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

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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 conspecificity 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 (EKWEILER & 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 EKWEILER & 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; EKWEILER & BOZANO 2011).
1775) and *H. lupina* (Costa, 1863) (Samodurov 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. galttscha* (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; Dincâ et al. 2015; Yang & Zhang 2015; Feng & Ning 2012; Lukhtanov & Novikova 2015; Dappporto et al. 2019). Although DNA barcode data alone has a limited value in taxonomy, 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. galttscha* in depth which will be considered in a later publication.

**Material and Methods**

**Museum work**

The Natural History Museum (London, UK) (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 Paleartic (BPAL) and Butterflies of Paleartic Part B (BPALB) at http://www.boldsystems.org/.

We also used 39 published COI sequences (Wahlberg et al. 2003; Lukhtanov et al. 2009; Dincâ et al. 2015; Yang & Zhang 2015; Feng & Ning 2012; Lukhtanov & Novikova 2015; Dappporto 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. 2015; Dincâ et al. 2015; Zhang & Dyke 2015; Feng & Ning 2012; Lukhtanov & Novikova 2015; Dappporto et al. 2019) which were downloaded from GenBank.
| 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 |                               | 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       | Fereydunshahr       | Iraq    |                               | This study                         |
| lupina          | 2005-LOWA-27 | 2005-LOWA-27 | FJ663648             | Kazakhstan | Ust-Kamenogorsk Region, Kurchum Mts | LUKHTANOV et al. 2009                |
| lupina          | 2005-LOWA-28 | 2005-LOWA-28 | FJ663647             | Kazakhstan | Ust-Kamenogorsk Region, Kurchum Mts | 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  | Akhaltsikhe                   | This study                         |
| lupina          | CCDB-17969_C06 | CCDB-17969_C06 | MW187186             | Iraq     | 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     | Saceqz                        | 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  | Akhaltsikhe                   | 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 | Harhorin                     | 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                         |
| 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 Martín de la Vega Comunidad de Madrid | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.08-M045 | RVcoll.08-M045 | GU676166              | Spain       | Gualda, La Alcarria Guadalajara Castilla-La Mancha | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.11-I530 | RVcoll.11-I530 | KP870656              | Spain       | SE of Cumbres Verdes (La Zuba), Granada, Andalusia | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.11-I531 | RVcoll.11-I531 | KP870552              | Spain       | SE of Cumbres Verdes (La Zuba), Granada, Andalusia | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.12-M632 | RVcoll.12-M632 | KP871143              | Spain       | Sotuelamos, El Bonillo Albacet Castilla-La Mancha | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.12-M633 | RVcoll.12-M633 | KP870843              | Spain       | Sotuelamos, El Bonillo Albacet Castilla-La Mancha | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.14-E221 | RVcoll.14-E221 | MN142116              | Spain       | Jaen, Cabanas al Nacimiento del Guadalquivir | DAPPORO et al. 2019 |
| *mauritanica* | RVcoll.14-D322 | RVcoll.14-D322 | MN138985              | Spain       | Valladolid, Castilla y Leon | DAPPORO et al. 2019 |
| *mauritanica* | RVcoll.290909HG13 | RVcoll.290909HG13 | JN278862              | Spain       | Castellon Comunidad Valenciana | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.08-L268  | RVcoll.08-L268  | JN278875              | Spain       | Castellon        | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.09-V504  | RVcoll.09-V504  | HM901411              | Spain       | Sierra de Alfarcar Granada Andalusia | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.08-L920  | RVcoll.08-L920  | HM901294              | Spain       | Escamilla Guadalajara Castilla-La Mancha | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.08-L921  | RVcoll.08-L921  | HM901309              | Spain       | Escamilla Guadalajara Castilla-La Mancha | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.08-M046  | RVcoll.08-M046  | HM901310              | Spain       | Budia, La Alcarria Guadalajara Castilla-La Mancha | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.08-M064  | RVcoll.08-M064  | HM901310              | Spain       | Escamilla Guadalajara Castilla-La Mancha | DINCÃ et al. 2015 |
| *mauritanica* | RVcoll.08-L921  | RVcoll.08-L921  | JN278877              | Spain       | Escamilla Guadalajara Castilla-La Mancha | DINCÃ 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* | HD-2005-71CI  | HD-2005-71CI  | JQ922050              | China       | Qinghai         | FENG & NING 2012 |
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).

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*;
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...
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 undersides (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. 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 (paralecotype of Epinephele Lycaon Rott. var. Catalampra Staudinger, 1895), Mongolia, Uliassutai; d-f – H. lycaon, female (paralecotype 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.](image)

![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”, “Кол. Вел. Кн. Николая Михайловича” [Collection of Grand Duke Nikolai Mikhailovich]; c – H. mauritanica, female, Spanien centr., Prov. Tenel, Albarracin, 1180 m, 22-26.07.1973, leg. P. Hofmann; d-e – H. lupina lupina, male, Kazakhstan, Turgoyaksky uzd, 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”.](image)
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 conspecificity 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 et 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 SAMODUROV 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* Samoudurov, 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 chitratala* Tytler, 1926; invalid as a homonym of *Maniola davendra chitratala* Evans, 1923) (= *tytleri* Charmeux & Desse, 2006, unnecessary replacement name for *Maniola cheena chitratala* Tytler, 1926; = *baltistana* Eckweiler & Bozano, 2011, synonymized by TSHIK-LOVETS & PAGES 2016)
   c) *H. cheena kashmirica* (Moore, 1893) (Note: considered as a distinct species by TSHIK-LOVETS & 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; = *turana* Heine, 1894; = *margelanica* Turati, 1909; = *nikokles* Fruhstorfer, 1910; = *transcaucasica* Jachontov, 1910; = *centralis* Riley, 1921; = *captus* Riley, 1921; = *magdalena* Hemming, 1928, = *lupinus* 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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