



Morphology and DNA barcoding reveal three cryptic species within the *Xylophanes neoptolemus* and *loelia* species-groups (Lepidoptera: Sphingidae)

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Abstract

Two species complexes within the genus *Xylophanes* are addressed using a combination of morphological study and analysis of DNA barcode sequences. The existence of two and three cryptic species respectively within the *X. loelia* and *X. neoptolemus* complexes is revealed following consideration of both adult habitus and genital morphology, and the results of a phylogenetic analysis of partial COI sequences—DNA barcodes—for 38 specimens. The taxonomic status of the available names is discussed and to clarify and stabilize the confused nomenclature of this group, a neotype for *Sphinx neoptolemus* Cramer, 1780, and lectotypes for *Choerocampa loelia* Druce, 1878 and *Chaerocampa trilineata* Walker, [1865], are designated. We describe three new species: *X. lolita* n. sp. Vaglia and Haxaire; *X. balcazari* n. sp. Haxaire and Vaglia; and *X. cthulhu* n. sp. Haxaire and Vaglia. The first is endemic to southeastern Brazil and closely allied to *X. loelia*; the second two are relatives of *X. neoptolemus*, of which the first is known only from Guerrero and Michoacán states in Mexico while the second is widely distributed in lowland forests of Central America.

Key words: COI, DNA barcodes, new species, South America, Central America

Introduction

Xylophanes Hübner, [1819] is the most speciose genus of the family Sphingidae (hawkmoths or sphinx moths), with 96 valid species and subspecies names listed in the most recent checklist (Kitching & Cadiou 2000). The present study focuses on a species complex that comprises taxa generally treated as *X. neoptolemus* (Cramer, 1780) and *X. loelia* (Druce, 1878) and represents a first step toward the elucidation of a larger complex that also includes *X. libya* (Druce) and *X. pearsoni* Soares & Motta. In their current sense, both *X. neoptolemus* and *X. loelia* are relatively common species widely distributed across Central and South America. However, it has become apparent, from variation in wingspan and subtle differences in wing pattern and genital structures that these names refer to a complex comprising more than two species. These initial observations are further examined and presented in this paper, and used in association with genetic data to distinguish and describe three new species within this complex. The DNA sequences used are part of a larger project that is assembling DNA barcodes—a part of the COI mitochondrial gene—for all species of sphingids (see <http://www.lepbarcoding.org>). One of the key features of this global campaign is the strong involvement of expert taxonomists and the integration of genetic data within a traditional taxonomic approach. The associ-

ation of taxonomic expertise with DNA barcoding results in an efficient and reliable identification tool applicable to any life stage. Simultaneously, the taxonomists benefit from an additional dataset by which to explore and describe diversity. This latter point is of special relevance to the discovery and characterization of cryptic species, whose general importance in biodiversity studies has recently received renewed emphasis (Bickford *et al.* 2007; Pfenninger & Schwenk 2007). DNA barcoding has already been used to uncover cryptic diversity in sphingid moths (Hajibabaei *et al.* 2006), but here the authors did not propose the formal description of any new species, reserving the task to expert taxonomists who would combine a traditional taxonomic approach with genetic results.

Abbreviations

Institutions

ZSM	Zoologische Staatssammlung München, Germany
BMNH	The Natural History Museum, London, UK
CNIN	Colección Nacional de Insectos, Universidad Nacional Autónoma de México, México City, Mexico
MNHN	Muséum national d'Histoire naturelle, Paris, France
RMCA	Royal Museum for Central Africa, Tervuren, Belgium
ZMA	Zoologisch Museum Amsterdam, The Netherlands
NNMN	Nationaal Natuurhistorisch Museum Naturalis, Leiden, The Netherlands
ZIRAS	Zoological Institute of the Russian Academy of Sciences, Saint-Petersburg, Russia
IM	Insectarium de Montréal, Montréal, Canada

Authors

JH	Jean Haxaire
TV	Thierry Vaglia

Material and methods

Morphological study

A total of 138 specimens in the collections of TV and JH formed the basis of this study, including 64 specimens of *X. loelia* from across the distribution range of this species. Of these 64, two individuals from Brazil (Pote, Minas Gerais), though closely resembling *X. loelia* in habitus, displayed an atypical wingspan and a slightly different wing shape. Seventy-four specimens of *X. neoptolemus* were also examined, including specimens that had been separated into three preliminary groups based on subtle differences in wing pattern and shape. For both species, an extensive survey of the variation in male genitalia was undertaken, with 63 specimens being dissected (28 *X. loelia*, 35 *X. neoptolemus*).

We examined the type specimens of *Choerocampa loelia* Druce, 1878, and *Choerocampa trilineata* Walker, [1865], preserved in the BMNH, and the type of *Xylophanes heinrichi* Closs, 1917, preserved in the ZSM. *C. trilineata* and *X. heinrichi* were listed by Kitching and Cadiou (2000) as junior subjective synonyms of *X. neoptolemus* and *X. loelia* respectively, and we here confirm those synonymies. We failed to locate the type material of *Sphinx neoptolemus* in any of those major institutions known potentially to have types of species described by Cramer in their collections (MNHN, BMNH, RMCA, ZMA, NNMN). Thus, the only evidence available relating to the type(s) of *X. neoptolemus* is the short original description and the accompanying painting (Cramer, 1780: plate 301, fig. F). The plates in the various published copies of Cramer

(1780) were copied by various artists from original paintings (sometimes referred to as the “pattern plates”, preserved in the Entomology Library of the BMNH) and although they are often somewhat stylized, the species represented are often clearly identifiable (Vane-Wright 1975; Chainey 2005). Nevertheless, the original paintings should always be consulted when making particularly critical determinations, as it is expected that these would generally be better matches for the type specimens than any of the published versions (Chainey 2005). We reproduce here in Fig. 1b the original painting of *X. neoptolemus*. Unfortunately, it is too imprecise to assess the subtle interspecific differences in this species complex. The falcate apices of the wings are striking at first sight, and it is a useful character in this group; however, as most of the illustrations by Cramer (1780) show such exaggerated acute apices, it is reasonable to question the accuracy of the representation of this character, which is more likely to have been due to the artistic considerations of that time than an accurate illustration of a specific morphological feature. Moreover, the abdomen lacks the typical longitudinal dorsal bands found in the *neoptolemus* complex, and as such the painting is more reminiscent of the pattern seen in *X. loelia*. Finally, the type locality, “Suriname”, is insufficiently precise to assist in determining the identity of the illustrated specimen. Therefore, we judge the illustration (Fig. 1b) to be an inadequate representation of a reliable type specimen of *X. neoptolemus*, and we therefore consider it necessary to designate a neotype to clarify and stabilize the nomenclature of this group (see below).

We have studied the holotype of *Chaerocampa* [sic] *brasiliensis* Schaufuss, 1870, another junior synonym of *X. neoptolemus*. (Note that *Chaerocampa* is an incorrect subsequent spelling of *Choerocampa* Duponchel that was widely used by many nineteenth century entomologists.) It is most likely to be in the ZIRAS, the only institution reported to date as having a type of a sphingid species described by Schaufuss from the Kaden collection (Cadiou 1995), but preliminary searches have so far failed to discover it. The description of Schaufuss (1870) is not detailed enough to allow the safe assignment of that name to any of the taxa treated herein. Moreover, we consider the stated type locality of *C. brasiliensis*, “Br.” (for “Brasilia”; that is, Brazil the country rather than Brasilia the city), to be meaningless because several other species referred by Schaufuss (1870) as coming from “Brasilia” have geographical ranges that do not intersect with even the broadest possible interpretation of this locality. For example, Schaufuss reported ‘Brasilia’ as a locality for *Sphinx oestri* (i.e. *Manduca sexta caestri* (Blanchard), endemic to Chile) and *S. celeus* (i.e., *M. quinquemaculatus* (Haworth), a species distributed from Canada to Guatemala). *Chaerocampa brasiliensis* must therefore be considered a *species inquirenda* (a species of doubtful identity requiring further study). We choose not to designate a neotype of *C. brasiliensis* because intensive searching of the ZIRAS collections may yet uncover the holotype and we do not want to restrict future options. So as not to clutter the taxonomy and nomenclature with dubious species, we elect for now to follow Kitching and Cadiou (2000) and maintain *C. brasiliensis* as a junior synonym of *Xylophanes neoptolemus*.

The holotype of *Xylophanes trinitatis* Closs, 1917 was studied by Kitching and Cadiou (2000), who concluded that it was also a junior synonym of *X. neoptolemus*; the findings presented herein, as well as the type locality of *X. trinitatis* (Trinidad), strongly support this decision, and re-examination of this type beyond examination of a color photograph was deemed unnecessary for the present work.

The relatively confused nomenclatural situation in this group, together with the need to fix the identities of some of the available names, requires that, in addition to a neotype for *Sphinx neoptolemus*, lectotypes for *Chaerocampa loelia* and *C. trilineata* be designated (see below). These, together with the holotype of *X. heinrichi*, are illustrated in the present paper.

Genetic study

Tissue samples (dry legs) were collected from 37 specimens (dry mounted moths) held in the collections of JH and TV, comprising eight specimens of *Xylophanes loelia* and 29 of *X. neoptolemus*, and including the morphological variants expected to represent new species. Among the data gathered in the context of the global DNA barcoding campaign for sphingids, we selected and used the sequences of three closely related taxa

as outgroups: *X. libya*, *X. aglaor* (Boisduval) and *X. cyrene* (Druce). Details of each specimen are given in Table 1, and are also available within the projects ‘Sphingidae - Type specimens’ (code SPTY), ‘Sphingidae - Haxaire collection PUBLISHED records’ (code JHPUB) and ‘Sphingidae - Vaglia collection PUBLISHED records’ (code TVPUB) in the Published Projects section of the Barcode of Life Data systems (BOLD; Ratnasingham & Hebert 2007; www.barcodinglife.org). Information on specimen vouchers (images, field data, GPS coordinates) and sequences (nucleotide composition, trace files) are found in these projects by following the ‘view all records’ link and clicking on the ‘specimen page’ or ‘sequence page’ links for each individual record.

Tissue samples were processed at the Canadian Centre for DNA Barcoding (CCDB). DNA was extracted from dry legs using a routine silica-based 96-well extraction automation protocol (Ivanova *et al.* 2006). The 658bp region of COI proposed for use as a ‘DNA barcode’ (Hebert *et al.* 2003) was amplified with the primer set LepF1/LepR1 (Hebert *et al.* 2004). The DNA extracts that did not amplify for the full-length DNA barcode were individually selected, reprocessed and re-amplified with the LepF1/EnhLepR1 (Hajibabaei *et al.* 2006) primer pair, targeting a 612 bp fragment of COI. All PCR amplifications were performed according to the standard PCR reaction protocol used in CCDB (Hajibabaei *et al.* 2005). PCR products were checked on a 2% E-gel[®] 96 Agarose (Invitrogen). Unpurified PCR fragments were sequenced in both directions using LepF1, LepR1 or EnhLepR1 primers depending upon the one used in the PCR reaction. The sequencing reactions followed CCDB protocols (Hajibabaei *et al.* 2005), with products subsequently purified using Agencourt[®] CleanSEQ protocol (Agencourt, Beverly, MA, USA). The sequences were managed in SeqScape version 2.1.1 (Applied Biosystems, Foster City, CA, USA) and Sequencher 4.5 (Gene Code Corporation, Ann Arbor, MI, USA) and aligned using Bioedit version 7.0.5.3 (Hall 1999) and MEGA4 (Tamura *et al.* 2007). For specimens that did not amplify after this procedure, DNA extraction was re-done in isolated tubes using a commercial extraction kit (NucleoSpin[®] tissue kit, Macherey-Nagel, Düren, Germany) and following the kit protocol. The same sets of primers were used as well as the additional primer pairs LepF1/MLepR1 and MLepF1/LepR1 targeting shorter DNA fragments and usually successfully amplifying specimens whose DNA was degraded with a high success rate (Hajibabaei *et al.* 2006). Regularly updated protocols used at the CCDB can be found at: <http://www.dnabarcoding.ca/pa/ge/research/protocols>.

TABLE 1. Details of the 41 specimens used in the genetic analysis. L = sequence length; Dep. = depository collection (DH&WH = collection of Dan Janzen and Winnie Hallwachs, University of Pennsylvania); F = female; M = male. Alt. = altitude in meters. SampleID and ProcessID are unique identifiers referring respectively to the voucher specimen and sequence information on BOLD. Holotypes of the species described in the present paper and the designated neotype of *Xylophanes neoptolemus* are in bold.

Sample ID	L	Identification	Dep.	GenBank#	Sex	Date Coll.	Country, province	Alt.
BC-Hax0904	658	<i>Xylophanes aglaor</i>	JH	FJ026855	M	15-Feb-1994	Brazil, Santa Catarina	210
BC-Hax4325	609	<i>Xylophanes balcazari</i>	JH	FJ026858	M	15-Aug-1992	Mexico, Guerrero	500
BC-Hax4326	609	<i>Xylophanes balcazari</i>	JH	FJ026857	M	15-Aug-1992	Mexico, Guerrero	500
BC-Hax4327	609	<i>Xylophanes balcazari</i>	BMNH	FJ026856	M	15-Aug-1992	Mexico, Guerrero	500
BC-Hax0759	651	<i>Xylophanes balcazari</i>	CNIN	FJ026861	M	15-Aug-1992	Mexico, Guerrero	-
BC-Hax0763	658	<i>Xylophanes balcazari</i>	JH	FJ026860	M	15-Aug-1992	Mexico, Guerrero	-
BC-Hax0764	658	<i>Xylophanes balcazari</i>	JH	FJ026859	M	15-Aug-1992	Mexico, Guerrero	-
BC-Hax0765	609	<i>Xylophanes cthulhu</i>	JH	FJ026871	M	21-Aug-1992	Mexico, Chiapas	50
BC-Hax0766	658	<i>Xylophanes cthulhu</i>	JH	FJ026870	M	24-Aug-1992	Mexico, Veracruz	1350
BC-Hax0767	658	<i>Xylophanes cthulhu</i>	JH	FJ026869	M	24-Aug-1992	Mexico, Veracruz	1350
BC-Hax0768	658	<i>Xylophanes cthulhu</i>	JH	FJ026868	M	20-Aug-1992	Mexico, Chiapas	1600
BC-Hax0769	658	<i>Xylophanes cthulhu</i>	JH	FJ026867	M	19-Jul-1995	Honduras, Yoro	1500

.....continue

TABLE 1. (continued)

Sample ID	L	Identification	Dep.	GenBank#	Sex	Date Coll.	Country, province	Alt.
BC-Hax0770	658	<i>Xylophanes cthulhu</i>	JH	FJ026866	M	02-Aug-2000	Nicaragua, Granada	-
BC-Hax4322	658	<i>Xylophanes cthulhu</i>	JH	FJ026865	M	01-Jul-2004	Guatemala, Izabal	80
BC-Hax4323	658	<i>Xylophanes cthulhu</i>	JH	FJ026872	M	04-May-2005	Costa Rica, Guanacaste	800
BC-Hax4324	658	<i>Xylophanes cthulhu</i>	JH	FJ026864	M	21-Jul-2004	Guatemala, Izabal	80
BC-Hax4328	658	<i>Xylophanes cthulhu</i>	JH	FJ026873	M	21-Jul-2004	Guatemala, Izabal	80
BC-Hax4329	658	<i>Xylophanes cthulhu</i>	JH	FJ026863	M	16-Jun-2002	Guatemala, Huehuetenango	1900
BC-Hax4423	658	<i>Xylophanes cthulhu</i>	JH	FJ026862	M	07-May-2007	Guatemala, Suchitepéquez	1040
BC-Hax0861	658	<i>Xylophanes cyrene</i>	JH	FJ026874	M	07-Jun-2002	Guatemala, Izabal	1100
BC-Hax0777	658	<i>Xylophanes libya</i>	JH	FJ026875	M	07-Aug-2003	Mexico, San Luis Potosi	860
VAG-377	307	<i>Xylophanes loelia</i>	TV	FJ026881	M	18-Dec-2002	Paraguay, Alto Parana	270
VAG-378	658	<i>Xylophanes loelia</i>	TV	FJ026880	F	18-Dec-2002	Paraguay, Alto Parana	270
BC-Hax0780	609	<i>Xylophanes loelia</i>	JH	FJ026878	M	24-Jul-2000	French Guiana	-
BC-Hax0783	609	<i>Xylophanes loelia</i>	JH	FJ026876	M	01-Feb-1991	Ecuador, Cañar	600
04-SRNP-42273	658	<i>Xylophanes loelia</i>	DH, WH	DQ276753	F	08-Oct-2004	Costa Rica, Alajuela	430
BC-Hax0779	658	<i>Xylophanes loelia</i>	JH	FJ026879	M	05-May-1999	Ecuador, Napo	1000
BC-Hax0782	658	<i>Xylophanes loelia</i>	JH	FJ026877	M	05-May-1999	Ecuador, Napo	1000
VAG-375	609	<i>Xylophanes lolita</i>	IM	FJ026883	M	30-Nov-2004	Brazil, Minas Gerais	50
BC-Hax0781	658	<i>Xylophanes lolita</i>	JH	FJ026882	M	16-Oct-2004	Brazil, Minas Gerais	700
BC-Hax0758	658	<i>Xylophanes neoptolemus</i>	JH	FJ026888	M	01-Nov-1990	Venezuela, Bolívar	800
BC-Hax4318	307	<i>Xylophanes neoptolemus</i>	JH	FJ026884	M	05-Sep-1983	Venezuela, Aragua	-
BC-Hax4319	658	<i>Xylophanes neoptolemus</i>	JH	FJ026893	M	05-Sep-1983	Venezuela, Aragua	-
BC-Hax4320	658	<i>Xylophanes neoptolemus</i>	JH	FJ026892	M	06-Jul-1983	French Guiana	-
BC-Hax4340	658	<i>Xylophanes neoptolemus</i>	JH	FJ026890	M	04-Aug-1986	Venezuela, Tachira	400
BC-Hax4321	574	<i>Xylophanes neoptolemus</i>	JH	FJ026891	M	06-Aug-1983	Venezuela, Bolívar	-
BC-Hax0762	594	<i>Xylophanes neoptolemus</i>	JH	FJ026885	M	22-Nov-1990	Venezuela, Bolívar	700
BC-Hax0760	658	<i>Xylophanes neoptolemus</i>	JH	FJ026887	M	03-Oct-2003	Venezuela, Trujillo	800
BC-Hax0761	658	<i>Xylophanes neoptolemus</i>	JH	FJ026886	M	01-Jul-1993	French Guiana	10
BC-Hax4339	658	<i>Xylophanes neoptolemus</i>	BMNH	FJ026894	M	30-Aug-1983	Venezuela, Aragua	-
BC-Hax4341	658	<i>Xylophanes neoptolemus</i>	JH	FJ026889	M	12-Aug-1983	Venezuela, Bolívar	-

In addition, we included in our analyses a sequence of *Xylophanes loelia* from the study of Hajibabaei *et al.* (2006) in the context of the biodiversity inventory of Lepidoptera in the Area de Conservación Guanacaste, Costa Rica (<http://janzen.sas.upenn.edu>). This sequence and specimen data are accessible on BOLD in the published project ‘Sphingidae of ACG1’ (code MHASA); it was chosen among the 34 identical barcodes already available for this species from ACG. It should be noted that only a single specimen of *X. neoptolemus* from Costa Rica was included in the present work as no significant genetic variation has been observed within the 28 full length barcodes also available from the ACG survey (these sequences exhibit a maximum K2P distance of 0.6 %, as implemented in BOLD). References to DNA barcode records in BOLD are given throughout the text in the following format: SampleID/ProcessID (e.g. VAG-###/SPTVA###), where SampleID and ProcessID are unique identifiers linked to the voucher specimen and to DNA data respectively.

Phylogenetic analysis of DNA sequences and calculation of genetic distances

Barcode sequences were first explored using the Neighbor-Joining reconstruction method implemented in BOLD, and further analyzed in a phylogenetic context using NONA 2.0 (Goloboff 1999) run from within WinClada 1.00.08 (Nixon 2002). A heuristic search using a branch swapping algorithm was performed using the following parameters: hold 100000, hold/100, mult*500 followed by max*. Branch support was estimated using the bootstrap, Bremer support (Bremer 1994) and rescaled Bremer support (RBS; Goloboff & Farris, 2001). Bootstrap values were calculated with 1000 pseudoreplicates analyzed using the same set of heuristic parameters given above. Bremer support and RBS were calculated using the commands 'bSupport' and 'bSupport*' in NONA following suboptimal tree searches. Among the selected outgroup taxa, *Xylophanes cyrene* is the most distantly related species and was chosen as the primary outgroup to root the trees. Distance calculations were performed using the Kimura 2-parameter (K2P) algorithm in MEGA4 (Kimura 1980; Tamura *et al.* 2007), including all sites with the pairwise deletion option, and assuming both a homogeneous pattern of divergence among lineages and a uniform rate of substitutions among sites.

Results

Differential morphological patterns—taxonomic diagnosis

Both *Xylophanes loelia* and *X. neoptolemus* were found to encompass divergent lineages with consistent, though subtle, morphological differences in both the habitus and male genitalia. In particular, the two specimens of *X. loelia* from southeastern Brazil (Pote, Minas Gerais; Figs. 5a, 5b) differ from typical *X. loelia* (Figs. 6a, 6b) in wingspan and wing shape: they are significantly larger than all other specimens studied (including *X. neoptolemus*) and have somewhat more elongated and less falcate wings. They are also unique in having a larger black discal spot on the forewing upperside and in the basal area of the hindwing upperside being black rather than the dark brown of *X. loelia*. Also, the pale median band of the hindwing upperside lacks the pinkish coloration typical of *loelia*. A closer examination of wing pattern details shows that the position of the oblique postmedian lines of the forewings is also different between these Brazilian specimens and other representatives of *X. loelia*: the first four lines are evenly spaced in the former, as they are in *X. neoptolemus* *sensu lato*, whereas the second and third are closer to each other than they are to the first and fourth respectively in *X. loelia*.

The *X. neoptolemus* complex was divided into three groups based on morphological studies. These groups are clearly separated geographically: the first (group 1) occupies lowland rain forest areas in South America (Figs. 3a, 3b) from northwestern Venezuela to French Guiana; the second (group 2) is restricted to the dry areas of Guerrero and Michoacán states in Mexico (Figs. 2a, 2b); and the third (group 3) is widely distributed in Central America (Figs. 4a, 4b) from Panama to southern Mexico. A number of differences were found among these three populations. In particular, specimens of group 3 can be immediately differentiated from the others by their large size, more brightly colored general appearance (especially the red median band of the hindwing upperside), the strongly falcate apex to the forewings and, on the underside, by a striking golden transverse band crossing the median area of the forewing. In contrast, specimens of group 2 have shorter and more rounded wings, as well as a different, paler coloration; the red median band of their hindwing upperside is significantly broader than in specimens from other geographical origins. All moths in group 1 are characterized by a very distinct dashed postmedian line on forewing and hindwing underside, highlighted by black vein dots.

The genital morphology of the species within the *loelia/neoptolemus* complex, and to some extent, within the larger complex including *X. libya* and *X. pearsoni*, is highly conservative and homogeneous. Consistent differences between species do exist, but they are very slight and subtle, requiring an attentive and meticulous

comparative study based on a series of specimens to appreciate their value as species-specific differences rather than intraspecific inter-individual variations. The male genitalia of the two specimens from southeastern Brazil (Figs. 9a–c) are very different in general appearance from those observed in other *X. loelia* specimens (Figs. 8a–c). Their general aspect is closer to that of *X. neoptolemus* sensu lato, but they are unique in the complex in having a much more massive harpe (Fig. 9b). The uncus (Fig. 9a) is thicker than that of typical *X. loelia* (Fig. 8a) but almost as straight as in that species.

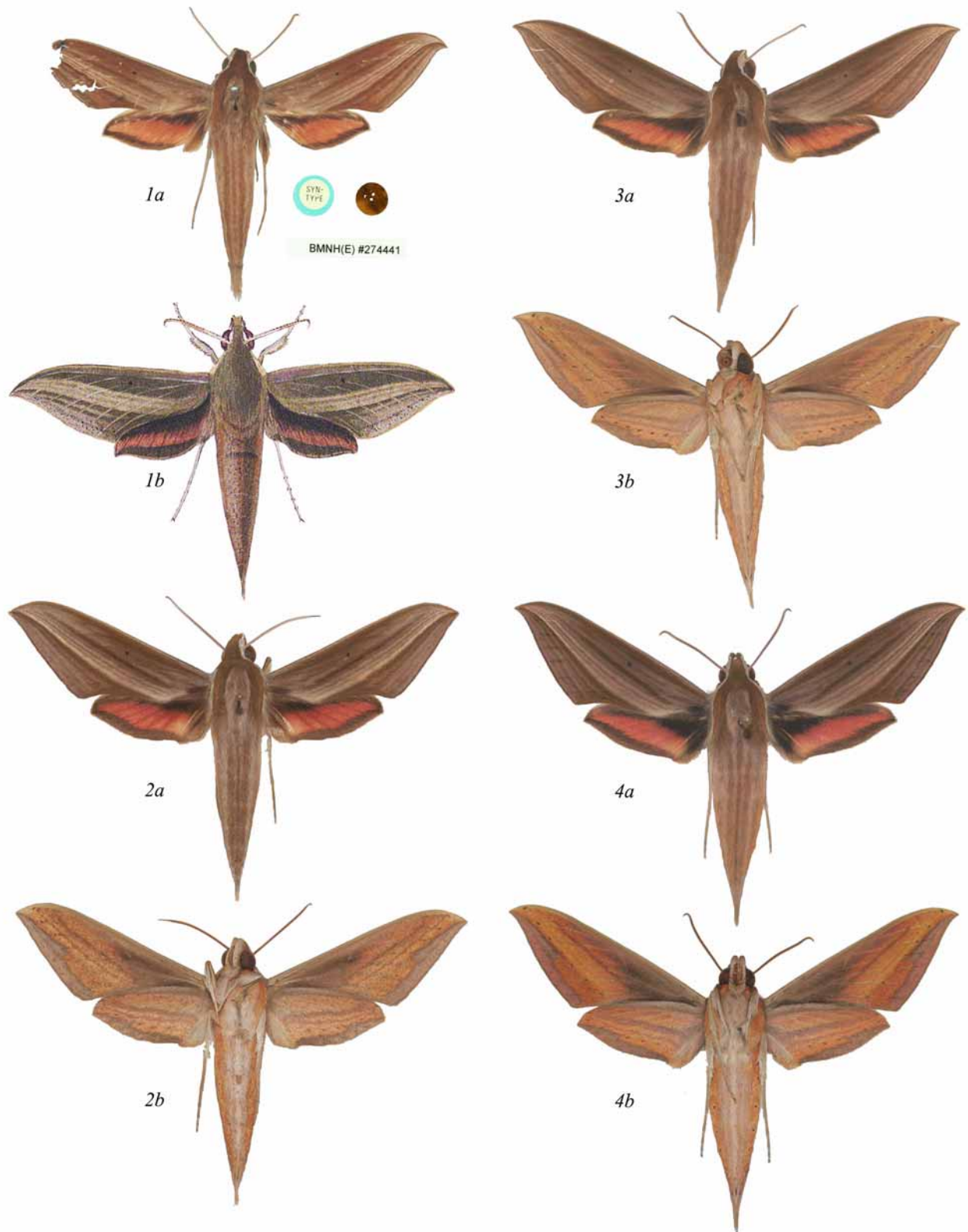
Within the *X. neoptolemus* complex, most differences concern the shape of the uncus and the harpe. The setigerous lobes of the uncus are more developed in specimens from Central America (group 2 and 3, as defined above; Figs. 11a, 12a), though less markedly in specimens from group 2. The latter have a slightly spatulate stout uncus apex (Fig. 11a), whereas it is thinner and strongly spatulate in group 1 (Fig. 10a). Except for the slight difference in the development of the setigerous lobes, the shape of the uncus is very stable across Central America (groups 2 and 3), though it is somewhat stouter and more bent ventrally in group 3 (Fig. 12a). The harpe is bent in specimens of groups 1 and 2, whereas in specimens of group 3 it is regularly curved and narrowing gradually to taper to a delicate and slightly bent apex (Fig. 12b). Some minute diagnostic features were also observed on the apical processes of the aedeagus; the right lobe is wider in specimens from group 2 and 3 (Figs. 11c, 12c), being especially stout in group 3. In this distinct population, the teeth of this lobe are long, evenly sized and mostly concentrated at the apex of the lobe (Fig. 12c). Specimens from group 2 show more unevenly distributed teeth (Fig. 11c).

Molecular divergence and species-level phylogeny

All of the 37 samples were eventually successfully sequenced using the different amplification procedures (see Material and Methods section), comprising 35 “full-length” barcodes (more than or very close to 600 bp) and two partial (307 bp) sequences from the LepF1/MlepR1 primer pair (see Table 1). Phylogenetic analyses were computed for both a dataset reduced to full-length sequences and for the complete dataset including the two short sequences, with alignment-gaps treated as missing data. We observed no deleterious effects of these short sequences on the final result. The dataset includes 64 parsimony informative characters (60 within the ingroup) and all observed substitutions are synonymous. The phylogenetic analysis resulted in nine most parsimonious trees, the strict consensus of which is shown in Fig. 13. *Xylophanes loelia* and *X. neoptolemus* are divided respectively in two and three strongly supported clades (bootstrap values ranging from 96 to 100%; Bremer support and RBS values ranging from 3 to 11 and 58 to 87 respectively). The genetic distances were calculated from the complete dataset; within and between clade distances are shown in Table 2. Intra-clade distances range from 0 to 0.2%, whereas inter-clade distances range from 2.6% (between the two *loelia* clades) to 6% (excluding outgroup taxa).

Discussion and species descriptions

The congruence in the discrimination of cryptic lineages by both morphological studies and phylogenetic analysis of the COI sequences is remarkable, and the perfect corroboration of the COI phylogeny by independent morphological features excludes any potential bias related to incomplete lineage sorting for this particular dataset. The *Xylophanes loelia/neoptolemus* complex thus appears to be a complex of five distinct species, which, though initially “cryptic”, are here clearly distinguished both morphologically and genetically. Taking the current nomenclatural and taxonomic status of the available names for this group into consideration, we describe below three new species and provide brief comments about the two previously valid names, *loelia* and *neoptolemus*, as well as their synonyms as listed by Kitching and Cadiou (2000).



FIGURES 1–4. 1a. *Chaerocampa trilineata* Walker, [1865], Lectotype, ♂, Venezuela; 1b. Watercolour of the original type of *Sphinx neoptolemus* Cramer, 1780; 2a and 2b. *Xylophanes balcazari* n. sp., Holotype, ♂, Mexico, Guerrero; 3a and 3b. *Sphinx neoptolemus*, Neotype, ♂, Venezuela, Aragua; 4a and 4b. *Xylophanes cthulhu* n. sp., Holotype, ♂, Guatemala, Izabal. (2a, 3a, 4a: dorsal view; 2b, 3b, 4b: ventral view).

TABLE 2. Mean Kimura 2-parameter distances (%) calculated within (shaded cells) and between each clade/species resulting from the analysis of the complete DNA barcode dataset. Standard error estimates are shown within brackets and were obtained by a bootstrap procedure (500 replicates) as implemented in MEGA4.

	<i>X. loelia</i> #1 (<i>X. lolita</i> n. sp.)	<i>X. loelia</i> #2	<i>X. neoptolemus</i> #2 (<i>X. balcazari</i> n. sp.)	<i>X. neoptolemus</i> #1	<i>X. neoptolemus</i> #3 (<i>X. cthulhu</i> n. sp.)
<i>X. loelia</i> #1 (<i>X. lolita</i> n. sp.)	0				
<i>X. loelia</i> #2	2.6 [0.6]	0.2 [0.1]			
<i>X. neoptolemus</i> #2 (<i>X. balcazari</i> n. sp.)	3.9 [0.9]	4 [0.9]	0		
<i>X. neoptolemus</i> #1	4.9 [0.9]	5.2 [0.9]	2.9 [0.7]	0.16 [0.11]	
<i>X. neoptolemus</i> #3 (<i>X. cthulhu</i> n. sp.)	5.3 [1]	6 [1]	3.9 [0.8]	3.1 [0.7]	0

Xylophanes loelia (Druce, 1878)

(Figs. 6a, 6b, 8a–c)

Xylophanes heinrichi Closs, 1917

Taxonomic Notes: *Xylophanes heinrichi* (Fig. 7a) was described by Closs (1917) from a single male collected by E. Christeller on 11.???.1912 (the month is unclear on the label) from “Amazonas”. This is an imprecise type locality, as it could refer to parts of Brazil, Venezuela, Colombia and even Peru. Of these, Brazil is perhaps somewhat more likely than the others but this is still not at all certain. However, regardless of where in “Amazonas” the moth was captured, the locality would be within the distribution of what we infer here to be *X. loelia*. The holotype, preserved in the ZSM, is illustrated in Fig. 7a. *X. heinrichi* is currently treated as a junior subjective synonym of *Xylophanes loelia*. *Choerocampa loelia* was described by Druce (1878) from an unstated number of specimens from Chiriquí, Panama, collected by Arcé. Only a single specimen with appropriate data and labels has been found in the BMNH and thus to stabilize the nomenclature, we hereby designate this specimen as the lectotype. The lectotype and its labels are illustrated in Figs 6a and 6b.

Distribution: This species is widely distributed in Central and South America. It is known to us from southern Brazil and Paraguay, north through Peru, Ecuador, French Guiana, Guyana, Venezuela, Trinidad and Colombia, and into Central America, through Costa Rica and Nicaragua, to Belize.

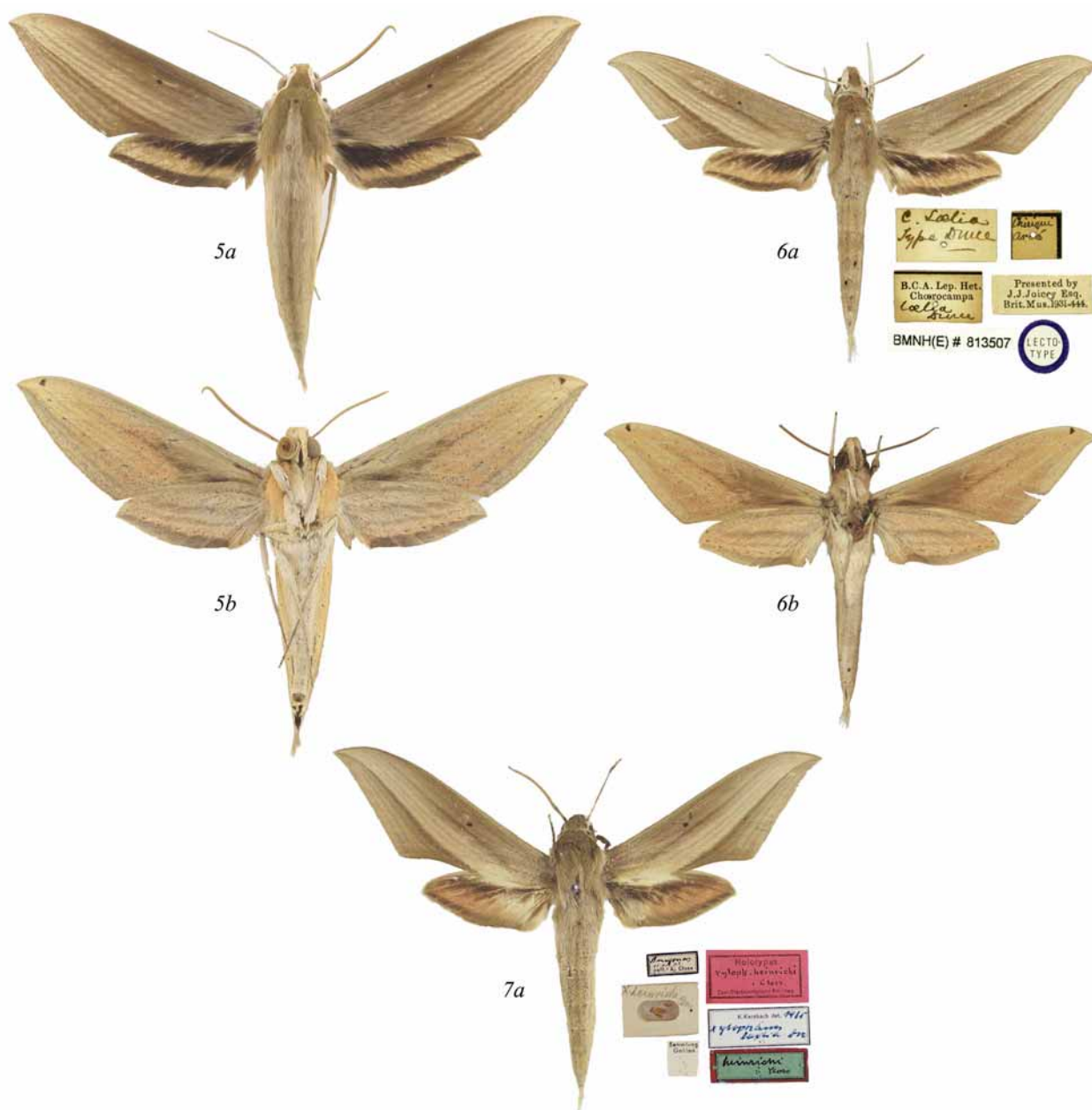
Genetic variation: Intraspecific variation for the part of COI we sequenced is very low (Table 2; Fig. 13, clade #2).

Xylophanes lolita Vaglia & Haxaire n. sp.

(Figs. 5a, 5b, 9a–c)

Type material: Holotype (Figs. 5a, 5b; in coll. TV, to be deposited in the Insectarium of Montréal, genital prep. #271104a, VAG-375/SPTVA739-07): ♂, Brazil, Minas Gerais, Pote, 540 m., 30.xi.2004, leg. Roney Alves dos Santos. Paratype (BC-Hax0781/SOWA788-06): 1 ♂, same data as holotype but 16.xi.2004.

Description: This species is described on the basis of combined evidence derived from morphology and genetic data (Fig. 13, clade #1; Table 2). Overall, it is very similar to *X. loelia* but is immediately distinguishable by its larger size, wing shape, and constant genital differences.



FIGURES 5–7. 5a and 5b. *Xylophanes lolita* n. sp., Holotype, ♂, Brazil, Minas Gerais; 6a and 6b. *Chaerocampa loelia* Druce, 1878, Lectotype, ♂, Panama, Chiriquí; 7a. *Xylophanes heinrichi* Closs, 1917, Holotype, ♂ “Amazonas”. (5a, 6a, 7a: dorsal view; 5b, 6b: ventral view).

Male (Figs. 5a, 5b). Head and body: Upperside of head, base of thorax and tegulae pale brown. Dorsal part of thorax and abdomen greyish-beige, with 11 longitudinal lines of unequal width; median thoracic line dark brown, thin, extending onto all abdominal segments, where it is bordered by a pair of thicker grey-brown lines, only slightly contrasting with the dorsal abdominal ground color. A pair of barely visible, black dots present immediately after these lines at the junction of each abdominal segment. Abdomen laterally with two pairs of alternately pale grey and brown lines; latero-ventrally orange-beige, with a pair of black dots on each segment; ventrally with five longitudinal lines, three cream coloured, interspaced with two pale brown lines. Thorax laterally orange-beige; ventrally pale brown, as are the legs and labial palpi. Forewing upperside: General coloration pale brown and beige. The wing is divided into four distinct areas. Basally pale brown, slightly greyish; costal margin, especially along the discal cell, deep brown. Median area pale beige, its distal

part becoming brown when approaching the costa, though less contrasted than the latter. Discal spot round and black. Full complement of six oblique postmedian and two submarginal lines present. First postmedian line brown, arising above the inner margin, a few millimeters from the wing base, straight, clearly defined, curving slightly toward the apex, but not reaching it (unlike the next three). Second postmedian line more diffuse and less contrasted. Third postmedian line almost as dark as the first, whereas the fourth is identical to the second, though thinner. These four lines parallel and evenly spaced. Fifth postmedian line wider and darker than others, followed by a somewhat similar, though narrower, sixth line. Between the fifth and sixth postmedian lines, the wing background color is slightly orange, especially close to the inner margin of the wing. First submarginal line diffuse and poorly defined; second submarginal line evanescent, only clearly visible near tornus. Fifth and sixth postmedian lines and both submarginal lines reach the apex. Hindwing upperside: Basal area dark brown, almost black. Median band wide, beige reaching the apex, with an irregular anterior margin and a smooth, clearly defined, posterior margin; Submarginal band dark brown (though less dark than the basal area) extending from tornus to apex. Forewing underside: Like the upperside, divided in four distinct areas: from base to median area, pale brown to grey-brown; median area orange brown; apical part beige; submarginal part of outer margin grey-brown. Postmedian and submarginal oblique lines, as described for the upperside, are apparent. First postmedian line dark brown, thick basally and narrowing toward costal margin, where it is reduced to a very thin trace. The next three postmedian lines grey-brown, contrasting little with the orange brown colour of this part of the wing; third postmedian line with a large black somewhat triangular spot between veins Rs_3 and Rs_4 ; fourth postmedian line with a small black vein dot on M_1 . Fifth postmedian line also grey-brown but wider than the preceding and bearing a row of distinct black vein dots on M_2 to CuA_2 . Sixth postmedian line mostly visible between apex and vein M_3 ; shortly beyond this vein, it disappears into the orange-beige ground coloration of the wing. First submarginal line clearly visible, almost as contrasted as the first postmedian line; the second submarginal line reduced to a suffusion of brown scales. Hindwing underside: Basally grey-beige, progressively becoming pinkish-orange towards the postmedian area. Median area crossed by two longitudinal pale brown lines, distal of which is an interrupted line with black vein dots. Submarginal band, from tornus to apex, dark brown though paler on the veins and towards the apex.

Female and pre-imaginal stages. Unknown.

Male genitalia (Figs. 9a–c). Overall, very similar to the other species of the complex. Uncus (Fig. 9a) relatively short, with well developed and setose lateral setigerous lobes; posterior third narrowed and down-curved, apex curved and spatulate. Harpe (Fig. 9b) short, basally very wide, bending and narrowing medially and becoming narrow and thinly upcurved apically; ventral part, especially basally, irregular and setose. Aedeagus (Fig. 9c), as in most *Xylophanes* species, with a transverse apical process bearing two distinct lobes; right lobe short, recurved, of even width only tapering at the apex, with about 20 tiny even teeth along its ventral and lateral margins; left lobe poorly developed, reduced to a plate following the left lateral margin of the aedeagus, thinly serrate with only few short scattered teeth from medially to the apex, where the serrations are slightly longer.

Distribution: This species is so far only known from Pote in Minas Gerais state, Brazil. It is likely to be restricted to this part of southeastern Brazil, which is known for its high level of endemism.

Genetic variation: The COI sequences for the two available specimens are identical.

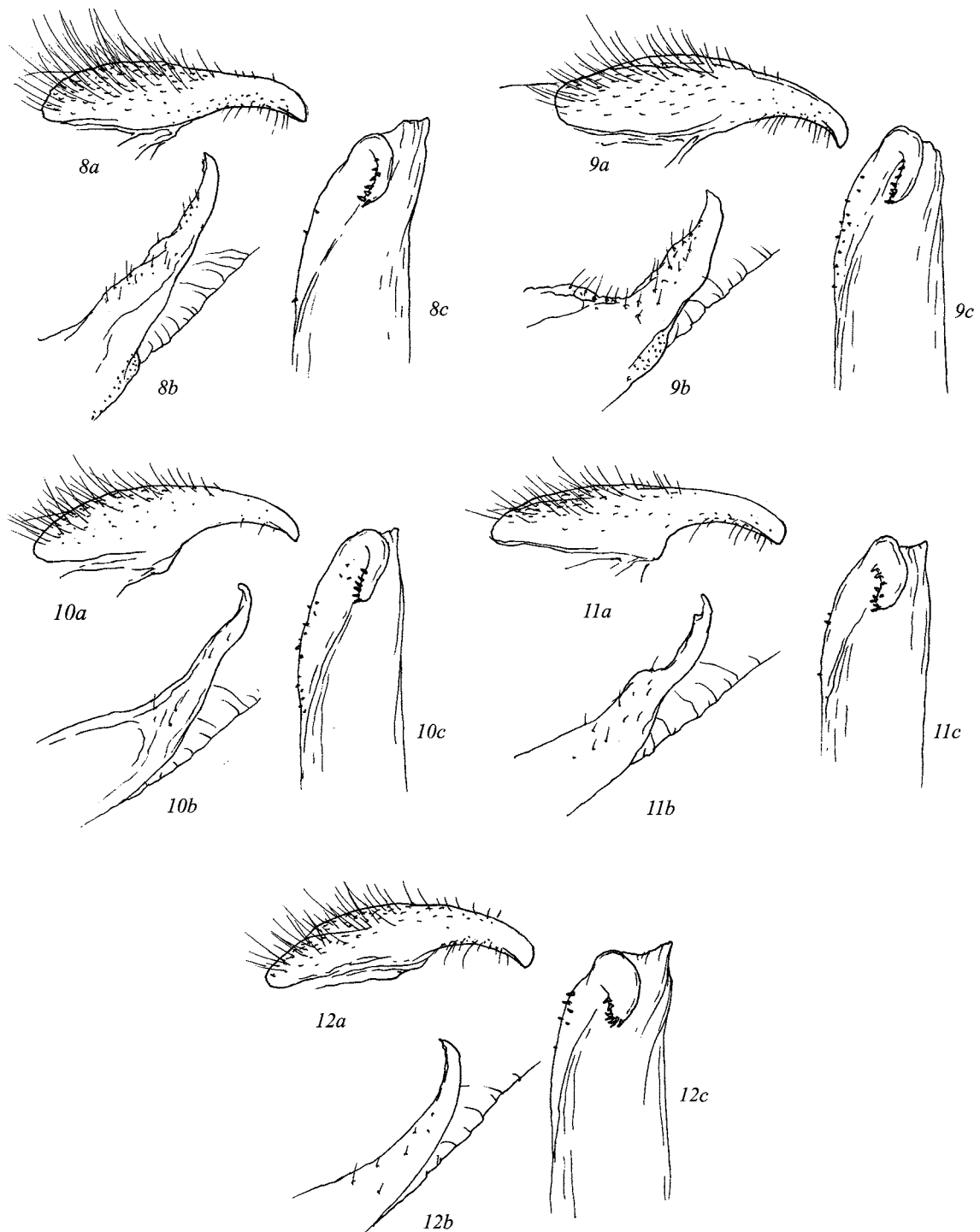
***Xylophanes neoptolemus* (Cramer, 1780)**

(Figs. 3a, 3b, 10a–c)

Chaerocampa trilineata (Walker, [1865])

Chaerocampa brasiliensis Schaufuss, 1870

Xylophanes trinitatis Closs, 1917



FIGURES 8–12. Drawings of diagnostic details of the male genitalia of *Xylophanes loelia* (Figs. 8a–c), *X. lolita* n. sp. (Figs. 9a–c), *X. neoptolemus* (Figs. 10a–c), *X. balcazari* n. sp. (Figs. 11a–c), and *X. cthulhu* n. sp. (Figs. 12a–c); a. uncus in lateral view, b. harpe, c. posterior end of the aedeagus.

Taxonomic Notes: As noted above, we failed to locate any type material for *Sphinx neoptolemus* in any of the major European museums that might have been expected to house it. Furthermore, the quality of the original painting is insufficient to determine the species that Cramer had before him. Although there is no taxonomic problem *per se* regarding *Sphinx neoptolemus* itself in “Suriname” (even if this term is used in its broader Eighteenth Century interpretation, which could include the West Indies), it may not represent the taxon we treat here as *Xylophanes neoptolemus* but another species of *Xylophanes* altogether. Thus, we do not consider

the painting to be useful as a representation of the type of *Sphinx neoptolemus*, although it is essentially consistent with our concept of this species. Therefore, with the express purpose of clarifying the taxonomic status of *Sphinx neoptolemus* and fixing the type locality, we hereby designate the following specimen as neotype (Figs. 3a, 3b; in coll. JH, to be deposited in the BMNH, BC-Hax4339/SOWE440-07): ♂, Venezuela, Aragua State, Maracay, station Rancho Grande, 30-31.viii.1983, leg. J. Haxaire & J.-Y. Rasplus. No specimens were available to us from Surinam and so we chose one from a locality that was as close as was practicable. Diagnostic features separating *Xylophanes neoptolemus* from its closest relatives, *X. balcazari* n. sp. and *X. cthulhu* n. sp., are given below in the descriptions of those two species and in the identification key.

Chaerocampa trilineata (Fig. 1a) was described from two specimens from “Venezuela” from the Dyson collection. In the original description, Walker ([1865]: 30) indicated that he had only males. However, we have located both specimens in the BMNH and found that one of the syntypes is actually a female. To stabilize the nomenclature, we hereby designate the male specimen (BMNH(E)#274441) as the lectotype. The lectotype and its original labels are illustrated in Fig. 1a. The pale blue-edged syntype label will be replaced with a purple-edged lectotype label. In addition to a pale blue-edged syntype label (which will be replaced with a pale blue-edged paralectotype label), circular locality label and a printed specimen register number label, the paralectotype female (BMNH(E)#274442) has a hand-written label stating “trilineata Wlk.” and a label comprising the words “CHÆROCAMPA TRILINEATA.” cut from a copy of Walker’s catalogue.

As noted above, we have been unable both to locate the holotype of *C. brasiliensis* and to determine the identity of the taxon. Therefore, we maintain *C. brasiliensis* as a synonym of *X. neoptolemus* pending discovery of type material.

Xylophanes trinitatis was described from a holotype male from Trinidad, deposited in the ZSM. We have examined a color photograph of the holotype and this, together with the type locality, confirms its synonymy with *X. neoptolemus*.

Distribution: Following the neotype designation above, our specimens from Venezuela and French Guiana represent *Xylophanes neoptolemus* (Fig. 13, group 1). Furthermore, our biogeographic results support the previous treatments of *X. trilineata* and *X. trinitatis* as junior subjective synonyms of *X. neoptolemus*. *X. neoptolemus* occurs in the lowland tropical rain forest of the northern part of South America. We know it from western Venezuela to French Guiana. In the latter country, it is only frequent in the so-called ‘zone 1’ region of St-Laurent and St-Jean-du-Maroni (Haxaire 1987), near the Surinam border. This moth becomes far less abundant southward. The furthest south it has been recorded is from the Jari Celulose S.A. landholding (Hawes 2005), between the Jari and Paru rivers, northwest of Monte Dourado in Pará state, Brazil, but it may not cross the Amazon river as it is not mentioned by Moss (1920) in his work on the sphingids of Belém.

Genetic variation: There is some diversity in the barcode region (Fig. 13, group 1) but intraspecific divergence is low, with the five different haplotypes differing from each other by only a single base pair.

***Xylophanes balcazari* Haxaire & Vaglia n. sp.**

(Figs. 2a, 2b, 11a–c)

Type material: Holotype (Figs. 2a, 2b; in coll. JH, to be deposited in CNIN; BC-Hax0759/SOWA766-06): ♂, Mexico, Guerrero, road from La Salitrera to Vallecitos de Zaragoza, km. 45, 500 m., 15.viii.1992, leg. D. Herbin & J. Haxaire. Paratypes (in coll. JH): 7 ♂♂ (5 with DNA Barcodes—BC-Hax0763/SOWA770-06, BC-Hax0764/SOWA771-06, BC-Hax4325/SOWE426-07, BC-Hax4326/SOWE427-07, BC-Hax4327/SOWE428-07), same data as holotype (one male to be deposited in the BMNH); 1 ♂ Mexico, Michoacán, road from Villa Victoria to Coalcoman, Los Laureles, 1538 m., 25.vi.2008, leg. J. Haxaire, O. Paquit & G. Nogueira; 4 ♂♂, Mexico, Michoacán, Coalcoman, Puerto La Zarzamora, Cerro El Laurel, 1635m., 26.vi.2008, leg. J. Haxaire, O. Paquit & G. Nogueira; 2 ♂♂, Mexico, Michoacán, Coalcoman, Puerto La

Zarzamora, Cerro El Laurel, 1635 m., 27.vi.2008, leg. J. Haxaire, O. Paquit & G. Nogueira; 2 ♂♂, Mexico, Michoacán, Coalcoman, Puerto La Zarzamora, Cerro El Laurel, 1635 m., 8.vii.2008, leg. J. Haxaire, O. Paquit & G. Nogueira.

Description: *Male* (Figs. 2a, 2b). Forewing length: 32 mm. Forewing upperside: General background color olive-beige. Discal spot small and black. Full complement of six oblique postmedian and two submarginal lines present, more or less easily distinguishable depending on specimen condition. First and fifth postmedian lines the most obvious, broader than the others. First postmedian line straight, stopping 2mm from costa, whereas the other lines all curve apically towards the wing apex. Area between first and fifth postmedian lines pale beige, crossed by the thin second, third and fourth postmedian lines; second postmedian line barely distinguishable except in its most apical part; third and fourth postmedian lines bend strongly beyond vein Rs_4 , becoming blurred toward the apex of the wing, although they do clearly reach it. The strongly marked fifth postmedian line is slightly sinuous, convex from inner margin to vein M_1 and then slightly concave from there to apex. Between this band and the next, the wing becomes reddish. Fifth and sixth postmedian lines 5 and 6 separate and parallel from about 1mm from the inner margin, but converging toward the apex and fused beyond Rs_4 . Submarginal area somewhat greyish, always dark; crossed by the barely visible, parallel first and second submarginal lines. Hindwing upperside: Basal area black, extending along inner margin toward tornus and merging into a dark brown band along the costa. Median area red, reaching neither tornus nor apex. Submarginal band brown, of almost equal width from tornus to apex. Tonal patch greyish, poorly contrasting. Forewing underside: Ground color reddish-yellow, with a thin suffusion of dark grey scales, giving the wing background a granular aspect. Basal area of the forewing grey-beige; first and fourth postmedian lines apparent; the first obvious, the fourth, though thinner, still distinct. Submarginal area pale grey, strongly dentate between M_2 and M_3 . Hindwing underside: Basal area whitish, extending along the costa; submarginal area pale gray, running from apex to vein 2A, where it merges into the basal area.

Female and pre-imaginal stages. Unknown.

Male genitalia (Figs. 11a–c). Uncus (Fig. 11a) relatively short and slightly produced dorsally; setigerous lobes distinct; distally somewhat quadrangular, its apex stout and weakly spatulate. Harpe (Fig. 11b) narrow, twisted and strongly bent medially; its apex is somewhat laminated and sclerotized, with an uneven internal margin possibly bent on itself or invaginated; internal margin bearing numerous setae with protruding bases, whereas the ventral side is only slightly setose. Right lobe of the apical process of the aedeagus (Fig. 11c) short and stout, dentate on its internal margin, with only a few, relatively long and unevenly distributed teeth present; left lobe poorly dentate, with very few and barely distinct small teeth.

Distribution: This new species is apparently restricted so far to Guerrero and Michoacán states in Mexico, but is likely also to be present in neighbouring states such as Colima and maybe Jalisco, where very similar and presumably favorable environments exist. It is remarkably isolated from the other Mexican representatives of the *X. neoptolemus* complex by large desert areas (especially in the Puebla state), and its biotope consists in forested areas with a strong Nearctic influence, clearly contrasting with Neotropical-like forests inhabited by *X. cthulhu* n. sp. (see below) in southern Mexico.

Genetic variation: The COI sequences for six specimens collected at the same locality in Guerrero state are all identical (Fig. 13 group 2).

Etymology: This species is dedicated to our colleague, Manuel A. Balcázar-Lara (University of Colima, Mexico), for his invaluable assistance to JH in his studies of Mexican sphingids.

***Xylophanes cthulhu* Haxaire & Vaglia n. sp.**

(Figs. 4a, 4b, 12a–c)

Type material: Holotype (Figs. 4a, 4b; deposited in coll. JH; BC-Hax4328/SOWE429-07): ♂, Guatemala, Iza-

bal department, track from Chocchoc to Cebol (Cevol), km. 2, Pueblo Cadenas, 76 m., 21.vii.2004, leg. O. Paquit & J. Haxaire. Paratypes: 2 ♂♂, same data as holotype (BC-Hax4322/SOWE423-07; BC-Hax4324/SOWE425-07); 1 ♂ (BC-Hax4423/SOWE524-07), Guatemala, Suchitepequez Dept., Reserve Tarrales, 1041 m., 7.v.2007, leg. M. Lauras & D. Herbin; 1 ♂, Guatemala, Baja Verapaz Dept., Reserve Santa Rosa, 1580 m., 23.v.2007, leg. M. Lauras & D. Herbin; 1 ♂ (BC-Hax4329/SOWE430-07), Guatemala, Huehuetenango Dept., Soloma, near Cruz Maltin, Aldea Crinolina, 1900 m., 16.v.2002, leg. J. Monzon Sierra; 1 ♂ (BC-Hax4323/SOWE424-07), Costa Rica, Guanacaste Prov., Area de Conservacion Guanacaste, Sector Pitilla, Estacion Biologica Pitilla, 800m., 4.v.2005, leg. J. Barbut & A. Lévêque; 1 ♂ (BC-Hax0765/SOWA772-06), genit. prep. #Hax171, Mexico, Chiapas State, road from Ococingo to Palenque, track to Salto de Agua, km. 10, 50m., 21.viii.1992, leg. J. Haxaire & D. Herbin; 1 ♂ (BC-Hax0768/SOWA775-06), genit. prep. #Hax169, Mexico, Chiapas State, Municipio Oxchuc, track to Pashtonticja, km. 4, 1600m., 20.viii.1992, leg. J. Haxaire & D. Herbin; 2 ♂♂, genit. prep. #Hax168, Mexico, Veracruz State, road from Coatepec-Teocelo to Los Altos, track to Chilchotla, km.4, 1350m., 3.viii.1992, leg. J. Haxaire & D. Herbin; 2 ♂♂ (BC-Hax0766/SOWA773-06; BC-Hax0767/SOWA-774-06), Mexico, Veracruz State, road from Coatepec-Teocelo to Los Altos, track to Chilchotla, km.4, 1350m., 24.viii.1992, leg. J. Haxaire & D. Herbin; 6 ♂♂ and 1 ♀, Mexico, Oaxaca, Sierra Juarez, 12-17.iii.1992, leg. local collectors; 8 ♂♂, Mexico, Veracruz, Dos Amates, 20.x.2005, leg. local collectors; 1 ♀, Mexico, Veracruz, Catemaco, 06.viii.2004, leg. local collectors; 1 ♂ (BC-Hax0770/SOWA777-06), Nicaragua, Granada, Mombacho Volcano, alt. 800 m., 02.viii.2000, leg. M. Laguerre; 3 ♂♂, Nicaragua, Nueva Segovia, Rio Mazarite, 10-12.xi.2000, leg. J.-M. Maes; 1 ♂, Panama, Coclé, Cerro Gaital, El Valle, 27.v.1994, leg. N. Smith & D. Mitchell; 1 ♂ (BC-Hax0769/SOWA776-06), Honduras, Yoro, Pijol Mountain, alt. 1500 m., 19.vii.1995, leg. T. Porion; 2 ♂♂, Panama, Panama Prov., Cerro Jofe, 900-1000m, 9-13.v.2007, leg. J. Touroult; 1 ♀, Panama, Chiriquí, 1980, leg. Moinier; all the above paratypes held in the collections of JH and TV, except 2 ♂♂ to be deposited in the CNIN and BMNH. In addition, 11 paratypes (6 ♀♀, 5 ♂♂) are designated from Costa Rica, Area de Conservacion Guanacaste: 1 ♂, Sector Cacao, Gongora Bananal, alt. 600 m., 05.viii.2004, leg. M. Pereira; 1 ♀, Sector Pitilla, Ingas, alt. 580 m., 29.i.2005, leg. M. Rios; 2 ♂♂ and 2 ♀♀, Sector Pitilla, Loaiciga, alt. 445 m., 10-26.i.2005, leg. M. Rios; 2 ♂♂ and 2 ♀♀, Sector Pitilla, Pasmonpa, alt. 440 m., 10-29.i.2005, leg. P. & M. Rios; 1 ♀, Rincon Rainforest, Camino Rio Francia, alt. 410 m., 16.viii.2004, leg. J.Perez. All of these 11 specimens are part of the dataset used in the DNA barcoding study by Hajibabaei et al. (2006); these specimens to be deposited in the Smithsonian Institution, Washington DC, USA, and specimen data as well as sequences are available from the public BOLD project 'Sphingidae of the ACG1' (code MHASA) or GenBank (accession numbers DQ276772 to DQ276782).

Description: This species is immediately distinguishable by its bright coloration and the acute and falcate apex of the forewings. It is widely distributed in Central America and was recorded as *X. neoptolemus* by Mooser (1940: 456) and Hoffmann (1943: 233) in their surveys of Mexican sphingids.

Male (Figs. 4a, 4b). Head and body: Dorsal part of body olive-brown; labial palpi, area above eyes and tegulae finely marked with grey. Tegula with a median longitudinal gold line. median dorsal area of thorax greyish-beige, contrasting with patagia and tegulae. Upperside of abdomen with five thin longitudinal dark beige lines. Forewing length: 35 mm. Forewing upperside: General background color olive-brown; crossed by six postmedian and two submarginal lines as in the previously described species. First and fifth postmedian lines the most heavily contrasted, delimiting between them a cream-colored band in the median area of the wing. General coloration beyond fifth postmedian line remains constant, submarginal area not strongly differentiated from this postmedian part; this region crossed by three even lines (sixth postmedian and the two submarginal). Apex acute, slightly to strongly falcate, especially in specimens from Veracruz State, Mexico. Hindwing upperside: Basal area pure black, extending toward tornus, where it turns pale grey with a white inner edge. Median band bright red, somewhat pinkish in fresher specimens; tapering progressively toward apex of wing, almost reaching it. Forewing and hindwing undersides: Contrasted but with few distinct markings; ground colour reddish yellow, of uniform aspect. Basal area of forewing grey-beige; first and fourth

postmedian lines apparent, first straight and strongly marked, fourth very narrow and only visible from inner edge to vein M_2 , beyond which it is replaced by small black vein dots. Between these two lines, the wing is golden-yellow, contrasting with the reddish tone of the rest of the wing; orange submarginal area dentate between M_2 and M_3 in most specimens. First submarginal line also usually apparent, running from the inner margin to the apex.

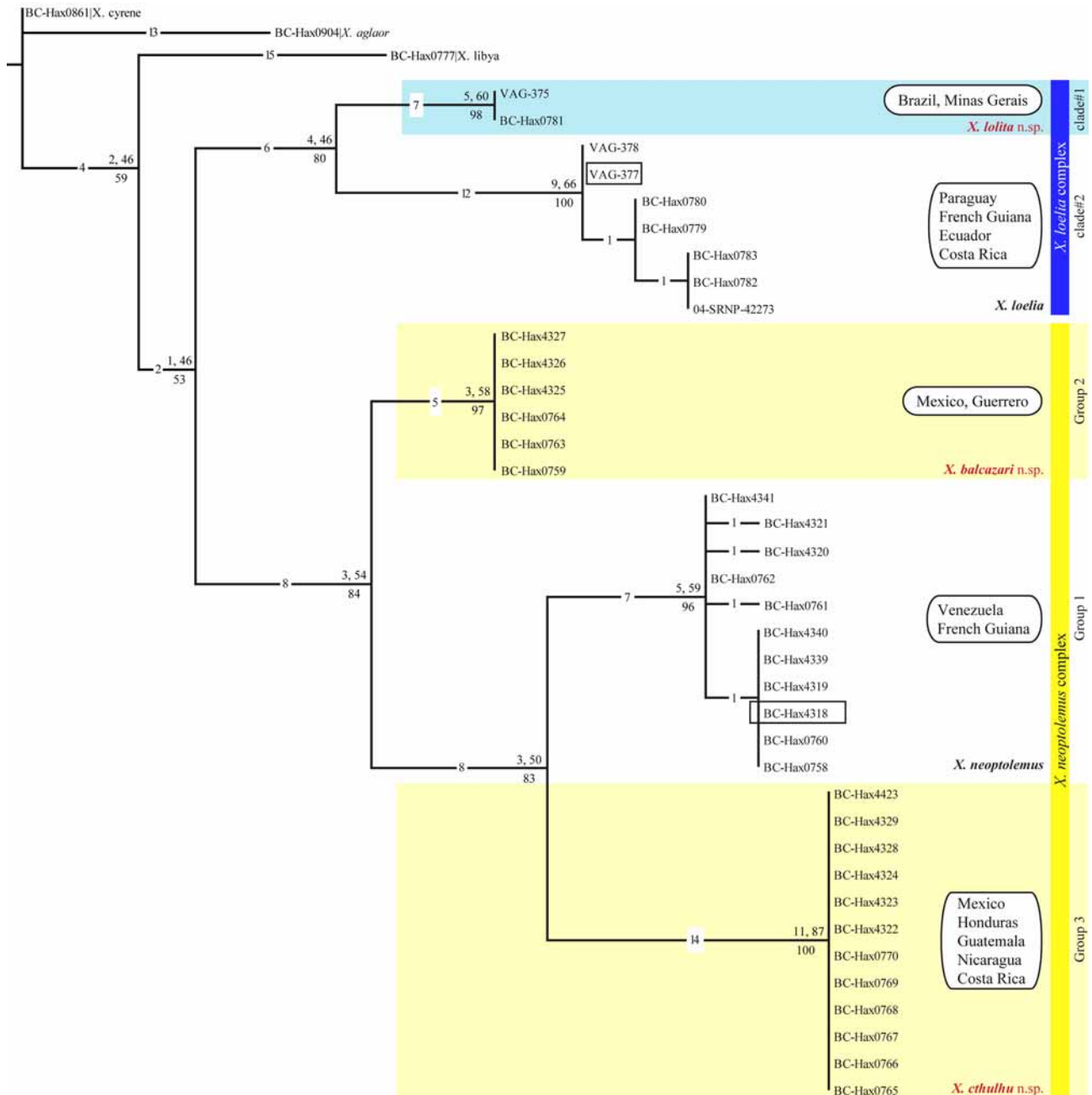


FIGURE 13. Strict consensus of the nine equally most-parsimonious cladograms (length=133, CI=0.78, RI=0.94) resulting from the phylogenetic analysis of the complete dataset of DNA barcode sequences for 38 specimens belonging to the *Xylophanes neoptolemus* (yellow) and *X. loelia* (blue) species complexes (*X. cyrene*, *X. aglaor* and *X. libya* are outgroup taxa). Each specimen is identified by its SampleID code (see Table 1), and the two specimens with short sequences are highlighted in boxes. The branch lengths are proportional to the number of changes (indicated on branches, optimized under FAST optimization); Bremer support and rescaled Bremer support values are given above the branches for each node, and bootstrap support values are indicated below. Clades within the *X. neoptolemus* complex are named after groups 1, 2 and 3 as described in the text.

Female. Forewing length: 37mm. Identical to the males in terms of general wing pattern and color, both upperside and underside, differing only in the normal differences between sexes in the genus *Xylophanes*, i.e. larger wingspan, broader and more rounded wings, and thinner antennae.

Male genitalia (Figs. 12a–c). Uncus stout; setigerous lobes developed and distinctly protruding; distal projection bent ventrally, its apex is slightly spatulate and truncate. Harpe short, thick basally and tapering progressively into a thin and slightly upcurved apex. The latter, together with the internal margin of the harpe, only slightly sclerotized. Setae on the harpe few, scattered, and present mostly on the ventral side. Right lobe of apical process of the aedeagus very stout, with long and uneven teeth grouped in the apical portion of its internal margin; left lobe barely distinct, bearing about 10 minute teeth on its lateral part.

Immature stages: A large number of rearing records for this species are reported on the ACG caterpillar inventory website (<http://janzen.sas.upenn.edu/>); when writing this article, more than 100 pictures of the caterpillar (penultimate and ultimate instars) and pupae of *Xylophanes cthulhu* were displayed on this site along with detailed collecting information, including food plant identifications. *X. cthulhu* is reported to feed exclusively on Rubiaceae of the genus *Spermacoce* L. (52 records on *S. exilis*, 6 on *S. ocymifolia* and 1 on *S. remota*).

Parasitoids: From the numerous rearing records of *X. cthulhu* in ACG, this species is reported as the host of the parasitoid wasps, *Cryptophion inaequalipes* (Hymenoptera: Ichneumonidae, Campopleginae. The reported parasitoid actually represents a species complex and a provisional name, *C. inaequalipes*DHJ02, is currently attached to the specimens reared out of *X. cthulhu* caterpillars) and *Charmedia chavarriai* (Ichneumonidae, Ichneumoninae), as well as the tachinid fly, *Drino incompta* (Diptera: Tachinidae).

Distribution. This species is widely distributed in Central America, inhabiting low to medium altitude forest areas from southern Mexico (Veracruz, Chiapas and Oaxaca states) to eastern Panama (Panama province). A possible contact zone with *X. neoptolemus* should be searched for in northern Colombia, where specimens of both species might be encountered.

Genetic variation: The lack of genetic variation of this species is remarkable, with a single haplotype found throughout the range from Mexico (Chiapas) to Panama (Fig. 13, group 3).

Identification key

The following key is primarily based on characters of the habitus, as differences in the genitalia are subtle and diagnosis from the habitus is more rapid and straightforward. Not all differences are listed in this key, and users wishing to investigate further differences between species are invited to use the figures and the more detailed descriptions and discussion of diagnostic characters in the first paragraph of the Results.

- 1 Hindwing upperside with a beige or pinkish-beige median band (Figs. 5a, 6a) 2
- Hindwing upperside with a red or dark pink median band (Figs. 2a, 3a, 4a)..... 3
- 2 Median band of hindwing upperside beige; first four postmedian lines of forewing upperside evenly spaced; harpe massive, strongly bent and apically curved *X. lolita* **n. sp.** (Figs. 5a, 5b, 9a–c) [This species is larger than *X. loelia*, with a wingspan >70 mm; the forewing has a larger black apical spot, is more elongate and less falcate than in *X. loelia*, the basal area of hindwing is black and the uncus is thicker than in *X. loelia*. *X. lolita* is a southeast Brazilian endemic, known to date only from Minas Gerais state]
- Median band of hindwing upperside pinkish-beige; postmedian lines of forewing upperside unevenly spaced, with second and third postmedian lines distinctly closer to one another *X. loelia* (Figs. 6a, 6b, 8a–c)
- 3 Underside of forewings with two clearly contrasting postmedian lines (Figs. 2b, 3b) 4

- Underside of forewings with a single postmedian line distinct, and crossed by a large golden band; harpe regularly curved *X. cthulhu* **n. sp.** (Figs. 4a, 4b, 12a–c)
[This species is more brightly coloured than its relatives, with bright red bands on hindwings and very contrasting forewing pattern; *X. cthulhu* is widely distributed in Central America, from Southern Mexico to Panama]
- 4 Forewing and hindwing undersides with a very distinct dashed postmedian line marked by black vein dots *X. neoptolemus* (Figs. 3a, 3b, 10a–c)
[This species is known from lowland rainforests in South America, from northwestern Venezuela to French Guiana and northern Pará state, Brazil]
- Forewing and hindwing undersides with dashed postmedian line on underside with barely distinct diffuse vein dots *X. balcazari* **n. sp.** (Figs. 2a, 2b, 11a–c)
[The wings are shorter and more rounded, and the coloration paler than in *X. neoptolemus* and *X. cthulhu*; the red median band of hindwing is larger; this species is known only from Guerrero state in Mexico.]

Concluding remarks

The exact congruence between slight but consistent morphological differences and the discrimination of genetic lineages in the two closely related species complexes addressed in this paper is remarkable. As morphology and mitochondrial DNA represent two independently evolving sets of characters, we interpret these results as demonstrating the existence of five distinct lineages, each having its own independent evolutionary trajectory and therefore worthy of being considered as distinct species. The phylogenetic relationships between these species, as inferred through the analysis of available sequences (Fig. 13), show a low level of homoplasy and an excellent resolution, opening interesting perspectives in term of exploring the biogeographical history and course and patterns of diversification of sphingid moths in the Neotropical region.

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References

- Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. & Das, I. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, 22, 148–155.
- Bremer, K. (1994) Branch support and tree stability. *Cladistics*, 10, 295–304.
- Cadiou, J.-M. (1995) Seven new species of Sphingidae (Lepidoptera). *Lambillionea*, 95, 499–515.

- Chainey, J.E. (2005) The species of Papilionidae and Pieridae (Lepidoptera) described by Cramer and Stoll and their putative type material in the Natural History Museum in London. *Zoological Journal of the Linnean Society*, 145, 283–337.
- Closs, A. (1917) *Xylophanes neoptolemus* Stoll, subspec. *neoptolemus trinitatis* subspec. nov. *Internationale Entomologische Zeitschrift*, 11, 96.
- Cramer, P. (1780) *Uitlandsche Kapellen, voorkomende in de drie Waereld-Deelen Asia, Africa en America, by een verzameld en bescreeven*. Barthelemy Wild, S.J. Baalde, Utrecht, 1–28, plates 289–304.
- Druce, H. (1878) Description of six new species of Sphingidae. *Entomologist's Monthly Magazine*, 14, 248–249.
- Goloboff, P.A. (1999) *NONA (NO NAME), Program and Documentation, Version 2.0*. Published by the author, Tucuman, Argentina.
- Goloboff, P.A. & Farris, J.S. (2001) Methods for quick consensus estimation. *Cladistics*, 17, S26–S34.
- Hajibabaei, M., deWaard, J. R., Ivanova, N. V., Ratnasingham, S., Dooh, R. T., Kirk, S. L., Mackie, P. & Hebert, P. D. N. (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society of London (Series B)*, 360, 1959–1967.
- Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Science USA*, 103, 968–971.
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- Hawes, J. (2005) *Impacts of landscape level disturbance on Amazonian moth assemblages*. MSc. thesis, University of East Anglia, Norwich, U.K. 45 pp.
- Haxaire, J. (1987) Le genre *Xylophanes* Hübner (1819) en Guyane Française. Quelques compléments (Lepidoptera Sphingidae). *Bulletin de la Société Sciences Nat.* 55, 12–19.
- Hebert, P.D., Cywinska, A., Ball, S.L. & deWaard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B Biological Sciences*, 270, 313–321.
- Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astrartes fulgerator*. *Proceedings of the National Academy of Science of the USA*, 101, 14812–14817.
- Ivanova, N.V., deWaard, J.R. & Hebert, P.D.N. (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, 6, 998–1002.
- Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
- Kitching, I.J. & Cadiou, J.-M. (2000) *Hawkmoths of the World. An annotated and illustrated revisionary checklist*. Cornell University Press, Ithaca, New York, 227 pp.
- Nixon, K.C. (2002) *WinClada, Ver. 1.00.08*. Published by the author, Ithaca, New York.
- Pfenninger, M. & Schwenk, K. (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, 7, 121.
- Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes*, 7, 355–364.
- Schaufuss, L.W. (1870) Die exotischen Lepidoptera Heterocera der Früher Kaden'schen Sammlung. *Nunquam Otiosus*, 1, 7–23.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599.
- Vane-Wright, R.I. (1975) The butterflies named by J. F. Gmelin (Lepidoptera: Rhopalocera). *Bulletin of the British Museum (Natural History) (Entomology)*, 32, 19–64.
- Walker, F. ([1865]) Supplement. *List of the Specimens of Lepidopterous Insects in the Collection of the British Museum*, 31, 1–321.