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Evoluce skupiny Parnassiinae

Evolutionary history of Parnassiinae

Bakalářská práce

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"Gothland ist es! Hier hat Linné gesammelt; den gothländischen aber hat er zuerst beschrieben; also einen insulären Apollo."

Felix Bryk, 1911

In: Der Linné'sche Apollo. *Internationale Entomologische Zeitschrift,* 5(20): 141-143.

"Die Nominatform habe ich aufgefunden, ich beschreibe sie so genau, daß kein Haar daran zu rütteln sein wird, bilde sie mehrmals ab; es bleibt mir also das freie Autorenrecht, diese bis jetzt unbekannte Rasse zu benennen. Um nun das etwaige Vorhaben der Benenner, den gotlandischen Apollo als Parnassius Apollo L. Apollo zu verzeichnen, zu vereiteln, benenne ich ihn Parnassius Apollo L. var. Linnei m."

Felix Bryk, 1913

In: „Parnassiana"'. VIII. Noch einmal über den Linnéschen Apollo. γ) Die nomenklatorische Behandlung des fennoskandischen Apollos. *Societas Entomologica,* 28(8): 31-34.

Contents

Abstract

This review is devoted to the evolutionary history of the Parnassiinae subfamily of the family Papilionidae and particularly to the nominate genus *Parnassius* which is the main group among the eight extant Parnassiinae genera. At first, the taxonomic concept, monophyly, and phylogenetic position of Parnassiinae are thoroughly discussed. It is shown that all its genera form three clades that are currently classified as tribes. Further, the estimated time of the divergence of Parnassiinae is given together with the description of all fossil taxa. The subsequent part is entirely focused on *Parnassius* whose internal phylogeny largely corroborates the traditional classification on eight subgenera, although the phylogenetic relationships among these clades remain mostly unresolved. All the clades are systematically discussed and interesting phylogenetic cases of species complexes and closely related taxa are analysed and supplemented by taxonomic remarks. The last three chapters are dedicated to the phylogeography of *P. mnemosyne*, *P. apollo*, and *P. phoebus* complex. Their evolutionary history is discussed in relation to glacial events in Pleistocene and warming of the climate in Holocene. The whole treatment attempts to integrate morphological, molecular, and biogeographical views together in order to provide a complete framework for a relevant phylogenetic and possibly taxonomic discussion.

Key words: Parnassiinae, *Parnassius*, molecular phylogeny, evolutionary history, phylogeography, semispecies, species complex, subspecies

Abbreviations

16S – 16S ribosomal RNA gene

BP – Before Present years

bp – Base Pair

COI – Cytochrome c Oxidase subunit I gene

COII – Cytochrome c Oxidase subunit II gene

E – East/eastern

EF-1α – Elongation Factor-1α gene

GTR – General Time-Reversible model of nucleotide substitution

Γ – gamma distribution in an evolution model

HKY – Hasegawa, Kishino and Yano model of nucleotide substitution

I – Invariants in an evolution model

IM – Isolation with Migration model

ICZN - International Commission on Zoological Nomenclature

JC – Jukes-Cantor model

ka – kiloannus (one thousand years)

LGM – Last Glacial Maximum

LSU – Large Subunit ribosomal RNA gene

Ma – megaannus (one million years)

MCMC – Markov Chain Monte Carlo

ML – Maximum Likelihood

MP – Maximum Parsimony

N – North/northern

ND1 – NADH dehydrogenase, subunit 1 gene

ND5 – NADH dehydrogenase, subunit 5 gene

NJ – Neighbor Joining method

S – South/southern

s. l. – *sensu lato*

s. str. – *sensu stricto*

tMRCA – Time to the Most Recent Common Ancestor

UPGMA – Unweighted Pair Group Method with Arithmetic Mean

W – West/western

Introduction

"Ich kümmere mich aber nicht um die Nomenklaturregeln und werde künftighin den südfinnischen Apollo var. nylandicus Rothsch. = finnmarchicus Rothsch. nennen." Felix Bryk, 1911, in: Der Linné'sche Apollo. (Fortsetzung.) [1]

All the entomologists, all the specialists in certain taxa as well as all the insect collectors believe that the group of their interest - whichever order, family or genus of insects they are dealing with - is the most interesting and the most attractive one among all the dull rest. No matter if they deal with birdwing butterflies of the genera *Ornithoptera* and *Troides*, no matter if they collect *Colias* or study large Lucanidae beetles of the Prioninae subfamily, all of them share something. All of them are absolutely WRONG, of course, with the only exception of parnassiologists. However, parnassiology is also the most decadent field of entomology as the kind reader might know or may reveal, hopefully, somewhere else than in this short review. It is entirely devoted to the unique clade of Parnassiinae and particularly to the astonishing butterflies of the genus *Parnassius*.

A pair of butterfly wings exhibit a symmetry termed bilateral symmetry in morphology whilst reflectional one in physics. By all means, it is neither this type of relation nor any symmetry element that relates the forewing and the hindwing of a butterfly. If any biological term should be used here, it is the homology, specifically, the iterative homology which brings us to its antipode being an analogy. The wings of a birdwing and the wings of a bird are a poetic example but we will offer another analogy to the reader. The analogy between the evolutionary history of *Parnassius* and the history of parnassiology. The *Parnassius* clade originated during the early Eocene and underwent a rapid radiation in the mountains of Central Asia and on the Tibetan Plateau. In Pleistocene the very plastic *P. apollo* (Linnaeus, 1758) behaved as a glacial invader in Europe and diversified here in multiple glacial refugia. Now Apollo is seriously decreasing, many populations of *P. apollo* became extinct and some of them were rather eradicated. The second act follows. In 1695 the apothecary James Petiver from London wrote [2]: "*Papilio Alpinus ex albo nigroque variegatus, Iride rubente oculatus. This Mr. Richard Wheeler brought me from Norway: the same I saw with the most celebrated Botanist Mr. John Ray, who long since observed it about the Alps.*" This is probably the first preserved note regarding the butterfly species which was later described by Carl von Linné as *Parnassius apollo* [3]. During the Golden Age of parnassiology more than 280 subspecies, many of them being synonyms, and hundreds of individual forms of *P. apollo* were described. Although only around fifty *Parnassius* species might exist, the total number of the proposed names for various *Parnassius* taxa far exceeds one thousand reflecting both their enormous geographic variability and popularity. Nonetheless, at present no professional entomologist would write a detailed treatment of all these names. *Nusquam est vere novus.* Hence, the general scheme for both the members of this analogy might be as follows: origination - radiation - extinction.

This review will focus on the evolutionary history of Parnassiinae with special regard to *Parnassius*. It is not a systematic treatment but a basic taxonomic framework will be indispensable. The group comprises well known butterflies of primarily Palaearctic distribution whose larvae feed predominantly on Fumariaceae, Aristolochiaceae, Crassulaceae, and Scrophulariaceae. Most of them are subalpine or alpine creatures with several taxa distributed above 5000 m. The number of species varies considerably around 70 due to different opinions on the status of many taxa of the *Parnassius* genus where around 50 species might exist.

Parnassiinae

Taxonomic concept of Parnassiinae

Among the three widely recognized subfamilies of the extant[1] Papilionidae Latreille, [1802] being the nominotypical subfamily Papilioninae Latreille, [1802], the monobasic Baroniinae Bryk, 1913 and the subfamily Parnassiinae Duponchel, [1835], the last one forms a very distinct group of butterflies comprising roughly one eighth of the species described within the Papilionidae family. At present, there is a general agreement on its taxonomic position as a subfamilly of Papilionidae, and the most recent studies based on the so-called total evidence approach clearly demonstrate its monophyly in the following sense. The current concept of Parnassiinae comprises eight extant genera: the type genus *Parnassius* Latreille, 1804; *Hypermnestra* Ménétriés, 1846; *Luehdorfia* Crüger, 1878; *Archon* Hübner, 1822; *Sericinus* Westwood, 1851; *Bhutanitis* Atkinson, 1873; *Zerynthia* Ochsenheimer, 1816, and *Allancastria* Bryk, 1934. Furthermore, two fossil genera are included: *Doritites* Rebel, 1898 and *Thaites* Scudder, 1875.

The taxon Parnassiinae was established in 1835 by Philogène Auguste Joseph Duponchel in the monumental work *L'Histoire naturelle des lépidoptères de France* of seventeen volumes that he wrote together with Jean Baptiste Godart. On the page 380 of the supplement to the first volume [5], he classified the genus *Parnassius* and the genus *Doritis* Fabricius, 1807 *sensu* Hübner, [1819] (= *Archon* Hübner, 1822; the genus name *Doritis* was used erroneously by Duponchel[2]) under the new taxon "*Parnassides*. Mihi". In the obsolete classification of those times Duponchel considered Parnassides as a "tribu" (= tribe in the French language) of the "famille" (= familly) Diurna Latreille, 1809 (= Rhopalocera) next to the "tribu *Papilionides*. Latr." (= Papilionidae). Interestingly, he placed the genus *Thais* Fabricius, 1807 (= *Zerynthia*) in Papilionides, and not in Parnassides.

The name Parnassiinae in its current form was mentioned for the first time by Swainson in 1840 in the book *On the History and Natural Arrangement of Insects* [8]; therefore, certain sources cite the authorship as Parnassiinae Swaison, 1840 instead of Parnassiinae Duponchel, [1835]. Swainson used a broad concept of Papilionidae that he subdivided on: "Papilioninae", "Pierinae", "Colianae", "Lycaeninae" (it should be Licininae)[3], and "Parnassiinae". Under the Parnassiinae subfamily he placed *Parnassius* and *Thais* (= *Zerynthia*). The Swainson's classification of butterflies might be surprising for us, but we have to keep in the mind that the early conception of taxonomic ranks was very different from the current approach. Thus, the original conception of even common families and

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[1] The Papilionidae family includes also the fossil subfamily Praepapilioninae Durden & Rose, 1978 that contains two known species, namely *Praepapilio colorado* Durden & Rose, 1978 and *P. gracilis* Durden & Rose, 1978. These taxa were described from the middle Eocene Florissant fossil beds in Colorado on the basis of single specimens [4].

[2] The genus name *Doritis* was misinterpreted by Hübner in 1819 [6] who placed here the species *Papilio thia* Hübner, [1806] which is a junior synonym of *Archon apollinus* (Herbst, 1789). Obviously, Hübner did not reflect that the type species of the genus *Doritis* is *Papilio apollo* Linnaeus, 1758. Therefore *Doritis* is a junior synonym of *Parnassius* and not a synonym of *Archon* [7]. However, many subsequent authors including Duponchel placed *Archon* spp. to the genus *Doritis*.

[3] The given use of the name Lycaeninae was probably only a typographic mistake albeit repeated through his work. Actually, Swainson was writing about a small group of butterflies comprising *Licinia amphione* (Cramer, [1779]) currently classified as *Dismorphia* Hübner, 1816.

genera is sometimes deviated from the present one. By all means, it should be noted that the Parnassiinae subfamily was treated by Swainson as a taxon separate from all other Papilionidae in the current sense.

The last mentioned view brings us to the taxonomic rank of Parnassiinae that was also considered as a distinct family by many earlier authors, e.g. by the most famous parnassiologist Felix Bryk [9, 10] who divided the family Parnassiidae into Parnassiinae and Zerynthiinae. Similar division of the current Parnassiinae subfamily into two separate subfamilies was assumed by E. B. Ford, but he kept both the Parnassiinae and Zerynthiinae in the family Papilionidae [11, 12]. Several entomologists went even further in designation of new families than F. Bryk, for example H. K. Clench [13] and F. Hemming [14] considered the tribes Parnassiini and Zerynthiini as distinct families Parnassiidae and Zerynthiidae [7]. Nevertheless, most of the later lepidopterological authorities dealing with butterflies or specifically with Papilionidae, like P. R. Ehrlich [15], E. Munroe [16] and P. R. Ackery [7] classified the Parnassiinae as a subfamily of Papilionidae with Zerynthiini included as its tribe. The modern entomologists with few exceptions[4] have not questioned this arrangement anymore. However, the traditional parnassiology, though represented by several outstanding experts, has been reluctant to fully accept these results. Even Curt Eisner (1890 - 1981), probably one of the most prolific authors in the field, treated Parnassiinae as a separate family Parnassiidae in his classical works published within the famous series *Parnassiana Nova* in *Zoologische Medelingen* (later *Zoologische Verhandelingen*), including his later reviews [20, 21].

Finally, the monotypic genus *Baronia* Salvin, 1893 was treated by some historical authors [22] as a member of the Parnassiinae subfamily (the Parnassiidae family at that time). The only species is the ancient relic *B. brevicornis* Salvin, 1893 occurring on a very restricted area in Mexico and showing many plesiomorphic traits. There is a general agreement in modern entomology that *Baronia* forms a separate subfamily which is a sister group of all the remaining Papilionidae.

Closing this part, we should shortly characterize the Parnassiinae subfamily which have been defined above only by an enumerative list of genera in order to distinguish them from other Papilionidae, and to show their common attributes. According to several authors [15, 23-25] the supposed synapomorphies of the Parnassiinae based on morphological characters are as follows. The third segment of palpi labiales is elongate. The pretarsi are asymmetrical. The wing venation of the forewing is characteristic by the incurved middle discocellular vein. The male genitalia contain a thin and extensively sclerotized (especially distally) aedeagus, while strongly sclerotized ostial region exhibiting a unique configuration is typical for females.

Monophyly and phylogenetic position of Parnassiinae

Although some alternative phylogenetic hypotheses have been suggested and despite the results of two recent molecular studies [24, 26] (*vide infra*), we will start by a statement that Baroniinae split from the common ancestor of the extant Papilionidae as the first branch while the remaining clade led to the sister groups

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[4] The following ones are worth of mentioning: L. G. Higgins elevated Zerynthiini again to the subfamily level in 1975 [17], C. L. Häuser suggested to remove *Hypermnestra* from Parnassiinae [18] while A. A. Stekolnikov and V. I. Kuznetsov have recently separated *Luehdorfia* to the subfamily Luehdorfiinae Tutt, 1896 on the basis of genitalia morphology [19].

Parnassiinae and Papilioninae [27]. This phylogeny is supported by a plethora of classical morphological studies as well as by the most recent works based on the total evidence approach which integrates molecular and morphological data. Specifically, the rigorous cladistic analyses of Hancock [28] and Miller [23] employed morphology. The former one was the first cladistic analysis of Papilionidae, and the latter one utilized extensive morphological datasets covering not only adult butterflies but also their preimaginal stages. Further, Tyler *et al.* [29] combined morphology with ecological and behavioural characters of both adults and juvenile stages. Finally, the detailed study by Simonsen *et al.* [27] employing 94 morphological characters and seven genes of the total length of 5616 bp provided especially convincing results regarding the phylogenetic position of Parnassiinae.

The monophyly of the subfamily has been supposed for a long time. An important contribution to its corroboration was done by Igarashi [30] who analysed the autapomorphies of Parnassiinae in the larvae and pupae. The strong support for the monophyly of Parnassiinae comes also from the morphological peculiarities of the genitalia as briefly described above. Opposite opinions have been very rare in modern phylogenetic studies of Papilionidae based on morphology [18, 19, 31]. De Jong *et al.* [31] suggested that the genus *Parnassius* might be the sister lineage to the remaining taxa of Papilionidae. However, this opinion is neither supported by molecular data nor in agreement with previous cladistic studies based on extensive morphological datasets. Further, Häuser [18] not only suggested to remove *Hypermnestra* from Parnassiinae, but he claimed that even the rest of the subfamily was not monophyletic.

Speaking about the molecular studies, the first ones, like Yagi *et al.* in 1999 [32] and Caterino *et al.* in 2001 [33] failed to support the monophyly of Parnassiinae. However, Yagi *et al.* used only a 784 bp fragment of just one mitochondrial gene, namely *ND5*, which might explain the results. According to this study Parnassiinae was found to be paraphyletic as Zerynthiini (represented by *Luehdorfia* and *Sericinus*) clustered with Papilioninae and not with Parnassiini (represented by *Parnassius*). The second study by Caterino *et al.* used DNA sequences of the total length of 3328 bp: mitochondrial *COI*, *COII* and nuclear *EF-1α*. They included *Parnassius* as the representative of Parnassiini while Zerynthiini were represented by *Zerynthia*, *Allancastria*, *Luehdorfia* and *Sericinus*. Further, *Baronia* was included and representatives of three Papilioninae tribes (Graphiini, Troidini, and Papilioniini]. They attempted to resolve the phylogeny using parsimony searches and likelihood analysis. The monophyly of Parnassiinae as a whole was favoured only in certain types of analyses (e.g. under the JC model). However, under more realistic models, especially the best-fitting one (GTR + *Γ* model) Parnassiinae seemed to be paraphyletic. The most likely topology supposed that *Parnassius* was a sister taxon to the clade Papilioninae + Zerynthiini. These two studies might be discussed as early molecular attempts that were, unfortunately, confined to molecular data. This is definitely not the case for the study of Parnassiinae phylogeny by Nazari *et al.* [24]. It is based on 236 morphological characters and seven genes, namely five mitochondrial genes: *16S*, *COI*, *COII*, *ND1,* and *ND5* and two nuclear ones: *EF-1α* and *wingles*s, of the total length of 5775 bp. The phylogenetic analyses were carried by means of MP, ML, and Bayesian methods (both the latter with GTR + *Γ* + I evolution models). The analyses included all the extant Parnassiinae genera (most of the non-*Parnassius* Parnassiinae spp. were included) and all the main *Parnassius* groups represented by eight spp. However, the surprising conclusion was that the

Parnassiinae + Baroniinae formed a clade which was the sister group to Papilioninae. Similar results were also presented by the molecular study of Michel *et al.* [26] who employed four mitochondrial genes: *LSU*, *ND1*, *ND5,* and *COI* of the total length of 2422 bp. The authors managed to include 65 Parnassiinae taxa and nearly all widely recognized *Parnassius* spp. The phylogenetic analyses were based on MP, ML, and Bayesian methods (both the latter with GTR models).

The remarkable phylogenetic study by Simonsen *et al.* [27] comprised 18 genera of Papilioninae, four genera of Parnassiinae (*Parnassius*, *Archon*, *Allancastria,* and *Luehdorfia*), and *Baronia*. Further, the molecular clock analyses (*vide infra*) were supplemented by sequences of the other four Parnassiinae genera. The morphological traits were based foremost on the list given in the detailed Miller's study [23] and the same seven genes as in the study by Nazari *et al.* were used (the total length of 5616 bp). The authors applied MP and partitioned Bayesian analyses, the latter one with the GTR $+ \Gamma +$ I model for the molecular data and a model analogous to the JC model for the morphological partition. The results might be used as illustrative example for textbooks of molecular phylogeny. Whereas the MP analysis of the molecular partition suggested that *Baronia* is a sister group to *Parnassius*; therefore, being an internal member of Parnassiinae, the Bayesian analysis of the sequence data provided the expected topology (Baroniinae, (Parnassiinae, Papilioninae)). These contradicting results could be explained by the long branch attraction artefact which is definitely a problem of the MP method in comparison to the Bayesian analysis. Naturally, the living fossil *B. brevicornis* forms a very long branch within the phylogeny of Papilionidae; therefore, posing a possible problem for MP. Moreover, the exclusive morphological analysis was leading to the same result as the mentioned Bayesian analysis of the molecular data and the same topology was also confirmed by the Bayesian analysis of the combined molecular and morphological datasets. Importantly, the authors identified morphological characters that supported the monophyly of the clade Parnassiinae + Papilioninae and the monophyly of Parnassiinae. The former clade is supported foremost by the absence of the third anal vein on the hindwing and by the presence of a ventral sclerite in the cervical membrane, while two characters are crucial for the monophyly of Parnassiinae: the thin and heavily sclerotized aedeagus in male and the strongly sclerotized ostial region in the female genitalia (*vide supra*) [27].

Now, we can finally interpret the previous results of Nazari *et al.* [24] and Michel et al. [26] who did not find Parnassiinae to be monophyletic. Nazari *et al.* did not employ certain morphological characters, namely, the presence/absence of a ventral sclerite in the cervical membrane and the lateral production of the prothoracic spinasternum at the spina that are both supporting the monophyly of Parnassiinae + Papilioninae [27]. Therefore the paraphyletic character of Parnassiinae with respect to *Baronia* resulted. This omission of morphologically important traits is not understandable since it is well known that these two characters are important for Parnassiinae. Moreover, both of them are discussed in the fundamental book on the Lepidoptera evolution by Kristensen [34]. The second study by Michel *et al.* was confined only to molecular data and the respective analyses were based on smaller dataset in comparison to the study by Simonsen *et al.*

Fossil taxa and divergence time of Parnassiinae

In the discussed study by Simonsen *et al.* [27], a relaxed Bayesian molecular clock analysis was carried out to estimate the times of divergences within Papilionidae using the fossil taxa *Praepapilio* and *Thaites* for the calibration. The analysis was run on an enforced Papilionidae topology obtained by the Bayesian analysis of the combined (molecular and morphological) dataset (*vide supra*). The root of the whole tree was placed to 100 ± 75 Ma ago. The minimum age of the clade Parnassiinae + Papilioninae was fixed to 50 Ma on the basis of the fossil *Praepapilio* which is dated to 48 Ma BP [4]. Further, the minimum age of the Parnassiini clade was set to 30 Ma reflecting the fossil record of *Thaites* from Oligocene [35]. One should mention that these assumptions comprise certain controversies albeit inevitable in studies of this kind[5]. The mean substitution rate across the Bayesian tree was evaluated to 0.019 substituion/(site.Ma). According to this result Parnassiinae started to diverge in the early Palaeocene around 64 Ma ago (95% confidence interval being 51 – 82 Ma BP).

Finally, let us briefly characterize the scarce fossil taxa included in Parnassiinae that might be of high phylogenetic significance. Unfortunately, both the described species are known probably only from single specimens whose current deposition and state is unknown as far as the author of these lines has been able to ascertain. Actually, all the references dealing with these taxa only cite the original descriptions and nobody has examined the material recently. *Thaites ruminiana* Scudder, 1875 was discovered in Aix-en-Provence, and the first record of its fossil was published by in 1861 by Heer [39] who only wrote on the page 205 of his treatment: "*Quatre genres* [...] *un cinquième (Thaites Ruminiana) est tres voisin du genre Thais qui appartient à la faune méditerranéene*", although he did not provide any specific description. The fossil taxon was later described by Scudder in 1875. The recent

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[5]The genus *Praepapilio* is traditionally classified as a member of the distinct subfamily Praepapilioninae [4]. Simonsen *et al.* did not emphasize this fact but they wrote: "*It seems unlikely that the fossil* [Praepapilio] *represents a stem group for Parnassiinae + Papilioninae, and we do not know if the Praepapilio lineage and Parnassiinae + Papilioninae lineage coexisted for a significant period of time*" and further : "*it [Praepapilio] also lacks several of the apomorphies that unite Parnassiinae and Papilioninae*". These comments are somewhat confusing. By all means, *Praepapilio* does not correspond to anything related to the Parnassiinae + Papilioninae clade. Therefore, it is quite doubtful to employ the fossil records of *Praepapilio* for estimating the minimum age of Parnassiinae + Papilioninae. *Praepapilio* might have originated in a far deeper divergence. In fact, its fossils are an illustrative example of the case when some apomorphic traits are shared with the extant taxa but at the same some plesiomorphic characters are retained. Two apomorphies typical for Papilionidae are present in *Praepapilio*: the cross-vein between Cu and 1A (so-called basal spur in the terminology of Miller [23]) in the forewing venation and the course of the 2A vein which is heading towards the hind margin of the forewing and not to the vein 1A. However, two other important characters exhibit plesiomorphic state: two anal veins are present in the hindwing venation and the forewing cubitus is not in line with the lower discocellular vein [36]. Therefore, *Praepapilio* should be definitely classified as the sister taxon of all extant Papilionidae. This further indicates the unsuitability of the *Praepapilio* fossils for estimating the age of Parnassiinae + Papilioninae as used by Simonsen *et al.* Interestingly, V. Nazari claimed that *Praepapilio* had got close relationship to the tribe Papilionini [37] according to the results of the phylogenetic study of Parnassiinae by Nazari *et al.* [24]. The authors of this study included *Praepapilio* in MP analysis but they obtained its morphological characters only from the original description and original figures of the two *Praepapilio* species. Unfortunately, these morphological data were very limited. The following MP analysis provided a topology where *Praepapilio* was a sister group of the monophyletic Papilioniini but with low support (bootstrap value = 59). One should conclude that this study could hardly challenge the above given interpretation of *Praepapilio* as the sister clade to all recent Papilionidae (we should avoid the incorrect term "the most basal taxon"). Moreover, certain authors even placed *Praepapilio* in a different family [38]. Another point relates to the fossil record of *Thaites* used to set the minimum age of Parnassiini to 30 Ma BP. Simonsen *et al.* wrote that its fossil came from the early Oligocene [27] probably according to the cited reference by de Jong [36]. However, the original description of *Thaites* mentioned "upper Eocene" [35]. More accurate specification of its possible age has not been published.

study by Nazari *et al*. [24] suggested that *Thaites* belonged either to Luehdorfiini or Zerynthiini. The second fossil taxon is *Doritites bosniackii* Rebel, 1898 which was described on the basis of a specimen from the Miocene deposits in Tuscany [40]. It is obviously related to *Archon* and the morphological analysis by Nazari *et al.* confirmed this earlier conclusion.

Internal classification and phylogenetic relationships within Parnassiinae

The discussion of the taxonomic concept and content of the Parnassiinae subfamily relates to Parnassiinae tribes as well. Among the classical works mainly the outstanding contributions by Munroe [16], Miller [23], Hancock [28], and Igarashi [30] should be emphasised. All of them assumed two monophyletic tribes for the extant taxa: Parnassiini (*Parnassius*, *Archon*, *Hypermnestra*) and Zerynthiini (= Luehdorfiini, including the remaining genera). However, the sister relationship of *Archon* and *Luehdorfia* is very well supported by the recent molecular studies [24, 26, 41, 42] in strong contrast to morphology and particularly to the external appearance of these butterflies. It is difficult to refuse the new results, and thus we should classify *Luehdorfia* and *Archon* together as the tribus Luehdorfiini *sensu* Nazari *et al.* [24]. The same studies evidenced also the sister relationship between *Parnassius* and *Hypermnestra*, both included in Parnassiini. Finally, the third monophyletic group with a high support is the trio of *Sericinus*, *Bhutanitis,* and *Zerynthia*, all of which constitute Zerynthiini. Nonetheless, the phylogenetic relationships between these three groups remain uncertain.

Phylogeny and internal classification of *Parnassius*

The monophyly of *Parnassius* is obvious from the morphology of Parnassiinae and the molecular studies have confirmed this fact (e.g. [26, 44]). Even the wing pattern of these butterflies is so distinct that no alternative phylogenetic structure can be suggested within the extent Parnassiinae. According to the study by Simonsen *et al.* [27], the divergence time of *Parnassius* was estimated to 47 Ma ago with the 95% confidence interval being 34 – 62 Ma BP. The most complete molecular phylogeny of the genus has been presented by Michel *et al.* [26]. However, most of the *Parnassius* taxa usually considered as *bona* species were already analysed in the first large study by Omoto *et al.* [41]. These authors sequenced a 777 bp fragment of the *ND5* gene and analysed the data by NJ method with a two-parameter model and MP (later they also published results of ML analysis [44]). The main clades identified in the studies by Michel *et al.* and Omoto *et al.* were identical and they corresponded, though with some discrepancies, to the traditionally recognized *Parnassius* subgenera[6]: *Parnassius s. str.*; *Sachaia* Korshunov, 1988; *Tadumia* Moore, 1902; *Lingamius* Bryk, 1935; *Kailasius* Moore, 1902; *Koramius* Moore, 1902; *Kreizbergius* Korshunov, 1990, and *Driopa* Korshunov, 1988. The results of the Bayesian and ML analyses by Michel *et al.* are summarized in Fig. 1. It should be noted that all these clades were statistically highly supported. An important feature of the topology in Fig. 1 as well as of the phylogenetic trees according to Omoto *et al.* [41, 44] is the basal divergence of *Parnassius s. str.* lineage and the clade of the remaining subgenera. Further, certain

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[6] Naturally, the agreement on the subgeneric classification of *Parnassius* has not been so wide. Certain authors have even preffered to split the genus on several genera and to define finer classification (the subgenera *Erythrodriopa*, *Quinhaicus* and *Eukoramius*). However, most of the subgenera in Fig. 1 belong to the traditional classification prevailing in the parnassiological literature.

sister relationships seem to be obvious, namely the relationship of *Kreizbergius* and *Driopa* and the relation between *Tadumia* and *Lingamius*. The phylogenetic position of the other clades has not been resolved sufficinetly. All the identified clades will be discussed in the following chapters linking them with the traditional classification.
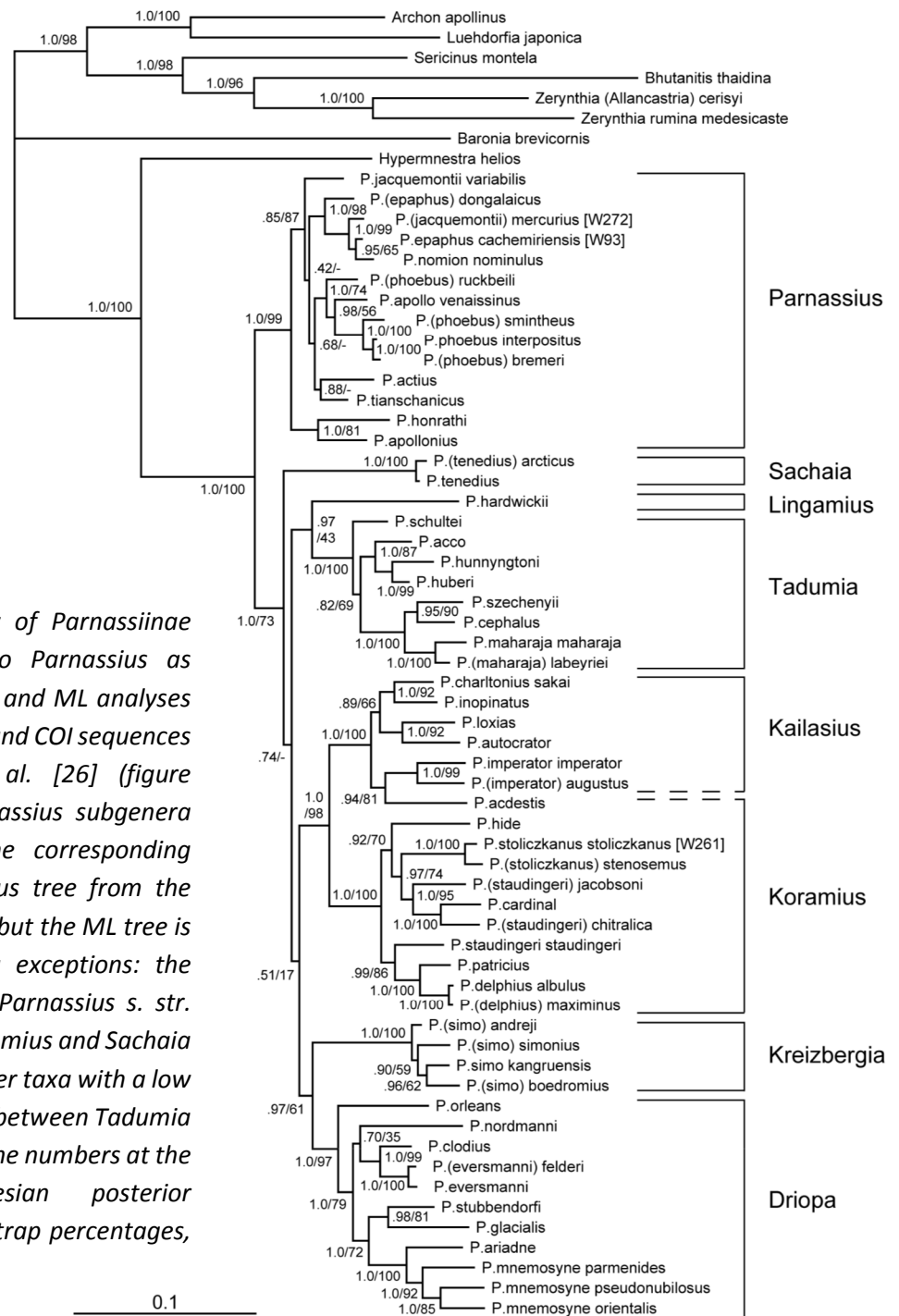

Fig. 1. Possible phylogeny of Parnassiinae with a special respect to Parnassius as inferred from the Bayesian and ML analyses of mitochondrial LSU, ND1 and COI sequences according to Michel et al. [26] (figure reprinted). The eight Parnassius subgenera are indicated next to the corresponding clades. Only the consensus tree from the Bayesian analysis is shown but the ML tree is similar with the following exceptions: the internal phylogeny of the Parnassius s. str. differs (see Fig. 2), the Lingamius and Sachaia subgenera appeared as sister taxa with a low support and were localized between Tadumia and the remaining clade. The numbers at the nodes represent Bayesian posterior probabilities and ML bootstrap percentages, respectively.

Parnassius sensu stricto

It is not necessary to introduce this group that comprises not only the nominate species *P. apollo* but also the well known taxa of the *P. phoebus* (Fabricius, 1793), *P. nomion* Fischer de Waldheim, 1823, *(P. epaphus* Oberthür, 1879)[7], and *P. jacquemontii* Boisduval, 1836 complexes (or supercomplexes). It is not necessary to state explicitly that a reasonable taxonomic treatment of this group, being inevitable before any serious phylogenetic account,

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[7] According to the recent molecular results it might be a part of the *P. nomion* supercomplex (*vide infra*).

would have been out of the limits of this brief review even if we had been allowed to cover ten times more pages than we may cover. However, it is necessary to confess the following weakness of the state of the art: although we abstract away from the internal classification and evolutionary history of the two most difficult cases being *P. apollo* and *P. phoebus* complex (both topics are treated in separate chapters), the phylogeny of the rest of *Parnassius s. str.* is not sufficiently resolved. These circumstances are obvious from the excellent and detailed molecular study by Michel *et al.* [26] who wrote: "*The two taxa* [*P. apollonius* Eversmann, 1847 and *P. honrathi* Staudinger, 1882], *whose sister-species status has long been recognized, have overlapping ranges in the mountains of western Central Asia. On the other hand, we were unable to come up with a stable phylogeny for the rest of the subgenus.*" The authors published two phylogenetic trees for *Parnassius s. str.* (see Fig. 2a, b) that were obtained via the Bayesian and ML analyses, respectively. The two methods provided considerably different topologies and several nodes of a high phylogenetic importance obtained rather low support. Nevertheless, some facts are clear and worth of a discussion.
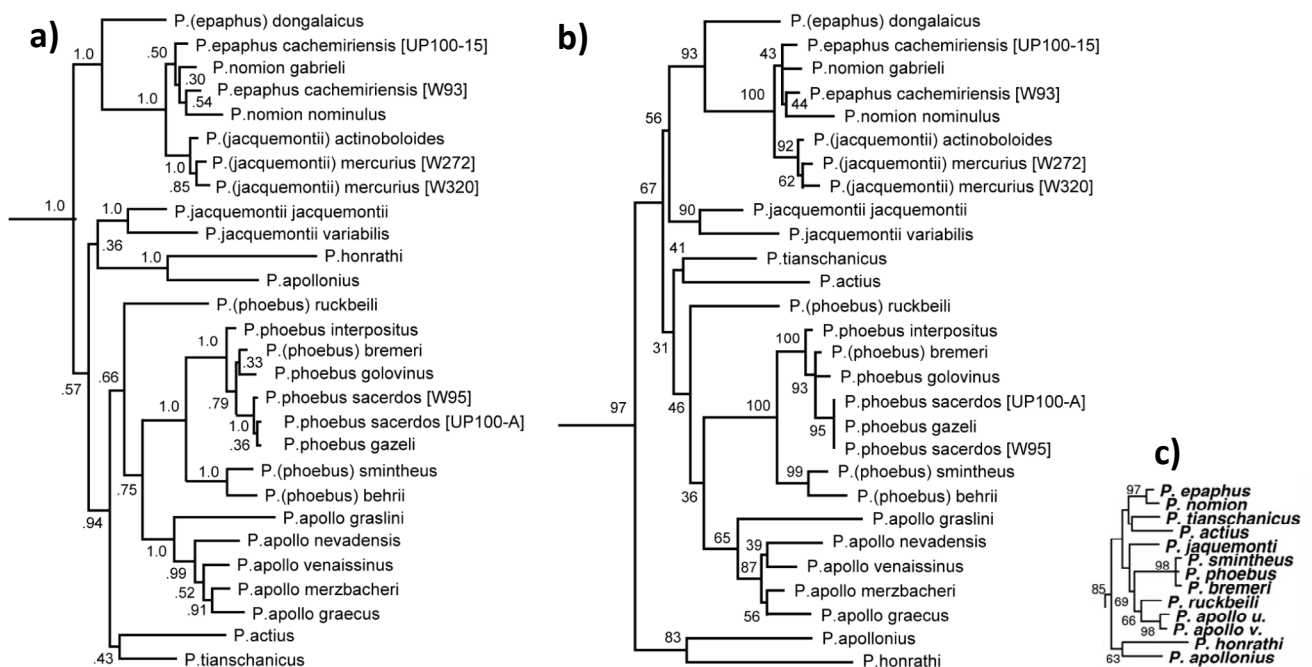

Fig. 2. Phylogenetic relationships within Parnassius subgenus. The Bayesian tree (a) and the ML tree (b) of the LSU, ND1 and COI sequences according to Michel et al [26]. The numbers give Bayesian posterior probabilities and ML bootstrap values, respectively. The parentheses are used to indicate taxa whose species status has been discussed recently. The last tree (c) was inferred via the ML analysis of the ND5 data by Omoto et al. [44], the numbers indicate bootstrap values higher than 50% (figures reprinted).

The sister relationship of *P. apollonius* and *P. honrathi* confirmed the previous conclusions based on morphology and observation of natural hybrids between these two species [45]. Further, the authors claimed that the clade of *apollonius + honrathi* had branched off from the main trunk in all their phylogenetic trees with the exception of the Bayesian one. The same result was achieved earlier by Omoto *et al.* by employing NJ, MP and ML methods [41, 44]. Not surprisingly, the *P. apollo* clade and the one of *P. phoebus* complex are also sister groups. This relationship was not detected in the trees by Omoto *et al.* where *P. ruckbeili* was branching in between, albeit with low support. It should be noted that a hybridization between *P. apollo* and the taxon *sacerdos* Stichel, 1906

of the *P. phoebus* complex occurs on suitable habitats in the Alps. The corresponding offspring is fertile [46] which further corroborates the close relation between the two lineages. In the study by Michel *et al. P. ruckbeili* occupies the sister position to the clade of *P. apollo* + *P. phoebus* complex and its nucleotide divergence is 2.4%. These results confirm its species status and indicate that *P. ruckbeili* is not included in the *P. phoebus* complex if the monophyly is required. Actually, the traditional entomology recognized even earlier that it is not a subspecies of *P. phoebus* (the classification used by Michel *et al.* is somewhat confusing as it might imply that *P. ruckbeili* was treated as a *P. phoebus* ssp. (see Fig. 2)). In fact, Weiss classified *P. ruckbeili* as a *bona* species in his monumental work [47] and its species status was suggested already by Eisner [21].

The study also confirmed the separation of the taxa *mercurius* Grum-Grshimailo, 1891 and *actinoboloides* Bang-Haas, 1928 from the *P. jacquemontii* group. Such an arrangement was suggested on the basis of the morphology and distribution. The species status of *P. actinoboloides* distributed in N China was primarily established by Weiss [47] while it had been previously treated as either a subspecies of *P. jacquemontii* (the original combination) or a subspecies of *P. actius* (Eversmann, 1843). The inconsistencies given by the classification of *mercurius* under the *P. jacquemontii* species were recognized even earlier. The range of *P. mercurius* includes N and central China [47] whereas *P. jacquemontii* is distributed in Central Asia, NE Afghanistan, N Pakistan, NW India and W China. According to Michel *et al.* both taxa *mercurius* and *actinoboloides* are closely related[8] and belong to a large and strongly supported clade which comprises the *P. nomion* supercomplex and *P. dongalaicus* Tytler, 1926. Michel *et al.* [26] showed that all these butterflies exhibit a unique mutation within *Parnassius*: A to C transversion at the position 252 of the *LSU* segment, i.e. within the LSU rRNA helix G3. The basal member of this clade is *P. dongalaicus*. It was described by Tytler who thought it "*may be a race of P. mercurius*" and further wrote: "*the keeled pouch* [= sphragis] [...] *places it in the Discobolus-Jacquemontii Mercurius group*" [50]. Only the female holotype was known and the position of *dongalaicus* remained enigmatic for long time. It was treated as a subspecies of *P. epaphus* or even as a hybrid of unknown origin [51]. Its status was resolved by Sugisawa [52] who revealed that this taxon is conspecific with the recently describ*ed P. rikihiroi* Kawasaki, 1995. The species status has been recently confirmed by the molecular data.

Comparing nearly any *P. nomion* subspecies, e.g. a specimen collected somewhere in lowlands of Siberia, with most of the *P. epaphus* taxa, distributed at high altitudes on the Tibetan plateau, one would not doubt these butterflies belong to different species. Nevertheless, Michel *et al.* indicated a very recent divergence of *nomion* and *epaphus* (nucleotide divergence 1.3%). Furthermore, some strange populations of *P. nomion* occurring in Qinghai resemble certain *epaphus* taxa, e.g. *P. nomion epaphoides* Bryk & Eisner, 1938 which is probably only a synonym of *P. nomion theagenes* O. Bang-Haas, 1928 [49][9]. We should note that the distribution of *P. nomion* and

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[8] Another interesting taxon which is closely related to *mercurius* and *actinoboloides* is *P. tibetanus* Ruhl, 1893 of an unclear status. Rose et Weiss [48] assume it has probably reached the stage of a "semi-species" while S. Kocman, one of the most experienced parnassiologists focused on the *Parnassius* of Tibet, considers *tibetanus* as a *P. mercurius* ssp. [49].

[9] Naturally, the situation is more complex. Several *nomion* taxa described in NE Qinghai resemble *P. epaphus*, namely *P. n. theagenes*; *P. n. sinchengi* Bryk & Eisner, 1938; *P. n. minschani* Bryk & Eisner, 1932 (the original spelling *P. n. minchani* was corrected by the authors in the same volume), *P. n. sinensis* O. Bang-Haas, 1927, *P. n. epaphoides*, *P. n. pax* Bryk & Eisner, 1934 all of which are treated as either obvious or probable synonyms of *P. n. theagenes* by Kocman [49]. Nevertheless, different opinions on the synonymy exist (see e.g. Rose *et* Weiss [48]). In order to illustrate the similarity of these populations

P. epaphus overlaps in the mentioned region. The author of these lines does not have enough determination to admit that *P. nomion* and *P. epaphus* might be conspecific since the butterflies from most of the range are extremely different. He will leave the problem of this complex unresolved until robust molecular data are available.

Sachaia

The unique taxon *P. arcticus* Eisner, 1968 from NE Yakutia is an enormously interesting case as well as the history of its original description and subsequent classification [10]. At the first glance one would say that *P. arcticus* is obviously a different species from *P. tenedius* Eversmann, 1851 because it is morphologically completely distinct. Moreover, *P. arcticus* exhibits also extreme sexual dimorphism in comparison to a moderate dimorphism of *P. tenedius*. However, there were some doubts in the past suggesting that *P. arcticus* could be a strongly deviated and dwarf form of *P. tenedius*. Already C. Eisner placed the taxon *arcticus* to *P. tenedius* as its subspecies correcting the original misplacement into *P. simo* G. Gray, 1853 ["1852"][11]. On contrary, the entomologist Z. Mráček, who personally collected *P. arcticus* in the field provided relevant information supporting the species status of *P. arcticus*. The genitalia of *arcticus* and *tenedius* are different: compared to the size of a butterfly they are relatively larger in the former. In addition, the uncus and the harpe are not as sclerotized and the dorsal groove in the saccus is less prominent in *P. arcticus* [53]. Further, *P. arcticus* is nearly sympatric with *P. t. britae* (Bryk, 1932) although it occurs at higher altitudes in very cold habitats. [48]. However, we should discuss also the low genetic distance between *P. arcticus* and *P. tenedius* indicated by molecular studies. At first, Omoto *et al.* [41] showed that the *ND5* sequences of *P. arcticus* and a specimen of *P. tenedius britae* from Artyk in NE Yakutia are virtually identical. Moreover, a detailed molecular study focused on *Sachaia* was published in 2004 by Chichvarkhin [57] who sampled a considerable number of specimens from five populations of *P. tenedius* and *P. arcticus* and sequenced two mitochondrial and two nuclear genes of the total length of 3573 bp. His analyses showed a low genetic variability within *Sachaia* and low genetic distance between *P. tenedius* and *P. arcticus*. Thus, Chichvarkhin

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to *P. epaphus,* we should mention that Weiss [47] regarded the type series of *P. n. epaphoides* as a mixture including *P. nomion* ssp. and *P. epaphus abruptus* O. Bang-Haas, 1927. However, S. Kocman did not agree with this conclusion. Interestingly, one subspecies of *P. epaphus* was named similarly as *P. e. nomionides* Schulte, 1992 but this name is obvious synonym of *P. e. hasegawai* Sugisawa, 1991 [49].

[10] The taxon was described by late C. Eisner who made several errors in the original description [53]. Firstly, he described the taxon as a subspecies of *P. simo* that, however, does not occur as far in the north as in Siberia. Secondly, C. Eisner confused two female specimens for a pair. The first male of *P. arcticus* was collected as late as in 1984. The species was rediscovered by Russian entomologists who provided the material to Korshunov. Not being familiar with the description of *arcticus* by Eisner, Korshunov described a new species *P. ammosovi* (Korshunov, 1988) on the basis of specimens collected in "РОССИЯ, Якутия, хребет Сунтар-Хаята, 180 км ВСВ поселка Хандыга, верховье реки Восточная Хандыга, 232 км трассы Хандыга-Магадан" (the data given for the holotype) [54].

[11] Most of the authors (including the most experienced parnassiologists) do not give the name of *P. simo* with a correct citation of the author and date in accordance with the ICZN Code [55]. The taxon was described in the first volume of the *Catalogue of lepidopterous insects in the collection of the British Museum* with an imprint of 1852 [56], but the work was published in 1853. The correct citation of the date could be one of the following possibilities: Gray, 1853; Gray, 1853 ["1852"]; Gray, 1853 ("1852") or Gray 1853 (imprint 1852) but definitely not Gray [1853]. The publication was written by the brothers and zoologists J. E. Gray and G. R. Gray but the taxon *simo* was described by George Robert Gray only. Therefore, it is better to use the initials although this fact is absolutely ignored in the modern literature. Nevertheless, the use of initials of an author is neither standardized nor excluded by the ICZN Code.

concluded that *P. arcticus* should be considered only a subspecies of *P. tenedius*. Further, Michel *et al.* evidenced a very recent divergence of *P. tenedius* and *P. arcticus* as both taxa exhibited only 0.3% nucleotide divergence in the combined *LSU*, *ND1* and *COI* data. Finally, Rose *et* Weiss [48] suggested that *P. arcticus* could be a local altitudinal form that had reached the stage of a "semi-species".

Tadumia

Four molecular studies have brought interesting results regarding the internal *Tadumia* phylogeny, namely the papers by Omoto *et al.* [41, 44], Katoh *et al.* [42], and Michel *et al.* [26], while morphological views were summarized by Rose *et* Weiss [48]. Already the first study by Omoto *et al.* [41] based on NJ and MP analyses of the *ND5* data showed that *P. szechenyii* Frivaldszky; 1886, *P. cephalus* Grum-Grshimailo, 1891, and *P. maharaja* complex (including the subgroup of *labeyriei* J.-C. Weiss & Michel, 1989) form a well-supported clade and that *P. hunnyngtoni* Avinoff, [1916][12] is related to *P. huberi* Paulus, 1999. These findings were later repeated in the ML analysis of the same data [44] and also confirmed by the analyses of the *16S* and *ND1* data, although *P. szechenyii* was not included [42]. Finally, the same phylogenetic relationships were observed in the most recent analyses of the combined *LSU*, *ND1,* and *COI* data by Michel *et al.* [26] (see Fig. 1). The previous morphological studies identified the monophyly of the first clade called as the *szechenyii* group. A particularly interesting case is the *maharaja* complex of the *szechenyii* group where twelve taxa have been described so far, from which nine were retained by Rose *et* Weiss [48]. Namely, the *labeyriei* subgroup[13] and the rare taxon *nosei* Watanabe, 1990 pose intriguing taxonomic issues. Both the *labeyriei* subgroup and *nosei* taxa have been frequently classified as separate species. The *labeyriei* subgroup is morphologically distinct and exhibits a geographically separated range quite distant from *P. maharaja* Avinoff, [1916] *s. str.* However, Rose *et* Weiss [48] suggested that it might be a "semi-species" within the *P. maharaja* complex. Actually, this conclusion was supported also by low genetic distance (nucleotide divergence of 1.7 %) between *labeyriei* and *maharaja* determined by Michel *et al.* At this point we have to emphasise that the genetic distances should not be the only and absolute measure of the species status. Typically, some mitochondrial genes are employed with an implicit assumption that they could differ between species. Another point is that such genes, like *COI* etc., are not directly related to any factor or character discussed in the usual definitions of a species. The strictly localized *nosei* is a different case than *labeyriei*. Actually, interesting forms of *nosei* have been collected recently, exhibiting well-developed and coloured ocelli. This wing pattern invokes a transition to *labeyriei* taxa which might suggest that *nosei* and *labeyriei* are conspecific [48]. Finally, according to Michel *et al.* [26] the interesting species *P. schultei* J.-C. Weiss & Michel, 1989 might be the sister species to all the remaining *Tadumia* taxa although the data published by Omoto *et al.* [41, 44] do not support this.

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[12] Not *hunningtoni* as it is given in certain sources including the cited references of Omoto *et al.* and Katoh *et al.*

[13] The labeyriei subgroup is represented by the following likely distinct taxa: *labeyriei s. str., sakyamuni* Kocman, 1995; *giacomazzoi* J.-C. Weiss, 1991; *naocoae* Morita, 1997; *natashae* Kawasaki & Tarasov; 1998 *kiyotakai* Sugiyama; 1992 and *holoydai* Kocman, 2003.

Lingamius

There is seemingly nothing to discuss since only one species *P. hardwickii* G. R. Gray, 1831 is placed to subgenus *Lingamius*. It seems to be even more obvious if we consider the conclusions of Weiss [58] (later confirmed by Rose *et* Weiss [48]) who put all the described subspecies of *P. hardwickii* to the synonymy with the nominate form. *P. hardwickii* is definitely an exceptional species among *Parnassius* butterflies taking into account its biology and patterns of variability. Originally five subspecies of *P. hardwickii* were recognized while other names were designated either for infrasubspecific taxa or as obvious synonyms. Consequently, the total number of the described taxa was 15 [58], which is still a low count for a *Parnassius* species that occupies a chain of mountains from N Pakistan across N India, Nepal, and Bhutan to S Tibet. However, it is a clear example of a species whose external appearance is very well correlated with its phenology and ecological factors. *P. hardwickii* is very variable and polymorphic, but the wing pattern is determined foremost by the order of a generation (it is bivoltine at lower altitudes and maybe even trivoltine in certain places[14]), climatic conditions (temperature and rainfall) and the altitude of the habitat. These factors dominate over its geographical variability [59]. Specifically, the first brood of butterflies is light-coloured with a reduced pattern and small ocelli, the second brood is better marked and more variable. Furthermore, certain populations in the eastern Himalayas are quite polymorphic with a frequent melanistic phenotype denoted as f. *nigerrima*. Several very distinct forms are known from the whole range of distribution, but they are only a demonstration of the individual variability.

It is interesting to mention molecular results, although they are based only on two specimens. The first butterfly coming from E Nepal was employed in the study by Omoto *et al.* [41], and the second one, collected in Deosai in Pakistan, was extracted by Michel *et al.* [26]. The later authors superimposed their distance matrix based on the *LSU*, *ND1,* and *COI* sequences with the *ND5* distance matrix based on the sequence data from Omoto *et al.* (the superimposition of distance matrices originating from different datasets is described together with evaluation of the differences in [60]). Interestingly, Michel *et al.* found that the agreement between the compared datasets was very good for all the *Parnassius* species employed in studies with the exception of *P. hardwickii*. This result indicates that the genetic distance of these two butterflies is probably quite large. The explanation is simple when the very distant localities of both the specimens are taken into account. However, the relevant question is how large the actual genetic variability of this alpine species is and, if any geographic patterns are present.

Kailasius

The beautiful and large butterflies *P. autocrator* Avinoff, 1913, *P. loxias* Pungeler, 1901, *P. charltonius* G. Gray, 1853 ["1852"][15], *P. inopinatus* Kotzsch, 1940 as well as the closely related taxa *P. imperator* Oberthür, 1883 and *P. augustus* Fruhstorfer, 1903 have been usually classified within the *Kailasius* subgenus although some of them were separated to *Eukoramius* subgenus or even placed to *Koramius* by certain authors. By all means, the traditional classification did not consider the small *P. acdestis* Grum-Grshimailo, 1891 as a species related to these

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[14] *P. hardwickii* is, apart from some populations of *P. eversmanni*, the only *Parnassius* sp. with more than one brood during a season [58].

[15] See the comment regarding the citation of the authorship and date given for *P. simo* in the footnote 11.

taxa. *P. acdestis* is an extremely variable and plastic species which was treated as a taxon related to the *P. delphius* complex whose females possess similar sphragis. According to such a view *P. acdestis* would be classified within the *Koramius* subgenus in our treatment. However, the molecular studies made the parnassiologists to reclassify it as a *Kailasius* member [48]. The first indication came in 2004 from Omoto *et al.* [41] who showed that the *ND5* sequence of *P. acdestis* clustered with sequences of typical *Kailasius* spp. Specifically the NJ and MP analyses showed that *P. acdestis* was probably a sister taxon of the *P. imperator* + *P. augustus* clade although the bootstrap values were not very convincing. The later molecular studies (*vide infra*), however, confirmed this phylogenetic arrangement.

In 2005 an incredible discovery was done in the range Moldo-Too of the Inner Tian Shan in Kyrgyzstan by Sergei Churkin who found a new and morphologically very distinct *Parnassius* species that belongs to the group of *P. charltonius*, *P. inopinatus*, *P. autocrator* and *P. loxias*. Hence, *P. davydovi* was described as species *nova* in 2006 causing a lot of sensation [61]. S. Churkin concluded that the species is closely related to *P. loxias*. He tried to discuss the phylogeny of this group mainly on the basis of genitalia and sphragis morphology, however, excluding *P. inopinatus* which occurs in Afghanistan due to the lack of sufficient material. He suggested that the last common ancestor of this group had divided into two lineages: one leading to *P. autocrator*, *P. loxias* and *P. davydovi* Churkin, 2006 and the other one to *P. charltonius* exhibiting some apomorphies in the male genitalia, namely, the absence of the distal process of the valva, somewhat flattened shape of the valvae and enlarged juxta and saccus. At the same time he argued that the relatively large range of *P. charltonius* indicated its better adaptation in comparison to the very limited distribution of the first three taxa suggesting they were relicts. Actually, these three species might be vicariants and S. Churkin even speculated about their possible historical distribution. At present *P. autocrator* is distributed in Hindukush Mts. of NE Afghanistan and in Pamir, *P. davydovi* is confined to Moldo-Too, and *P. loxias* occurs in the extreme E Kyrgyzstan in the Sary-Dzhaz Mts. and north of Aksu in NW China[16]. Thus it is likely that the ranges of *P. davydovi* and *P. loxias* were connected in the past which was only possible before all glacial periods. At that time the climate was warmer than now and it is possible that suitable habitats for *Kailasius* butterflies existed in the Inner Tian Shan at those times. The subsequent isolation of the populations in Naryn valleys on one side and Aksu valleys on the other side was definitely caused by climatic conditions in the high mountains of Tian Shan and it has persisted to present times. Therefore these taxa have to be much older than the typical "glacial" taxa like some *Erebia* and *Melitaea* spp. [61].

Later Omoto *et al.* presented a complete study of *Kailasius* [44] involving all the species of the subgenus as it covered also *P. davydovi*. The authors employed *ND5* sequences determined in the previous study [41], added new sequences of *P. davydovi* as well as some other Parnassiinae and carried out phylogenetic analyses. They performed ML analysis with the GTR + Γ + I model. The same model was used in the Bayesian analysis. Finally, NJ method was applied using the MCL (Maximum Composite Likelihood) + Γ model. All the analyses provided

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[16] In addition, the mysterious *P. loxias raskemensis* Avinoff, 1916 was described on the basis of one female specimen from "*the ramifications of the Raskem Mountains, namely, from the Kiliang* Pass [...]" [62]. The type locality is found in the western part of the Kunlun Mts.. No other material is known than the holotype [48].

essentially the same topology illustrated by the ML tree in Fig. 3 that shows also different types of sphragis that occur in the subgenus *Parnassius*. The first important result is a clear genetic difference between *P. davydovi* and *P. loxias*. The authors attempted to estimate the time of divergence of *P. davydovi* and *P. loxias* as well, assuming validity of the molecular clock with a constant rate within the *Parnassius* genus. The calibration was based on the assumption that the rapid radiation of different *Parnassius* clades had occurred 24.3 Ma ago when the uplift of the Tibetan plateau was accelerated, which probably provided new suitable niches for *Parnassius* butterflies. According to this picture the divergence time of the *P. loxias* and *P. davydovi* is 10.8 Ma ago and the corresponding 95% confidence interval is 7.5 – 14.7 Ma BP.

The morphological diversity of a sphragis in the *Kailasius* clade is surprising; four types occur here in contrast to a single type found in other *Parnassius* subgenera [44]. As already mentioned, the sphragis of *P. acdestis* with two brownish horns is of the same type as that found in the *Koramius* subgenus, which indicates its plesiomorphic state. In contrast, the clade *P. autocrator* + *P. loxias* + *P. davydovi* is characterised by a rather large and yellowish sphragis of a roughly square shape. *P. charltonius* and *P. inopinatus* have got a rolled light-brown sphragis that is larger in *P. charltonius* and somewhat reduced in *P. inopinatus*. The closely related taxa *P. imperator* and *P. augustus* exhibit a brownish fan-like sphragis. Despite its variability the sphragis morphology supports at least certain phylogenetic relationships found in the *Kailasius* tree (see Fig. 3).
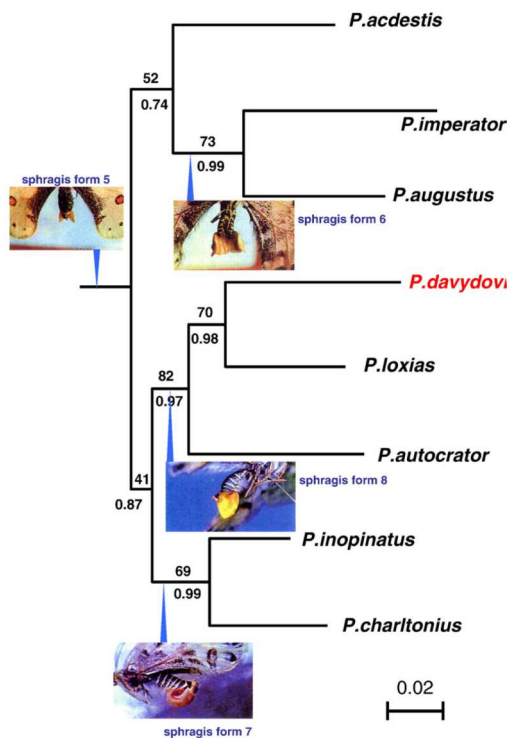

Fig. 3. Phylogenetic relationships and morphology of sphragis in Kailasius according to Omoto et al. [44] (figure reprinted). The tree was reconstructed by ML analysis of the ND5 data. The numbers above the branches give bootstrap values for the ML analysis while posterior probabilities of the Bayesian analysis are below.

Importantly, exactly the same topology of the *Kailasius* tree (including *P. acdestis*) was obtained by Michel *et al.* [26] on the basis of the *LSU*, *ND1* and *COI* sequences (see Fig. 1). Further, the same phylogenetic relationships were observed by Katoh *et al.* who included *P. charltonius*, *P. autocrator*, *P. acdestis*, and *P. imperator* from the *Kailasius* subgenus and carried out analyses of their *16S* and *ND1* sequences. Thus, the position of *P. acdestis* in the *Kailasius* clade is well supported. A final note is dedicated to one interesting taxon among more than 50 described subspecies of *P. acdestis* (26 were retained by Rose *et* Weiss [48]). This particular subspecies occurs in the vicinity of the Tshurpu monastery 50km west of Lhasa [49] and its wing pattern somewhat resembles *P. imperator* [26]. Therefore, it was named *P. acdestis imperatoides* J.-C. Weiss & Michel, 1989. Nonetheless, this kind of correspondence could be rather a random case taking into account the large diversity of wing patterns in the enormously variable *P. acdestis*.

Koramius

If anything certain can be written about this difficult group, it is the obvious fact that from the viewpoint of taxonomy it is the most challenging *Parnassius* clade, whose classification is far from any consensus. The number of recognized species fluctuates between two and ten among the recent authors [26]. It is easily understandable why Rose *et* Weiss [48] used the category of "semi-species" so frequently within the *Koramius* subgenus. Undoubtedly, there is a large number of morphologically and geographically very distinct taxa distributed through Central Asia, Kashmir, Himalayas, and on the Tibetan Plateau although their phylogenetic relationships are mostly unresolved and the systematic position and status unclear. The situation is further complicated by large variability, polymorphism (e.g. in various subspecies of *P. delphius* Eversmann, 1843 *s. str.*) as well as by hybridization [63]. Interestingly, several taxa traditionally recognized as species (*P. delphius*; *P. maximinus* Staudinger, 1891; *P. staudingeri* Bang-Haas, 1882; *P. cardinal* Grum-Grshimailo, 1887; *P. stoliczkanus* C. & R. Felder, [1865], and *P. stenosemus* Honrath, 1890) are easily identified on the basis of the external appearance, but they cannot be separated reliably (or easily in certain cases) by means of the genitalia morphology [64].

It is definitely out of the limits of this brief review to describe all different classification schemes suggested for the involved taxa and to explain various conceptions of the species as *P. delphius*, *P. staudingeri* etc. as they were interpreted by different authors. Unfortunately, a considerable confusion has evolved in the literature regarding the content of these species. Soma taxa have been repeatedly placed in different species by different authors, while the other authors either considered them as separate species or claimed that they are just a demonstration of an ecologically determined variability of no taxonomic meaning. By all means, anyone with a sufficient understanding of this group and reasonable field experience from Central Asia would agree that these butterflies could be hardly discussed just within the traditional ranks of a species, a subspecies, and an individual form. The populations forming this clade pose much higher complexity. One might speculate about an early phylogenetic state of many of these taxa that might be still evolving. Thus, there is only one step to the controversial concept of the so-called semi-species[17].

The first interesting results inferred from molecular data were published by Omoto *et al.* [41] who showed that the *ND5* sequence of *maximinus* Staudinger, 1891, treated traditionally as a *bona* species, is essentially identical with the sequences of *P. delphius juldussicus* Bang-Haas, 1915 and *P. delphius albulus* Honrath, 1889 (the former is probably a synonym of the latter [65]). Thus *maximinus* might be considered rather as a subspecies of *P. delphius* although it is easily recognized on the basis of wing coloration and pattern: it is whitish and the wing pattern is specific at the first glance. Its species status was advocated by Kreuzberg in his important work "*Parusniki grupp delphius, charltonius, simo (Lepidoptera, Papilionidae) fauny SSSR*" [66] where he pointed out that even the larvae and host plants of *maximinus* and *delphius* were different. He assumed that *maximinus* larvae fed on *Corydalis ledebouriana* and *C. darwasica* in comparison to *delphius* whose host plant was *Cysticorydalis*. In fact, *P. delphius* has got two morphs of larvae differing in their morphology and host plants: one is greenish with orange spots and

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[17] The author of these lines does not want to suggest its use, but during the last years he entirely resigned to employ only the conservative ranks within the *P. delphius* supercomplex (*vide infra*).

feeds on *Cysticorydalis fedtschenkoana*, and the other one is blackish with yellow spots and feeds on *Corydalis gortschakovi* [67]. According to Churkin [63], Kreuzberg compared larvae of *maximinus* and *delphius* that belonged to these different morphs which made him to believe that these taxa were distinct *bona* species. Finally, the specific status of *maximinus* was strongly supported by its sympatric occurrence with *delphius* in Chatkal according to Kreuzberg who did not find any hybrids in the material available to him. However, Churkin described [63] how he had been observing and collecting *maximinus* and *delphius* at the Chanach Pass. According to his report there were more than 15 % of hybrids in the series of several hundred specimens collected at the given locality[18]. Moreover, he claimed that the females of both *maximinus* and *delphius* had utilized using the same host plant since he observed how both the species had laid eggs close to the plants of *Corydalis gortschakovi*. In addition, he contradicted the note by Toropov and Zhdanko [67] that *Cysticorydalis* was the host plant of *maximinus* in Chatkal. On the basis of these facts and taking into account the morphological similarity (rather an identity) of the genitalia of both the taxa and their low genetic distances, he concluded that *maximinus* is a subspecies of *P. delphius*. Rose *et* Weiss [48] treat *maximinus* as a "semi-species" similarly to many other *Koramius* taxa.

Only the molecular study by Michel *et al.* [26] involved more than few *Koramius* taxa including several important ones. The phylogenetic relationships among them are depicted in Fig. 4. Generally, the branching events are not especially well supported considering the low bootstrap values and ambiguous position of *P. hide* Koiwaya, 1987. However, this species still appears a rather basal member of the *Koramius* clade. The close relation of *maximinus* and *delphius* is obvious from these trees as well. In details, there are only three nucleotide positions on the whole dataset of *LSU*, *ND1* and *COI* where *maximinus* differs from both the analysed *delphius* specimens. Further, it is well supported that *P. patricius* Niepelt, 1911 is a sister taxon of *P. delphius*. There is no doubt that *P. patricius* is a separate species due to its different morphology, ecology as well as sympatric distribution and simultaneous occurrence with *P. delphius* on several localities in Kyrgyzstan and Xinjiang. Michel *et al.* noted that their nucleotide divergence is only 2.1 % which corresponds to a relatively recent common ancestor. These species might have originated through the parapatric speciation when *P. patricius* started to occupy higher altitudes within the geographical range of *P. delphius*.

Further, it is interesting to compare the genetic distance between *P. patricius* and *P. delphius* on one side with the distance between *P. staudingeri staudingeri* Bang-Haas, 1882 and the cluster of the taxa *illustris* Grum-Grshimailo, 1888, *kiritshenkoi* Avinoff, 1910, and *darvasicus* Avinoff, 1916 on the other side (see also Fig. 4). If *P. patricius* and *P. delphius* are treated as distinct species, the given results would support a classification where two species are recognized in the latter group as well. On contrary, all the taxa of the second group together with several others were treated as various subspecies of *P. staudingeri* in the classical review by Kreuzberg [66]. Generally, recent parnassiologists assume there is a large *P. staudingeri* complex comprising nine groups: *staudingeri*; *infernalis* Elwes, 1886; *kiritschenkoi*; *elegans* Bryk, 1932; *ruth* Kotzsch, 1936; *jacobsoni* Avinoff, 1913; *hunza* Grum-Grshimailo, 1886; *mamaievi* Bang-Haas, 1915, and *cardinal* [64]. These groups are supposed to be

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[18] Unfortunately, some people think that such way of collecting is all right.

either clades of taxa (formally subspecies) belonging to *P. staudingeri s. l.* or even separate species, while Rose *et Weiss* term them "semi-species" in the most recent treatment [48]. Specifically, Kreuzberg classified this complex as three distinct species: *P. staudingeri*, *P. infernalis*, and *P. jacobsoni*. Taking into account the present results of Michel *et al.* (Fig. 4), the *P. staudingeri* complex is polyphyletic since it comprises two separate mitochondrial lineages: the clade of the true *staudingeri* and the clade containing the beautiful taxon *cardinal* Grum-Grshimailo, 1887. These clades exhibit different ranges, the *staudingeri* lineage is found on the north while the *cardinal* lineage is distributed on the south, but both of them overlap in Pamir. The given phylogenetic relationships are surprising, but they become understandable in the view of geographical distribution and ecological differences among the separated taxa. Unfortunately, it is not possible to analyse here all the relationships within the *cardinal* lineage, but some of them are definitely worth of mentioning, e.g. the close relation between *ruth* Kotzsch, 1936 and *cardinal* (nucleotide divergence 1.1 %) contrasting with the large differences in their wing patterns. Closing this part we should emphasise that the *P. staudingeri* complex comprising 39 described taxa forms a considerable part of the so-called *P. delphius* supercomplex[19]. The other two groups included here are: the *P. delphius* complex with 14 described taxa (only the main two discussed above) and the *P. stoliczkanus* group with 24 described taxa [48].
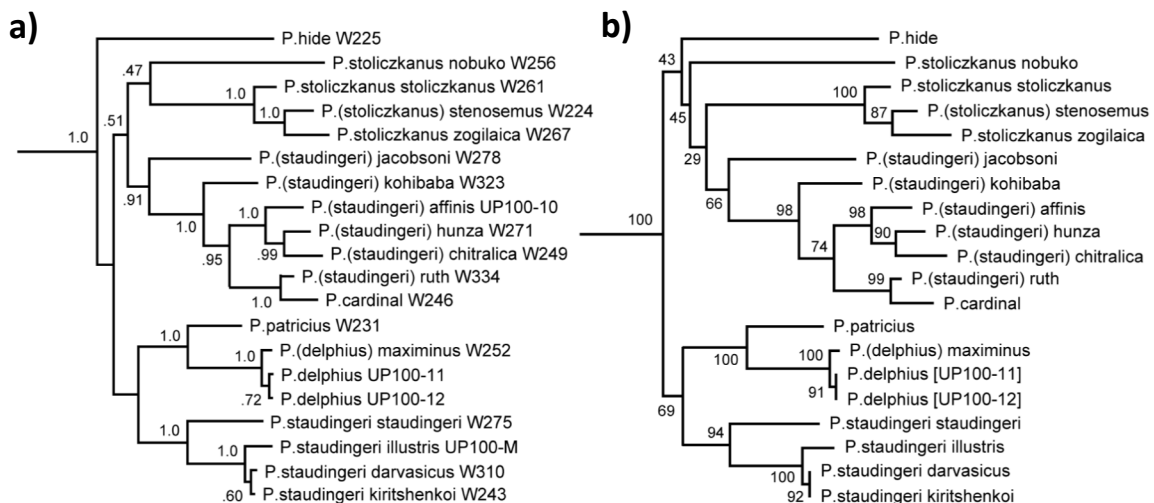

Fig. 4. Phylogenetic relationships within Koramius subgenus inferred via the Bayesian analysis (left) and ML analysis (right) of LSU, ND1 and COI sequences according to Michel et al. [26] (figures reprinted). The numbers give Bayesian posterior probabilities and bootstrap values, respectively. The parentheses are used to indicate taxa where species status has been discussed recently.

The *P. stoliczkanus* group comprises very attractive butterflies that were traditionally classified as two species, *P. stoliczkanus* and *P. stenosemus*, that differ considerably in the wing pattern. Moreover, both the species occur in several places together, namely, in Zanskar *P. stoliczkanus* flies together with *P. stenosemus* on clay slopes at around 5000 m [64], e.g. at the Rohtang Pass or Baralacha Pass [26]. Thus it should be clear that these species are well separated although the situation is not as straightforward as presented here. The whole *P. stoliczkanus* group contains 24 described taxa [48]. Interestingly, some of them are seemingly intermediate and have been classified

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[19] The *P. delphius* supercomplex *sensu* Rose *et* Weiss [48] does not include *P. hide* and *P. patricius*; therefore, it is also far from being monophyletic considering the suggested phylogenetic trees.

either as *P. stoliczkanus* or *P. stenosemus*, e.g. the taxon *atkinsoni* (Moore, [1902]) from N Kashmir which is probably synonymous with *zogilaicus* Tytler, 1926 [48]. Furthermore, Michel *et al.* [26] found that the genetic distance between *P. stoliczkanus* from Stok in Ladakh and *P. stenosemus* from Rangdum in Zanskar is quite low with the nucleotide divergence of 1.1 %. At the same time the authors showed that *atkinsoni* with the transitive phenotype belongs to *P. stenosemus* as otherwise *P. stoliczkanus* would be paraphyletic. The very important result of this study is the phylogenetic position of *nobuko* Ohya, 1996 represented by a specimen from Mahakali Tata in Nepal, which is on the east end of the range of the *P. stoliczkanus* group. Its deeper divergence and large genetic distance are obvious. It would be interesting to confirm its basal position with regards to the *stenosemus* and *stoliczkanus* clades, but the current support of the topology is too low for any serious discussion. By all means, the discovery of *nobuko* as such together with the molecular results led to a reclassification of the *P. stoliczkanus* group. As a result, the species status of *P. kumaonensis* Riley, 1926 was re-established, and the related taxon *nobuko* was placed as its subspecies [48]. Importantly, *P. kumaonensis* is sympatric with *P. stoliczkanus* since the taxon *harutai* Omoto et Kawasaki, 1998 (considered as a synonym of *P. stoliczkanus florenciae* Tytler, 1926 by Rose *et* Weiss [48]) was collected on the type locality of *nobuko* (*vide infra*), which further corroborates its species status.

Rose *et* Weiss [48] revised also the status of the mysterious *P. nandadevinensis* D. Weiss, 1990 known only from a single male collected on the Nanda Devi Mt. in Uttar Pradesh at 4200 – 4500 m on 15.6.1978. This butterfly is very unusual and evokes an impression of a very distinct species dissimilar to the others. It was described as a *bona* species, but no other specimens have been collected so far. The literature contains various speculations that mostly assume it is an extreme aberration of either *P. acdestis*, *P. stoliczkanus* or *P. stenosemus* [64, 68]. By all means, neither clear evidence nor detailed morphological analysis supporting the synonymy have been published. However, Rose *et* Weiss considered the new records of the *P. stoliczkanus* group in the region around Nanda Devi. The taxon *nobuko* was described from the Tata area [69] located below the Tinkar Pass [68] what is even further to the east than Nanda Devi. On the same place another taxon was collected and later described by Omoto *et* Kawasaki as *P. stoliczkanus harutai* [68]. These findings obviously influenced Rose *et* Weiss who suggested that *nandadevinensis* belongs to *P. kumaonensis* since its submarginal line on the hindwings and its small ocelli somewhat resemble the wing pattern of *P. kumaonensis*. Naturally, they added that it is not possible to resolve the status of *nandadevinensis* on the basis of a single specimen as it might be either an individual form or a distinct taxon.

Kreizbergius

In 1990 Korshunov described the genera *Kreizbergius*[20] and *Quinhaicus* placing *P. simo* complex into the former one and *P. szechenyii*, *P. cephalus*, and *P. maharaja* into the later one [71]. Although the efforts to split *Parnassius* on several smaller genera are strange, there are definitely certain morphological peculiarities, foremost, the very specific morphology of the male genitalia which rationalize the classification of the *P. simo* complex a separate

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[20] Not "*Kreuzbergia*" as certain sources give, e.g. [48, 70].

subgenus of *Parnassius*. In contrast, the idea of *Quinhaicus* Korshunov, 1990 could have hardly found any other reaction than synonymization with *Parnassius* [72]. The classification of this tremendously interesting complex has been accompanied with a lot of problems and contradicting opinions. In spite of that, we will state straight away that the group contains probably four *bona* species: *P. simo*, *P. andreji* Eisner, 1930, *P. simonius* Staudinger, 1889, and *P. boedromius* Pungeler, 1901. Such conclusion might be controversial taking into account molecular findings, but these four species are supported very well by the traditional entomology. The key study was published by Kreuzberg in 1985 [66] evidencing the species status of the three following taxa: *simo*, *simonius*, and *boedromius*. Kreuzberg showed the differences in their male genitalia, in the seventh tergite and also summarized their different wing patterns. The very clear difference in male genitalia is found namely between *P. simo* and the others. Its ventral side of the distal end of the uncus does not bear a pair of blade-like structures like in *P. simonius* and *P. boedromius*, its aedeagus is shorter and thicker and its valvae are heavily sclerotized and strongly curved [70]. The differences in male genitalia between *P. simonius* and *P. boedromius* are confined to the uncus. The size of the excavations at the base of the uncus, its shape and thickness and the steric configuration of the blade-like structures at the distal end are significantly different in these two species (for extensive details see the work by Stekolnikov *et* Kuznetsov [73] and further comments by Churkin [70]).

The fourth species *P. andreji* is distributed exclusively in China and was enigmatic for long time due to the limited amount of material and unknown distribution and biology. As China became open to foreigners, more specimens were available and new populations were found. Thus it was possible to clarify the differences and to add important biogeographical views. The male genitalia of *P. andreji* are characterized by a very short uncus possessing the blade-like projections in contrast to *P. simo.* Its valvae are similar to *P. simo,* but they end with a curve apex [53]. The wing pattern of *P. andreji* is characterized by several specific features. The subcostal marks on the forewings are uniformly dark without any red colouration in comparison to the geographically close taxa of *P. simo*, i.e. the subspecies from the north-eastern areas of the Tibetan plateau possessing red centres or at least traces of red in these marks. Similarly, the dark dusting at the base of hindwing undersides in *P. andreji* does not exhibit any red pattern while at least some traces of red are found in *P. simo* [48][21].

The morphological and biogeographical views on the phylogeny of the *P. simo*, *P. simonius*, and *P. boedromius* were provided by Churkin [70]. He assumes that the last common ancestor of these species split into the lineage leading to *P. simo* without any blade-like projections at the distal end of the uncus and the lineage which later provided *P. simonius* and *P. boedromius* that possess larger uncus with blade-like projections. The molecular results by Michel *et al.* [26] (see the tree in Fig. 1) indicate that all four species are closely related as the nucleotide

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[21] It should be noted that the above given discussion is simplified as it covers only the four main taxa considered to be species. However, all of them are constituted from geographically separated populations that exhibit obvious differences in the wing pattern. These populations are classified as different subspecies. More than 50 infraspecific taxa have been described for *P. simo*, and 23 of them are recognized as valid geographic subspecies by Rose *et* Weiss [45]. In *P. andreji* they recognize 6 sspp., but altogether 13 sspp. have been described. In *P. simonius* they consider 3 sspp. to be distinct among 7 described taxa and in *P. boedromius* 3 out of 9 sspp. are probably valid. However, these numbers given by Rose *et* Weis might be underestimated and do not describe the geographical variability within these species appropriatly as the diversity of *P. andreji* on the Tibetan plateau shows [64].

divergence based on *LSU*, *ND1* and *COI* is ranging 1.7 – 2.2 %, but we cannot let the morphological and biological views to be ignored[22]. Moreover, at least the sympatric occurrence of *P. simo* and *P. andreji* is confirmed [74] supporting that they are separate species.

Driopa

Two years before describing the genus *Kreuzbergius* Korshunov established also *Driopa* as a new genus with the type species *P. mnemosyne* (Linnaeus, 1758), and he divided the genus into two subgenera: the nominate *Driopa* Korshunov, 1988 and *Erythrodriopa* Korshunov, 1988 [75]. *P. mnemosyne* was classified together with *P. stubbendorfii* Ménétriés, 1847 (and obviously also together with *P. glacialis* Butler, 1866) in the former one while *Erythrodriopa* included *P. nordmanni* Ménétriés, 1850, *P. clodius* Ménétriés, 1852, *P. eversmanni* Ménétriés, 1850, and *P. felderi* Bremer, 1861 in addition to its type species *P. ariadne* Lederer, 1889. Korshunov's internal classification of *Driopa* neither reflects current opinions nor is in accordance with molecular phylogenetic studies. However, the concept of *Driopa* seems to be useful as a subgenus. Interestingly, Rose *et* Weiss [48] included *P. hardwickii* as another member of the *Driopa* subgenus, but such classification is not consistent with the recent molecular findings [42, 76]. The concept of the subgenus *Driopa* employed in our treatment corresponds to the original arrangement of Korshunov but includes also *P. orleans* Oberthür, 1890 [48] and keeps *P. (Lingamius) hardwickii* in the separate subgenus.
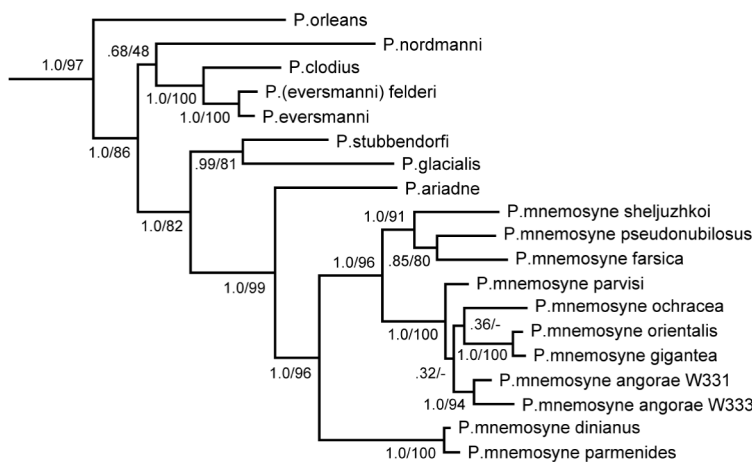

Fig. 5. The combined Driopa tree based on the two consensus trees obtained via Bayesian and ML analyses of LSU, ND1 and COI sequences according to Michel et al. [26] (figure reprinted). The topology of the initial Bayesian and ML trees was the same with the exception of the poorly resolved clade comprising P. m. parvisi, P. m. angorae and taxa from Central Asia. The numbers give the Bayesian posterior probabilities and bootstrap percentages.

The phylogenetic structure of the *Driopa* clade according to Michel *et al.* is shown in Fig. 5. Both the molecular studies by Katoh *et al.* [42] and by Michel *et al.* [26] indicated that *P. orleans* Oberthür, 1890 is the sister taxon to all remaining *Driopa* members. From the morphological viewpoint its wing pattern is definitely plesiomorphic in comparison to the other members of the *Driopa* subgenus as the blue marginal ocelli are present only on hindwings of *P. orleans*. It is distributed mainly on the Tibetan Plateau and in the neighbouring regions of SE Kansu, S Shaanxi and also in the northernmost areas of Burma. In the whole range 25 subspecies have been described so far, but only 13 of them were retained by Rose *et* Weiss [48]. An authoritative treatment of 22 subspecies from Tibet and adjacent territories was given by S. Kocman who recognized 10 of them as distinct. Unfortunately, no molecular data regarding the diversity of *P. orleans* are available.

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[22] On the other hand, Rose *et* Weiss [48] did not want to refuse the suggestions of molecular entomologists completely, and thus they were using the term "semi-species" for all the four main taxa of the *P. simo* complex.

The most derived species is *P. mnemosyne* that will be discussed in a separate chapter below. Now we should only point out that its sister taxon is *P. ariadne* Lederer, 1889 according to the results of all molecular studies including both the species [26, 41, 42]. This clear finding might be surprising for the traditional parnassiology since *P. mnemosyne* was usually classified together with *P. stubbendorfii* and *P. glacialis* to constitute so-called *P. mnemosyne* group. In contrast, *P. ariadne* was classified together with *P. nordmanni* Ménétriés, 1850 and *P. clodius* Ménétriés, 1852 to the separate *P. ariadne* group [58]. This arrangement corresponds to the external appearance as the wing pattern is generally reduced in the *P. mnemosyne* group while more typical *Parnassius* pattern with developed red ocelli is found in the *P. ariadne* group. The range of *P. ariadne* is basically limited to Altai, the neighbouring Tarbagatai Mts. and their eastern continuation, the Saur Mts. Only four subspecies of *P. ariadne* have been described, one of which is obviously a synonym of the nominate subspecies[23]. Interestingly, the last described taxon *P. a. erlik* Yakovlev, 2009, discovered in the Chikhacheva Mts., possesses largely reduced ocelli that can be completely black or exhibit only weak orange centres which suggests certain similarity with the extensive reduction in the allied taxa. The other species of the original *P. ariadne* group do not pose any serious taxonomic problems. The range of the very distinct species *P. nordmanni* is limited to the Caucasus and Kaçkar Mts. where it forms four or five different subspecies[24]. *P. clodius* is distributed in the North American mountains from Alaska to California where 12 subspecies have been described, six of which were retained by Rose *et* Weiss [48]. The remaining taxa of the subgenus *Driopa*, speaking about the *P. eversmanni* complex and *P. stubbendorfii* complex to which *P. glacialis* is related, are taxonomically challenging, and their phylogenetic and well resolved phylogeographical analyses would be extremely interesting.

P. stubbendorfii is a complex of taxa distributed from Altai across N Mongolia and SE Siberia to Korea and Japan, on south spreading through NE and central China to N Szetschwan and northern parts of the Tibetan plateau. They are mostly considered as different subspecies of *P. stubbendorfii s. l.* although the eastern island populations are frequently considered as a *bona* species. As a result, the complex might be divided into four species: *P. stubbendorfii s. str.* with the continental distribution, somewhat larger *P. hoenei* Schweitzer, 1912 from Japan, possessing more rounded apices, smaller and lighter *P. esakii* Nakahara, 1926 from Sakhalin Islands, and *P. tateyamai* Fujioka, 1997 described from the minute Rishiri Island near the north tip of Hokkaido being smaller and with strongly marked veins [48]. With the exception of *P. tateyamai*, possessing extremely small range, all the mentioned taxa were further subdivided[25]. The close relation of *P. glacialis* to *P. stubbendorfii* is very clear from their morphological similarity that made earlier authors to consider *P. glacialis* as a subspecies of *P. stubbendorfii*. Their phylogenetic relationship was also evidenced by a number of molecular studies [26, 41, 42, 78] showing that

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[23] *P. a. jiadengyuensis* Huang & Murayama, 1992 is a synonym of *P. a. ariadne* Lederer, 1889 [48, 77].

[24] The number depends on the status of *P. n. bogosi* O. Bang-Haas, 1934 whether it is treated as a distinct subspecies or as a junior synonym of *P. n. nordmanni*.

[25] This relates particularly to *P. stubbendorfii s. str.* with more than 20 described sspp. half of which could be considered to exhibit some morphological differences while the rest are probably synonyms. Four subspecies have been established within the taxon *hoenei* with three subspecies currently recognized as valid. In contrast, the taxon *esakii* is considered to be rather homogeneous although Asahi attempted to separate the population occurring on the Moneron Island as a subspecies different to the nominate form.

P. glacialis is the sister group of *P. stubbendorfii*. *P. glacialis* occurs in Japan, eastern and central China and reaches even the east edge Tibetan Plateau.

Rich material of both the *P. stubbendorfii* complex and *P. glacialis* was analysed by Yagi *et al.* in the study published in 2001 [78]. Specifically, the *P. stubbendorfii* complex was represented by several taxa from Kazakhstan, Russia, and China and by both the Japanese taxa *hoenei* and *esakii*[26]. Representatives of *P. glacialis* included samples from Shikoku, Honshu, and Hokkaido and two Chinese populations in Hubei and Sichuan. The mitochondrial *ND5* sequence of the length 805 bp was used, and several phylogenetic methods were employed: NJ, ML, UPGMA, and also MP. According to the authors all the methods provided trees essentially of the same topology which is illustrated by the NJ tree in Fig. 6. The phylogeny as well as the pairwise genetic distances based on the Kimura's approach showed that the continental populations on one side and the Japanese together with Sakhalin populations on the other side are considerably divergent in both *P. stubbendorfii s. l.* and *P. glacialis*. In contrast, the genetic differences and phylogenetic signal in the *ND5* data was too weak to distinguish the populations or to reconstruct their evolutionary history. Although this study being one of the first molecular approaches to *Parnassius* phylogeny might be interpreted as a methodologically early attempt, it is important to emphasize the absolutely correct conclusions made by the authors on the genetic differences. Yagi *et al.* wrote that: "*In P. stubbendorfii, many subspecies were described in the continent, but differences in ND5 sequences among the subspecies were unexpectedly too small to distinguish these subspecies. Genes with higher evolutionary rate should be used for analysing the reliable phylogenetic relationships among these geographical groups.*" Their data did not show that the continental populations (or Japanese) were taxonomically homogenous or that these populations were not variable enough. The *ND5* data only proved to be insufficient to resolve the subspecies described within continental Asia or Japanese archipelago. It would be necessary to employ more variable loci for the sake of such a study. This conclusion seems to be trivial, however, some authors systematically ignore this elementary fact. For example there have been attempts to synonymize various subspecies, albeit with geographically separated distribution and constant morphological differences, on the basis of rather short fragments of mitochondrial genes.

Yagi *et al.* [78] also tried to estimate the divergence times of the continental and Japanese populations. They established the time calibration on the assumption that the simultaneous radiation of *Parnassius* butterflies occurred during the uplift of the Tibetan plateau at 3.5 – 4.5 Ma BP. This would lead to the following equivalence between the Kimura's two-parameter distance and time: 0.01 of the distance corresponding to 0.75 Ma (see Fig. 6). According to the UPGMA tree the divergence of the respective populations occurred in both *P. stubbendorfii* and *P. glacialis* at the same, at around 1.7 − 2.0 Ma BP. Thus, following phylogeographic scenario may be suggested. The ancient Japan separated from the Asian continent at about 15 Ma ago, and the proto-form of the Japanese archipelago was formed at about 5 Ma ago after the opening of the Sea of Japan, followed by the submergence and upheaval of the islands [79, 80]. During the glaciation at 2 Ma ago, the archipelago was largely

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[26] The authors classified the material from Sakhalin as well as the butterflies from Hokkaido as *P. stubbendorfii hoenei*, not considering the *esakii* taxon at all.

connected to the continent in consequence of the land bridges that appeared in Korea-Tsushima channel on the south-west and in the Strait of Tartary and Soya channel on the north-east [81]. This was the moment when the spread of *P. stubbendorfii* and *P. glacialis* from Siberia and China was enabled, which corresponds to the estimated time of the divergence of 1.7 – 2.0 Ma BP. Due to the following rise of the sea level the butterflies became isolated in Japan and Sakhalin although the sea level was altering even thereafter and consequently, the Japanese archipelago was getting periodically connected to the continent. Further, another connection via the narrow Tatar land bridge existed during last LGM. In spite of this fact *P. glacialis* does not occur on the Korean Peninsula. Although it is distributed in S Japan, it did not immigrated to the Korean Peninsula via the mentioned land bridges. By all means, the described scenario supposes only a single immigration wave for *P. stubbendorfii* and *P. glacialis*, what might be too simplified taking into account that the periodically dry sea floor provided possible migration corridors. Nevertheless, the *ND5* data do not support more complex sequence of evolutionary events since the phylogeographic signal is too weak.
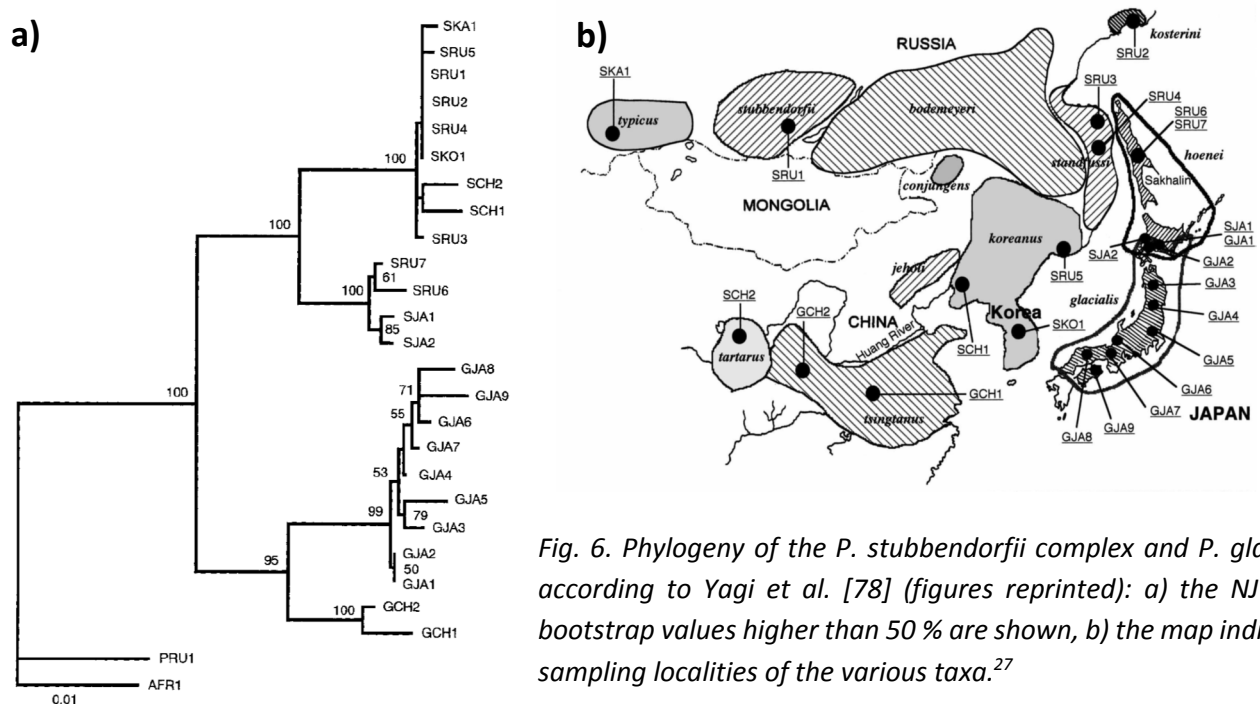

Fig. 6. Phylogeny of the P. stubbendorfii complex and P. glacialis according to Yagi et al. [78] (figures reprinted): a) the NJ tree, bootstrap values higher than 50 % are shown, b) the map indicates sampling localities of the various taxa.[27]

The last note should address the genetic distances between the continental and Japanese/Sakhalin populations. For example the nucleotide difference in *ND5* sequences between *P. g. glacialis* from Hobetsu in southern Hokkaido (sample GJA1) and *P. g. nankingi* O. Bang-Haas, 1927 from Shennongjia in Hubei, treated as *P. g. tsingtauus* O. Bang-Haas, 1910 by the authors (sample GCH1), is 2.5 %. Interestingly, Yagi *et al.* pointed out that such genetic distance is even higher than the values observed for the taxa traditionally recognized as a separate species, e.g. *P. bremeri* Bremer, 1864 *vs. P. phoebus* or *P. epaphus* vs. *P. nomion* (see the discussion above), and they suggested that the compared populations may be classified as different species. The only possible comment to this issue is that cross-breeding of the respective populations should be tested together with the fertility of

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[27] Actually, the current classification of several taxa involved in the study is different than the authors employed. It is caused by very varied opinions on the synonymy and classification within *P. stubbendorfii* complex and *P. glacialis* that disable to speak clearly about the individual taxa if the exact localities are not given.

their offspring. Rose *et* Weiss [48] wrote about the taxa *hoenei*, *esakii*, and *tateyamai* that the insular isolation had brought certain genetic differences to them in comparison to *stubbendorfii s. str.*, but such assumption did not allow the affirmation that they had reached the stage of species. This opinion implicitly evokes the idea of the semi-species whose concept, though abandoned by taxonomists and ICZN, could offer a suitable framework for the discussion of such populations. The author of these lines does not want to re-introduce old ghosts of systematics back to modern classification, but he thinks that the mentioned concept could be useful in the phylogenetic discussion of certain *Parnassius* clades.

The last sentences relate also to another interesting group which is the *P. eversmanni* complex. Its closest relative is *P. clodius* according to the molecular data [26], but their sister relationship is also logical from the biogeographical viewpoint since the range of *P. eversmanni* reaches North America where *P. clodius* exclusively occurs. The *P. eversmanni* complex has been a difficult case for the traditional parnassiology, and the first and very limited molecular results did not help to shed more light on these butterflies. It comprises three important taxa (in fact three groups of taxa): *P. eversmanni*, *P. maui* Bryk, 1915, and *P. felderi*. The major discussion is whether *P. felderi* is just a divergent subspecies of *P. eversmanni* or a separate species. According to the recent molecular data they are only weakly differentiated [26, 41].

Evolutionary history of *Parnassius mnemosyne*

Parnassius mnemosyne

The dissertation of Isaas Uddman issued in 1753 [82] contains a description of a butterfly species that is headed by a descriptive title[28]: "*Papilio hexapus supra niger; alis omnibus ordine macularum transversali albo: inferioribus dentatis.*" and "*montes in Tavastia*" is given as its habitat. The Uddman's thesis is cited by Linnaeus in the 10th edition of the *Systema naturae* under the description of the *Papilio* species no. 42 being "*Papilio (Heliconius) mnemosyne*". The lectotype was designated by Honey *et* Scoble [83] who pointed out that they selected a specimen from the Linnaean material which exhibited a similar preparation style as some other specimens originating from the Udmann's collection. Considering these facts, Honey *et* Scoble specified the type locality of *Parnassius mnemosyne* (Linnaeus, 1758) to be the mountains in Tavastland. Actually, the original information (the type locality according to the original description) by Linnaeus was very general: "*Habitat in Finlandia*". Nevertheless, the population in Tavastland is already extinct, and the nearest place where the species occurs is roughly 100 miles away [83].

Diversity of *Parnassius mnemosyne*

The limited wing pattern of *P. mnemosyne,* which is further reduced in certain populations, does not allow such enormous external variability as the wing pattern of *P. apollo* and most of other *Parnassius* species. Not even this obstacle prevented entomologists to split *P. mnemosyne* on a plethora of subspecies. It might be symptomatic for earlier parnassiology, however, that many of these subspecies are not even based on minor differences

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[28] In the pre-Linnean era of biology short Latin descriptions were often used instead of standardized nomenclature.

resulting from individual variability. Actually, a certain number of these subspecies was established just on the basis of more or less distant type localities. Taking into account that among other *Parnassius* spp. of the *Driopa* subgenus *P. mnemosyne* has got particularly large distribution spanning from Pyrenees to Tian Shan, it is not surprising that the total number of described subspecies is around 200, not speaking about infrasubspecific names. Unfortunately, no serious revision of *P. mnemosyne* has been published so far.

The first studies regarding the genetic diversity of *P. mnemosyne* utilized allozyme data which proved to be suitable for the analysis of genetic differences among populations, possible gene flow, isolation of local colonies and geographical distribution of diversity [84, 85]. In spite of the high significance of the allozyme studies for the mentioned issues, we could argue that DNA sequences offer more robust data for detailed phylogenetic reconstructions. Interesting results regarding the genetic diversity of *P. mnemosyne* were reported by Michel *et al.* in the aforementioned study based on the *LSU*, *ND1* and *COI* sequences. The authors included several subspecies from distant localities of the whole range. These taxa are listed together with their sampling sites[29] in order to prevent any geographical confusion that might arise from the unresolved subspecific taxonomy and unstable synonymy of *P. mnemosyne* sspp. Unfortunately, the sampling in Europe was limited only taxa from Alpes Maritimes and Greece. Both the Bayesian and the ML analyses provided nearly identical topology (see Fig. 5). The studied populations formed three clades: I) *P. m. pseudonubilosus*, *P. m. farsica,* and *P. m. sheljuzhkoi* from Iran and southern Turkey; II) *P. m. parvisi* from Greece and *P. m. angorae* from Central Turkey clustered together with *P. mnemosyne* taxa from Central Asia, and III) both the populations from Alpes Maritimes formed a distinct clade. One should point out that these clades were separated by considerable genetic distances since the nucleotide divergence for the clades I and III was 3.2 % and the divergence between representatives I and II was 2.6 %. Indeed, it might fall still within the intraspecific variability, but it is surprising. The authors considered a possibility that the genetic divergence within *P. mnemosyne* had been overestimated in their analyses. Therefore, they compared the number of substitutions in *P. mnemosyne* clade to the other *Driopa* taxa using other *Parnassius* subgenera as outgroups. There was no excess of substitutions in *ND1* and *COI* genes at all while only *LSU* sequences showed higher number of substitution events for the *P. mnemosyne* clade.

Although a complex morphological discussion of *P. mnemosyne* within the whole area of distribution would exceed the possible extent of this summary (in fact, a meaningful discussion of the *P. mmenosyne* morphology would go beyond the current state of the art of parnassiology), it is important to mention that there are clear differences between populations from clade I and from the other two clades. All the three analysed subspecies of the clade I belong to the so-called *nubilosus* subgroup which comprises 16 subspecies according to J. C. Weiss

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[29] *P. m. dinianus* Fruhstorfer, 1908 (Montagne de Lachens, Alpes Maritimes, France); *P. m. parmenides* Fruhstorfer, 1908 (Boréon, Alpes Maritimes, France); *P. m. ochracea* Austaut, 1891 (Tachtakaratscha Pass, Zerafschanskyi Mts, Tadjikistan); *P. m. sheljuzhkoi* Bryk, 1912 (Hasanbeyli, Adana Prov., Turkey); *P. m. orientalis* Vérity, 1907 (S. Zailijsky Mts., Kyrgyzstan); *P. m. gigantea* Staudinger, 1886 (Chirchik, Chatkalski Mts., Uzbekistan); *P. m. angorae* Bryk & Eisner, 1931 (2 specimens: W331 - Kizilcahamam, Ankara Prov., Turkey; W333 - Ilgazdagi Geçidi, Kastamonu Prov., Turkey); *P. m. parvisi* Turati, 1919 (Pelkofi to, Grammos, Greece); *P. m. farsica* O. Bang-Haas, 1938 (N-O Ardakan, Fars, Iran), and *P. m. pseudonubilosus* Vérity, 1909 (Likbin, Ourmia Lake, Iran).

[58]. This is one of the three large subgroups recognized within *P. mnemosyne*. At the first glance the external appearance of these butterflies, distributed mainly in Iran, S and NE Turkey, W Armenia, Syria[30], Lebanon, and also spanning to southern Turkmenistan, is completely different from the others[31]. Their wings are very light-coloured, the forewings can be described as more angular with more pointed apex, and the sexual dimorphism is more pronounced. At least in males the hyaline marginal area of the forewings comprises well developed white markings called lunulae that might form a continuous white row. The cellular marks are substantially reduced in males, but the maculae in females are rather developed [58].

Michel *et al.* [26] even suggests that the mitochondrial sequences of *P. mnemosyne* from Western Alps and the sequences from the southern areas of the range are so different that they might belong to different species. This speculation is especially attractive with regards to the case of *Zerynthia polyxena (Denis & Schiffermüller, 1775)* and *Z. cassandra* Geyer, 1828, which might be considered as an interesting precedent [76]. The authors recommended to locate the contact between the clades II and III in order to check the viability and fertility of the corresponding hybrids [26].

Pleistocene evolutionary history of Central and Eastern Europe populations

The geographical patterns of the *P. mnemosyne* genetic diversity were definitely shaped by the Pleistocene climatic changes when cold and arid periods were striking. The glacial cycles had to induce changes in the species distribution which was contracting or expanding if glaciation or warming of the climate occurred. The corresponding evolutionary dynamics of *P. mnemosyne* in Central and Eastern Europe and phylogeographic patterns were analysed in a thorough study by Gratton *et al.* [86]. It is important to note that the previous work by Gratton [87] demonstrated that the populations from the studied region formed a clade. The subsequent study by Gratton *et al.* [86] was covering 92 locations in this region and employed 225 *P. mnemosyne* specimens. The mitochondrial *COI* gene was utilized[32] and all the 225 sequences were collapsed into 53 haplotypes. The complete alignment had the length of 931 bp. The phylogenetic reconstruction was carried out by the analysis with the HKY + Γ + I model of evolution, and the genealogical relationships among the populations were also probed by MJ (Median-Joining) method. An important feature of the study is the attempt to determine the intraspecific time-dependent rate μ using calibration based on palaeoecological and geological data. The tMRCA (time to most recent common ancestor) was estimated by the Bayesian coalescent approach with the HKY + Γ + I model under the assumption of the simple strict clock model. The Bayesian coalescent approach was also used to determine the parameters of the IM (Isolation with Migration) model of population divergence where possible gene flow between the two descendant populations is supposed [89].

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[30] In fact, some populations of *P. mnemosyne* in Syria exhibit the *mnemosyne*-subgroup phenotype.

[31] The other large subgroups are: the *mnemosyne* subgroup containing most of the taxa distributed across Europe to Caucasus and Urals and the *giganteus* subgroup including central Asia [58].

[32] In 2007 Konopiński published a set of four short overlapping amplicons that were developed for the amplification and sequencing of a 1016 bp *COI* fragment in *Parnassius* species [88]. His report showed that the new primers worked very well for *COI* even in very old museum specimens of *P. mnemosyne* (complete sequence was obtained from 15 of 42 samples older than 80 years including specimens more than 100 years old). Gratton *et al.* took advantage of this methodical progress.

The ML tree of the studied haplotypes is depicted in Fig. 7a where eight clades can be recognized. In spite of low support these clusters are geographically well defined indicating their phylogeographical relevance. The most divergent haplogroup A is distributed in the southern part of Eastern Alps. The two haplotypes designated as B and T form probably strictly localized and isolated lineages, the former one being found in S Austria while the later one in Peloponnese. Other Greek populations and those from Macedonia are characterized by haplotypes clustering to G, although with lower support. Several localities in Eastern Alps of the neighbouring countries Austria, Italy and Slovenia house haplogroup C. The remaining vast regions of the area covered by the Michel's study are dominated by two geographically separated clades with only very narrow overlap. The haplogroup W is distributed in the western part: NE Italy, Slovenia, Hungary, Austria, Czech Republic, W Slovakia, Germany, and Åland Islands (consider the phylogeographic meaning), whilst the large clade E comprises the populations spreading from Bulgaria and Croatia to the north and north-east. Two distinct subgroups of the E clade exhibit meaningful support: the haplogroup EN from the Baltic region and N Russia but also found in central Hungary, and the haplogroup EE distributed from Hungary and northern and eastern parts of Carpathian system and through Eastern Europe. Actually, the same clades were identified in the MJ network where also an additional subgroup of the diverse lineage E was indicated being the haplogroup ER comprising Russian colonies.

The discussed geographical patterns can provide a basic insight into the Pleistocene history of *P. mnemosyne* whose profound genetic diversity is found within a restricted area of Eastern Alps and through the Pannonian Plain along the Balkan range to southern Greece. In contrast, the vast areas north of Alps and north and east of the Carpathian system are populated by rather genetically uniform populations. This is consistent with an explanation that refugia of *P. mnemosyne* were located in the perialpine region and on the Balkan Peninsula during glaciations, and later the species expanded to the north and east (see Fig. 7c).

The EN lineage was employed by Michel *et al.* for the molecular clock calibration. The authors assumed that the diversity of haplotypes of EN in the Baltic region originated in loco after the postglacial colonization. Specifically the authors supposed that tMRCA of the EN lineage had been 10 – 11 ka ago, what was introduced as the prior hypothesis on the coalescent time. The argumentation is based on geological and palaeoecological findings. In Europe *P. mnemosyne* inhabits various woody ecotones or places with trees found at least in the vicinity where the host plants *Corydalis* spp.[33] grow. Thus the authors concluded that *P. mnemosyne* probably had not arrived to the Baltic region before typical pioneer trees, such as *Alnus* spp. The most ancient pollen of *Alnus* spp. found the Baltic area was determined to be approximately 11 ka old while the most ancient arboreal pollen in the region should not be older than 12 ka [91]. These facts are in agreement with the time duration of the Scandinavian ice sheet in the eastern Baltic region that lasted till 14.5 – 12.0 ka BP. These assumptions are not as strong as one would wish, but in the case of butterflies where fossils or subfossils are extremely rare. Therefore, it is probably the only way how to handle the calibration of the molecular clock. The critical comment should focus on the assumption regarding the ecology/palaeoecology of *P. mnemosyne*. In fact, *P. mnemosyne* in Central Asia, Turkey,

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[33] With regards to host plants of *P. mnemosyne* in Europe, one should mention that the host plant *Corydalis lutea* is currently classified as *Pseudofumaria lutea*.

but also in southern Europe, occurs on quite dry alpine meadows and not on the typical woodland ecotones with moist soil where the common *Corydalis* spp. grow [58]. The subsequent Bayesian coalescent analyses described the molecular evolution rate μ by a marginal posterior probability distribution with the median of 0.096 substitutions/(site.Ma), the mean value of 0.107 and the 95 % credibility interval (0.029, 0.210). Such a rate is very high in comparison to the traditional mitochondrial μ = 0.01/(site.Ma) that was supposed, e.g., for *P. smintheus* Doubleday, 1847 in the Rocky Mts. in the classical study by DeChaine et Martin [92] as well as in many other studies. However, the approach of Michel *et al.* seems to be well supported by the underlying idea of time dependency of μ that probably decreases with an increasing time depth [93]. Based on the given value Michel *et al.* suggested that the whole eastern clade E had arisen approximately 30 ka ago, and the most recent ancestor of all studies haplotypes could have been dated to 60 – 70 ka BP. Further, the IM model led to the estimation of the divergence time of the W and E clade to be 19 ka ago with 95 % credibility interval (65.0, 49.5) ka BP. It is clear that this estimate coincides with the LGM.
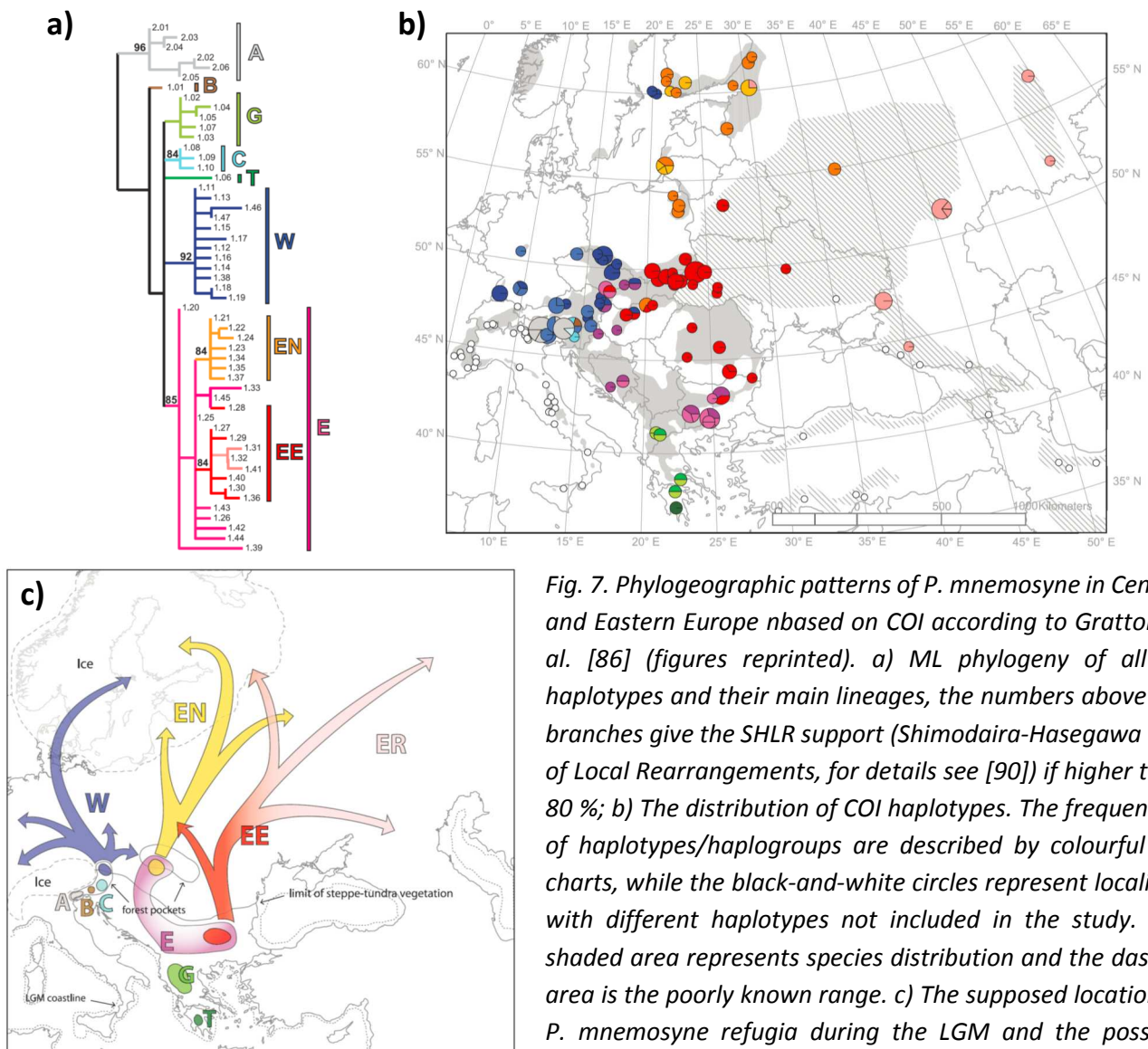

Fig. 7. Phylogeographic patterns of P. mnemosyne in Central and Eastern Europe nbased on COI according to Gratton et al. [86] (figures reprinted). a) ML phylogeny of all 53 haplotypes and their main lineages, the numbers above the branches give the SHLR support (Shimodaira-Hasegawa test of Local Rearrangements, for details see [90]) if higher than 80 %; b) The distribution of COI haplotypes. The frequencies of haplotypes/haplogroups are described by colourful pie charts, while the black-and-white circles represent localities with different haplotypes not included in the study. The shaded area represents species distribution and the dashed area is the poorly known range. c) The supposed location of P. mnemosyne refugia during the LGM and the possible routes of postglacial recolonization.

The estimated tMRCA of all the haplotypes indicates that the last common ancestor lived during the glacial stage of the Würm period (maximum glaciation around 65 ka BP). The fragmentation into different lineages occurred probably during the LGM in consequence of the genetic isolation of refugia in the perialpine region and on the Balkan Peninsula. The high genetic diversity in Bulgarian, Hungarian, and Slovakian populations indicates that localities along Balkan and Carpathian ranges could have acted as a meta-refugium exhibiting certain interconnections. The situation between the earlier Würm period and LGM cannot be addressed directly on the basis of these results, but the current model assumes that there was some degree of connectedness since the present lineages probably arose just during the LGM. The large regions north of the Alps and north and east of the Carpathians are occupied by three lineages with low admixing which suggests a considerable founder-effect during the postglacial colonization.

Evolutionary history of *Parnassius apollo*

The first molecular phylogeographic study of *P. apollo* was published in 2010 by Todisco *et al.* [94] who attempted to employ *COI* sequences of specimens from the whole range of distribution to explore its historical biogeography and to identify the patterns of its genetic variability. The authors of the mentioned study, entitled "*Phylogeography of Parnassius apollo: hints on taxonomy and conservation of a vulnerable glacial butterfly invader*", wrote that they had analysed the *COI* variation also to provide the first genetic basis for a revision of the subspecific taxonomy of *P. apollo*. With all due respect, it should be noted that the subspecific taxonomy is based predominantly on morphological differences and high degree of geographical isolation and not on the variation of mitochondrial DNA represented by *COI*. *COI* seems to be a suitable marker for a species separation, albeit rough and far from perfect, but it has not been used in any relevant study for the discrimination of individual subspecies. One cannot speculate that *COI* mutations would influence the wing pattern in butterflies or that they would be just a reasonable measure for the intraspecific variation of those genes which are responsible for morphological characters. Such genes are strongly affected by the natural and sexual selection. This fundamental fact completely decouples any analogy with the *COI* gene particularly in small and isolated populations. In spite of the great merit of the study by Todisco *et al.* the discussed ambitions regarding subspecific taxonomy are going far beyond the employed data.

By all means, the study utilized reasonable material of 201 specimens from 78 localities to analyse the phylogeographic processes. The employed fragment of *COI* was 869 bp long. All the sequences provided 71 haplotypes whose phylogenetic relationships were reconstructed by means of ML analysis with the GTR + Γ + I model of evolution and the resulting phylogram is shown in Fig. 8c. At the same time the MJ network was calculated (see Fig. 8d). Both the results are very consistent and reveal strong geographical patterns. The identified lineages are characterized by rather low bootstrap values, but their existence is supported by their well-defined geographical distribution (see Fig. 8) which is exactly the same situation we saw in the similar analysis of *P. mnemosyne*. The clade I possessing a reasonable support comprises several lineages or clusters distributed from European Russia to northern Europe and across Eastern Europe to Turkey. Specifically, the lineage Ir is described from European Russia, the lineage Ig occurs in southern Balkan Peninsula excluding Peloponnese.

Further, the Anatolian samples with the exception of far eastern populations form a separate lineage Ia. The other lineages from northern part of the Balkan Peninsula, Carpathians and Scandinavia are clustered into the paraphyletic basal group Ie. The butterflies from NE Turkey, Armenia and Caucasus are related forming the lineage C while the only subspecies from Peloponnese *P. apollo atrides* Dils & van der Poorten, 1986 is quite a divergent from the discussed clades I and E occupying the separate lineage R. The populations from the Alps, Massif Central and butterflies from the eastern parts of the Appenine Peninsula as far as to Aspromonte in southern Italy form lineage A. Several distinct lineages are found in various mountain ranges: N in Central Apennines, S in Sicilian Madonie, and P in Pyrenees. The haplogroups from central and southern Spain form a monophyletic lineage E with a strong support. Finally, the populations from Central Asia are clustered in a separate haplogroup K which mixes with the Ir lineage in the Urals. The phylogenetic relationships among the different lineages are not very clear from the ML analysis due to shallow divergence of the *COI* sequences. Similarly, the MJ network contains also an unresolved central loop to which the mentioned lineages are connected. Nevertheless, the authors mentioned that the Asian sequences had been found to be basal ones according to ML analysis. This implies an idea that *P. apollo* might have originated in Central Asia [26], which is in agreement with the idea that centre of diversity is there, and five of the eight subgenera are confined to Central Asia.

Further, Todisco *et al.* described the problems related to the application of a molecular clock and mentioned that a fully dated reconstruction of the *P. apollo* evolutionary history could be hardly resolved from the given data, but still they attempted to analyse it. They employed the ML approach and tested both the global and local molecular clocks. The optimal model was then transformed to the time scale using two alternative substitution rates as lower and higher estimates. The lower one was based on the conventional interspecific substitution rate used in entomological studies μ_1 = 0.01 substitution/(site.Ma^{-1}), and the higher one was approximately ten times higher being μ_2 = 0.096 substitution/(site.Ma^{-1}). The second rate is equal to the value suggested by Gratton *et al.* in the recent phylogeographic study of *P. mnemosyne*. The LRT (likelihood ratio test) indicated that the global clock model should be rejected when compared to a model with independent substitution rates. This might reflect the differences in the evolutionary dynamics of the main lineages that were separated for long time and were subjected to different selection and demographic processes. Thus the authors favoured a local clock model with a different substitution rate for the clade I that exhibited longer branch in comparison to the others. The deepest divergence among all the haplotypes and thus the maximum age of all the involved *COI* variability was younger than 1.5 Ma (μ_1) or 100 ka (μ_2).
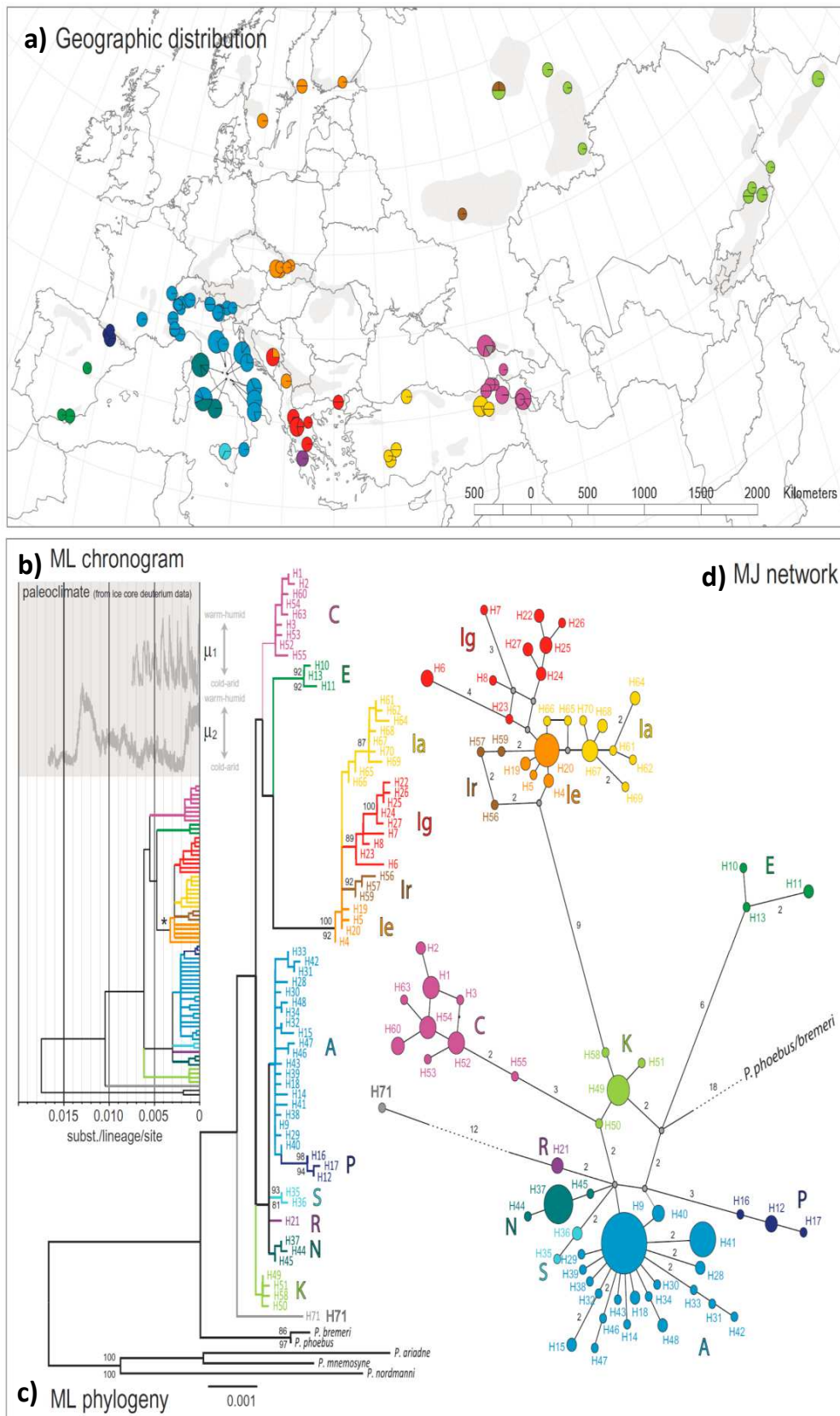
Fig. 8. Mitochondrial lineages of P. apollo according to Todisco et al. (reprinted): a) geographic distribution of the haplogroups whose frequency is described by pie charts, b) ML chronogram of the haplotypes based on a local molecular clock (branch with a separate rate marked by an asterisk), the upper part shows palaeoclimatic data scaled according to μ₁ and μ₂ rate, c) ML tree of all haplotypes with bootstrap values higher than 80% and d) MJ network with nucleotide substitutions described by the numbers next to the connections.

According to the faster rate μ_2, the species reached its current distribution not earlier than 60 ka ago while the slower estimate μ_1 leads to the age of 500 – 600 ka. The latter time frame would probably provide more resolved phylogeny due to the five complete glacial-interglacial cycles, but the phylogeny is not as much resolved. Therefore the results obtained under the assumption of faster μ_2 could be considered. The scenario suggested by the authors (see Fig. 9) assumes that the spread of *P. apollo* from Central Asia to Europe was rapid and occurred between 70 – 100 ka ago concurrently with the expansion of open habitats after Riss-Würm interglacial. The following glaciation caused a diversification in southern areas (see Fig. 9a) when glaciation prevented gene flow through central Europe. The secondary expansion in southern Europe (see Fig. 9a), corresponding for example to the coalescence within haplogroup I, was enabled by the spread of cold and arid zones in consequence of the LGM 30 ka ago (see Fig. 9b). The last expansion was leading to the north (see Fig. 9b) and occurred later than 10 – 11 ka BP when the ice was disappearing from the Baltic and Scandinavian regions. The geographical distribution of the broad lineage I shows that the populations from northern Europe are related to those ones in the Carpathians and on the Balkan Peninsula. The interesting feature is mixing of K and Ir lineages in the Urals which could be explained by a secondary contact of the original lineage from central Asia and the butterflies once originated in the Balkan Peninsula.
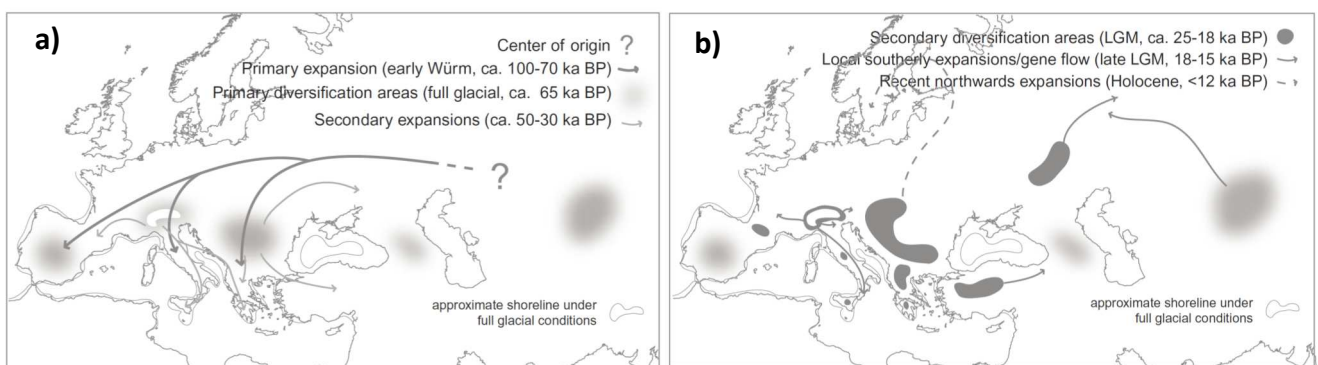

Figure 9. Possible expansion and evolution of P. apollo according to Todisco et al. (figures reprinted): diversifications centres are supposed to be found where exclusive COI lineages occur, while some primary centres are only assumed as geographical centres of clades with wider distribution.

Parnassius phoebus complex

This group includes the following morphologically and geographically very distinct taxa that are traditionally considered as different species: *P. phoebus* with a large range spanning from the Urals across Central Asia and N China, Far East and crossing the Bering Strait to Alaska and Yukon, *P. bremeri* distributed in E Siberia, neighbouring eastern regions of China and in Korea, *P. sacerdos* found only in the European Alps, *P. smintheus* from the western chain of mountains in Canada and USA, *P. behrii* Edwards, 1870 from Sierra Nevada in California[34], and *P. rueckbeili* Deckert, 1909 restricted to the Barkul Mts. in N China. The difference and possible phylogenetic position of the last mentioned species was already discussed.

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[34] There is a thrilling mystery related to the potential distribution of this species in Mexico. Three specimens of a butterlfy resembling *P. behrii* were reportedly collected at around 450 m near Ciudad Victoria in Tamaulipas on the 3rd of july, 1952 by A. H. Moeck [47, 95].

Earlier the very distinct taxon *P. bremeri* was not considered to be a true member of the *P. phoebus* complex [47], although now it seems to be genetically very close to *P. phoebus s. str.*, and the recent molecular studies have classified it as a *P. phoebus* ssp. Omoto *et al.* [41] claimed that the *ND5* sequences of *P. phoebus* and *P. bremeri* were essentially identical. Employing *LSU*, *ND1* and *COI* sequences Michel *et al.* [26] showed that *P. bremeri* from Sout Korea fit between *P. phoebus interpositus* Herz, 1903 and *P. p. golovinus* Holland, 1930 (see also Fig. 2). Therefore, these authors concluded that *P. bremeri* represented only a morphologically distinct form of *P. phoebus*. On contrary, from the morphological viewpoint one would not hesitate to classify *P. bremeri* separately due to its very distinct wing pattern (prominent veins suffused by black scales) and due to the different antennae (in *P. bremeri* the antennal stalks are black or rarely grey with black rings while in *P. phoebus* spp. the stalks are greyish due to the presence of white scales and further ringed with black) [7]. Nevertheless, its genitalia are not very different from that of *P. phoebus s. str.* taxa. Moreover, transitive populations exist, e.g. the taxon *amgunensis* Shlejuzhko, 1928 that was originally described as a *P. bremeri* ssp. and later placed to the *P. phoebus*. The taxon *amgunensis* is probably identical to the taxon *gulshenkoi* Iwamoto, 1997 which was, interestingly, described as a subspecies of *P. phoebus*. Finally, we should remark the phylogenetic trees in Fig. 2 might be somewhat confusing with respect to *P. sacerdos* since the predominant view of the populations in the Alps is that they all belong to the species *P. sacerdos* which was further split on more than 15 subspecies with *gazeli* Praviel, 1936 being the very distinct ssp. among them.

The butterflies of *P. phoebus* complex occurring on alpine and subalpine habits have attracted attention of molecular scientists also thanks to their large Holarctic distribution and potential significance as a suitable model for the phylogeographic history of alpine fauna in relation to Pleistocene-Holocene climate changes. Four molecular studies focusing on these butterflies have been published so far, dealing with the phylogeography of *P. smintheus* [92, 96], *P. smintheus*, and *P. behrii* [97] and the evolutionary history of the whole *P. phoebus* complex within the Holarctic region [98]. The last mentioned study by Todisco *et al.* [98] integrated also all the sequences from the previous authors and analysed them again within one large dataset. The study was based on the *COI* gene whose 824 bp fragment was sequenced for 203 specimens from 72 sites located mainly in Palaearctic region, and these data were further combined with 498 sequences from the previous studies providing an overlap of 512 bp with the new ones. In total 178 unique haplotypes were identified. The phylogenetic analysis was carried out by means of ML with GTR + I + Γ, GTR, and GTR + I + Γ models for the first, second and third codon position, respectively, as the best fitting models. The authors also employed a complex analysis under the framework of the IM model estimating its parameters by the MCMC approach. These simulations were run for several pairs of neighbouring population units under different priors, either enabling migration or disabling it. The respective results for the pair of *P. phoebus* populations in Siberia and Alaska were used in the calibration of the molecular clock. Specifically, the results obtained under the assumption of the non-migration model were used to evaluate the *COI* substitution rate μ considering that the opening of the Bering Strait occurred before 10 ka.

The ML tree with the exception of few nodes exhibited low support, but it indicated the reciprocal monophyly of the Eurasian-Alaskan populations of *P. phoebus* complex on one side and the North American clade of *P.*

smintheus and *P. behrii* on the other side. The mean nucleotide divergence between the clades was 3.0 %. These two lineages were obviously separated by the North American ice sheets during the last glacial period. Further, there is a reasonable support for the haplotypes of *P. smintheus* from Klamath Mts. to be the basal group of the North American clade. The monophyly of *P. behrii* with respect to *P. smintheus* was not supported by the ML analysis of *COI* haplotypes, however, it does not share any haplotype with *P. smintheus*. The current distribution of *P. smintheus* in Yukon and British Columbia likely resulted from the northward expansion of the southern populations after LGM as the same haplotypes occur in Washington and Montana. According to the authors no significant phylogeographical signal was found among the mitochondrial haplotypes of the Eurasian-Alaskan populations. Nevertheless, some geographic patterns of the genetic variability were clear, e.g. populations from the Alps and the Urals possess mostly their own haplotypes. In summary, the situation indicates that the divergence of the studied taxa has been very recent.

The authors evaluated the rate μ to be 0.086 substitutions/(site.Ma with the 90 % confidence interval being 0.039 – 0.139 substitutions/(site.Ma). Such a rate is rather high, four to twenty times higher than the conventional phylogenetic rate (see also the above discussed study of *P. mnemosyne* by Gratton *et al.*). This fast rate is leading to divergence times within regional population pairs between 10 to 35 ka (70 ka within the confidence intervals) which is well after the last interglacial period at 130 ka. Further, tMRCA of the N American clade should not be higher than 125 ka. The tMRCA is higher for the Eurasian-Alaskan clade being 100 – 200 ka, however, only due to one sample from the Khangai Mts. in Mongolia. After its removal tMRA for this clade decreases below 100 ka. The given sequence from Khangai Mts. might be just a unique relic of the variability older than the last interglacial period. In contrast, all remaining polymorphism resulted from a common ancestor which expanded during the Würm/Wisconsin glacial phase (50-18 ka) and its range was subsequently fragmented due to the Holocene warming causing the diversification.

Conclusions

Closing this treatment we are supposed to summarize the present state of the art and to suggest relevant questions or even anticipate their answers. We may fail in the latter and we might not suggest feasible approaches to important issues, however, we will definitely fail in preventing the author of these lines to state some critical comments while summarizing as follows.

Unfortunately, the current molecular studies are rather determined by the availability of sequences in databases and by the ease of sequencing evergreens like *COI* than by the actual needs of the intended studies. Although it is quite clear that the mitochondrial DNA neither rules nor determines the diversity of species and their mutual delimitations, people still believe that they might use even short fragments of mitochondrial genes to disentangle taxonomically complicated issues without considering morphology, biogeography, and ecology. Such blind faith in the power of the celebrated *COI* or similar entities could rather lead to mitochondrial obscurantism than to a real progress in taxonomy. Not only sophisticated methods of computational analysis and reasonable sampling, both of which appear to be accomplished in recent studies, but also relevant sequence data are necessary to shed

some light on Parnassiinae, a group exhibiting profound complexity and variability. Otherwise the molecular aficionados would not differ from the early enthusiasts who described a plethora of taxa and infrasubspecific forms, burdening the field with methodically wrong results. Identification and use of suitable sequences both for the phylogenetic and phylogeographic/genealogical studies seems to be a major obstacle before entering the new area of the *Parnassius* research being the molecular parnassiology. Moreover, at present several authors deplorably FAIL to understand the nature of phylogenetic analyses in the following sense. If a certain phylogenetic structure is not supported (is not resolved) in our tree, it does not mean that the phylogenetic (and taxonomic structure) does not exist and should be rejected. In fact, it is just not supported by the employed data that might be completely useless or inappropriate for the purpose of our analysis.

The author is not optimistic regarding the future of the traditional parnassiology for several reasons, and he is not even optimistic regarding the future of these amazing butterflies. Several dark facts are related to *Parnassius* collecting. The extent of the data falsification is particularly enormous in *P. apollo* since ridiculous collectors are keen to pay considerable amounts of money for specimens reputedly coming from certain extinct populations. There were even EVIL efforts to eradicate certain *Parnassius* taxa in order to increase their price on the market. The extinction has been a threat not only for many *P. apollo* subspecies, a lot of *P. mnemosyne* populations including several described subspecies have disappeared as well and even certain populations of other *Parnassius* species have been completely lost. Instead of losing the last lines with trivial conclusions it is rather important to warn that we are currently losing huge genetic diversity hidden in scattered and isolated colonies of these butterflies belonging to the most spectacular living creatures.

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