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NEW SPECIES OF THE GENUS *YPSOLOPHA* LATREILLE, 1796 (LEPIDOPTERA: YPSOLOPHIDAE) FROM THE SOUTH OF THE RUSSIAN FAR EAST

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Summary. An analysis of the mtCOI sequences in the species from the genus *Ypsolopha* Latreille, 1796 made it possible to confirm a new species for science, *Y. occultatella* **sp. n.**, morphologically similar to the East Asian species *Y. yasudai* Moriuti, 1964. The genetic distance between the mtCOI sequences in *Y. occultatella* **sp. n.** and *Y. yasudai* is 0,066–0,069 (6,6–6,9 %). However, the minimal genetic distance, 0,038–0,042 (3,8–4,2 %), was determined between the mtCOI sequences of the new species and *Y. blandella* (Christoph, 1882), while these species differ well in the forewing pattern.

Key words: Ypsolophidae, *Ypsolopha*, new species, molecular analysis, COI, genetic distance, Russian Far East.

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Резюме. Анализ последовательностей митохондриального фрагмента COI у комплекса видов рода *Ypsolopha* Latreille, 1796 позволил выявить новый для науки вид, *Y. occultatella* **sp. n.**, морфологически близкий восточноазиатскому виду *Y. yasudai* Moriuti, 1964. Генетическая дистанция между последовательностями mtCOI у *Y. occultatella* **sp. n.** и *Y. yasudai* составляет 0,066-0,069 (6,6-6,9%). Однако, наименьшая генетическая дистанция, 0,038-0,042 (3,8-4,2%), установлена между mtCOI последовательностями у нового вида и *Y. blandella* (Christoph, 1882), однако эти виды хорошо отличаются рисунком переднего крыла.

INTRODUCTION

The present study is devoted to the ypsolophid species inhabiting in the boreal landscape with *Larix*, *Betula*, *Lonicera*, *Ledum* and *Vaccínium* as aspecting plants. Of them the *Lonicera* spp. only is a host plant for moths from the genus *Ypsolopha* Latreille, 1796. From 28 species recorded in the Russian Far East (Ponomarenko, 2016; Ponomarenko & Sinev, 2019) the complex of 4 species usually was collected in such biotopes. 3 species of them (*Y. dentella* (Fabricius, 1775), *Y. falcella* ([Denis et Schiffermüller], 1775) and *Y. yasudai* Moriuti, 1964) trophically linked with honeysuckle. The fourth species is *Y. blandella* (Christoph, 1882), inhabiting in the same biotopes although its host plant is unknown yet.

After primary analysis of collected material it was revealed more species than it was known before. Furthermore, some ypsolophid species habitually looked heterogeneous and there were difficulties to separate the series of close species, when specimens are shabby and lacking typical elements in pattern of forewings. Taking into account the weak species divergence in morphology of the male and female genitalia in this group, on the one hand, and some intraspecific variability, on the other hand, it was reason to perform the molecular-phylogenetic analysis.

The listed above four species together with specimens the identification of which was uncertain were outlined as nuclear group for comparative genetic analysis. Besides, the species *Y. leuconotella* (Snellen, 1884) was included into examined group as potential inhabitant of the same biotopes since the *Y. lonicerella* Stöckl, 1922, described from *Lonicera nigra*, was considered a junior synonym for the former (Baraniak & Walczak, 2014). Also the *Lonicera* sp. was indicated as host plant for European populations of *Y. vittella* (Linnaeus, 1758) and was not confirmed for Far Eastern one yet, but it is not reason for excluding this species from the study. The rest species (*acerella*, *acuminata*, *amoenella*, *costibasella*, *parallela*, *parenthesella*, *yangi*) from the genus were included into the analysis to estimate the interspecific genetic distances within the genus *Ypsolopha* Latreille.

Additionally, to original data received in present study, the data from GenBank (NCBI) and BOLD system on mentioned species were included in molecular study.

The vouchers of sampled specimens and type material of a new species are kept in Federal Scientific Center of East Asia Terrestrial Biodiversity (FSC Biodiversity FEB RAS).

MATERIAL AND METHODS

Species sampled. The material on ypsolophid species was collected in the south of the Russian Far East – in Primorskii krai (PK) and Khabarovskii krai (KhK). Moths were collected by net at daytime and attracted on the light at night. The specimens for genetic research were preserved in 96% ethanol and other specimens were processed as dry collection for morphological study.

In total, 14 species from the genus *Ypsolopha* Latreille were included in present study. The comparative molecular analysis based on 38 sequences of mtCOI, 17 of them for 11 species sampled in Russian Far East, were received in present study. The list of species, sampled for genetic study, locality of their collecting and number of vouchers are in the Table 1. Additionally, for species with East Asian, transpalaearctic and transholarctic distribution the available sequences of representatives from South Korean, European and from North American populations, respectively, were taken from GenBank (NCBI) and BOLD system. Species, for which the nucleotide sequences of mtCOI fragments were taken from GenBank (NCBI) and BOLD systems (public data portal), are enumerated in Table 2.

Molecular study. The target fragment mtCOI (658 bp) were selected for estimation of genetic distances within complex of ypsolophid species and for phylogenetic analysis with reconstruction of the tree.

Total genomic DNA from thoracic and abdominal muscle was extracted following the protocol Purification of Genomic DNA from insects appended to Qiagen DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). For each sample of genomic DNA was used one individual of every species. Since genitalia in micromoths have diagnostic significance every sample was no grinded before lysis to save copulative apparatus for voucher. PCR-sequencing of target fragment was made by the Sanger method described in detail by Ponomarenko and Chernikova (2018) with the changes as followings. For polymerase chain reaction (PCR) of the COI fragment the primer combinations LCO1490: 5'-GGTCAACAAATCATA AAGATATTGG-3' and HCO2198: (5'- TAAACTTCAGGGTGACCAAAAAATCA-3' after Folmer *et al.* (1994) was used.

Table 1. List of species from the genus *Ypsolopha* sampled for molecular study (specimens were collected and identified by author, except for the specimen with V-MP-152)

Species	Locality	Voucher Nos.
Y. occultatella **sp. n.**	PK, Chuguevskii distr., "Zov Tigra" Nat. Park, 16 km SE Yasnoe vill., "Pobedinskaya polyana", 31.07.2012	V-MP-150
Y. occultatella **sp. n.**	PK, Chuguevskii distr., "Zov Tigra" Nat. Park, 16 km SE Yasnoe vill., "Pobedinskaya polyana", 31.07.2012	V-MP-236
Y. occultatella **sp. n.**	KhK, Vaninskii distr., Lidoga-Vanino track, 62 km W Vanino, 30.07.2019	V-MP-520
Y. falcella ([Denis et Schiffermüller], 1775)	PK, Chuguevskii distr., "Zov Tigra" Nat. Park, 24.5 km SE Yasnoe vill., 17.07.2010	V-MP-142
Y. falcella ([Denis et Schiffermüller], 1775)	PK, Lasovskii distr., Kievka vill., 07.2010 (Zinchenko coll.)	V-MP-152
Y. falcella ([Denis et Schiffermüller], 1775)	PK, Chuguevskii distr., "Zov Tigra" Nat. Park, 16 km SE Yasnoe vill., "Pobedinskaya polyana", 31.07.2012	V-MP-209
Y. falcella ([Denis et Schiffermüller], 1775)	PK, Khasanskii distr., 36 km S Slavyanka Telyakovskogo Bay, 7-9.07.2016	V-MP-348
Y. yasudai Moriuti, 1964	PK, Vladivostok, 5 km SEE Okeanskaya st., Bogataya Griva ridge, 15.07.2016	V-MP-345
Y. blandella (Christoph, 1882)	PK, Chuguevskii distr., "Zov Tigra" Nat. Park, 16 km SE Yasnoe vill., Pobedinskaya polyana, 31.07.2012	V-MP-151
Y. blandella (Christoph, 1882)	PK, Chuguevskii distr., "Zov Tigra" Nat. Park, 16 km SE Yasnoe vill., Pobedinskaya polyana, 31.07.2012	V-MP-234
Y. costibasella (Caradja, 1939)	PK, Chuguevskii distr., 39 km E Yasnoe vill., Snezhnaya Mt., alt. 1230 m, 31.07-01.08.2012	V-MP-235
Y. acuminata (Butler, 1878)	PK, Oktyabrskii distr., 17 km W Pokrovka vill., middle course of riv. Orlikha, 25-28.05.2011	V-MP-201
Y. amoenella (Christoph, 1882)	PK, Chuguevskii distr., "Zov Tigra" Nat. Park, 24.5 km SE Yasnoe vill., 17.07.2010	V-MP-210
Y. acerella Ponomarenko, Sohn et Zinchenko, 2011	PK, Khankaiskii distr., 2.5 km NNE Dvoryanka vill., 2-5.07.2009	V-MP-217
Y. parallela (Caradja, 1939)	PK, Khankaiskii distr., 2.5 km NNE Dvoryanka vill., 2-5.07.2009	V-MP-218
Y. parenthesella (Linnaeus, 1761)	PK, Oktyabrskii distr., 17 km W Pokrovka vill., middle course of riv. Orlikha, 24-26.06.2010	V-MP-204
Y. yangi Ponomarenko et Sohn, 2011	PK, Oktyabrskii distr., 17 km W Pokrovka vill., middle course of riv. Orlikha, 24-26.06.2010	V-MP-203

Table 2. List of species from genus *Ypsolopha* for which the nucleotide sequences of mtCOI are taken from GenBank and BOLD system

Name of species (as identified in GenBank and BOLD)	**Country of sample origin**	**Voucher**	**GenBank Accession Nos.**	**BOLD Sequence ID**
Y. falcella ([Denis et Schiffermüller], 1775)	Finland	MM17333	JF853784.1	
Y. falcella ([Denis et Schiffermüller], 1775)	Germany	BC ZSM Lep 25347	HM391765.1	
Y. falcella ([Denis et Schiffermüller], 1775)	Germany	BC ZSM Lep 23050	HM391704.1	
Y. falcella ([Denis et Schiffermüller], 1775)	Austria	TLMF Lep 08241	KM573686.1	
Y. blandella (Christoph, 1882)	South Korea	JCS-08-1052	HM377862	LTOLB470-09.COI-5P
Y. dentella (Fabricius, 1775)	Finland	MM08913	HM874106.1	
Y. dentella (Fabricius, 1775)	Italy	TLMF Lep 11998	MG522656.1	
Y. dentella (Fabricius, 1775)	Canada	BIOUG<CAN>:2005-ONT-1092	GU091492.1	
Y. dentella (Fabricius, 1775)	Germany	BC ZSM Lep 25706	GU707030	FBLMU196-09.COI-5P
Y. leuconotella	Italy	TLMF Lep 03652	JN287247.1	
Y. leuconotella	Italy	TLMF Lep 03653	JN287248.1	
Y. vittella (Linnaeus, 1758)	Italy	TLMF Lep 11856		LEATE444-13.COI-5P
Y. vittella (Linnaeus, 1758)	Finland	MM12142	HM875200	LEFIF515-10.COI-5P
Y. vittella (Linnaeus, 1758)	Germany	BC ZSM Lep 28343	HM391875	FBLMV363-09.COI-5P
Y. vittella (Linnaeus, 1758)	Norway	NorBOL LepVM46		LEPVM046-12.COI-5P
Y. yasudai Moriuti, 1964	South Korea	JCS-06-0147	KF523861.1	LTOL887-08.COI-5P
Y. yasudai Moriuti, 1964	South Korea	JCS-08-1048	KF523862.1	LTOLB055-08.COI-5P
Y. yasudai Moriuti, 1964	South Korea	JCS-08-1049	GU696062.1	LTOLB467-09.COI-5P
Y. yasudai Moriuti, 1964	South Korea	JCS-08-1050	GU696063.1	LTOLB468-09.COI-5P
Y. yasudai Moriuti, 1964	South Korea	JCS-08-1051	GU696064.1	LTOLB469-09.COI-5P
Y. yasudai Moriuti, 1964	Russia: PK			GMRSE187-14.COI-5P

Each PCR amplification was performed in a total volume of 20 µl of PCR mixture consisting of 10 µl GoTaq® Green Master Mix, 1 µl 2.5 µM/µl of each forward and reverse primers, 0.5 µl 25 mM $MgCl_2$, 6.5 µl deionized water and 1 µl of DNA for each sample. PCR products were cleaned by using a Thermo Scientific FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific, USA) and then amplified for sequencing using the protocol of BigDye Terminator v.3.1 Cycle Sequencing Kit. PCR fragments were sequenced in Genetic Analyser 3130xl, (Applied Biosystems, USA). The visualization sequences, export them for edit and alignment were made with Program SequenceScanner v 1.0. The amplified sequences of mtCOI (625-720 bp) were aligned and analyzed with the software packages FinchTV 1.4.0. and MEGA-7 (Kumar *et al*., 2016).

Phylogenetic analysis. The phylogenetic tree with inferred relationships between ypsolophid species was constructed on the base of mtCOI fragment by Neighbor-Joining statistical method, Kimura 2-parameter model with using software packages MEGA-7. The genetic divergence (evolutionary distance) between nucleotide sequences of the mtCOI fragments in ypsolophid species was estimated by the Pairwise Distance method, using Kimura 2-parameter model (Kimura, 1980).

Morphological study. The male and female genitalia were prepared by traditional lepidopterological techniques (Falkovich & Stekolnikov, 1978). The genitalia were studied using a stereomicroscope Nikon SMZ-10. The genitalia of both sexes after studying were mounted in the Euparal following the technique described by Robinson (1976). The membranous parts in the female genitalia were stained by Chlorazol Black. For genitalia slides the acronyms MP (prepared by M.G. Ponomarenko) was used. Photographs of adults and genitalia slides were taken using Olympus SZX16 microscope with an incorporated digital camera DP74.

RESULTS

Phylogenetic analysis

The comparative genetic analysis based on barcoding fragment mtCOI for 38 sequences belonging to 14 ypsolophid species was performed.

The reconstructed phylogenetic tree is shown on the Fig. 1. As result all samples of the indeterminate species united into clade **1** with intraspecific genetic distance 0,00-0,004 (0,4%). The comparative genetic analysis is confirmed the presence of a new species for science – *Y. occultatella* sp. n. The new species and *Y. blandella* combined into one clade with bootstrap support 97%. The minimal genetic distance of *Y. occultatella* with *Y. blandella* – 0,038-0,042 (3,8-4,2 %), from which the new species well differs in pattern of forewing (Figs. 2, 5), whereas between new species and habitually similar *Y. yasudai* evolutionary distance 0,066-0,069 (6,6-6,9%) (Table 3). The genetic divergence between new species and other ypsolophid species within the interval 0,062-0,12 (6,2-12%).

The samples of the *Y. yasudai* from Russian Far East and South Korea united into clade **2** (bootstrap 100%, p-distance – 0%) and opposited to large clade **3** with samples from Europe, Russian Far East and South Korea. Both clades 2 and 3 united with bootstrap 100%. The genetic distances between European and East Asian *Y. falcella* – 0,017- 0,019 (1,7-1,9 %) with samples from Russian Far East and 0,015 (1,5%) with samples from South Korea. In the clade **3** all samples (3 from South Korea, and 1 from Russia, Primorskii krai) taken in GenBank and Bold system were registered there as *Y. yasudai*, but their genetic divergence with *Y. falcella* (less 2%) indicates that East Asian population is conspecific with European one, and identification of samples from South Korea and Russian Primorskii krai should be re-considered (Table 4). That is supported by pattern of forewings on the photographs of South Korean samples in the Bold system, which is similar to European *Y. falcella*.

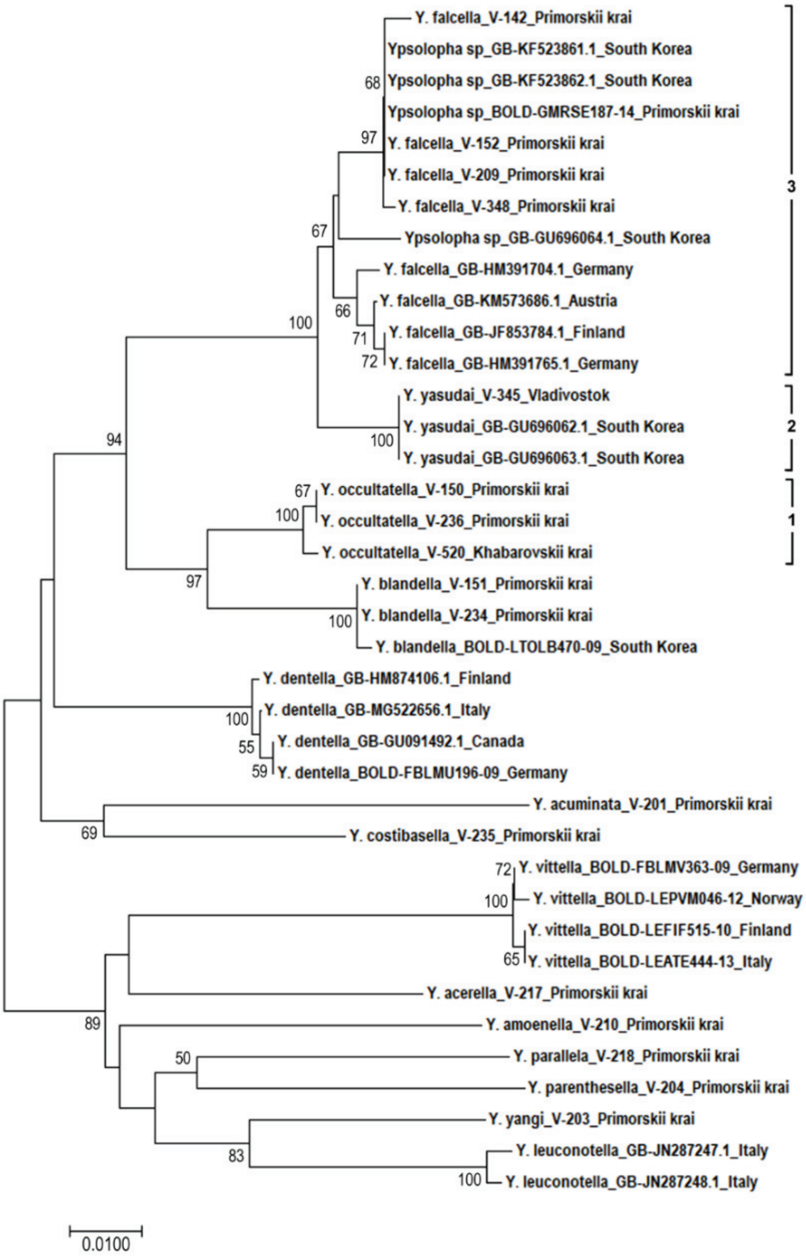


Fig. 1. Phylogenetic relationships of species from the genus *Ypsolopha* Latreille inferred using Neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown from 50%.

DESCRIPTION OF A NEW SPECIES

Ypsolopha occultatella Ponomarenko, sp. n.

http://zoobank.org/NomenclaturalActs/7013C45B-3343-4778-B704-4D7EF849C04E
Figs 2, 6–10

TYPE MATERIAL. Holotype: ♂, **Russia**: Khabarovskii krai, Vaninskii distr., Lidoga-Vanino track, 62 km W Vanino, 49°03'14" N, 139°25'05" E, 680 m above sea level, 30.07. 2019, gen. slide 139 MP (Ponomarenko leg.). Paratypes: 9 ♂, 3 ♀, same locality, date and

Table 3. Estimates of evolutionary divergence between sequences of mtCOI fragment in representatives of ypsolophid species

No	Sample	1	2	3	4	5	6	7	8
1	occultatella_V-150_Primorskii_krai								
2	occultatella_V-236_Primorskii_krai	0,000							
3	occultatella_V-520_Khabarovskii_krai	0,004	0,004						
4	falcella_V-142_Primorskii_krai	0,066	0,066	0,066					
5	falcella_V-152_Primorskii_krai	0,062	0,062	0,062	0,004				
6	falcella_V-209_Primorskii_krai	0,062	0,062	0,062	0,004	0,000			
7	falcella_V-348_Primorskii_krai	0,060	0,060	0,060	0,006	0,002	0,002		
8	falcella_GB-JF853784.1_Finland	0,062	0,062	0,062	0,019	0,015	0,015	0,017	
9	falcella_GB-HM391765.1_Germany	0,062	0,062	0,062	0,019	0,015	0,015	0,017	0,000
10	falcella_GB-HM391704.1_Germany	0,064	0,064	0,064	0,017	0,013	0,013	0,015	0,006
11	falcella_GB-KM573686.1_Austria	0,060	0,060	0,060	0,017	0,013	0,013	0,015	0,002
12	Ypsolopha_sp_BOLD-GMRSE187-14_Prim	0,062	0,062	0,062	0,004	0,000	0,000	0,002	0,015
13	Ypsolopha_sp_GB-GU696064.1_South_Ko	0,064	0,064	0,064	0,019	0,015	0,015	0,017	0,015
14	Ypsolopha_sp_GB-KF523862.1_South_Ko	0,062	0,062	0,062	0,004	0,000	0,000	0,002	0,015
15	Ypsolopha_sp_GB-KF523861.1_South_Ko	0,062	0,062	0,062	0,004	0,000	0,000	0,002	0,015
16	yasudai_V-345_Vladivostok	0,066	0,066	0,066	0,024	0,020	0,020	0,022	0,020
17	yasudai_GB-GU696062.1_South_Korea	0,066	0,066	0,066	0,024	0,020	0,020	0,022	0,020
18	yasudai_GB-GU696063.1_South_Korea	0,066	0,066	0,066	0,024	0,020	0,020	0,022	0,020
19	dentella_GB-GU091492.1_Canada	0,064	0,064	0,064	0,082	0,078	0,078	0,078	0,072
20	dentella_GB-MG522656.1_Italy	0,062	0,062	0,062	0,080	0,076	0,076	0,076	0,070
21	dentella_GB-HM874106.1_Finland	0,062	0,062	0,062	0,080	0,076	0,076	0,076	0,070
22	dentella_BOLD-FBLMU196-09_Germany	0,064	0,064	0,064	0,082	0,078	0,078	0,078	0,072
23	blandella_V-151_Primorskii_krai	0,036	0,036	0,036	0,070	0,066	0,066	0,064	0,068
24	blandella_V-234_Primorskii_krai	0,036	0,036	0,036	0,070	0,066	0,066	0,064	0,068
25	blandella_BOLD-LTOLB470-09_South_Ko	0,038	0,038	0,038	0,072	0,068	0,068	0,066	0,070
26	leuconotella_GB-JN287247.1_Italy	0,106	0,106	0,106	0,130	0,126	0,126	0,123	0,126
27	leuconotella_GB-JN287248.1_Italy	0,108	0,108	0,108	0,125	0,121	0,121	0,119	0,126
28	acerella_V-217_Primorskii_krai	0,097	0,097	0,102	0,115	0,110	0,110	0,108	0,108
29	acuminata_V-201_Primorskii_krai	0,105	0,105	0,105	0,121	0,116	0,116	0,119	0,114
30	amoenella_V-210_Primorskii_krai	0,120	0,120	0,120	0,119	0,115	0,115	0,117	0,120
31	costibasella_V-235_Primorskii_krai	0,076	0,076	0,076	0,097	0,093	0,093	0,090	0,086
32	parallela_V-218_Primorskii_krai	0,109	0,109	0,109	0,129	0,125	0,125	0,125	0,125
33	parenthesella_V-204_Primorskii_krai	0,119	0,119	0,119	0,134	0,130	0,130	0,128	0,128
34	vittella_BOLD-FBLMV363-09_Germany	0,110	0,110	0,115	0,133	0,129	0,129	0,126	0,119
35	vittella_BOLD-LEFIF515-10_Finland	0,113	0,113	0,117	0,135	0,131	0,131	0,129	0,122
36	vittella_BOLD-LEATE444-13_Italy	0,113	0,113	0,117	0,135	0,131	0,131	0,129	0,122
37	vittella_BOLD-LEPVM046-12_Norway	0,112	0,112	0,117	0,135	0,131	0,131	0,128	0,122
38	yangi_V-203_Primorskii_krai	0,108	0,108	0,108	0,130	0,126	0,126	0,123	0,128

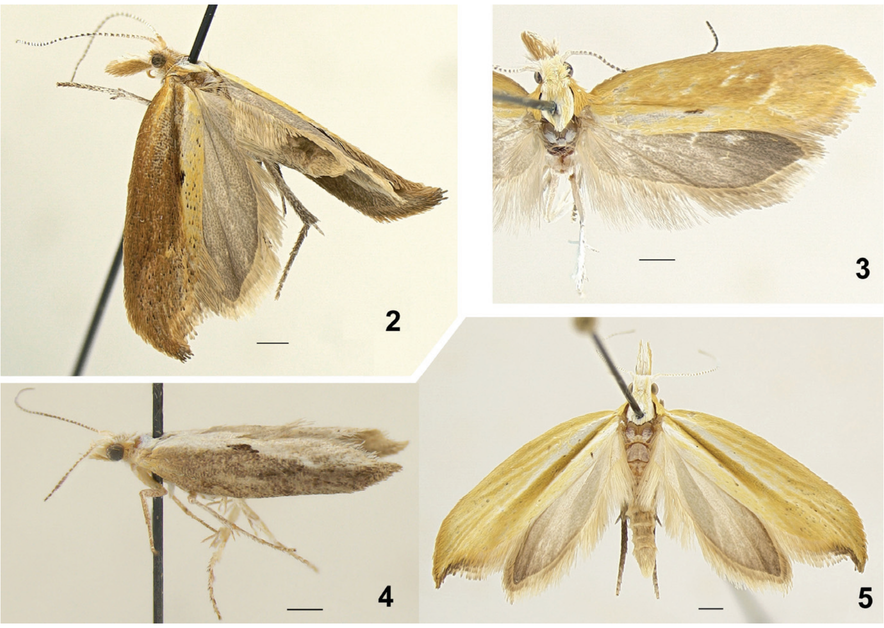
Table 3. Continued

No	9	10	11	12	13	14	15	16	17	18	19	20	21	22
9														
10	0,006													
11	0,002	0,007												
12	0,015	0,013	0,013											
13	0,015	0,017	0,017	0,015										
14	0,015	0,013	0,013	0,000	0,015									
15	0,015	0,013	0,013	0,000	0,015	0,000								
16	0,020	0,022	0,019	0,020	0,022	0,020	0,020							
17	0,020	0,022	0,019	0,020	0,022	0,020	0,020	0,000						
18	0,020	0,022	0,019	0,020	0,022	0,020	0,020	0,000	0,000					
19	0,072	0,072	0,070	0,078	0,080	0,078	0,078	0,078	0,078	0,078				
20	0,070	0,070	0,068	0,076	0,078	0,076	0,076	0,078	0,078	0,078	0,002			
21	0,070	0,070	0,068	0,076	0,078	0,076	0,076	0,076	0,076	0,076	0,002	0,004		
22	0,072	0,072	0,070	0,078	0,080	0,078	0,078	0,078	0,078	0,078	0,000	0,002	0,002	
23	0,068	0,068	0,066	0,066	0,068	0,066	0,066	0,070	0,070	0,070	0,072	0,070	0,070	0,072
24	0,068	0,068	0,066	0,066	0,068	0,066	0,066	0,070	0,070	0,070	0,072	0,070	0,070	0,072
25	0,070	0,070	0,068	0,068	0,070	0,068	0,068	0,072	0,072	0,072	0,074	0,072	0,072	0,074
26	0,126	0,128	0,124	0,126	0,130	0,126	0,126	0,125	0,125	0,125	0,106	0,104	0,104	0,106
27	0,126	0,123	0,123	0,121	0,125	0,121	0,121	0,123	0,123	0,123	0,105	0,103	0,103	0,105
28	0,108	0,108	0,106	0,110	0,110	0,110	0,110	0,108	0,108	0,108	0,101	0,099	0,099	0,101
29	0,114	0,114	0,112	0,116	0,114	0,116	0,116	0,112	0,112	0,112	0,106	0,104	0,104	0,106
30	0,120	0,117	0,117	0,115	0,110	0,115	0,115	0,115	0,115	0,115	0,111	0,109	0,109	0,111
31	0,086	0,086	0,084	0,093	0,095	0,093	0,093	0,092	0,092	0,092	0,067	0,065	0,065	0,067
32	0,125	0,123	0,123	0,125	0,127	0,125	0,125	0,120	0,120	0,120	0,098	0,096	0,096	0,098
33	0,128	0,128	0,126	0,130	0,127	0,130	0,130	0,121	0,121	0,121	0,103	0,101	0,101	0,103
34	0,119	0,122	0,117	0,129	0,128	0,129	0,129	0,122	0,122	0,122	0,104	0,101	0,101	0,104
35	0,122	0,124	0,120	0,131	0,131	0,131	0,131	0,124	0,124	0,124	0,106	0,104	0,104	0,106
36	0,122	0,124	0,120	0,131	0,131	0,131	0,131	0,124	0,124	0,124	0,106	0,104	0,104	0,106
37	0,122	0,124	0,119	0,131	0,130	0,131	0,131	0,124	0,124	0,124	0,106	0,103	0,103	0,106
38	0,128	0,128	0,126	0,126	0,125	0,126	0,126	0,125	0,125	0,125	0,095	0,093	0,093	0,095

Table 3. Continued

No	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
23															
24	0,000														
25	0,002	0,002													
26	0,110	0,110	0,113												
27	0,110	0,110	0,112	0,006											
28	0,100	0,100	0,102	0,108	0,106										
29	0,124	0,124	0,126	0,126	0,126	0,144									
30	0,125	0,125	0,127	0,113	0,109	0,103	0,126								
31	0,084	0,084	0,087	0,112	0,112	0,124	0,092	0,131							
32	0,121	0,121	0,118	0,110	0,107	0,103	0,141	0,103	0,109						
33	0,117	0,117	0,119	0,097	0,101	0,104	0,140	0,104	0,102	0,089					
34	0,134	0,134	0,136	0,110	0,110	0,094	0,133	0,109	0,112	0,112	0,104				
35	0,136	0,136	0,138	0,108	0,108	0,096	0,131	0,107	0,114	0,110	0,106	0,002			
36	0,136	0,136	0,138	0,108	0,108	0,096	0,131	0,107	0,114	0,110	0,106	0,002	0,000		
37	0,136	0,136	0,138	0,112	0,112	0,096	0,135	0,111	0,114	0,114	0,106	0,002	0,004	0,004	
38	0,108	0,108	0,106	0,066	0,070	0,086	0,120	0,097	0,112	0,097	0,085	0,104	0,102	0,102	0,106

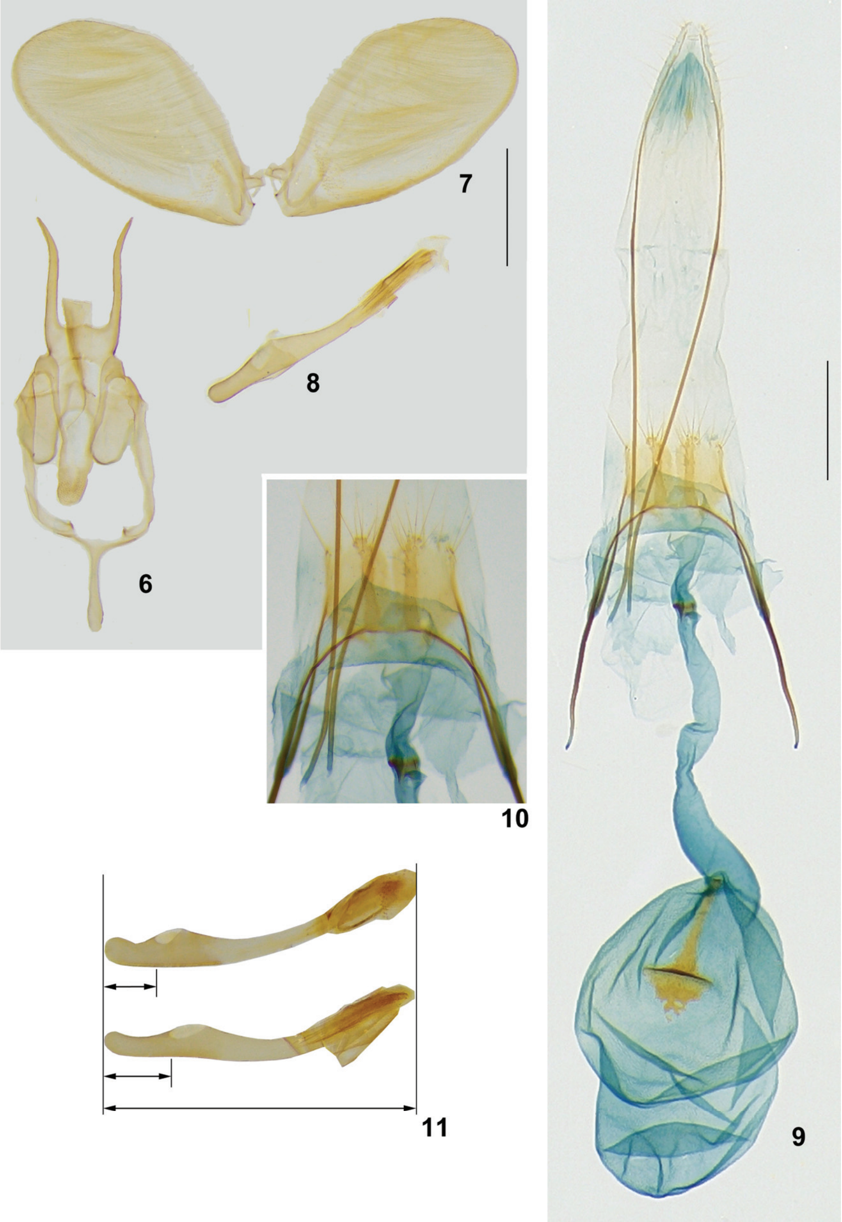
collector, gen. slides 141 (m), 140 (f), 142 (f) MP; 3 ♂, same locality and collector, 9.08. 2020; **Primorskii krai**: 3 ♂, 2 ♀, Dalnegorskii distr., 15 km NW Dalnegorsk, 3 km NE Taiga vill., 18.07.2000, gen slides 143–145 (m), 146-147 (f) MP; Chuguevskii distr., "Zov Tigra" Nat. Park, 1 ♀, 24,5 km SE Yasnoe vill., 16.07.2010, gen slide 148 (m) MP, 1 ♂, 3 ♀, 16 km SE Yasnoe vill., "Pobedinskaya polyana", 2.08.2012 (Ponomarenko leg.).



Figs 2–5. *Ypsolopha* spp., adults. 2 – *Y. occultatella* sp. n., 3 – *Y. yasudai*, 4 – *Y. falcella*, 5 – Y. *blandella*. Scale bar 0.1 mm.

DIAGNOSIS. The new species is similar in pattern of forewing with *Y. yasudai* (Fig. 3) and *Y. falcella* (Fig. 4), but can be distinguished by dark greyish-brown costal part of forewing from the first and greyish-yellow dorsal part and smaller longitudinal dark spot at near 1/3 of wing length from the second. In the genitalia a new species is similar to *Y. yasudai*, but most easy it differs by length and width of coecum in the male genitalia. The coecum is about 1/4 of total aedeagus length and narrow like middle part of aedeagus tube (Fig. 11). In the female genitalia a new species distinguishable by slightly diverging sclerotized bands in ventral part of VIII segment. *Y. yasudai* has the aedeagus with coecum is about 1/6 of its total length and thicker than middle part of aedeagus tube in the male genitalia and almost parallel sclerotized band in ventral part of VIII segment in the female genitalia.

Adult (Fig. 2). Head mainly white on the frons and vertex, with greyish-brown scales around eyes. Antenna with white scapus and pedicellus, flagellum with dark ring on the each segment distally. Basal segment and short third one in labial palpus white; the second segment with large tuft, 2 times as long as head length, greyish-brown laterally and white on inner side and upper margin. Thorax white anteriorly ang light-yellow posteriorly. Tegula greyish-brown. Legs light-grey with scattered dark scales, tarsi darker. Forewing length 8.5-9.5 mm (n = 12), subtrapezoidal, with apex produced and falcate, and sinuate termen. In venation of



Figs 6–10. *Ypsolopha occultatella* sp. n., genitalia. 6–8 – male genitalia: 6 – uncus, tegumen and vinculum, ventral view; 7 – valvae, ventral view; 8 - aedeagus, lateral view. 9, 10 – female genitalia: 9 – ventral view, 10 – sternal part of VIII segment. 11 – comparison of relative length of coecum and aedeagus in *Y. yasudai* (upper) and *Y. occultatella* sp. n. (lower). Scale bar for male and female genitalia 0.5 mm.

the forewing R_4 and R_5 are stalked, Cu_1 and Cu_2 are short–stalked from the angle. Costal part of forewing from costa to the fold dark greyish-brown, with yellow longitudinal basal spot and light-yellow small sport beyond 2/3 of wing length; white narrow indistinct line just below fold from the base to before the middle; the dark, almost black longitudinal sport at near 1/3 of forewing length; dorsal part of forewing greyish-yellow; dark-brown scales scattered along the wing. The apex of forewing with dark almost black scales. Fringe brown along the distal part of costa and termen, the basal scales along the latter light brown. Hindwing grey, with light-grey fringe. In venation of the hindwing M_2 and M_3 are remote. Abdomen brownish grey dorsally, with light brown scales terminally.

Male genitalia (Figs 6–8). Uncus as rectangular plate, its width 2.5 times larger its length, with posterior margin angularly convex; socii 1.5 times longer than saccus, almost straight, with parallel sides on basal 2/3, tapering to pointed apex and slightly curved outwardly at distal 1/3. Gnathos with cup-shaped median plate, almost 2.5 times narrower than uncus width. Tegumen with a U-shaped median excision on anterior margin deeper than mid-length of tegumen, lateral lobes rounded anteriorly. Valva obovate, 2 times longer than maximal width, considerably narrowed to base, rounded distally; costa strong sclerotized, reaching 2/3 of valvar length. Vinculum band-like, arched laterally, with saccus dilated to the apex. Aedeagus slightly curved beyond the middle, with coecum 1/4 as long as whole aedeagus, almost equal to middle part of aedeagus in width; cornuti as four long spines, about 1/3 of aedeagus length.

Female genitalia (Figs 9, 10). Papilla analis slightly sclerotized; ovipositor telescopic, membrane between IX and VIII segments more than 4 times as long as VIII segment. Apophysis posterioris slender, reaching apex of papilla analis, thickened anteriorly, 4.2 times longer than apophysis anterioris; apophysis anterioris with Y–shaped base, it's shorter and thinner branch strengthens anterior margin of VIII segment lateroventrally. Ventral sclerotization of VIII segment with two slightly diverging sclerotized bands, every with rounded lobe posteriorly, and bearing long setae on the apex. Ostium near posterior margin of VII segment; antrum cone-shaped, separated from ductus bursae by lateral narrowing and ring-like sclerotization concave inward dorsally. Ductus bursae tubular, slightly longer than corpus bursae, dilated towards the corpus bursae; bulla seminalis much smaller than corpus bursae, with ductus seminalis shorter than bulla seminalis, arising from sclerotization of the ductus bursae. Corpus bursae with anterior inflation, membranous; signum at posterior 1/3 of corpus bursae, triangular anteriorly, with transverse ridge, and elongated into narrow band to the place where from ductus bursae arising.

DISTRIBUTION. Russia (south part of Far East).

ETYMOLOGY. The species name, *occultatella*, is derived from Latin *occultatum*, meaning "hidden" and refers to the described cryptic new species.

DISCUSSION

Last two decades, the revision of biodiversity has been carried out using molecular techniques. Following the fundamental work of Hebert *et al*. (2003), which proposed a preliminary threshold for an interspecific genetic distance of 3%, in various groups of invertebrates, including insects, a large number of cryptic species (sibling species) were discovered. Studies have shown that the 3% threshold is not universal for interspecies genetic distance in different groups. In addition, within one group, the intervals of intraspecific genetic variability and interspecies genetic distance overlap. Thus, within insects the overlap between intra– and interspecific genetic variability for congeneric sequences is from 0% to15,5% (Cognato, 2006; Meier *et al.*, 2006). Therefore the deciding on the taxon independence should be supported by strong arguments, including morphological differences. In the present study, the discovery of a new species *Y. occultatella* sp. n., which minimal genetic distance from *Y. blandella* 0,038–0,042 (3,8–4,2%) is sufficient to describe a new taxon of the species rank, moreover, that is reliably supported by the morphological differences discussed above. *Y. occultatella* **sp. n**. is belonging to the *mucronellus*-group (after Moriuti, 1977) together with involved in present study representatives of the latter – *Y. blandella*, *Y. dentella* and *Y. falcella*. The interspecific genetic distances within that group from 3,8% to 6,6%. The genetic distances of described new species with representatives of the *vittella*–group reach from minimal 9,7% (*Y. acerella*) to maximal 12% (*Y. amoenella*), and with *acuminata*–group 10,5% (*Y. acuminata*) (Table 3).

Table 4. Reconsideration of the samples identification in the Gen Bank and BOLD system

GenBank Accession No.	**BOLD Sequence ID**	**Country of origin**	**Registered identification**	**Re-considered identification**
KF523861.1	LTOL887-08.COI-5P	South Korea	*Y. yasudai* Moriuti, 1964	*Y. falcella* ([Denis et Schiffermüller], 1775)
KF523862.1	LTOLB055-08.COI-5P	South Korea	*Y. yasudai* Moriuti, 1964	*Y. falcella* ([Denis et Schiffermüller], 1775)
GU696064.1	LTOLB469-09.COI-5P	South Korea	*Y. yasudai* Moriuti, 1964	*Y. falcella* ([Denis et Schiffermüller], 1775)
	GMRSE187-14.COI-5P	Russia: Far East	*Y. yasudai* Moriuti, 1964	*Y. falcella* ([Denis et Schiffermüller], 1775)

United clustering of the sequences of East Asian and European samples of *Y. falcella* in clade **3** on the tree with a genetic distance of 0,017–0,019 (1,7–1,9%) indicated their probable conspecificity. The latter is supported by a similar colour of the forewing with dark grey-brown costal and light-grey slightly yellowish dorsal parts, as well as an almost white thorax in the adults. Samples from South Korea rooted together with *Y. falcella* in clade **3** have the same colour of the forewing in the photographs in the BOLD system, that allows to re-consider their identification (Table 4). That also concerns to the specimen caught in Primorskii krai, which is rooted in the same clade with European samples. The small genetic distance between the species *Y. yasudai* and *Y. falcella* has already been indicated in the Bold system, partly using incorrectly identified samples. In the present study, clade **2** with *Y. yasudai* is opposited to clade **3** with 100% bootstrap support and genetic distance 0,019–0,02 (1,9-2%) with *Y. falcella* samples from Europe and 0,02–0,024 (2–2,4%) with samples from East Asia. It should be noted that the samples from South Korea were shared into two clades - **2** and **3** with a genetic distance of 0,02–0,024 (2–2,4%) without a clinal transition. That indicates a sympatric habitat in East Asia of two species *Y. yasudai* and *Y. falcella*, habitually distinguishable on fresh material and difficult separable, if specimens are shabby and lacking typical elements in pattern of forewings.

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