

1 *Maculinea* or *Phengaris*? New insights from genitalia morphometry

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## 12 **Abstract**

13 The main goal of our research was to complete the previous studies on the taxonomy of the

14 *Phengaris-Maculinea* complex applying a geometric morphometric approach on male

15 genitalia. Strong phylogenetic signal was detected in the shape of valva. *Phengaris* s.str. and

16 *Maculinea* s.str. could not be separated perfectly owing to the intermediate position of

17 *Phengaris xiushani* having ‘*Maculinea*-like’ valva shape. Our investigation emphasizes the

18 need of a more comprehensive phylogenetic survey including all *Phengaris* species. At the

19 same time, it also suggests that the synonymization of the two genera seems to be reasoned

20 under the name *Phengaris* as senior synonym.

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22 **Key words:** geometric morphometry, phylogenetic signal, valva shape

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25           The obligatory myrmecophilous blues of the genus *Maculinea* Van Eecke, 1915  
26 (Lepidoptera, Lycaenidae) belong to the most intensively studied butterflies in Europe  
27 (Settele et al., 2005) primarily owing to their extraordinary life cycle. Their larval survival  
28 depends on the dual presence of their specific initial food plant and host ant species. Due to  
29 their specific life history, these butterflies are highly endangered throughout their European  
30 range, and have been the focus of intense conservation research and action plans (Munguira  
31 and Martin, 1999; Thomas, 1995; Van Swaay et al., 2012).

32           Despite the fact that several detailed studies have been executed on the phylogeny of the  
33 genus *Maculinea* in the past two decades (Als et al., 2004; Fric et al., 2007; Pech et al., 2004;  
34 Ugelvig et al., 2011), a lively debate has been emerged concerning the valid name of the  
35 genus. The most closely-related relatives of *Maculinea* butterflies proved to be the members  
36 of the genus *Phengaris* Doherty, 1892 from the eastern Palaearctic region (*Phengaris* sensu  
37 stricto) whose caterpillars also use specific initial food plants and host ant species as dual  
38 resources for their survival (Igarashi and Fukuda, 2000; Jean, 1996; Uchida, 1995). The  
39 previous phylogenetic studies showed that *Maculinea* species form a monophyletic clade  
40 sister to *Phengaris atroguttata* and *P. albida* (Als et al., 2004; Ugelvig et al., 2011). However,  
41 *P. daitozana* has been separated from these latter two species and found as the most basal  
42 branch of the phylogenetic tree making the genus *Phengaris* paraphyletic. At the same time,  
43 the newly discovered *P. xiushani* (Wang and Settele, 2010) has not been included in these  
44 previous studies.

45           On the contrary, the study based on numerous morphological and ecological characters  
46 showed that *Phengaris* is a monophyletic group inside the *Maculinea* clade, that is, *Maculinea*  
47 is a paraphyletic group in this case (Pech et al., 2004). However, the combined use of  
48 molecular markers as well as numerous discrete morphological and ecological traits resulted  
49 in the paraphyly of the genus *Phengaris* (Fric et al., 2007). Therefore, the synonymization of

50 these genus names has been initiated and the use of the name *Phengaris* over *Maculinea* has  
51 been proposed as the senior synonym (Fric et al., 2007). This initiation has generated a  
52 taxonomic debate which has not been closed yet. A proposal to retain the name *Maculinea* in  
53 use over *Phengaris* has been published (Balletto et al., 2010) but counter arguments have also  
54 been presented (Fric et al., 2010) while Ugelvig et al. (2011) have recommended the delay of  
55 the debate until irrefutable evidence is provided.

56 Here, we reveal further information on the relation of the two genera applying  
57 geometric morphometric approach on the male genitalia which may contribute to closing of  
58 the on-going taxonomic debate.

59 Altogether 68 individuals from the genera *Maculinea* and *Phengaris* (s.str.) were used  
60 in our survey as well as 18 specimens from the out-group taxa (Supplementary Table S1). The  
61 preparation of male external genitalia was performed following the procedure described in  
62 Bereczki et al. (2014). Genital slides were digitized by combining an Olympus camera and a  
63 Wild Heerbrugg M420 Microscope. The genital photos of *Phengaris* (s.str.) species from  
64 Wang and Settele (2010) were also used. Since we found only few real landmarks on valva,  
65 we recorded a close curve on it using TpsDig v. 2.10. For the analysis of the outlines elliptic  
66 Fourier analysis was used (Giardina and Kuhl, 1977; Kuhl and Giardina, 1982). The  
67 algorithm fits Fourier series on x and y-coordinates as functions of the curvilinear abscissa  
68 (Claude, 2008). For the analysis we used these Fourier coefficients.

69 The measure of phylogenetic signal in the shape of valva was determined by the  
70 multivariate version of K-statistics (Adams, 2014) using the average valva shape of each  
71 species and the phylogenetic tree reconstructed by Ugelvig et al. (Ugelvig et al., 2011). K-  
72 value evaluates the degree of phylogenetic signal in a dataset relative to what is expected  
73 under a Brownian motion model of evolution. A significance test was carried out using 10

74 000 permutation of the shape data among the tips of the phylogeny. R computing environment  
75 was used for calculations (R Development Core Team, 2014).

76 Principal component analysis (PCA) was performed to visualise the morphological  
77 relationships among taxa using the average valva shape of each species. To get the average  
78 valva shapes we used the group means of the Fourier coefficients. Individual-based PCA was  
79 also performed to present the intraspecific variability of the valva (Supplementary Fig. S1).  
80 PCA was carried out using PAST 2.17 (Hammer et al., 2001).

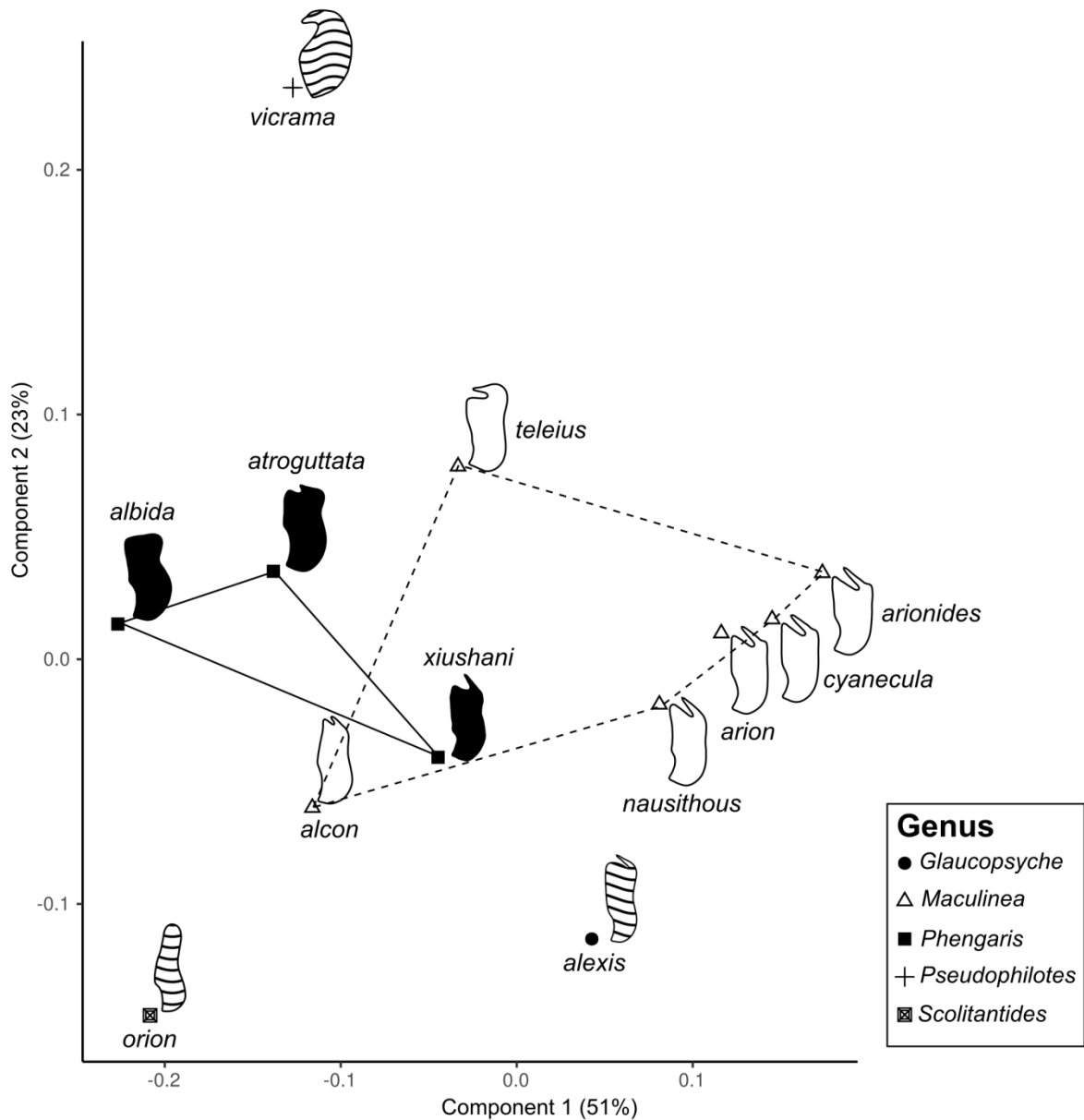
81 Significant phylogenetic signal was detected in our dataset ( $K=0.65$ ,  $p<0.05$ ) indicating  
82 strong phylogenetic structure in the shape of valva of different taxa. The PCA plot showed  
83 that *Phengaris* s.str. and *Maculinea* s.str. could not be separated perfectly owing to the  
84 intermediate position of *Phengaris xiushani* having ‘*Maculinea*-like’ valva shape (Fig.1).

85 Previous studies have demonstrated that genital traits are highly suitable to serve as  
86 taxonomic characters in several groups (Dapporto, 2008; Simonsen, 2005; Tóth and Varga,  
87 2011). Moreover, it has been shown that the shape of valva could exhibit strong phylogenetic  
88 signal (Tóth et al., 2014) just like in our dataset. It is remarkable that *M.alcon* was located  
89 nearest to *Phengaris* s. str. in the morphometric space similarly to the pattern shown by the  
90 previous phylogenetic analyses (Als et al., 2004; Fric et al., 2007; Ugelvig et al., 2011). Based  
91 on the position of *P. xiushani* in the morphometric space it is expected to cluster among  
92 *Maculinea* species in a DNA-based phylogenetic reconstruction given the strong phylogenetic  
93 signal in valva shape.

94 That is, our investigation emphasizes the need of a more comprehensive phylogenetic  
95 survey including all *Phengaris* species. Simultaneously, it also suggests that the  
96 synonymization of the two genera seems to be reasoned under the name *Phengaris* as senior  
97 synonym.

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 102 Zsolt Bálint (Hungarian Natural History Museum) for valuable museum specimens.



103

104 **Fig. 1.** The results of PCA with the average valva shapes.

105

106 **Supplementary Table S1.** Specimens used for the study.

| Individual ID | Species  | Sampling site                                | Location                                     |
|---------------|--|--|--|
| ZSM M-1       | <i>Phengaris atroguttata</i><br>Oberthür, 1876             | Sichuan, China                               | Zoological State Collection (Munich)         |
| ZSM M-3       |  |  | Zoological State Collection (Munich)         |
| ZSM M-4       |  |  | Zoological State Collection (Munich)         |
| ZSM M-26      |  |  | Zoological State Collection (Munich)         |
| ZSM M-27      |  |  | Zoological State Collection (Munich)         |
| ZSM M-2       |  | Tibet, China                                 | Zoological State Collection (Munich)         |
| ZSM M-30      |  | Tibet, China                                 | Zoological State Collection (Munich)         |
| ZSM M-29      |  | Naga Hills, Myanmar                          | Zoological State Collection (Munich)         |
| M225          |  | Hualien county, Taiwan                       | Hungarian Natural History Museum (Budapest)  |
| PAT           |  | Yunnan, China                                | see in Wang & Settele 2010                   |
| ZSM M-25      |  | <i>Phengaris albida</i><br>Leech, 1893       | Tibet, China                                 |
| PAL           | China  |  | see in Wang & Settele 2010                   |
| PXI           | <i>Phengaris xiushani</i><br>Wang & Settele, 2010          | Yunnan, China                                | see in Wang & Settele 2010                   |
| FU4           | <i>Maculinea alcon</i><br>([Denis & Schiffermüller], 1775) | Fülesd, Hungary                              | The collection of the University of Debrecen |
| FU7           |  |  | The collection of the University of Debrecen |
| FU10          |  |  | The collection of the University of Debrecen |
| FU14          |  |  | The collection of the University of Debrecen |
| FU20          |  |  | The collection of the University of Debrecen |
| NM2           |  | Nagymező, Hungary                            | The collection of the University of Debrecen |
| NM5           |  |  | The collection of the University of Debrecen |
| NM6           |  |  | The collection of the University of Debrecen |
| NM8           |  |  | The collection of the University of Debrecen |
| NM14          |  |  | The collection of the University of Debrecen |
| TSI1          |  |  | Szin, Hungary                                |
| TSI3          |  | The collection of the University of Debrecen |  |
| TSI4          |  | The collection of the University of Debrecen |  |
| TSI5          |  | The collection of the University of Debrecen |  |
| TSI7          | The collection of the University of Debrecen               |  |  |
| NSI2          | The collection of the University of Debrecen               |  |  |
| NSI10         | The collection of the University of Debrecen               |  |  |
| NSI12         | The collection of the University of Debrecen               |  |  |

|       |   |   |  |
|-------|---|---|--|
| NSI15 |   |   | The collection of the University of Debrecen |
| NSI17 |   |   | The collection of the University of Debrecen |
| M221  | <i>Maculinea arionides</i><br>(Staudinger, 1887)      | unknown   | Hungarian Natural History Museum (Budapest)  |
| M222  |   | localitas ac datum dubiosa<br>vide No. 863-029  | Hungarian Natural History Museum (Budapest)  |
| M223  |   |   | Hungarian Natural History Museum (Budapest)  |
| M224  |   |   | Hungarian Natural History Museum (Budapest)  |
| ZSM31 |   | Mandshuria, China                               | Zoological State Collection (Munich)         |
| M172  |   | <i>Maculinea cyanecula</i><br>(Eversmann, 1848) | Central Aimak, Mongolia                      |
| M174  | Hungarian Natural History Museum (Budapest)           |   |  |
| M176  | Hungarian Natural History Museum (Budapest)           |   |  |
| M177  | Hungarian Natural History Museum (Budapest)           |   |  |
| M188  | Hungarian Natural History Museum (Budapest)           |   |  |
| M190  | Hungarian Natural History Museum (Budapest)           |   |  |
| M192  | Hungarian Natural History Museum (Budapest)           |   |  |
| M194  | Hungarian Natural History Museum (Budapest)           |   |  |
| M195  | Hungarian Natural History Museum (Budapest)           |   |  |
| M196  | Hungarian Natural History Museum (Budapest)           |   |  |
| TEL1  | <i>Maculinea teleius</i><br>(Bergsträsser, 1779)      | Aggtelek, Hungary                               | The collection of the University of Debrecen |
| TEL2  |   |   | The collection of the University of Debrecen |
| TEL3  |   |   | The collection of the University of Debrecen |
| TEL4  |   |   | The collection of the University of Debrecen |
| TEL7  |   |   | The collection of the University of Debrecen |
| TEL9  |   |   | The collection of the University of Debrecen |
| TEL11 |   |   | The collection of the University of Debrecen |
| TEL12 |   |   | The collection of the University of Debrecen |
| TEL13 |   |   | The collection of the University of Debrecen |
| TEL15 |   |   | The collection of the University of Debrecen |
| NAU1  | <i>Maculinea nausithous</i><br>(Bergsträsser, [1779]) | Kétvölgy, Hungary                               | The collection of the University of Debrecen |
| NAU4  |   |   | The collection of the University of Debrecen |
| NAU5  |   |   | The collection of the University of Debrecen |

|       |  |                           |  |
|-------|--|---------------------------|--|
| NAU6  |  |                           | The collection of the University of Debrecen |
| NAU7  |  |                           | The collection of the University of Debrecen |
| NAU9  |  |                           | The collection of the University of Debrecen |
| NAU10 |  |                           | The collection of the University of Debrecen |
| NAU11 |  |                           | The collection of the University of Debrecen |
| NAU12 |  |                           | The collection of the University of Debrecen |
| NAU13 |  |                           | The collection of the University of Debrecen |
| GA1   | <i>Glaucopsyche alexis</i><br>(Poda, 1761)   | Szin, Hungary             | The collection of the University of Debrecen |
| GA4   |  |                           | The collection of the University of Debrecen |
| GA5   |  |                           | The collection of the University of Debrecen |
| GA8   |  |                           | The collection of the University of Debrecen |
| GA9   |  |                           | The collection of the University of Debrecen |
| GA10  |  |                           | The collection of the University of Debrecen |
| GA11  |  |                           | The collection of the University of Debrecen |
| GA12  |  |                           | The collection of the University of Debrecen |
| GA13  |  |                           | The collection of the University of Debrecen |
| GA14  |  |                           | The collection of the University of Debrecen |
| ORI1  | <i>Scolitantides orion</i><br>(Pallas, 1771) | Bükk Mountains, Hungary   | The collection of the University of Debrecen |
| ORI2  |  |                           | The collection of the University of Debrecen |
| ORI3  |  |                           | The collection of the University of Debrecen |
| ORI4  |  |                           | The collection of the University of Debrecen |
| SCH1  | <i>Pseudophilotes vicrama</i> (Moore, 1865)  | Pirin Mountains, Bulgaria | The collection of the University of Debrecen |
| SCH2  |  |                           | The collection of the University of Debrecen |
| SCH3  |  |                           | The collection of the University of Debrecen |
| SCH4  |  |                           | The collection of the University of Debrecen |

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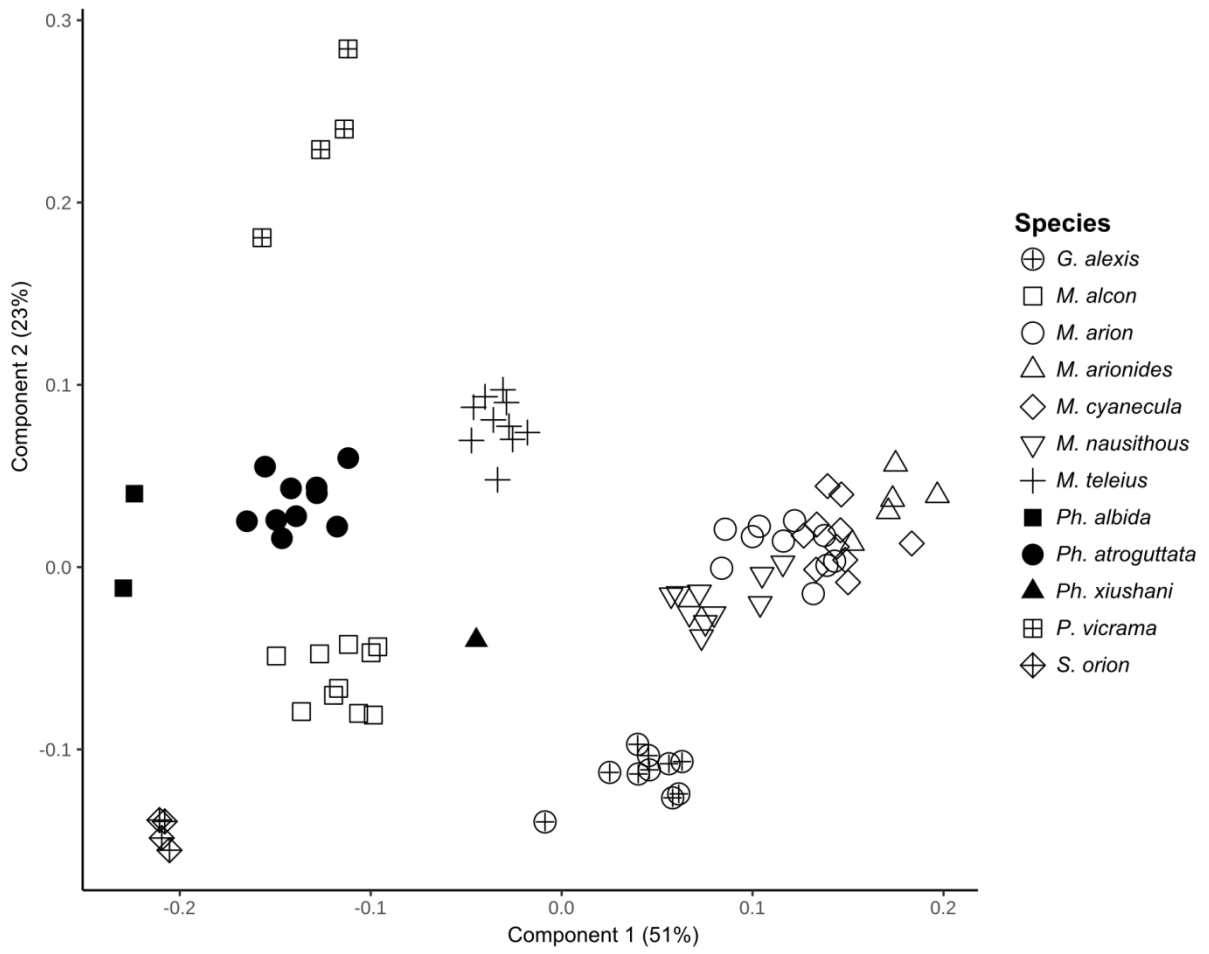
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111 **Supplementary Figure S1.** PCA plot based on valva outlines.



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