

Taxonomic diversity of the genus *Cynaeda* Hübner, [1825] (Lepidoptera: Crambidae) in Central Asia based on the molecular data

Таксономическое разнообразие рода *Cynaeda* Hübner, [1825] (Lepidoptera: Crambidae) в Средней Азии на основе молекулярных данных

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КЛЮЧЕВЫЕ СЛОВА: огнёвки, таксономия, Центральная Азия, молекулярные исследования, цитохромоксидаза субъединица I.

ABSTRACT. First results of the DNA studies with in the *Cynaeda* representatives from Central Asia are submitted. The status of recently described taxon *cobaini* raised to the species rank: *Cynaeda cobaini* Korb, 2019, **stat.n.** The interspecific genetic distance within the genus *Cynaeda* is 0.038. The status of the recently described taxon *C. forsteri komarovi* remain unclear; due to its interspecific genetic distance it can be the separated species or the subspecies of *C. cobaini*; this question can be resolved only when the COI sequence of nominate *C. forsteri* will obtained.

РЕЗЮМЕ. Предлагаются первые результаты молекулярных исследований представителей рода *Cynaeda* в Центральной Азии. Описанному недавно таксону *cobaini* придан видовой ранг: *Cynaeda cobaini* Korb, 2019, **stat.n.**, что обосновано значением межвидовой генетической дистанции по фрагменту COI 0.038. Статус недавно описанного таксона *C. forsteri komarovi* остается неясным; согласно имеющимся различиям в последовательности COI он может оказаться как самостоятельным видом, так и подвидом *C. cobaini*. Этот вопрос может быть разрешён только после того, как будет получена последовательность COI номинативной *C. forsteri*.

Introduction

The genus *Cynaeda* Hübner, [1825] is a compact group of snout moths of the family Crambidae with very characteristic checkerspot wing pattern. External fea-

tures of its species are very variable; genitalia are little bit better key to determine the species but also very variable and structural uniform. Thus mainly the species identification within this group is possible basing on the statistically solid material. It makes the species identification within this group quite difficult. Actually most of the species can be determined with certainty by their distribution (including not only geographical range but also biotope preferences). It was nicely described for Europe and adjacent areas in the recently published book of Slamka [2006]. For Central Asia we have only some data mostly from the revisional work of de Lattin [1951]. Basically all published data about Central Asiatic *Cynaeda* can be summarized within two phrases. First, the genus *Cynaeda* represented in Central Asia by only one species (*C. furiosa* Hampson, 1900). Second, there are possibly some other species represented on this territory.

Starting from 2009 I made a series of moths collecting trips on the territories of Kazakhstan, Kyrgyzstan and Tajikistan. It gave me possibility to add into the Central Asiatic fauna of Lepidoptera at least three more species of *Cynaeda*: *C. dentalis* ([Denis et Schiffermüller], 1775), *C. forsteri* de Lattin, 1951 and *C. gigantea* (Staudinger, 1878) [Korb, 2019; Korb, Sinev, 2019]; both have been described from this region as separate subspecies. At the same time I made a DNA sampling of the *Cynaeda* representatives from the studied territory. The analysis of mtCOI fragments in representatives of *Cynaeda* DNA sampling showed up that at least one of the described by me taxon have rank of separate species; the status of second one needs further examination. Results of the

DNA sampling I present in this paper the results of analysis of mtCOI fragments in Central Asiatic *Cynaeda*.

Materials and methods

For this work I used DNA samples of the Cytochrome Oxidase Subunit I sequence (COI) both mined from the online databases (GenBank and BOLD) as far as my own. The following four DNA samples have been mined from the online databases: PHLSA611 (BOLD) *C. gigantea* Italy, PN Majella, Taranta Peligna, Pian di Valle; LON4094 (BOLD) *C. pustulalis* (Hübner, 1823) Sweden, Oeland, Borgholm, Kolstad; KX071115 (GenBank) *C. dentalis* ([Denis et Schiffermüller], 1775) Germany, Bavaria, Oberpfalz; LEATA458 (BOLD) *C. dentalis* Austria, Tirol, Nordtirol.

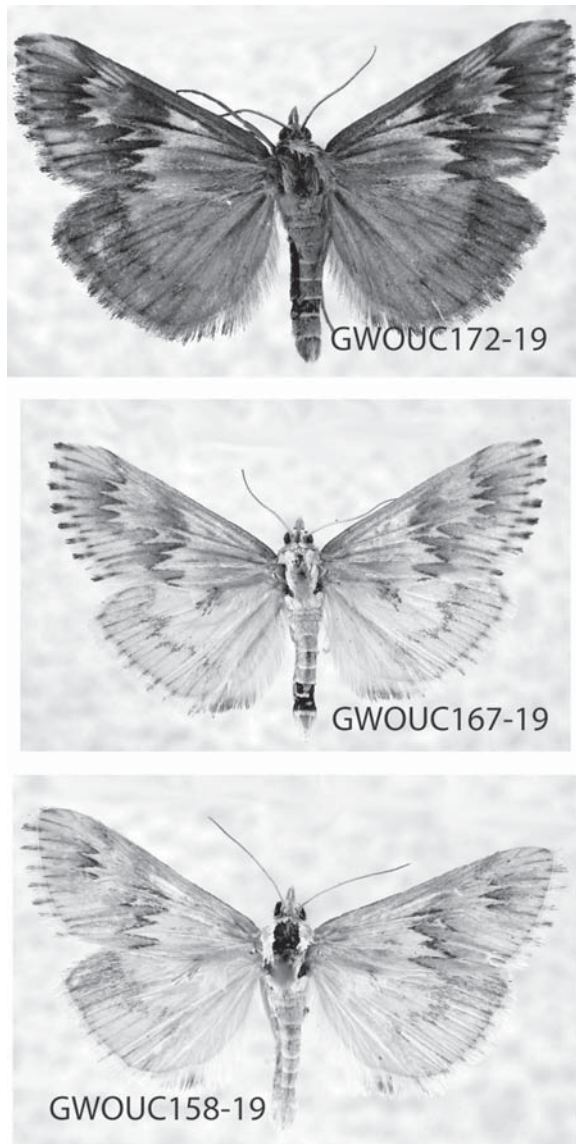


Fig. 1. COI-sampled Central Asiatic *Cynaeda* specimens.

Рис. 1. Экземпляры *Cynaeda* из Центральной Азии, у которых изучены образцы COI.

The following three DNA samples have been processed from my data (all BOLD) (Fig. 1): GWOU158 — *C. forsteri komarovi* Korb, 2019, West Tian-Shan, Talas Mts., Kara-Buura river; GWOU167 — *C. gigantea cobaini* Korb, 2019, Alai Mts., Kyzyl-Eshme valley; GWOU172 — *C. furiosa*, Inner Tian-Shan, Dzhumgaltoo Mts., Sary-Kaiky.

As outgroup for phylogenetic reconstruction the sequence of *Udea decripetalis* (Herrich-Schäffer, 1848) (ABOLA783, BOLD) is used.

DNA sampling and sequencing was processed in the University of Guelph (Canada) by the BOLD program [Ratnasingham, Hebert, 2007]; it was made using processes and protocols described in Huemer et al. [2014]. The length of COI sequences obtained for this analysis is 658 sites.

In total 103 specimens have been studied for comparison with Central Asian ones from Europe, Asia Minor and Near East (see Comparative material examined section below). These ones have been studied morphologically, both external and genitalia (its differences described in detail in Korb [2019] so here are not mentioned). 20 specimens of Central Asiatic *Cynaeda* have been dissected by the methods, described by Stradomsky [2005].

Comparative material examined.

Cynaeda forsteri de Lattin, 1951. 6 ♂, 2728.07.2019, Kyrgyzstan, Talas Prov., Talas Mts., Kara-Buura river coast, 31 km S of Kluchevka (leg. Korb); 1 ♂, 10.07.2014, Moldo-Too Mts., Koro-Goo Pass environs (leg. Korb); 1 ♂, 12.08.2014, Kirghizsky Mts., Bishkek environs, Arashan village (leg. Korb) (paratypes of *komarovi* Korb, 2019); 2 ♂, 2 ♀, 1.06.2017, Kazakhstan, Charyn Relic Forest Nature Reserve (leg. Korb).

Cynaeda dentalis ([Denis et Schiffermüller], 1775). 1 ♂, 13.07.1999, Kyrgyzstan, Northern slope of Issyk-Kul lake, Bosteri (leg. Korb); 1 ♂, 14.07.2004, Kyrgyzstan, Naryn (leg. Korb); 1 ♂, 15.07.2004, Kyrgyzstan, Talass Mts., Chichkan valley (leg. Korb); 1 ♂, 12–13.07.2015, Kyrgyzstan, Bishkek environs, Arashan (leg. Korb); 1 ♂, 13.07.2016, Kyrgyzstan, Toktogul reservoir south shore, Tortkol (leg. Korb); 1 ♂, 21.07.2016, Kyrgyzstan, Dzhumgaltoo Mts., West Karakol river valley 7 km S of Suusamyр (leg. Korb); 3 ♂, 10 ♀, 22.07.2016, Kyrgyzstan, Suusamyрtoо Mts., Kekemerен river valley (leg. Korb); 3 ♂, 12.07.1973, Russia, Crimea, Simferopol environs (leg. Potopolsky); 1 ♂, 1 ♀, 2.08.2008, Finland, Uusimaa, Sipo (leg. Korb); 5 ♂, 10.09.2013, Germany, near Dresden (leg. Korb); 12 ♂, 20.06.2011, Turkey, Chanakkale Prov., Kayarlar (leg. Korb).

Cynaeda cobaini Korb, 2019, **stat.n.** 5 ♂, 14.07.2015, Kyrgyzstan, Alai Mts., Katta-Karakol valley (leg. Korb); 3 ♂, 21.22.07.2019, Kyrgyzstan, Osh Prov., Alai Mts., 6,25 km NNE Kyzyl-Eshme, (leg. Korb) (holotype and paratypes).

Cynaeda furiosa Hampson, 1900. 26 ♂, 12 ♀, 17.07.2015, 21.07.2016, Kyrgyzstan, Dzhumgaltoo Mts., West Karakol river valley 7 km S of Suusamyр (leg. Korb); 2 ♂, 2 ♀, 22.07.2016, Kyrgyzstan, Suusamyрtoо Mts., Kekemerен river valley (leg. Korb); 2 ♂, 10.07.2014, Kyrgyzstan, Moldo-Too Mts., Koro-Goo pass (leg. Korb).

The following software have been used for the DNA analysis: BioEdit ver. 7.2.5, MEGA ver. X.

Results

The phylogenetic tree (Fig. 2) clearly show that all Central Asian representatives of *Cynaeda* form their

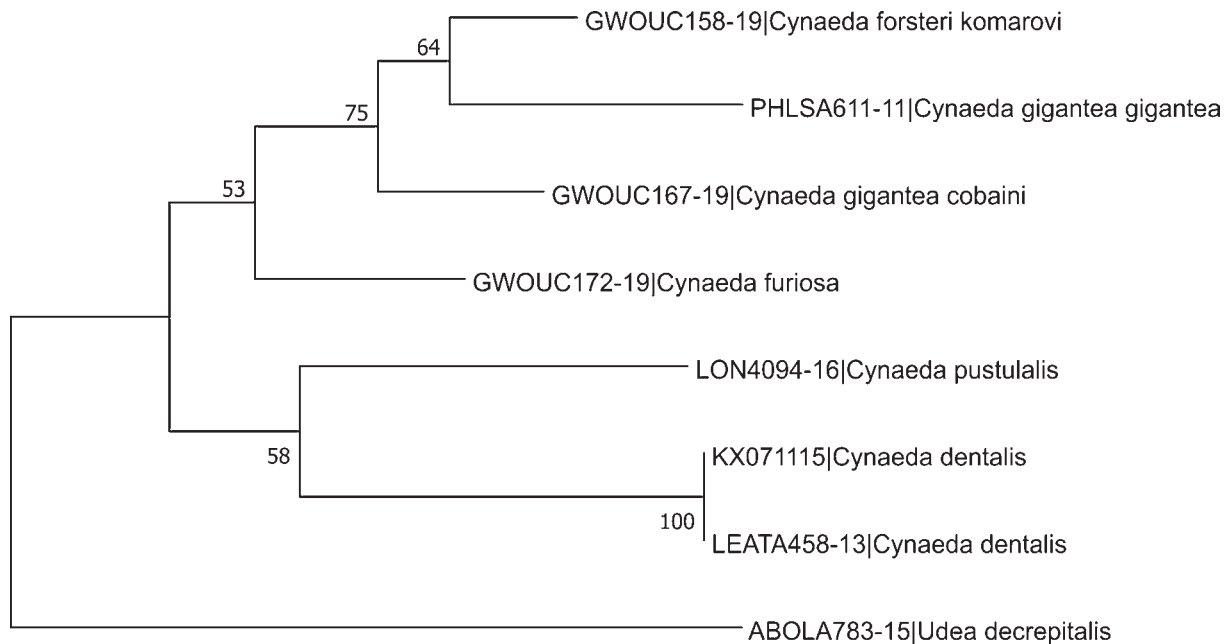


Fig. 2. A phylogenetic tree of the some Palaearctic representatives of *Cynaeda* based on the COI sequence. The tree was reconstructed by using the Maximum Likelihood method based on the Tamura-Nei-parameter model, 10000 bootstrap-replications. The percentage of trees in which the associated taxa clustered together is shown below/over the branches.

Рис. 2. Филогенетическое дерево некоторых палеарктических представителей *Cynaeda* на основе последовательности COI. Дерево было реконструировано с использованием метода максимального сходства, основанного на модели параметров Тамура-Ней, 10000 бутстрап-репликаций. Процент деревьев, в которых связанные таксоны сгруппированы вместе, показан ниже / над ветвями.

own cluster branching in which has bootstrap support 5375%. with p-distances over 0.038. As result of phylogenetic analysis of nucleotide sequences of COI in some representatives of the genus *Cynaeda* the tree was reconstructed. The cluster with two European taxa is opposite to the cluster joined Central Asiatic taxa. The taxon *cobaini* which status is reconsidered in present work is represented by separate branch opposed to *C. forsteri komarovi* + *C. gigantea gigantea* cluster with bootstrap support 75%.

According the DNA barcoding testing data [Huemer et al., 2014], in the majority of cases 2% of COI difference mean a separate species; sometimes it can be more or less of this exact value and it is shown in many papers (for example of small COI differences: Burns et al. [2007] considered genetic distances in 13 nucleotides only enough to separate some skipper butterflies species; for example of big COI differences: Ullah et al. [2017] determined the COI difference between the closely related species of the snout moths within the genus *Tylostega* Meyrick, 1894 as 3.277.60%). In case of Central Asiatic *Cynaeda* it is good to consider not only the phylogenetic tree, but also the result of pairwise analysis of COI sequences, because it (especially in comparison with definitely separate species) is more demonstrable.

In the Table 1 I submit the p-distances of COI sequences for the species of *Cynaeda*. First of all, if we consider the interspecific differences in *C. furiosa*, *C. pustulalis* and *C. dentalis*, the p-distance is 0.071

between *C. furiosa* *C. dentalis* and 0.070 between *C. furiosa* *C. pustulalis*. The differences between the taxa within Central Asia are lesser than the differences between mentioned species figured above; these parameters between *C. furiosa* and both recently described *C. gigantea cobaini* and *C. forsteri komarovi* are 0.049 and 0.052 respectively. The smallest genetic distances we received between *C. gigantea cobaini* and *C. forsteri komarovi* (0.038) and *C. gigantea gigantea* (0.052).

Discussion

The differences between European species of *Cynaeda* and Central Asiatic ones are correspond to different intervals. The p-distance vary in the range between 0.079 and 0.084 for Europe; and it is in the range 0.038–0.052 between *Cynaeda* spp. from Central Asia. Between Central Asiatic and European species the differences are in the range 0.044–0.071. Thus, the minimal interspecific genetic distance within this genus based on current data is 0,038 (3,8%). The p-distance between *C. gigantea gigantea* and *C. gigantea cobaini* is 0.052, and this is clear evidence that the last taxon is a separate species. Basing on this evidence I raise here the rank of recently described taxon *C. gigantea cobaini* to the species: *Cynaeda cobaini* Korb, 2019, **stat.n.** The morphological differences of this taxon from its closely related *C. gi-*

Table. Estimates of evolutionary divergence between sequences COI in species of *Cynaeda*
Таблица. Оценки эволюционной дивергенции между последовательностями COI у видов *Cynaeda*

Sample	KX071115 <i>dentalis</i>	LEATA458 <i>dentalis</i>	LON4094 <i>pustulalis</i>	GWOU158 <i>forsteri komarovi</i>	GWOU167 <i>cobaini</i>	GWOU172 <i>furiosa</i>
KX071115 <i>dentalis</i>	-					
LEATA458 <i>dentalis</i>	0,000					
LON4094 <i>pustulalis</i>	0,079	0,079				
GWOU158 <i>forsteri komarovi</i>	0,074	0,074	0,081			
GWOU167 <i>cobaini</i>	0,079	0,079	0,076	0,038		
GWOU172 <i>furiosa</i>	0,071	0,071	0,070	0,049	0,052	
PHLSA611 <i>gigantea</i>	0,084	0,084	0,088	0,044	0,052	0,065

gantea were described in details in its original description: 1) from the internal side of the postmedial line on the forewing in *cobaini stat.n.* the brown field is located; in *gigantea* this field is white; 2) postmedial line of the hindwing in *cobaini stat.n.* with unclear short dents, in *gigantea* this line have clear, sharp and relatively long dents; 3) *cobaini stat.n.* in general darker than *gigantea*; 4) postmedial line of the hindwing in *cobaini stat.n.* is gray, in *gigantea* it is brown. The male genitalia differences were not found; the female genitalia were not studied.

The p-distance between *C. cobaini* and *C. forsteri komarovi* is 0,038; this parameter is enough to manage both taxa as the separate species, but as we have not enough information to make this judgement uniformly with the above described case, we must wait until at least COI-sequence of nominate *C. forsteri* will be received.

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