# A second new *Hyalophora* from Mexico: *Hyalophora leonis* sp. n. from Nuevo León in the Sierra Madre Oriental (Lepidoptera: Saturniidae, Attacini)

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Abstract: A new species of the genus Hyalophora, H. leonis sp. n., is described. Male and female specimens from the Mexican federal state of Nuevo León as well as male genitalia are illustrated; a distribution map is included. The male holotype is deposited in Colección Nacional de Insectos, Universidad Nacional Autónoma de México, Ciudad México, Mexico. Both sexes of the new species are known. The new species is compared with other Hyalophora species, including the recently described H. mexicana Nässig, Nogueira & Naumann, 2014. The description of the new species is based on studies of imaginal morphology including male genitalia and mtDNA (COI barcode). H. leonis sp. n. is a medium-sized to large species within the genus. It is unique in the genus by the combination of its carmine red ground colour, the relatively wide white ante- and postmedian lines and marginal area, the small, somewhat rounded forewing ocellular patches in combination with drop-like patches of the hindwing, the huge blue portion of the subapical ocellus, the striking white tufts on both male and female abdomen; and in male genitalia the shape of the valves, especially the ventral part, appears to be diagnostic. In addition, some other problems in Hyalophora are discussed.

Keywords: *Hyalophora leonis* sp. n., Mexico, Nuevo León, Coahuila, Sierra Madre Oriental.

# Eine zweite neue *Hyalophora* aus Mexiko: *Hyalophora leonis* sp. n. aus Nuevo León in der Sierra Madre Oriental (Lepidoptera: Saturniidae, Attacini)

Zusammenfassung: Es wird eine neue Art der Gattung Hyalophora, H. leonis sp. n., beschrieben. Männchen und Weibchen aus dem mexikanischen Bundesstaat Nuevo León sowie männliche Genitalien werden abgebildet; dazu eine Verbreitungskarte. Der männliche Holotypus befindet sich in der Colección Nacional de Insectos, Universidad Nacional Autónoma de México, Ciudad México, Mexiko. Beide Geschlechter der neuen Art sind bekannt. Sie wird verglichen mit anderen Hyalophora-Arten einschließlich der kürzlich neubeschriebenen H. mexicana Nässig, Nogueira & Naumann, 2014. Die Beschreibung basiert auf Studien der Imagines und der männlichen Genitalmorphologie sowie dem COI-"Barcode" der mtDNA. H. leonis sp. n. ist eine mittelgroße bis große Art der Gattung. Sie zeichnet sich aus durch die Kombination folgender Merkmale: die teilweise karminrote Grundfarbe der Flügel mit den recht breiten weißen Ante- und Postmedianlinien sowie dem Marginalfeld, eher gerundete Vorderflügelaugenflecken zusammen mit fast tropfenförmigen Flecken auf den Hinterflügeln, dem großen Anteil blauer Schuppen im Vorderflügel-Subapikalocellus, den auffälligen weißen Haarbüscheln auf dem Hinterleib beider Geschlechter, sowie im männlichen Genital der Valvenform, insbesondere ventral. Dazu werden einige weitere Probleme bei Hyalophora diskutiert.

Una segunda *Hyalophora* de México, nueva en su especie: *Hyalophora leonis* sp. n. de Nuevo León en la Sierra Madre Oriental (Lepidoptera: Saturniidae, Attacini)

Resumen: Se describe una nueva especie del género Hyalophora, H. leonis sp. n. Se ilustra con especímenes machos y hembras de la entidad federativa de Nuevo León, México, así como con los genitales machos. Además, el artículo viene con un mapa de distribución. El holotipo macho se halla en la Colección Nacional de Insectos de la Universidad Nacional Autónoma de México, Ciudad de México, México. Ambos sexos de la nueva especie son conocidos. La nueva especie se compara con otras especies de Hyalophora, incluyendo la recientemente descrita H. mexicana Nässig, Nogueira & Naumann, 2014. La descripción de la nueva especie está basada en el estudio de la morfología imaginal que incluye los genitales del macho así como ADNmt (código de barras COI). H. leonis sp. n. es una especie de tamaño medio hasta grande dentro del género. Es única en su género por la combinación de su rojo carmín como color de base, sus líneas blancas ante y posmedianas y área marginal relativamente anchas. Asimismo, es única por las pequeñas manchas ocelares algo redondas situadas en las alas anteriores en combinación con las manchas gotiformes en las alas posteriores así como por la gran parte de azul en el ocelo subapical y los impresionantes mechones blancos en el abdomen, tanto de los machos como de las hembras. La forma de las válvulas en los genitales machos, especialmente en la parte ventral, parece ser diagnóstica. Además, el artículo trata de otros aspectos de Hyalophora.

#### Introduction

The genus *Hyalophora* Duncan, 1841 is a genus of north American Attacini which, according to actual literature (e.g., Ferguson 1972, Lemaire 1978, 1996, Tuskes et al. 1996, Collins 1997, Powell & Opler 2009, Nässig et al. 2014), presently contains four generally accepted species:

- *Hyalophora cecropia* (Linnaeus, 1758) in the eastern and central area of the US and Canada.
- *H. columbia* (S. I. Smith, 1865) with two (or more) so-called subspecies, *H. columbia columbia* (and perhaps *H. c. nokomis* (Brodie, 1894)?) in central to eastern Canada and *H. c. gloveri* (Strecker, 1872) in the Rocky Mountains/Great Basin area; this latter taxon was listed as a separate species by Ferguson (1972), with a red "form" in southern Arizona (Ferguson 1972: 257, Peigler & Opler 1993, Powell & Opler 2009: 241).

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<sup>&</sup>lt;sup>2</sup> 84th contribution to the knowledge of the Saturniidae (83th contribution: Nässig, W. A., Nogueira G., G., & Naumann, S. (2014): A new species of the genus *Hyalophora* Duncan, 1841 from Central Mexico (Lepidoptera: Saturniidae, Attacini). – Journal of Research on the Lepidoptera, 47: 49–63).

• H. euryalus (Boisduval, 1855) [= rubra Neumoegen & DYAR, 1894] in the West, primarily along the Pacific Coast (Peigler & Opler 1993, Tuskes et al. 1996), with probably a subspecies on Cedros Island, Baja California, Mexico. Populations in the interior of British Columbia, Canada, and in the Bitterroot Mts. of Idaho and Montana (USA), named as subspecies kasloensis Cockerell [in Packard 1914], have usually been treated as a hybrid intergrade between *euryalus* and gloveri (SWEADNER 1937, TUSKES et al. 1996, COL-LINS 1997, 2007), or alternatively as a subspecies of euryalus (e.g., Ferguson 1972).

The fourth species was described recently:

• H. mexicana Nässig, Nogueira G. & Naumann, 2014 from the Sierra Madre Occidental (Federal States of Zacatecas and Guanajuato) in Central Mexico.

These four species are rather closely related, and, for the northern species, most authors agree that local hybridisation and introgression often occurs in areas where they meet naturally (Tuskes et al. 1996, Collins 1997, 2007, Collins & Rawlins 2014). This aspect is not yet studied for the Mexican species.

In our recent paper (Nässig et al. 2014) we discussed our results indicating a more complex structure of the genus (with possibly more than these 4 species). Also, we cited some specimens from northeastern (or eastern central) Mexico (especially from the Sierre Madre Oriental, in the federal states of Nuevo León and Coahuila, see map in Fig. 1) received only recently during the time of submission and revision of the manuscript of our 2014 publication. However, at that time we did not yet receive any COI barcode results of this population (the first legs of these specimens sent to Canada did not result in any DNA data); so these specimens were illustrated as white dots (= "unidentified population, no barcode") on the map in Nässig et al. (2014: 51). In the meanwhile, with some additional specimens, we have succeeded to get barcode sequences indicating that this NE Mexican population apparently is also such well-defined both in morphology and barcode that it requires being described as a separate taxon on equal rank as species.

There are still taxonomic problems, especially for the northern populations. Based on our present knowledge, apparently not every population which presently is called "Hyalophora columbia gloveri" truely represents that species (respectively, subspecies); see discussion.

#### Material and methods

Data of the specimens which were used for the mtDNA analysis are listed in Table 1. The analysis of sequence data was conducted using MEGA5 (TAMURA et al. 2011); see Fig. 2. The COI barcode data of 44 Hyalophora plus the 3 Callosamia specimens used in our analysis as outgroup were either generated in Guelph, Ontario, by Bold (2014) or provided by Jim Fetzner, John Rawlins and Michael M. Collins. We used only sequences that were more than

600 base pairs (bp) long. Shorter sequences as well as those from specimens of doubtful origin (especially most possible hybrids from reared specimens) were discarded. For further details, see in Nässig et al. (2014).

We believe, in accordance with, e.g., Zwick (2009: 148), that the preservation of the undistorted three-dimensional structure of the genitalia is essential for the understanding of their function. Here, we tried to photograph the genitalia from a genital in fluid (70% ethanol).

#### Abbreviations used

BCBarcode [no.].

**CCMC** Collection Carlos C. G. MIELKE, Curitiba, Brazil.

**CDHP** Collection Daniel Herbin, Pechabou, France.

CMNHCarnegie Museum of Natural History, Pittsburgh, PA, USA.

CRRR Collection Rodolphe Rougerie, Rouen, France.

**CSNB** Collection Stefan Naumann, Berlin, Germany, now part of the Rainer Seegers Foundation, to be incorporated in ZMHU Berlin.

Forewing. fw.

Forewing length; measured from base to tip of apex; all fwl. measurements right specimen side.

GP Genitalia dissection [no.].

НТ Holotype.

hw. Hindwing.

hwl. Hindwing length; measured from base to the most distant part of the hw. margin.

IBUNAM Instituto de Biología (Colección Nacional de Insectos), Universidad Nacional Autónoma de México, Ciudad México (Mexico City), Mexico.

PT Paratype.

SMFL Senckenberg-Museum, Lepidoptera collection, Frankfurt am Main, Germany (including coll. W. A. Nässig).

UAG Universidad Autónoma de Guadalajara collection, Zapopan, Jalisco, México.

**ZMHU** Museum für Naturkunde, Berlin (formerly Zoologisches Museum der Humboldt-Universität), Germany.

## Results

## Hyalophora leonis sp. n.

Holotype &: México, Nuevo León, Galeana, 24.4713° N, 100.0305° W, 2100 m, July 2013, leg. local collectors; BC SNB 5191, CSNB; to be deposited in coll. IBUNAM, Ciudad México.

Paratypes (7  $\eth \eth$ , 2  $\circlearrowleft$ ), all Mexico: Nuevo León: 1  $\eth$ , 1  $\circlearrowleft$ , same locality and date as HT; & GP 2400/14 WAN; SMFL. 1  $\mathcal{E}$ , 1  $\mathcal{Q}$ , same locality and date as HT,  $\mathcal{E}$  BC SNB 5190,  $\mathcal{Q}$ BC SNB 5192, CSNB. 1 &, Laguna de Sanchez, 25.2048° N, 100.1654° W, 2180 m, vII. 2013, leg. local collector, ♂ GP 2399/14 WAN, SMFL. 1 ♂, same locality and date, BC SN 5189, CSNB. 1 ♂, same locality (but more precisely: "8 km W Laguna de Sanchez, GPS 252048, 1001654, 1900 m"), vi. 2014, leg. local collector, CSNB. 1 ♂, Santiago, 25°21' N, 100°18' W, 1760 m, leg. V. O. Becker (no. 120703), BC SNB 4560 (no result), CCMC. – Coahuila: 1 ♂, Chapultepec, 25°13' N, 100°57' W, 2210 m, 22. vii. 2009, leg. G.N.G., SMFL. Etymology: Named after the Mexican Federal State of

**Table 1:** Data of the specimens of *Hyalophora* (44 specimens) and *Callosamia* (3 specimens, included as outgroup) used for the mtDNA sequence analysis. — Additional abbreviations: bp = [mtDNA] base pairs; GBAC = GenBank Access Code; HT = holotype; PT = paratype; SL = Sequence Length (data from BOLD or simple count of bp); — = information not available. — In the same order of taxa and specimens as in the tree graph, Fig. 2.

Species	Sample-ID	Process-ID	GBAC	SL	Deposition	Locality of Origin	
H. "gloveri a"	SNB 1699	SASNB699-09	GU702999	658[0n]bp	CSNB	USA, Arizona, Cochise Co., Guadelupe Canyon	
H. "gloveri a"	SNB 1863	SASNB768-10	HQ579817	658[0n]bp	CSNB	USA, Arizona, Cochise Co., Guadelupe Canyon	
H. "gloveri a"	MGS 721	_	KJ865744	658 bp	CMNH	USA, Arizona, Cochise Co., Huachuca Mtns.	
H. "gloveri a"	MGS 723	_	KJ865745	658 bp	CMNH	USA, Arizona, Cochise Co., Huachuca Mtns.	
H. "gloveri a"	MGS 734	_	KJ865746	658 bp	CMNH	USA, Arizona, Cochise Co., Huachuca Mtns.	
H. "gloveri a"	MGS 233	_	KJ865741	658 bp	CMNH	USA, Arizona, Gila Co., Payson	
H. "gloveri a"	MGS 569 = 570	_	KJ865742	658 bp	CMNH	USA, Arizona, Hualapai	
H. "gloveri a"	MGS 707	_	KJ865743	658 bp	CMNH	USA, Arizona, Graham Co., Pinaleno Mtns.	
H. "columbia a"	SNB 1861	SASNB766-10	KM287184	658[0n]bp	CSNB	USA, Colorado, Colorado Springs	
H. "columbia a"	SNB 1860	SASNB765-10	HQ579815	658[0n]bp	CSNB	USA, Colorado, Colorado Springs	
H. "columbia a"	SNB 5211	SASNC2747-13	KM995810	658[0n]bp	CSNB	Canada, Alberta	
H. "columbia a"	B3218-wn-C05	SAWNA027-09	GU703464	658[0n]bp	SMFL	Canada, Ontario, Norland	
H. "columbia a"	B3218-wn-C06	SAWNA028-09	GU703465	658[0n]bp	SMFL	Canada, Ontario, Norland	
H. "columbia a"	SNB 5210	SASNC2746-13	KM995808	658[0n]bp	CSNB	Canada, Alberta	
H. columbia	SNB 1698	SASNB698-09	HM383529	658[0n]bp	CSNB	Canada, Ontario, Haliburton Highlands	
H. columbia	SNB 1857	SASNB762-10	HQ579814	658[0n]bp	CSNB	Canada, Ontario	
H. columbia (hybr. with cecropia?)	SNB 1866	SASNB771-10	HQ579818	658[1n]bp	CSNB	Canada, Ontario, Haliburton Highlands	
H. cecropia	SNB 3227	SASNC1238-11	KM287185	658[0n]bp	CSNB	USA, New Jersey, Beachwood	
H. cecropia	SNB 3237	SASNC1248-11	KM287195	658[0n]bp	CSNB	USA, Wisconsin, Portage Co.	
H. columbia nokomis (hybr. with cecropia?)	SNB 1859	SASNB764-10	KM287193	658[0n]bp	CSNB	Canada, Ontario, Mafeking	
H. cecropia	SNB 1869	SASNB774-10	HQ579819	658[0n]bp	CSNB	USA, Texas, Bexar Co., San Antonio	
Н. сесторіа Н. сесторіа	B3218-wn-B11	SAWNA022-09	GU703463	658[0n]bp	SMFL	Canada	
•			KM287183		CSNB		
H. cecropia	SNB 3228	SASNC1239-11	KM287193	658[0n]bp	CSNB	USA, New Jersey, Beachwood	
H. cecropia	SNB 1867	SASNB772-10		658[0n]bp		Canada, Ontario, Haliburton Highlands	
H. cecropia H. leonis PT	SNB 1871	SASNB776-10	KM287190	658[0n]bp	CSNB CSNB	USA, Colorado, Denver	
	SNB 5189	SASNC2725-13	KM995811	658[0n]bp	IBUNAM	Mexico, Nuevo León	
H. leonis HT	SNB 5191	SASNC2727-13	KM995807	658[0n]bp		Mexico, Nuevo León	
H. leonis PT	SNB 5192	SASNC2728-13	KM995809	658[0n]bp	CSNB	Mexico, Nuevo León	
H. leonis PT	SNB 5190	SASNC2726-13 SASNB694-09	KM995812	658[0n]bp	CSNB	Mexico, Nuevo León	
H. euryalus	SNB 1694		GU703001	658[0n]bp	CSNB	USA, California, San Diego, vic. Escondido	
H. euryalus H. euryalus	B3218-wn-C02 B3218-wn-C01	SAWNA024-09 SAWNA023-09	GU703536 GU703535	658[0n]bp 658[0n]bp	SMFL SMFL	USA, California, Monterey USA, Washington, Chelan Co., vic. Leavenworth	
H. euryalus kasloensis	CND 1606	SASNID (O) (O)	C11702000	(5050m llam	CCNID		
	SNB 1696	SASNB696-09	GU703000	658[0n]bp	CSNB	Canada, Brit. Columbia, Okanagan Valley	
H. euryalus H. euryalus	SNB 1695	SASNB695-09	GU703002 KM287188	658[0n]bp	CSNB CSNB	USA, California, Nevada Co. USA, California, Nevada Co.	
	SNB 1858	SASNB763-10				, ,	
H. "gloveri b"	B3218-wn-C03	SAWNA025-09	GU703533	658[0n]bp	SMFL	USA, Utah, Box Elder Co.	
H. "gloveri b" H. "gloveri b"	B3218-wn-C04 SNB 1864	SAWNA026-09	GU703534 KM287189	658[0n]bp	SMFL CSNB	USA, [reared, no data] USA, Utah	
		SASNB769-10		L 21		<u>'</u>	
H. mexicana PT	SNB 1686	SASNB686-09	GU703009	658[0n]bp	CSNB	Mexico, Zacatecas, Tlaltenango de Sánchez	
H. mexicana PT	B3218-wn-B07	SAWNA018-09	GU703460	658[0n]bp	SMFL	Mexico, Guanajuato, Sierra de Santa Rosa	
H. mexicana HT	B3218-wn-B08	SAWNA019-09	GU703461	658[0n]bp	IBUNAM	Mexico, Zacatecas, La Manchada	
H. mexicana PT H. mexicana PT	B3218-wn-B09 BC-Roug1230	SAWNA020-09 SATWB181-11	GU703462 KM287191	658[0n]bp 633[0n]bp	UAG	Mexico, Zacatecas, La Manchada  Mexico, Zacatecas, 'dirt road' Momax to San Lovonzo, ofter San Lovonzo	
H. mexicana PT	BC-Her2360	SDHC360-08	_	658[0n]bp	CDHP	Lorenzo, after San Lorenzo  Mexico, Zacatecas, 'dirt road' Momax to San Lorenzo, after San Lorenzo	
C. promethea	SNB 1856	SASNB761-10	HQ579813	658[0n]bp	CSNB	Canada, Quebec	
•			-				
C. angulifera	SNB 1853	SASNB758-10	HQ579812	658[0n]bp	CSNB	USA, Pennsylvania	
C. securifera	SNB 1852	SASNB757-10	KM287187	633[1n]bp	CSNB	USA, Florida, Lake Co.	

## Description and diagnosis

Generally, a medium-sized to large species within the genus. As given in detail below, it is unique in the genus by the combination of its carmine red ground colour; the relatively wide, white ante- and postmedian lines and marginal area; the small, somewhat rounded forewing ocellular patches in combination with drop-like patches of the hindwing; the huge blue portion of the subapical ocellus; the striking white tufts on both male and female abdomen; and in male genitalia the shape of the valves, especially the ventral part, which appears to be diagnostic.

Colour and wing pattern. Ground colour on dorsal side intensive carmine red, very similar to fresh specimens of *H. columbia gloveri*. Antennae black. Head, thorax and segments of abdomen in ground colour, between head and thorax a wide white collar, thorax and abdomen separated by a wide band of long white hair, and whole abdomen with wide intersegmental white tufts, most intense for the genus.

Forewing in ground colour, with broad white antemedian and postmedian line, both bordered black to median area, in 2 specimens these black parts are even confluent. The forewing ocellular patch round and elongated, with short tip to the wing margin, white with yellowish and black margin. Postmedian area greyish, suffused with some carmine scales, in the apical parts more violet, in marginal parts becoming ochreous. Marginal area separated from this part by a thin greyish black line with rounded indentions between the veins, of very light creamy white colour with darkened outer margin. The round subapical ocellus black with wide halfmoon (widest for the genus), consisting of blue scales.

Hindwing of same colouration as forewing, but basal area completely white. Hindwing discoidal patch larger than on forewing, of typical drop-like form. Postmedian area of same colour as on forewing, the ochreous outer portion has a row of grey patches and is separated from the outer margin by an intensive greyish black line.

On ventral side almost same ornamentation, but antemedian line of both fore- and hindwing missing. Both fore- and hindwing are strongly suffused with white and black

scales, only median area of the forewing with somewhat carmine character, and give that ventral side a very dark colouration. Thorax with legs and abdomen in ground colour, but abdomen with a longitudinal row of white hair in the ventral center.

d genitalia (Figs. 8a-8f): As expected, very similar to the other species of the genus; a safe determination via genitalia requires direct comparison and more than one specimen per species. Generally perhaps more delicate in comparison to the larger species as, e.g., H. mexicana. The bifid uncus distally with narrow, finger-like processes, less curved ventrally and less triangular or claw-like at the very tip than in most other species. The phallus is somehow more delicate and slightly bent, not straight. Perhaps the best differentiating character appears to be the shape of the valves: sacculus (with a very big fold to inner side) rather long, straight and ending in a nearly rectangular, sharp bend to the distal edge of the valve, which again is rather long and runs quite straight up to the finger-like process (harpe), which forms together with the dorsal part of the valve a rather narrow indention with parallel edges. Dorsal part of the valves in relation to ventral part smaller than in most other Hyalophora.

Q (Figs. 4a-4b): Measurements, 2 QQ paratypes: fwl. 68.5 and 65.0 mm, fw. ocellular patch largest diameter 7.0 mm (diagonally to wing veins) and 10 mm (parallel to veins), subapical ocellus largest diameter 6.5 and 6.0 mm, hwl. 51 and 49 mm, hw. eyespot largest diameter 13 mm (n=1, parallel to veins), antenna with 29 segments, quadripectinate to its tip, ca. 14.0 mm (second Q without antennas) long, longest rami ca. 1.8 mm.

Aside from typical sexual dimorphic characters, the Q of H. leonis sp. n. shares all characters of the  $\partial \mathcal{J}$ . It is a little larger than  $\partial \mathcal{J}$ , has more rounded wings, rounded eyespots on all wings, and a larger abdomen, and the last abdominal segment is covered with greyish white tufts; the white tufts on the abdomen are generally more intensive than in males.

**Q** genitalia not studied.

Preimaginal instars, ecology and larval foodplants: There is no knowledge about the preimaginal instars and larval foodplants of *H. leonis* sp. n. At the collecting sites in Nuevo León (Galeana and Laguna de Sanchez, both at altitudes of around 2000 m), there are semi-dry pine and oak forests with pecan trees (*Carya* sp., Juglandaceae); potential larval foodplants of the genera *Arbutus* (e.g., *A. xalapensis*, Ericaceae) and *Ceanothus* (e.g., *C. caeroleus*, *C. buxifolius*, Rhamnaceae), which are used by other *Hyalophora* species, can be found widely among the other plants. Most rain is observed normally between late May and October, temparatures do not fall under –3°C during winter time (H. Arellano Garcia, pers. comm.).

The collecting site in Coahuila, almost at same altitude as in Nuevo León, is an open pine forest (*Pinus cembroides* Zucc., Pinaceae) mixed with other elements such as *Juniperus* (Cupressaceae), *Quercus* sp. (Fagaceae), *Yucca* 

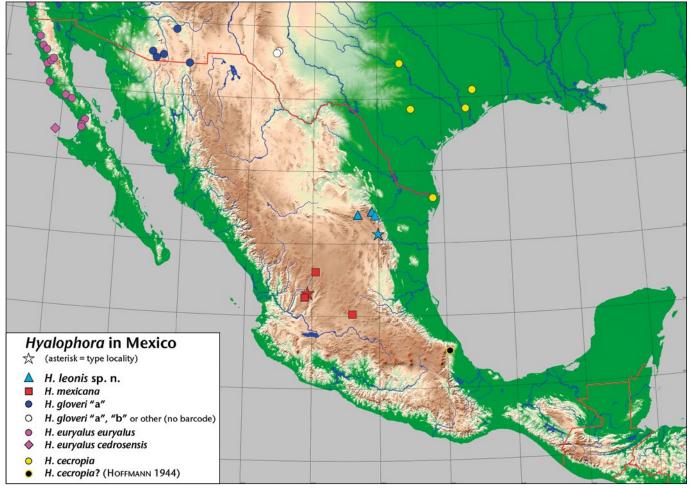


Fig. 1 (Map): Distribution of Hyalophora leonis sp. n. and related species of Hyalophora in Mexico and bordering states of the USA (species codes see insertion). One dot may represent more than one locality if in close proximity; we have not located every label or published data on the map. Many more or less inexact records of (usually) "H. columbia gloveri" from northern Mexico and southern USA in general literature or old collection specimens have not been included or (when exact locality data was available) are represented as white dots, as they have not been identified respectively confirmed by barcode. — Map created with Map Creator 2.0 Personal Edition, © 2003–2007 primap software, modified and localities added (W.A.N.).

sp. and *Dasylition* sp. (both Liliaceae); the climate classification would also be arid to semiarid forest. The locality is found in an open canyon of limestone which leads from West to East (G.N.G., pers. obs.).

#### Discussion

Hyalophora leonis sp. n.

The new species *Hyalophora leonis* externally shows quite some similarity to the large specimens with large proportions of red of *H.* "gloveri a" from Arizona (USA) and surrounding areas, and probably also to other gloveri-like specimens with reddish parts of the wing upperside colouration, but is well differing from these in some morphological details and in the barcode (1.8% difference over sequence pairs between groups, see Tab. 2), and a bootstrap result of 94% in the barcode (NJ-tree, Fig. 2). Without doubt, the status of *H. leonis* as a valid species is not different from the other taxa in the genus presently considered to be separate species. — In this case, again the COI barcode proved helpful to see structures within the complex genus *Hyalophora*.

The Mexican Sierra Madre Oriental is separated from the central and western mountain ranges by desert-like landscape (the large Chihuahua desert and similar arid areas), and thus probably there is no genetic exchange between *H. leonis* and other Mexican species at present. The existence of the populations at rather high altitudes in the mountains suggests a refugial distribution from more humid times. For *H. cecropia* in the northeast, see discussion below.

Compared with the other species, the more western taxa (*H. euryalus* and *H. mexicana*) are rather similar to each other externally (very large proportion of red), while the easternmost species (*H. cecropia*, the Canadian *H. c. columbia* and *H. leonis* sp. n.) are more similar with the larger proportion of blackish ground colour, the latter two also by their carmine colouration.

The present description raises the number of described species of the genus *Hyalophora* to 5 species, without considering the identities of Strecker's taxon *gloveri*, *H.* "*gloveri* b" and possibly some other populations.

### Comments and notes about other Hyalophora

Especially for the northern populations in Canada and the USA, our research was restricted to a small number of specimens from a small number of populations of the different *Hyalophora* species. Recent, well-labelled mate-

Table 2: Estimates of divergence [in %] over sequence pairs between groups. The number of base substitutions per site from averaging over all sequence pairs between groups are shown. Standard error estimates are shown above the diagonal [in square brackets and italics]. Analyses were conducted using the Maximum Composite Likelihood model (TAMURA et al. 2004). The rate variation among sites was modelled with a gamma distribution (shape parameter = 3). The differences in the composition bias among sequences were considered in evolutionary comparisons (TAMURA & KUMAR 2002). The analysis involved 47 nucleotide sequences (= specimens) in groups of populations (= possibly species or subspecies) or species (3 species of Callosamia united in one group). There were a total of 658 positions (= base pairs) in the final dataset. Analyses were conducted in MEGA5 (TAMURA et al. 2011).

Groups/taxa		1	2	3	4	5	6	7	8	9
H. "gloveri a"	1		[0,003]	[0,003]	[0,005]	[0,005]	[0,006]	[0,006]	[0,014]	[0,005]
H. "columbia a"	2	0,007		[0,002]	[0,004]	[0,005]	[0,006]	[0,006]	[0,014]	[0,005]
H. columbia	3	0,009	0,004		[0,004]	[0,005]	[0,005]	[0,006]	[0,014]	[0,005]
H. cecropia	4	0,017	0,015	0,014		[0,004]	[0,005]	[0,006]	[0,014]	[0,004]
H. euryalus	5	0,019	0,017	0,016	0,015		[0,005]	[0,006]	[0,013]	[0,005]
H. "gloveri b"	6	0,023	0,021	0,017	0,016	0,017		[0,006]	[0,013]	[0,005]
H. mexicana	7	0,026	0,027	0,023	0,023	0,025	0,022		[0,013]	[0,005]
Callosamia	8	0,099	0,100	0,095	0,098	0,092	0,096	0,095		[0,014]
H. leonis sp. n.	9	0,018	0,017	0,016	0,014	0,017	0,017	0,017	0,102	

rial, collected in the wild (and not being the result of obscure rearing and hybridisation experiments of amateur breeders not indicated on the labels) and suitable for barcoding (or other DNA analysis), is generally rather rare in European collections. We present here new questions from our studies, and hope that colleagues in North America with better access to fresh specimens can solve these later.

# The complex of "H. columbia/gloveri (sensu lato)"

There are three populations of what usually is called "gloveri" and/or "columbia" more or less clearly separated by COI barcode (see the barcode tree in Fig. 2). We have provisionally named two of these populations "gloveri a" and "gloveri b" here, because they are so distinct from each other (disclaimer: all these provisional names are not created for the purpose of Zoological Nomenclature, ICZN 1999):

• H. "gloveri a" are the usually carmine red, only rarely dominantly grey specimens from southern Arizona (and likely New Mexico and also adjacent northern Mexico); the geographical limits of this population are not yet clear.

Most interestingly, this population links in quite well with two population groups presently subsumed under "*H. columbia columbia*" within the NJ graph (Fig. 2), showing only a groupwise difference between 0.7 and 0.9% to these (see Tab. 2).

Under the populations presently subsumed under "*H. columbia columbia*" there are two groups with some slight, but distinguishable differences in barcode. However, the differences between these populations (called here *columbia* and "*columbia* a") are only minor (only 0.4%, Tab. 2) and most likely do not require separate naming as subspecies.

 Some specimens from Colorado (vicinity of Colorado Springs) keyed out in the barcode tree together with some of the Canadian "true" columbia; they have a similar colouration as "gloveri b" from Utah, but are much smaller than these (provisionally named here "columbia a"; these had been called "columbia 'grey like gloveri" in Nässig et al. 2014). We think that these specimens are really southern columbia, and the synonymy of gloveri (to be precise, "gloveri a" only) and columbia in present literature appears to be fully justified based on these populations only.

Interestingly, two sampled specimens look very similar externally (sample-IDs no. SNB 1859 and SNB 1866, see Table 1); but one of these clearly keyed out in the mtDNA barcode with *H. columbia*, the other one with *H. cecropia*. These might possibly represent hybrids between these two species (which would also explain their blackish colouration in the median field with a clear reddish shade along the outside of the postmedian white line), but with different sexes of the ancestors.

The following population appears to be quite separate from the others and shows a much larger difference (between 1.6 and 2.3% pairwise group difference, see Tab. 2, and a bootstrap support of 99%, see Fig. 2) towards all other *Hyalophora* studied — in this dissimilarity aspect second only to *H. mexicana*.

• *H.* "gloveri b" is a more greyish (especially distally of the postmedians), large form known to us from Utah only so far. Here a lot of further research is required, as we do not know much about this apparently well-isolated population. Its place within the barcode tree suggests that it requires a full species status as well, but under which name?

Anyway: Revealing the true identity of *gloveri* is the necessary basis for any further study and still requires a thorough critical study and comparison of the Strecker (1872) description and original types of *gloveri* (type locality as written by Strecker: "Arizona" — not too helpful now, over 140 years later) with the other populations. We suppose, based on Strecker's illustrations of his types of *gloveri* (see Nässig et al. 2014: fig. 5, scanned from the original printed plate), that either the more northern "*gloveri* b" or the Colorado form of "*columbia* a" might be the true *gloveri* Strecker, 1872, but perhaps not the southern, dominantly carmine red "*gloveri* a". At

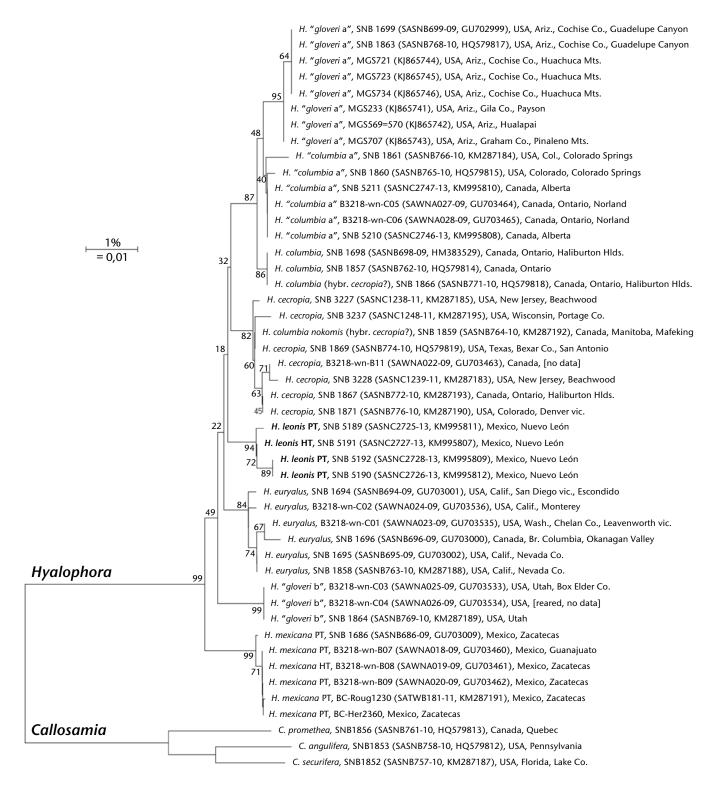


Fig. 2. The tree of *Hyalophora* taxa was inferred using the Neighbour-Joining method (SAITOU & NEI 1987). The optimal tree with the sum of branch length = 0.20221233 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2500 replicates) are shown next to the branches (FELSENSTEIN 1985). Evolutionary distances were computed using the Maximum Composite Likelihood method (TAMURA et al. 2004). The rate variation among sites was modeled with a gamma distribution (shape parameter = 3). The differences in the composition bias among sequences were considered in evolutionary comparisons (TAMURA & KUMAR 2002). The analysis involved 47 nucleotide sequences (= specimens, see Tab. 1). There were a total of 653 positions in the final dataset. Analyses were conducted in MEGA5 (TAMURA et al. 2011).

least one of Strecker's syntypes is deposited in the Field Museum, Chicago (see under "Search: Lepidoptera" 2013), and should be studied in detail; possibly other syntypes might be deposited there as well.

BOYD & BOYD (2012) described and illustrated a "unique larval phenotype" of "Hyalophora columbia gloveri" (sensu lato). It was discovered within the Spring Moun-

tain Range in Clark County, Nevada. All three larvae observed by these authors were found crawling on the ground looking for a pupation site. Many saturniid caterpillars in prepupal stage exhibit a colour different from the usual colours of feeding larvae caused by hormonal changes during prepupal development and partial loss of the waxy cover of the cuticle during this stage which might be responsible for "strange" larval colours.

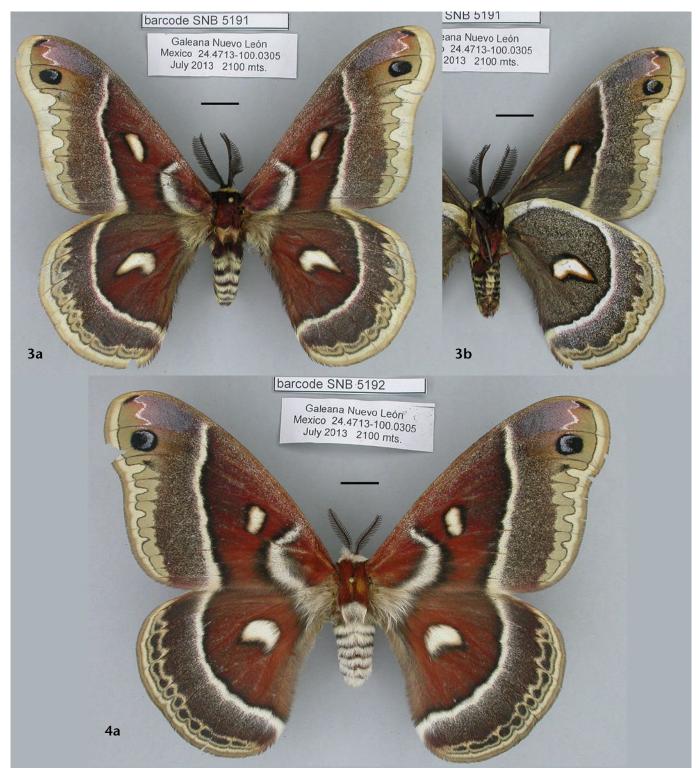


Plate 1, Figs. 3—4a: Specimens of *Hyalophora leonis* sp. n.; a = uppersides, b = undersides. Figs. 3a, b: *H. leonis*, HT ♂. Fig. 4a: *H. leonis*, PT ♀. — Scale bars = 1 cm; *i.e.*, approximately natural size.

However, according to Collins (pers. comm.), the scoli colour of *Hyalophora* caterpillars rarely changes during prepupal stage. Another possible interpretation might be shown by Collins (1999); he documented a hostplant-induced larval polyphenism in *H. euryalus*.

On the other side, when there is indeed more than one species presently included within this complex of "*Hyalophora columbia gloveri* (*sensu lato*)" as indicated above, this "aberrant colouration" observed by BOYD & BOYD might just as well represent the normal colouration of

one of these other populations as a another possible explanation. Anyway, the difference is not too large compared to the picture shown by Collins (1997: fig. 1a, as *Hyalophora "kasloensis"* from Montana). Perhaps barcode analyses of the two specimens which hatched from the three larvae and of the adult moth found by Boyd & Boyd might solve this question most easily.

In a recent publication Collins (2013) figures 3 specimens of 4 columbia gloveri (sensu lato) from Inyo and Mono counties and an intergrade specimen (natural



Plate 2, Figs. 4b–7: Specimens of *Hyalophora leonis* sp. n.; a = uppersides, b = undersides. Fig. 4b: *H. leonis*, PT ♀. Fig. 5a, 5b: *H. leonis*, PT ♂. Fig. 6a, 6b: *H. leonis*, PT ♂. Fig. 7: *H. leonis*, PT ♂. upperside, without scale (specimen greasy). — Scale bars = 1 cm; *i.e.*, smaller than natural size (approx. half natural size) and not to the same scale as in the first plate. — All specimen photos S.N. — Figs. 8a—8f: *H. leonis*, PT ♂ genitalia, GP CWAN/SMFL 2399/14, Laguna de Sanchez; photos taken in 70% ethanol, unflattened, without distortion; phot. W.A.N. Figs. 8a—b: genitalia with phallus removed; 8a: caudoventral view; 8b: lateral view. Figs. 8c—f: phallus; 8c: dorsal view; 8d: ventral; 8e: right lateral; 8f: left lateral view. — Scale bar = 5 mm (for all genitalia pictures).

hybrid of *H. columbia gloveri* with *H. euryalus*), also from Mono County, all eastern California near the border to Nevada. Also for those specimens barcode results would be interesting.

• Those further *Hyalophora* specimens from NE Mexico (Sierra Madre Oriental) recently received and described here above as *H. leonis* sp. n. clearly do not belong to the recently described *H. mexicana*, nor do they belong to any other population which we have studied. In contrast, they show a groupwise difference to all other *Hyalophora* between 1.4 and 1.8% (see Table 2) and also have a high bootstrap support of 94% in the NJ tree (Fig. 2).

The recent discovery of this population in the eastern federal states of Nuevo León and Coahuila raises the question whether the *Hyalophora* listed by Hoffmann (1942) from Xalapa (Veracruz) may not just as well be a misidentified member of this population (see below).

# The Veracruz record of "Hyalophora cecropia"

The status of the *Hyalophora* population reported by Hoffmann (1942) from Mexico: Veracruz, Jalapa (recent spelling: Xalapa, see black dot in map) was not resolved by us due to lack of material; the cited specimen was not found in Hoffmann's collection today deposited in IBUNAM, Ciudad México, during a search by G.N.G., and we did not see any recent material from Veracruz. If it is

not just a misidentification of an unidentified population of "H. gloveri (sensu lato)" (compare Tuskes et al. 1996), or the result of an artificial introduction of H. cecropia as indicated by Hoffmann (1942), or a misidentified Veracruz record of the northeastern Mexican H. leonis sp. n. as indicated above, it may alternatively reflect a continuous distribution of H. cecropia through the lowlands and/or lower slopes of the Sierra Madre Oriental along the Caribbean (eastern) coastline of Mexico – perhaps only temporarily or formerly? The presently closest recorded locality is Brownsville in Texas at the Rio Grande borderline between the USA and Mexico (Fer-GUSON 1972: 247). This is, in a bent line along the Caribbean coastline, approximately 750 km from Xalapa in Veracruz state. Beutelspacher Baigts & Balcázar-Lara (1994: 19) suppose that H. cecropia also occurs in the Mexican state of Tamaulipas (close to the Texas border), but records from there have evidently not been published so far. The apparent average rarity and often low population densities of at least H. cecropia (compare, e.g., Tuskes et al. 1996: 204) might be responsible for the lack of further observations. Ecologically, H. cecropia, being primarily a species of warm-temperate clima, might not really be expected today in these subtropical areas along the Caribbean coastline (Collins, pers. comm.).

## Notes on Hyalophora euryalus

Except specimens in the hybrid zones with H. colombia (sensu lato) (e.g., kasloensis), H. euryalus appears to be morphologically rather homogeneous across its range, except some differences in size (on average smaller in the North). However, the isolated insular population from Cedros Isl. west of the Californian Peninsula appears to be well distinguished and apparently requires at least (based on morphology of imagines and larvae) a status as separate subspecies (Smith & Wells 1993, Beutelspacher BAIGTS & BALCÁZAR-LARA 1994). On the other side, Cedros Isl. is not far away from Baja California peninsula; the direct distance is only about 25 km, and the small Isla Natividad might be used as a stepstone in between, which all would allow some genetic interchange over longer periods, especially at times of lower sea water levels during the glaciation periods. A mtDNA barcode analysis (or any other DNA study) of H. euryalus cedrosensis Cockerell [in Packard], 1914 and comparison with H. e. euryalus appears to be lacking so far.

The population of *H. euryalus* at least of Baja California Norte (Mexico) appears to be genetically not different from southern Californian (US) specimens, based on COI barcode data from Baja California Norte provided by J. Fetzner and J. Rawlins for comparison, which shows an identical sequence to our specimen from San Diego (SNB 1694), but is not included here in the present graph.

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