Chromosomal and molecular evidence for presence of Polyommatus (Agrodiaetus) poseidon (Lepidoptera, Lycaenidae) in Caucasus region

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Abstract
We show how combination of chromosomal and molecular markers can be applied for proper species identification in Agrodiaetus Hübner, 1822 blue butterflies. Using this approach we provide first evidence for presence of P. (A.) poseidon (Herrich-Schäffer, [1851]) in Georgia.

Keywords
Agrodiaetus, chromosome number, COI, karyotype, Lycaenidae, Polyommatus

Introduction
The blue butterfly subgenus Agrodiaetus Hübner, 1822 belongs to the genus Polyommatus Latreille, 1804 (Talavera et al. 2013). In the last years, this group become a model system for study of speciation and chromosome evolution (Lukhtanov et al. 2015, Vershinina et al. 2015). Despite this, its taxonomy is still poorly elaborated and identification of individual species is difficult due to their morphological similarity. Species within the subgenus are mostly uniform and exhibit few differences in
characters traditionally used in classification, such as wing pattern and/or aspects of the male and female genitalia (Lukhtanov et al. 2006, Vila et al. 2010). The genus was estimated to have originated very recently (Kandul et al. 2004) and, thus, many *Agrodiaetus* species may have not had sufficient time to acquire extensive genetic differences. In particular, COI barcode gap is low or even absent between numerous closely related species of *Polyommatus* (*Agrodiaetus*) (Wiemers and Fiedler 2007). In opposite to majority of other butterflies and moths (Lukhtanov 2014), many *Agrodiaetus* species have evolved distinctive karyotypes. They show one of the highest interspecific karyotypic diversities known in the animal kingdom with haploid chromosome numbers ranging from $n = 10$ to $n = 134$ (Lukhtanov et al. 2005). Therefore, karyotypic features provide important identification characters for many described species that are virtually indistinguishable by their morphology. However, it should be noted that in few cases the chromosome number may be identical in different species (see Results and Discussion).

Here we show how combination of chromosomal and molecular markers can be applied for proper species identification in *Agrodiaetus*. Using this approach we provide first evidence for presence of *P. (A.) poseidon* (Herrich-Schäffer, [1851]) in Georgia.

**Material and methods**

The samples used for molecular and chromosomal analysis were collected in Georgia (Akhaltsikhe, 41.60N, 43.06E, 1000 m alt., 18 July 2014, V. Lukhtanov et V. Tikhonov leg., samples 2014VL56, 2014VL57, 2014VL58, 2014VL62, 2014VL63, 2014VL64, 2014VL65, 2014VL68, 2014VL69, 2014VL70). The methods of DNA sequencing, chromosomal analysis and phylogenetic inference were described previously (Lukhtanov and Dantchenko 2002a, Lukhtanov et al. 2008, 2014, Vershinina and Lukhtanov 2010, Przybyłowicz et al. 2014). Additional samples of *Polyommatus* belonging to *P. (A.) poseidon* species complex (Kandul et al. 2007) were used for comparison.

**Results and discussion**

The species *P. (A.) poseidon* (= *Lycaena poseidon* var. *mesopotamica* Staudinger, 1892, synonymized with *P. poseidon* by Schurian et. 1992) is known to be an endemic of the Middle East sporadically distributed from Kütahya in West Turkey to Artvin in North-East Turkey (Hesselbarth et al. 1995). Phenotypically similar, but chromosomally distinct species *P. (A.) putnami* (Lukhtanov & Dantchenko, 2002) was described from East Turkey (provinces Erzurum and Ağrı) (Lukhtanov and Dantchenko 2002a). The last taxon is allopatric in distribution with *P. (A.) poseidon* and differs from *P. poseidon* by chromosome number and karyotype structure (Lukhtanov and Dantchenko 2002b). *P. (A.) poseidon* has relatively low haploid chromosome number (from n=19 on the south and east of the distributional range to n=21 in the north), all the chro-
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Males of P. (A.) poseidon have plesiomorphic (Kandul et al. 2004, Lukhtanov et al. 2005) blue colouration of the upper side of the wings with no specific morphological characters. Therefore their morphological discrimination from phenotypically similar P. (A.) caeruleus (Staudinger, 1871), P. (A.) damocles and P. (A.) damonides (Staudinger, 1899) is difficult. With respect to mitochondrial genes COI and COII it is very distant from P. (A.) poseidon and was shown to be a subspecies of P. (A.) damocles (Herrich-Schäffer, [1844]) (Lukhtanov et al. 2005, Kandul et al. 2007).

Figure 1. Polyommatus (Agrodiaetus) poseidon from Akhaltsikhe, Georgia. a male, upperside b male, underside.
et Eckweiler, 2001 and *P. (A.) pierceae* (Lukhtanov & Dantchenko, 2002) (Kandul et al. 2007, Lukhtanov et al. 2014).

A population of blue butterflies which were morphologically similar to *P. (A.) poseidon* (Fig. 1) was discovered near Akhaltsikhe in Georgia in 2013 by V.Tikhonov and I. Kostyuk. In 2014 the locality was visited again in order to collect material available for molecular and chromosomal study. Molecular analysis of this material revealed

**Figure 2.** Bayesian tree of the species close to *Polyommatus (Agrodiaetus) poseidon* inferred from COI sequences. Posterior probability values >50% are shown.

**Figure 3.** Male karyotype of *Polyommatus (Agrodiaetus) poseidon* from Georgia. a) sample 2014VL57, metaphase I, n = 19 b) sample 2014VL62, metaphase II, n = 19. Bar = 10 μm.
that COI barcodes were completely identical or nearly identical (barcode gap from 0 to 0.6%) in population from Akhaltsikhe and other populations of P. (A.) poseidon and P. (A.) putnami (Fig. 2).

The haploid chromosome number n=19 was found in MI and MII cells of three studied individuals (2014VL57, 2014VL58, 2014VL62) (Fig. 3). All chromosome elements formed a gradient size row. The karyotype contained no exceptionally large or small chromosomes. In this respect, the population from Akhaltstikhe is indistinguishable from populations of P. (A.) poseidon from Amasya (de Lesse 1963) and Artvin (Kandul and Lukhtanov 1997), but differs from P. (A.) putnami (n=26) (Lukhtanov and Dantchenko 2002b).

Thus, although in the studied case neither the DNA barcodes nor chromosomal numbers are species-specific characters, their combination clearly indicates that the population from Akhaltsikhe should be identified as P. (A.) poseidon. This is the first evidence of P. (A.) poseidon for Georgia and for Caucasus region at whole.

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