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The Taxa of the *Hyponephele lycaon – H. lupina* Species Complex (Lepidoptera, Nymphalidae, Satyrinae): Deep DNA Barcode Divergence despite Morphological Similarity

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Original article

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The genus *Hyponephele* includes about 40 species distributed throughout the southern part of the Palaearctic area. Within this genus, the taxa of the *H. lycaon – H. lupina* species complex are similar with respect to the wing pattern and genitalia structure. Here we revise this group using analysis of butterfly morphology, DNA barcodes, and study of the type material. We show that, with a few exceptions, the species in this group are allopatric in distribution. Allopatry in combination with phenotypic similarity may be theoretically interpreted as evidence for the conspecifity of these taxa. Here we falsify this hypothesis by using DNA barcode analysis. We show that the species of this complex are genetically very distant and cannot be combined together as a polytypic species. We also demonstrate that *H. lupina* consists of two deeply diverged allopatric clades, *H. lupina s.s.* and *H. mauritanica* comb. & stat. nov. The barcode p-distance between these taxa (3.4-4.9%) is significantly higher than the generally accepted 'standard' minimum interspecific divergence (2.0-3.0%) threshold. These two clades can also be distinguished by the color of the upperside of the wing in males (brown with conspicuous golden reflection in *H. lupina*; dark brown without golden reflection in *H. mauritanica*) and by details in male genitalia and male androconia structures. Syntypes of *Hyponephele sifanica*, *H. cheena cheena*, *H. cheena iskander,* and *H. cheena kashmirica* are studied and figured.

Key words: Lepidoptera, Nymphalidae, Satyrinae, *Hyponephele*, phylogeny, DNA barcode, COI, morphology, androconia.

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The species-rich genus *Hyponephele* Muschamp, 1915 comprises about 40 species distributed in the Palaearctic region, from N. Africa and Portugal to Far Eastern Russia and China, with the center of the species diversity in Central Asia (ECKWEILER & BOZANO 2011). Morphologically, this is a very homogenous group. Interspecific differences in male genitalia structure are not pronounced or absent (HIGGINS 1975; SAMODUROW *et al.* 1995, 1996, 1997, 1999a, 1999b, 2000, 2001). The male wing pattern is similar in many species, and the female wing pattern can be characterized as extremely similar in many species (see the figures in ECKWEILER & BOZANO 2011).

Therefore, it is not surprising that there are numerous unresolved taxonomic problems within the genus. Several partially overlapping and partially alternative taxonomic hypotheses have been suggested to describe the species and subspecies diversity in the group of species close to *H. lycaon* (Rottemburg, 1775) and *H. lupina* (Costa, 1863) (SAMODUROW *et al.* 1995; ECKWEILER & BOZANO 2011).

1775) and *H. lupina* (Costa, 1863) (SAMODUROW *et al.* 1995; ECKWEILER & BOZANO 2011).

The species of this group were considered as a separate section (in fact, as a subgenus *Hyponephele* (*Hyponephele*) Muschamp, 1915 by KOÇAK and KEMAL (2001). ECKWEILER and BOZANO (2011) did not accept the division of the genus *Hyponephele* into subgenera and sections; however, the taxa close to *H. lycaon* and *H. lupina* are presented in their book as a single block of species consisting of *H. lycaon*, *H. lycaonoides* D. Weiss, 1978, *H. maroccana* (Blachier, 1908), *H. galtscha* (Grum-Grshimailo, 1893), *H. sifanica* (Grum-Grshimailo, 1891), *H. cheena* (Moore, 1865), *H. lupina,* and *H. interposita* (Erschoff, 1874).

We are not aware of any attempts to solve taxonomic problems in the genus *Hyponephele* using molecular data, although GenBank has scattered information on the mitochondrial DNA barcodes of some species (WAHLBERG *et al*. 2003; LUKHTANOV *et al*. 2009; DINCĂ *et al*. 2015; YANG & ZHANG 2015; FENG & NING 2012; LUKHTANOV & NOVIKOVA 2015; DAPPORTO *et al*. 2019). Although DNA barcode data alone has a limited value in taxonomy (DASMAHAPATRA *et al*. 2010; PAZHENKOVA & LUKHTANOV 2019), combining the morphological and DNA barcode data is an efficient method for creating new and testing old species-level taxonomic hypotheses (LUKHTANOV *et al.* 2016).

In this work, we attempt (1) to obtain and analyze a more complete and systematized set of DNA barcodes for representatives of the group of species close to *H. lycaon* and *H. lupina*, and then (2) to use these DNA barcodes in combination with data on morphology and geographic distribution to test the previously formulated hypotheses on taxonomic relationships in the *H. lycaon – H. lupina* species complex.

We pay special attention to the taxa *H. maroccana*, *H. sifanica*, *H. cheena*, *H. lupina,* and *H. interposita* and argue that *H. mauritanica* (Oberthür, 1881) comb. & stat. nov. is a distinct species (although it can also be interpreted as an extremely diverged subspecies of *H. lupina*). Here we do not analyze the taxonomic structure of the species *H. lycaon*, *H. lycaonoides,* and *H. galtscha* in depth which will be considered in a later publication.

Material and Methods

Museum work

The Natural History Museum (London, UK) (BMNH) was visited in order to study the type material of *Hyponephele sifanica*, *H. cheena cheena*, *H. cheena iskander* Hemming, 1941, and *H. cheena kashmirica* (Moore, 1893). The collections of BMNH, of the Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) (ZIN) and the McGuire Center for Lepidoptera and Biodiversity (University of Florida, Gainesville, Florida, USA) (MGCL) were used for analysis of butterfly morphology and geographic distribution.

Morphological analysis

For genitalia preparation, adult abdomens were soaked in hot (90°C) 10% KOH for 3-10 min. They were then transferred to water, and the genitalia were carefully extracted and macerated under a stereomicroscope with the help of a pair of preparation needles or with the help of a needle and watchmaker's tweezers. Once cleansed of all unwanted elements, they were transferred and stored in glycerin. The cleansed genital armatures were handled, studied, and photographed while immersed in glycerin, free from the pressure they would have been subjected to if mounted and, therefore, free from the ensuing distortion. Photographs of the genitalia were taken with a Leica M205C binocular microscope equipped with a Leica DFC495 digital camera, and processed using the software Leica Application Suite, v.4.5.0.

DNA barcode analysis

Standard *COI* barcodes (658-bp 5' segment of mitochondrial *cytochrome oxidase subunit I*) were studied. *COI* sequences were obtained from 30 specimens representing the *H. lycaon – H. lupina* species complex (Table 1). Legs were sampled from these specimens, and sequence data from the DNA barcode region of the *COI* were obtained at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) using protocols described in HAJIBABAEI *et al*. (2005), IVANOVA *et al*. (2006) and DEWAARD *et al*. (2008). The examined specimens were deposited at ZIN and at MGCL. Photographs of these specimens, as well as their collecting data are available in the Barcode of Life Data System (BOLD), projects Butterflies of Palearctic (BPAL) and Butterflies of Palearctic Part B (BPALB) at http://www.boldsystems.org/.

We also used 39 published *COI* sequences (WAHLBERG *et al*. 2003; LUKHTANOV *et al*. 2009; DINCĂ *et al*. 2015; YANG & ZHANG 2015; FENG & NING 2012; LUKHTANOV & NOVIKOVA 2015; DAPPORTO *et al*. 2019) which were downloaded from GenBank (Table 1). The genus *Cercyonis* was found to be a sister to *Hyponephele* (ZHANG *et al*. 2020); therefore, *C. pegala* was used as an outgroup to root the phylogeny.

Sequences were aligned using the software BioEdit (HALL 1999) and edited manually. Phylogenetic hypotheses were inferred using the Bayesian approach (BI) as described previously (PRZYBYŁOWICZ *et al*.

Table 1

Material used in this study

Species	Voucher ID	Sample ID	NCBI accession number	Country	Locality	Reference
Cercyonis pegala	isolate 8-2	isolate 8-2	AY218239	USA		WAHLBERG *et al.* 2003
cheena kashmirica	CCDB-05788_F04	BPAL1964-12	MW288153	India	Kashmir	This study
cheena kashmirica	CCDB-05788_F02	BPAL1962-12	MW288154	India	Kashmir	This study
cheena kashmirica	CCDB-05788_F03	BPAL1963-12	MW288155	India	Kashmir	This study
cheena kashmirica	CCDB-05788_F05	BPAL1965-12	MW288156	India	Kashmir	This study
cheena kashmirica	CCDB-05788_F07	BPAL1967-12	MW288157	India	Kashmir	This study
interposita	CCDB-03033 D06	BPAL1657-12	MW288158	Iran	Quchan	This study
interposita	2005-LOWA-188	2005-LOWA-188	FJ663637	Kazakhstan	Ust-Kamenogorsk Region, Kurchum Mts	LUKHTANOV *et al.* 2009
interposita	2005-LOWA-189	2005-LOWA-189	FJ663636	Kazakhstan	Ust-Kamenogorsk Region, Kurchum Mts	LUKHTANOV *et al.* 2009
interposita	2005-LOWA-190	2005-LOWA-190	FJ663635	Kazakhstan	Ust-Kamenogorsk Region, Kurchum Mts	LUKHTANOV *et al.* 2009
interposita	2005-LOWA-599	2005-LOWA-599	FJ663634	Uzbekistan	Jizzax Viloyati, Nuratau Mts	LUKHTANOV *et al.* 2009
lupina	CCDB-17968_B11	BPAL2683-14	KT864689	Israel	Hermon	LUKHTANOV & NOVIKOVA 2015
lupina	CCDB-17968_E11	BPAL2719-14	KT864688	Israel	Hermon	LUKHTANOV & NOVIKOVA 2015
lupina	LI-90-NSh-10-26-114	LI-90	MW187281	Iran	Fereydunshahr	This study
lupina	2005-LOWA-27	2005-LOWA-27	FJ663648	Kazakhstan	Tienschan, Kurdai Pass	LUKHTANOV *et al.* 2009
lupina	2005-LOWA-28	2005-LOWA-28	FJ663647	Kazakhstan	Tienschan, Kurdai Pass	LUKHTANOV *et al.* 2009
lupina	2005-LOWA-597	2005-LOWA-597	FJ663646	Uzbekistan	Dzhizakskaya_obl., Zarmitan	LUKHTANOV *et al.* 2009
lupina	CCDB-17969_B10	CCDB-17969_B10	MW187180	Georgia	Akhalcikhe	This study
lupina	CCDB-17969_C06	CCDB-17969_C06	MW187186	Iran	Kuh-e-Tamandar	This study
lupina	CCDB-17969_C08	CCDB-17969_C08	MW187187	Iran	Darecht Tash	This study
lupina	CCDB-17969_C09	CCDB-17969_C09	MW187188	Iran	Nahavand	This study
lupina	CCDB-17969_F06	CCDB-17969_F06	MW187215	Iran	Saqqez	This study
lupina	RVcoll. 12-R176	RVcoll. 12-R176	MN144764	Italy	Sicily	DAPPORTO *et al.* 2019
lupina	RVcoll.10-C625	RVcoll.10-C625	MN142660	Italy	Sicily	DAPPORTO *et al.* 2019
lupina	RVcoll.12-R115	RVcoll.12-R115	MN141090	Italy	Sicily	DAPPORTO *et al.* 2019
lupina	RVcoll.LD-2974	RVcoll.LD-2974	MN139329	Italy	Sicily	DAPPORTO *et al.* 2019
lupina	RVcoll.LD-3015	RVcoll.LD-3015	MN138850	Italy	Sicily	DAPPORTO *et al.* 2019
lupina	RVcoll.14-V871	RVcoll.14-V871	MN138754	Italy	Calabria	DAPPORTO *et al.* 2019
lupina	CCDB-25454_B04	CCDB-25454_B04	MW187258	Israel	Hermon	This study
lupina	CCDB-17969_B06	CCDB-17969_B06	MW187176	Georgia	Akhalcikhe	This study
lycaon	2005-LOWA-832	2005-LOWA-832	FJ663650	Kazakhstan	Ust-Kamenogorsk	LUKHTANOV *et al.* 2009
lycaon	2005-LOWA-318	2005-LOWA-318	FJ663651	Kazakhstan	Ust-Kamenogorsk	LUKHTANOV *et al.* 2009
lycaon	2005-LOWA-317	2005-LOWA-317	FJ663652	Kazakhstan	Ust-Kamenogorsk	LUKHTANOV *et al.* 2009
lycaon	CCDB-17969_D01	CCDB-17969_D01	MW187191	Mongolia	Hishig Ongor	This study
lycaon	CCDB-17969_D02	CCDB-17969_D02	MW187192	Mongolia	Harchorin	This study
lycaon	CCDB-17969_E03	CCDB-17969_E03	MW187202	Greece	Karpenisi	This study
lycaon	CCDB-17969_E04	CCDB-17969_E04	MW187203	Greece	Karpenisi	This study

Table 1 Cont.

Species	Voucher ID	Sample ID	NCBI accession number	Country	Locality	Reference
lycaon	CCDB-17969_E05	CCDB-17969_E05	MW187204	Greece	Kalavrita	This study
lycaon	CCDB-17969_E06	CCDB-17969_E06	MW187205	Greece	Kalavrita	This study
lycaon	CCDB-17969_E07	CCDB-17969_E07	MW187206	Greece	Kalavrita	This study
lycaon	CCDB-17969_E08	CCDB-17969_E08	MW187207	Greece	Parnassos	This study
lycaon	CCDB-17969_E09	CCDB-17969_E09	MW187208	Greece	Parnassos	This study
lycaon	CCDB-17969_E10	CCDB-17969_E10	MW187209	Greece	Katara pass	This study
lycaon	CCDB-17969_E11	CCDB-17969_E11	MW187210	Greece	Falakro	This study
lycaon	CCDB-17969_F01	CCDB-17969_F01	MW187211	Italy	Salmona	This study
lycaon	CCDB-17969_F07	CCDB-17969_F07	MW187216	Kazakhstan	Ust-Kamenogorsk	This study
lycaon	CCDB-17969_F08	CCDB-17969_F08	MW187217	Kazakhstan	Ust-Kamenogorsk	This study
lycaon	61026_EP	61026_EP	MW187263	Russia	Samara	This study
maroccana	CCDB-03030_D12	CCDB-03030_D12	KT864703	Morocco	Oukaimeden	LUKHTANOV & NOVIKOVA 2015
maroccana	CCDB-03030_D11	CCDB-03030_D11	KT864704	Morocco	Oukaimeden	LUKHTANOV & NOVIKOVA 2015
maroccana	CCDB-03030_D10	CCDB-03030_D10	KT864705	Morocco	Oukaimeden	LUKHTANOV & NOVIKOVA 2015
mauritanica	RVcoll.08-M019	RVcoll.08-M019	GU676180	Spain	San Martin de la Vega Comunidad de Madrid	DINCĂ *et al*. 2015
mauritanica	RVcoll.08-M045	RVcoll.08-M045	GU676166	Spain	Gualda, La Alcarria Guadalajara Castilla-La Manc	DINCĂ *et al*. 2015
mauritanica	RVcoll.11-I530	RVcoll.11-I530	KP870656	Spain	SE of Cumbres Verdes (La Zubia), Granada, Andalusia	DINCĂ *et al*. 2015
mauritanica	RVcoll.11-I531	RVcoll.11-I531	KP870552	Spain	SE of Cumbres Verdes (La Zubia), Granada, Andalusia	DINCĂ *et al*. 2015
mauritanica	Rvcoll.12-M632	Rvcoll.12-M632	KP871143	Spain	Sotuelamos, El Bonillo Albacete Castilla-La Mancha	DINCĂ *et al*. 2015
mauritanica	Rvcoll.12-M633	Rvcoll.12-M633	KP870843	Spain	Sotuelamos, El Bonillo Albacete Castilla-La Mancha	DINCĂ *et al*. 2015
mauritanica	RVcoll.14-E221	RVcoll.14-E221	MN142116	Spain	Jaen, Cabanas al Nacimiento del Guadalquivir	DAPPORTO *et al*. 2019
mauritanica	RVcoll.14-D322	RVcoll.14-D322	MN138985	Spain	Valladolid, Castilla y Leon	DAPPORTO *et al*. 2019
mauritanica	RVcoll.290909HG13	RVcoll.290909HG13	JN278862	Spain	Castellon Comunidad Valenciana	DINCĂ *et al*. 2015
mauritanica	Rvcoll.08-L268	Rvcoll.08-L268	JN278875	Spain	Castellon	DINCĂ *et al*. 2015
mauritanica	RVcoll.09-V504	RVcoll.09-V504	HM901411	Spain	Sierra de Alfacar Granada Andalusia	DINCĂ *et al*. 2015
mauritanica	RVcoll.08-L920	RVcoll.08-L920	HM901294	Spain	Escamilla Guadalajara Castilla-La Mancha	DINCĂ *et al*. 2015
mauritanica	RVcoll.08-M046	RVcoll.08-M046	HM901309	Spain	Gualda, La Alcarria Guadalajara Castilla-La Mancha	DINCĂ *et al*. 2015
mauritanica	RVcoll.08-M064	RVcoll.08-M064	HM901310	Spain	Budia, La Alcarria Guadalajara Castilla-La Mancha	DINCĂ *et al*. 2015
mauritanica	Rvcoll.08-L921	Rvcoll.08-L921	JN278877	Spain	Escamilla Guadalajara Castilla-La Mancha	DINCĂ *et al*. 2015
sifanica	n/a	n/a	KM111621	China	Qinghai	YANG & ZHANG 2015
sifanica	CCDB-05788_G08	BPAL1980-12	MW187171	China	NE Qinghai, Chiaotou	This study
sifanica (‘*dysdora*’)	HD-2005-71CI	HD-2005-71CI	JQ922050	China	Qinghai	FENG & NING 2012

2014; LUKHTANOV *et al*. 2014, 2019). The Bayesian analysis was performed using the program MrBayes 3.2 (RONQUIST *et al*. 2012). Two runs of 10,000,000 generations with four chains (one cold and three heated) were performed. We checked runs for convergence and proper sampling of parameters [effective sample size (ESS) 200] using program Tracer v1.7.1 (RAMBAUT *et al*. 2018). The consensus of the obtained trees was visualized using FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). The *COI* uncorrected p-distances between the samples (Supplementary Material) were calculated using MEGA version X (KUMAR *et al*. 2018).

Results and Discussion

Geographic distribution

Three species of the complex, *H. lycaon*, *H. lupina,* and *H. interposita,* occur together (sympatrically) in the northern part of Central Asia in Kazakhstan, Kyrgyzstan and W. China. Three species, *H. galtscha*, *H. lupina,* and *H. interposita,* occur sympatrically in Tajikistan. Two species, *H. lupina* and *H. interposita*, occur sympatrically in Uzbekistan, Turkmenistan, NE Iran, Afghanistan, and N. Pakistan. Two species, *H. lupina* and *H. lycaon*, occur sympatrically in S. Europe, Anatolia, Levant, the Caucasus, and Iran. Two species, *H. mauritanica* and *H. maroccana,* occur sympatrically in N. Africa. Two species, *H. mauritanica* and *H. Lycaon,* occur sympatrically in the Iberian Peninsula (Figs 1, 2).

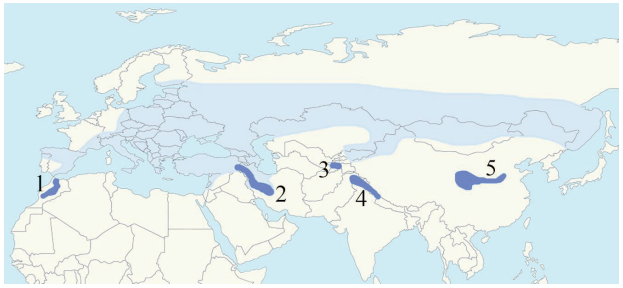

Fig. 1. Distribution of *H. lycaon* (gray fill), *H. maroccana* (1), *H. lycaonoides* (2), *H. galtscha* (3), *H. cheena* (4), and *H. sifanica* (5).

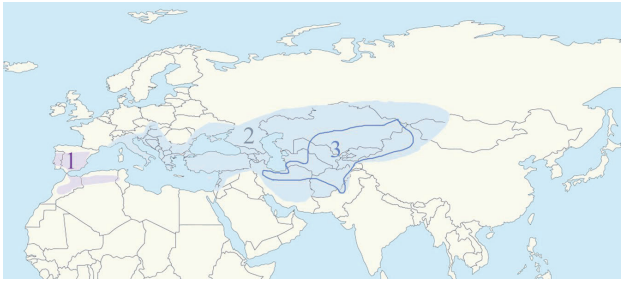

Fig. 2. Distribution of *H. mauritanica* (1, purple filling), *H. lupina* (2, gray filling), and *H. interposita* (3, circled in blue).

On the periphery of the distribution of the complex, the species are found in allopatry: *H. lycaon* in Central Europe, S. Siberia, and the Russian Far East; *H. sifanica* in Central China and *H. cheena* in N. Pakistan and N. India (Fig. 1).

Wing pattern and male androconial patch

The shape of the male androconial patch is the most important morphological trait to distinguish between the species of *Hyponephele* (SAMODUROV *et al.* 1995). Although it is variable, sometimes in an individual or at the subspecies level, it is mostly species-specific. We selected four types of androconial patches in the *H. lycaon – H. lupina* group:

(1) the patch is long and narrow (*H. lycaon* type) (Fig. 3a), found in *H. lycaon*, *H. maroccana,* and *H. sifanica*;

(2) the patch is long and broad (*H. lupina* type) (Fig. 3b-d), found in *H. lupina* (Fig. 3c and *H. mauritanica* (Fig. 3c,d);

(3) the patch is very broad, trapezoidal (*H. interposita* type) (Fig. 3e), found in *H. interposita*;

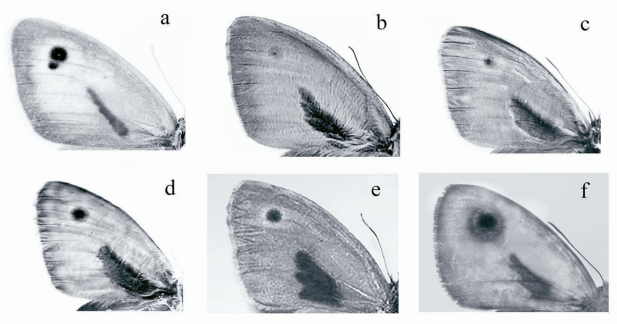

Fig. 3. Male androconial patch. a – *H. lycaon* type (forewing of *H. maroccana* is figured); b –*H. lupina* type (*H. lupina lupina*, Kazakhstan); c – *H. lupina* type (*H. mauritanica,* "Mauretania"); d – *H. lupina* type (*H. mauritanica,* Algeria); e – *H. interposita* type (*H. interposita*, Kazakhstan); f – *H. cheena* type (*H. cheena kashmirica*).

(4) the patch is toothed; in width, it is intermediate between those of *H. lycaon* and *H. lupina* (*H. cheena* type) (Fig. 3f), found in *H. cheena*.

The three species with the *H. lycaon* type of male androconial patch (Fig. 3a) are also similar in respect to their wing pattern (ECKWEILER & BOZANO 2011) (Fig. 4).

The androconial patch is similar, but not identical, in *H. lupina* and *H. mauritanica*. In *H. mauritanica* (Fig. 3c, d), it is slightly wider and more convex than

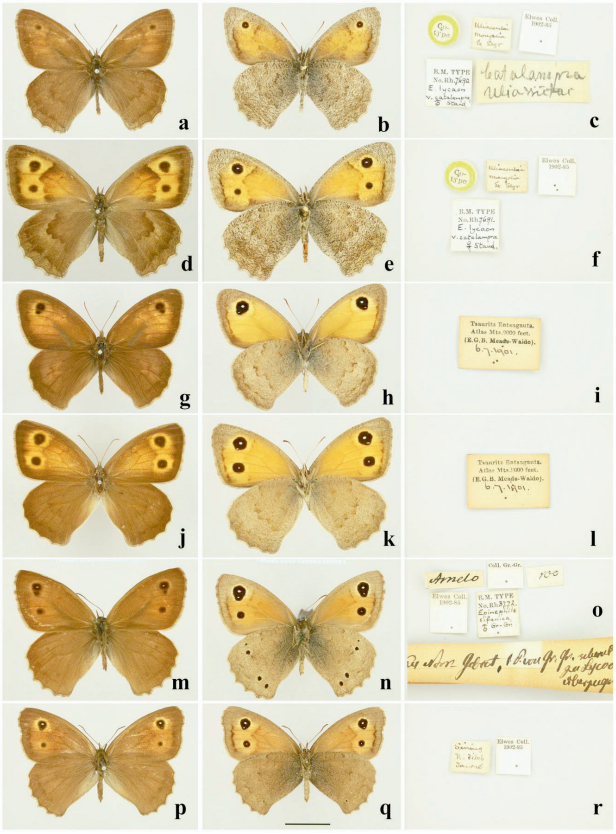

Fig. 4. *Hyponephele lycaon, H. maroccana,* and *H. sifanica* (all specimens in coll. BMNH). Photo: V. Lukhtanov. Scale bar corresponds to 10 mm. a-c – *H. lycaon*, male (paralectotype of *Epinephele Lycaon* Rott. var. *Catalampra* Staudinger, 1895), Mongolia, Uliassutai; d-f – *H. lycaon*, female (paralectotype of *Epinephele Lycaon* Rott. var. *Catalampra* Staudinger, 1895), Mongolia, Uliassutai; g-i – *H. maroccana*, male, Morocco, High Atlas; j-l – *H. maroccana*, female, Morocco, High Atlas; m-o – *H. sifanica*, male (syntype of *Epinephele Sifanica* Grum-Grshimailo, 1891), China, Amdo; p-r – *H. sifanica*, female, China.

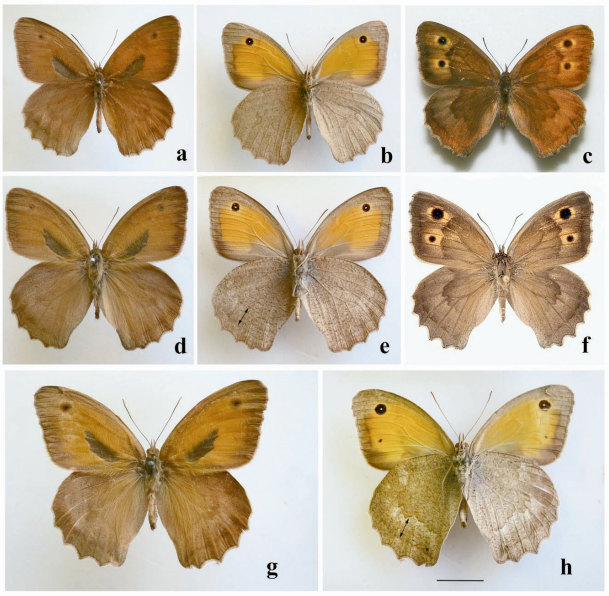

Fig. 5. *Hyponephele mauritanica* and *H. lupina.* Specimens in coll. ZIN (a, b, d, e, g, h), MGCL (c), and coll. Tikhonov (Pyatogorsk, Russia). Photo: V. Lukhtanov (a-e, g. h) and V. Tikhonov (f). Scale bar corresponds to 10 mm. Postdiscal light band is shown by arrows. a-b – *H. mauritanica*, male, "Mauret"[ania], "Stgr" [received from O. Staudinger], "749", "Колл. Вел. Кн. Николая Михайловича" [Collection of Grand Duke Nikolai Mikhailovich]; c – *H. mauritanica*, female, Spanien centr., Prov. Teruel, Albarracin, 1180 m, 22-26.07.1973, leg. P. Hofmann; d-e *H. lupina lupina*, male, Kazakhstan, Turgoyaksky uezd, 2nd Naurzumskaya volost, 19.06.1908, I. Krasheninnikov (coll. S.Tschetverikov); f – *H. lupina lupina*, female, Azerbaijan, East Caucasus, near Gilazi, 600 m, V. Tikhonov leg., 17.06.12 (TIKHONOV *et al*. 2021); g-h – *H. lupina rhamnusia,* male, Italy, Sicily, "v. Rhamnusia 30".

in *H. lupina* (Fig. 3b). These two taxa are also differentiated in respect to other characteristics of wing color and pattern (Fig. 5). The wing color of male *H. mauritanica* is dark brown without any golden reflection (Fig. 5a). The wing color of male *H. lupina* is brown with a conspicuous golden reflection (Fig. 5d); this reflection is especially strong in *H. lupina rhamnusia* (Freyer, 1845) (Fig. 5g). In males of *H. mauritanica*, the hindwing underside has uniform pattern and color, with no postdiscal light band (Fig. 5b), while in *H. lupina* this light band is usually better developed (Fig. 5e). In females of *H. mauritanica,* the wing upperside (Fig. 5c) is much darker than in *H. lupina* (Fig. 5f).

With respect to the wing pattern and androconial shape, *H. cheena* is intermediate between *H. lycaon* and *H. lupina* (Figs 3-6).

In the *H. lycaon – H. lupina* species complex, *Hyponephele interposita* has the most derived wing pattern with a much broader androconial patch in males (Fig. 3e) and without a second postdiscal black ocellus on the forewing in females (see ECKWEILER & BOZANO 2011).

Male genitalia

The male genitalia of the *H. lycaon – H. lupina* complex were studied by HIGGINS (1975) (the species *H. mauritanica*, *H. lycaon*, *H. maroccana*), WEISS (1978) (*H. lycaon*, *H. lycaonoides*), SAMODUROW *et al*. (2001) (*H. lycaon*, *H. lupina*, *H. interposita*, *H. galtscha*), ECKWEILER and BOZANO (2011) (*H. sifanica*, *H. cheena*). *Hyponephele lycaon*, *H. lycaonoides*, *H. maroccana*, *H. lupina,* and *H. interposita* were reported by these authors to have a species-specific shape of valve, uncus, and subunci. As can be seen from the comparison of figures in these works, closely related taxa *H. mauritanica* from Spain (HIGGINS 1975) and *H. lupina* (SAMODUROW *et al*. 2001) differ in the shape of valve, which is shorter at the base in *H. mauritanica*. We checked these differences by comparing the genitals of *H. mauritanica* from Africa with those of *H. lupina lupina* and *H. lupina rhamnusia*

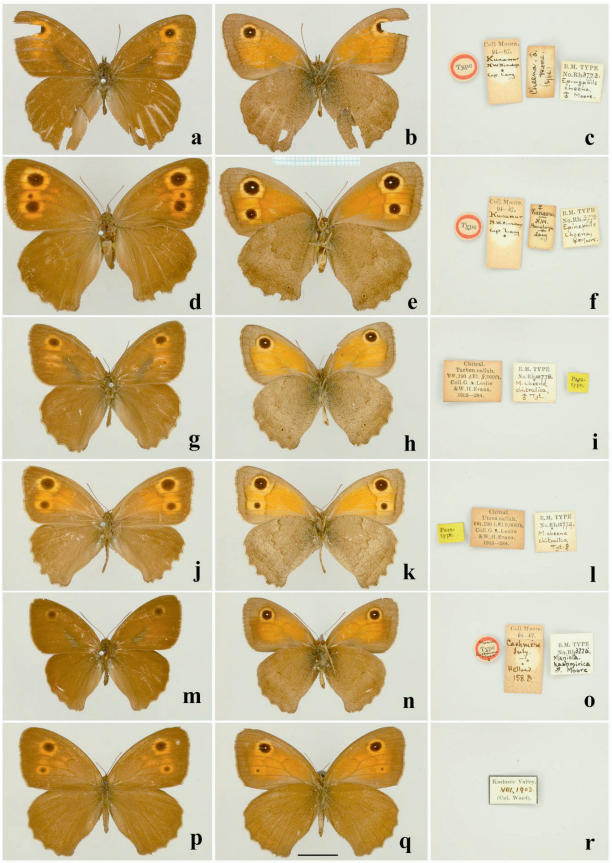

Fig. 6. *Hyponephele cheena* (all specimens in coll. BMNH). Photo: V. Lukhtanov. Scale bar corresponds to 10 mm. a-c – *H. cheena cheena*, male (lectotype of *Epinephile* [sic!] *cheena* Moore, 1865), Kunawur; d-f – *H. cheena cheena*, female (paralectotype of *Epinephele* [sic!] *cheena* Moore, 1865), Kunawur; g-i – *H. cheena iskander* Hemming, 1941, male (syntype of *Maniola cheena chitralica* Tytler, 1926), Chitral, Tarben nallah; j-l – *H. cheena iskander* Hemming, 1941, female (syntype of *Maniola cheena chitralica* Tytler, 1926), Chitral, Utzen nallah; m-o – *H. cheena kashmirica*, male (syntype of *Maniola kashmirica* Moore, 1893); p-r – *H. cheena kashmirica*, female, Kashmir Valley.

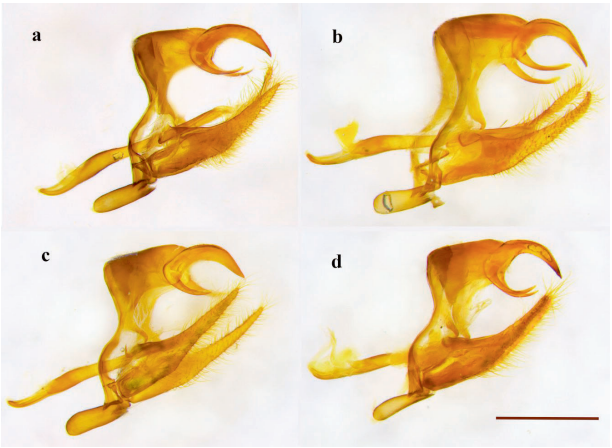

Fig. 7. Male genitalia of *Hyponephele lupina* and *H. mauritanica*, lateral view (all specimens in coll. ZIN). Scale bar corresponds to 1 mm. a – *H. lupina lupina*, Kazakhstan, Turgoyaksky uezd, 2nd Naurzumskaya volost, 19.06.1908, I. Krasheninnikov (coll. S.Tschetverikov); b – *H. lupina rhamnusia,* male, Italy, Sicily, "v. Rhamnusia 30". c – *H. mauritanica*, "Mauret"[ania], "Stgr" [received from O. Staudinger], "749", "Колл. Вел. Кн. Николая Михайловича" [Collection of Grand Duke Nikolai Mikhailovich]; d – *H. mauritanica*, Algeria, coll. Deckert.

(Fig. 7). Our data confirm that the valve of *H. mauritanica* is slightly wider at the base. In addition, the point of inflection of the dorsal margin of the valve from the basal part, which is directed horizontally, to the apical part, which is directed slightly upward, has a different position in these two taxa. In *H. lupina*, it is shifted to the middle of the valve, while in *H. mauritanica*, it is shifted to the base of the valve.

DNA barcode analysis

Allopatry in combination with phenotypic similarity may be theoretically interpreted as evidence for the conspecifity of the taxa *H. lycaon*, *H. maroccana*, *H. sifanica,* and *H. cheena*. In our research, we tested this hypothesis by using DNA barcode analysis. A phylogenetic analysis of this marker revealed seven strongly supported monophyletic groups within the studied species complex (Fig. 8). These groups correspond to the known taxa: *H. sifanica*, *H. interposita*, *H. lupina*, *H. mauritanica*, *H. maroccana*, *H. lycaon,* and *H. cheena*.

The constructed tree is mostly unresolved at the level of basal clades, and the hierarchy of these discovered clades remains unclear. The exceptions are taxa *H. lupina* and *H. mauritanica*, which form a clade with support of 1. *Hyponephele interposita* appears to be a sister to this clade with intermediate support (0.91) while *Hyponephele lycaon* and *H. maroccana* show as sister groups, but with low support (0.7).

The calculation of the uncorrected DNA barcode p-distances showed that the species in the complex are genetically very distant and cannot be combined together as a polytypic species. In the group of allopatric taxa *H. lycaon – H. maroccana – H. sifanica – H. cheena*, the uncorrected p-distances ranged from 4.0% (between *H. sifanica* and *H. cheena*) to 10.3% (between *H. lycaon* and *H. cheena*) (Supplementary Material).

We also demonstrated that the divergence between allopatric clades, *H. lupina* (S. Europe, Anatolia, Levant, the Caucasus, Iran, Central Asia) and *H. mauritanica* (Iberian Peninsula) (Fig. 8) is deep, confirming the conclusion of HINOJOSA *et al*. (2018). The barcode p-distance between these taxa ranges from 3.4% (minimum divergence) to 4.9% (maximum divergence). Thus, the minimum divergence (3.4%) is higher than the generally accepted 'standard' minimum interspecific divergence (2.0-3.0%) threshold (HEBERT *at al*. 2013; LUKHTANOV *et al*. 2016).

Taxonomic conclusion

The distinct DNA barcode gap between *H. lupina* and *H. mauritanica* that we reported is correlated with a morphological hiatus (see above). Previously, we

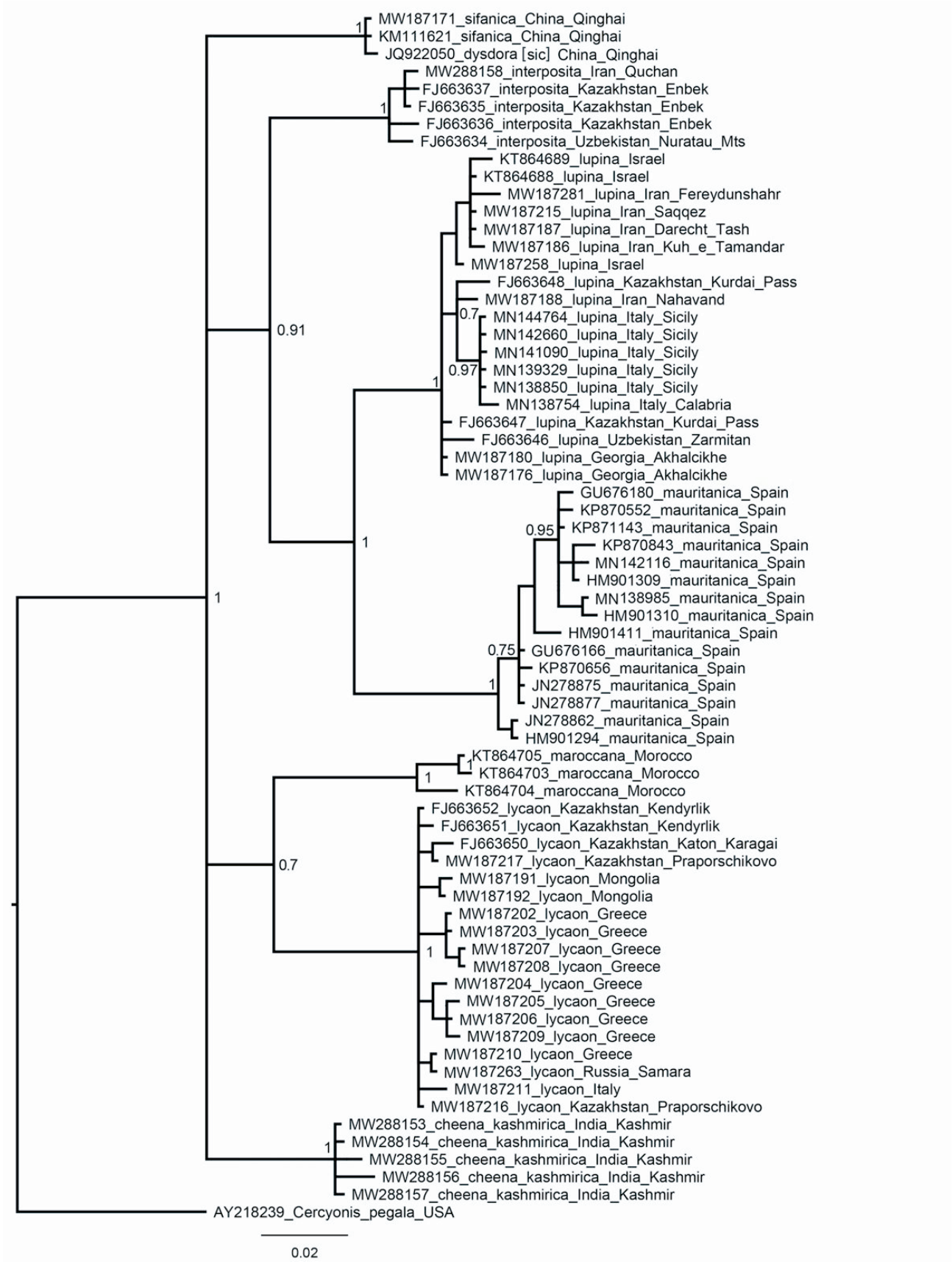

Fig. 8. BI 50% major consensus tree of the studied samples based on *COI* barcodes. Supports less than 0.7 are not shown.

argued that two or more allopatric clusters of individuals can be classified as different species if the presence of a distinct DNA barcode gap between them is correlated with a morphological hiatus, and if the *COI* genetic distance between them is deeper than the 'standard' DNA-barcode species threshold (2.0-3.0%) (LUKHTANOV *et al*. 2016). In accordance with these criteria, *H. lupina* and *H. mauritanica* can be considered as two distinct species (although they can be also interpreted as two extremely diverged subspecies of *H. lupina*).

It should be noted that, with regard to the DNA barcodes, *H. lupina* is very uniform throughout the entire range, with the exception of southern Italy (Sicily, Calabria). The latter population forms a well-supported cluster on the tree. Thus, DNA barcodes do not support the subspecies *H. lupina intermedia* (Staudinger, 1886) from Central Asia (see SAMODUROW *et al.* 2001), but they do support *H. lupina rhamnusia* (described from Sicily and larger than other subspecies, with a more intense light golden reflection on the wing upperside). There are no molecular data for *H. lupina cypriaca* Riley, 1921, but it is morphologically distinct, with a darker wing color in both males and females and characteristic dark, dusty yellow markings on the wings of females (ECKWEILER & BOZANO 2011). Therefore, the subspecies status of *H. lupina cypriaca* Riley, 1921 should be preserved.

We propose the following taxonomic arrangement of the *H. lycaon – H. lupina* species group.

1. *Hyponephele interposita* (Erschoff, 1874) (= *depressa* Korolew, 1995; = *mimonovi* Samodurow, 1995).

2. *Hyponephele sifanica* (Grum-Grshimailo, 1891) (= *deiphobe* Leech, 1894; = *nordoccidentalis* Chou, Yuan & Zhang, 2001) (Note: the sequence JQ922050 of *H. sifanica* is placed in GenBank under the name *H. dysdora*).

3. *Hyponephele cheena* (Moore, 1865)

a) *H. cheena cheena* (Moore, 1865) (Note: the figured "holotype" (BOZANO & ECKWEILER 2011) is not a holotype in fact, since the species description is based on at least two specimens; the holotype was not fixed by original designation (ICZN 73.1.1)

b) *H. cheena iskander* Hemming, 1941 (replacement name for *Maniola cheena chitralica* Tytler, 1926; invalid as a homonym of *Maniola davendra chitralica* Evans, 1923) (= *tytleri* Charmeux & Desse, 2006, unnecessary replacement name for *Maniola cheena chitralica* Tytler, 1926; = *baltistana* Eckweiler & Bozano, 2011, synonymized by TSHIKOLOVETS & PAGES 2016)

c) *H. cheena kashmirica* (Moore, 1893) (Note: considered as a distinct species by TSHIKOLOVETS & PAGES 2016).

4. *Hyponephele lupina* (Costa, 1863) (Note: the spelling *H. lupinus* can be found in scientific literature, e.g. in ECKWEILER & BOZANO 2011. Here we use the spelling *H. lupina* as suggested in WIEMERS *et al*. 2018)

a) *H. lupina lupina* (= *lanata* Alpheraky, 1876; = *intermedia* Staudinger, 1886; = *turanica* Heine, 1894; = *margelanica* Turati, 1909; = *nikokles* Fruhstorfer, 1910; = *transcaucasica* Jachontov, 1910; = *centralis* Riley, 1921; = *captus* Riley, 1921; = *magdalena* Hemming, 1928, = *lupinulus* Turati & Fiori, 1930; = *florentia* Verity, 1937; = *herata* Howarth & Povolny, 1976)

b) *H. lupina rhamnusia* (Freyer, 1845)

c) *H. lupina cypriaca* Riley, 1921.

5. *H. mauritanica* (Oberthür, 1881) (= *najera* Fruhstorfer, 1910; = *celtibera* Sagarra, 1924).

6. *Hyponephele maroccana* (Blachier, 1908) (= *nivellei* Oberthür, 1920).

7. *Hyponephele lycaon* (Rottemburg, 1775).

8. *Hyponephele lycaonoides* D. Weiss, 1978.

9. *H. galtscha* (Grum-Grshimailo, 1893).

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Author Contributions

Research concept and design: V.A.L.; Collection and/or assembly of data: V.A.L., E.A.P.; Data analysis and interpretation: E.A.P.; Writing the article: E.A.P.; Critical revision of the article: V.A.L., E.A.P.; Final approval of article: V.A.L., E.A.P.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary Material to this article can be found online at: http://www.isez.pan.krakow.pl/en/folia-biologica.html

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