



## Description of *Nola estonica* sp. nov., with comparison to *N. aerugula* and *N. atomosa* stat. rev. (Lepidoptera, Nolidae, Nolinae)

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### Abstract

*Nola estonica* Õunap **sp. nov.** (Lepidoptera, Nolidae, Nolinae) is described based on type material from Estonia. The lectotype is designated for *Glaphyra atomosa* Bremer, 1861, which is reinstated from a subspecies of *Nola aerugula* (Hübner, [1793]) to a full species: *Nola atomosa* (Bremer, 1861) **stat. rev.** The status of these three taxa as separate species is supported by the results of phylogenetic analysis of DNA barcodes, as well as external and genital morphology of adult specimens. Two new synonyms are established as follows: *Nola atomosa* (Bremer, 1861) = *Nola candidalis* Staudinger, 1892 **syn. nov.** and *Nola shin* Inoue, 1982 **syn. nov.** *N. estonica* occurs sympatrically with *N. aerugula* in Estonia, and with *N. atomosa* in South Korea and easternmost Russia. While the available data suggest a disjunct distribution of *N. estonica* (eastern Europe and the temperate Far East), it appears highly possible that the species has a wide transpalearctic distribution.

**Key words:** *Nola*, taxonomy, Palaearctic, DNA barcode

### Introduction

The diverse genus *Nola* Leach, [1815] has a worldwide distribution (Fibiger *et al.* 2009), with more than 200 species known from Eurasia and Africa (Hacker *et al.* 2012, László *et al.* 2014). In the taxonomically less comprehensively studied parts of the world, new *Nola* species are still regularly discovered (e.g. László *et al.* 2014, Da *et al.* 2021). Even after compiling their major revision of African Nolini, Hacker *et al.* (2012) predicted that tens of African species still await description. In Europe, however, the species composition of *Nola* is better understood. The most recently described European species is *N. ronkayorum* Beshkov, 2006. Subsequently, Nupponen & Fibiger (2006) reported the presence of an overlooked species, *N. crambiformis* Rebel, 1902 on the eastern margin of Europe. Further changes in the list of European *Nola* have resulted from revising already known taxa. As a result of their major taxonomic work, Fibiger *et al.* (2009) reported 17 *Nola* species from Europe, while subsequent revision by Hacker *et al.* (2012) elevated this number to 18. To our knowledge, no changes in the list of European *Nola* have been made thereafter.

The present study was initiated when one of the authors (TT) noticed unusual light-coloured specimens of *Nola* of Estonian origin, which he suspected to be distinct from *Nola aerugula* (Hübner, [1793]), a common species in the country. Besides the deviating external appearance, it was considered notable that such moths had always been collected as singletons with no typically looking *N. aerugula* present in the catch. DNA barcoding (Hebert *et al.* 2004) revealed that these moths carried a COI haplotype strongly differing from that of *N. aerugula*. A subsequent search revealed that this unusual form is abundant in at least one locality in southeastern Estonia, where numerous specimens were collected in late July and early August 2020, together with several individuals of *N. aerugula*.

We thereafter performed a comprehensive literature search covering all known Palaearctic species to clarify the status of these moths. The only taxon resembling the light Estonian specimens was *N. aerugula atomosa* (Bremer, 1861), the eastern subspecies of *N. aerugula*, which is distributed in the Russian Far East, Northern China, Korea, and Japan (Oh 2001, Matov 2019). Therefore, a few specimens of *N. aerugula atomosa* from South Korea were also subjected to DNA barcoding, which revealed that these significantly differed from both *N. aerugula* and the problematic Estonian taxon. Moreover, DNA barcodes demonstrated that the unknown taxon from Estonia occurs in South Korea as well. To resolve the taxonomic status of the two species present in the Eastern Palaearctic region, the type material of *Glaphyra atomosa* Bremer, 1861 was examined.

The purpose of the current article is to describe the problematic taxon as a new species, and to revise the status of *Glaphyra atomosa*, which is currently treated as a subspecies of *Nola aerugula*.

## Material and methods

### Specimens

As the initial results of DNA barcoding indicated that the unknown taxon from Estonia is related to *N. aerugula*, we concentrated on this species and taxa morphologically closest to it. In total, about 200 specimens were studied. The material was obtained from the following collections

IZBE: Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences (former Institute of Zoology and Botany), Tartu, Estonia

MNUEE: Insect collection in the Environmental Education lab, Mokpo National University, South Korea

RCEÕ: Research Collection of Erki Õunap, Tartu, Estonia

RCAT: Research Collection of Andro Truuverk, Tartu, Estonia

RCIT: Research Collection of Imre Taal, Tartu, Estonia

RCTT: Research Collection of Toomas Tammaru, Tartu, Estonia

TUZ: Natural History Museum, University of Tartu.

ZISP: Zoological Institute RAS, St. Petersburg, Russia

ZMH: Zoological Museum Hamburg, Germany

### Morphological study

Imagoes from Estonian collections were photographed using a Canon EOS 700D DSLR camera equipped with a Canon EF 100mm f/2.8L USM IS objective. Helicon Remote software was used for a series of multiple gradually focused images, which were thereafter stacked together using the Helicon Focus version 7.6.1 software. Specimens from coll. MNUEE were photographed using a Nikon D300 DSLR camera equipped with an AF-S Micro-Nikkor 105mm f/2.8G IF-ED objective. Material from coll. ZISP was photographed with a Canon PowerShot A495 digital camera with its standard objective. Images were edited and compiled into plates using Adobe Photoshop CS3 software.

Abdomens of the moths selected for dissection ( $n = 33$ ) were detached from the thorax and macerated in a 15% solution of KOH for 20 hours at room temperature or 20 minutes by boiling, followed by neutralization and washing in distilled water. Thereafter, abdomens were cut open along pleurites and genitalia were separated. Scales and loose hairs were brushed off, and both genitalia and abdominal sclerites were inserted into 96% ethanol for 5 minutes before being mounted into euparal. Slides were photographed several weeks later when euparal had solidified. The built-in digital camera of a Leica S9i stereomicroscope was used to photograph genitalic slides of the material from Estonian collections and coll. MNUEE. Genitalic slides from coll. ZISP were photographed using a Leica MZ95 stereomicroscope equipped with a Leica DFC290 digital camera. Images were edited and compiled into plates using Adobe Photoshop CS3 software. Terminology used follows Hacker *et al.* (2012) and Kristensen (1999).

### Molecular study

Altogether 44 specimens from 9 localities were subjected to molecular study, 38 from Estonia (6 localities) and 6 from South Korea (3 localities). Genomic DNA was extracted from one or two crushed legs of each specimen using DNEasy Blood & Tissue Kit (Quiagen N.V., Venlo, Netherlands) following the manufacturer's instructions. A standard barcoding fraction (658 bp) of the mitochondrial COI gene (Hebert *et al.* 2004) was sequenced, with reaction protocols being different for Estonian and South Korean material.

Estonia: PCR was performed in a total volume of 20 µl, with the reaction mixture containing 1X BD Advantage 2 PCR buffer, 1U BD Advantage 2 Polymerase mix (BD Biosciences, San Jose, USA), 0.2 mM dNTP (Thermo Scientific, Pittsburgh, USA), 5 pmol of primers cov-1f (5'-TCGCTTATTATTCAGCCATTTTATT-3') (Õunap *et al.* 2008) and nan (5'-CCCGTAAAATTAATAATAAACT-3') (Õunap *et al.* 2005), and 1-2 ng of purified genomic DNA. One sample failed to amplify with primers cov-1f and nan, and the latter was replaced by cov-1r (5'-CTGCACCATTTTCTACAATTCTTCT-3') (Õunap *et al.* 2008) to obtain a 306 bp fragment of COI from the 5' end of the barcoding fraction. PCR was performed on a T1 thermocycler (Biometra, Göttingen, Germany) and the cycling parameters were: a 2-min denaturing step at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 50°C and 60 s at 68°C with a subsequent 7-min final extension at 68°C. PCR products were visualized on a 1.6% agarose gel and 10 µl of the PCR solution was treated with FastAP thermosensitive alkaline phosphatase and exonuclease I (Thermo Scientific). One unit of both enzymes was added to the PCR solution, which was incubated for 15 min at 37°C, followed by 15 min inactivation at 80°C. The DNA cycle sequencing was performed in a total volume of 10 µl using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Cycling conditions were: 33 cycles of 20 s at 95°C, 20 s at 45°C and 60 s at 60°C. Both DNA strands were sequenced with 2 pmol of primers and sequences were resolved by 3730xl DNA Analyzer automated sequencer (Applied Biosystems) in Estonian Biocentre (Tartu, Estonia).

South Korea: For amplification, a standard pair of barcoding primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994) was used. The PCR was conducted using FastMix Frenche PCR kits (i-Taq; iNtRon Biotechnology, Korea) under the following conditions: initial denaturation for 3 min at 94°C, followed by 30 cycles of 94°C for 1 min, 50°C for 30 sec, and 72°C for 1 min, with a subsequent final 7 min extension at 72°C. Gel electrophoresis for visualizing PCR products was carried out using 1× TAE buffer on 1% agarose gel with Top Green Nucleic Acid Gel Stain (LED; Genomic Base, Korea) for 15 min at 135V. The obtained PCR products were purified with a PCR purification kit (iNtRON) and were sequenced with forward and reverse primers (GenoTech Korea).

### Phylogenetic analysis

Consensus sequences were created with Geneious R7 (Biomatters Ltd, Auckland, New Zealand). In addition to the DNA barcodes of 44 specimens originally sequenced in this study, a dataset comprising 146 specimens of 11 Palaearctic *Nola* species was downloaded from the public data portal of Barcode of Life Data Systems (<http://www.boldsystems.org/index.php>) (referred to as BOLD hereinafter). *Meganola albula* (Denis & Schiffermüller, 1775) and *M. strigula* (Denis & Schiffermüller, 1775) were used as outgroup, thus the total size of the data matrix was 192 DNA barcodes. The BOLD sequence IDs and GenBank accession codes for sequences used are presented in Table 1. All sequences were assembled into a data matrix in BioEdit 7.0.5.2 (Hall 1999) and aligned using ClustalW (Thompson *et al.* 1994). Both uncorrected pairwise genetic distances between the studied specimens and phylogenetic tree implementing neighbor-joining algorithm were constructed using MEGA6 (Tamura *et al.* 2013).

**TABLE 1.** DNA barcodes used in this study. BOLD: sequence ID in BOLD Systems database. GenBank: sequence accession code in NCBI GenBank.

Species	BOLD	GenBank	Sample ID	Country
<i>Meganola albula</i>	ABOLB409-15		KLM Lep 02594	Austria
<i>Meganola strigula</i>	ABOLA529-14		TLMF Lep 16216	Austria
<i>Nola aerugula</i>	ABOLD146-16		KLM Lep 06131	Austria
<i>Nola aerugula</i>	ABOLD280-16		TLMF Lep 20883	Austria
<i>Nola aerugula</i>	ABOLD329-16		TLMF Lep 20932	Austria
<i>Nola aerugula</i>	ABOLD350-16		TLMF Lep 20953	Austria
<i>Nola aerugula</i>	DEEUR713-16		TLMF Lep 19143	Austria
<i>Nola aerugula</i>	GBLAB623-13		BC ZSM Lep 75394	Germany
<i>Nola aerugula</i>	GBLAF588-14		BC ZSM Lep 82294	Germany
<i>Nola aerugula</i>	GWOSI793-10	JN284309	BC ZSM Lep 49199	Germany

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**TABLE 1. (Continued)**

<b>Species</b>	<b>BOLD</b>	<b>GenBank</b>	<b>Sample ID</b>	<b>Country</b>
<i>Nola aerugula</i>	GWOTD002-12	KX040562	BC ZSM Lep 48218	Hungary
<i>Nola aerugula</i>	GWOTD003-12	KX041117	BC ZSM Lep 48219	Hungary
<i>Nola aerugula</i>	GWOTD198-12	KX040866	BC ZSM Lep 48414	Germany
<i>Nola aerugula</i>	LEASS852-17		TLMF Lep 22500	Austria
<i>Nola aerugula</i>	LEAST280-17		TLMF Lep 22878	Austria
<i>Nola aerugula</i>	LEATH348-14		TLMF Lep 15560	Italy
<i>Nola aerugula</i>	LEATH352-14		TLMF Lep 15564	Italy
<i>Nola aerugula</i>	LEFIA1248-10	GU828670	MM01776	Finland
<i>Nola aerugula</i>	LEFIC652-10	HM872473	MM04599	Finland
<i>Nola aerugula</i>	LEFID584-10	HM873349	MM06590	Finland
<i>Nola aerugula</i>	LON4192-16		NHMO-DAR-10136	Norway
<i>Nola aerugula</i>	NLLEA1277-14		RMNH.INS.538621	Netherlands
<i>Nola aerugula</i>	NOENO375-17		BC_LSNOE_Lep_00375	Austria
<i>Nola aerugula</i>	ODOPE702-11	KX040566	BC ZSM Lep 50343	Germany
<i>Nola aerugula</i>	TTNFS200-09		FG200	Serbia
<i>Nola aerugula</i>	NOLAE001-21	OL539556	IZBE1137193	Estonia
<i>Nola aerugula</i>	NOLAE002-21	OL539557	IZBE1137194	Estonia
<i>Nola aerugula</i>	NOLAE003-21	OL539558	IZBE1137195	Estonia
<i>Nola aerugula</i>	NOLAE004-21	OL539559	IZBE1137196	Estonia
<i>Nola aerugula</i>	NOLAE005-21	OL539560	IZBE1137197	Estonia
<i>Nola aerugula</i>	NOLAE006-21	OL539561	IZBE1137198	Estonia
<i>Nola aerugula</i>	NOLAE007-21	OL539562	IZBE1137199	Estonia
<i>Nola aerugula</i>	NOLAE008-21	OL539563	TUZ300202	Estonia
<i>Nola aerugula</i>	NOLAE009-21	OL539564	TUZ300203	Estonia
<i>Nola aerugula</i>	NOLAE010-21	OL539565	TUZ300204	Estonia
<i>Nola aerugula</i>	NOLAE011-21	OL539566	TUZ300205	Estonia
<i>Nola aerugula</i>	NOLAE012-21	OL539567	TUZ300206	Estonia
<i>Nola atomosa</i>	NOLAE013-21	OL539568	MNU NE2	South Korea
<i>Nola atomosa</i>	NOLAE014-21	OL539569	MNU NE3	South Korea
<i>Nola cicatricalis</i>	ABOLC094-16		TLMF Lep 20127	Austria
<i>Nola cicatricalis</i>	ABOLC132-16		TLMF Lep 20165	Austria
<i>Nola cicatricalis</i>	DEEUR714-16		TLMF Lep 19144	Austria
<i>Nola cicatricalis</i>	EII636-15		LC01128-26837-F06	Czech Republic
<i>Nola confusalis</i>	ABOLB407-15		KLM Lep 02592	Austria
<i>Nola confusalis</i>	CGUKA089-09		UKLB1H07	United Kingdom
<i>Nola confusalis</i>	CGUKA097-09		UKLB2A03	United Kingdom
<i>Nola confusalis</i>	CGUKB759-09		UKLB19F08	United Kingdom
<i>Nola confusalis</i>	CGUKD203-09	KX043376	UKLB35A10	United Kingdom
<i>Nola confusalis</i>	GBLAA1140-15		BC ZSM Lep 86646	Germany
<i>Nola confusalis</i>	GBLAC1055-13		BC ZSM Lep 77346	Germany
<i>Nola confusalis</i>	GBLAF286-14		BC ZSM Lep 81992	Germany
<i>Nola confusalis</i>	GBLGC180-12		BC ZSM Lep R 21130	Germany
<i>Nola confusalis</i>	GBLGC188-12		BC ZSM Lep R 21138	Germany

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**TABLE 1. (Continued)**

<b>Species</b>	<b>BOLD</b>	<b>GenBank</b>	<b>Sample ID</b>	<b>Country</b>
<i>Nola confusalis</i>	GWOR3846-09	JF415813	BC ZSM Lep 21130	Germany
<i>Nola confusalis</i>	GWOR3851-09	JF415814	BC ZSM Lep 21135	Germany
<i>Nola confusalis</i>	GWOR3854-09	JF415815	BC ZSM Lep 21138	Germany
<i>Nola confusalis</i>	GWOR3975-09	JF415816	BC ZSM Lep 21259	Germany
<i>Nola confusalis</i>	GWOR4211-09	JF415812	BC ZSM Lep 21495	Germany
<i>Nola confusalis</i>	GWORZ089-10	HM913995	BC ZSM Lep 30445	Italy
<i>Nola confusalis</i>	LEAFN669-13		RMNH.INS.544520	Netherlands
<i>Nola confusalis</i>	LEATA182-13		TLMF Lep 09599	Austria
<i>Nola confusalis</i>	LEFIG103-10	HM875783	MM13887	Finland
<i>Nola confusalis</i>	NLLEA1244-14		RMNH.INS.538567	Netherlands
<i>Nola confusalis</i>	NLLEA318-12	KX049324	RMNH.INS.538933	Netherlands
<i>Nola confusalis</i>	NOENO503-17		BC_LSNOE_Lep_00503	Austria
<i>Nola confusalis</i>	PHLAC648-10	JF860215	TLMF Lep 02683	Italy
<i>Nola confusalis</i>	PHLAE329-11	JN284314	TLMF Lep 04644	Austria
<i>Nola confusalis</i>	ABOLD281-16		TLMF Lep 20884	Austria
<i>Nola confusalis</i>	CGUKA295-09	KX043750	UKLB4B02	United Kingdom
<i>Nola confusalis</i>	CGUKA627-09	KX043854	UKLB7F05	United Kingdom
<i>Nola confusalis</i>	CGUKD263-09	KX042890	UKLB35F10	United Kingdom
<i>Nola confusalis</i>	FBLMS297-09	GU654850	BC ZSM Lep 23242	Germany
<i>Nola confusalis</i>	FBLMU519-09	HM391846	BC ZSM Lep 27169	Germany
<i>Nola confusalis</i>	GBLAA1019-14		BC ZSM Lep 83675	Germany
<i>Nola confusalis</i>	GBLAA1020-14		BC ZSM Lep 83676	Germany
<i>Nola confusalis</i>	GBLAC522-13		BC ZSM Lep 78143	Germany
<i>Nola confusalis</i>	GBLGC185-12		BC ZSM Lep R 21135	Germany
<i>Nola confusalis</i>	GWOR3976-09	JF415810	BC ZSM Lep 21260	Germany
<i>Nola confusalis</i>	GWOR4210-09	JF415811	BC ZSM Lep 21494	Germany
<i>Nola confusalis</i>	GWOR503-09	GU655899	BC ZSM Lep 21833	Germany
<i>Nola confusalis</i>	GWOTD208-12	KX041113	BC ZSM Lep 48424	Germany
<i>Nola confusalis</i>	LEATA183-13		TLMF Lep 09600	Austria
<i>Nola confusalis</i>	LEFIC298-10	HM872142	MM03773	Finland
<i>Nola confusalis</i>	LEFID957-10	HM873707	MM07911	Finland
<i>Nola confusalis</i>	LENOA1188-11	KX045073	LN-BD0711	France
<i>Nola confusalis</i>	LENOA1189-11	KX047376	LN-BD0712	France
<i>Nola confusalis</i>	LON4191-16		NHMO-DAR-10135	Norway
<i>Nola confusalis</i>	LON7218-18		KBE 2018484	Croatia
<i>Nola confusalis</i>	PHLAA672-09	HM426073	TLMF Lep 00712	Italy
<i>Nola cristatula</i>	ABOLB411-15		KLM Lep 02596	Austria
<i>Nola cristatula</i>	DEEUR712-16		TLMF Lep 19142	Austria
<i>Nola cristatula</i>	FBLMU065-09	HQ955220	BC ZSM Lep 25575	Germany
<i>Nola cristatula</i>	FBLMU521-09	HM391847	BC ZSM Lep 27171	Germany
<i>Nola cristatula</i>	FBLMV291-09	GU707341	BC ZSM Lep 28271	Germany
<i>Nola cristatula</i>	GBLAC722-13		BC ZSM Lep 77773	Germany
<i>Nola cristatula</i>	GBLAD431-14		BC ZSM Lep 77957	Germany

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**TABLE 1. (Continued)**

<b>Species</b>	<b>BOLD</b>	<b>GenBank</b>	<b>Sample ID</b>	<b>Country</b>
<i>Nola cristatula</i>	GBLAD543-14		BC ZSM Lep 78069	Germany
<i>Nola cristatula</i>	GBLGC186-12		BC ZSM Lep R 21136	Germany
<i>Nola cristatula</i>	GWOR3852-09	JF415817	BC ZSM Lep 21136	Germany
<i>Nola cristatula</i>	GWORE2040-09	HM393511	BC ZSM Lep 22438	Germany
<i>Nola cristatula</i>	GWORK523-09	JF415400	BC ZSM Lep 21853	Germany
<i>Nola cristatula</i>	GWORL464-09	GU686839	BC ZSM Lep 22366	Germany
<i>Nola cristatula</i>	GWORL465-09	GU686840	BC ZSM Lep 22367	Germany
<i>Nola cristatula</i>	GWOTS579-17		BC ZSM Lep 94635	Germany
<i>Nola cucullatella</i>	ABOLB410-15		KLM Lep 02595	Austria
<i>Nola cucullatella</i>	CGUKA296-09	KX043052	UKLB4B03	United Kingdom
<i>Nola cucullatella</i>	CGUKB131-09	MF102756	UKLB13A02	United Kingdom
<i>Nola cucullatella</i>	CGUKB135-09	MF102445	UKLB13A06	United Kingdom
<i>Nola cucullatella</i>	CGUKC005-09	MF102776	UKLB22C07	United Kingdom
<i>Nola cucullatella</i>	CGUKD503-09	KX044188	UKLB38C01	United Kingdom
<i>Nola cucullatella</i>	FBLMU514-09	HM391843	BC ZSM Lep 27164	Germany
<i>Nola cucullatella</i>	FBLMU515-09	HM391844	BC ZSM Lep 27165	Germany
<i>Nola cucullatella</i>	GBLAB621-13	MF102573	BC ZSM Lep 75392	Germany
<i>Nola cucullatella</i>	GBLAD420-14	MF102502	BC ZSM Lep 77946	Germany
<i>Nola cucullatella</i>	GWOR4216-09	JF415818	BC ZSM Lep 21500	Germany
<i>Nola cucullatella</i>	GWOTD196-12	KX041077	BC ZSM Lep 48412	Germany
<i>Nola cucullatella</i>	GWOTL132-13	MF102763	BC ZSM Lep 67063	Germany
<i>Nola cucullatella</i>	IBLAO574-12	MF102442	AOC Lep 00669	Spain
<i>Nola cucullatella</i>	IBLAO739-12	MF102562	AOC Lep 00834	Spain
<i>Nola cucullatella</i>	LEFIC237-10	HM872081	MM03640	Finland
<i>Nola cucullatella</i>	LEFIC788-10	HM872607	MM04925	Finland
<i>Nola cucullatella</i>	LEFIE701-10	HM874422	MM09683	Finland
<i>Nola cucullatella</i>	LENOA1192-11	KX044672	LN-BD0715	France
<i>Nola cucullatella</i>	LON4206-16		NHMO-DAR-10150	Norway
<i>Nola cucullatella</i>	LON452-08	KX048888	NHMO-08102	Norway
<i>Nola cucullatella</i>	NLLEA585-12	KX049047	RMNH.INS.540777	Netherlands
<i>Nola cucullatella</i>	PHLAE252-11	JN284313	TLMF Lep 04472	Austria
<i>Nola cucullatella</i>	RDNMD724-06	MF102744	CNCNoctuoidea13056	Denmark
<i>Nola cucullatella</i>	RDNMD746-06	MF102644	CNCNoctuoidea13078	Denmark
<i>Nola cucullatella</i>	ABOLB717-15		KLM Lep 03377	Austria
<i>Nola cucullatella</i>	CGUKB555-09	MF102488	UKLB17E04	United Kingdom
<i>Nola cucullatella</i>	CGUKC424-09	MF102752	UKLB26G02	United Kingdom
<i>Nola cucullatella</i>	CGUKD373-09	KX043083	UKLB36H01	United Kingdom
<i>Nola cucullatella</i>	GWOTL131-13	MF102701	BC ZSM Lep 67062	Germany
<i>Nola cucullatella</i>	LEATB524-13	MF102661	TLMF Lep 10701	Austria
<i>Nola cucullatella</i>	NOENO502-17		BC_LSNOE_Lep_00502	Austria
<i>Nola dresnayi</i>	GWOTD193-12	KX041014	BC ZSM Lep 48409	France
<i>Nola dresnayi</i>	GWOTD215-12	KX040047	BC ZSM Lep 48431	France
<i>Nola estonica</i>	NOLAE015-21	OL539570	EÕ1484	Estonia

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**TABLE 1. (Continued)**

<b>Species</b>	<b>BOLD</b>	<b>GenBank</b>	<b>Sample ID</b>	<b>Country</b>
<i>Nola estonica</i>	NOLAE016-21	OL539571	EÕ1488	Estonia
<i>Nola estonica</i>	NOLAE017-21	OL539572	EÕ1489	Estonia
<i>Nola estonica</i>	NOLAE018-21	OL539573	EÕ1490	Estonia
<i>Nola estonica</i>	NOLAE019-21	OL539574	EÕ1529	Estonia
<i>Nola estonica</i>	NOLAE020-21	OL539575	EÕ1550	Estonia
<i>Nola estonica</i>	NOLAE021-21	OL539576	EÕ1552	Estonia
<i>Nola estonica</i>	NOLAE022-21	OL539577	TUZ300207	Estonia
<i>Nola estonica</i>	NOLAE023-21	OL539578	TUZ300208	Estonia
<i>Nola estonica</i>	NOLAE024-21	OL539579	TUZ300209	Estonia
<i>Nola estonica</i>	NOLAE025-21	OL539580	TUZ300210	Estonia
<i>Nola estonica</i>	NOLAE026-21	OL539581	TUZ300211	Estonia
<i>Nola estonica</i>	NOLAE027-21	OL539582	TUZ300212	Estonia
<i>Nola estonica</i>	NOLAE028-21	OL539583	TUZ300213	Estonia
<i>Nola estonica</i>	NOLAE029-21	OL539584	TUZ300214	Estonia
<i>Nola estonica</i>	NOLAE030-21	OL539585	TUZ300215	Estonia
<i>Nola estonica</i>	NOLAE031-21	OL539586	TUZ300255	Estonia
<i>Nola estonica</i>	NOLAE032-21	OL539587	TUZ300256	Estonia
<i>Nola estonica</i>	NOLAE033-21	OL539588	TUZ300257	Estonia
<i>Nola estonica</i>	NOLAE034-21	OL539589	TUZ300285	Estonia
<i>Nola estonica</i>	NOLAE035-21	OL539590	TUZ300286	Estonia
<i>Nola estonica</i>	NOLAE036-21	OL539591	TUZ300298	Estonia
<i>Nola estonica</i>	NOLAE037-21	OL539592	TUZ300299	Estonia
<i>Nola estonica</i>	NOLAE038-21	OL539593	TUZ300300	Estonia
<i>Nola estonica</i>	NOLAE039-21	OL539594	TUZ300301	Estonia
<i>Nola estonica</i>	NOLAE040-21	OL539595	TUZ300309	Estonia
<i>Nola estonica</i>	NOLAE041-21	OL539596	MNU NE1	South Korea
<i>Nola estonica</i>	NOLAE042-21	OL539597	MNU NE7	South Korea
<i>Nola estonica</i>	NOLAE043-21	OL539598	MNU NE8	South Korea
<i>Nola estonica</i>	NOLAE044-21	OL539599	MNU NE10	South Korea
<i>Nola fraterna</i>	MAMTJ917-12	KX862465	BIOUG02380-D07	Pakistan
<i>Nola fraterna</i>	GMPBK2144-18		BIOUG40824-A02	Pakistan
<i>Nola fraterna</i>	GMPBK2154-18		BIOUG40824-A12	Pakistan
<i>Nola fraterna</i>	GMPBK2215-18		BIOUG40824-G01	Pakistan
<i>Nola fraterna</i>	GMPBY012-18		BIOUG41154-F06	Pakistan
<i>Nola fraterna</i>	GMPBZ006-18		BIOUG41266-C10	Pakistan
<i>Nola fraterna</i>	GMPBZ027-18		BIOUG41266-E07	Pakistan
<i>Nola karelica</i>	GWOTD209-12	KX040278	BC ZSM Lep 48425	Finland
<i>Nola karelica</i>	LEFIF276-10	HM874969	MM11058	Finland
<i>Nola karelica</i>	LEFIF275-10	HM874968	MM11057	Finland
<i>Nola karelica</i>	LEFIF277-10	HM874970	MM11059	Finland
<i>Nola karelica</i>	LEFIG991-10	HQ570421	MM15855	Finland
<i>Nola karelica</i>	LON918-12	KX047688	RV090	Norway
<i>Nola squalida</i>	GWOTD199-12	KX040254	BC ZSM Lep 48415	Spain

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TABLE 1. (Continued)

Species	BOLD	GenBank	Sample ID	Country
<i>Nola subchlamydula</i>	FBLMW301-10	HQ563546	BC ZSM Lep 37402	Germany
<i>Nola subchlamydula</i>	GWOSF580-10	KX040184	BC ZSM Lep 41576	Spain
<i>Nola subchlamydula</i>	LEATD009-13		TLMF Lep 12656	Italy
<i>Nola subchlamydula</i>	LEATD010-13		TLMF Lep 12657	Italy
<i>Nola subchlamydula</i>	LEATJ095-15		TLMF Lep 18525	Italy
<i>Nola subchlamydula</i>	LON7216-18		KBE 2018482	Croatia
<i>Nola subchlamydula</i>	LON7217-18		KBE 2018483	Croatia
<i>Nola subchlamydula</i>	PHLAF585-11		TLMF Lep 05755	Italy
<i>Nola subchlamydula</i>	PHLAG082-12	KX046876	KLM Lep 00272	Italy
<i>Nola thymula</i>	GWOTD203-12	KX040180	BC ZSM Lep 48419	Spain

## Results

### Morphological study

Analysis of the external and genitalic characters of *N. aerugula aerugula*, *N. aerugula atomosa* and the third, unknown taxon revealed that there are constant differences suggesting these taxa to be separate species. Below, we describe the unknown taxon as *Nola estonica* Õunap **sp. nov.** and reinstate *Glaphyra atomosa* Bremer, 1861 from a subspecies of *Nola aerugula* (Hübner, 1793) to species level as *Nola atomosa* (Bremer, 1861) **stat. rev.**

### *Nola estonica* Õunap **sp. nov.**

(Figures 1–18, 43–44, 49–51, 55)

### Type material

Holotype: ♀, ESTONIA, Piusa Railway Station, at light, 57°50'20.9"N 27°28'15.0"E, 03.08.2020, leg. E. Õunap, TUZ300299.

Paratypes, 82♂♂, 53♀♀.

### ESTONIA

1♂, Põlvamaa, Värskaa, 57°58'N 27°37'E, 19.09.2001, leg. T. Ruben/A. Lindt, IZBE1137190.

1♂, Põlvamaa, Korela, 57°53'N 27°44'E, 01.-15.07.2010, leg. T. Ruben, IZBE1137191.

1♂, Värskaa, Õrsava, 57°56'46"N 27°37'54"E, 19.07.2011, leg. T. Tammaru, DNA voucher EÕ1488, RCTT.

1♀, Piusa Railway Station, 57°50'30"N 27°27'26"E, 20.07.2011, leg. T. Tammaru, DNA voucher EÕ1489, RCTT.

1♂, Harjumaa, Mustjõe, 59°19'N 25°28'E, 01.-19.07.2012, leg. T. Ruben, IZBE1137192.

1♀, Mäe-Palo, 57°37'06"N 27°07'26.5"E, 04.07.2012, leg. E. Õunap, DNA voucher EÕ1490, RCEÕ.

1♂, Piusa, 57°50'30"N 27°27'18"E, 03.08.2017, leg. I. Taal & T. Tasane, RCIT.

1♂, Karilatsi 1 km W, 58°07'25"N 26°54'05"E, 07.09.2018, leg. T. Tammaru, DNA voucher EÕ1484, RCTT.

1♂, Parmu, at light, 57°33'53.3"N 27°19'15.4"E, 20.07.2020, leg. E. Õunap, RCEÕ.

6♂♂, 2♀♀, Piusa Railway Station, 57°50'21"N 27°28'14"E, 27.07.2020, leg. I. Taal & A. Truuverk (incl. 2♂♂, DNA vouchers EÕ1550, EÕ1552, used for genetic study), RCIT.

5♂♂, 5♀♀, Piusa Railway Station, 57°50'21"N 27°28'14"E, 27.07.2020, leg. I. Taal & A. Truuverk, RCAT.

48♂♂, 30♀♀, Piusa Railway Station, 57°50'21"N 27°28'14"E, 27.07.2020, leg. I. Taal & A. Truuverk, 2♂♂, 2♀♀ dissected, TUZ300207–TUZ300284.

3♂♂, 3♀♀, Piusa Railway Station, at light, 57°50'20.9"N 27°28'15.0"E, 03.08.2020, leg. E. Õunap (incl. 1♂, DNA voucher EÕ1529, used for genetic study) RCEÕ.

13♂♂, 11♀♀, Piusa Railway Station, at light, 57°50'20.9"N 27°28'15.0"E, 03.08.2020, leg. E. Õunap, 2♂♂, 5♀♀ dissected, TUZ300285–TUZ300298, TUZ300300–TUZ300309.



## Other material examined

### RUSSIA

1 ♀, Primorsky region, Kedrovaja Pad, V, L[ight], 43°06'N 131°29'E, 2-17.08.1997, leg. Laanetu & Viidalepp, dissected, IZBE0106558.

1 ♂, Amurskaja region, Svobodnenski district, Iverskii zakaznik, 18.06.-01.07.2010, leg. A. Barbarich, A. Streltsov, P. Osipov, dissected, slide Matov 0589, ZISP.

### SOUTH KOREA

6 ♀♀, Mt. Samaksan, Deokduwon-ri, Seo-myon, Chuncheon, Gangwon-do Province, at light, 37°50'11"N 127°37'30"E, 25.06.2016, leg. S. S. Kim, 2 ♀♀ dissected, MNU genital slides no. 1172 and 1173, MNU 5-MNU 10.

1 ♂ Haesan, Hwacheon-gun, Gangwon-do Province, at light, 38°11'15"N 127°47'18"E, 24.06.2017, leg. S. S. Kim, dissected, MNU genital slide no. 1170, MNU NE1.

## Description

**External morphology.** Wingspan 15.2-18.1 (average  $16.4 \pm 1.0$  SD,  $n = 18$ ) mm in males 15.4-19.0 (average  $17.2 \pm 1.0$  SD,  $n = 16$ ) mm in females. **Head** white, antennae covered with white scales. Male antennae bipectinate, bearing numerous sensilla on the ventral side. The length of sensilla exceed the diameter of the flagellum. Female antennae filiform. Labial palpi porrect, elongated, more than two times longer than the diameter of the eye, intermixed with light and dark scales on the lateral side, but only white scales present on the medial side. Proboscis present. **Thorax** white. **Forewing** elongated, apex rounded. Upperside white. Three tufts of raised scales present along the anterior edge of the cell, the medial and distal tuft always containing at least some dark scales, the proximal tuft sometimes completely white. Subbasal line present as a brown costal blotch, sometimes completely absent. Antemedial line, if present, usually brown, rarely black, jagged, forming an irregular curve towards the termen. A large brown blotch sometimes present on costa proximal to the antemedial line. Medial line absent. Postmedial line brown, rarely black, parallel to costa in the subcostal region, but turns towards inner margin at an acute angle on  $R_5$ . Postmedial line almost straight between  $R_5$  and inner margin, with clear darker spots on veins, sometimes proximally accompanied by a light brown band. Subterminal line undulating, light brown to light grey, sometimes completely absent. Terminal line light brown to light grey, sometimes hardly visible, sometimes interrupted by a row of white or yellowish dots on veins. Fringes usually unicolourous, white, light beige or light grey, rarely slightly lighter on veins. Pattern reduced in many specimens, sometimes represented only by a few dark scales on subcostal hair tufts, and as a row of small dark dots referring to postmedial line. Underside unicolourous dark grey in males, white with most veins dark grey and some grey scales diffused between the veins in females. **Hindwing** with evenly curved termen, apex rounded. Upperside white, subcostal region light grey. In darker specimens wings gradually darkening from white to light grey in subterminal area. Discal spot very weak, formed by a small number of dark scales. Terminal line light grey, interrupted by a row of white or yellowish dots on veins, sometimes hardly visible. Fringes white, light beige or light grey. Underside white, with diffused grey scales mostly present on the anterior half of the wing and on the subterminal area. Discal spot grey. **Legs** white or grey, darker in males than in females, one pair of tibial spurs present in midlegs, and two pairs in hindlegs of both sexes. **Abdomen** dorsally light yellowish grey, posterior edges of segments visible as a row of lighter scales. Ventral side of the abdomen light yellowish grey suffused with small number of black scales.

**Male genitalia.** Uncus absent. Tegumen narrow, 1.5 times longer than vinculum. Saccus short and very wide, with rounded tip. Scaphium with two extremely long, parallel, stick-like, sclerotized structures. Valva long, bilobed, costa and ventral margin heavily sclerotized, rounded at both tips. Tip of the ventral lobe of valva extended to a tiny hook. Harpe strong, triangular, spine-like, with a pointed tip. Editum present as a rounded protuberance bearing a number of tiny papilles carrying thin setae, positioned close to base of costa. Transtilla narrow, heavily sclerotized. Juxta plate-like, laterally extended as two arms to dorsal side. Aedeagus almost straight, three times longer than wide, apex ventrally elongated as a thin triangular slat, coecum absent. Vesica straight, slightly wider and longer than aedeagus, with one cornutus. Cornutus short and wide, with a prominent central ridge extending beyond its posterior edge. Eighth tergite with two narrow anterior projections located wide apart from each other, posterior edge of the heavily sclerotized area rounded.

**Female genitalia.** Ovipositor short, very wide; posterior apophyses approximately as long as ovipositor. Anterior apophyses short, their length approximately 2/3 of the length of posterior apophyses. Ostium bursae heavily sclerotized, genital orifice oval, wider than long. Antrum region very short, membranous. Posterior part of ductus bursae moderately sclerotized, the sclerotized region wider than long, its length about 1/5 of the total length of ductus bursae. Middle part of ductus bursae membranous, two times longer than wide, the membrane slightly wrinkled, sometimes with irregular patches of sclerotization. Anterior part of ductus bursae heavily sclerotized, dilated, sclerotization present as irregular longitudinal folds. Corpus bursae ovoid, elongate, 2.5 times longer than wide, with one signum. The posterior part of signum bursae bearing a heavily sclerotized thorn pointing towards the lumen of corpus bursae.

**Diagnosis.** *N. estonica* (Figures 1–18) differs from *N. atomosa* by its rather straight postmedial line which is darker on veins and often divided into a row of dark spots. The postmedial line of *N. atomosa* (Figures 19–30) is strongly undulating and almost unicolourous. Even in very light specimens of *N. atomosa* the postmedial line is not interrupted into separate spots located on veins. In *N. atomosa*, fringes are chequered, being white on tips of the veins, and light grey between the veins. Male genitalia of *N. estonica* (Figures 43ab, 44ab) and *N. atomosa* (Figures 45ab, 46ab) are very similar and cannot be used for reliable identification. However, the 8th tergite of *N. estonica* has narrow anterior projections that are situated apart from each other (Figures 43c, 44c), while that of *N. atomosa* usually has wide anterior projections that are located much closer to each other (Figures 45c, 46c). Females of *N. estonica* can easily be separated from *N. atomosa* by genitalia dissection, as this species has only one signum in bursa copulatrix, which is located ventrolaterally (Figures 49–51). *N. atomosa* has an additional smaller signum on the opposite side of bursa copulatrix (Figures 52–53), though the latter may be small, almost transparent and therefore hard to notice. A fine detail characteristic of *N. estonica* is an inward-pointing thorn on the posterior edge of signum (Figure 55). Though the posterior edge of the larger signum of *N. atomosa* is also bent inwards (Figure 56), it does not form a distinct narrow thorn. The sclerotized posterior part of ductus bursae is wider than long in *N. estonica*, but almost rectangular in *N. atomosa*.

*N. aerugula* (Figures 31–42) can usually be separated from *N. estonica* by its much darker colouration. Even in very light specimens of *N. aerugula* the ground colour of forewings is often yellowish, not white, as opposed to the pure white ground colour of *N. estonica*. Though the postmedial line of *N. aerugula* is sometimes almost as straight as that of *N. estonica*, it is not distinctly darker on veins nor divided into a row of spots. The hindwings of *N. aerugula* are almost unicolourous and darker than those of *N. estonica*: dark grey in the darkest specimens, light grey in the lightest ones. Male genitalia of *N. aerugula* (Figures 47a, 48a) differ from those of *N. estonica* (Figures 43a, 44a) by shorter vinculum, which has length/width ratio of about 0.5 (as opposed to at least 0.6 in *N. estonica*), and by very short and narrow saccus. There are, however, no differences in the shape of the aedeagus of *N. estonica* (Figures 43b, 44b) and *N. aerugula* (Figures 47b, 48b). The 8th tergite of *N. estonica* has narrow anterior projections that are situated apart from each other (Figures 43c, 44c), while that of *N. aerugula* usually has wide anterior projections that are located much closer to each other (Figures 47c, 48c). Females of *N. estonica* can easily be separated from *N. aerugula* by genitalia dissection, as this species has only one ventrolateral signum on bursa copulatrix (Figures 49–51), but *N. aerugula* has an additional smaller signum on the opposite side of bursa copulatrix (Figure 54). However, the latter may be small, almost transparent and therefore hard to notice. The larger signum of *N. aerugula* is often just a flat patch of sclerotization on the wall of bursa copulatrix which is thicker on its posterior edge (Figure 57), but sometimes its posterior edge is bent inwards. Even in the latter case it does not form a distinct narrow inward-pointing thorn which is characteristic to *N. estonica*. The sclerotized posterior part of ductus bursae is wider than long in *N. estonica*, but almost rectangular in *N. aerugula*.

**Note.** Though the hitherto known European and Far Eastern populations of *N. estonica* are separated by at least 6000 kilometers, we have not found any consistent differences in their morphology. The South Korean and Russian specimens fit well within the intraspecific variation of the Estonian material.

**Biology.** *N. estonica* appears to be locally common in southeastern Estonia. The majority of the type series were collected from a dry, narrow meadow stripe in the railway corridor that penetrates a landscape dominated by dry pine forest on sandy soil. Whether the species prefers woodland or open habitat is yet unknown, as though the moths were captured on a meadow, they may have flown to light from the nearby forest only 15–20 meters away. In South Korea, the moths were collected in mountainous woodland with mixed coniferous and deciduous trees, and the single contemporary specimen from Russian Far East was taken from mixed forest adjacent to large xerophytic meadows. Most of the hitherto known specimens have been collected in July and early August, but two records from

September suggest that partial second brood may exist. Other details of the life cycle and larval foodplants are not known.

**Etymology.** The name *estonica* refers to Estonia, as the species was first discovered in this country, which is also the area of origin of the type series.

### ***Nola atomosa* (Bremer, 1861) stat. rev.**

(Figures 19–30, 45–46, 52–53, 56)

*Glaphyra atomosa* Bremer, 1861, Bulletin de l'Académie Impériale des sciences de St-Petersbourg 3: 491. LT: Amur, Russian Federation

= *Nola candidalis* Staudinger, 1892, Mémoires sur les Lépidoptères 6: 258. TL: Amur, Russian Federation **syn. nov.**

= *Nola shin* Inoue, 1982, Moths of Japan: 661. TL: Shibechea, Kushiro, Hokkaido, Japan **syn. nov.**

### **Type material examined.**

Lectotype of *Glaphyra atomosa* Bremer, 1861, hereby designated: ♂, RUSSIA, dark brown label „Ussuri“, „Maack“, white label „coll. Acad. Petrop.“, red label „*Glaphyra atomosa* Bremer, 1861, LECTOTYPE, des. Matov & Ōunap 2021“, white label „slide Matov 0588“, ZISP.

Paralectotypes, hereby designated: 1♂, 1♀, RUSSIA, dark brown label „Ussuri“, „Maack“, white label „coll. Acad. Petrop.“, red label „*Glaphyra atomosa* Bremer, 1861, PARALECTOTYPE, des. Matov & Ōunap 2021, ZISP.

### **Other material examined**

NORTH KOREA

1♂, [North] Korea, 20/6 [20.06.][1903?], leg. Herz, ZISP.

RUSSIA

1♂, Primorsky region, Ussuriisk (as Nikolsk-Ussuriisk on the label), Baranovskij polygon, 1913, leg. Andrievskii, ZISP.

2♂♂, Primorsky region, Spassk district, Yakovlevka, 13.07.1926, leg. Djakonov & Filipjev, ZISP.

1♂, Primorsky region, Spassk district, Yakovlevka, 14.07.1926, leg. Djakonov & Filipjev, dissected, slide Matov 0590, ZISP.

2♂♂, 2♀♀, Primorsky region, Spassk district, Yakovlevka, 17.07.1926, leg. Djakonov & Filipjev, ZISP.

1♂, 2♀♀, Primorsky region, Spassk district, Yakovlevka, 23.07.1926, leg. Djakonov & Filipjev, ZISP.

1♀, Primorsky region, Spassk district, Yakovlevka, 29.07.1926, leg. Djakonov & Filipjev, ZISP.

1♂, Primorsky region, Suchan, 26.07.1928, leg. Kurentsov, ZISP.

1♀, Primorsky region, Suchan, 27.07.1928, leg. Kurentsov, ZISP.

5♂♂, Primorsky region, Vinogradovka, 19.-20.07.1929, leg. Djakonov & Filipjev, 1♂ dissected, slide Matov 0592, ZISP.

1♀, Primorsky region, vic. Vladivostok, Chernaya Rechka, 02.08.1931, leg. Moltrecht, ZISP.

1♂, Primorsky region, Suchansk, rudnik, 16.08.1933, leg. Palshikov, dissected, slide Matov 0591, ZISP.

1♂, Primorsky region, Khasan, at light, 14.07.1959, leg. A. Zemlina, ZISP.

1♀, Primorsky region, Ugolnaja Ussuri region, 31.07.1960, leg. L. Anufriev, ZISP.

1♂, Primorsky region, Kaimanovka, 23.07.1961, leg. A. Ivanov, ZISP.

1♂, 2♀♀, Khabarovsk, leg. Graeser, ex coll. Dieckmann, 1♂ dissected, slide Matov 0593, ZISP.

1♂, Khabarovsk, L[ight], 07.07.1973, leg. Viidalepp, Kullman, Tiivel, IZBE0106553.

1♀, Primorsky region, Valley of Izvilinka River, 43°54'N 133°54'E, 08.07.1976, leg. J. Viidalepp, IZBE0106555.

1♀, Primorsky region, Tshugujevsk district, Valley of Izvilinka River, DRL, 43°48'N 133°54'E, 08.-09.07.1976, leg. Metsaviir, Viidalepp, dissected, slide J. Viidalepp 2402, IZBE0106556.

1♀, Primorsky region, Kedrovaja Pad, V, L[ight], 43°06'N 131°29'E, 02.-17.08.1977, leg. Talve & Metsaviir, dissected, IZBE0106557.

1♂, Khabarovsk region, Nelma, 47°40'N 139°10'E, 16.-26.07.1977, leg. Viidalepp, Laanetu, Talve, IZBE0106554.

1♀, Kunashir Island, Alekhino, 43°55'N 145°32'E, 19.07.1980, leg. T. Ruben, dissected, slide J. Viidalepp 3372, IZBE0106559.

1♂, Amur region, Svobodnenski district, Iverskii zakaznik, 18.06.-01.07.2010, leg. A. Barbarich, A. Streltsov, P. Osipov, ZISP.

#### SOUTH KOREA

1♂, 2♀♀, Jindong-ri, Inje-gun, Gangwon-do Province, at light, 37°59'31"N 128°29'38"E, 20.06.2017, leg. S. S. Kim, 1♀ dissected, MNU genital slide no. 1171, MNU 2-MNU 4.

**Diagnosis.** Wingspan 15-19 mm. *N. atomosa* (Figures 19-30) is best characterized by the heavily undulating postmedial line and chequered fringes of the forewing, though the most light-coloured specimens (Figure 30) can be almost without any pattern. Further morphological details allowing to distinguish it from *N. estonica* have been discussed above. *N. aerugula* (Figures 31–42) has a straighter postmedial line than *N. atomosa*, and the fringes of its forewings are almost always completely unicolourous. The hindwing of *N. atomosa* is light, unicolourous or gradually darkening in subterminal area, but never completely dark as that of many specimens of *N. aerugula*. Male *N. aerugula* (Figures 47a, 48a) can be separated by its shorter vinculum and saccus but the shape of aedeagus and 8th tergite of this species (Figures 47bc, 48bc) are similar to those of *N. atomosa* (Figures 45bc, 46bc). Females of both *N. atomosa* (Figures 52, 53) and *N. aerugula* (Figure 54) have two signa in the opposite sides of bursa copulatrix. The shape of the larger (ventrolateral) signum, however, is variable. In *N. atomosa* the posterior edge of the larger signum bends inwards towards the lumen of bursa copulatrix (Figure 56), though it does not form as distinct thorn as in *N. estonica*. In many specimens of *N. aerugula* both signa are just flat patches of sclerotization on the bursa wall, though the larger one is somewhat thickened on its posterior edge (Figure 57). However, we have also observed that in some specimens of *N. aerugula* the posterior edge of the larger signum is also bent inwards. Therefore the shape of the signum alone should not be used to distinguish this species from *N. atomosa*.

**Note.** *N. atomosa* has been considered to be an eastern subspecies of *N. aerugula* (Oh 2001, Matov 2019). Consequently, additional eastern Palaearctic taxa *Nola candidalis* Staudinger, 1892 and *Nola shin* Inoue, 1982 which are conspecific with *N. atomosa*, have been treated earlier as synonyms of *N. aerugula* (e. g. Oh 2001, Fibiger *et al.* 2009, Sasaki 2011, Hacker *et al.* 2012). As *N. atomosa* is reinstated as a valid species in this article, neither *N. candidalis* or *N. shin* can no longer be treated as synonyms of *N. aerugula*. Therefore, we hereby establish the priority of *N. atomosa* over junior synonyms as follows: *Nola atomosa* (Bremer, 1861) = *Nola candidalis* Staudinger, 1892 **syn. nov.** and *Nola shin* Inoue, 1982 **syn. nov.**

### *Nola aerugula* (Hübner, [1793])

(Figures 31–42, 47–48, 54, 57)

*Phalaena Bombyx aerugula* Hübner, [1793], Sammlung Auserlesener Vögel und Schmetterlinge, mit ihrem Namen Herausgegeben auf Hundert nach der Natur Ausgemalten Kupfern: 11, pl. 61. LT: [Europe]

= *Pyralis centonalis* Hübner, 1796, Sammlung Europäischer Schmetterlinge 6: pl. 3, fig. 15. LT: [Europe]

= *Hercyna scabralis* Eversmann, 1842, Bulletin de la Société Impériale des Naturalistes de Moscou, 15: 562. LT: Russia

= *Nola littoralis* Paux, 1901, Bulletin scientifique de la France et de la Belgique 35: 479. LT: Dunkerque, France

#### Type material examined

Holotype of *Celama centonalis* f. *alfkeni* Warnecke, 1938: ♂, GERMANY, white label „♂“, white label „Borkum, Auf. VII. 1924, am licht“, red label „n. f. Alfkeni, Warnecke, Type“, white label „photographiert, Warnecke 1933“, white label „Slg-G Warnecke, Eing Nr 5 1949“, white label „ZMH 833303“, ZMH.

Paratypes of *Celama centonalis* f. *alfkeni* Warnecke, 1938: ♂, GERMANY, white label „26.8.35, St.“, white label „Insel Borkum, 1935 Struve“, red label „f. Alfkeni Warn., Paratype“, white label „Sammlung, G. Warnecke, Eing. Nr. 5, 1949“, white label „Slg-G Warnecke, Eing Nr 5, 1949“, white label „ZMH 833304“, ZMH; ♂, GERMANY, white label „7.7.34, Bl“, white label „Ins. Borkum, 1935 Struve“, red label „f. Alfkeni Warn., Paratype“, white label „Sammlung, G. Warnecke, Eing. Nr. 5, 1949“ white label „ZMH 833305“, ZMH.

Lectotype of *Hercyna scabralis* Eversmann, 1842: ♀, RUSSIA, white label „Simb.“ [Simbirsk], white label “coll. Eversmann”, red label “LECTOTYPUS ♀, *Hercyna scabralis*, Eversmann, 1942, design. S. Yu. Sinev, 2015”, ZISP.

Paralectotypes of *Hercyna scabralis* Eversmann, 1842: 15♂♂, 1♀, RUSSIA, white label “Spask”, ZISP; 1♀, RUSSIA,



white label “Orb” [Orenburg], ZISP; 1 ♂, RUSSIA, white label “Kas” [Kazan], ZISP; 1 ♂, RUSSIA, white label “Menzelinsk”, ZISP; 1 ♂, 1 ♀, no label with geographic name, ZISP.

### Other material examined

#### ESTONIA

- 1 ♀, 19.06.1881, leg. F. Sintenis, IZBE1136185.  
1 ♀, sph. Sonda, 03.07.1937, leg. D. Kuskov, dissected, IZBE1136200.  
1 ♂, Kohala, 59°25'N 26°32'E, 13.07.1991, leg. E. Mäe, TUZ407496.  
1 ♀, Teenuse, 58°48'52.2"N 24°11'23.3"E, L[ight], 12.07.1992, leg. R. Ülemaante, dissected, IZBE1136225.  
1 ♂, Kohala, 59°25'N 26°32'E, 18.07.1992, leg. E. Mäe, TUZ407489.  
1 ♂, Kohala, 59°25'N 26°32'E, 23.07.1992, leg. E. Mäe, TUZ407494.  
1 ♀, Kohala, 59°25'N 26°32'E, 16.07.1994, leg. E. Mäe, TUZ407485.  
1 ♀, Vanajärvesoo north to Lake Tüandre, 57°58'16"N 25°36'57"E, 06.07.1995, leg. E. Õunap, dissected, RCEÕ.  
1 ♂, Verhulitsa, at light, 14.07.2003, leg. E. Õunap, RCEÕ.  
1 ♀, Verhulitsa, at light, 21.07.2004, leg. E. Õunap, RCEÕ.  
3 ♂♂, Nigula Nature Reserve, light trap, 58°01'01.7"N 24°43'08.7"E, 24.-30.06.2019, leg. M. Leivits, 1 ♂ dissected, IZBE1137193–IZBE1137195.  
1 ♂, Piilse, light trap, 59°14'10.5"N 26°59'49.2"E, 14.-20.07.2019, leg. A. Täpsi, dissected, IZBE1137196.  
1 ♂, Nigula Nature Reserve, light trap, 58°01'01.7"N 24°43'08.7"E, 22.-28.06.2020, leg. M. Leivits, dissected, IZBE1137197.  
1 ♂, Piilse, light trap, 59°14'10.5"N 26°59'49.2"E, 28.06.-04.07.2020, leg. A. Täpsi, dissected, IZBE1137198.  
4 ♂♂, 1 ♀, Piusa Railway Station, 57°50'21"N 27°28'14"E, 27.07.2020, leg. I. Taal & A. Truuverk, TUZ300202–TUZ300206.  
1 ♀, Piilse, light trap, 59°14'10.50"N 26°59'49.20"E, 02.-08.08.2020, leg. A. Täpsi, dissected, IZBE1137199.

#### GEORGIA

- 1 ♂, Georgia, Poti, 06.08.1939, leg. G. Lvov, ZISP.

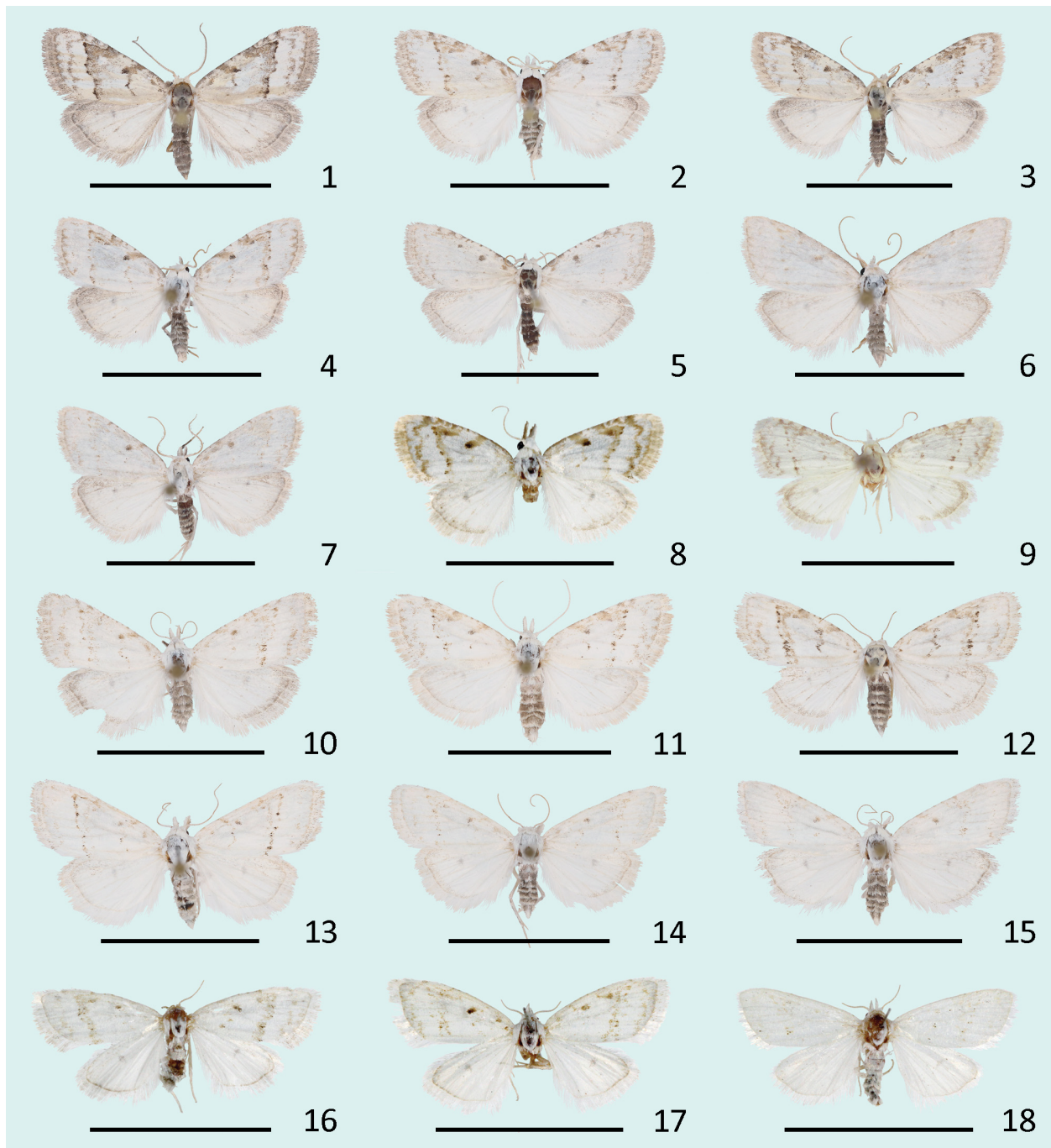
#### RUSSIA

- 1 ♀, Irkutsk Oblast, 12.07.1916, leg. Markova, ZISP.  
1 ♂, St. Petersburg vic., Privetninskoe, 05.07.1917, leg. A. Djakonov, ZISP.  
1 ♀, St. Petersburg vic., Sablino, 09.07.1922, leg. A. Djakonov, ZISP.  
2 ♂♂, Krasnojarsk region, Minusinsk district, 06.07.1924, ZISP.  
1 ♂, Krasnojarsk region, Minusinsk district, 14.07.1926, ZISP.  
1 ♂, Krasnojarsk region, Enisejsk, 23.07.1930, leg. Kvetmanov, ZISP.  
1 ♂, Kemerovo region, Vaganovo, 26.06.1955, leg. Falkovitsh, ZISP.  
1 ♂, 1 ♀, Sverdlovsk, 01.07.1931, leg. S. Tshetverikov, ZISP.  
1 ♂, Sverdlovsk, 05.07.1931, leg. S. Tshetverikov, ZISP.  
1 ♂, Tuva Republic, Kyzyl, Kaa-Hem, L[ight], 08.07.1972, leg. Ruben, Viidalepp, IZBE0106551.  
1 ♂, Tuva Republic, Kyzyl, Kaa-Hem, L[ight], 01.08.1972, leg. Ruben, Viidalepp, IZBE0106552.  
1 ♂, Leningrad region, Dubocki station 28.06.1983, leg. V. Mironov, ZISP.  
1 ♀, Altai region, Zonalnoje, on tree trunk, 20.07.1989, leg. T. Tammaru, RCTT.  
1 ♂, [Saint-Petersburg], Karelian isthmus, Komarovo, Sphagnum bog, 27.06.[19]53, [leg. I. Kozhanchikov], ZISP.

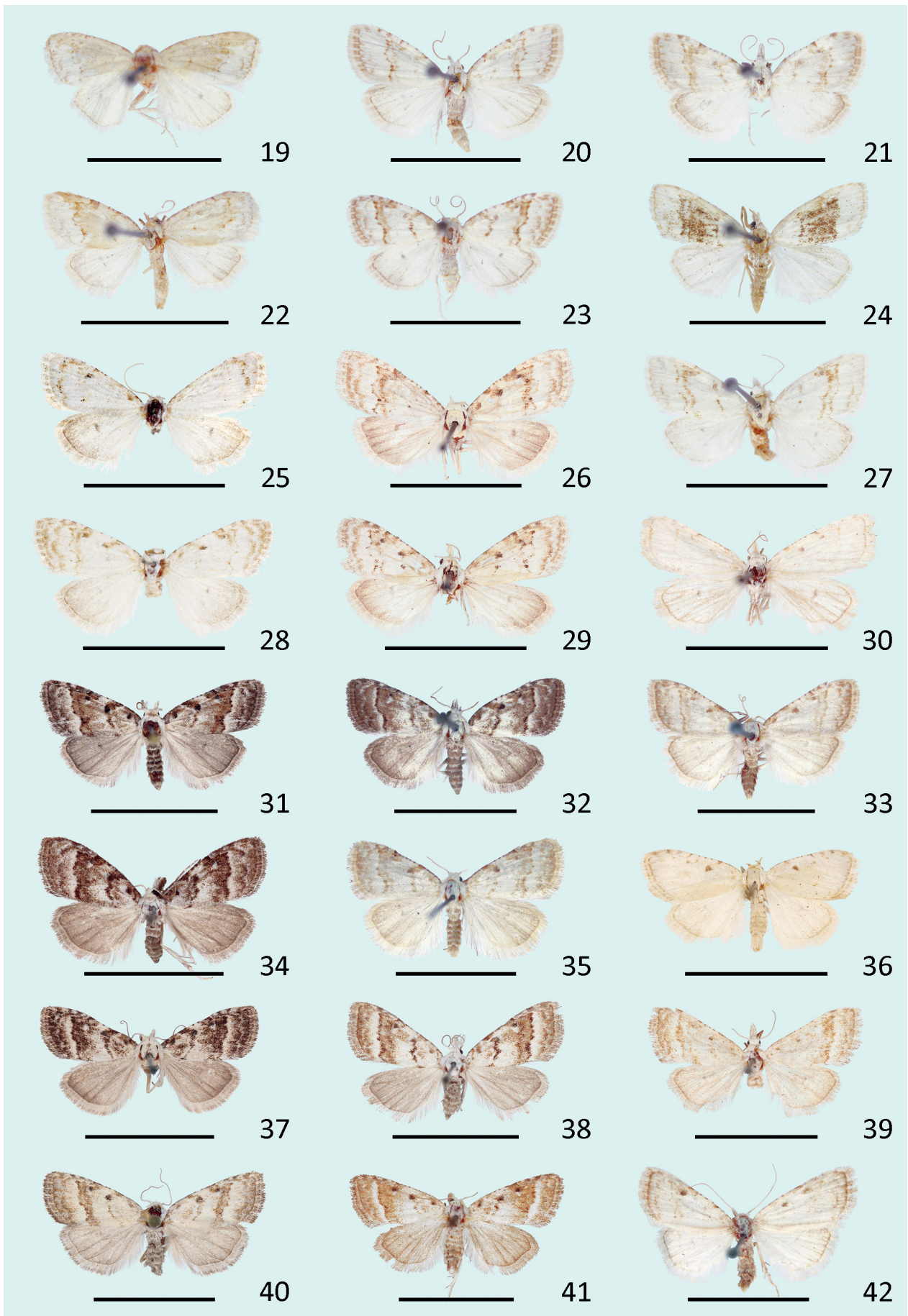
**Diagnosis.** Wingspan 16-19 mm. In most cases, *N. aerugula* can easily be separated from *N. estonica* and *N. atomosa* by its well expressed dark pattern on the forewings and unicolourous grey hindwings. Further morphological details allowing to distinguish these species have been discussed above.

**Note.** *N. aerugula* is known for rather extensive variation in the intensity of markings on the wings (de Freina & Witt 1987, Fibiger *et al.* 2009), ranging from individuals with almost completely pale yellowish white forewings that have hardly any markings (f. *alfkeni* Warnecke, 1938) to very dark specimens with entirely brown forewings (f. *fumosa* Berger, 1918). Interestingly, the lighter forms seem to be dominant in the eastern part of its range, as all Siberian specimens of *N. aerugula* in coll. ZISP are rather light.





**FIGURES 1–18.** External habitus of *Nola estonica* sp. nov. Dorsal view. 1. ♂, PARATYPE, Estonia, Piusa Railway Station, 27.07.2020, TUZ300207 (barcoded). 2. ♂, PARATYPE, Estonia, Piusa Railway Station, 27.07.2020, RCIT (barcoded, sample ID EÕ1550). 3. ♂, PARATYPE, Estonia, Piusa Railway Station, 27.07.2020, TUZ300208 (barcoded). 4. ♂, PARATYPE, Estonia, Parmu, 20.07.2020, RCEÕ. 5. ♂, PARATYPE, Estonia, Piusa Railway Station, 27.07.2020, RCIT. 6. ♂, PARATYPE, Estonia, Piusa Railway Station, 03.08.2020, RCEÕ. 7. ♂, PARATYPE, Estonia, Piusa Railway Station, 03.08.2020, RCEÕ. 8. ♂, South Korea, Haesan, 26.06.2017, coll MNU (barcoded) 9. ♂, Russia, Ivesrkii zakaznik, 18.06.-01.07.2010, coll. ZISP (dissected). 10. ♀, HOLOTYPE, Estonia, Piusa Railway Station, 03.08.2020, TUZ300299, (barcoded, dissected). 11. ♀, PARATYPE, Estonia, Mäe-Palo, 04.07.2012, RCEÕ (barcoded, sample ID EÕ1490). 12. ♀, PARATYPE, Estonia, Piusa Railway Station, 27.07.2020, TUZ300255 (barcoded). 13. PARATYPE, Estonia, Piusa Railway Station, 03.08.2020, TUZ300309, (barcoded). 14. ♀, PARATYPE, Estonia, Piusa Railway Station, 03.08.2020, RCEÕ. 15. ♀, PARATYPE, Piusa Railway Station, 03.08.2020, TUZ300308. Estonia, 16. ♀, South Korea, Mt. Samaksan, 25.06.2016, coll MNU (barcoded). 17. ♀, South Korea, Mt. Samaksan, 25.06.2016, coll MNU (barcoded, dissected, MNU genital slide no. 1172). 18. ♀, South Korea, Mt. Samaksan, 25.06.2016, coll MNU (barcoded). Scale: 1 cm.





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**FIGURES 19–42.** External habitus of *Nola* spp. Dorsal view. 19–30. *Nola atomosa* (Bremer) **stat. rev.** 31–42. *Nola aerugula* (Hübner). 19. ♂, LECTOTYPE of *Glaphyra atomosa* Bremer, Russia, Ussuri, coll. ZISP (dissected, slide Matov 0588). 20. ♂, Russia, Yakovlevka, 13.07.1926, coll. ZISP. 21. ♂, Russia, Yakovlevka, 14.07.1926, coll. ZISP (dissected, slide Matov 0590). 22. ♂, PARALECTOTYPE of *Glaphyra atomosa* Bremer, Russia, Ussuri, coll. ZISP. 23. ♂, Russia, Vinogradovka, 19.-20.07.1929, coll. ZISP. 24. ♂, Russia, Yakovlevka, 23.07.1926, coll. ZISP. 25. ♀, South, Korea, Jindong-ri, 20.06.2017, coll. MNU (barcoded, dissected, MNU genital slide no. 1171). 26. ♀, Russia, Valley of Izvilinka River, 08.-09.07.1976, IZBE0106556 (dissected, slide J. Viidalepp 2402). 27. ♀, Russia, Yakovlevka, 29.07.1926, coll. ZISP. 28. ♀, South, Korea, Jindong-ri, 20.06.2017, coll. MNU (barcoded). 29. ♀, ♀, Russia, Valley of Izvilinka River, 08.-07.1976, IZBE0106555. 30. ♀, Russia, Alekhino, 19.07.1980, IZBE0106559 (dissected, slide J. Viidalepp 3372). 31. ♂, Estonia, Nigula Nature Reserve, 24.-30.06.2019, IZBE1137193. 32. ♂, Russia, Komarovo, 27.06.1953, coll. ZISP. 33. ♂, Russia, Enisejsk, 23.07.1930, coll. ZISP. 34. ♂, Estonia, Kohala, 13.07.1991, TUZ407496. 35. ♂, Russia, Minusinsk, 14.07.1926, coll. ZISP. 36. ♂, HOLOTYPE of *Celama centonalis* f. *alfkeni* Warnecke, Germany, Borkum, 07.1924, ZMH 833303 (dissected). 37. ♀, Estonia, Teenuse, 12.07.1992, IZBE1136225 (dissected). 38. ♀, Estonia, Kohala, 16.07.1994, TUZ407485. 39. ♀, Estonia, Sonda, 03.07.1937, IZBE1136200 (dissected). 40. ♀, Estonia, Verhulitsa, 21.07.2004, RCEÕ. 41. ♀, Estonia, 19.06.1881, IZBE1136185. 42. ♀ (f. *alfkeni*), Russia, Irkutsk, 12.07.1916, coll. ZISP. Scale: 1 cm.

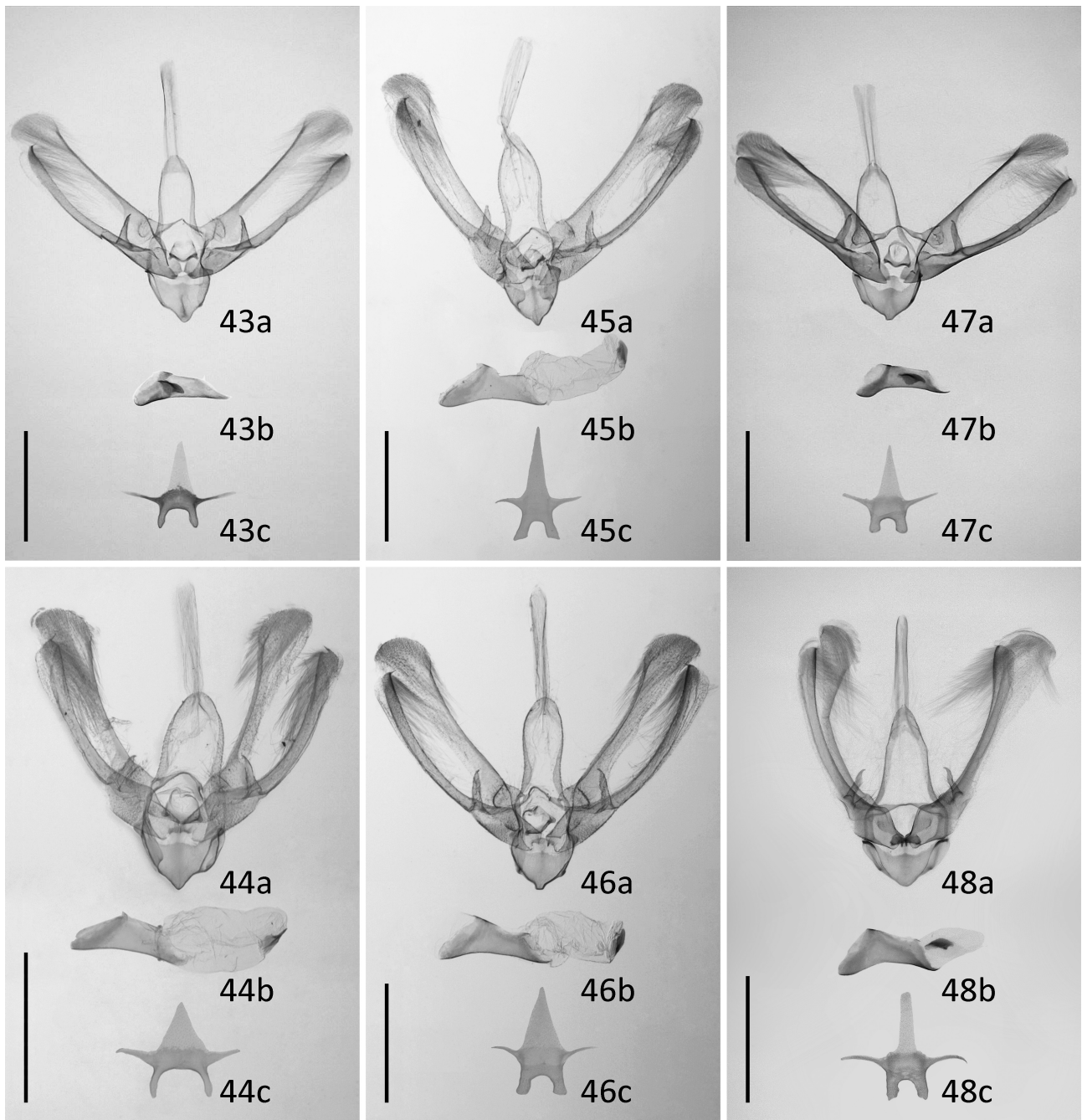
Sinev *et al.* (2017) recently demonstrated that the type series of *Hercyna scabralis* Eversmann, 1824 in coll. ZISP actually is conspecific with *N. aerugula*, as the moths have all characters typical for that species. Therefore, lectotype was designated for *H. scabralis* and illustrated, and this taxon was synonymized with *N. aerugula* (Sinev *et al.* 2017). Eversmann (1824) had described *H. scabralis* as a taxon belonging to Pyralidae, which is the likely reason why researchers focusing on Noctuoidea had overlooked this taxon for so long.

## DNA barcoding

All three target species (*N. aerugula*, *N. atomosa*, *N. estonica*) were recovered as monophyletic based on the analysis of DNA barcoding data (Figure 58). Despite being closely related, taken together, they nevertheless do not form an exclusively monophyletic entity, as widespread but morphologically very different Asian species *N. fraterna* (Moore, 1888) appeared as sister taxon to *N. estonica*, and West European endemic *N. dresnayi* (Warnecke, 1946) as sister taxon to *N. aerugula*, respectively (Figure 58).

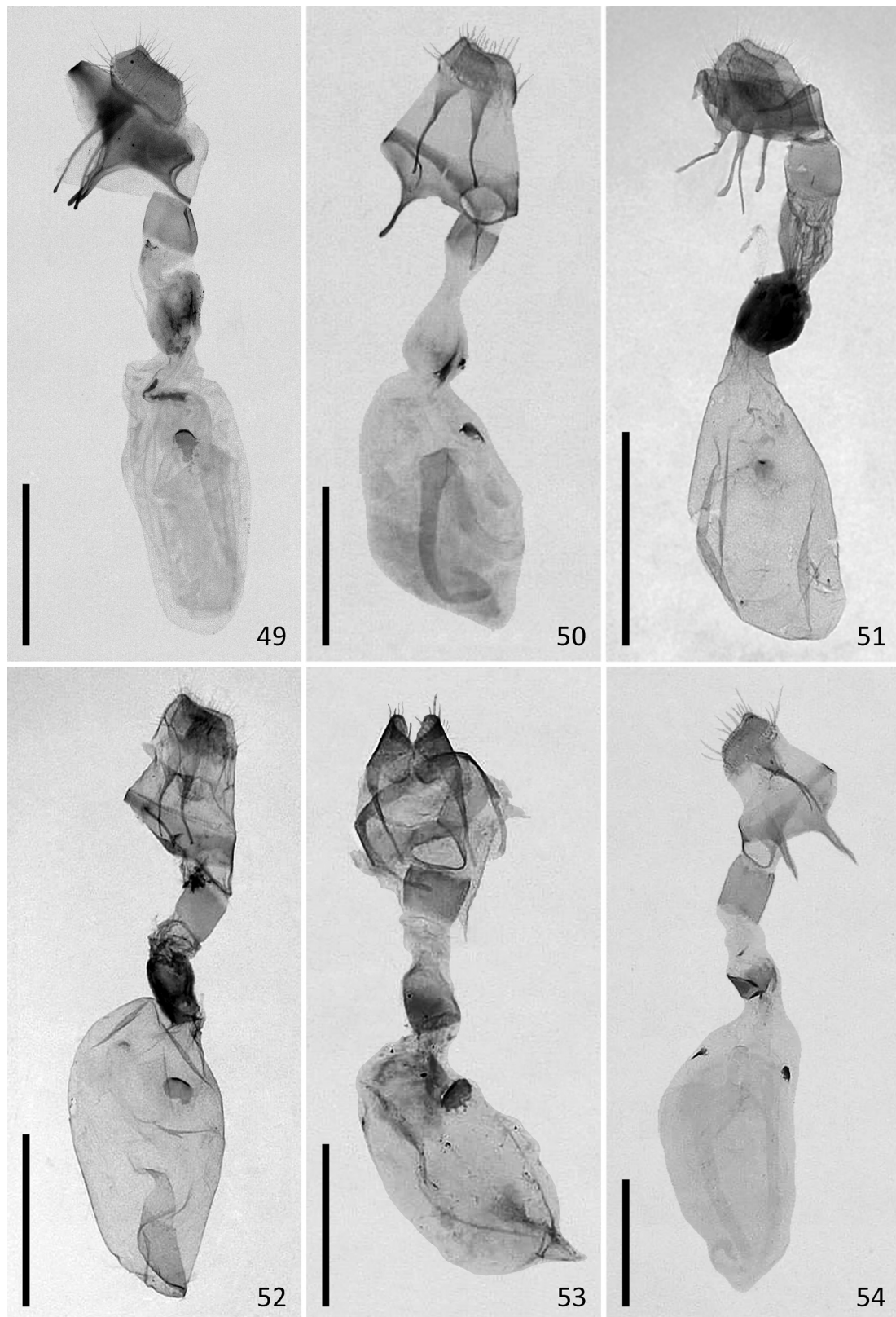
The intraspecific genetic variation varied from 0.000 to 0.011 (average 0.003±0.002 SD) in *N. aerugula*, and from 0.000 to 0.012 (average 0.001±0.003 SD) in *N. estonica*, whereas the two sequenced specimens of *N. atomosa* differed by just one substitution (p-distance = 0.002). In each interspecific pairwise comparison, the genetic distance exceeded the intraspecific genetic distances presented above at least twice: 0.031–0.04 (average 0.035±0.002 SD) between *N. aerugula* and *N. atomosa*, 0.037–0.055 (average 0.042±0.003 SD) between *N. aerugula* and *N. estonica*, and 0.02–0.028 (average 0.022±0.002 SD) between *N. atomosa* and *N. estonica*.

Further analysis of the data matrix demonstrated that these differences are comparable or even exceed the interspecific genetic distances between our target group and several other *Nola* species. For example, the genetic distance between *N. dresnayi* and *N. aerugula* (0.031–0.036, average 0.034±0.01) is almost identical to that between *N. aerugula* and *N. atomosa*. Moreover, *N. dresnayi* is the species closest to *N. atomosa* judging on barcode data only (p-distance 0.019–0.02, average 0.02±0.001). A similar pattern is seen in *N. estonica*, as the results of pairwise interspecific comparisons reveal both *N. dresnayi* (p-distance 0.028–0.033, average 0.028±0.001 SD) and *N. fraterna* (p-distance 0.028–0.033, average 0.029±0.001 SD) to be genetically closer to it than is *N. aerugula*. We therefore conclude that treating all three taxa considered in the present paper (*N. aerugula*, *N. atomosa* and *N. estonica*) as distinct species is fully justified also from the genetic point of view.



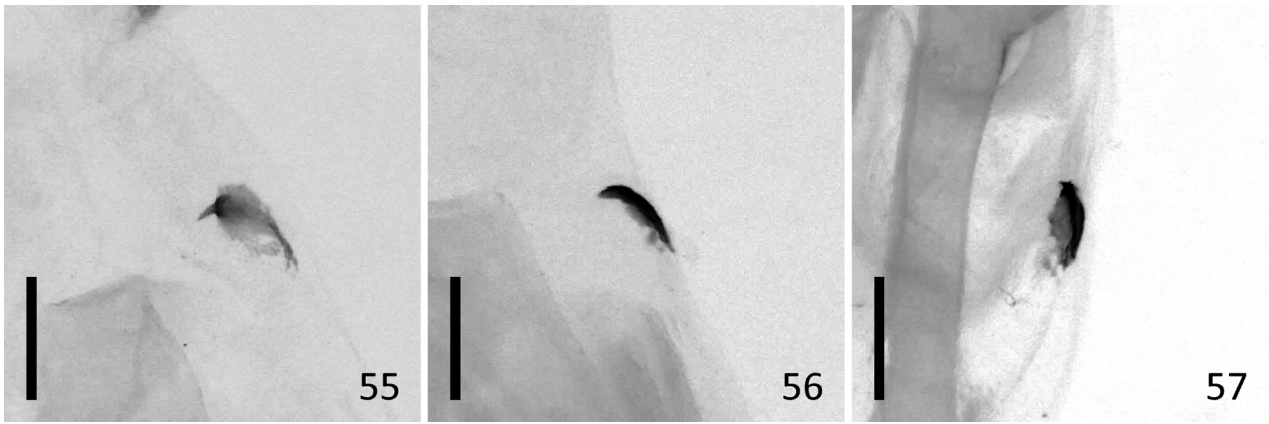
**FIGURES 43–48.** Male genitalia of *Nola* spp. 43. *Nola estonica* **sp. nov.**, PARATYPE, Estonia, Piusa Railway Station, 03.08.2020, TUZ300286 (barcoded). 44. *Nola estonica* **sp. nov.**, Russia, Iverskii zakaznik, 18.06.-01.07.2010, coll. ZISP. 45. *Nola atomosa* **stat. rev.**, LECTOTYPE, Russia, Amur region, slide Matov 0588, coll. ZISP. 46. *Nola atomosa* **stat. rev.**, Russia, Vinogradovka, 19.-20.07.1929, slide Matov 0592, coll. ZISP. 47. *Nola aerugula*, Estonia, Piilse, 14.-20.07.2019, IZBE1137196. 48. *Nola aerugula*, HOLOTYPE of *Celama centonalis* f. *alfkeni*, Germany, Borkum, 07.1924, ZMH 833303. a. genital armature. b. aedeagus (with vesica everted in 44–46, semi-everted in 48). c. 8th tergite. Scale: 1 mm.



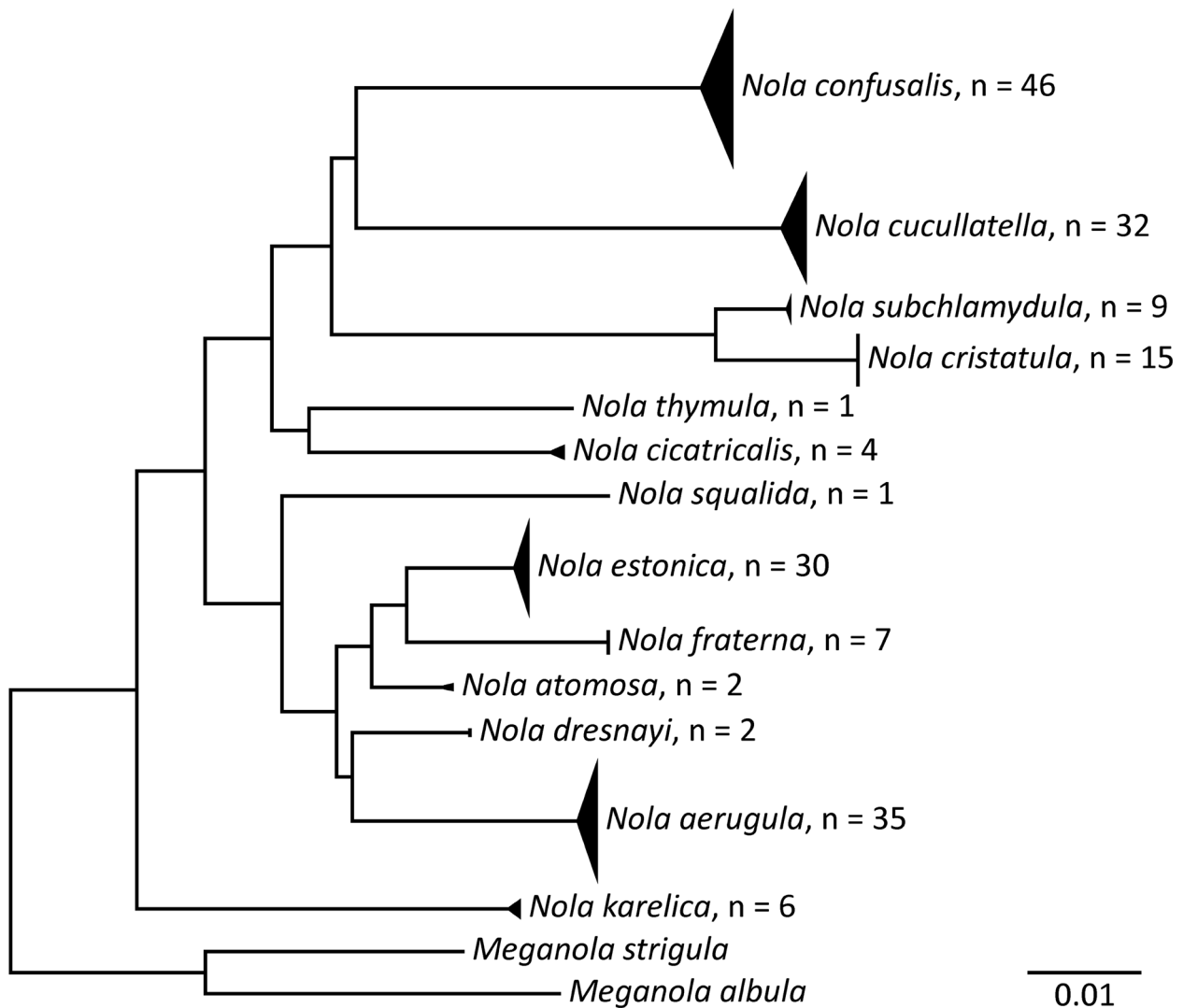


**FIGURES 49–54.** Female genitalia of *Nola* spp. 49. *Nola estonica* **sp. nov.**, HOLOTYPE, Estonia, Piusa Railway Station, 03.08.2020, TUZ300299. 50. *Nola estonica* **sp. nov.**, PARATYPE, Estonia, Piusa Railway Station, 03.08.2020, TUZ300300. 51. *Nola estonica* **sp. nov.**, South Korea, Mt. Samaksan, 25.06.2016, MNU genital slide no. 1172, coll. MNU (barcoded). 52. *Nola atomosa* **stat. rev.**, South Korea, Jindong-ri, 20.06.2017, MNU genital slide no. 1171, coll. MNU (barcoded). 53. *Nola atomosa* **stat. rev.**, Russia, valley of Izvilinka River, 08.–09.07.1976, IZBE0106556, slide J. Viidalepp 2402. 54. *Nola aerugula*, Estonia, Sonda, 03.07.1937, IZBE1136200. Scale: 1 mm.





**FIGURES 55–57.** Ventral signum of female *Nola* spp. 55. *Nola estonica* **sp. nov.**, PARATYPE, Estonia, Piusa Railway Station, 03.08.2020, TUZ300300. 56. *Nola atomosa* **stat. rev.**, Russia, Kedrovaja Pad, 02.–17.08.1977, IZBE0106557. 57. *Nola aerugula*, Estonia, Vanajärvesoo, 06.07.1995, RCEÕ. Scale: 200 µm.



**FIGURE 58.** Neighbor-joining tree of DNA barcode data of *Nola* spp.

## Discussion

The present study shows that there are three species within the paraphyletic entity previously united under the name *Nola aerugula*. In Europe, *N. aerugula* coexists with the previously unrecognized *N. estonica*. In the Far East, *N. estonica* is sympatric with *N. atomosa*, the latter taxon being here reinstated to the status of a species distinct from *N. aerugula*. While it appears clear that the taxon described here as *N. estonica* has not earlier been recognized in such a taxonomic and geographical scope as presented in the current paper, it cannot be *a priori* excluded that the type material of some other taxon may belong to this species. Such taxa may currently be treated as synonyms of other species or may have been assigned an intraspecific rank. Indeed, for the externally variable *N. aerugula* numerous aberrations have been described, and possible confusion with some other species should also be considered.

First and foremost, forms with light-coloured wings and reduced pattern are primary suspects for possible confusion with *N. estonica*. *Celama centonalis* f. *alfkeni* Warnecke, 1938, currently treated as a form of *N. aerugula*, is one such taxon. According to Warnecke's (1938) description (forewings clear white without any transverse lines; only dark tufts of scales in the middle area and brown stripes or dots on costa are visible), f. *alfkeni* may be suspected to be conspecific with *N. estonica*. In external appearance, the type material of f. *alfkeni* (Figure 36) is indeed remarkably similar to some forms of *N. estonica* with a very strongly reduced wing pattern (Figures 5, 15). Nevertheless, the genitalia of the male holotype of f. *alfkeni* unequivocally demonstrate that this taxon is different from *N. estonica*, and appears conspecific with *N. aerugula*. In particular, its vinculum (Figure 48a) is much shorter than that of *N. estonica* (Figures 43a, 44a), and the anterior projections of the 8th tergite of f. *alfkeni* are wide and located close to each other (Figure 48c), not narrow and apart from each other as in *N. estonica* (Figures 43c, 44c). Moreover, though we were not able to secure fresh material of particularly light-coloured specimens of *N. aerugula* for sequencing DNA barcodes, public data downloaded from the BOLD database indicate that such individuals have been studied elsewhere. In particular, specimen RMNH.INS.538621, collected from the western coast of the Netherlands, fully matches the description of *C. centonalis* f. *alfkeni*, but despite that, it has COI haplotype typical to *N. aerugula*.

Other synonyms or infrasubspecific subdivisions of *N. aerugula* we are aware of do not match with *N. estonica* having a different external morphology: *Pyralis centonalis* Hübner, 1796 has forewings suffused with pale yellow and the transverse lines are much more clear than in *N. estonica*; *Hercyna scabralis* Eversmann, 1842 has as strong and dark pattern as typical *N. aerugula*; *Nola littoralis* Paux, 1901 has much darker wings than *N. estonica*; *Nola centonalis* ab. *fasciata* Rebel, 1910 has the entire medial area unicolourous light brown; *Nola centonalis* ab. *fumosa* Berger, 1918 and *Nola centonalis* ab. *spitzi* Schawerda, 1921 have completely unicolourous brown forewings; in *Celama centonalis* f. *aurea* Lucas, 1959 all normally brown markings of the forewing have been replaced by yellowish brown ones.

*Nola holsatica* Sauber, 1916 is a sister taxon of *N. aerugula*, which has a rather restricted range on the coastal parts of Belgium, the Netherlands, northwestern Germany and southwestern Denmark (Fibiger *et al.* 2009). The taxonomic status of *N. holsatica* has been disputed since its description. Originally it was described as *N. centonalis* var. *holsatica* (Sauber, 1916), differing from typical form by smaller size, sharper forewing apex and a dark medial line on the upperside of the hindwings. Subsequent authors have sometimes treated *holsatica* as a subspecies of *aerugula* (e. g. Heydemann 1934, Fibiger & Karsholt 1998) but the more widely accepted view is that it is a distinct species (Lempke 1938, de Freina & Witt 1987, Fibiger *et al.* 2009, Hacker *et al.* 2012). The very dark and well expressed pattern of the forewing of *N. holsatica* (for figures including the lectotype, see Fibiger *et al.* 2009) strongly differs from that of *N. estonica*. The genitalia of *N. holsatica* have been figured by e.g. de Freina & Witt (1987) and Fibiger *et al.* 2009, the latter authors explicitly stating that the females possess two signa in the bursa copulatrix. As there is just one signum in the bursa copulatrix of *N. estonica* (Figures 49–51), it appears undeniable that this species cannot be conspecific with *N. holsatica*. This view is further supported by the report by Aarvik *et al.* (2017) that the DNA barcodes of *N. holsatica* and *N. aerugula* are 'almost identical', whereas there is a large (in average 0.042) genetic distance between the DNA barcodes of *N. aerugula* and *N. estonica*.

As is the case with the closely related *N. aerugula*, several intraspecific divisions of *N. holsatica* have been described. First, *Celama centonalis* ssp. *contrarialis* Heydemann, 1934 is currently treated as a synonym of *N. holsatica* (Fibiger *et al.* 2009). These moths are characterized by the absence of the dark transverse line on the upperside of the hindwing, but the pattern of the forewings is as clear and dark as that of typical *N. holsatica* (for figures, see Heydemann 1934). Second, three infrasubspecific subdivisions, published as aberrations of *Celama*

*holsatica*, are also known: ab. *reducta* Lempke, 1938, ab. *fasciata* Lempke, 1938 and ab. *obscura* Lempke, 1938. The original publication by Lempke (1938) is illustrated with photos of material which indicate that all these forms have a much darker pattern on the forewings than *N. estonica*, and have a transverse band on the upperside of the hindwings that is lacking in *N. estonica*. Therefore, we see no reason to suspect that any of those taxa are conspecific with *N. estonica*.

For *N. atomosa*, which is sympatric with *N. estonica* in the eastern part of the range of the latter species, we are aware of two synonyms. Specimens with a conspicuously dark brown medial area of the forewing were described as a separate species on two occasions: as *Nola candidalis* Staudinger, 1892 and as *Nola shin* Inoue, 1982. To our knowledge, forms with a completely dark medial area of the forewing (Fig. 24) do not occur in *N. estonica*, thus neither *N. candidalis* nor *N. shin* can be conspecific with this newly described species.

*Nola dresnayi* appeared as a sister taxon to *N. aerugula* in the phylogenetic analysis of DNA barcodes (Figure 58), though based on actual genetic distances its closest relative is *N. atomosa*. *N. dresnayi* has recently been figured by both Fibiger *et al.* (2009) and Hacker *et al.* (2012). The grey ground colour of the forewings differentiates *N. dresnayi* from *N. estonica*, and its completely grey hindwings are markedly different from the white hindwings of the latter. Clear differences can be found also in the genitalia: the long, spine-like cornutus of male *N. dresnayi* significantly differs from the short and wide cornutus of *N. estonica*. According to Fibiger *et al.* (2009), the females of *N. dresnayi* are characterized by having just one signum in the bursa copulatrix, sharing this character with *N. estonica*. However, the posterior strongly sclerotized part of ductus bursae of *N. dresnayi* is about two times longer than wide (Fibiger *et al.* 2009), whereas in *N. estonica* it is shorter than wide (Figures 49–51).

*Nola duercki* (Zerny, 1935) is a northwestern African species that has been figured by de Freina & Witt (1987) and more recently by Hacker *et al.* (2012). This species is characterized by pale fore- and hindwings, with a rather straight dark postmedial line of the forewing being the most prominent element of the wing pattern. The male genitalia of *N. duercki*, figured by Hacker *et al.* (2012), are easily distinguished from those of *N. estonica* by distinctly shorter valvae, which have both dorsal and ventral lobes wider than those of the latter species. Females of *N. duercki* are unknown (de Freina & Witt 1987, Hacker *et al.* 2012).

*Nola enphaea* (Hampson, 1901) is a little known Chinese species that resembles *N. dresnayi*. The original description (Hampson 1901) did not illustrate the species, but the subsequent treatment by Hampson (1914) is accompanied by a colour drawing that matches the description well. The grey colouration of the body and both fore- and hindwings of the moths indicates that *N. enphaea* cannot be confused with *N. estonica*.

In the analysis of genetic data, *Nola fraterna* clustered as sister to *N. estonica* (Figure 58). Such a placement was surprising, as *N. fraterna* is classified as belonging to the *pumila-fraterna* group *sensu* László *et al.* (2014). This unexpected result may be explained by the fact that DNA barcodes alone are unsuitable for large-scale phylogenetic analyses due to saturation (summarized in DeSalle & Goldstein 2019), though their usefulness in solving species-level taxonomic questions is beyond doubt (Hebert & Gregory 2005, Miller *et al.* 2016). In any case, the fact that *N. fraterna* clustered as sister to *N. estonica* further supports our conclusion that the latter taxon truly is a species distinct from both *N. aerugula* and *N. atomosa* that had so far remained undetected. The possibility that *N. fraterna* is conspecific with *N. estonica* can be excluded beyond reasonable doubt: in addition to the large genetic distance (on average 0.029), these species are very different externally. Specifically, the wings of *N. fraterna* are dark grey with a black, strongly curved antemedial line of the forewing being the most prominent element of the wing pattern (for illustrations, see Hampson 1914, Inoue 1998, Holloway 2003), whereas the wings of *N. estonica* are white and the antemedial line on its forewing is never darker than the postmedial line (Figures 1–18).

In summary, we are currently unaware of any previously described taxa potentially synonymous with *N. estonica*. The possibility that we have overlooked a described taxon which is synonymous with *N. estonica* appears to be low, considering the number of thorough revisions incorporating the genus *Nola* that have become available within the last few decades (Fibiger *et al.* 2009, Hacker *et al.* 2012, László *et al.* 2014).

In the Far East, based on specimens examined, *N. estonica* appears to be rather widely distributed from South Korea to the Amur region of Russia. Moreover, in NCBI GenBank there is a COI sequence of *Nola* from an undisclosed locality in China (MT785500), which, being almost identical to haplotypes of *N. estonica* from South Korea, should be attributed to this species. Nevertheless, as far as it can be judged, *N. estonica* is less common than *N. atomosa* in the Primorsky Region of Russia. The data for South Korea are currently too preliminary to tell which species is more widespread, but both are known to occur in the northeastern part of the country.

The known European distribution of *N. estonica* is currently limited to Estonia. All specimens but one have

been recorded in the southeastern part of the country, where the existence of permanent populations is beyond doubt. However, some of the known localities are situated just a few kilometers away from northeastern Latvia and the western border of the Pskov Region of Russia, making the presence of *N. estonica* in those regions very likely.

The data currently available thus point at the possibility that *N. estonica* may have a disjunct distribution, with the East European and the East Asian populations being separated by at least 6000 kilometers. Such a biogeographic pattern is not unprecedented, represented by e.g. *Catocala electa* (Vieweg, 1790) (Erebidae) and *Craniophora ligustri* ([Denis & Schiffermüller], 1775) (Noctuidae). However, the lepidopterans showing such a distribution pattern tend to be nemoral species feeding on hardwood trees, which follow the disjunct distribution of their host plants. The known habitat preference of *N. estonica* in Estonia (pine forest or dry meadow on sandy soil) does not support this scenario. It therefore appears more likely that *N. estonica* has a broad transpalaeartic distribution and its occurrence in the vast territory separating the known populations just remains to be confirmed.

The oldest available Estonian record of *N. estonica* dates from 2001, and the species could thus be a newcomer in the Estonian fauna. This does not appear impossible, as, in addition to the influx of southern lepidopterans associated with the climate change (Tiitsaar *et al.* 2019, Jürivete and Õunap 2020), some eastern moth species—such as *Eversmannia exornata* (Eversmann, 1837) (Uraniidae) and *Calyptra thalictri* (Borkhausen, 1790) (Erebidae)—have also colonized the country during the past 20 years. Nevertheless, it is premature to judge the history of *N. estonica* in the region before all historical collections in Estonia and neighboring countries have been thoroughly studied. We thus encourage lepidopterists to carefully examine unusually light specimens of *N. aerugula* and *N. atomosa* to reveal the actual distribution of *Nola* spp. in both the Western and Eastern Palaearctic region, respectively. If DNA barcoding would appear impractical due to e.g. the age of the specimen, female genitalia provide the most reliable means of identification.

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## References

- Aarvik, L., Bengtsson, Å., B., Elven, H., Ivinskis, P., Jürivete, U., Karsholt, O., Mutanen, M. & Savenkov, N. (2017) Nordic-Baltic Checklist of Lepidoptera. *Norwegian Journal of Entomology*, Supplement 3, 1–236.
- Da, W., Wang, M. & Hu, Y.-Q. (2021) Description of a new species and a new record of the genus *Nola* Leach, 1815 (Lepidoptera, Nolidae, Nolinae) from Tibet, China. *Zootaxa*, 4926 (2), 293–295.  
<https://doi.org/10.11646/zootaxa.4926.2.9>
- De Freina, J.J. & Witt, T.J. (1987) *Die Bombyces und Sphinges der Westpalaearktis (Insecta, Lepidoptera). Band 1*. Edition Forschung & Wissenschaft Verlag GmbH, München, 708 pp.
- DeSalle, R. & Goldstein, P. (2019) Review and interpretation of trends in DNA barcoding. *Frontiers in Ecology and Evolution*, 7, 302.  
<https://doi.org/10.3389/fevo.2019.00302>
- Eversmann, E. (1842) Quaedam lepidopterorum species novae, in Rossia orientali observatae, nunc describuntur et depictae. *Bulletin de la Société Impériale des Naturalistes de Moscou*, 15, 543–565.
- Fibiger, M. & Karsholt, O. (1998) First records of *Nola harouni* from Europe and comments on the taxonomic status of *N. centonalis holsatica* (Nolidae). *Nota Lepidopterologica*, 21, 194–205.
- Fibiger, M., Ronkay, L., Steiner, A. & Zilli, A. (2009) *Noctuidae Europaeae. Vol. 11. Pantheinae, Dilobinae, Acronictinae*,



- Eustrotiinae, Nolinae, Bagisarinae, Acontiinae, Metoponiinae, Heliiothinae, and Bryophilinae*. Entomological Press, Sorø, 504 pp.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Hampson, G.F. (1901) New species of *Syntomidae* and *Arctiidae*. *The Annals and Magazine of Natural History, including Zoology, Botany and Geology*, 45, 165–186.
- Hampson, G.F. (1914) *Catalogue of the Lepidoptera Phalaenae in the British Museum. Supplement. Vol. 1. Catalogue of the Amatidae and Arctiidae (Nolinae and Lithosianae) in the collection of the British Museum*. Printed by Order of the Trustees, London, 858 pp.
- Hacker, H., Schreier, H.-P. & Goater, B. (2012) Revision of the tribe Nolini of Africa and Western Palaearctic Region (Lepidoptera, Noctuoidea, Noctuidae, Nolinae). *Esperiana*, 17, 1–614.
- 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.
- Hebert, P.D., 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 *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 14812–14817.  
<https://doi.org/10.1073/pnas.0406166101>
- Hebert, P.D.N. & Gregory, T.R. (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology*, 54, 852–859.  
<https://doi.org/10.1080/10635150500354886>
- Heydemann, F. (1934) Einige für Schleswig-Holstein beachtenswerte oder neue Lepidopteren. *Internationale Entomologische Zeitschrift*, 27, 417–422.
- Holloway, J. D. (2003) The Moths of Borneo. Part 18. Nolidae. Southdene Sdn. Bhd. Kuala Lumpur, 279 pp.
- Inoue, H. (1998) Nolinae (Noctuidae) from Nepal. Moths of Nepal. Part 5. *Tinea*, 15 (Supplement 1), 89–98.
- Jürivete, U. & Õunap, E. (2020) *Estonian Lepidoptera. Catalogue*. Eesti Lepidopteroloogide Selts, Tallinn, 192 pp.
- Kristensen, N.P. (1999) 4. Skeleton and muscles: adults. In: Kristensen, N.P. (Ed.), *Lepidoptera, Moths and Butterflies. Vol. 2. Morphology, Physiology and Development. Handbook of Zoology IV. Arthropoda: Insecta. Part 36*. Walter de Gruyter, Berlin and New York, pp. 39–131.  
<https://doi.org/10.1515/9783110893724.39>
- László, G.M., Ronkay, G. & Ronkay, L. (2014) Taxonomic studies on the genus *Nola* Leach, 1815 (Lepidoptera, Noctuoidea, Nolidae, Nolini). *Fibigeriana Supplement*, 2, 201–262.
- Lempke, B.-J. (1938) *Celama centonalis* Hb. et *Celama holsatica* Sauber. *Lambillionea*, 38, 27–38.
- Matov, A. Yu. (2019) Nolidae. In: Sinev, S. Yu. (Ed.), *Catalogue of the Lepidoptera of Russia*. Zoological Institute RAS. St. Petersburg. pp. 317–319.
- Miller, S.E., Hausmann, A., Hallwachs, W. & Janzen, D.H. (2016) Advancing taxonomy and bioinventories with DNA barcodes. *Philosophical Transactions of the Royal Society B – Biological Sciences*, 371, 20150339.  
<https://doi.org/10.1098/rstb.2015.0339>
- Nupponen, K. & Fibiger, M. (2006) Additions and corrections to the list of Bombyces, Sphinges and Noctuidae of the Southern Ural Mountains. Part I. (Lepidoptera: Lasiocampidae, Lemonidae, Sphingidae, Notodontidae, Noctuidae, Pantheidae, Lymantriidae, Nolidae, Arctiidae). *Esperiana*, 12, 167–195.
- Oh, S.-H. (2001) A Review of the Subfamily Nolinae (Lepidoptera, Noctuidae) in Korea (I): Genus *Nola* Leech. *Insecta Koreana*, 18, 123–137.
- Õunap, E., Viidalepp, J. & Saarma, U. (2005) Phylogenetic evaluation of the taxonomic status of *Timandra griseata* and *T. comae* (Lepidoptera: Geometridae: Sterrhinae). *European Journal of Entomology*, 102, 607–615.  
<https://doi.org/10.14411/eje.2005.085>
- Õunap, E., Viidalepp, J. & Saarma, U. (2008) Systematic position of Lythriini revised: transferred from Larentiinae to Sterrhinae (Lepidoptera, Geometridae). *Zoologica Scripta*, 37, 405–413.  
<https://doi.org/10.1111/j.1463-6409.2008.00327.x>
- Sauber, A. (1916) *Nola centonalis* Hb. n. var. *holsatica*. *Internationale Entomologische Zeitschrift*, 10, 97.
- Sasaki, A. (2011) Nolidae. In: Kishida, Y. (Ed.), *The Standard of Moths in Japan II*. Gakken Education Publishing, Tokyo, pp. 170–189.
- Sinev, S.Yu., Anikin, V.V. & Zolotuhin, V.V. (2017) Volgo-Ural Pyraloidea and Pterophoridae described by E. Eversmann. In: Anikin, V.V., Sachkov, S.A. & Zolotuhin, V.V. (Ed.), “Fauna Lepidopterologica Volgo-Uralensis”: from P. Pallas to present days. *Proceedings of the Museum Witt Munich*, 7, pp. 380–386.
- Tamura, K., Stecher, G., Peterson, D., Filipinski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.  
<https://doi.org/10.1093/molbev/mst197>
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.  
<https://doi.org/10.1093/nar/22.22.4673>
- Tiitsaar, A., Valdma, D., Õunap, E., Remm, J., Teder, T. & Tammaru, T. (2019) Distribution of butterflies (Lepidoptera:



Papilionoidea) in Estonia: Results of a systematic mapping project reveal long-term trends. *Annales Zoologici Fennici*, 56, 147–185.

<https://doi.org/10.5735/086.056.0114>

Warnecke, G. (1917) Über einige Probleme der Schmetterlingsfauna der ostfriesischen Inseln. *Abhandlungen des naturwissenschaftlichen Vereins Bremen*, 30, 118–125.