Taxonomic Position and Status of *Polyommatus (Agrodiaetus) iphigenia* (Lepidoptera, Lycaenidae) from the Peloponnese, Southern Greece*

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In our study we use a 690 bp fragment of the COI gene to analyze a taxon from southern Greece, usually treated as *Polyommatus (Agrodiaetus) iphigenia nonacriensis* (Brown, 1977). The previous conclusions on taxonomy and nomenclature of *P. (A.) iphigenia nonacriensis* were not supported by molecular or cytological data, therefore the problem of identity of this taxon has remained unsolved. We found that with respect to COI haplotypes, *P. (A.) iphigenia nonacriensis* from Greece is similar to the studied populations of *P. (A.) iphigenia* from Turkey and Armenia. Thus, we confirm that the only Greek *Agrodiaetus* butterfly with blue wing coloration in males actually belongs to the species *P. (A.) iphigenia*.

Key words: *Agrodiaetus* butterflies, Lepidoptera, Lycaenidae, molecular marker, Greece, Peloponnese.

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The subgenus *Agrodiaetus* Hübner, 1822 is a species-rich monophyletic lineage within the genus *Polyommatus* (Latreille, 1804) (TALAVERA et al. 2013) and one of the most complicated and troublesome groups of the Palaearctic Lepidoptera (COUTSIS 1986; VILA et al. 2010; LUKHTANOV et al. 2015a). It includes numerous species, subspecies, and forms with uncertain taxonomic status, and the majority of these taxa are weakly differentiated with respect to genitalia structure, wing colour and wing pattern (HESSELBARTH et al. 1995; LUKHTANOV et al. 2008, 2014, 2015b). Within this subgenus, FORSTER (1960) (who considered *Agrodiaetus* as a valid genus) located a polytypic species *Agrodiaetus iphigenia* (Herrich-Schäffer, 1847) that included the following subspecies: *A. iphigenia iphigenia* (Herrich-Schäffer, 1847), *A. iphigenia barthae* (Pfeiffer, 1932), *A. iphigenia araratensis* de Lesse, 1957, *A. iphigenia iphidamon* (Staudinger, 1889), *A. iphigenia iphigenides* (Staudinger, 1886), *A. iphigenia juludus* (Staudinger, 1886) and *A. iphigenia ruckbeili* Forster, 1960. At a later time, the only Greek *Agrodiaetus* butterfly with blue wing coloration in males was found in northern Peloponnese (Mt. Chelmos, Greece) and provisionally identified as *A. damone* (Eversmann, 1841) (BROWN & DE WORMS 1975). Later it was described as subspecies *A. iphigenia nonacriensis* (Brown, 1977). Recently two new taxa from Turkey were described: *P. (A.) iphigenia ipicarmon* from Taurus mountains (Isparta province) (ECKWEILER & ROSE 1993) and *P. (A.) iphigenia manuelae* from Hakkari province (ECKWEILER & SCHURIAN 2013).

Recent DNA-based phylogenetic analyses and karyotype studies demonstrated that *P. (A.) iphigenia sensu lato* was an artificial polyphyletic assemblage consisting of several species (WIEMERS 2003; KANDUL et al. 2004, 2007; LUKHTANOV et al. 2005). In particular, these approaches proved that

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P. (A.) juldusus, P. (A.) iphigenides and P. (A.) iphidamon are distinct species, not even closely related to P. (A.) iphigenia. *Polyommatus* (A.) juldusus and P. (A.) iphigenides were recognized as members of the P. (A.) *damone* species-group which is the sister lineage to the P. (A.) *iphigenia* species-group. *Polyommatus* (A.) *iphidamon* was recognized within the P. (A.) *carmon* (Herrick-Schäffer, 1851) species complex which is not even distantly related to the P. (A.) *iphigenia* group. Finally, P. (A.) *iphicarmon* was recognized as a distinct species within the P. (A.) *iphigenia* species-group.

Thus, the taxonomic significance of the morphological features is low in *Agrodiaetus*. Therefore, descriptions which were based mainly on external morphological characters alone (e.g. FORSTER 1960), did not clarify the phylogenetic position and identity of taxa from the P. (A.) *iphigenia sensu* FORSTER 1960 assemblage. Unlike the abovementioned taxa, P. (A.) *iphigenia nonacriensis* has never been studied genetically, therefore its identification, taxonomic status and phylogenetic position have remained unverified. Our paper addresses a detailed analysis of this taxon, which is apparently present only in Greece (Peloponnesian peninsula).

### Material and Methods

Two females of *P. (A.) iphigenia nonacriensis* were collected in 2008 in southern Greece (see Table 1 and Fig. 1). A fragment of the mitochondrial cytochrome oxidase I gene (*COI*) (first 690 positions) was used as a molecular marker. For DNA amplification for *COI* we used primers K698 and Nancy (CATERINO & SPERLING 1999). Sequencing of the double-stranded product was carried out at the Research Resource Center for Molecular and Cell Technologies (Saint-Petersburg State University). Collected specimens are kept in the Zoological Institute of the Russian Academy of Science (St. Petersburg).

### Table 1

<table>
<thead>
<tr>
<th>Sample ID or Genbank number</th>
<th>Taxon</th>
<th>COI haplotype group</th>
<th>Country</th>
<th>Locality and coordinates</th>
<th>Altitude</th>
<th>Date</th>
<th>Collectors</th>
<th>References</th>
</tr>
</thead>
<tbody>
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<td>iphigenia</td>
<td>Greece</td>
<td>Katafava, N 38°01’37”; E 22°13’23”</td>
<td>1700 m</td>
<td>17 July 2008</td>
<td>NS, LR, VL</td>
<td>current study</td>
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<tr>
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<td>Katafava, N 38°01’37”; E 22°13’23”</td>
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<td>17 July 2008</td>
<td>NS, LR, VL</td>
<td>current study</td>
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<td>AY946756</td>
<td><em>iphigenia aracalensis</em></td>
<td>iphigenia</td>
<td>Turkey</td>
<td>Van Province, Çatuk</td>
<td>–</td>
<td>July 2001</td>
<td>NK, VL, KANDUL et al. 2004</td>
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<tr>
<td>AY946755</td>
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<td>–</td>
<td>June 2000</td>
<td>AD</td>
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<td>EF104609</td>
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<td>iphigenia</td>
<td>Turkey</td>
<td>Izmit Province, Vanişar bombeli</td>
<td>–</td>
<td>July 2004</td>
<td>–</td>
<td>KANDUL et al. 2007</td>
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<td>iphigenia</td>
<td>Turkey</td>
<td>Gümüşhane Province, Gümüşhane</td>
<td>–</td>
<td>July 2001</td>
<td>VL, AD KANDUL et al. 2004</td>
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<td>1700 m</td>
<td>21 July 1998</td>
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<td>iphigenia</td>
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<td>Van Province, 25-22 km N Çatak</td>
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<td><em>beytopi</em></td>
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<td>Turkey</td>
<td>Van Province, Güzeldere Gedik, Baskale</td>
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<td>19 July 1999</td>
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<td>Turkey</td>
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<td>MW WIEBERS 2003</td>
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<td>Van Province, Güzeldere Gedik, Baskale</td>
<td>2500 m</td>
<td>July 2001</td>
<td>VL LUKHTANOV et al. 2005</td>
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<tr>
<td>AY556977</td>
<td><em>novshari</em></td>
<td>iphigenia</td>
<td>Iran</td>
<td>Abarbyan-e Sharqi Province, Dargaran, 30 m NE Murand</td>
<td>2000 m</td>
<td>15 July 2000</td>
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<td>AY557149</td>
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<td>Iran</td>
<td>Abarbyan-e Sharqi Province, Mahmoodab, W Kafrbas</td>
<td>2200-2400 m</td>
<td>20 July 2002</td>
<td>WE WIEBERS 2003</td>
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</table>
Bayesian approach was used for estimating the phylogeny. Since Polyommatus icarus (Rottemburg, 1775) and P. stempferi (Brandt, 1938) were earlier inferred as outgroups to the subgenus Agrodiaetus (TalaVera et al. 2013), we used them to root the phylogram. A complete list of specimens included in this study is given in Table 1. A Bayesian approach was used for estimating the phylogeny. Bayesian analyses were
performed using the program MrBayes 3.1.2 with the nucleotide substitution model GTR+G+I. jModelTest was used to determine optimal substitution models for Bayesian inference (BI) analysis (Posada 2008). TRACER, version 1.4 was used for summarizing the results of the Bayesian phylogenetic analyses (http://beast.bio.ed.ac.uk/Tracer).

Results and Discussion

Analysis of a dataset of 49 specimens recognized *P. (A.) iphidamon* as a highly differentiated lineage with a basal position. All other specimens constituted two major clades: the *P. (A.) damone* lineage, and the *P. (A.) iphigenia* lineage (Fig. 2).

Fig. 1. Sampling localities of specimens used in present study: *P. (A.) iphigenia nonacriensis* (red circle) and *P. (A.) iphigenia* s. str. (blue circles).

Fig. 2. The Bayesian tree of the *Polyommatus (Agrodiaetus)* damone group (highlighted in pink) and *Polyommatus (Agrodiaetus)* iphigenia group (highlighted in blue) based on analysis of COI gene from 49 specimens. Numbers at nodes indicate Bayesian posterior probability. The position of *P. (A.) iphigenia nonacriensis* is shown in red.

We also found that in our previous studies (Kandul et al. 2004, 2007; Lukhtanov & Budashkin 2007), an error was made during reassembling 4 separate sequence reads of P. (A.) pljushchti (the representative of the P. (A.) damone species-group) into one contig. Three of them were assembled correctly, while the second fragment was erroneously taken from the other species P. (A.) damon (GenBank accession number AY496733). Thus, the contig of P. (A.) pljushchti available from GenBank under accession number AY496774 actually represents a chimeric nucleotide sequence. This resulted in erroneous phylogenetic reconstructions where P. (A.) pljushchti occupied an isolated basal position within the complex of P. (A.) damone s.l. (Kandul et al. 2004, 2007; Lukhtanov & Budashkin 2007). Here, in our analysis, we used the corrected sequence of the taxon pljushchti. We demonstrate that this taxon is closely related to P. (A.) damone and should be considered as a subspecies P. (A.) damone pljushchti, as it is already treated by Tshikolovets (2011).

In our phylogenetic reconstruction, two Greek specimens, which were identified in the field as females of P. (A.) iphigenia nonacriensis, formed a well-supported cluster with other sequences of P. (A.) iphigenia s.str. (Fig. 2). Genetic divergence of the Greek samples as compared with other specimens of P. (A.) iphigenia from Turkey and Armenia is low (0.86-1.01%) and is based on seven nucleotide substitutions in the studied COI fragment. Interestingly, specimens of P. (A.) iphigenia from the province Isparta (Turkey) also differ from all other representatives of P. (A.) iphigenia s.str. by fixed nucleotide substitutions and by having a unique chromosome number n=15 (Wiemers 2003; Kandul et al. 2007).

In the light of the data obtained, and taking into account the genetic and morphological similarity of the taxa nonacriensis and iphigenia, we consider nonacriensis unlikely to be a separate species. It should be synonymized with P. (A.) iphigenia or, at most, considered as a weakly differentiated subspecies of the latter. Thus, our work clarifies the taxonomic status of the Greek Agrodiaetus butterfly, which is considered in the literature as P. (A.) iphigenia nonacriensis, and the position of which was under debate.

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