculinea)-populations regionally adapted to different *Myrmica* hosts are not able to survive with non-host *Myrmica* species in periods of stress and deprivation (Thomas *et al.* 2013, Elmes *et al.* 2004). Thus, they are ecologically not exchangeable. According to Crandall *et al.* (2000) ecological non-exchangeability is an important criterion for the definition of Evolutionarily Significant Units (ESUs). Host specificity has been evaluated as most relevant criteria

for the definition of ESUs in *Phengaris (Maculinea)* regardless of their genetic differentiation (Casacci *et al.* 2014, Sielezniew *et al.* 2012). Especially in *P. (M.) teleius* it is often not appropriate to define a primary host, as it is harboured by several *Myrmica* hosts in similar frequencies (e.g., Witek *et al.* 2008; see also chapter 4.2.2.). Thus, ecological information currently available in *P. (M.) teleius* is not discriminative enough for the definition of ESUs. Also in *P. (M.) nausithous* it remains an open question if far distantly located populations from different genetic entities, but all hosted by *Myrmica rubra*, a genetically highly diverse species, are ecologically exchangeable and thus, may belong to a single CU despite belonging to several phylogeographic microsatellite clusters. As rearing *Phengaris (Maculinea)* caterpillars within artificial ant nests under laboratory conditions is very difficult and labor-intensive (Wardlaw *et al.* 1998) there is no robust experimental information on ecological exchangeablity between *P. (M.) teleius* as well as *P. (M.) nausithous* populations from different COI haplogroups or microsatellite clusters available. Further, it is even more difficult to keep ant colonies and adopted/integrated caterpillars alive for a sufficient period of time (over >10 months!) and in a statistically representative manner (own observations). This is essential for a reliable comparison of integration rates, developmental rates, and survival rates of adopted caterpillars and, based on that, for an evaluation of local adaptation and ecological exchangeability between far distantly located *P. (M.) teleius* as well as *P. (M.) nausithous* populations.

Instead, the collection and comparison of chemo-taxonomic information of *Phengaris (Maculinea)* caterpillars may be a more promising technique, as chemo-taxonomy has been identified as a faster, easier, cheaper, and more accurate method than existing genetic or morphological methods, e.g., to differentiate between *Myrmica sabuleti* (host of *P. (M.) arion*), and *M. scabrinodis* (non-host) (Guillem *et al.* 2012), to differentiate between *M. schenckii* and *M. sabuleti* adapted *P. (M.) alcon* (ecotype *"rebeli"*) populations (Schlick-Steiner *et al.* 2004), or to differentiate between common European *Myrmica*-species and explain host specificity of *P. (M.) alcon* (ecotype *"rebeli"*) (Elmes *et al.* 2002). Thus, the method bears promising potential to help understand host specificity of *Phengaris* butterflies throughout their geographical range. Further, the method may be straightforward to re-evaluate preliminary ESU-classifications presented herein. The applicability of chemo-taxonomic CHC profiling in *Myrmica* hosts and their *Phengaris (Maculinea)* parasites over large Eurasian species ranges need to be intensively explored in future for taxonomic and species conservation purposes!

8.2 Implications for species conservation

All European *Phengaris (Maculinea)* species are regarded as indicator species of high biodiversity in their declining habitats (Thomas *et al.* 2005, Maes and van Dyck 2005) and have became flagship species for conservation in Europe (Settele *et al.* 2011, Thomas *et al.* 2009). Numerous studies exist dealing with management measures and recommendations for species and habitat conservation strategies (e.g., Bubová *et al.* 2015, Romo *et al.* 2015, Thomas *et al.* 2013, Filz *et al.* 2013, van Swaay *et al.* 2012, Guillem *et al.* 2012, Casacci *et al.* 2011, Wynhoff *et al.* 2011, Musche *et al.* 2008, Johst *et al.* 2006, Jansen *et al.* 2006, Settele *et al.* 2005, Maes *et al.* 2004, Griebeler and Seitz 2002, Meyer-Hozak 2000, Munguira and Martin 1999, Wynhoff 1998b, Elmes and Thomas 1992, Settele and Geissler 1988), and by now the taxon *Maculinea* is one of

the best examples for insect conservation biology (Thomas *et al.* 2009). Further, habitat management strategies to create long-term optimum habitats for the butterfly and their specific host ant in the face of climate change are outlined roughly (Settele and Kühn 2009). Based on these recommendations, several management actions at local scales are performed in European Union member states not to loose more and more *Phengaris (Maculinea)* populations, but to keep them stable. Moreover, successful re-introduction programs has been carried out in Western Europe (Andersen *et al.* 2014, Thomas *et al.* 2009, Wynhoff 1998b) and (re-) introduction of *Phengaris (Maculinea)* populations in suitable habitats of yet uncolonized areas is recommended (Romo *et al.* 2015).

8.2.1 Implications for species conservation: *Wolbachia*

The present study delivers some important implications for species conservation. The presence of *Wolbachia* may constitute a risk for the stability of *Phengaris (Maculinea)* populations, as an introduction of *Wolbachia* infected individuals into small populations to restock them might lead to a selective sweep and to full fixation of the *Wolbachia* infection and the associated genotype within the population (Jansen *et al.* 2008). This may lead to the elimination of the locally adapted genetic composition and diversity due to selective sweeps, as already observed in *Phengaris (Maculinea)* and other butterfly species (Sielezniew *et al.* 2012, Narita *et al.* 2006). In the worst case it may lead to *Wolbachia*-caused population extinctions, as described for a male-killing strain in *Acraea*-butterflies (Hassan and Idris 2013). Consequently, in order not to introduce possibly detrimental elements into small and already threatened populations it is absolutely essential to include comprehensive *wsp*-screenings and *wsp* PCR-blottings (Schneider *et al.* 2013) for the detection of *Wolbachia* presence in the source population *by standard* prior to future re-introduction or population augmentation programs for endangered insect species (Nice *et al.* 2009) like *Phengaris (Maculinea)*!

8.2.2 Phylogeographic clusters and CUs

Phylogeographic clusters identified here have been combined with available information on ecological and subspecific differentiation to preliminary delineate several ESUs in order to depict regions of special conservation concern for each species. East Asia (China, Japan, Mongolia) is highly important to conserve the maximum evolutionary potential in *P. (M.) teleius*. Numerous special protected areas should be established there to conserve the species in its diversification and glacial survival centres. If necessary, region specific management strategies should be developed and applied to keep *P. (M.) teleius* populations in a good status. It is not clear yet, if Asian *P. (M.) teleius* experience losses in abundance and population number as observed in Europe since decades. Thus, long-term monitoring schemes according to Van Swaay *et al.* (2015b) in order to investigate population trends need to be installed also in Asia. For the protection of *P. (M.) nausithous´* genetic diversity Europe is the more important region (including umbrella effects for co-occuring *P. (M.) teleius*). Of course, to consider all genetic, ecological, and morphological differentiated entities species need to be protected in its total range. Protection should occur in a network of special protected areas applying management strategies adapted to the particular regional habitat requirements, as outlined in the Natura 2000 network

of the European Union. Much had been understood about the species biology mainly in Europe, much more is unexplored yet, and awaits discovery, especially in Central and Eastern Asia. Of course, as long as detailed ecological information is lacking from large parts of distributional ranges, suggested ESUs presented herein has to be taken with caution. Nevertheless, as long as no other information is available, re-introduction of the species into extinct populations should include individuals from the same ESU, in order not to introduce possibly maladapted individuals.

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ca.	circa
etc.	et cetera
mya/MYA	million years ago
e. g.	for example
BSC	biological species concept
CU	conservation unit
ESU	evolutionarily significant unit
GEU	geminate evolutionary unit
MU	management unit
Fig.	figure
Tab.	table
ssp.	subspecies
pop. no.	population number(s)
CHC	cuticular hydrocarbon

Appendix: Supplemental material

1. Genetic material

Table 4: *Phengaris (Maculinea)* material used for analysis. N: sample size; w+: *Wolbachia* infection status; ?: *Wolbachia* infection unknown; n.i.: Barcode sequences which were not included in haplotype network construction and phylogenetic analyses due to low coverage with our sequences; * : Coordinates of these populations were inferred from locality information using Google Earth and are only approximate. Shading color on the left table part corresponds to subspecific membership of the species (cyan: nominate species, peach: *P. (M.) teleius obscurata*, blue: *P. (M.) t. euphemia*, orange: *P. (M.) t. sinalcon*, pink: *P. (M.) t. ogumae*, yellow: *P. (M.) t. kazamoto*, green: *P. (M.) t. hosonoi*, violet: *P. (M.) t. daisensis*) and on the right table part to COI haplogroup membership resulting from network analyses (see Fig. 2).

Specimen voucher	Species	Collecting locality	Latitude	Longitude	Pop code	Sex	w+	Haplotypes	COI clade	GenBank accession numbers	Source
SR006.01	*P. teleius euphemia*	C Mongolia, Hustai mountains	47°38'N	105°52'E	06		no	**T41**	*P. teleius* I	JX311049	Present thesis
SR006.03	*P. teleius euphemia*	C Mongolia, Hustai mountains	47°38'N	105°52'E	06		no	**T41**	*P. teleius* I	JX311050	Present thesis
SR006.05	*P. teleius euphemia*	C Mongolia, Hustai mountains	47°38'N	105°52'E	06		no	**T42**	*P. teleius* I	JX311051	Present thesis
SR006.06	*P. teleius euphemia*	C Mongolia, Hustai mountains	47°38'N	105°52'E	06	♂	yes	**T41**	*P. teleius* I	JX311052	Present thesis
SR006.07	*P. teleius euphemia*	C Mongolia, Hustai mountains	47°38'N	105°52'E	06		no	**T41**	*P. teleius* I	JX311053	Present thesis
SR006.08	*P. teleius euphemia*	C Mongolia, Hustai mountains	47°38'N	105°52'E	06		no	**T41**	*P. teleius* I	JX311054	Present thesis
SR006.10	*P. teleius euphemia*	C Mongolia, Hustai mountains	47°38'N	105°52'E	06		no	**T07**	*P. teleius* I	JX311055	Present thesis
SR008.01	*P. teleius euphemia*	C Mongolia, Hustai mountains	47°45'N	105°53'E	08		no	**T40**	*P. teleius* I	JX311056	Present thesis
SR009.01	*P. teleius euphemia*	C Mongolia, Hustai mountains	47°44'N	105°52'E	09		no	**T37**	*P. teleius* I	JX311057	Present thesis
SR010.01	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		no	**T07**	*P. teleius* I	JX311058	Present thesis
SR010.14	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		no	**T35**	*P. teleius* I	JX311059	Present thesis
SR010.15	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		no	**T39**	*P. teleius* I	JX311060	Present thesis
SR010.17	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		yes	**T30**	*P. teleius* Wolbachia	JX311061	Present thesis
SR010.20	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		no	**T38**	*P. teleius* I	JX311062	Present thesis
SR010.21	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		no	**T38**	*P. teleius* I	JX311063	Present thesis
SR010.23	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		yes	**T30**	*P. teleius* Wolbachia	JX311064	Present thesis
SR010.32	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		no	**T37**	*P. teleius* I	JX311065	Present thesis
SR010.38	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		no	**T36**	*P. teleius* I	JX311066	Present thesis
SR010.46	*P. teleius euphemia*	N Mongolia, Chentej mountains	49°05'N	107°17'E	10		no	**T35**	*P. teleius* I	JX311067	Present thesis
SR019.21	*P. teleius obscurata* #	Russia, Novosibirsk, near Akademgorodok	54°49'N	83°06'E	19		no	**T34**	*P. teleius* I	JX311069	Present thesis
SR021.44	*P. teleius obscurata* #	Russia, Novosibirsk, near Akademgorodok	54°50'N	83°08'E	21		no	**T07**	*P. teleius* I	JX311075	Present thesis
SR022.04	*P. teleius obscurata* #	Russia, near Barnaul	53°22'N	84°03'E	22		no	**T07**	*P. teleius* I	JX311078	Present thesis
SR023.09	*P. teleius obscurata* #	Russia, Altai mountains, around Cherga	51°33'N	85°33'E	23		no	**T33**	*P. teleius* I	JX311083	Present thesis
SR023.10	*P. teleius obscurata* #	Russia, Altai mountains, around Cherga	51°33'N	85°33'E	23		no	**T07**	*P. teleius* I	JX311084	Present thesis
SR023.33	*P. teleius obscurata* #	Russia, Altai mountains, around Cherga	51°33'N	85°33'E	23		no	**T33**	*P. teleius* I	JX311086	Present thesis

Specimen voucher	Species	Collecting locality	Latitude	Longitude		Pop code	Sex	w+	Haplotypes	COI clade	GenBank accession numbers	Source
SR024.01	*P. teleius obscurata* #	Russia, Altai mountains, Choya-District, near Levinka	51°57'N	86°23'E		24		no	**T48**	*P. teleius* I	JX311087	Present thesis
SR025.04	*P. teleius obscurata* #	Russia, Altai mountains, Turochak District, near Verkh Biisk	52°02'N	87°04'E		25		no	**T32**	*P. teleius* I	JX311093	Present thesis
SR025.12	*P. teleius obscurata* #	Russia, Altai mountains, Turochak District, near Verkh Biisk	52°02'N	87°04'E		25		no	**T32**	*P. teleius* I	JX311096	Present thesis
SR027.01	*P. teleius obscurata* #	Russia, Altai mountains, Ongutai District, near Kurota	50°49'N	85°60'E		27		no	**T07**	*P. teleius* I	JX311100	Present thesis
SR027.03	*P. teleius obscurata* #	Russia, Altai mountains, Ongutai District, near Kurota	50°49'N	85°60'E		27		yes	**T07**	*P. teleius* I	JX311101	Present thesis
SR027.04	*P. teleius obscurata* #	Russia, Altai mountains, Ongutai District, near Kurota	50°49'N	85°60'E		27		yes	**T31**	*P. teleius* Wolbachia	JX311102	Present thesis
SR027.08	*P. teleius obscurata* #	Russia, Altai mountains, Ongutai District, near Kurota	50°49'N	85°60'E		27		yes	**T30**	*P. teleius* Wolbachia	JX311103	Present thesis
SR027.12	*P. teleius obscurata* #	Russia, Altai mountains, Ongutai District, near Kurota	50°49'N	85°60'E		27		no	**T07**	*P. teleius* I	JX311104	Present thesis
SR028.01	*P. teleius obscurata* #	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		yes	**T30**	*P. teleius* Wolbachia	JX311105	Present thesis
SR028.02	*P. teleius obscurata* #	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		yes	**T30**	*P. teleius* Wolbachia	JX311106	Present thesis
SR028.07	*P. teleius obscurata* #	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		no	**T29**	*P. teleius* I	JX311107	Present thesis
SR028.15	*P. teleius obscurata* #	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		yes	**T30**	*P. teleius* Wolbachia	JX311108	Present thesis
SR028.16	*P. teleius obscurata* #	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		yes	**T30**	*P. teleius* Wolbachia	JX311109	Present thesis
SR028.18	*P. teleius obscurata* #	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		yes	**T30**	*P. teleius* Wolbachia	JX311110	Present thesis
SR028.21	*P. teleius obscurata* #	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		yes	**T30**	*P. teleius* Wolbachia	JX311111	Present thesis
SR028.26	*P. teleius obscurata* #	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		yes	**T30**	*P. teleius* Wolbachia	JX311113	Present thesis
SR028.28	*P. teleius obscurata* #	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		yes	**T30**	*P. teleius* Wolbachia	JX311114	Present thesis
SR029.03	*P. teleius obscurata* #	Russia, Altai mountains, Kyrlyk river, near Kyrlyk	50°42'N	84°58'E		29		yes	**T30**	*P. teleius* Wolbachia	JX311116	Present thesis
SR029.05	*P. teleius obscurata* #	Russia, Altai mountains, Kyrlyk river, near Kyrlyk	50°42'N	84°58'E		29		no	**T29**	*P. teleius* I	JX311117	Present thesis
SR029.07	*P. teleius obscurata* #	Russia, Altai mountains, Kyrlyk river, near Kyrlyk	50°42'N	84°58'E		29		no	**T29**	*P. teleius* I	JX311118	Present thesis
SR029.09	*P. teleius obscurata* #	Russia, Altai mountains, Kyrlyk river, near Kyrlyk	50°42'N	84°58'E		29		no	**T29**	*P. teleius* I	JX311119	Present thesis
SR029.10	*P. teleius obscurata* #	Russia, Altai mountains, Kyrlyk river, near Kyrlyk	50°42'N	84°58'E		29		no	**T07**	*P. teleius* I	JX311120	Present thesis
SR029.12	*P. teleius obscurata* #	Russia, Altai mountains, Kyrlyk river, near Kyrlyk	50°42'N	84°58'E		29		yes	**T30**	*P. teleius* Wolbachia	JX311121	Present thesis
SR029.13	*P. teleius obscurata* #	Russia, Altai mountains, Kyrlyk river, near Kyrlyk	50°42'N	84°58'E		29		no	**T29**	*P. teleius* I	JX311122	Present thesis
SR032.01	*P. teleius teleius*	Germany, Westerwald, around Montabaur	50°25'N	07°48'E		32		no	**T07**	*P. teleius* I	JX311126	Present thesis
SR032.03	*P. teleius teleius*	Germany, Westerwald, around Montabaur	50°25'N	07°48'E		32		no	**T52**	*P. teleius* I	JX311128	Present thesis
SR033.01	*P. teleius teleius*	Germany, Westerwald, around Montabaur	50°29'N	07°55'E		33		no	**T28**	*P. teleius* I	JX311129	Present thesis
SR033.02	*P. teleius teleius*	Germany, Westerwald, around Montabaur	50°29'N	07°55'E		33		no	**T07**	*P. teleius* I	JX311130	Present thesis
SR035.01	*P. teleius teleius*	France, Rhône-Alpes, Divonne-les-Bains, Ain	46°22'N	06°10'E	*	35		no	**T25**	*P. teleius* I	JX311134	Present thesis
SR036.01	*P. teleius teleius*	France, Rhône-Alpes, Ceyzérieu, Ain	45°50'N	05°45'E	*	36		yes	**T26**	*P. teleius* I	JX311135	Present thesis
SR036.02	*P. teleius teleius*	France, Rhône-Alpes, Ceyzérieu, Ain	45°50'N	05°45'E	*	36		no	**T27**	*P. teleius* I	JX311136	Present thesis
SR036.04	*P. teleius teleius*	France, Rhône-Alpes, Ceyzérieu, Ain	45°50'N	05°45'E	*	36		no	**T26**	*P. teleius* I	JX311137	Present thesis
SR038.01	*P. teleius teleius*	France, Lorraine, Vergonge, Jolivet	48°36'N	06°30'E	*	38		no	**T25**	*P. teleius* I	JX311138	Present thesis
SR038.03	*P. teleius teleius*	France, Lorraine, Vergonge, Jolivet	48°36'N	06°30'E	*	38		no	**T25**	*P. teleius* I	JX311139	Present thesis
SR047.19	*P. teleius obscurata* #	Russia, S Ural mountains, Cheljabinsk region, near Snezhinsk	56°05'N	60°44'E	*	47		no	**T24**	*P. teleius* I	JX311157	Present thesis
SR050.01	*P. teleius teleius*	SE Ukraine, Kharkov region, near Mohnach	49°59'N	36°12'E	*	50		no	**T05**	*P. teleius* I	JX311162	Present thesis
SR050.03	*P. teleius teleius*	SE Ukraine, Kharkov region, near Mohnach	49°59'N	36°12'E	*	50		no	**T05**	*P. teleius* I	JX311163	Present thesis
SR050.05	*P. teleius teleius*	SE Ukraine, Kharkov region, near Mohnach	49°59'N	36°12'E	*	50		no	**T07**	*P. teleius* I	JX311164	Present thesis
SR051.01	*P. teleius teleius*	SE Ukraine, Lugansk region, near Ilienko	48°34'N	39°18'E	*	51		no	**T05**	*P. teleius* I	JX311165	Present thesis
SR053.01	*P. teleius obscurata* #	Russia, S Ural mountains, Cheljabinsk region, Itkul lake	56°21'N	62°11'E	*	53		no	**T53**	*P. teleius* I	JX311167	Present thesis
SR053.02	*P. teleius obscurata* #	Russia, S Ural mountains, Cheljabinsk region, Itkul lake	56°21'N	62°11'E	*	53		no	**T07**	*P. teleius* I	JX311168	Present thesis
SR053.03	*P. teleius obscurata* #	Russia, S Ural mountains, Cheljabinsk region, Itkul lake	56°21'N	62°11'E	*	53		no	**T07**	*P. teleius* I	JX311169	Present thesis
SR062.01	*P. teleius teleius*	Germany, Saxony, near Schkeuditz	51°23'N	12°11'E		62		no	**T51**	*P. teleius* I	JX311182	Present thesis
SR062.04	*P. teleius teleius*	Germany, Saxony, near Schkeuditz	51°23'N	12°11'E		62		no	**T51**	*P. teleius* I	JX311183	Present thesis
SR063.01	*P. teleius teleius*	Germany, Bavaria, Lake Constance, near Constance	47°39'N	09°10'E	*	63		no	**T23**	*P. teleius* I	JX311189	Present thesis
SR063.03	*P. teleius teleius*	Germany, Bavaria, Lake Constance, near Constance	47°39'N	09°10'E	*	63		no	**T23**	*P. teleius* I	JX311190	Present thesis
SR065.02	*P. teleius teleius*	C Poland, Wolka	52°16'N	20°42'E		65		no	**T22**	*P. teleius* I	JX311193	Present thesis
SR065.03	*P. teleius teleius*	C Poland, Wolka	52°16'N	20°42'E		65		no	**T22**	*P. teleius* I	JX311194	Present thesis

Specimen voucher	Species	Collecting locality	Latitude	Longitude		Pop code	Sex	w+	Haplotypes	COI clade	GenBank accession numbers	Source
SR065.04	*P. teleius teleius*	C Poland, Wolka	52°16'N	20°42'E		65		no	**T22**	*P. teleius* I	JX311195	Present thesis
SR065.05	*P. teleius teleius*	C Poland, Wolka	52°16'N	20°42'E		65		no	**T22**	*P. teleius* I	JX311196	Present thesis
SR065.06	*P. teleius teleius*	C Poland, Wolka	52°16'N	20°42'E		65		no	**T21**	*P. teleius* I	JX311197	Present thesis
SR066.02	*P. teleius teleius*	E Poland, Kosyn	51°23'N	23°34'E		66		no	**T65**	*P. teleius* I	JX311198	Present thesis
SR066.03	*P. teleius teleius*	E Poland, Kosyn	51°23'N	23°34'E		66		no	**T07**	*P. teleius* I	JX311199	Present thesis
SR066.04	*P. teleius teleius*	E Poland, Kosyn	51°23'N	23°34'E		66		no	**T19**	*P. teleius* I	JX311200	Present thesis
SR066.05	*P. teleius teleius*	E Poland, Kosyn	51°23'N	23°34'E		66		no	**T20**	*P. teleius* I	JX311201	Present thesis
SR066.06	*P. teleius teleius*	E Poland, Kosyn	51°23'N	23°34'E		66		no	**T19**	*P. teleius* I	JX311202	Present thesis
SR067.02	*P. teleius teleius*	S Poland, Wiesiółka	50°25'N	19°21'E		67		no	**T18**	*P. teleius* I	JX311207	Present thesis
SR067.03	*P. teleius teleius*	S Poland, Wiesiółka	50°25'N	19°21'E		67		no	**T17**	*P. teleius* I	JX311208	Present thesis
SR067.04	*P. teleius teleius*	S Poland, Wiesiółka	50°25'N	19°21'E		67		no	**T17**	*P. teleius* I	JX311209	Present thesis
SR067.05	*P. teleius teleius*	S Poland, Wiesiółka	50°25'N	19°21'E		67		no	**T16**	*P. teleius* I	JX311210	Present thesis
SR067.06	*P. teleius teleius*	S Poland, Wiesiółka	50°25'N	19°21'E		67		no	**T16**	*P. teleius* I	JX311211	Present thesis
SR068.02	*P. teleius teleius*	SE Poland, Widacz	49°38'N	21°50'E		68		no	**T07**	*P. teleius* I	JX311213	Present thesis
SR068.03	*P. teleius teleius*	SE Poland, Widacz	49°38'N	21°50'E		68		no	**T15**	*P. teleius* I	JX311214	Present thesis
SR068.04	*P. teleius teleius*	SE Poland, Widacz	49°38'N	21°50'E		68		no	**T07**	*P. teleius* I	JX311215	Present thesis
SR068.05	*P. teleius teleius*	SE Poland, Widacz	49°38'N	21°50'E		68		no	**T15**	*P. teleius* I	JX311216	Present thesis
SR068.06	*P. teleius teleius*	SE Poland, Widacz	49°38'N	21°50'E		68		no	**T14**	*P. teleius* I	JX311217	Present thesis
SR070.02	*P. teleius teleius*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**T13**	*P. teleius* I	JX311223	Present thesis
SR070.08	*P. teleius teleius*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**T07**	*P. teleius* I	JX311225	Present thesis
SR070.09	*P. teleius teleius*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**T12**	*P. teleius* I	JX311226	Present thesis
SR070.13	*P. teleius teleius*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**T46**	*P. teleius* I	JX311227	Present thesis
SR070.16	*P. teleius teleius*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**T11**	*P. teleius* I	JX311229	Present thesis
SR070.19	*P. teleius teleius*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**T46**	*P. teleius* I	JX311230	Present thesis
SR070.28	*P. teleius teleius*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**T10**	*P. teleius* I	JX311231	Present thesis
SR070.31	*P. teleius teleius*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**T61**	*P. teleius* I	JX311232	Present thesis
SR075.04	*P. teleius sinalcon*	China, SW Gansu, Qinling mountains, valley SE of Bola (Hezuo)	33°55'N	101°40'E	*	75		no	**T63**	*P .teleius* IV	JX311248	Present thesis
SR075.08	*P. teleius sinalcon*	China, SW Gansu, Qinling mountains, valley SE of Bola (Hezuo)	33°55'N	101°40'E	*	75		no	**T62**	*P. teleius* IV	JX311249	Present thesis
SR077.02	*P. teleius teleius*	Belarus	51°48'N	30°15'E	*	77		no	**T50**	*P. teleius* Wolbachia	JX311250	Present thesis
SR077.03	*P. teleius teleius*	Belarus	51°48'N	30°15'E	*	77	♂	yes	**T47**	*P. teleius* Wolbachia	JX311251	Present thesis
SR078.02	*P. teleius teleius*	Slovenia, Celje	46°14'N	15°15'E	*	78		no	**T09**	*P. teleius* I	JX311254	Present thesis
SR078.03	*P. teleius teleius*	Slovenia, Celje	46°14'N	15°15'E	*	78		no	**T07**	*P. teleius* I	JX311255	Present thesis
SR078.10	*P. teleius teleius*	Slovenia, Celje	46°14'N	15°15'E	*	78		no	**T45**	*P. teleius* I	JX311260	Present thesis
SR079.03	*P. teleius teleius*	Slovenia, Ilirska Bistrica	45°34'N	14°14'E	*	79		no	**T07**	*P. teleius* I	JX311261	Present thesis
SR079.04	*P. teleius teleius*	Slovenia, Ilirska Bistrica	45°34'N	14°14'E	*	79		no	**T07**	*P. teleius* I	JX311262	Present thesis
SR079.06	*P. teleius teleius*	Slovenia, Ilirska Bistrica	45°34'N	14°14'E	*	79		no	**T07**	*P. teleius* I	JX311263	Present thesis
SR079.07	*P. teleius teleius*	Slovenia, Ilirska Bistrica	45°34'N	14°14'E	*	79		no	**T07**	*P. teleius* I	JX311264	Present thesis
SR080.01	*P. teleius teleius*	Slovenia, Nova Gorika	45°57'N	13°39'E	*	80		no	**T07**	*P. teleius* I	JX311265	Present thesis
SR080.02	*P. teleius teleius*	Slovenia, Nova Gorika	45°57'N	13°39'E	*	80		no	**T07**	*P. teleius* I	JX311266	Present thesis
SR080.03	*P. teleius teleius*	Slovenia, Nova Gorika	45°57'N	13°39'E	*	80		no	**T08**	*P. teleius* I	JX311267	Present thesis
SR080.05	*P. teleius teleius*	Slovenia, Nova Gorika	45°57'N	13°39'E	*	80		no	**T07**	*P. teleius* I	JX311268	Present thesis
SR081.02	*P. teleius teleius*	Ukraine, Transcarpathia, Mukachero District	48°26'N	22°43'E	*	81		no	**T04**	*P. teleius* I	JX311269	Present thesis
SR081.06	*P. teleius teleius*	Ukraine, Transcarpathia, Mukachero District	48°26'N	22°43'E	*	81		no	**T07**	*P. teleius* I	JX311270	Present thesis
SR081.09	*P. teleius teleius*	Ukraine, Transcarpathia, Mukachero District	48°26'N	22°43'E	*	81		no	**T06**	*P. teleius* I	JX311271	Present thesis
SR081.13	*P. teleius teleius*	Ukraine, Transcarpathia, Mukachero District	48°26'N	22°43'E	*	81		no	**T05**	*P. teleius* I	JX311272	Present thesis
SR081.14	*P. teleius teleius*	Ukraine, Transcarpathia, Mukachero District	48°26'N	22°43'E	*	81		no	**T04**	*P. teleius* I	JX311273	Present thesis
SR087.01	*P. teleius euphemia*	Russia, Chabarovsk, near Voronezhskoe	48°28'N	135°05'E	*	87		no	**T05**	*P. teleius* I	JX311275	Present thesis
SR091.03	*P. teleius sinalcon*	China, Shaanxi, Qinling mountains, Taibai Shan	34°02'N	107°18'E		91		no	**T01**	*P. teleius* IV	JX311279	Present thesis

Specimen voucher	Species	Collecting locality	Latitude	Longitude		Pop code	Sex	w+	Haplotypes	COI clade	GenBank accession numbers	Source
ZF-LY-000370	*P. teleius ogumae*	Japan, Hokkaido, Nakasatsunai-mura, Kasai-gun, Moto-sarabetsu	42°36'N	143°06'E	*	15		no	**T55**	*P. teleius* IV	JX311300	Present thesis
ZF-LY-000371	*P. teleius ogumae*	Japan, Hokkaido, Nakasatsunai-mura, Kasai-gun, Moto-sarabetsu	42°36'N	143°06'E	*	15		no	**T02**	*P. teleius* I	JX311301	Present thesis
ZF-LY-000373	*P. teleius ogumae*	Japan, Hokkaido, Nakasatsunai-mura, Kasai-gun, Moto-sarabetsu	42°36'N	143°06'E	*	15		no	**T02**	*P. teleius* I	JX311302	Present thesis
ZF-LY-000374	*P. teleius ogumae*	Japan, Hokkaido, Nakasatsunai-mura, Kasai-gun, Moto-sarabetsu	42°36'N	143°06'E	*	15		no	**T02**	*P. teleius* I	JX311303	Present thesis
ZF-LY-000378	*P. teleius ogumae*	Japan, Hokkaido, Nakasatsunai-mura, Kasai-gun, Moto-sarabetsu	42°36'N	143°06'E	*	15		no	**T55**	*P. teleius* IV	JX311304	Present thesis
ZF-LY-000379	*P. teleius ogumae*	Japan, Hokkaido, Nakasatsunai-mura, Kasai-gun, Moto-sarabetsu	42°36'N	143°06'E	*	15		?	**?**	?	?	?
ZF-LY-000384	*P. teleius ogumae*	Japan, Hokkaido, Nakasatsunai-mura, Kasai-gun, Moto-sarabetsu	42°36'N	143°06'E	*	15		no	**T02**	*P. teleius* I	JX311305	Present thesis
ZF-LY-000386	*P. teleius ogumae*	Japan, Hokkaido, Nakasatsunai-mura, Kasai-gun, Moto-sarabetsu	42°36'N	143°06'E	*	15		no	**T02**	*P. teleius* I	JX311306	Present thesis
ZF-LY-000387	*P. teleius ogumae*	Japan, Hokkaido, Nakasatsunai-mura, Kasai-gun, Moto-sarabetsu	42°36'N	143°06'E	*	15		no	**T54**	*P. teleius* IV	JX311307	Present thesis
ZF-LY-000343	*P. teleius daisensis*	Japan, Honshu, Shimane pref., Okuizumo-cho, Nita-gun, Oh Pass	35°09'N	133°02'E		11		no	**T03**	*P. teleius* II	JX311290	Present thesis
ZF-LY-000344	*P. teleius daisensis*	Japan, Honshu, Shimane pref., Okuizumo-cho, Nita-gun, Oh Pass	35°09'N	133°02'E		11		no	**T03**	*P. teleius* II	JX311291	Present thesis
ZF-LY-000345	*P. teleius daisensis*	Japan, Kyushu, Oita pref., Kokonoe-machi, Kusu-gun, Jizohara	33°10'N	131°11'E		12		no	**T43**	*P. teleius* III	JX311292	Present thesis
ZF-LY-000346	*P. teleius daisensis*	Japan, Kyushu, Oita pref., Kokonoe-machi, Kusu-gun, Jizohara	33°10'N	131°11'E		12		no	**T59**	*P. teleius* III	JX311293	Present thesis
ZF-LY-000347	*P. teleius daisensis*	Japan, Kyushu, Oita pref., Kokonoe-machi, Kusu-gun, Jizohara	33°10'N	131°11'E		12		no	**T43**	*P. teleius* III	JX311294	Present thesis
ZF-LY-000348	*P. teleius daisensis*	Japan, Kyushu, Oita pref., Kokonoe-machi, Kusu-gun, Jizohara	33°10'N	131°11'E		12		?	**?**	?	?	
ZF-LY-000349	*P. teleius daisensis*	Japan, Kyushu, Oita pref., Kokonoe-machi, Kusu-gun, Jizohara	33°10'N	131°11'E		12		no	**T59**	*P. teleius* III	JX311295	Present thesis
ZF-LY-000354	*P. teleius kazamoto*	Japan, Honshu, Yamanashi pref., Fujiyoshida City, Kitafuji Square	35°26'N	138°47'E		13		no	**T64**	*P. teleius* II	JX311296	Present thesis
ZF-LY-000364	*P. teleius daisensis*	Japan, Honshu, Hiroshima pref., Shobara City, Takano-cho, Kenashiyama Farm	35°03'N	132°56'E		14		no	**T56**	*P. teleius* II	JX311297	Present thesis
ZF-LY-000366	*P. teleius daisensis*	Japan, Honshu, Hiroshima pref., Shobara City, Takano-cho, Kenashiyama Farm	35°03'N	132°56'E		14		no	**T70**	*P. teleius* II	JX311298	Present thesis
ZF-LY-000367	*P. teleius daisensis*	Japan, Honshu, Hiroshima prefecture, Yamagata-gun, Kitahiroshima-cho, Mt. Ungetsu	34°41'N	132°32'E	*	16		no	**T58**	*P. teleius* II	JX311299	Present thesis
ZF-LY-000339	*P. teleius kazamoto*	Japan, Honshu, Yamanashi pref., Shimohagihara, Koshu City, Enzan	35°42'N	138°45'E		7		no	**T60**	*P. teleius* II	JX311286	Present thesis
ZF-LY-000340	*P. teleius kazamoto*	Japan, Honshu, Yamanashi pref., Shimohagihara, Koshu City, Enzan	35°42'N	138°45'E		7		no	**T49**	*P. teleius* II	JX311287	Present thesis
ZF-LY-000341	*P. teleius kazamoto*	Japan, Honshu, Yamanashi pref., Shimohagihara, Koshu City, Enzan	35°42'N	138°45'E		7		no	**T49**	*P. teleius* II	JX311288	Present thesis
ZF-LY-000342	*P. teleius kazamoto*	Japan, Honshu, Yamanashi pref., Shimohagihara, Koshu City, Enzan	35°42'N	138°45'E		7		no	**T49**	*P. teleius* II	JX311289	Present thesis
ZF-LY-000336	*P. teleius kazamoto*	Japan, Honshu, Nagano pref., Matsumoto, Yoriaido, Nagawa	36°03'N	137°41'E		5		no	**T57**	*P. teleius* IV	JX311285	Present thesis
ZF-LY-000316	*P. teleius hosonoi*	Japan, Honshu, Gifu pref., Ono-gun, Mt. Ohchozan	36°10'N	136°51'E		2		no	**T44**	*P. teleius* IV	JX311282	Present thesis
ZF-LY-000317	*P. teleius hosonoi*	Japan, Honshu, Gifu pref., Yoshinodani-mura, Hakusan	36°27'N	136°39'E		3		no	**T44**	*P. teleius* IV	JX311283	Present thesis
ZF-LY-000333	*P. teleius ogumae*	Japan, Honshu, Aomori pref., Ohma-machi, Shimokita-gun, Okoppe	41°29'N	140°54'E		4		no	**T55**	*P. teleius* IV	JX311284	Present thesis
MG02N009	*P. teleius teleius* $	Romania, Transylvania, Cluj-Napoca	46°46'N	23°35'E	*	93		?	**T07**	*P. teleius* I	AY675418, HQ918140	Als *et al.* 2004
TDA99Q975	*P. teleius teleius* $	Poland, Domaszowice	50°52'N	20°40'E	*	94		?	**T67**	*P. teleius* I	AY675428, HQ918139	Als *et al.* 2004
TDA99Q976	*P. teleius teleius* $	Poland, Krakow	50°03'N	19°56'E	*	95		?	**T69**	*P. teleius* I	AY675429	Als *et al.* 2004
UK99W801	*P. teleius kazamoto* $	Japan, Honshu, Yamanashi Pref., Nirasaki	35°42'N	138°27'E	*	96		?	**T68**	*P. teleius* II	AY675437, HQ918138	Als *et al.* 2004
UK99W809	*P. teleius kazamoto* $	Japan, Honshu, Yamanashi Pref., Kuromori	35°54'N	138°32'E	*	97		?	**T66**	*P. teleius* II	AY675440, HQ918035	Als *et al.* 2004
TERU5	*P. teleius teleius* $	Russia				98		?	**T07**	*P. teleius* I	HQ918159	Ugelvig *et al.* 2011
RV07E460	*P. teleius teleius* $	Romania, Transylvania, Brasov, Dumbrava Vandului	45°47'N	25°07'E	*	99		?	**T07**	*P. teleius* I	HQ918167	Ugelvig *et al.* 2011
UK08J627	*P. teleius euphemia* $	Mongolia, Ulaanbaatar, Bogt Uul	47°48'N	106°59'E	*	120		?	**T30**	*P. teleius* Wolbachia	HQ918161	Ugelvig *et al.* 2011
TEJA1	*P. teleius daisensis* $	Japan, Honshu, Hiroshima prefecture, Candelo	34°N	132°E	*	121		?	**T71**	*P. teleius* II	HQ918158	Ugelvig *et al.* 2011
TESL6	*P. teleius teleius* $	Slovakia, Trencin	48°54'N	18°02'E	*	122		?	**T72**	*P. teleius* I	HQ918160	Ugelvig *et al.* 2011
RV-06-M891	*P. teleius teleius* $	Romania, Transylvania, Bihor	46°56'N	22°32'E		n.i.		?	**T04**	*P. teleius* I	HQ004727	Dincă *et al.* 2011b
RV-06-M964	*P. teleius teleius* $	Romania, Transylvania, Brasov, Racos	46°02'N	25°22'E		n.i.		?	**T04**	*P. teleius* I	HQ004729	Dincă *et al.* 2011b
RVcoll.07-C168	*P. teleius teleius* $	Romania, Transylvania, Cluj-Napoca	46°50'N	23°38'E		n.i.		?	**T04**	*P. teleius* I	HQ004724	Dincă *et al.* 2011b
RVcoll.07-C163	*P. teleius teleius* $	Romania, Transylvania, Cluj-Napoca	46°50'N	23°38'E		n.i.		?	**T04**	*P. teleius* I	HQ004725	Dincă *et al.* 2011b
RV-07-C162	*P. teleius teleius* $	Romania, Transylvania, Cluj-Napoca	46°50'N	23°38'E		n.i.		?	**T04**	*P. teleius* I	HQ004726	Dincă *et al.* 2011b
RVcoll.06-M885	*P. teleius teleius* $	Romania, Transylvania, Bogomaia	46°50'N	23°39'E		n.i.		?	**T04**	*P. teleius* I	HQ004723	Dincă *et al.* 2011b
BC ZSM Lep 21847	*P. teleius teleius* $	Germany, Bavaria, Diessen	47°56'N	11°05'E		n.i.		?	**T23**	*P. teleius* I	JF415712	Hausmann *et al.* 2011

Specimen voucher	Species	Collecting locality	Latitude	Longitude		Pop code	Sex	w+	Haplotypes	COI clade	GenBank accession numbers	Source
BC ZSM Lep 28464	*P. teleius teleius* $	Germany, Bavaria, Saulburg	48°59'N	12°32'E		n.i.		?	**T23**	*P. teleius* I	HM391889	Hausmann *et al.* 2011
BC ZSM Lep 30669	*P. teleius teleius* $	Germany, Bavaria, Passau, near Egling				n.i.		?	**T23**	*P. teleius* I	HQ957211	Hausmann *et al.* 2011
MT-Nagano1	*P. teleius kazamoto* $	Japan, Nagano Pref., Chino City				n.i.		?	**T66**	*P. teleius* II	AB457755	Yago *et al.* (unpubl.)
SR019.11	*P. nausithous*	Russia, Novosibirsk, near Akademgorodok	54°49'N	83°06'E		19		no	**N22**	*P. nausithous* I	JX311068	Present thesis
SR020.02	*P. nausithous*	Russia, Novosibirsk, near Akademgorodok	54°49'N	83°07'E		20		no	**N34**	*P. nausithous* I	JX311070	Present thesis
SR020.03	*P. nausithous*	Russia, Novosibirsk, near Akademgorodok	54°49'N	83°07'E		20		no	**N21**	*P. nausithous* I	JX311071	Present thesis
SR021.01	*P. nausithous*	Russia, Novosibirsk, near Akademgorodok	54°50'N	83°08'E		21		no	**N13**	*P. nausithous* I	JX311072	Present thesis
SR021.03	*P. nausithous*	Russia, Novosibirsk, near Akademgorodok	54°50'N	83°08'E		21		no	**N13**	*P. nausithous* I	JX311073	Present thesis
SR021.38	*P. nausithous*	Russia, Novosibirsk, near Akademgorodok	54°50'N	83°08'E		21		no	**N13**	*P. nausithous* I	JX311074	Present thesis
SR022.01	*P. nausithous*	Russia, near Barnaul	53°22'N	84°03'E		22		no	**N13**	*P. nausithous* I	JX311076	Present thesis
SR022.03	*P. nausithous*	Russia, near Barnaul	53°22'N	84°03'E		22		no	**N13**	*P. nausithous* I	JX311077	Present thesis
SR022.05	*P. nausithous*	Russia, near Barnaul	53°22'N	84°03'E		22		no	**N13**	*P. nausithous* I	JX311079	Present thesis
SR022.06	*P. nausithous*	Russia, near Barnaul	53°22'N	84°03'E		22		no	**N20**	*P. nausithous* I	JX311080	Present thesis
SR022.07	*P. nausithous*	Russia, near Barnaul	53°22'N	84°03'E		22		no	**N13**	*P. nausithous* I	JX311081	Present thesis
SR023.03	*P. nausithous*	Russia, Altai mountains, around Cherga	51°33'N	85°33'E		23		no	**N39**	*P. nausithous* I	JX311082	Present thesis
SR023.31	*P. nausithous*	Russia, Altai mountains, around Cherga	51°33'N	85°33'E		23		no	**N19**	*P. nausithous* I	JX311085	Present thesis
SR024.03	*P. nausithous*	Russia, Altai mountains, Choya-District, near Levinka	51°57'N	86°22'E		24		no	**N30**	*P. nausithous* I	JX311088	Present thesis
SR024.08	*P. nausithous*	Russia, Altai mountains, Choya-District, near Levinka	51°57'N	86°22'E		24		no	**N18**	*P. nausithous* I	JX311089	Present thesis
SR024.36	*P. nausithous*	Russia, Altai mountains, Choya-District, near Levinka	51°57'N	86°22'E		24		no	**N13**	*P. nausithous* I	JX311090	Present thesis
SR025.01	*P. nausithous*	Russia, Altai mountains, Turochak District, near Verkh Biisk	52°02'N	87°04'E		25		no	**N32**	*P. nausithous* I	JX311091	Present thesis
SR025.02	*P. nausithous*	Russia, Altai mountains, Turochak District, near Verkh Biisk	52°02'N	87°04'E		25		yes	**N13**	*P. nausithous* I	JX311092	Present thesis
SR025.07	*P. nausithous*	Russia, Altai mountains, Turochak District, near Verkh Biisk	52°02'N	87°04'E		25		no	**N16**	*P. nausithous* I	JX311094	Present thesis
SR025.10	*P. nausithous*	Russia, Altai mountains, Turochak District, near Verkh Biisk	52°02'N	87°04'E		25		no	**N17**	*P. nausithous* I	JX311095	Present thesis
SR026.05	*P. nausithous*	Russia, Altai mountains, around Ulus-Cherga	51°31'N	85°27'E		26		no	**N16**	*P. nausithous* I	JX311097	Present thesis
SR026.06	*P. nausithous*	Russia, Altai mountains, around Ulus-Cherga	51°31'N	85°27'E		26		no	**N38**	*P. nausithous* I	JX311098	Present thesis
SR026.53	*P. nausithous*	Russia, Altai mountains, around Ulus-Cherga	51°31'N	85°27'E		26		no	**N16**	*P. nausithous* I	JX311099	Present thesis
SR028.24	*P. nausithous*	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		no	**N16**	*P. nausithous* I	JX311112	Present thesis
SR028.32	*P. nausithous*	Russia, Altai mountains, Shebalino District, near Ilyianka	51°20'N	85°10'E		28		no	**N16**	*P. nausithous* I	JX311115	Present thesis
SR031.01	*P. nausithous*	N Kazakhstan, Akmola Prov., around Kokshetau	53°16'N	69°24'E	*	31		no	**N36**	*P. nausithous* I	JX311123	Present thesis
SR031.02	*P. nausithous*	N Kazakhstan, Akmola Prov., around Kokshetau	53°16'N	69°24'E	*	31		no	**N36**	*P. nausithous* I	JX311124	Present thesis
SR031.03	*P. nausithous*	N Kazakhstan, Akmola Prov., around Kokshetau	53°16'N	69°24'E	*	31		yes	**N41**	*P. nausithous* Wolbachia	JX311125	Present thesis
SR032.02	*P. nausithous*	Germany, Westerwald, around Montabaur	50°25'N	07°48'E		32		no	**N15**	*P. nausithous* I	JX311127	Present thesis
SR034.01	*P. nausithous*	SE France, Rhône-Alpes, Lavours Ain	45°48'N	05°45'E	*	34		no	**N31**	*P. nausithous* I	JX311131	Present thesis
SR034.02	*P. nausithous*	SE France, Rhône-Alpes, Lavours Ain	45°48'N	05°45'E	*	34		no	**N14**	*P. nausithous* II	JX311132	Present thesis
SR034.03	*P. nausithous*	SE France, Rhône-Alpes, Lavours Ain	45°48'N	05°45'E	*	34		no	**N14**	*P. nausithous* II	JX311133	Present thesis
SR039.01	*P. nausithous*	E France, Lorraine, Mortagne, Rambervilles	48°20'N	06°37'E	*	39		no	**N37**	*P. nausithous* I	JX311140	Present thesis
SR039.02	*P. nausithous*	E France, Lorraine, Mortagne, Rambervilles	48°20'N	06°37'E	*	39		no	**N02**	*P. nausithous* I	JX311141	Present thesis
SR040.01	*P. nausithous*	E France, Lorraine, Moselotte, Saint-Amé	47°59'N	06°45'E	*	40		no	**N26**	*P. nausithous* I	JX311142	Present thesis
SR041.01	*P. nausithous*	E Germany, Chemnitz, Heinersdorfer Teiche	50°52'N	12°53'E	*	41		no	**N03**	*P. nausithous* II	JX311143	Present thesis
SR041.03	*P. nausithous*	E Germany, Chemnitz, Heinersdorfer Teiche	50°52'N	12°53'E	*	41		no	**N03**	*P. nausithous* II	JX311144	Present thesis
SR042.01	*P. nausithous*	E Germany, Saxony, near Schkeuditz	51°23'N	12°13'E		42		no	**N03**	*P. nausithous* II	JX311145	Present thesis
SR042.02	*P. nausithous*	E Germany, Saxony, near Schkeuditz	51°23'N	12°13'E		42		no	**N03**	*P. nausithous* II	JX311146	Present thesis
SR042.03	*P. nausithous*	E Germany, Saxony, near Schkeuditz	51°23'N	12°13'E		42		no	**N03**	*P. nausithous* II	JX311147	Present thesis
SR043.01	*P. nausithous*	SW Germany, Palatinate, near Landau	49°12'N	08°05'E		43		no	**N02**	*P. nausithous* I	JX311148	Present thesis
SR043.02	*P. nausithous*	SW Germany, Palatinate, near Landau	49°12'N	08°05'E		43		no	**N02**	*P. nausithous* I	JX311149	Present thesis
SR044.01	*P. nausithous*	SW Germany, Palatinate, Queichhambach	49°13'N	07°58'E		44		no	**N02**	*P. nausithous* I	JX311150	Present thesis

Specimen voucher	Species	Collecting locality	Latitude	Longitude		Pop code	Sex	w+	Haplotypes	COI clade	GenBank accession numbers	Source
SR044.02	*P. nausithous*	SW Germany, Palatinate, Queichhambach	49°13'N	07°58'E		44		no	**N02**	*P. nausithous* I	JX311151	Present thesis
SR045.13	*P. nausithous*	SW Germany, Palatinate, near Zeiskam	49°13'N	08°14'E		45		no	**N02**	*P. nausithous* I	JX311152	Present thesis
SR047.08	*P. nausithous*	Russia, S Ural mountains, Cheljabinsk region, near Snezhinsk	56°05'N	60°44'E	*	47		no	**N13**	*P. nausithous* I	JX311153	Present thesis
SR047.11	*P. nausithous*	Russia, S Ural mountains, Cheljabinsk region, near Snezhinsk	56°05'N	60°44'E	*	47		no	**N13**	*P. nausithous* I	JX311154	Present thesis
SR047.17	*P. nausithous*	Russia, S Ural mountains, Cheljabinsk region, near Snezhinsk	56°05'N	60°44'E	*	47		no	**N35**	*P. nausithous* I	JX311155	Present thesis
SR047.18	*P. nausithous*	Russia, S Ural mountains, Cheljabinsk region, near Snezhinsk	56°05'N	60°44'E	*	47		no	**N13**	*P. nausithous* I	JX311156	Present thesis
SR048.01	*P. nausithous* §	Russia, Volgograd region, near Vodnyi village	48°43'N	44°30'E	*	48		no	**N12**	*P. nausithous* I	JX311158	Present thesis
SR048.02	*P. nausithous* §	Russia, Volgograd region, near Vodnyi village	48°43'N	44°30'E	*	48		no	**N12**	*P. nausithous* I	JX311159	Present thesis
SR048.06	*P. nausithous* §	Russia, Volgograd region, near Vodnyi village	48°43'N	44°30'E	*	48		no	**N12**	*P. nausithous* I	JX311160	Present thesis
SR048.09	*P. nausithous* §	Russia, Volgograd region, near Vodnyi village	48°43'N	44°30'E	*	48	♀	yes	**N11**	*P. nausithous* Wolbachia	JX311161	Present thesis
SR052.01	*P. nausithous* §	Russia, Volgograd region, near Vodnyi village	48°43'N	44°30'E	*	52		no	**N11**	*P. nausithous* Wolbachia	JX311166	Present thesis
SR053.08	*P. nausithous*	Russia, S Ural mountains, Cheljabinsk region, Itkul lake	56°21'N	62°11'E	*	53		no	**N13**	*P. nausithous* I	JX311170	Present thesis
SR054.01	*P. nausithous* §	Russia, Volgograd region, Kalach-na-Dour-district, Ryumino village	48°43'N	43°37'E		54	♀	yes	**N11**	*P. nausithous* Wolbachia	JX311171	Present thesis
SR055.01	*P. nausithous*	E Germany, south of Kahla, Elsterwerda, Schwarze Elster	51°28'N	13°33'E	*	55		no	**N03**	*P. nausithous* II	JX311172	Present thesis
SR055.02	*P. nausithous*	E Germany, south of Kahla, Elsterwerda, Schwarze Elster	51°28'N	13°33'E	*	55		no	**N03**	*P. nausithous* II	JX311173	Present thesis
SR055.03	*P. nausithous*	E Germany, south of Kahla, Elsterwerda, Schwarze Elster	51°28'N	13°33'E	*	55		no	**N03**	*P. nausithous* II	JX311174	Present thesis
SR055.04	*P. nausithous*	E Germany, south of Kahla, Elsterwerda, Schwarze Elster	51°28'N	13°33'E	*	55		no	**N03**	*P. nausithous* II	JX311175	Present thesis
SR055.05	*P. nausithous*	E Germany, south of Kahla, Elsterwerda, Schwarze Elster	51°28'N	13°33'E	*	55		no	**N03**	*P. nausithous* II	JX311176	Present thesis
SR058.01	*P. nausithous*	Bulgaria, near Sofia, Mt. Lyulin	42°39'N	23°05'E	*	58		no	**N25**	*P. nausithous* I	JX311177	Present thesis
SR058.02	*P. nausithous*	Bulgaria, near Sofia, Mt. Lyulin	42°39'N	23°05'E	*	58		no	**N25**	*P. nausithous* I	JX311178	Present thesis
SR059.01	*P. nausithous*	Spain, Soria province, near Abejar	41°48'N	2°47'W		59		no	**N10**	*P. nausithous* II	JX311179	Present thesis
SR060.02	*P. nausithous*	Spain, Madrid Province, Oteruelo del Valle	40°55'N	3°51'W		60		no	**N10**	*P. nausithous* II	JX311180	Present thesis
SR060.05	*P. nausithous*	Spain, Madrid Province, Oteruelo del Valle	40°55'N	3°51'W		60		no	**N10**	*P. nausithous* II	JX311181	Present thesis
SR062.05	*P. nausithous*	E Germany, Saxony, near Schkeuditz	51°23'N	12°11'E		62		no	**N03**	*P. nausithous* II	JX311184	Present thesis
SR062.06	*P. nausithous*	E Germany, Saxony, near Schkeuditz	51°23'N	12°11'E		62		no	**N03**	*P. nausithous* II	JX311185	Present thesis
SR062.07	*P. nausithous*	E Germany, Saxony, near Schkeuditz	51°23'N	12°11'E		62		no	**N03**	*P. nausithous* II	JX311186	Present thesis
SR062.08	*P. nausithous*	E Germany, Saxony, near Schkeuditz	51°23'N	12°11'E		62		no	**N03**	*P. nausithous* II	JX311187	Present thesis
SR062.09	*P. nausithous*	E Germany, Saxony, near Schkeuditz	51°23'N	12°11'E		62		no	**N03**	*P. nausithous* II	JX311188	Present thesis
SR064.04	*P. nausithous*	S Germany, Lake Constance, near Constance	47°39'N	09°10'E	*	64		no	**N09**	*P. nausithous* II	JX311191	Present thesis
SR064.05	*P. nausithous*	S Germany, Lake Constance, near Constance	47°39'N	09°10'E	*	64		no	**N09**	*P. nausithous* II	JX311192	Present thesis
SR066.11	*P. nausithous*	E Poland, Kosyn	51°23'N	23°34'E		66		no	**N33**	*P. nausithous* II	JX311203	Present thesis
SR066.12	*P. nausithous*	E Poland, Kosyn	51°23'N	23°34'E		66		no	**N24**	*P. nausithous* I	JX311204	Present thesis
SR066.13	*P. nausithous*	E Poland, Kosyn	51°23'N	23°34'E		66		no	**N24**	*P. nausithous* I	JX311205	Present thesis
SR066.14	*P. nausithous*	E Poland, Kosyn	51°23'N	23°34'E		66		no	**N24**	*P. nausithous* I	JX311206	Present thesis
SR067.08	*P. nausithous*	S Poland, Wiesiółka	50°25'N	19°21'E		67		no	**N40**	*P. nausithous* II	JX311212	Present thesis
SR068.11	*P. nausithous*	SE Poland, Widacz	49°38'N	21°50'E		68		no	**N08**	*P. nausithous* I	JX311218	Present thesis
SR068.13	*P. nausithous*	SE Poland, Widacz	49°38'N	21°50'E		68		no	**N06**	*P. nausithous* I	JX311219	Present thesis
SR068.14	*P. nausithous*	SE Poland, Widacz	49°38'N	21°50'E		68		no	**N07**	*P. nausithous* I	JX311220	Present thesis
SR068.15	*P. nausithous*	SE Poland, Widacz	49°38'N	21°50'E		68		no	**N06**	*P. nausithous* I	JX311221	Present thesis
SR069.01	*P. nausithous*	SE Germany, Traunstein, Kirchanschöring	47°56'N	12°56'E	*	69		no	**N42**	*P. nausithous* I	JX311222	Present thesis
SR070.05	*P. nausithous*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**N28**	*P. nausithous* I	JX311224	Present thesis
SR070.14	*P. nausithous*	Croatia, Bedekovičeve Grabe	46°26'N	16°24'E	*	70		no	**N05**	*P. nausithous* I	JX311228	Present thesis
SR072.01	*P. nausithous*	E Germany, Saxony, Leipzig/Wachau	51°16'N	12°26'E		72		no	**N04**	*P. nausithous* II	JX311233	Present thesis
SR072.02	*P. nausithous*	E Germany, Saxony, Leipzig/Wachau	51°16'N	12°26'E		72		no	**N04**	*P. nausithous* II	JX311234	Present thesis
SR073.01	*P. nausithous*	E Germany, Thuringia, Jena	50°55'N	11°35'E		73		no	**N03**	*P. nausithous* II	JX311235	Present thesis
SR073.02	*P. nausithous*	E Germany, Thuringia, Jena	50°55'N	11°35'E		73		no	**N03**	*P. nausithous* II	JX311236	Present thesis
SR073.03	*P. nausithous*	E Germany, Thuringia, Jena	50°55'N	11°35'E		73		no	**N23**	*P. nausithous* II	JX311237	Present thesis
SR073.04	*P. nausithous*	E Germany, Thuringia, Jena	50°55'N	11°35'E		73		no	**N03**	*P. nausithous* II	JX311238	Present thesis

Specimen voucher	Species	Collecting locality	Latitude	Longitude		Pop code	Sex	w+	Haplotypes	COI clade	GenBank accession numbers	Source
SR073.05	*P. nausithous*	E Germany, Thuringia, Jena	50°55'N	11°35'E		73		no	**N23**	*P. nausithous* II	JX311239	Present thesis
SR073.06	*P. nausithous*	E Germany, Thuringia, Jena	50°55'N	11°35'E		73		no	**N03**	*P. nausithous* II	JX311240	Present thesis
SR074.02	*P. nausithous*	SW Germany, Palatinate, Neuburg/Rhein	48°59'N	08°15'E		74		no	**N02**	*P. nausithous* I	JX311241	Present thesis
SR074.03	*P. nausithous*	SW Germany, Palatinate, Neuburg/Rhein	48°59'N	08°15'E		74		no	**N02**	*P. nausithous* I	JX311242	Present thesis
SR074.04	*P. nausithous*	SW Germany, Palatinate, Neuburg/Rhein	48°59'N	08°15'E		74		no	**N02**	*P. nausithous* I	JX311243	Present thesis
SR074.05	*P. nausithous*	SW Germany, Palatinate, Neuburg/Rhein	48°59'N	08°15'E		74		no	**N02**	*P. nausithous* I	JX311244	Present thesis
SR074.07	*P. nausithous*	SW Germany, Palatinate, Neuburg/Rhein	48°59'N	08°15'E		74		no	**N02**	*P. nausithous* I	JX311245	Present thesis
SR074.08	*P. nausithous*	SW Germany, Palatinate, Neuburg/Rhein	48°59'N	08°15'E		74		no	**N02**	*P. nausithous* I	JX311246	Present thesis
SR074.09	*P. nausithous*	SW Germany, Palatinate, Neuburg/Rhein	48°59'N	08°15'E		74		no	**N02**	*P. nausithous* I	JX311247	Present thesis
SR077.05	*P. nausithous*	Belarus	51°48'N	30°15'E	*	77		no	**N27**	*P. nausithous* I	JX311252	Present thesis
SR077.06	*P. nausithous*	Belarus	51°48'N	30°15'E	*	77		no	**N46**	*P. nausithous* I	JX311253	Present thesis
SR078.04	*P. nausithous*	Slovenia, Celje	46°14'N	15°15'E	*	78		no	**N05**	*P. nausithous* I	JX311256	Present thesis
SR078.06	*P. nausithous*	Slovenia, Celje	46°14'N	15°15'E	*	78		no	**N29**	*P. nausithous* I	JX311257	Present thesis
SR078.07	*P. nausithous*	Slovenia, Celje	46°14'N	15°15'E	*	78		no	**N05**	*P. nausithous* I	JX311258	Present thesis
SR078.08	*P. nausithous*	Slovenia, Celje	46°14'N	15°15'E	*	78		no	**N05**	*P. nausithous* I	JX311259	Present thesis
SR083.01	*P. nausithous* [§]	Romania, Transylvania, Cluj-Napoca	46°54'N	23°46'E		83		no	**N44**	*P. nausithous* I	JX311274	Present thesis
SR088.01	*P. nausithous*	Slovakia, Abrod	48°42'N	17°10'E	*	88	♀	yes	**N43**	*P. nausithous* Wolbachia	JX311276	Present thesis
SR088.02	*P. nausithous*	Slovakia, Abrod	48°42'N	17°10'E	*	88		no	**N43**	*P. nausithous* Wolbachia	JX311277	Present thesis
SR089.01	*P. nausithous*	Slovakia, Stará Turá, Drgonova dolina	48°46'N	17°40'E	*	89		no	**N43**	*P. nausithous* Wolbachia	JX311278	Present thesis
SR101.01	*P. nausithous*	Czech Republic, Straznice	48°54'N	17°19'E		1		no	**N01**	*P. nausithous* Wolbachia	JX311280	Present thesis
SR101.02	*P. nausithous*	Czech Republic, Straznice	48°54'N	17°19'E		1	♀	yes	**N01**	*P. nausithous* Wolbachia	JX311281	Present thesis
AD00P068	*P. nausithous*	Russia, Tula	54°12'N	37°37'E	*	90		?	**N47**	*P. nausithous* I	AY675403, HQ918134	Als *et al.* 2004
TDA99Q966	*P. nausithous*	SW Poland, Swidnica	50°50'N	16°28'E	*	128		?	**N45**	*P. nausithous* II	AY675427, HQ918033	Als *et al.* 2004
ZD99S301	*P. nausithous*	Slovakia, Abrod	48°42'N	17°10'E	*	92		?	**N01**	*P. nausithous* Wolbachia	AY675446, HQ918135	Als *et al.* 2004
NACZ1	*P. nausithous*	Czech Republic, Mecichov	49°21'N	13°49'E	*	123		?	**N02**	*P. nausithous* I	HQ918151	Ugelvig *et al.* 2011
RV08L884	*P. nausithous*	Spain, Soria province, near Abejar	41°48'N	2°47'W	*	124		?	**N10**	*P. nausithous* II	HQ918150	Ugelvig *et al.* 2011
NAGE2	*P. nausithous*	Germany, Bavaria				125		?	**N13**	*P. nausithous* I	HQ918152	Ugelvig *et al.* 2011
RV07C331	*P. nausithous*	Romania, Moldavia, Suceava, Radauti	47°47'N	25°54'E	*	126		?	**N48**	*P. nausithous* I	HQ918166	Ugelvig *et al.* 2011
RV06M889	*P. nausithous*	Romania, Transylvania, Cluj-Napoca	46°50'N	23°40'E	*	127		?	**N49**	*P. nausithous* I	HQ918165	Ugelvig *et al.* 2011
RVcoll.07-C334	*P. nausithous*	Romania, Moldavia, Suceava, Radauti	47°47'N	25°54'E		n.i.		?	**N13**	*P. nausithous* I	HQ004715	Dincă *et al.* 2011b
RV-07-C116	*P. nausithous*	Romania, Transylvania, Cluj-Napoca	46°50'N	23°38'E		n.i.		?	**N13**	*P. nausithous* I	HQ004720	Dincă *et al.* 2011b
RVcoll.07-C164	*P. nausithous*	Romania, Transylvania, Cluj-Napoca	46°50'N	23°39'E		n.i.		?	**N13**	*P. nausithous* I	HQ004717	Dincă *et al.* 2011b
RVcoll.06-M917	*P. nausithous*	Romania, Transylvania, Cluj-Napoca	46°50'N	23°39'E		n.i.		?	**N13**	*P. nausithous* I	HQ004718	Dincă *et al.* 2011b
RVcoll.07-C167	*P. nausithous*	Romania, Transylvania, Cluj-Napoca	46°50'N	23°38'E		n.i.		?	**N13**	*P. nausithous* I	GU675622	IBoL unpubl.
RVcoll.07-C161	*P. nausithous*	Romania, Transylvania, Cluj-Napoca	46°50'N	23°38'E		n.i.		?	**N13**	*P. nausithous* I	GU675623	Dincă *et al.* 2011b
RVcoll.06-M890	*P. nausithous*	Romania, Transylvania, Cluj-Napoca	46°50'N	23°39'E		n.i.		?	**N49**	*P. nausithous* I	GU675624	Dincă *et al.* 2011b
RVcoll.07-C165	*P. nausithous*	Romania, Transylvania, Cluj-Napoca	46°50'N	23°39'E		n.i.		?	**N49**	*P. nausithous* I	HQ004716	Dincă *et al.* 2011b
RV-07-C332	*P. nausithous*	Romania, Moldavia, Suceava, Radauti	47°47'N	25°54'E		n.i.		?	**N50**	*P. nausithous* I	HQ004722	Dincă *et al.* 2011b
BC ZSM Lep 28463	*P. nausithous*	Germany, Bavaria, Saulburg	48°59'N	12°32'E		n.i.		?	**N51**	*P. nausithous* I	HM391888	Hausmann *et al.* 2011
BC ZSM Lep 30498	*P. nausithous*	Germany, Bavaria, Zellwies Koenigsdorf				n.i.		?	**N52**	*P. nausithous* I	GU688438	Hausmann *et al.* 2011
2005-LOWA-542	*P. nausithous*	Russia	50°54'N	106°00'E		n.i.		?	**Chimaera**[and]		FJ663755	Lukhtanov *et al.* 2009
SY03A500	*P. albida*	China, Jiangjin						?			AY675423, HQ918037	Als *et al.* 2004
SY03A501	*P. atroguttata*	Taiwan, Heping						?			AY675424, HQ918038	Als *et al.* 2004

Specimen voucher	Species	Collecting locality	Latitude	Longitude	Pop code	Sex	w+	Haplotypes	COI clade	GenBank accession numbers	Source
SY03A503	*P. daitozanus*	Taiwan, Heping					?			AY675425, HQ918039	Als *et al.* 2004
TDA99Q985	*P. alcon*	Denmark					?			AY675431, HQ918119	Als *et al.* 2004
TDA99Q989	*P. arion*	Sweden					?			AY675433, HQ918130	Als *et al.* 2004
MW99045	*P. arion*	Turkey, Erzurum, Köşkköy	40°05'N	41°26'E			?			AY557034	Wiemers and Fiedler 2007

\# These individuals were assigned to ssp. *obscurata* according to Tshikolovets *et al.* 2002, 2009a, and 2009b although the range limits of this subspecies are debated.

$ These individuals retrieved from Genebank were not assigned to a certain subspecies. Our assignment is based on Tshikolovets *et al.* 2009a, 2009b, and Sibatani *et al.* 1994

§ These individuals were assigned as subspecies "*kijevensis*" by the specimen collector.

& This barcode sequence of *Phengaris (Maculinea) nausithous* from Russia differs strongly (more than 6.8%) from all other *Phengaris* sequences and turned out to be a chimaera of *Phengaris (Maculinea) teleius* (5' half) and *Brenthis ino* (3' half). It was therefore excluded from further analysis. Its voucher specimen is figured in BOLD (2005-LOWA-542) and actually represents *P. teleius*. Females of *P. teleius* can be very dark in Transbaikalia and are therefore easily confused with *P. (M.) nausithous* which does not seem to occur in the area. Earlier records from Transbaikalia (Amazar) were questioned (Dubatolov and Kosterin 1999). The 3' half of the sequence was identical to two sequences of *Brenthis ino* used in the same study (FJ663337 and FJ663338; Lukhtanov *et al.* 2009) and whose vouchers had been caught at the same location and on the same date as the *P. (M.) teleius* specimen.

2. Phylogenetic analyses

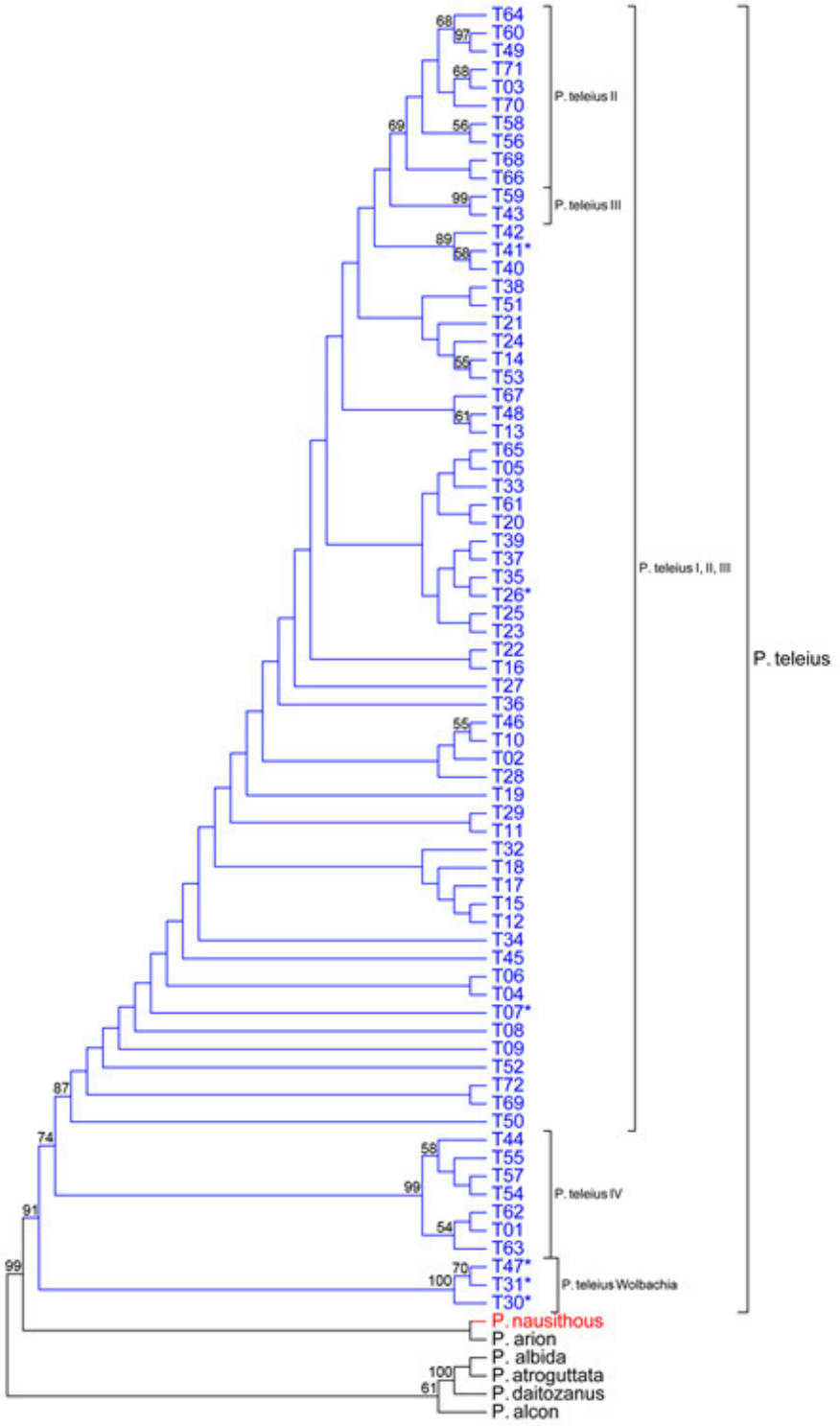

Figure 5: ML cladogram using the TrN+I+G model of nucleotide substitution (-logL = 2105.87) and depicting relationships among haplotypes of *P. (M.) teleius* (blue). Haplotypes for *P. (M.) nausithous* (red) are collapsed. Bootstrap values in percent (>50%) are given. Origin of haplotypes according to Tab. 4; * haplotypes associated with *Wolbachia* infected individuals.

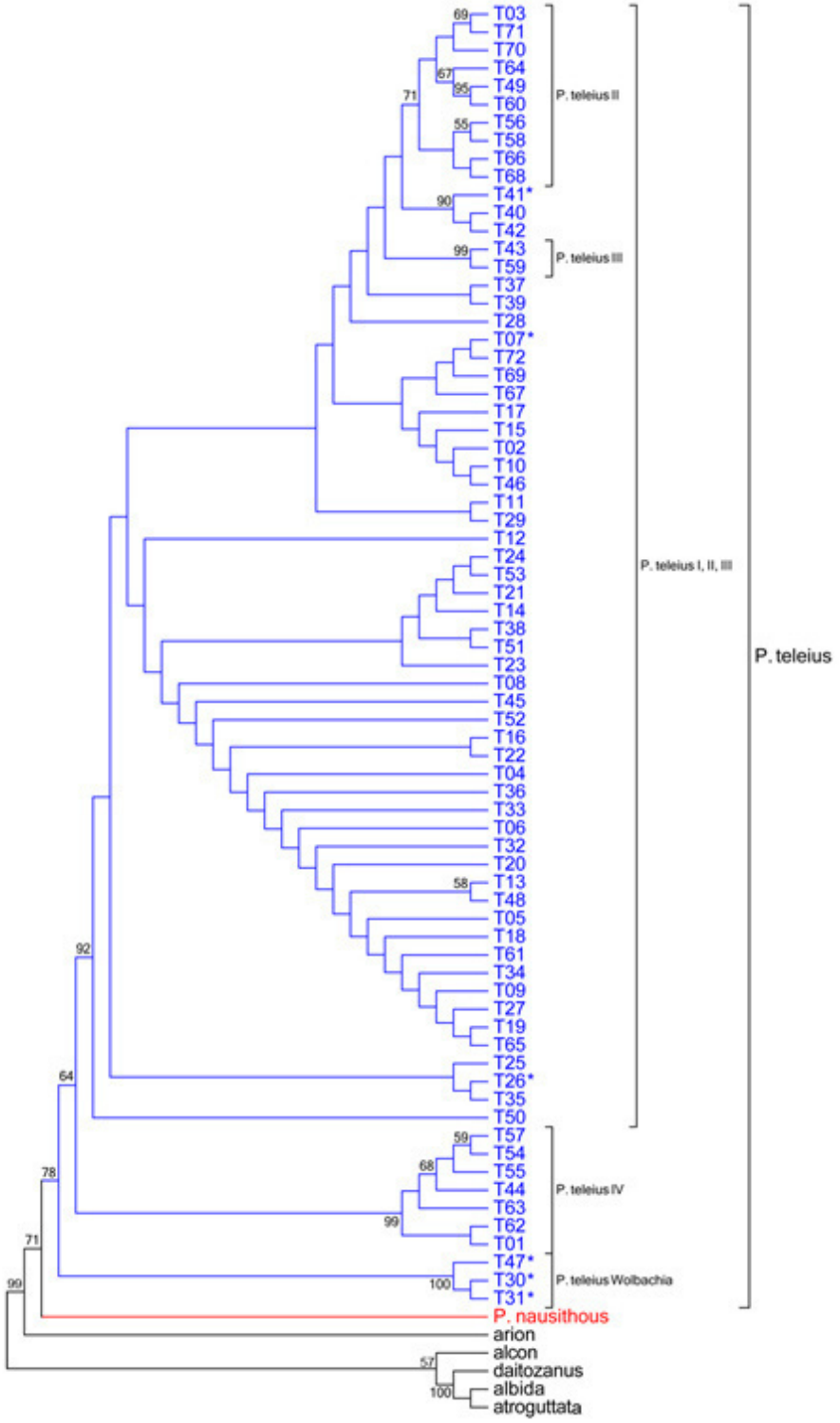

Figure 6: Cladogram of tree #1 out of 131 most parsimonious trees (length = 687) depicting relationships among haplotypes of *P. (M.) teleius* (blue). Haplotypes for *P. (M.) nausithous* (red) are collapsed. Bootstrap values in percent (>50%) are given. Origin of haplotypes according to Tab. 4; * haplotypes associated with *Wolbachia* infected individuals.

3. Microsatellite analyses

Table 5: Microsatellite genotypes of *Phengaris (Maculinea) teleius* and *P. (M.) nausithous* (light grey shade) used for analysis (loci according to Zeisset *et al.* 2005).

Specimen voucher	1a	1b	3a	3b	7a	7b	15a	15b	8a	8b	16a	16b	9a	9b	11a	11b
SR006.01	99	99	118	118	114	135	135	135			343	343	168	173	188	188
SR006.03	99	99			135	135	126	135			343	343	156	156	188	188
SR006.05	99	99	112	112	114	135	126	135			335	337	156	156	188	188
SR006.06	99	99	112	112	135	135	126	135	213	214	343	347	156	170	188	188
SR006.07	99	99			135	135	126	135			345	345	156	172	188	188
SR006.08	102	102	112	112	135	135	126	135	216	216	331	331	170	172	188	188
SR006.10	117	128	110	124	114	114	131	139	186	186	273	353	156	156	188	188
SR008.01	99	99	112	112	135	135	135	135	186	193			156	156	188	188
SR009.01									186	196	273	273	156	156	188	188
SR010.01	119	123	110	124	114	114	128	137	196	196	363	367	156	156	188	188
SR010.14	111	125	110	124	114	114	137	137	196	196	337	339	156	156	188	188
SR010.15	121	121	110	110	114	114	137	137	186	188	310	349	156	156	189	189
SR010.17	111	125	110	110	114	114	137	139	186	186	308	343	156	158	188	188
SR010.21	125	125	110	112	114	114	137	137	186	196	308	328	156	158	188	188
SR010.32	123	134	110	110	114	114	128	137	186	193	315	320	156	156	188	188
SR010.38	121	139	110	110	114	114	137	139	186	186	308	343	173	173	188	188
SR010.46	117	137	110	110	114	114	137	139	192	198	308	310	156	156	188	188
SR019.21	104	123	110	124	114	114	137	139	186	186	308	308	156	158	188	188
SR021.44	121	134	110	110	114	114	137	141	186	190	339	343	156	156	188	188
SR022.04	113	142	110	110	114	114	141	141	188	198	308	317	156	173	188	188
SR023.09	117	125	110	124	114	114	137	139	182	186	308	315	156	156	188	188
SR023.10	140	140	110	110	114	114	139	139	186	186	308	326	156	156	188	188
SR023.33	142	152	110	124	114	114	137	137	186	196	359	361	156	156	188	188
SR024.01	137	140	110	110	114	114	137	137	186	186	308	310	156	168	188	189
SR025.04	125	134	124	124	114	114	137	137	186	186	308	308	156	156	188	188
SR025.12	125	140	110	124	114	114	137	139	186	186	310	315	156	156	188	188
SR027.01	123	132	110	124	114	114	137	137	186	188	308	308	156	156	188	188
SR027.03	128	130	110	124	114	114	137	139	186	188	308	332	156	156	188	188
SR027.04	104	125	110	124	114	114	137	141	186	186	310	347	175	177	188	188
SR027.08	121	142	110	110	114	114	137	141	186	188	308	328	175	177	188	188
SR027.12	121	132	110	124	114	114	137	137	186	186	308	343	156	156	188	188
SR028.01	117	121	124	124	114	114	137	137	188	196	343	349	156	156	188	188
SR028.02	132	152	110	124	114	114	137	137	186	188	343	355	156	173	188	188
SR028.07	117	134	110	124	114	114	137	139	188	188	308	343	156	156	188	188
SR028.15	130	134	110	112	114	114	137	141	188	196	308	345	156	173	188	188
SR028.16									186	186	308	315	156	173	188	188
SR028.18	119	123	110	124	114	114	137	137	186	186	308	313	156	173	188	188
SR028.21	125	139	110	116	114	114	137	137	186	188	308	310	156	156	188	188
SR028.26	111	134	124	124	114	114	137	139	186	188	308	339	156	156	188	188
SR028.28	125	142	110	110	114	114	137	141	162	196	315	315	156	156	188	189
SR029.03	121	128	110	110	114	114	137	139	186	186	308	308	155	156	188	188
SR029.05	104	125	110	110	114	114	137	141	186	186	308	315	150	156	188	188
SR029.07	123	128	110	124	114	114	137	137	186	186	349	353	150	150	188	189
SR029.09	104	128	110	110	114	114	137	137	186	186	308	315	150	156	180	188
SR029.10	115	121	110	124	114	114	137	137	186	186	308	308				
SR029.12	121	134	110	110	114	114	128	137	186	186	308	308	156	156		

Specimen voucher	1a	1b	3a	3b	7a	7b	15a	15b	8a	8b	16a	16b	9a	9b	11a	11b
SR029.13	127	132	110	124	114	114	128	137	186	188	308	353	156	156	188	188
SR032.01	119	119	110	110	114	114	123	137	186	188	349	349	149	156	188	189
SR032.03	119	119	110	124	114	114	137	137	186	186	349	349	156	158	188	188
SR033.01	119	123	110	110	114	114	137	137	186	186	292	349	150	156	188	188
SR033.02	119	119	110	124	114	114	128	139	186	192	339	347	150	156	184	188
SR035.01	125	144	110	124	114	114	137	137	186	196	357	357	156	158	188	190
SR036.01	132	134	110	124	114	114	137	139	186	196	308	351	156	156	188	188
SR036.02	144	146	110	124	116	116	139	139	186	196	351	353	150	156	148	188
SR036.04	134	134	110	110	114	114	137	137	186	196	308	357	150	156	148	148
SR038.01	121	121	110	110	114	114	137	137	186	186	347	347	156	156	148	188
SR038.03	121	127	110	124	114	114	133	137	186	186	347	347	150	156	148	148
SR047.19	115	117	110	110	114	114	137	139	186	198	308	308	156	156	188	188
SR050.01	119	123	110	124	114	114	137	137	186	186	345	347			188	188
SR050.03	119	130	110	110	114	114	137	139	186	196	310	347	156	156		
SR050.05	115	119	110	110	114	114	137	137	186	196	308	308	156	156		
SR051.01	117	117	110	110	114	114	137	139	186	196	270	308	170	183	190	190
SR053.01	119	119	110	124	114	114	137	141	186	186	313	349	183	183	190	190
SR053.02	119	119	110	110	114	114	137	137	186	188	308	349	168	175	190	190
SR053.03	113	127	124	124	114	114	137	137	186	186	306	351	172	172	190	190
SR062.01	123	123	124	124	114	114	131	137	186	186	355	355	172	173	190	190
SR063.01	115	123	110	124	114	114	137	137	186	196	351	351	172	183	190	190
SR063.03	115	115	124	124	114	114	128	137	196	198	308	367	168	173	190	190
SR065.02	106	106	110	124	114	114	133	137	184	186	280	324	168	170	190	190
SR065.03	113	113	110	110	116	116	139	139	186	186	302	355	168	177	190	190
SR065.04	119	121	110	110	114	114	137	137	186	186	302	308	170	172	190	190
SR065.05	123	123	110	124	114	114	133	137	186	200	347	355	170	170	190	190
SR065.06	121	140	110	110	114	114	137	139	186	186	302	343	168	168	188	188
SR066.02	121	127	110	124	114	114	137	141	186	186	308	315	156	173	188	188
SR066.03	125	128	110	110	114	114	137	137	186	186	345	349	156	172	188	188
SR066.04	119	119	110	110	114	114	135	137	186	186	347	349	156	156	188	188
SR066.05	108	128	110	110	114	114	137	137	186	186	345	361	156	172	188	188
SR066.06	111	121	112	126	116	116	139	139	186	186	308	353	156	156	188	188
SR067.02	104	119	124	124	114	114	137	137	186	196	343	361	156	168	188	188
SR067.03	121	134	112	112	116	116	139	139	186	186	347	347	156	156	188	188
SR067.04	117	121	110	110	114	114	135	139					156	156	188	188
SR067.05	104	123	110	110	114	114	137	137	186	193	357	361	156	156	188	188
SR067.06	117	117	110	110	114	114	137	137	186	193	347	353	156	156	188	188
SR068.02	123	123	110	110	114	114	128	128	196	196	308	353			184	190
SR068.03	121	121	112	126	116	116	139	139	186	186	308	361	156	156	188	188
SR068.04	119	119	110	126	116	116	139	139	186	192	345	345	156	172	188	188
SR068.05	119	125	110	124	114	114	137	141	186	186	351	355	156	156	188	188
SR068.06	117	132	110	110	114	114	137	139	186	186	308	353	156	156	188	188
SR070.02	119	130	110	124	114	114	137	137	186	186	351	351	156	168	188	188
SR070.08	132	137	124	124	114	114	137	137	186	186	345	351	156	158	188	188
SR070.09	104	119	124	124	114	114	137	137	186	186	345	353	168	168	188	188
SR070.13	119	137	110	124	114	114	137	137	186	196	345	355	156	168	188	188
SR070.16	130	132	110	110	114	114	135	137	186	192	347	353	156	168	188	188
SR070.19	104	121	126	126	116	116	139	139	186	186	371	373	155	168		
SR070.28	111	119	110	110	114	114	137	137	186	186	308	347	168	168	188	188
SR070.31	121	121	124	124	114	114	137	137	186	196	343	345	168	168	188	188
SR075.04	99	108	110	110	114	149	161	161			273	273				
SR075.08	104	108	110	110	149	149	161	182			273	273	156	156	188	188
SR077.02	119	134	124	124	114	114	139	139	186	196	308	351	156	156		
SR077.03	123	130	110	110	114	114	137	137	186	186	332	332	156	168	188	188

Specimen voucher	1a	1b	3a	3b	7a	7b	15a	15b	8a	8b	16a	16b	9a	9b	11a	11b
SR078.02	119	121	124	124	114	114	137	137	196	198	345	345	156	158	188	188
SR078.03	104	123	110	124	114	114	137	137	186	186	353	353				
SR078.10	123	128	110	110	114	114	137	141	186	198	343	355	156	168	188	188
SR079.03	119	121	110	124	114	114	137	137	186	188	345	355	156	168		
SR079.04	119	128	110	110	114	114	137	137	186	188	353	359	156	168	188	188
SR079.06	128	128	110	110	114	114	137	137	186	188	345	359	156	168	188	188
SR079.07	111	132	126	126	116	116	139	139	188	196	345	345	168	168		
SR080.02	104	113	110	110	114	114	137	137	186	188	351	353	156	156	188	188
SR080.03	130	130	110	110	114	114	137	137	186	186	353	353	156	156		
SR080.05	113	113	110	110	114	114	137	137	186	186	351	353	156	156	188	188
SR081.02	104	121	124	124	114	114	137	137	186	186	326	339	156	156	188	188
SR081.06	121	142	110	124	114	114	137	137	186	186	308	308	156	156	188	188
SR081.09	119	119	110	124	114	114	137	137	186	186	349	353	156	156	188	188
SR081.13	119	121	110	110	114	114	137	137	186	186	313	353	156	156	188	188
SR087.01	130	130	110	110	114	114	137	154	186	186	273	273	156	162	188	190
SR091.03	119	121	110	110	135	135	135	144	188	188	294	297	156	156	164	189
ZF-LY-000370	99	99	110	110	135	135	141	141	186	200	300	300	156	158	189	189
ZF-LY-000371	100	102	110	110	135	135	139	146	188	188	369	371	156	158	189	189
ZF-LY-000373									200	201	306	373	158	158	189	189
ZF-LY-000374	100	102	110	110	114	135	141	148	200	200	359	371	158	158	189	189
ZF-LY-000378	100	100	110	110	114	135	141	141	200	201	306	306	158	158	189	189
ZF-LY-000379	100	102	110	110	135	135	141	141	184	184			156	158	189	189
ZF-LY-000384	100	100	110	110	135	135	141	141	186	200	359	359	158	158	189	189
ZF-LY-000386									200	200	357	359	158	158	189	189
ZF-LY-000387	100	100	110	110	112	135	141	141	200	200	306	306	156	158	189	189
ZF-LY-000343	102	102	102	102	114	135	152	154			335	337	156	156	148	188
ZF-LY-000344	102	102			114	114	152	158								
ZF-LY-000345	102	102	102	102	135	135			186	186	343	347	156	156	188	188
ZF-LY-000346	102	102			135	135	156	170					156	156	188	188
ZF-LY-000347	102	102	102	102	135	135	156	170	186	186	313	313				
ZF-LY-000348	99	99	124	124	135	135	172	172	165	216	339	339				
ZF-LY-000349	99	99	110	110	112	135	158	158	186	186	337	339	156	156	148	188
ZF-LY-000354	99	99	110	110	114	135	135	144	165	165	308	339	156	156	188	189
ZF-LY-000364	102	102	110	110	135	135	144	152	165	165	339	339	156	156	148	148
ZF-LY-000366	102	102	110	110	135	135	154	154			328	328	156	156	188	188
ZF-LY-000367	102	108			135	135	178	180			320	320	156	156	148	188
ZF-LY-000339	99	102	102	106	114	135	135	187	186	186	300	300	156	156	148	188
ZF-LY-000340	102	102	102	102	114	114							156	156	148	188
ZF-LY-000341	102	102	104	104	114	114	187	187	186	186			156	156	148	184
ZF-LY-000342	100	102	102	102	114	114	135	199	186	186			156	156	148	148
ZF-LY-000336	102	102	110	110	112	135	156	202	186	186	278	292	156	156	188	188
ZF-LY-000316	99	106	110	114	114	114	137	144			297	297	156	156	189	189
ZF-LY-000317	100	104	110	110	114	114	137	152	190	190	302	302	156	156	174	184
ZF-LY-000333	100	100	110	110	135	135	128	137	184	186			160	160	188	189
SR019.11	104	106	142	148	120	122	135	139	190	203	267	292	164	172	190	190
SR020.02	104	104	161	163	118	120	133	133	192	192	271	349	183	217	190	190
SR021.01	104	104	145	150	120	120	131	139	200	204	287	291	190	206	190	190
SR021.03	104	104	145	150	120	122	133	139	200	204	276	291	190	204	190	190
SR021.38	104	106	145	161	120	120	133	133	196	204	263	291	172	172	190	190
SR022.01	104	104	150	163	122	122	133	133	190	204	271	326	170	200	190	190
SR022.03	104	104	142	150	122	122	125	133	190	203	271	326	172	198	190	190
SR022.05	104	104	152	161	120	122	133	139	190	190	263	270	185	206	190	190
SR022.07	104	106	157	157	118	120	125	133	169	182	267	271	172	185	190	190
SR023.03	104	104	143	145	120	122	133	139	190	190	276	320	173	194	190	190

Specimen voucher	1a	1b	3a	3b	7a	7b	15a	15b	8a	8b	16a	16b	9a	9b	11a	11b
SR023.31	106	106	143	145	122	133	133	133	176	186	292	334	210	212	190	190
SR024.03	104	106	143	161	122	133	125	131	184	201	267	285	172	172	190	190
SR024.08	104	104	157	163	122	132	133	133	186	198	270	321	198	200	190	190
SR024.36	106	108	157	173	120	122	121	126	190	196	263	291	172	173	190	190
SR025.01	104	106	152	159	122	122	125	133	193	226	287	291	170	177	190	190
SR025.02	104	104	152	169	118	122	125	133	196	226	267	343	164	173	190	190
SR025.07	106	106	139	159	118	118	133	133	200	226	326	328	172	172	190	190
SR025.10	104	104	143	169	122	122	133	133	176	226	267	287	177	196	190	190
SR026.05	104	106	142	159	120	122	126	133	200	237	266	267	173	175	190	190
SR026.06	104	106	159	161	122	122	133	139	206	220	267	331	168	168	190	190
SR026.53	104	106	139	165	118	122	133	133	192	196	266	289	183	200	190	190
SR028.24	104	106	152	154	120	122	133	139	188	193	276	304	172	172	190	190
SR028.32	104	104	143	157	118	120	133	139	188	190	263	326	172	175	190	190
SR031.01									182	192	263	267	183	185	190	190
SR031.02	104	106	135	145	120	122	125	133	182	192	263	270	183	185	190	190
SR031.03	106	106	135	159	118	120	133	133	190	200	326	341	172	172	190	190
SR032.02	163	169	130	142	120	120	123	131	226	230	273	273	172	172	190	190
SR034.01			154	157	122	122	135	135	145	145	220	222	190	192	190	190
SR034.02			157	157	122	122	135	135	145	145	210	212	170	192	190	190
SR034.03	119	119	157	157	122	122	135	135	145	145	210	220	190	192	190	190
SR039.01	171	179	130	165	120	120	131	135	200	200	204	273	158	158	192	192
SR039.02	159	195	130	142	120	120	135	139	200	228	273	273	170	177	192	192
SR040.01	163	165	139	139	116	116	117	123	200	208	173	287	158	170	192	192
SR041.01	159	162	145	145	120	120	135	144	160	188	335	335	170	181	190	190
SR041.03	104	104	154	161	120	120	133	137	160	220	202	335	179	181	190	192
SR042.01			143	152	120	120	133	139	175	198	206	270	170	170	190	192
SR042.02	104	104	143	152	120	120	135	137	162	198	177	270	173	181	190	190
SR042.03	159	162	145	161	120	120	133	139	206	210	192	196	164	170	190	190
SR043.01	154	155	130	130	120	120	126	135	175	200	200	271	170	172	192	192
SR043.02	167	169	130	142	120	122	131	135	243	245	200	202	170	172	192	192
SR044.02	148	163	130	130	120	120	131	135	224	228	273	273	170	170	190	190
SR045.13	146	148	130	130	120	120	135	139	200	231	271	273	177	177	190	192
SR047.08	104	106	143	150	122	122	133	133	198	204	263	324	173	173	190	190
SR047.11	104	106	143	161	120	120	126	133	200	214	263	328	172	173	190	190
SR047.17	104	106	130	157	120	122	126	133	190	190	263	328	168	179	190	190
SR047.18	104	106	145	171	120	120	139	139	188	190	263	337	172	172	190	190
SR048.01	104	106	150	159	120	125	133	133	169	188	263	266	156	173	188	188
SR048.02	104	106	145	148	120	120	133	133	186	190	263	331	156	156	188	188
SR048.06	108	115	150	159	120	132	133	133			266	266				
SR048.09	106	115	145	154	120	122	133	133	216	220	263	263	168	168		
SR052.01	104	115	157	157	120	132	139	139	186	190	263	263	156	156	188	188
SR053.08	106	106	130	145	122	132	133	133	190	206	263	328	172	172	190	190
SR054.01									186	200	263	263	156	156	188	188
SR055.01	113	137	143	143	120	122	135	135	160	175	171	171	170	175	190	190
SR055.02	140	142	167	167	116	120	135	144	175	188	171	192	170	224	190	190
SR055.03			145	145	116	120	135	144	160	160	171	171	226	228	190	190
SR055.04	104	104	143	143	116	120	135	144	160	160	171	331	170	170	190	190
SR055.05	106	117	152	152	116	120	135	144	160	206	171	171	170	170	190	190
SR058.01	130	130	169	171	135	137	139	139	201	206	263	263	168	168	190	190
SR058.02	130	130	145	163	135	139	139	141	201	210	263	263	168	168	190	190
SR059.01	102	106	128	148	120	120	133	139	196	203	308	313	170	177	190	190
SR060.02									196	196	306	306	170	170	190	190
SR060.05	106	106	150	150	120	120	133	139	196	196	306	306	170	170	190	190
SR062.05	104	104	145	150	120	120	133	139	160	166	202	270	173	179	190	190

Specimen voucher	1a	1b	3a	3b	7a	7b	15a	15b	8a	8b	16a	16b	9a	9b	11a	11b
SR062.06					120	120	133	137	190	208	171	196	170	181	190	190
SR062.07	198	200	145	150	120	122	131	137	160	160	198	331	170	175	190	190
SR062.08	104	104	145	145	120	120	133	137	160	190	194	270	170	179	190	190
SR062.09	104	104	145	145	120	120	131	139	190	198	190	194	175	181	190	190
SR064.04	159	196	137	139	122	122	117	123	233	235	208	210	170	172	190	190
SR064.05	117	117	137	137	120	122	123	123	188	188	208	210	170	172	190	190
SR066.11	115	115	152	159	120	120	117	123	226	235	285	291	173	173	190	190
SR066.12	128	144	152	152	120	120	135	144	184	190	192	304	172	172	190	190
SR066.13	119	128	145	171	120	120	126	141	180	198	292	334	172	188	190	190
SR066.14	104	108	154	169	120	120	117	123	180	184	285	285	172	177	190	190
SR067.08	106	128	148	148	120	120	119	126	186	201	194	196	172	172	190	190
SR068.11	198	200	148	150	120	120	123	135	184	192	190	331	172	172	190	190
SR068.13	115	115	148	150	120	120	126	126	184	190	326	331	172	172	190	190
SR068.14	100	130	148	150	120	120	135	135	201	201	190	190	172	179	190	190
SR068.15	115	134	148	148	120	120	126	139	184	192	198	266	172	172	190	190
SR069.01	121	121	137	154	118	120	123	144	200	235	188	190	170	172	190	190
SR070.05	121	121	135	139	120	120	135	135	210	210	181	186	172	172	190	190
SR070.14	121	123	135	145	120	122	126	135	188	200	186	331	172	175	190	190
SR072.01	104	104	161	161	122	122	141	141	200	200	192	200	170	172	190	190
SR072.02	104	104	161	161	122	122	141	141	200	212	192	200	170	170	190	192
SR073.01	188	190	133	152	124	124	137	139	192	212	171	196	172	177	190	190
SR073.02	104	104	145	152	120	120	137	139	162	162	196	202	170	175	190	190
SR073.03	104	104	152	161	118	118	139	139	160	160	202	204	170	173	190	192
SR073.04	106	106	137	159	124	124	139	139	192	198	171	202	170	177	190	190
SR073.05	104	104	161	161	120	120	139	139	160	198	202	204	170	170	190	192
SR073.06	104	104	145	145	120	120	139	139	160	188	194	204	173	173	190	190
SR074.02	206	208	130	142	120	120	135	139	200	200	271	323	170	177	190	190
SR074.03	196	198	135	142	120	120	135	146	200	224	321	321	177	177	190	190
SR074.04									224	226	271	297	172	172	190	192
SR074.05	140	142	135	142	120	120	135	135	224	226	271	323	170	177	190	190
SR074.07	140	140	135	142	120	120	135	146	200	200	271	323	177	177	190	190
SR074.08	140	140	130	142	120	120	123	135	200	224	196	323	170	170	190	190
SR074.09	198	200	130	135	120	120	123	135	200	200	196	323	170	177	190	190
SR077.05	106	106	148	150	120	120	126	133	184	190	320	335	190	192	190	190
SR077.06	142	142	145	145	122	122	133	146	188	193	282	294	160	187	190	192
SR078.04	119	119	122	122	120	122	133	135	212	216	246	250	172	173	190	190
SR078.06	119	121	145	148	120	122	135	135	200	216	240	242	156	172	188	188
SR078.07	121	121	148	148	120	120	133	135	216	216	181	181	156	156	188	188
SR078.08	121	121	122	122	120	122	133	133	200	216	240	242	156	156	188	188
SR083.01	106	115	152	157	122	122	146	148	200	206	263	263	170	183	190	190
SR088.01	123	134	139	139	120	120	133	133	198	198	192	194	156	156	188	188
SR088.02	123	134	152	165	120	122	133	133	190	196	175	175	156	156	188	188
SR089.01	142	142	148	148	120	120	133	133	184	192	171	171	156	156	188	188
SR101.01	106	106	139	139	120	120	133	133	186	186	181	184	181	183	190	190
SR101.02	106	106	139	139	120	120	133	144	186	186	270	335			190	190

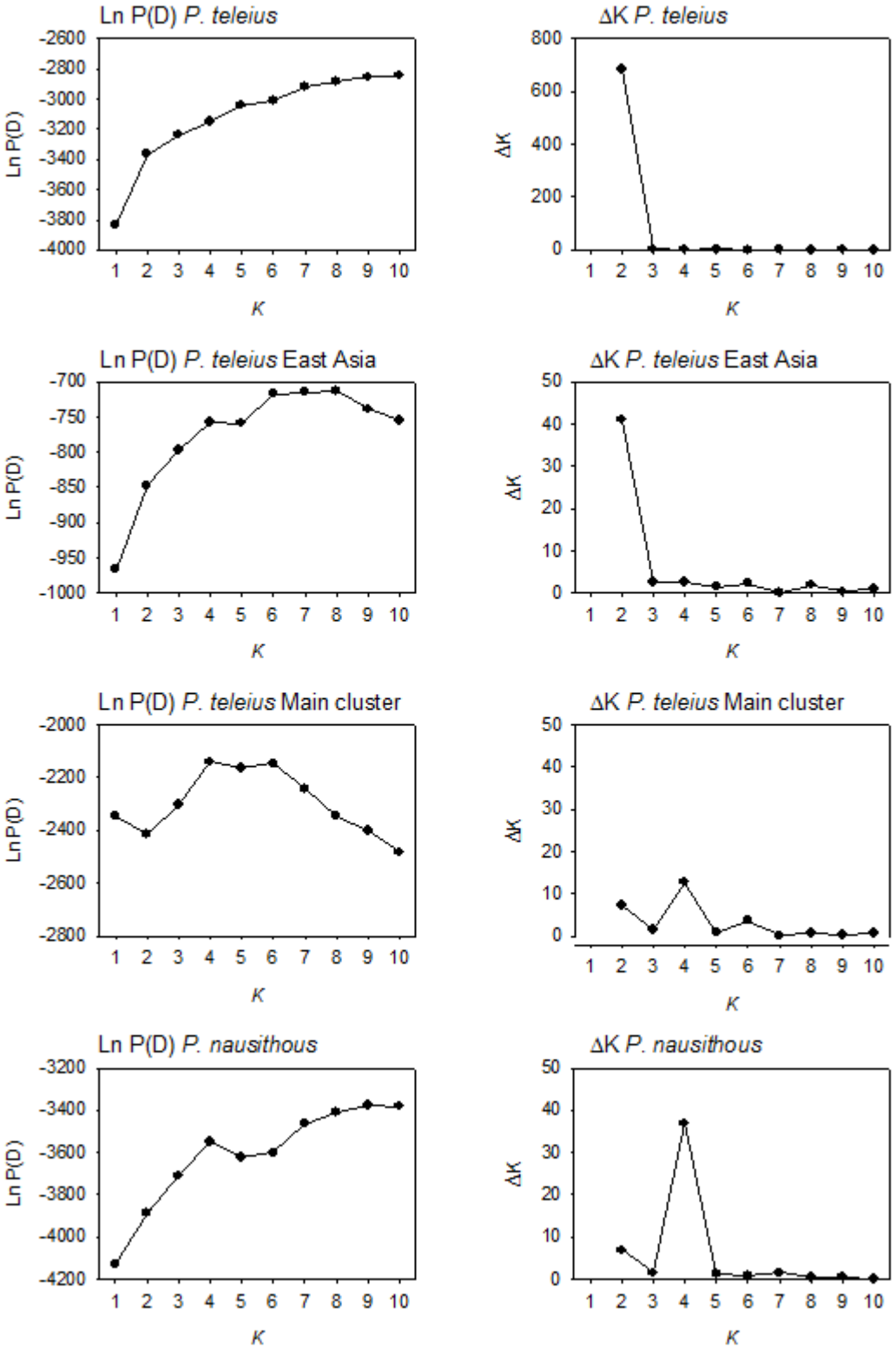

Figure 7: Determination of the most likely *K* of the STRUCTURE analyses according to Evanno *et al.* (2005). Mean values of Ln P(D) and Δ*K* as a function of *K* for different data sets of *P. (M.) teleius* and of *P. (M.) nausithous.*

Erklärung über den persönlichen Anteil

Nachfolgende Tabelle gibt einen Überblick über meinen persönlichen Anteil an der wissenschaftlichen Publikation mit Co-Autorenschaft "*Wolbachia* infections mimic cryptic speciation in two parasitic butterfly species, *Phengaris teleius* and *P. nausithous* (Lepidoptera: Lycaenidae)" (Ritter *et al.* 2013), dessen Inhalte in vorliegende Dissertation eingegangen sind und von mir um weitere, bisher nicht veröffentlichte, weiterführende Informationen, Interpretationen und Empfehlungen ergänzt worden sind:

	SR (%)	**SGM**	**JS**	**MW**	**ZFF**	**MSi**	**MSa**	**YR**	**WD**
Idea	X (95)		x						x
Sampling	X (50)		x	x	x	x	x	x	
Data gathering in Lab	X (95)	x							x
Analysis	X (95)	x		x					x
Figures	X (95)	x		x					
Manuscript	X (95)	x	x	x					x

SR = Sylvia Ritter, **SGM** = Stefan G. Michalski, **JS** = Josef Settele, **MW** = Martin Wiemers, **ZFF** = Zdenek F. Fric, **MSi** = Marcin Sielezniew, **MSa** = Martina Šašić, **YR** = Yves Rozier, **WD** = Walter Durka

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Eidesstattliche Versicherung

Hiermit versichere ich, Sylvia Ritter, geboren am 01.12.1978, an Eides statt, dass ich die vorliegende Dissertationsschrift mit dem Titel „Are there really cryptic species within the myrmecophilous butterfly species *Phengaris (Maculinea) teleius* and *P. (M.) nausithous* (Lepidoptera: Lycaenidae)? Analyses across Eurasian distribution ranges, confusing effects of the endosymbiotic bacterial parasite *Wolbachia*, and implications for *Phengaris (Maculinea)* conservation“ selbständig und ohne fremde Hilfe verfasst und keine anderen als die angegebenen Quellen oder Hilfsmittel benutzt habe. Die Stellen der Arbeit, die wörtlich oder inhaltlich anderen Arbeiten entnommen wurden, sind in jedem Fall unter Angabe der Quelle(n) kenntlich gemacht. Die Arbeit ist noch nicht in anderer Form oder zu einem früheren Zeitpunkt als Prüfungsleistung der Naturwissenschaftlichen Falkultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg oder einer anderen wissenschaftlichen Einrichtung zur Promotion vorgelegt worden. Darüber hinaus erkläre ich, dass ich mich hiermit erstmalig um einen Doktorgrad bewerbe.

Bingel, 11.01.2017

Ort, Datum

Sylvia Ritter

Unterschrift