

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).

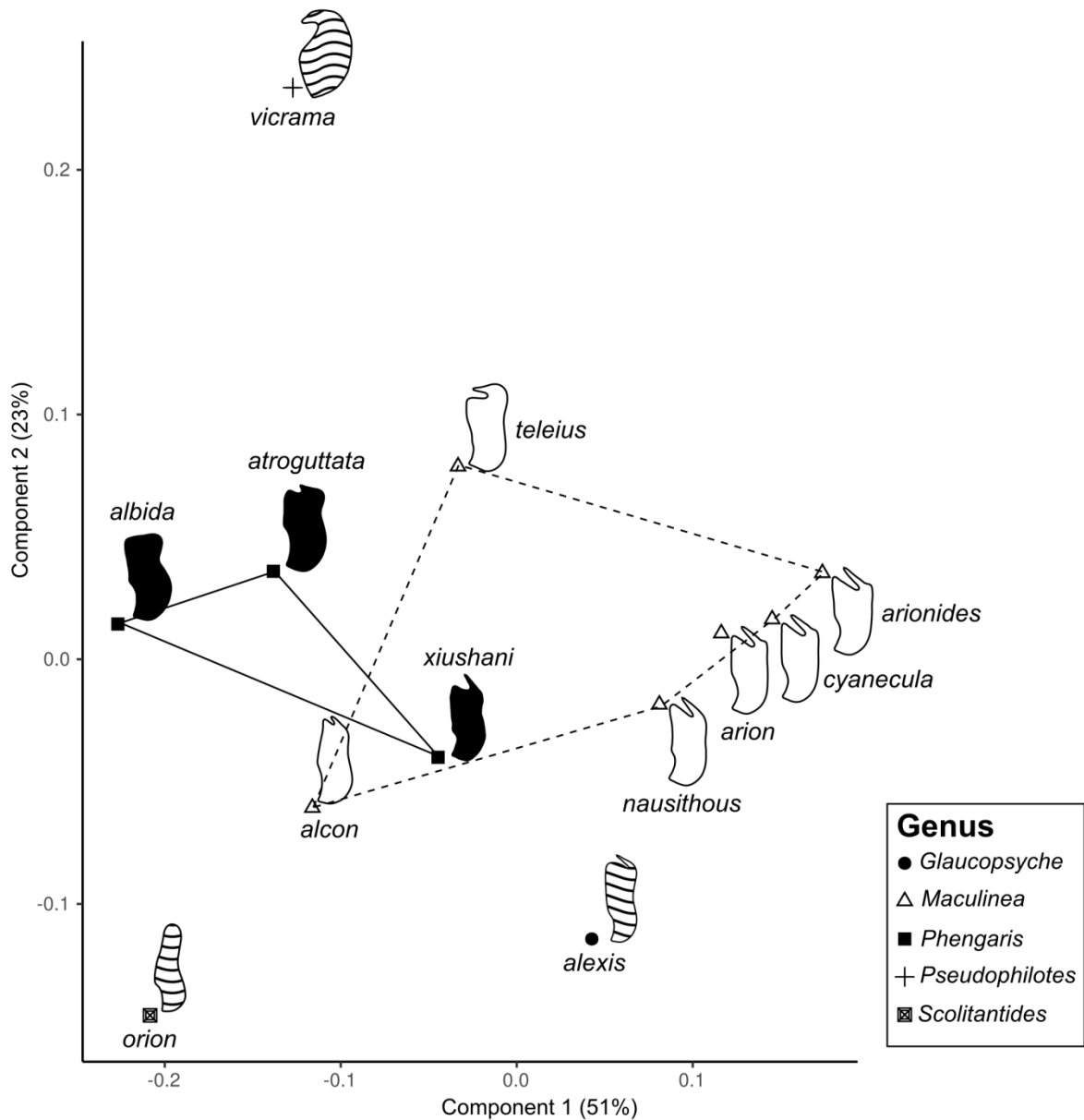
81 Significant phylogenetic signal was detected in our dataset ( $K=0.65$ ,  $p<0.05$ ) indicating  
82 strong phylogenetic structure in the shape of valva of different taxa. The PCA plot showed  
83 that *Phengaris* s.str. and *Maculinea* s.str. could not be separated perfectly owing to the  
84 intermediate 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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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> 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> Leech, 1893	Tibet, China
PAL	China		see in Wang & Settele 2010
PXI	<i>Phengaris xiushani</i> Wang & Settele, 2010	Yunnan, China	see in Wang & Settele 2010
FU4	<i>Maculinea alcon</i> ([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> (Staudinger, 1887)	unknown	Hungarian Natural History Museum (Budapest)
M222		localitas ac datum dubiosa 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> (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> (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> (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> (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> (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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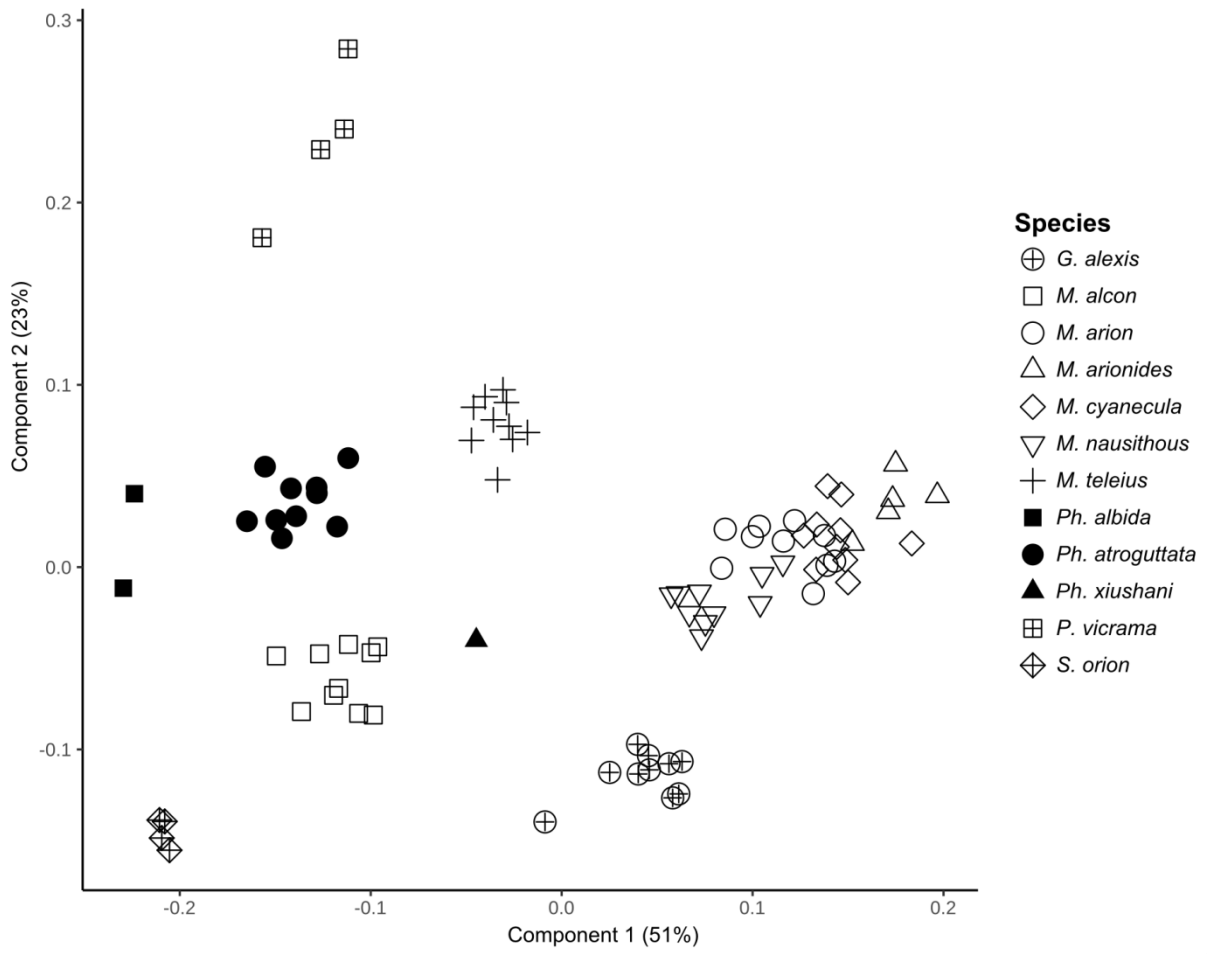
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111 **Supplementary Figure S1.** PCA plot based on valva outlines.



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