

## *Bombyx pseudohuttoni* sp.n. from southern Laos and Vietnam (Lepidoptera: Bombycidae)

*Bombyx pseudohuttoni* sp.n. — новый вид шелкопрядов  
из южного Лаоса и Вьетнама (Lepidoptera: Bombycidae)

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КЛЮЧЕВЫЕ СЛОВА: Аннамские горы, ДНК-баркодинг, криптические виды, центры биоразнообразия.

**ABSTRACT.** In this paper, we describe *Bombyx pseudohuttoni* sp.n., a new species from Laos and Vietnam. This species is closely related to *Bombyx incomposita* (van Eecke, 1929) (Sundaland) and *Bombyx huttoni* Westwood, 1847. The latter species was described from India, and its distribution is probably limited by India, China, Nepal, Bhutan, and northern Thailand. The previous records of *B. huttoni* from Vietnam belong to the new species. The uncorrected *COI* p-distance between these taxa is 3.37–3.85%, that is comparable with distances between other species of the genus.

**РЕЗЮМЕ.** В этой статье мы описываем новый вид из семейства шелкопрядов — *Bombyx pseudohuttoni* sp.n. из Лаоса и Вьетнама. Новый вид близок к *Bombyx incomposita* (van Eecke, 1929) (Сундаланд) и *Bombyx huttoni* Westwood, 1847, который был описан из Индии и ареал которого, вероятно, ограничен Индией, Китаем, Непалом, Бутаном и северной частью Таиланда. Предыдущие находки *B. huttoni* из Вьетнама принадлежат новому виду. Ге-

нетическая дистанция между этими двумя таксонами составляет 3.37–3.85%, что сопоставимо с дистанциями между остальными видами рода.

### Introduction

The genus *Bombyx* Linnaeus, 1758 contains six species [Wang *et al.*, 2015] distributed from the Kurile Islands (Russia) in the north to Java in the south [Holloway, 1987; Wang *et al.*, 2015; Rybalkin, 2020; Spitsyna, Spitsyn, 2023]. Until recently, *Rotunda rotundapex* (Miyata et Kishida, 1990) and its junior synonym, *Bombyx shini* Park et Sohn, 2002, belonged to the genus *Bombyx*. However, Wang *et al.* [2015] transferred these taxa to the genus *Rotunda* Wang, X. et Zolotuhin, 2015. At first, it was assumed *Bombyx huttoni* Westwood, 1847 (Fig. 1A–B) to have a wide range, from the Himalayas to the Greater Sunda Islands, but then based on small morphological differences Holloway [1987] proved there was a separate species *Bombyx incomposita* (van Eecke, 1929) in Sumatra, Borneo, and Peninsular Malaysia. Later it

was also confirmed by means of the DNA barcoding approach [Lin *et al.*, 2021]. In this paper, another new cryptic species belonging to *Bombyx huttoni-incomposita* species-group is described from Laos and Vietnam. The previous records of *B. huttoni* from Vietnam belong to the new species [Zolotuhin, Witt, 2009].

## Materials and Methods

The type specimens are deposited in the Russian Museum of Biodiversity Hotspots (RMBH), N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia), Zoologische Staatssammlung (ZSM; Munich, Germany) and private collections of Evgeny Koshkin (Khabarovsk, Russia), Günter Müller (Freising, Germany), and Siegfried Ihle (Filderstadt, Germany). The genitalia were dissected, mounted on temporary glass slides with 70% ethanol. The genitalia are kept in a micro-tube with glycerin pinned to the specimen. The images of the specimens were taken using a Canon EOS 7D camera with a Canon EF 100mm f/2.8L Macro IS USM lens (Canon Inc., Tokyo, Japan) (Figs 1A, B) and a Sony SLT-A65 camera with a Sony 2.8/50 Macro lens (Sony Group Corporation, Tokyo, Japan) (Fig. 1C, D).

The DNA extraction and the *cytochrome c oxidase subunit I (COI)* gene sequencing were performed as described in Bolotov *et al.* [2018]. Supplementary sequence data was obtained from BOLD and GenBank (Table 1). The alignment of

the sequences was performed using the MUSCLE algorithm implemented in MEGA7 [Kumar *et al.*, 2016]. The maximum likelihood phylogenetic analysis was based on the *COI* dataset of *Bombyx* species with 13 unique haplotypes. This analysis was carried out with an online version of IQ-TREE v1.6.11 [Trifinopoulos *et al.*, 2016] using an ultrafast bootstrap algorithm [Hoang *et al.*, 2017] and an automatic identification of the most appropriate substitution models [Kalyaanamoorthy *et al.*, 2017]. Sequences of *Ocinara albicollis* (Walker, 1862) and *Triuncina daii* Wang, X. *et al.* Zolotuhin, 2015 were used as outgroup.

## Taxonomy

### *Bombyx pseudohuttoni* Spitsyn *et al.* Koshkin *sp.n.* Figs 1–4.

TYPE MATERIAL. Holotype male RMBH Sph1039: LAOS, Bolaven Plateau, Sekong province, Thateng town, mountain tropical forest, 15°24'16"N, 106°22'58"E, 22–25.12.2022, E. Spitsyna & V. Spitsyn leg.; paratypes RMBH Sph1040, Sph1075–1084: the same locality, date, and collectors — 1♂ 1♀; the same locality and collectors, 21–25.07.2023 — 7♂♂ 1♀; LAOS, Bolaven Plateau, Champasak province, Paksong town, mountain tropical forest and pine plantations, 15°10'54"N, 106°14'25"E, 2–4.07.2023, E. Spitsyna & V. Spitsyn leg. — 1♂; paratypes ZSM: VIETNAM, Cuc. Phuong, 60 km SW Hanoi, 20°15'N, 105°20'E, 400 m, 18.11–

**Table 1.** Additional *COI* sequences of *Bombyx* species used in this study with BOLD and GenBank accession codes.

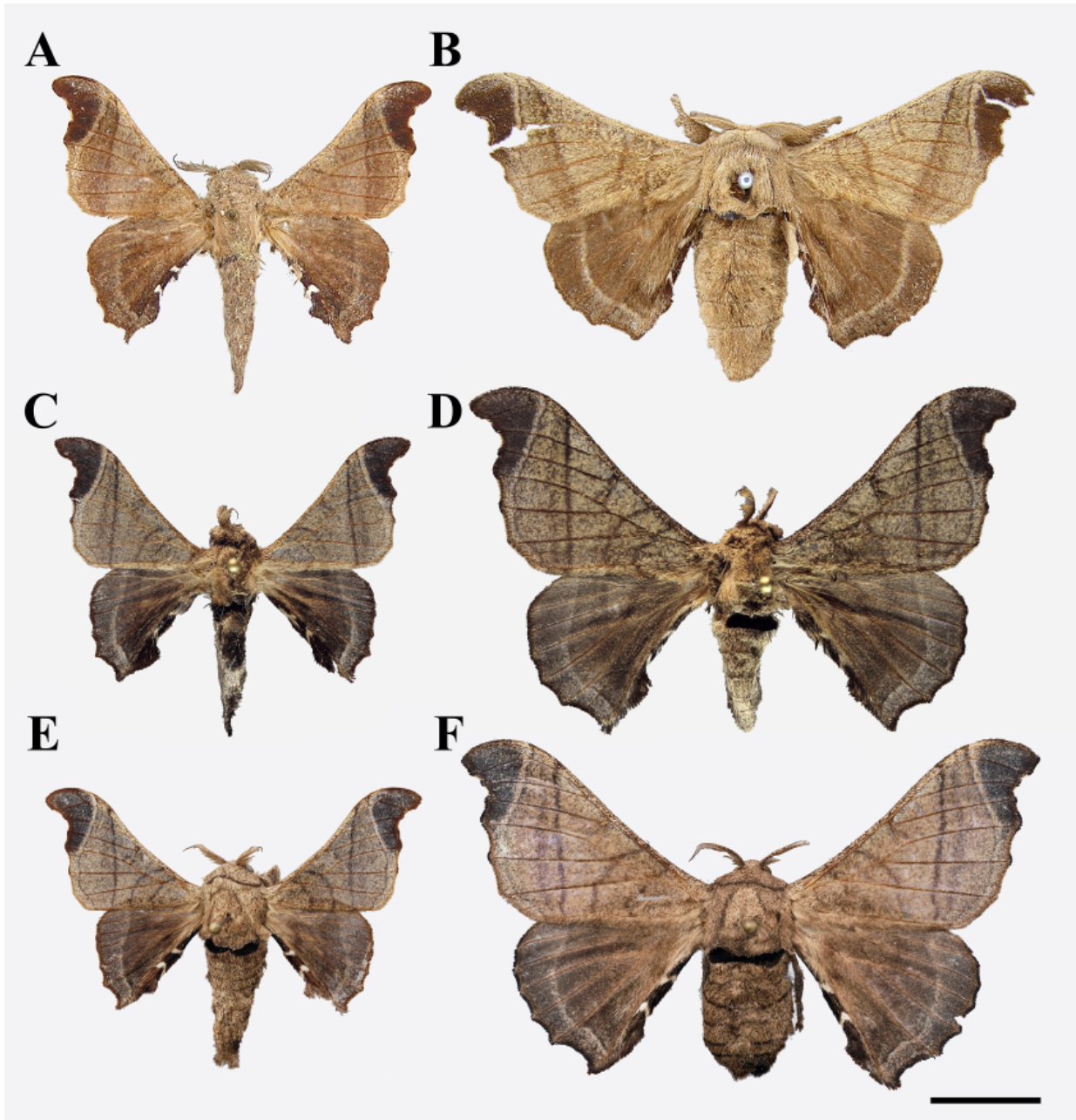
**Таблица 1.** Дополнительные последовательности *COI* видов рода *Bombyx*, использованные в данном исследовании, с номерами BOLD и GenBank.

Species	COI haplotype code	COI GenBank acc. no.	COI BOLD acc. no.	Locality	References
<i>Bombyx huttoni</i>	Bh1	KF491596	LTOL787-07	Bhutan	[NCBI GenBank/BOLD]
<i>B. huttoni</i>	Bh2	NC026518	SPMIS085-22	China	[Peng <i>et al.</i> , 2016]
<i>B. huttoni</i>	Bh2	KP216766	GBMNA10015-19	China	[Peng <i>et al.</i> , 2016]
<i>B. huttoni</i>	Bh3	MH817457	GBMNA21948-19	China	[NCBI GenBank/BOLD]
<i>B. incomposita</i>	Bi1	MW898927	N/A	Malaysia	[Lin <i>et al.</i> , 2021]
<i>Bombyx</i> sp.	Bsp1	KX861956	MAMOT1294-11	Pakistan	[Ashfaq <i>et al.</i> , 2017]
<i>Bombyx</i> sp.	Bsp1	KX861165	MAMOT2958-12	Pakistan	[Ashfaq <i>et al.</i> , 2017]
<i>Bombyx</i> sp.	Bsp1	KX862799	MAMOT2959-12	Pakistan	[Ashfaq <i>et al.</i> , 2017]
<i>Bombyx</i> sp.	Bsp1	KX863015	MAMOT1297-11	Pakistan	[Ashfaq <i>et al.</i> , 2017]
<i>B. lemeepauli</i>	Ble1	NC037149	GBGL25784-19	China	[NCBI GenBank/BOLD]
<i>B. lemeepauli</i>	Ble1	KY620270	GBMNA10016-19	China	[Liu <i>et al.</i> , 2017]
<i>B. lemeepauli</i>	Ble2	MH817452	GBMNA21943-19	China	[NCBI GenBank/BOLD]
<i>B. mandarina</i>	Bma1	AF167277	GBGL0178-06	Korea	[NCBI GenBank/BOLD]
<i>B. mandarina</i>	Bma2	MK251840	N/A	China	[NCBI GenBank]
<i>B. mandarina</i>	Bma2	FJ384796	GBGL8177-12	China	[Hu <i>et al.</i> , 2010]
<i>B. mandarina</i>	Bma3	MW960642	N/A	China	[NCBI GenBank]
<i>B. mandarina</i>	Bma4	JN305965	LTOLB952-11	Taiwan	[NCBI GenBank/BOLD]
<i>B. mori</i>	Bmo1	MK295814	N/A	China	[Li, Zhu, 2019]
<i>B. mori</i>	Bmo1	KP192478	GBGL17382-15	China	[Zhang, Wu, 2016]
<i>B. mori</i>	Bmo1	MG797555	GBGL25920-19	China	[Li <i>et al.</i> , 2018]
<i>B. mori</i>	Bmo1	KM875545	GBGL18354-15	China	[Zhang, Zhou, 2014]
<i>B. mori</i>	Bmo1	KM347743	GBGL18353-15	China	[Li <i>et al.</i> , 2014]
<i>B. mori</i>	Bmo1	KM279431	GBGL18352-15	China	[Zhang <i>et al.</i> , 2014]
<i>Ocinara albicollis</i> *	–	NC038087	GBGL25924-19	China	[NCBI GenBank/BOLD]
<i>Triuncina daii</i> *	–	KY091643	GBMNA10018-19	China	[Zhao <i>et al.</i> , 2017]

N/A — not available; \* — these species were used as outgroups for maximum likelihood analysis.

03.12.1992, Sinaev & Simonov leg. — 1♂; VIETNAM, Lai Châu province, Cha-pa [Sa Pa district], Mt. Fan-si-pan, N. Seite [northern slope], primär. Urwald [primary virgin forest], 22°17'N, 103°44'E, 1525 m, 07–10.07.1994, Brechlin & Schintlmeisted leg. — 1♂; VIETNAM, Lai Châu province, Cha-pa [Sa Pa district], Mt. Fan-si-pan, N-Seite, Cha-pa

(= Sapa), 1600 m, 22°17'N, 103°44'E, 05.1995, primär Urwald, Sinaev & einh. Sammler [local collector] leg. — 1♂; VIETNAM, Tam Dao, 60 km NW Hanoi, 21°34'N, 105°20'E, 950 m, 17.10.1995, Sinaev leg. — 1♂; VIETNAM, Lai Châu province, Cha-pa [Sa Pa district], Mt. Fan-sipan, W. Seite [western slope], primär. Nebelwald [primary cloud forest],

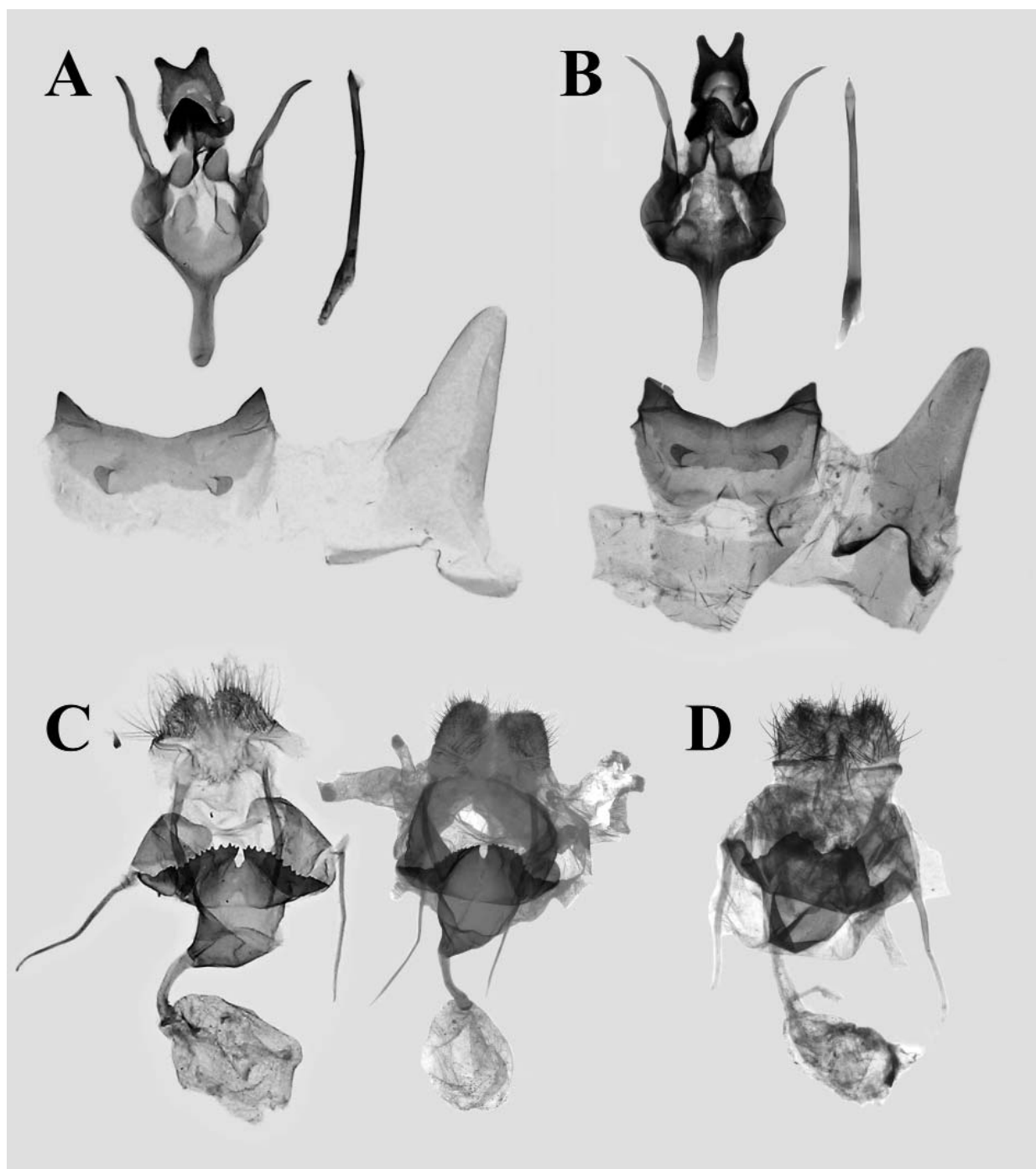


**Fig. 1.** *Bombyx* spp.: A–B — *Bombyx huttoni* Westwood, 1847: A — male (ZSM, India, Kumaon – Himalaya Dist., Nainital), B — female, holotype (Oxford University, India, Mussooree); C–F — *Bombyx pseudohuttoni* Spitsyn et Koshkin, **sp.n.**: C — male, holotype (RMBH, Laos, Sekong province, Thateng town), D — female, paratype (RMBH, Laos, Sekong province, Thateng town), E — male, paratype (Evgeny Koshkin's private collection, Vietnam, Kon Tum province), F — female, paratype (Evgeny Koshkin's private collection, Vietnam, Kon Tum province). Scale bar 10 mm.

**Рис. 1.** *Bombyx* spp.: A–B — *Bombyx huttoni* Westwood, 1847: A — самец (Индия, Кумаон – Гималаи, Найнитал), B — самка, голотип (Индия, Массури); C–F — *Bombyx pseudohuttoni* Spitsyn et Koshkin, **sp.n.**: C — самец, голотип (Лаос, провинция Секонг, Тхатенг), D — самка, паратип (Лаос, провинция Секонг, Тхатенг), E — самец, паратип (Вьетнам, провинция Контум), F — самка, паратип (Вьетнам, провинция Контум). Масштабная линейка 10 мм.

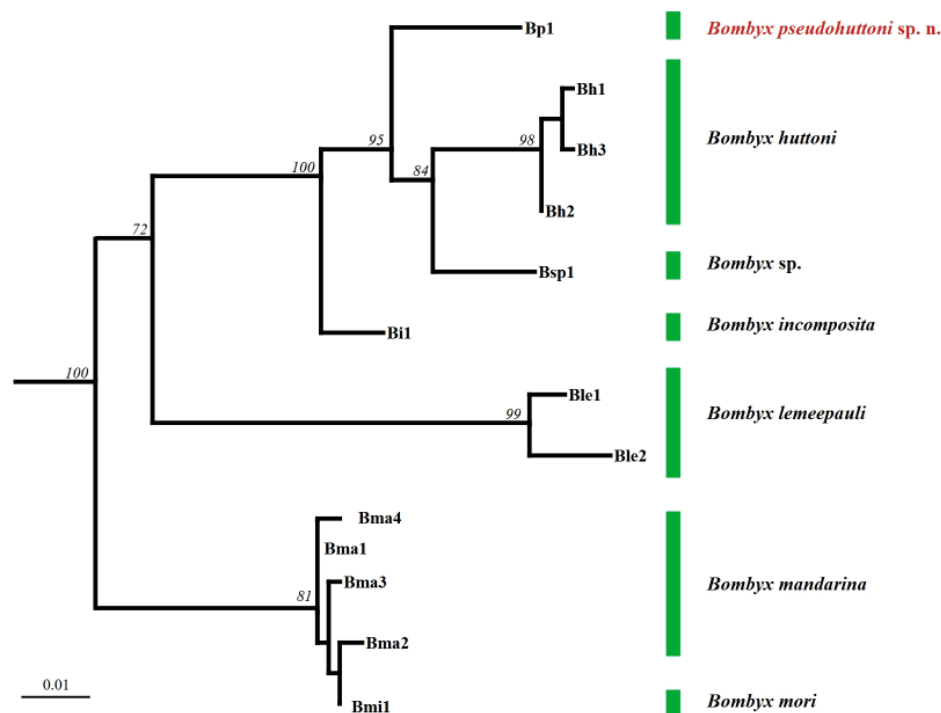
22°15'N, 103°45'E, 2000 m, 05.07.1994, Brechlin & Schintlmeister leg. — 1♂; VIETNAM, Lai Châu province, Cha pa [Sa Pa district], Mt. Fan-si-pan, 22°15'N, 103°46'E, 2400 m, 8–29.05.1993, Sinaiev & Simonov leg. — 1♂; VIETNAM,

Lai Châu province, Cha pa [Sa Pa district], Mt. Fan-si-pan, 22°15'N, 103°46'E, 1700 m, 04.1994, Sinaiev & einh. Sammler [local collector] leg. — 1♂; VIETNAM, Lai Châu province, Cha pa [Sa Pa district], Mt. Fan-si-pan (west), Sek. Wald/



**Fig. 2.** Genitalia of *Bombyx* spp.: A, C — *Bombyx huttoni*: A — male, ZSM genitalia slide 32.549 (India, Kumaon – Himalaya Dist., Nainital), C — females, ZSM genitalia slides 32.550 and 34.081 (India, Kumaon – Himalaya Dist., Nainital); B, D — *Bombyx pseudohuttoni* Spitsyn et Koshkin, **sp.n.**: B — male, holotype, RMBH genitalia slide Sph1039 (Laos, Sekong province, Thateng town), D — female, paratype, RMBH genitalia slide Sph1084 (Laos, Sekong province, Thateng town).

**Рис. 2.** Гениталии *Bombyx* spp.: A, C — *Bombyx huttoni*: A — самец, препарат гениталий ZSM 32.549 (Индия, Кумаон – Гималаи, Найнитал), C — самки, препараты гениталий ZSM 32.550 и 34.081 (Индия, Кумаон – Гималаи, Найнитал); B, D — *Bombyx pseudohuttoni* Spitsyn et Koshkin, **sp.n.**: B — самец, голотип, препарат гениталий RMBH Sph1039 (Лаос, провинция Секонг, Тхатенг); D — самка, паратип, препарат гениталий RMBH Sph1084 (Лаос, провинция Секонг, Тхатенг).



**Fig. 3.** Maximum likelihood phylogeny of *Bombyx* species based on the *COI* barcode sequence dataset. *Bombyx pseudohuttoni* Spitsyn et Koshkin, sp.n. is red. Numbers near nodes are bootstrap support values. Scale bar on the tree indicates the branch lengths. Outgroup taxa (*Ocinara albicollis* and *Triuncina daii*) are not shown.

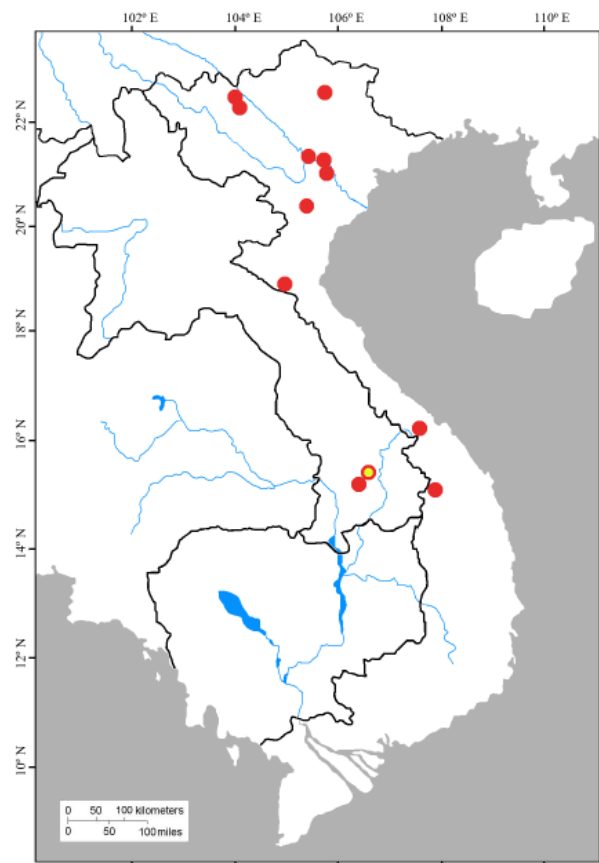
**Рис. 3.** Филогенетическое дерево, построенное по методу максимального правдоподобия для представителей рода *Bombyx* на основе гена *COI*. *Bombyx pseudohuttoni* Spitsyn et Koshkin, sp.n. выделен красным цветом. Числа возле узлов представляют значения поддержки бутстрапа. Масштабная линейка показывает процентное расхождение последовательностей отдельных видов, наложенное на длину ветвей. В качестве аутгруппы использовались *Ocinara albicollis* и *Triuncina daii*, которые не представлены на дереве.

Kulturland [secondary forest / agricultural land], 22°20'N, 103°40'E, 1600–1800 m, 10.06–6.07.1994, Siniav & einh. Sammler [local collector] leg. — 5♂♂; VIETNAM, Lai Châu province, Cha-pa [Sa Pa district], Mt. Fan-si-pan (West), 22°20'N, 103°40'E, 1600–1800 m, 30.04–12.07.1994, Schintlmeister leg. — 2♂♂; VIETNAM, Lai Châu province, Cha-pa [Sa Pa district], Mt. Fan-si-pan (West), Sek. Wald/Kulturland [secondary forest / agricultural land], 22°20'N, 103°40'E, 1600–1800 m, 11.1994, Siniav & einh. Sammler [local collector] leg. — 1♂; VIETNAM, Lai Châu province, Cha-pa [Sa Pa district], Mt. Fan-sipan, W-side, 22°20'N, 103°40'E, 1600–1800 m, 15–25.04.1995, Siniav & local collector leg. — 1♂; VIETNAM, Lai Châu province, Cha-pa [Sa Pa district], Mt. Fan-si-pan, secondary forest, 22°20'N, 103°40'E, 1600–1800 m, 05–06.1995, local collectors leg. — 3♂♂ 1♀; paratypes from the collection of Evgeny Koshkin: VIETNAM, Kon Tum province, Dak Glei district, 1 km NW Dak Man village, mountain tropical forest, 15°13'24"N, 107°43'51"E, 1080 m, 11–12.05.2017, E. S. Koshkin leg. — 1♂; the same locality and collector, 14.05.2017 — 1♂ 2♀♀; the same locality and collector, 19.05.2017 — 1♂; the same locality and collector, 24.05.2017 — 1♀; paratype from the collection of Günter C. Müller: VIETNAM, Lai Châu province, Cha pa [Sa Pa district], Mt. Fan-si-pan, W-side, Sek. Wald [secondary forest], 22°20'N, 103°40'E, 1600–1800 m, 10.06–6.07.1994, Siniav leg. — 1♂; paratypes from the collection of Siegfried Ihle:

VIETNAM, Thai Nguen province, 30 km E from Thai Nguen, Mo Ba, 10.2009, Hoa Bihn Nguen leg. — 1♂; VIETNAM, Vinh Phuc province, ca. 20 km N from Vinhuyen, Tam Bao, 09.2007, Thomas Ihle leg. — 1♂.

ADDITIONAL MATERIAL (See DNA BARCODING below). VIETNAM, Vinh Phuc province, Ngoc Tanh village, Me Linh biostation, 21°23'N, 105°43'E, 60 m, 10.12.2009, A. Solovyev leg., barcode LBEO181-10 — 1♂; VIETNAM, Cao Bang province, Nguen Binh county, Thanh Cang, Phia Den, 22°34'N, 105°52'E, 1030 m, 10.11.2009, V. Zolotuhin leg., barcode LBEO180-10 — 1♂; VIETNAM, Lai Chau, Fan-Si-Pan Mt, Sa Pa National Park, 5 km N Sa Pa, 22°21'N, 103°49'E, 1500 m, 16.05.2006, V. Zolotuhin leg., barcode LBEO457-10 — 1♂; VIETNAM, Nghe An province, Pu Mat National Park, Phuc Sn village, 18°49'N, 104°58'E, 130 m, 1.10.2008, V. Zolotuhin leg., barcode LBEO460-10 — 1♂; VIETNAM, Vinh Phuc province, Thua Thien Hue, Hue, Bihn Tri Thien, Bach Ma National Park, 16°12'N, 107°51'E, 1250 m, 21.04.2009, V. Zolotuhin leg., barcode LBEO466-10 — 1♂.

DIAGNOSIS. Compared to *B. huttoni*, the new species has no significant differences in colouration of adults (compare Fig. 1A–B and 1C–F) but has slight differences in genitalia morphology, namely in longer saccus in males (compare Figs 2A and 2B) and specific shape of antevaginal plate in females (compare Fig. 2C and 2D). Additionally, the new species can be accurately distinguished by the DNA barcoding (Fig. 3)



**Fig. 4.** Collecting localities of *Bombyx pseudohuttoni* Spitsyn et Koshkin, **sp.n.** The yellow circle indicates the type locality.  
**Рис. 4.** Места сбора *Bombyx pseudohuttoni* Spitsyn et Koshkin, **sp.n.** Желтый круг указывает типовое местообитание.

and its limited distribution (Fig. 4). The species differs from *B. huttoni* by 21 fixed nucleotide substitutions in the *COI* gene sequences: 44G, 50T, 51T, 143C, 170T, 248T, 264T, 267C, 291A, 293T, 320C, 356C, 392C, 401T, 455C, 461T, 464T, 596C, 614C, 635C, 642C.

**DESCRIPTION. Male morphology** (Fig. 1C, E): Wingspan 31–36 mm ( $n = 13$ ), forewing length 17–20 mm ( $n = 13$ ). Eye black-gray. Antenna short, bipectinate, gray-brown. Head gray-brown. Labial palpus very short, gray-brown. Thorax, patagium, tegula gray-brown. Legs gray-brown. Upperside of forewing gray; apex elongated, curved, almost black; basal and antemedial lines strongly curved, almost black; postmedial line straight or slightly curved, almost black; marginal line S-shaped, almost black. Underside of forewing similar to that of upperside, but differs by having slightly discernible basal and antemedial lines. Upperside of hindwing dark gray; postmedial line white-gray, curved; medial line dark, slightly discernible; anal margin darker, with two small white spots. Underside of hindwing similar to that of upperside, but differs by having a little lighter colouration and more discernible medial band. Abdomen gray-brown, except for first segment, which is black dorsally. **Male genitalia** (Fig. 2B): Typical structure for *Bombyx huttoni-incomposita* species-group with slender valvae, saccus and aedeagus. **Female morphology** (Fig. 1D, F): Wingspan 45–52 mm ( $n = 5$ ), forewing length 25–27 mm ( $n = 5$ ). Female is externally similar to male, but differs in significantly wider wings and shorter antennal rami. **Female genitalia** (Fig. 2D): Typical structure for *Bombyx huttoni-incom-*

*posita* species-group with inverted lateral abdominal gland at base of papillae anales. Antevaginal plate has irregular shape of distal margin.

**DNA BARCODING.** Uncorrected *COI* p-distance between *Bombyx pseudohuttoni* **sp.n.** and *B. huttoni* is 3.37–3.85%, and between *B. pseudohuttoni* **sp.n.** and *B. incomposita* — 3.53%. These distances equal or exceed uncorrected *COI* p-distance between *B. huttoni* and *B. incomposita* which is 3.37%. The maximum intraspecific distance of *B. pseudohuttoni* **sp.n.** is equal to 1.13%. The BOLD database contains nine sequences of the new species from different provinces of Vietnam, five of them were sent to BOLD by the late Dr Vadim Zolotuhin and were analyzed by us. These five barcoded specimens are listed above in the additional material and supposed to be stored in Dr Zolotuhin's collection but were not found (pers. comm. by Alexey Prozorov), thus they are not included into the type series, but their collecting localities are added to the distribution map of *B. pseudohuttoni* **sp.n.**

**REFERENCE *COI* BARCODE SEQUENCE.** GenBank acc. no. PP028818 (holotype).

**DISTRIBUTION.** Laos, Vietnam (Fig. 4).

**ETYMOLOGY.** The name of the new species reflects its morphological similarity to *Bombyx huttoni* Westwood, 1847.

**Competing interests.** The authors declare no competing interests.

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