University of South Bohemia in České Budějovice
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Phylogeny of Brimstone butterflies (genus *Gonepteryx*): The evolution of colour pattern in UV spectrum and geographical area

Master thesis

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Annotation

Phylogeny, phylogeography and evolution of UV reflecting patterns were studied in 12 species of the genus *Gonepteryx*. Sequences of one mitochondrial (COI) and one nuclear gene (Wingless) were used for phylogenetic analyses and reconstruction of the biogeographical events. The results were later compared with the extent of UV reflecting pattern to construct the ancestral situation and evolution of the UV pattern within the genus.

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Dana Hanzalová

Declaration & Acknowledgement

As the supervisor of Dana Hanzalová, I hereby declare that she played a major role in the process leading to the manuscript presented here as her Master thesis. Dana conducted all the phylogenetic laboratory work, participated in analyses of both phylogenetic and UV data, and wrote the manuscript. The material was collected by me and our collaborators and the photographs of UV pattern were produced by Pavel Pecháček (Charles University in Prague). Dana thus contributed to the overall work by approximately 65%.

RNDr. Zdeněk Faltýnek Fric, PhD.

I would like to thank my supervisor, Zdeněk Faltýnek Fric, for his time, patience and his infinite kindness. I would like to thank Pavel Pecháček for a great contribution to this study. I would also like to thank my colleagues, especially Jana Marešová, Alena Bartoňová and Michal Rindoš for helping me and never abandoning me in the time of need, my friends and family for their support and my fiancé for always standing by my side. Finally I would like to thank every person not mentioned above who contributed to this study, and the Faculty of Science of University of South Bohemia for allowing me to carry on my research. This study would never exist without all of you. Thank you.

Contents

Abstract

The ultraviolet pattern is a common and well known trait amongst the Lepidoptera. That includes the genus *Gonepteryx*, where the males of majority species possess a certain amount of largely variable UV reflecting pattern on the dorsal surfaces of their wings, which evolutionary history we decided to inspect. We used the sequences of one mitochondrial (COI) and one nuclear gene (Wingless) for reconstruction of phylogeny and biogeography of the genus *Gonepteryx*, the connection with the presence of the UV reflecting pattern and its evolution. We inferred that the genus *Gonepteryx* is monophyletic as well as the origin of the UV reflecting pattern in the genus. The ancestor possessed a moderate UV reflecting area on both forewings and hindwings, which later grew larger, approximately the same size, or secondarily disappear in the descendant species. There is a significant correlation between the variability of the UV pattern and the number of species occurring in an area.

Introduction

Sensitivity to ultraviolet light in animals was for the first time observed in 1882 by John Lubbock, the 1st Baron Avebury (Lubbock 1882). Since then, many studies of ultraviolet perception in animals were published in all major animal groups (Tovée 1995): both vertebrates such as reptiles (Fleishman et al. 1993), birds (Burkhardt 1982, 1989; Bennett & Cuthill 1994), mammals (Jacobs et al. 1991), and invertebrates including spiders (Heiling et al. 2003, 2005), beetles (Pope and Hinton 1977) or butterflies (Lutz 1933a; Mazokhin-Porshnyakov 1957; Nekrutenko 1965; Silberglied and Taylor 1978; Silberglied 1979; Eguchi and Meyer-Rochow 1983; Brunton and Majerus 1995; Kemp 2005). Especially the last group, Lepidoptera, has been an object of particular interest for many researchers through the past decades.

One of the best examined families in butterflies regarding ultraviolet reflection is the family Pieridae (Duponchel, 1835), which was an object of several studies (Brunton 1998; Makino et al. 1952; Mazokhin-Porshniakov 1957; Nekrutenko 1964; Obara & Hidaki 1968; Primož 2011), including genus *Gonepteryx* (Leach, 1815). The ultraviolet reflecting pattern appearing on the wings of male butterflies tend to be consistent in the genus *Gonepteryx* (with the exceptions of few species, such as *Gonepteryx rhamni*; Linnaeus, 1758) and therefore can be used as a taxonomic tool, although not the only one (Kudrna 1975). UV reflectance in *Gonepteryx* genus has been studied by Nekrutenko (1964, 1968, 1970),

Brunton et al. (1996), Pecháček et al. (2014) and it was recently explored also in a monograph by Bozano et al. (2016).

The genus *Gonepteryx* consists of 16 middle-sized to large species (Bozano et al. 2016). Majority of them can be found across the Palearctic region with the exception of *Gonepteryx taiwana* (Paravicini, 1913), *Gonepteryx amintha formosana* (Fruhstorfer, 1908) and *Gonepteryx amintha burmensis* (Tytler, 1926) entering Oriental region in southern Himalayas, southern China and Taiwan, respectively (Bozano et al. 2016). The most widespread and possibly the best known species of the genus is *Gonepteryx rhamni* inhabiting a wide area from Northwestern Africa through Mediterranean and boreal Eurasia to the mountains of Central Asia.

Large variability in UV pattern between different species of the genus *Gonepteryx* and also variability inside of the species *G. rhamni* described by Pecháček et al. (2014) led us to a conclusion that it is a proper time to inspect evolutionary history of the UV reflectance pattern in the genus.

The main object of this study was to construct a phylogenetic tree of *Gonepteryx* genus with possibly all the existing species, examine the phylogenetic and biogeographical relationships across the genus and connect them with the presence of the ultraviolet reflecting pattern and its evolution. We hypothesised that the evolution of the UV patterns correlates with the phylogeny of the genus and that there is a wider range of the UV patterns in the areas with two and more species living sympatrically.

Methods

Sampling

Samples were collected directly or obtained from private collections. We gathered and isolated DNA from a total number of 97 samples of 12 species of the genus *Gonepteryx*: 3 samples of *Gonepteryx acuminata* (C. & R. Felder, 1862), 10 samples of *Gonepteryx amintha* (Blanchard, 1871), 9 samples of *Gonepteryx aspasia* (Menetries, 1859), 2 samples of *Gonepteryx cleobule* (Hübner, 1824), 18 samples of *Gonepteryx cleopatra* (Linnaeus, 1767), 2 samples of *Gonepteryx eversi* (Rehnelt, 1974), 6 samples of *Gonepteryx farinosa* (Zeller, 1847), 3 samples of *Gonepteryx maderensis* (Felder, 1862), 3 samples of *Gonepteryx maxima* (Butler, 1885), 9 samples of *Gonepteryx nepalensis* (Doubleday, 1847), 2 samples of *Gonepteryx palmae* (Stamm 1963), 30 samples of *Gonepteryx rhamni* and 3 samples of *Gonepteryx* sp. which are probably *G. rhamni* females but could not be identified properly

due to the absence of UV reflecting pattern (Appendix 1). The sampling covered majority of the area of the genus *Gonepteryx*.

As outgroups, we selected three species of related genera: *Catopsilia florella* (Fabricius, 1775), *Dercas gobrias* (Hewitson, 1864) and *Eurema lisa* (Boisduval & LeConte, 1829).

DNA extraction, PCR amplification and sequencing

Samples used for the analyses were already desiccated. We extracted the DNA from butterfly legs by grinding the dry tissue and used Genomic DNA Mini Kit (Tissue) (Geneaid) for the extraction, following the instructions from the producer. The isolated DNA was kept in microtubes in aproximately -20 °C.

The fragments of DNA were amplified using PCR. We run the PCR for one mitochondrial (cytochrome c oxidase subunit I, COI) and one nuclear (Wingless) marker. We used the protocols described by Wahlberg & Wheat (2008). The primers used for the amplification were: HybLCO-HybHCO and HybRon-HybHCO for the first part of COI (COIa) and HybLepWG1-HybLepWG2 for Wingless (Table 1). The reaction was prepared in 12.5 μl of PPP Mastermix (Top-Bio), 8.6 μl of H2O, 1 μl of reverse primer, 1 μl of forward primer a 1.9 μl of DNA. The following PCR protocol was used: 5 minutes of 95 °C (beginning); 30 seconds of 94 °C, 30 seconds of 50 °C and 90 seconds of 72 °C (35 cycles); 10 minutes of 72 °C (final extension). The presence of products was tested with gel electrophoresis.

The successful samples were sequenced in one-way in Macrogen, South Korea (http://www.macrogen.com/eng/index/). We manually aligned the final sequences in Geneious v. 9.0.4. (Kearse et al. 2012). The total length of each sequence was 487 bp for COI and 412 bp for Wingless.

Table 1. Primers used for PCR amplification.

Marker	**Part**	**Type**	**Primer**	**Sequence**
COI	1	F	HybLCO	5' GGTCAACAAATCATAAAGATATTGG 3'
	1	R	HybHCO	5' TAAACTTCAGGGTGACCAAAAAATCA 3'
	1	F	HybRon	5' GGAGCYCCWGATATAGCTTTCCC 3'
	1	R	HybHCO	5' TAAACTTCAGGGTGACCAAAAAATCA 3'
Wingless	1	F	HybLepWG1	5' GGARTGYAARTGYCAYGGYATGTCTGG 3'
	1	R	HybLepWG2	5' ACTICGCARCACCARTGGAATGTRCA 3'

Phylogenetic and biogeographical analyses

We used 97 samples of the genus *Gonepteryx* from which we successfully obtained the sequences for both molecular markers and 3 samples of related species as outgroups. We used Partition Finder2 (Lanfear et al. 2016) for the selection of the best substitution model. The selected model for the concatenated dataset was GTR+G (Generalised time-reversible model plus gamma distribution).

For the construction of phylogenetic trees we used Maximum Likelihood (RAxML; Stamatakis 2014) and Bayesian analysis (MrBayes 3.2; Ronquist & Huelsenbeck 2003) with sample frequency = 1000, temperature = 0.2, number of chains = 2 and number of runs = 4; 5,000,000 mcmc generations. The rest of the setting was left as default.

Dating of phylogenetic events was conducted in BEAST 1.8.0. (Drummond et al. 2012). For calibration of the tree, we defined 3 nods, based on the study by Edger et al. (2015). The first one was the separation of *Gonepteryx* genus and outgroups from the rest of Pieridae family (55,6 mil years; standard deviation = 2,5), the second one was the separation of the branch of *Gonepteryx* and *Dercas* from *Eurema* (38,5 mil years; standard deviation = 1,8), and finally the third one was the separation of *Gonepteryx* genus (34,9 mil years; standard deviation = 1,4). The molecular clock was set to uncorrelated log-normal relaxed clock. The coalescent model was set to Constant. The length of chain was 50,000,000 generations.

For the biogeographical analysis, we defined 7 areas: Canary Islands + Madeira (A), Northwestern Africa (B), Boreal Eurasia (C), Mediterranean (D), Central Asian mountains (E), East Asia (F) and Taiwan (G) (Fig. 1). The reconstruction of ancestral area was computed in RASP 4.0 (Yu et al. 2015) using BioGeoBears script (an R package) which compares several alternative biogeographical scenarios and performs inference of biogeographic history on phylogenies, and also model testing and model choice of the many different possible models (dispersal, vicariance, founder-event speciation, DEC, DIVA, BAYAREA, etc.) (Matzke 2013).

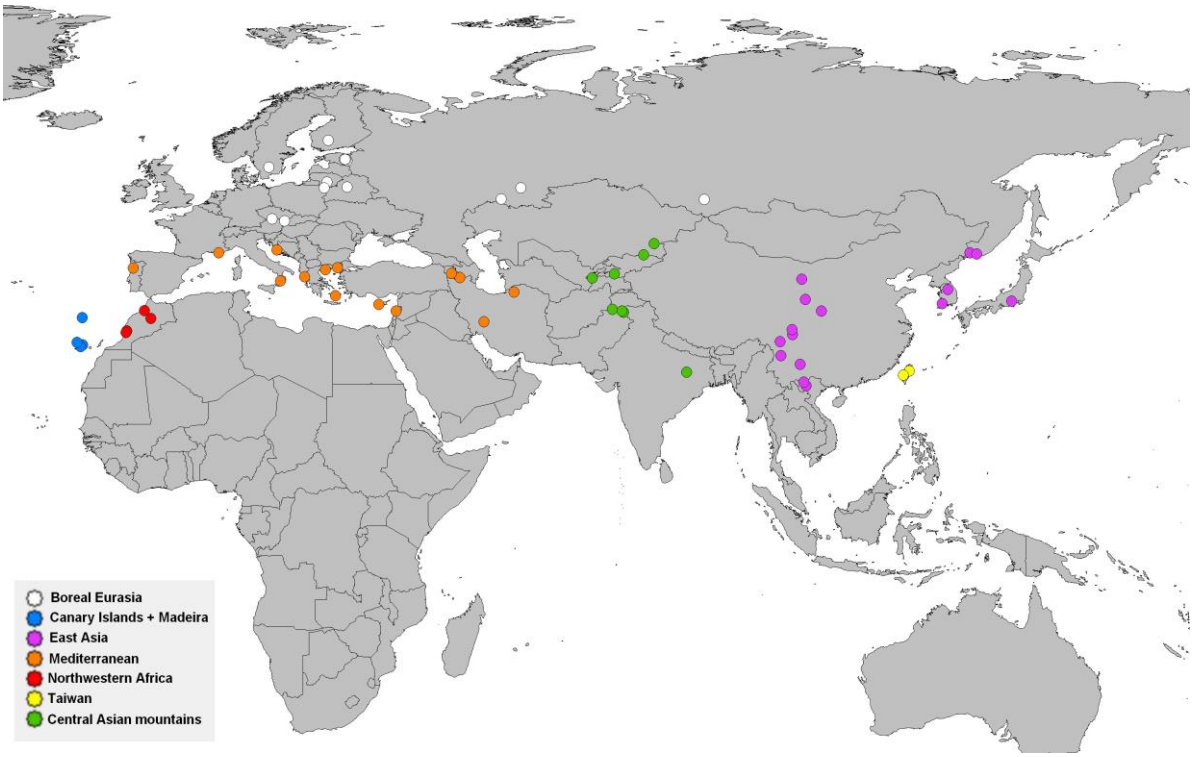

Figure 1. Distribution of *Gonepteryx* spp. samples assigned to the geographical areas used for biogeographical analysis.

Analysis of the ultraviolet reflectance

For the purposes of examining the ancestral situation and evolution of the UV reflectance in *Gonepteryx* genus we put each sample into a table according to presence of the the UV reflectant pattern (present/ absent) and according to the size of the wing area covered with UV reflectant pattern (scale 1-6). The strength of the ultraviolet reflectance was not taken into consideration. The table was prepared separately for the forewings and the hindwings. For taking photographs in the UV wavelength range, we used a FujiFilm IS Pro digital camera suitable for UV photography due to its broad sensitivity spectrum, which spans from 330 to 900 nm. The camera was equipped with an uncoated UV-transmitting lens (Helios 44-2 58mm f/2 lens). We used photographical filters B+W 403 (which blocks the visible spectrum 400–700nm) and B+W BG 53 (which blocks the IR light $\lambda > 700$nm). As a result, only the UV light ($\lambda < 400$nm) is transmitted through the lens. For illumination of the photographed objects, we used a UVP MRL-58 multiple-Ray-Lamp (8-watt, 230V-50Hz, 0.16A) equipped with a mercury fluorescent lamp 8w F8T5 long-wave 365nm. All objects were illuminated under the angle of 45° and photographed in a standardized position (dorsal view). The following setting of the FujiFilm IS Pro camera was used for all specimens: ISO

400, shutter time 15′, aperture of 3.5. All images were standardized, using 18% gray card, Kodak colour separation guide, and a 15 cm length scale (Pecháček et al. 2014).

Reconstruction of the ancestral state was computed in R 3.3.1 (R Core Team 2013) using fastAnc algorithm (phytools package; Revell 2012), as well as other calculations like simple regressions.

We counted presence of different number of UV patterns per the 7 biogeographical areas and correlated them with a number of species occurring in the area (Table 2). Phylogenetic signal in the UV reflectance was calculated separately for the forewing and the hindwing using Blomberg's K (Bloomerg et al. 2007) and Pagel's λ statistics (Pagel 1999). The K values compares variance-covariance effects of the data on the phylogeny with a Brownian motion model. The value of λ is a transformation of the phylogeny that ensures the best fit of trait data to a Brownian Motion mode. In both measures, values close to zero means no phylogenetic signal, whereas values close 1 indicates strong Brownian motion. The values were calculated in R (phylosig in package geiger with 1000 simulations). The values of the variables were compared using Freckleton and Harvey Node-Height Test (nh. test in geiger) (Freckleton & Harvey 2006).

Table 2. Number of species of the genus *Gonepteryx* and number of different types of UV pattern on forewings and hindwings.

Area	**Number of species**	**Different UV patterns on forewing**	**Different UV patterns on hindwing**
Canary Islands + Madeira	2	2	2
Northwestern Africa	2	2	2
Boreal Eurasia	1	1	1
Mediterranean	3	3	3
Central Asian mountains	3	2	1
East Asia	5	4	2
Taiwan	1	1	1

Results

Phylogenetic analyses

The results of the three phylogenetic methods, Maximum Likelihood in RaxML (Appendix 2-4), Bayesian Interference in MrBayes and Beast (Appendix 5) were congruent. The final result is mostly driven by COI since the separate analysis of Wingless is inconclusive and does not reflect the evolution of the studied genus properly. Genus *Gonepteryx* is monophyletic with *Dercas* as the most closely related genus of the chosen outgroups. All species are well defined (Fig. 2).

The origin of *Gonepteryx*, i.e. its split from the common ancestor of *Gonepteryx* and *Dercas*, is estimated around 35 mya (Edger et al. 2015). The first division of *Gonepteryx* genus to the branch containing Eastern Asian species *G. aspasia* and *G. acuminata* and the rest of the species occurred around 22 mya. The rest of Eastern Asian species, namely *G. nepalensis*, *G. amintha* and *G. maxima* separated from the Middle and Western Palearctic branch 16 mya. *G. nepalensis* is a sister species to the other two and the division occurred approximately 10 mya.

The Eurasian group was divided into two main branches 14 mya, when the three species endemic to Canary Islands separated from the branch containing *G. rhamni*, *G. farinosa*, *G. cleopatra* a *G. maderensis*. *G. palmae* is a sister species to *G. eversi* and *G. cleobule* and separated approximately 5 mya.

The most widespread species, *G. rhamni*, separated from the rest of the group around 12 million years ago. Finally, the rest is divided into a branch of *G. farinosa*, which is sister to the branch of *G. cleopatra* and *G. maderensis* and separated 7 mya.

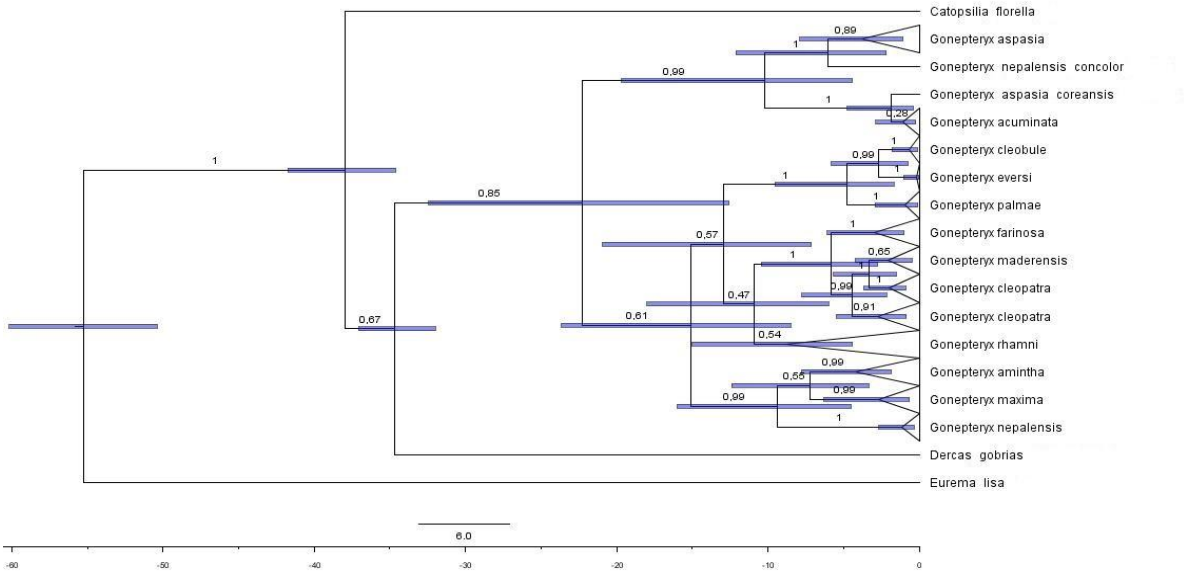

Figure 2. Chronogram showing the phylogeny of the genus *Gonepteryx* based on COI and Wingless and calculated in BEAST 1.8.0. The molecular clock is calibrated by outgroups according to Edger et al. (2015). The branch labels show the posterior probabilities and the time scale is in mya.

Biogeographical analysis

Most favored biogeographical model according the BioGeoBears was DIVALIKE+J (likelihood version of the DIVA model: Ronquist, 1997) (Table 3), which is a likelihood-based model of dispersal-vicariance with additional "j" parameter (founder event/ jump speciation) allowing descendant lineages to have a different area from the direct ancestor (Matzke, 2013; Vasconcelos et al., 2017). Biogeographical analysis (Fig. 3) shows that the common ancestor of *Gonepteryx* genus inhabited a wide area of Central Asian mountains and Eastern Asia including Taiwan and reaching to the Canary Islands. The most frequent biogeographic events were dispersals (26 events) followed by vicariance (18 events). The branch containing *G. aspasia* and *G. acuminata* firstly dispersed into Boreal Eurasia and then separated from the rest of the genus, followed by Central and Eastern Asian branch of *G. amintha* and *G. maxima* and also *G. nepalensis* from Taiwan. The three species show a vicariant occurrence.

The rest of the genus dispersed across the Eurasian area with *G. rhamni* as the most widespread species with the largest area of occurrence. The group of *G. maderensis*, *G. eversi*, *G. cleobule* and *G. palmae* separated from the rest of the species and remained in the area of the Canary Islands.

Table 3. Parameters of biogeographical models for biogeographical analysis of the genus *Gonepteryx* conducted in BioGeoBears. According to the lowest AIC, the DIVALIKE+J model was selected.

	LnL	**numparams**	**d**	**e**	**j**	**AICc**	**AICc_wt**
DEC	-123.5	2	0.0090	1.0e-12	0	251.1	3.6e-13
DEC+J	-94.7	3	1.0e-12	1.0e-12	0.020	195.6	0.40
DIVALIKE	-117.9	2	0.012	1.0e-12	0	239.8	1.0e-10
DIVALIKE+J	-94.34	3	1.0e-12	1.0e-12	0.020	194.9	0.58
BAYAREALIKE	-168.4	2	0.0086	0.14	0	340.9	1.2e-32
BAYAREALIKE+J	-97.59	3	1.0e-07	1.0e-07	0.021	201.4	0.022

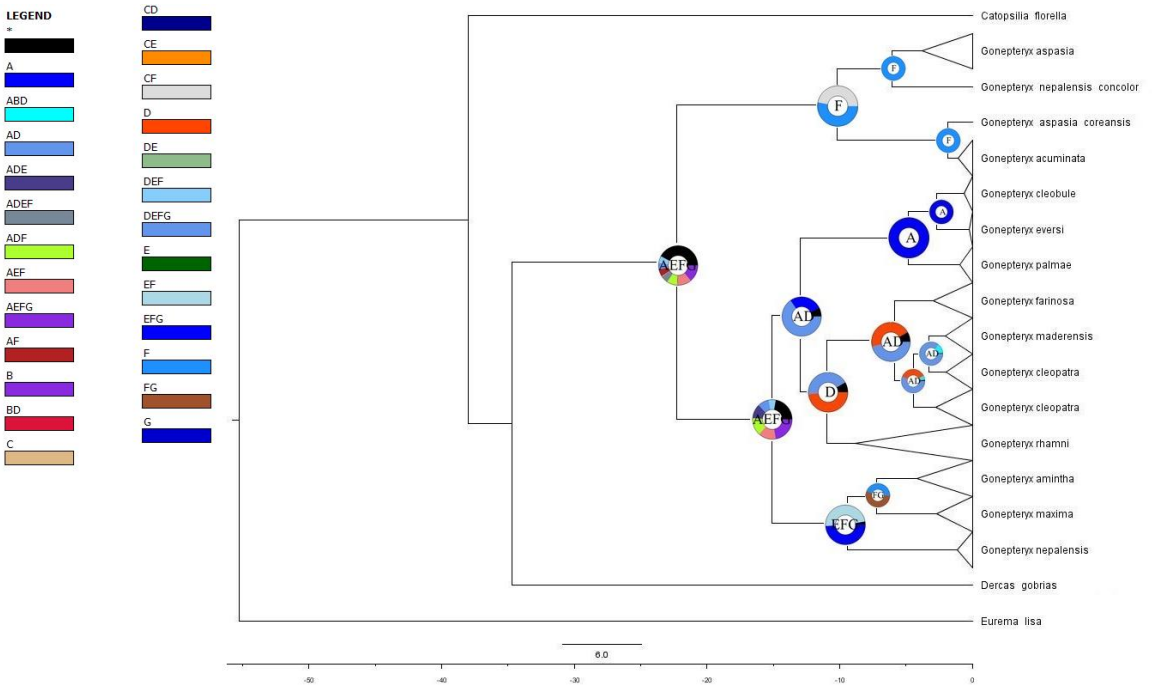

Figure 3. Distribution of the genus *Gonepteryx* in the geographical area in the course of evolution. Time scale is in mya. The regions are (A) Canary Islands + Madeira, (B) Northwestern Africa, (C) Boreal Eurasia, (D) Mediterranean, (E) Central Asian mountains, (F) East Asia and (G) Taiwan.

Ultraviolet patterns

The evolution of ultraviolet patterns in male *Gonepteryx* butterflies shows that the common ancestor possessed a certain amount of ultraviolet reflectance on forewings (Fig. 4) but only a little amount of reflectance on hindwings (Fig. 5).

The surface of forewings covered with ultraviolet pattern grew independently in the branch of Canarian species (*G. maderensis*, *G. eversi*, *G. cleobule* and *G. palmae*) and in Eastern Asian branch of *G. amintha* and *G. maxima*, while in *G. cleopatra* the UV reflecting area grew only a little. The UV reflectance on forewings disappeared completely in *G. farinosa*. For the rest of the species the occurrence and size of UV pattern on forewings did not change.

The presence of UV pattern on hindwings is not as common as on forewings. The most eminent pattern developed in three Canarian species (*G. eversi*, *G. cleobule* and *G. palmae*) while the Madeiran *G. maderensis* and Mediterranean *G. cleopatra* show slightly smaller area of UV reflectance on hindwings but still significantly larger than in the common ancestor. A small area of UV pattern – similar in size to the common ancestor – occurs also in species *G. maxima*, and subspecies *G. amintha formosana* and *G. rhamni meridionalis*. The rest of the species of *Gonepteryx* genus show no sign of UV reflection presence on hindwings.

The relationship between number of species and the variability of the forewing UV pattern was highly significant ($F=53.57$; $df=1, 5$; $p=0.0007$), there was no significant difference in the species number and the variability of the UV pattern on hindwings ($F=1.28$; $df=1, 5$; $p=0.31$).

The Bloomberg's K values for the UV pattern on forewings were equal to 0.261 ($p<0.001$) and for hindwings equal to 2.227 ($p<0.001$). The Pagel's λ values for forewings were 0.95 ($p<<0.001$) and for hindwings 1.004 ($p<<0.001$). It means that the UV pattern has strong phylogenetic signal, however, the low value of K for forewings indicates a presence of a constrain. The UV patterns of both fore- and hindwing were strongly correlated with phylogeny (forewing: intercept=5.30, SE=3.20, t=1.65, p=0.101, estimate=-0.72, SE=0.16, t=-4.51, $p<<0.001$; hindwing: intercept=4.51, SE=2.31, t=1.96, p=0.053, estimate=-0.73, SE=0.12, t=-6.38, $p<<0.001$).

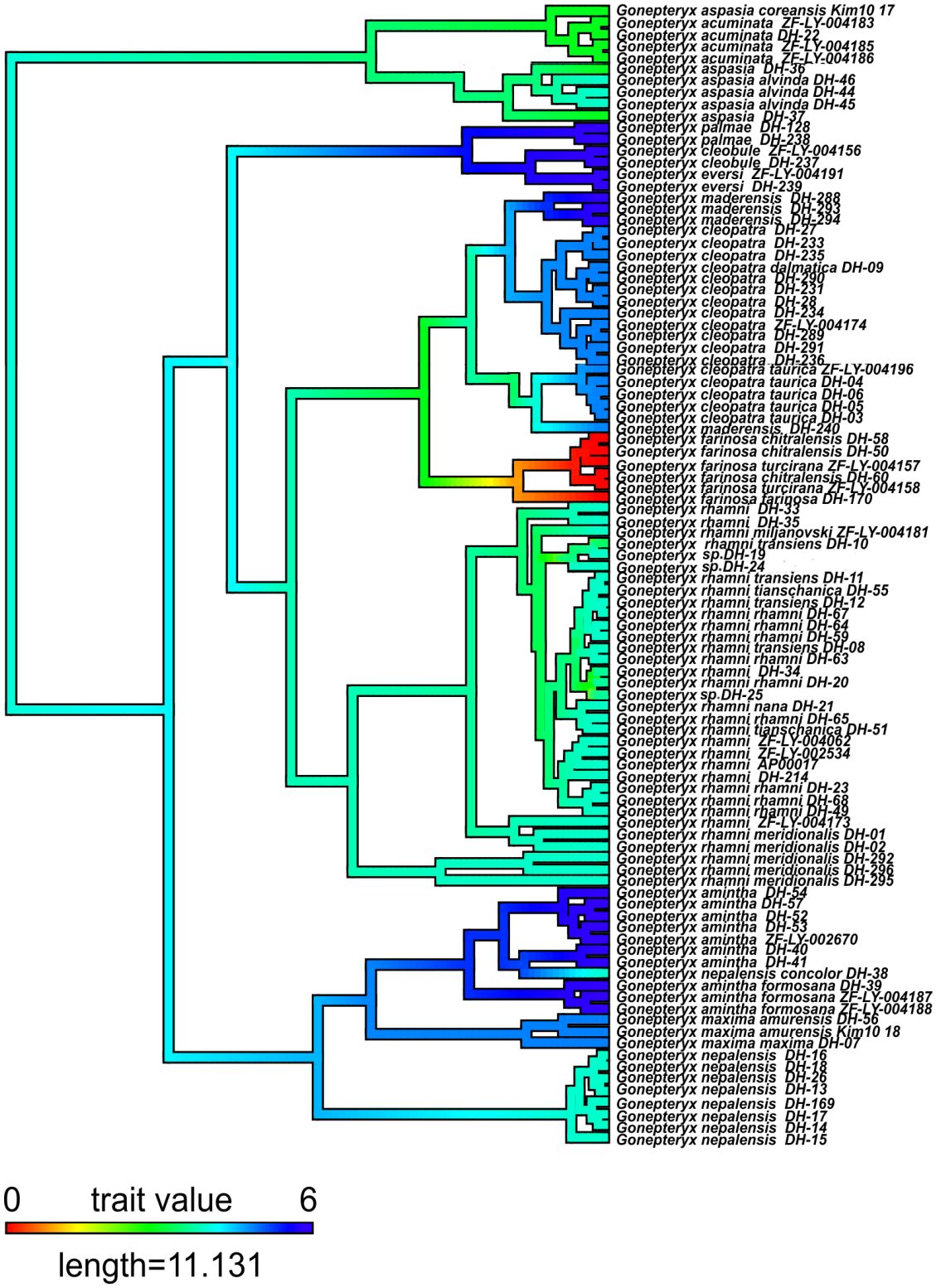

Figure 4. Evolution of UV reflecting pattern in the genus *Gonepteryx* – forewings. Trait value defines the size of UV pattern, where 0 = no UV pattern and 6 = largest area covered with UV pattern.

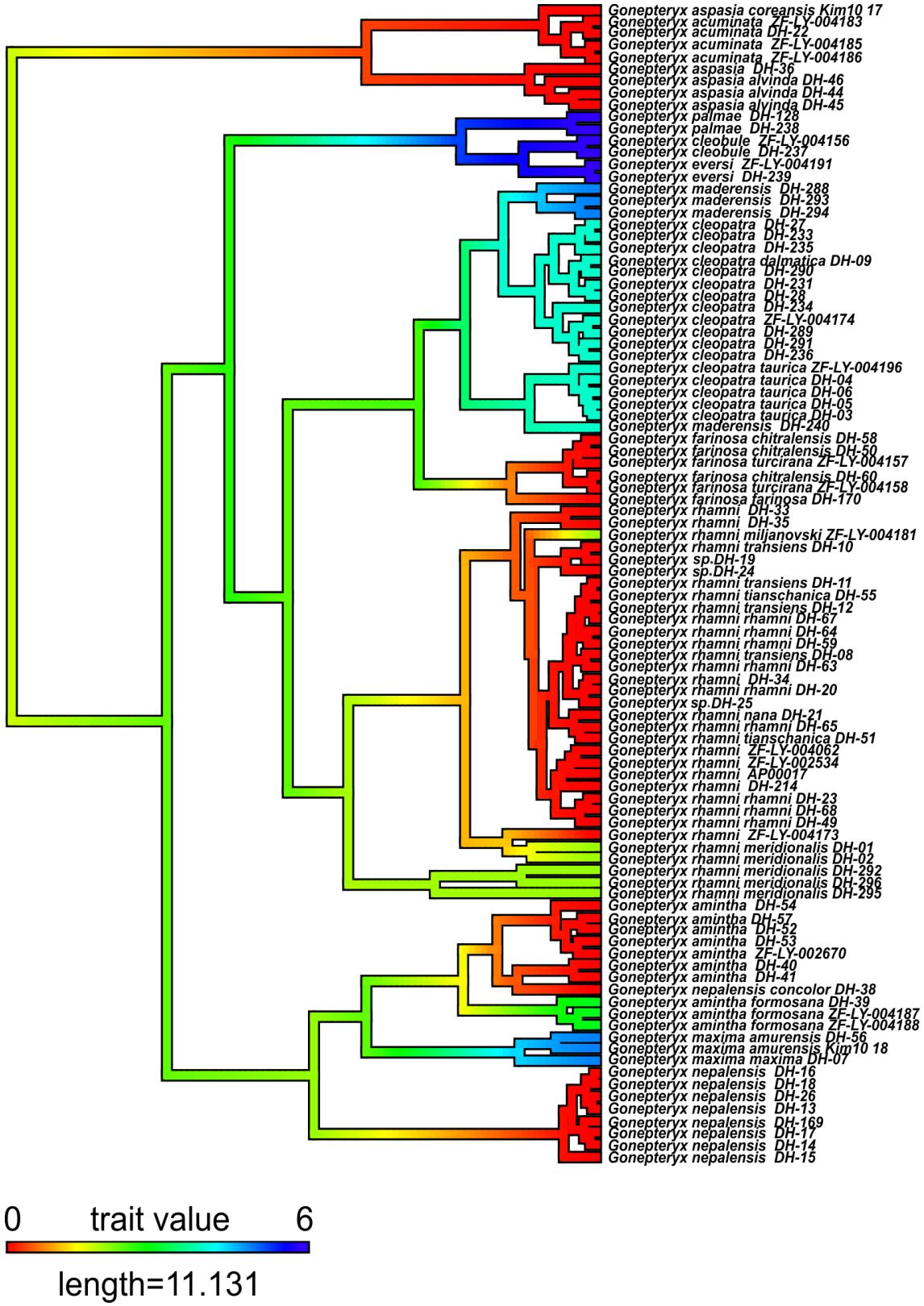

Figure 5. Evolution of UV reflecting pattern in the genus *Gonepteryx* – hindwings. Trait value defines the size of UV pattern, where 0 = no UV pattern and 6 = largest area covered with UV pattern.

Discussion

We examined the phylogeny of the butterfly genus *Gonepteryx* according to its wing UV reflecting pattern. The study proved that genus *Gonepteryx* is monophyletic and separated from the common ancestor with the species *Dercas* approximately 35 mya (cf. Edger et al. 2015). It is divided into two main groups corresponding with their geographical areas: Eastern and Central Asian group including *G. aspasia*, *G. acuminata*, *G. nepalensis*, *G. amintha* and *G. maxima*; and Eurasian group containing the rest of the species, which could be furthermore divided into the Canarian branch and the rest.

While most species are well defined, there seems to be a problem within a few species. Interesting case is the one of *G. aspasia* and *G. acuminata*. The study of Bozano et al. (2016) works with *G. acuminata* as a subspecies of *G. aspasia*. Our study supports the older interpretation of them being two separate species. However, the situation on the Korean Peninsula has yet to be examined closer since the Korean sample of *G. aspasia* obtained from GenBank clearly belongs to the group of *G. acuminata*. Another problematic species is *G. nepalensis*, which creates two separate branches: *G. nepalensis concolor* and *G. n. nepalensis* in different position. It seems that the subspecies *G. nepalensis concolor* belongs to *G. amintha*. Moreover, our findings that *G. nepalensis* is not a subspecies of *G. rhamni*, support the findings by Bozano et al. (2016). The situation of *G. cleopatra* is different, according to our data, *G. (cleopatra) taurica* represents a separate species. In addition, the earliest separated branch of *Gonepteryx rhamni*, i.e. *meridionalis*, may represent a distinct species.

The common ancestor of the genus *Gonepteryx* occurred in a wide area from Eastern Asia including Taiwan and the mountains of Central Asia to Canary Islands. The genus later split up between Asia and the Mediterranean. The genus *Gonepteryx* is most diverse in the terms of species in Asia. Similar pattern can be observed in other butterfly genera, such as *Coenonympha* (Hübner, 1819) studied by Kodandaramaiah & Wahlberg (2009) and also other various groups of flora and fauna (Sanmartín et al., 2001, and references therein). The situation can be explained by the fact that the Pleistocene glaciations were more severe in Europe (which was almost all covered in ice sheets in glacial maxima) than in Asia, causing impoverishment of European biota (Pielou, 1979; Sanmartín et al., 2001). The colonization of Canary Islands and later Mediterranean is rather old and happened before the Messinian Salinity Crisis (7-5.3 mya) when the Mediterranean Sea dried up partially or completely as a result of the closure of its connection with the Atlantic Ocean (Hsü et al., 1973; Krijgsman,

2002; Duggen et al., 2003; Rouchy & Caruso, 2006). However, the refilling of the Mediterranean Sea probably resulted in the vicariant speciation in the Canary Islands which timing correlates with the geographical events.

The origin of UV reflecting pattern in *Gonepteryx* genus is monophyletic. The ancestor possessed a certain amount of UV reflecting pattern on both forewings and hindwings with the UV reflecting area on hindwings being much smaller than on forewings. The UV pattern on both forewings and hindwings is also present in other genera of the family Pieridae, such as *Eurema* Hübner, 1819 (Yata 1989). The UV pattern area later grew significantly larger in the species inhabiting Canary Islands, Mediterranean and Northwestern Africa (both wings) and separately also in the branch containing *G. amintha* and *G. maxima* (forewing only). The UV reflecting pattern on forewings tend to stay the same size or larger during the course of evolution, with the only exception of *G. farinosa* where it disappeared completely. On the other hand, there is rather a disappearing tendency in the hindwing pattern which was already much smaller even in the ancestor.

The study of *G. rhamni* by Pecháček (2014) declares the possibility of connection between variation in the UV pattern and environmental conditions. However, our study did not confirm this possible pattern on the level of species. The correlation of the forewing UV pattern and the number of species occurring in the area indicates that the UV patterns in the genus *Gonepteryx* might play a role in interspecies communication and recognition.

The possible future studies may concentrate on uncovering relationships between phylogeny and ultraviolet reflectant patterns in other genera of Pieridae family and thus putting this study into a wider context. Another interesting topic might be a deeper exploration of taxonomic position of the few problematic species such as *G. nepalensis*, *G. cleopatra* or the situation of *G. aspasia* and *G. acuminata* on Korean Peninsula.

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Appendices

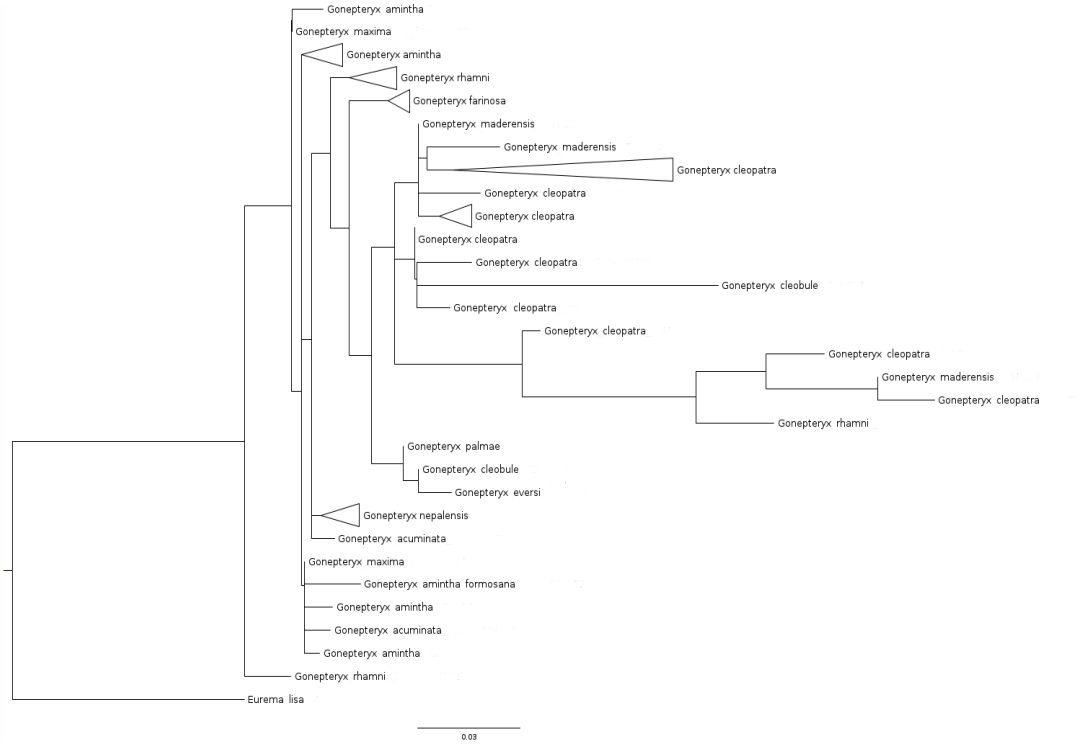
Appendix 1: Table of samples used in the study of wing UV pattern in the genus *Gonepteryx*.

Name	**Code**	**Country**	**Collection date**
G. acuminata	DH-22	Russia	-
G. acuminata	ZF-LY-004185	China	10. 7. 2010
G. acuminata	ZF-LY-004186	China	10. 7. 2010
G. amintha	DH-39	China	28. 4. 2015
G. amintha	DH-40	China	26. 6. 2013
G. amintha	DH-41	China	26. 6. 2013
G. amintha	DH-52	Vietnam	-
G. amintha	DH-53	Vietnam	-
G. amintha	DH-54	Vietnam	-
G. amintha	DH-57	Vietnam	-
G. amintha	ZF-LY-002670	Vietnam	1. 8. 2013
G. amintha	ZF-LY-004187	Taiwan	22. 6. 2016
G. amintha	ZF-LY-004188	Taiwan	1. 5. 2002
G. aspasia	DH-36	China	3. 7. 2009
G. aspasia	DH-44	China	-
G. aspasia	DH-45	China	18. 7. 2007
G. aspasia	DH-46	China	23. 7. 2007
G. aspasia	Kim10_17	Korea	-
G. aspasia	ZF-LY-004183	China	9. 7. 2010
G. cleobule	ZF-LY-004156	Spain	7. 3. 2013
G. cleobule	DH-237	Spain	22. 2. 2010
G. cleopatra	DH-03	Lebanon	27. 5. 2015
G. cleopatra	DH-04	Lebanon	24. 4. 2016
G. cleopatra	DH-05	Lebanon	13. 3. 2016
G. cleopatra	DH-06	Lebanon	9. 5. 2016
G. cleopatra	DH-09	Greece	13. 6. 2008
G. cleopatra	DH-27	Greece	14. 6. 2008
G. cleopatra	DH-28	Greece	13. 6. 2008
G. cleopatra	ZF-LY-004196	Cyprus	26. 5. 2014
G. cleopatra	ZF-LY-004174	Morocco	15. 4. 2010
G. cleopatra	DH-231	Morocco	-

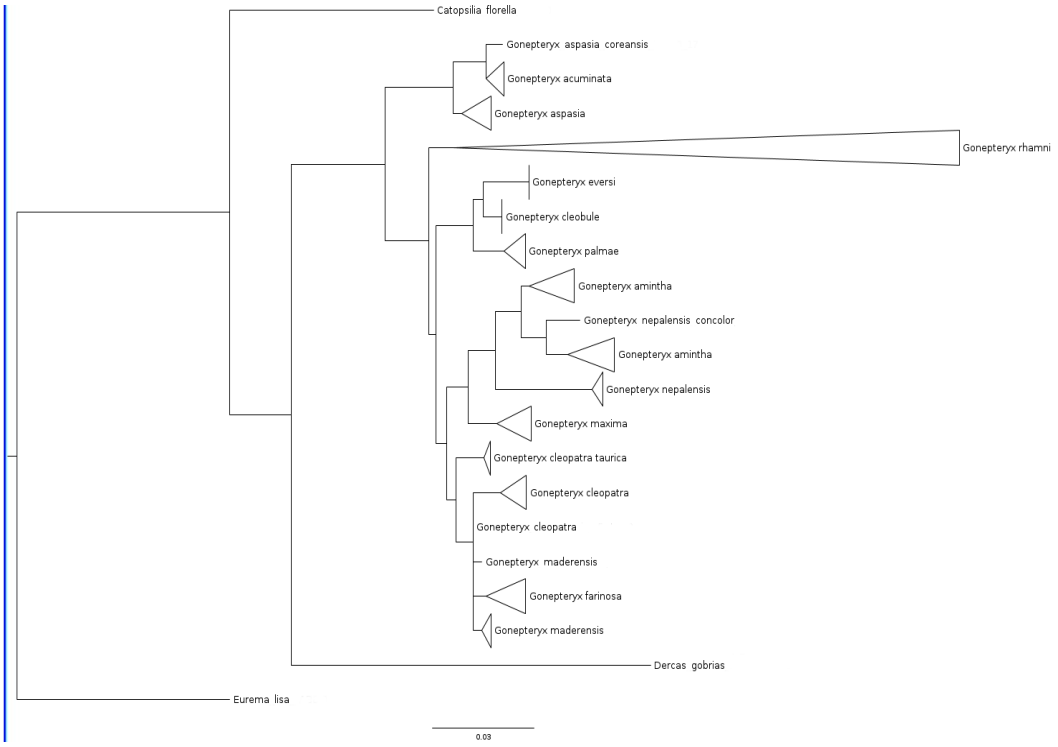
G. cleopatra	DH-233	France	-
G. cleopatra	DH-234	Italy	-
G. cleopatra	DH-235	Croatia	-
G. cleopatra	DH-236	Greece	6. 6. 2012
G. cleopatra	DH-240	Portugal	28. 7. 2011
G. cleopatra	DH-289	Morocco	-
G. cleopatra	DH-290	Morocco	-
G. cleopatra	DH-291	Morocco	-
G. eversi	ZF-LY-004191	Spain	6. 10. 2007
G. eversi	DH-239	Spain	14. 7. 2015
G. farinosa	DH-50	Tajikistan	7. 7. 2015
G. farinosa	DH-58	Kyrgyzstan	5. 7. 2012
G. farinosa	DH-60	Kyrgyzstan	5. 7. 2012
G. farinosa	ZF-LY-004157	Iran	7. 7. 2016
G. farinosa	ZF-LY-004158	Iran	7. 7. 2016
G. farinosa	DH-170	Greece	13. 4. 2011
G. maderensis	DH-288	Madeira	-
G. maderensis	DH-293	Madeira	-
G. maderensis	DH-294	Madeira	-
G. maxima	DH-07	Japan	3. 8. 1997
G. maxima	DH-56	Russia	1. 5. 2015
G. maxima	Kim10_18	Korea	-
G. nepalensis	DH-13	Pakistan	25. 8. 2011
G. nepalensis	DH-14	Pakistan	22. 8. 2011
G. nepalensis	DH-15	Pakistan	26. 8. 2011
G. nepalensis	DH-16	Pakistan	21. 8. 2011
G. nepalensis	DH-17	Pakistan	1. 8. 2011
G. nepalensis	DH-18	Pakistan	28. 8. 2011
G. nepalensis	DH-26	Pakistan	22. 8. 2011
G. nepalensis	DH-38	China	28. 6. 2012
G. nepalensis	DH-169	India	18. 7. 2014
G. palmae	DH-128	Spain	14. 7. 2013
G. palmae	DH-238	Spain	14. 7. 2013
G. rhamni	DH-01	Lebanon	2. 4. 2016
G. rhamni	DH-02	Lebanon	2. 4. 2016

G. rhamni	DH-08	Greece	22. 6. 2000
G. rhamni	DH-10	Greece	22. 6. 2000
G. rhamni	DH-11	Russia	4. 5. 2013
G. rhamni	DH-12	Russia	4. 5. 2013
G. rhamni	DH-20	Sweden	15. 7. 2006
G. rhamni	DH-21	Russia	27. 8. 2003
G. rhamni	DH-23	Sweden	15. 7. 2006
G. rhamni	DH-33	Austria	14. 8. 2016
G. rhamni	DH-34	Austria	14. 8. 2016
G. rhamni	DH-35	Austria	14. 8. 2016
G. rhamni	DH-49	Belarus	7. 7. 1990
G. rhamni	DH-51	Kazakhstan	26. 6. 2008
G. rhamni	DH-55	Kazakhstan	27. 7. 2014
G. rhamni	DH-59	Belarus	20. 8. 2000
G. rhamni	DH-63	Estonia	2. 7. 2016
G. rhamni	DH-64	Estonia	6. 7. 2016
G. rhamni	DH-65	Estonia	6. 7. 2016
G. rhamni	DH-67	Estonia	23. 7. 2016
G. rhamni	DH-68	Estonia	6. 7. 2016
G. rhamni	ZF-LY-004062	Czech Republic	16. 7. 2016
G. rhamni	ZF-LY-002534	Finland	-
G. rhamni	AP00017	Russia	2. 7. 2014
G. rhamni	ZF-LY-004181	Armenia	23. 6. 2006
G. rhamni	ZF-LY-004173	Morocco	26. 4. 2014
G. rhamni	DH-214	Lithuania	30. 6. 2007
G. rhamni	DH-292	Armenia	11. 7. 2010
G. rhamni	DH-295	Armenia	11. 7. 2010
G. rhamni	DH-296	Armenia	11. 7. 2010
G. sp.	DH-19	Iran	13. 5. 2016
G. sp.	DH-24	Greece	23. 6. 2008
G. sp.	DH-25	Greece	22. 6. 2008

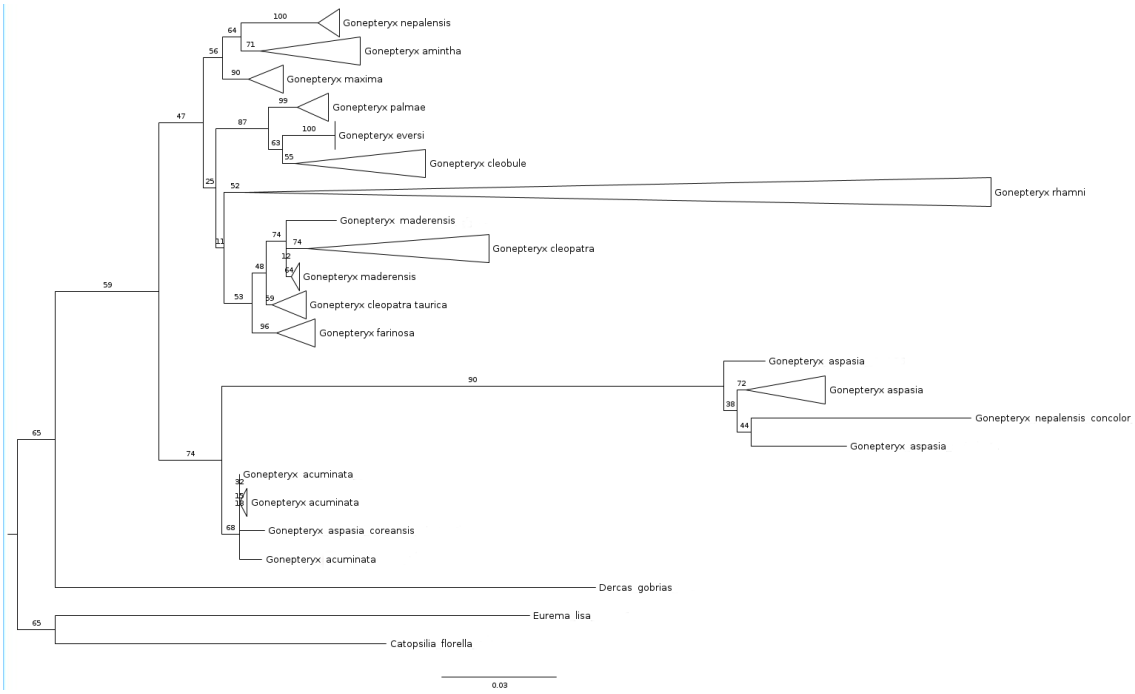
Appendix 2: Results of Maximum Likelihood: Phylogeny of the genus *Gonepteryx* based on COI, computed in RaxML.



Appendix 3: Results of Maximum Likelihood: Phylogeny of the genus *Gonepteryx* based on Wingless, computed in RaxML.



Appendix 4: Results of Maximum Likelihood: Phylogeny of the genus *Gonepteryx* based on both COI and Wingless, computed in RaxML.



Appendix 5: Phylogeny of the genus *Gonepteryx* based on both COI and Wingless, computed in BEAST 1.8.0.