Taxonomy 2.0: Sequencing of old type specimens supports the description of two new species of the *Lasiocampa decolorata* group from Morocco (Lepidoptera, Lasiocampidae)

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**Abstract**

The type of *Lasiocampa decolorata* (KLUG, 1830), collected in 1820, was successfully barcoded to generate a 658bp COI-fragment after 194 years. The resulting molecular data allowed the description of two closely related species from Morocco: *Lasiocampa hannae* SPEIDEL, MOOSER & WITT sp. n. from the Anti Atlas and *Lasiocampa editae* SPEIDEL, MOOSER & WITT sp. n. from the High Atlas.

**Key words:** DNA barcode, *Lasiocampa decolorata*, *Lasiocampa hannae*, *Lasiocampa editae*, *Lasiocampa staudingeri*, new species, taxonomy, Morocco, Egypt, North Africa

**Introduction**

The use of sequence data from a standardized region in the mitochondrial genome (COI 5’ ‘DNA barcode region’) has been proposed as a tool for species identification (HEBERT et al. 2003) and for descriptive (alpha-) taxonomy (e.g. HEBERT et al. 2004). After controversial discussions about the suitability of such molecular data for taxonomy (cf. e.g. TAUTZ et al. 2002, 2003, WILL et al. 2005), there is now broad acceptance of the “integrated taxonomic approach” which combines molecular and morphological data (e.g. TELETCHEA 2010; PADIAL et al. 2010, GOLDSTEIN & DeSALLE 2011, HAUSMANN 2011). Although this approach, recently dubbed ‘taxonomy 2.0’ (JÖRGER & SCHRÖDL 2014), is now widely accepted, the crucial point, i.e. the correct linkage of molecular data with type specimens as the name-bearing key vouchers is still infrequent. Following initial success in sequencing old specimens with rather demanding, time-consuming methods (HAUSMANN et al. 2009a, 2009b), a different approach which focused on the recovery of a short 164bp amplicon from the centre of the COI 5’ barcode gene fragment yielded a very high success rate (80%) for thousands of geometrid type specimens. Here we report a recent breakthrough in recovering the full 658bp COI barcode fragment from a 194 year old type specimen (*Lasiocampa decolorata*) in one single step through next-generation sequencing (NGS).
The poorly known *Lasiocampa decolorata* (KLUG, 1830) is rarely mentioned in modern literature (LEWANDOWSKI & FISCHER 2005, 2008, 2012), and the few existing reports have only led to small advances in the knowledge of this enigmatic species. Initially described in 1830 from Lower Egypt (near Alexandria, erroneously indicated as ‘Upper Egypt’ in De FREINA & WITT 1987), several populations of this species were discovered a few decades ago in Algeria and Tunisia, as well as a single questionable record from Morocco (De FREINA & WITT 1987). In this report, the species was exclusively found in habitats located in desert plains at higher elevation (De FREINA & WITT 1987), contrasting with the wide spectrum of environments inhabited by the *Lasiocampa decolorata* group. Indeed, the analysis of the currently known localities of the *Lasiocampa decolorata* species-group with several recently detected populations in Morocco shows that it inhabits areas ranging from about sea-level to more than 2,000 m, exhibiting an elevational range which is quite remarkable for a single species.

The typical *Lasiocampa decolorata* from Egypt remained nearly unknown, and the Algerian, Tunisian and Libyan populations that had been described under separate taxon names were never compared to the nominotypical Egyptian population. It was essential, therefore, to obtain more information about the type specimen of *Lasiocampa decolorata* which was found in an area now close to the harbour of metropolitan Alexandria (EHRENBERG 1828, BAKER 1997, LEWANDOWSKI & FISCHER 2012), where it is probably extinct. Since only a single female type specimen (holotype by monotypy) is known from this locality, morphological analysis does not reliably clear up the identity of the taxon *decolorata* KLUG, 1830 and the identity of the Moroccan populations because of (1) the absence of valuable differential traits in females and (2) the absence of females in the available material from the Moroccan populations. The successful recovery of the full COI barcode fragment from the 194 year old holotype (collected in 1820), however, finally allows an unambiguous taxonomic analysis in this group.

**Material and methods**

**Abbreviations**

ZSM Bavarian State Collection of Zoology, Munich  
CCDB Canadian Centre for DNA Barcoding  
MWM Museum Witt, Munich  
BOLD Barcode of Life Data Systems  
BIN Barcode Index Number (Global Unique Identifier)  
COI mitochondrial cytochrome c oxidase I (COI) gene, region near the 5’ terminus (barcode fragment, 658 bp)

**Sampling.** Some 500 Palearctic specimens of the genus *Lasiocampa* have been examined at the Museum Thomas WITT (MWM) and the Bavarian State Collection of Zoology (ZSM). For identification, 50 male and female dissections were made, using standard procedures (ROBINSON 1976).

DNA sequences for 105 Palearctic specimens in the genus *Lasiocampa* were available for analysis, 11 belonging to the North African *Lasiocampa decolorata* species-group. These sequences were obtained from DNA extracts obtained from a single leg from dried specimens in various museums and private collections. Barcode sampling strategy aims to gain comprehensive coverage across the distribution of each species. At the present stage, potential sampling bias due to insufficient geographical coverage is expected to play a negligible role.

**DNA Analysis.** PCR amplification and DNA sequencing was performed at the CCDB, for most specimens, following standard high-throughput protocols (IVANOVA et al. 2006; DeWAARD et al. 2008) that can be accessed under http://www.dnabarcoding.ca/pa/ge/research/protocols. PCR amplification with a single pair of primers consistently recovered a 658 bp region near the 5’ terminus of the mitochondrial cytochrome c oxidase I (COI) gene that included the standard 648 bp barcode region for the animal kingdom (HEBERT et al. 2003). DNA extracts are stored at both the CCDB and in the DNA-Bank facility of the ZSM (see http://www.zsm.mwn.de/dnabank/). Another publication (PROSSER et al. 2015) will describe lab protocols and data analysis for the 194 years old type specimen, submitted to NGS sequencing (Torrent PGM316).
All sequences are deposited in GenBank according to the iBOL data release policy. Complete specimen data including images, voucher deposition, GenBank accession numbers, GPS coordinates, sequence and trace files can easily be accessed in the Barcode of Life Data System (RATNASINGHAM & HEBERT 2007) in the public dataset DS-LASIONA.

**Data analysis.** Sequence divergences for the barcode region were calculated using the Kimura-2-Parameter model, employing the analytical tools on BOLD (RATNASINGHAM & HEBERT 2007) and MEGA 6 (TAMURA et al. 2013). Genetic distances between species are reported as minimum pairwise distances, while intraspecific variation is reported as maximum pairwise distances.

**Systematic part**

*Lasiocampa decolorata* (KLUG, 1830)

*Gastropacha decolorata* KLUG, 1830, in Hemprich & Ehrenberg, Symbolae Physicae, pars Zoologicca 2 (series 1 Insecta, Decas 2): unpaginated [9], pl. 20, fig. 1. Locus typicus: ad puteos Dscheil el achterie prope Alexandriam [Egypt]

**Synonyms:**

*Bombyx datini* MABILLE, 1888, Annls Soc. ent. Fr. (6) 8 (Bull.): xlii (Locus typicus: Gabès [Tunisia]), redescribed as *Bombyx datini* OBERTHÜR, 1890, Études Ent. 13: 29, pl. 6, fig. 31, 32 (Locus typicus: Gabès).


**Material examined.** Holotype ♀: *Decolorata*, N, Tscheila Ehrenb, 9018, BC ZSM Lep 81612. Museum für Naturkunde Berlin; 1 ♂ Syntype *Lambessa decolorata sordidior* Type Rothsch., syntype; Guelt-es-Stel, C. Algeria, September 1913 (V. Faroult), Rothschild Bequest 1939-1;


Ehrenberg and Hemprich accompanied the famous Minutoli expedition to Egypt for the discovery of antiquities in order to collect Natural History objects and discovered, in 1820, a single female specimen (holotype) of this species. Under the leadership of Ehrenberg and Hemprich (EHRENBERG, 1828) some members of this expedition left Dscheil el Achterie near Alexandria en route to Cyrenaica but it was never reached. According to LEWANDOWSKI & FISCHER (2012) the collecting date can be reconstructed as either September 23 or 28, 1820, and according to the label, the moth was collected by Ehrenberg.

**Re-description.** The species was described after a female specimen. The male characters are taken from the taxon *sordidior*, which the molecular genetic analysis places near *L. decolorata*. Male: Wing-shape and antenna-structure corresponding to *L. trifolii* (DENIS & SCHIFFERMÜLLER, 1775). As in all species of the group unicolourous yellowish brown. The Algerian populations with a greyish tinge. Female: The winged female holotype is very pale, but this is not likely a result of bleaching because the original coloured figure already shows a very pale moth. The population from Egypt is known only by a single specimen collected in 1820 in an area which is now part of the metropolitan harbour area of Alexandria.

Male genitalia (genitalia slide 19874, fig. 1): No morphological description seems to exist in literature for the male genitalia of *Lasiocampa*. A general, tentative description is given here for explaining the terminology of the below given differential analyses. Male genitalia of *Lasiocampa* are very modified, strongly diverging from the lepidopteran groundplan. They are situated almost on the ventral side of the abdomen, not at its caudal end as usual. This position is caused by a slight extension of the tergal abdominal part which is bent downwards at the end. The vinculum is quite enlarged, with two lateral processes - rolled in in some preparations. The valvae are upwards directed in our figures, but, in natural position, they are pointing caudad. The tegumen is membranous, the valvae consist of two lobes, the inner ones probably representing the sacculus. The inner lobes are weaker sclerotized than the external ones and are easily damaged when the valvae are brought to a standard position, as they are fused to some extent. The external lobes are individually quite variable in shape in all species dissected in numbers (e.g. *Lasiocampa trifolii*), whereas the shape of the inner ones seems to be specific. In *Lasiocampa decolorata*, the inner lobe of valva is pointed, covered with setae. Vesica dorsolaterally everted, aedeagus with a strong, sclerotized projection at its ventral side.

**Distribution.** Only known with certainty from the type locality, but morphology and barcode data suggest Tunisian and Algerian records are also attributed to this species. While records from Palestine are erroneous (see
discussion), further analysis should ascertain if populations from Libya and eastern lowland Morocco also represent this species.

**FIGURE 1.** *Lasiocampa decolorata* (KLUG, 1830). Genitalia slide Heterocera Nr. 19874 MWM "Algeria c., Guelt es Stel, [19]31": Aedeagus Genitalia slide SP 1522 same locality. ZSM.


**FIGURE 3.** *Lasiocampa editae* sp. n. Genitalia slide Heterocera Nr. 19872 MWM "Morocco, High Atlas, Oukaimeden, NW slopes, 2,0–2,300 m, 11.–20. 08. 2012, G. Muller, E. Revay et al., BC ZSM Lep 76653, Museum Witt München".

LASIOCampa decolorata
Ecology. A photograph of the environment west of the type locality is given by LEWANDOWSKI & FISCHER 2012 (page 101). Flight period is September.

Discussion. The species was formerly believed to occur across North Africa in scattered populations which almost all have been described under separate subspecies or species-names, but were later regarded as synonyms. The following additional taxa were placed in synonymy with *L. decolorata* by DE FREINA & WITT 1987: *Lambessa virago* ROTHSCCHILD, 1912, Novit. zool. 19: 118 (Locus typicus: Col de Sfa, Biskra, S. Algeria), and *Lambessa siniscalchi* TURATI, 1926, Atti Soc. Ital. Sci. nat. 65: 29, figured (Locus typicus: Cirene; Derna (Cirenaica) [Libya]). No material of these taxa could be examined and, therefore, they are tentatively left in synonymy with *Lasiocampa decolorata*, following DE FREINA & WITT 1987. The status of these taxa will be clarified, when material from their type localities becomes available for molecular analysis. The taxon *Lambessa decolorata* ssp. *rubrescens* WILTSHIRE, 1986, Fauna of Saudi Arabia 8: 268. Locus typicus: Wadi Sarawin, 610 m (Saudi Arabia) is now viewed as a subspecies of the Asian *Lasiocampa puengeleri* STERTZ, 1915 (LEWANDOWSKI & FISCHER 2012). A few specimens recorded as *L. decolorata* from Um Arad (Sinai) and a few erroneous records from ‘Palestine’ (cf. LEWANDOWSKI & FISCHER 2012) are probably also referable to *L. puengeleri*, the specimens from Sinai were stated to be “redder than KLUG’s type” (WILTSHIRE 1948, 228).
Other records of *L. decolorata* from Egypt are also thought to be erroneous (WILTSHIRE 1948, 228). One male in bad condition was doubtfully recorded as “*L. decolorata*” by RUNGS 1981 (page 428) from eastern Morocco, Kenitra (elevation 25 m). It would be the only record for Morocco and the most western one, if verified.

*Lambessa virago* ROTHSCILD, 1912 from Biskra, Col de Sfâ (Algeria) may represent a specimen of *Lasiocampa decolorata* modified by the influence of breeding abroad. It also seems likely that the taxon *siniscalchi* is correctly placed as a synonym judging from its geographical origin between Algeria and Egypt.

**Genetic data.** BIN: BOLD: ABW5023 (n=3, 658bp), including the Egyptian holotype and two specimens from Tunisia (identified as “subsp. *datini*”), intraspecific variation: 0.6%, suggesting conspecificity of the populations from Egypt and Tunisia. In addition, one very short (94bp) DNA sequence from an Algerian specimen (Guelt es Stel, “subsp. *sordidior*”), at 0.6% distance from the other sequences of BIN BOLD:ABW5023, supporting the hypothesis of conspecificity. Closest neighbouring species: *Lasiocampa editae* with a genetic distance of 3.5%, *Lasiocampa hannae* with 3.0% divergence. The (short 164bp) barcode-fragment of a wingless female of *L. staudingeri* (Tunisia) only shows a divergence of 1.2%, but this value may change when the whole barcode fragment of that species will be sequenced.

**Lasiocampa hannae** SPEIDEL, MOOSER & WITT sp. n.
(Figs 8, 17–19)


**Description.** Wingspan 36–40 mm, forewing length 16–19 mm. Wing-shape and structure of antenna corresponding to that of *L. trifolii*. Easily distinguished from the pale taxa of the *L. decolorata* complex by its reddish-brown wing color, very similar to *L. editae* sp. n. but with a smaller average wingspan and a slightly paler color.

Male genitalia (genitalia slide 19873 (MWM), fig. 2): Valvae very modified, like in all *Lasiocampa* species in a hanging position, not laterally movable. The genitalia are easily damaged if one tries to move the valvae to a standardized position and if the three–dimensional (steric) structures are too strongly flattened. Valva bifid. The exterior lobe of the valva variable in size, strongly sclerotized. Interior lobe much stronger than in *L. editae*, terminally ending truncate, broader. Vesica dorsolaterally everted, with the sclerotized zone stronger developed than in *L. editae*.

**Genetic data.** BIN: ABX3741 (n=2), including holotype. Intraspecific variation: 0.0%. Closest neighbouring species: *Lasiocampa decolorata* at a genetic distance of 3.0%, *Lasiocampa hannae* at 3.2%.

**Etymology.** Named in honor of Mrs. Hanna Sander-Mooser.

**Ecology.** Flight period September, at medium elevations of 1200–1600 m a.s.l. in an isolated area of the Anti Atlas Mountains.

**Lasiocampa editae** SPEIDEL, MOOSER & WITT sp. n.
(Figs 9, 20–22)

**Material.** Holotype ♂: Morocco, High Atlas, Oukaimeden, NW slopes, 2,0–2,300 m, 11.–20. 08. 2012, G. Muller, E. Revay et al., BC ZSM Lep 76653, Genitalpräparat Heterocera Nr. 19872 Museum Witt München.

Description. Male wingspan 38–46 mm, forewing length 19–22 mm. Wing-shape and antenna-structure corresponding with that of other members of the *L. trifolii* group, but without traces of fasciae. Wings reddish brown, superficially indiscriminable from *L. hannae* sp. n., but on average slightly larger and darker than the latter.

Male genitalia (genitalia slides 19867-19872 (MWM), fig. 3): Valvae very modified, like in all *Lasiocampa* species in a dorsal position in the standard preparations, not laterally movable. The genitalia are easily damaged, if one tries to move the valvae to a standardized position and if the three-dimensional structures are too strongly flattened. Valva bifid. The exterior lobe of the valva is variable in size, strongly sclerotized. Interior lobe weaker, rounded and easily destroyed during preparation. Vesica dorsolaterally everted.

Genetic data. BIN: ACL1902 (n=6), including holotype. Intraspecific variation: 0.0%. Closest neighbouring species: *Lasiocampa decolorata* at a genetic distance of 3.5%, *Lasiocampa hannae* at 3.2%.

Etymology. Named in honour of Prof. Dr. Dr. Edita Revay.

Ecology. The species is found in the Central High Atlas Mts. above the tree line, adults fly in July and August. For habitat pictures see Figs 26 and 27.

Discussion. The females of the two new species are so far unknown. Females of the *Lasiocampa trifolii* group are readily attracted to light, and *Lasiocampa decolorata* also possesses winged females that are phototrophic. Thus, the females of the present species may show low affinity to light or even may prove to be wingless, like the allied species *Lasiocampa staudingeri* which ranges from Libya to Morocco. However, males of the latter taxon are easily discriminated by its paler yellow rather than reddish-brown wing colour. As well, adults are only found in autumn and there are no records from the alpine region.

*Lasiocampa staudingeri* (BETHUNE-BAKER, 1885) (Figs 11, 23–25)

*Bombyx staudingeri* BETHUNE-BAKER, 1885, Entomologist's mon. Mag. 21: 242. Locus typicus: Lambessa (Algeria) [author name only Baker in the original publication]


Material. 2 ♂ Algeria, Lambessa. MWM, 1 ♂ Algeria (no further data); 1 ♂ Tunis, 11. A. Faller, Fbg, coll. Th. Witt, München/Weiden, Abgebildet de Freina & Witt, Bombyces & Sphinges der Westpalaearktis Bd. 1, Taf. 28, Fig. 39, staudingeri Bak. MWM; 1 ♀ Tunis e. l., [Kurt John], Franz Daniel; 1 ♀ e. l. 11. x. 71, Tunisia, vic. Hammamet, G. Hesselbarth leg., Sammlung de Freina München, coll. Th. Witt München/Weiden, Abgebildet de Freina & Witt, Bombyces & Sphinges der Westpalaearktis Bd. 1, Taf. 28, Fig. 41, BC ZSM Lep 84742.

Male: The male adults are similar to the other species described here, although the ground colour is distinctively paler than in the other species of the *L. staudingeri*-group, unicolourous pale yellowish brown. Female with only minute traces of wings, almost wingless.

Male genitalia (genitalia slide 8691 (MWM), fig. 4). The aedeagus is slightly curved, whereas it is straight in the other species. Vesica more or less terminally everted. It is likely that cross-breeding with *L. decolorata* and allied species is improbable due to different form of the aedeagus and orientation of the everted vesica.

Distribution. The species is known from Libya to Morocco (De FREINA & WITT 1987), but many of the localities require confirmation, as males can be confused with *Lasiocampa decolorata*.

Genetic data: A 164bp COI-fragment was obtained from a wingless female from northern Tunisia. It showed 1.2% divergence from Tunisian/Algerian *Lasiocampa decolorata*, 2.5% from *L. hannae* sp. n., and 3.7% from *L. editae* sp. n., supporting species status of the last two.
FIGURE 26. Morocco, Toubkal, Ait El Qad 2200–2600 m. Typical habitat of *L. editae* on the southern slopes of the High Atlas Mts. Here Dr. Revay collecting *Coenonympha vaucheri*, *Berberia abdelkader* and *Hipparchia hansii*, typical butterfly species associated with *L. editae*.

FIGURE 27. Morocco, High Atlas, Oukaimeden, Tisrafene south facing slopes, 2,800–3,000 m.
FIGURE 28. NJT tree (Kimura 2 parameter, built with MEGA6) for 10 species in the genus *Lasiocampa* based on sequence variation at COI. Sequence length of the holotype of *Lasiocampa decolorata*: 658bp. Width of triangles represent sample size, depth the genetic variation within the cluster. Source: DNA Barcode data from BOLD (Barcode of Life Database, cf. RATNASINGHAM & HEBERT 2007).

**Discussion.** The present species is surprisingly similar to *Lasiocampa decolorata* in its COI fragment. It seems questionable whether *L. staudingeri* is a separate species, when only considering the small genetic divergence of 1,2% which concerns, however, just the short length of the 164bp fragment. There may be more informative nucleotide sites in the rest of the barcode region. Moreover, *Lasiocampa decolorata* has full-winged females whereas those of *L. staudingeri* have strongly reduced wings. Finally there are subtle morphological differences as given above. We conclude that there are good arguments for tentatively retaining the two taxa as different species. More data are needed for a definitive decision about the status of *L. staudingeri* as a different biospecies or not, but in any case the putative close relationship of species/populations with winged and almost wingless females is surprising.

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