Reassessment of *Poecilocampa navalagamellae* Expósito, 2004 stat. nov. and its distribution in the Iberian Peninsula (Lepidoptera: Lasiocampidae, Poecilocampinae)

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Abstract. *Poecilocampa navalagamellae* Expósito, 2004 stat. nov., endemic to Spain, is characterized and elevated to species rank. This taxon was originally described as a subspecies of *Poecilocampa alpina* (Frey & Wullschlegel, 1874) with insufficient diagnosis based on two male specimens only. Its southern populations are described as *Poecilocampa navalagamellae turdetana* ssp. nov. Notes on external morphology of the imago, genitalia, chorionic structure of the egg, habitat and host plant are presented herein, as well as ab ovo breeding results of *P. n. turdetana*. An actualised distribution map is provided. According to the results obtained from the study of mitochondrial DNA barcoding, *P. navalagamellae* belongs to a lineage well differentiated from the rest of the European *Poecilocampa* Stephens, 1828 taxa. It also differs from these in the wing pattern, the male genitalia, the chorionic structure of the egg and the larval external traits.

Resumen. Se redescribe y se eleva al rango de especie *Poecilocampa navalagamellae* Expósito, 2004 stat. nov., endémica de España. Este taxón fue originalmente descrito de forma escueta como una subespecie de *Poecilocampa alpina* (Frey & Wullschlegel, 1874) en base a dos machos y sin el estudio de los genitalia. Sus poblaciones meridionales se describen como *Poecilocampa navalagamellae turdetana* ssp. nov. Se presentan también observaciones relativas a los adultos, genitalia, corion de los huevos, hábitat y plantas nutricias, así como los resultados de cría *ab ovo* de *P. n. turdetana*. Se proporciona un mapa de distribución actualizado. De acuerdo con los resultados obtenidos del estudio del ADN mitocondrial (código de barras genético), *P. navalagamellae* pertenece a un linaje bien diferenciado del resto de los taxones de *Poecilocampa* Stephens, 1828 europeos. También se distingue de ellos en el patrón alar, los genitalia masculinos, la estructura del corion del huevo y el aspecto de la larva.

Zusammenfassung. Die in Spanien endemische *Poecilocampa navalagamellae* Expósito, 2004 stat. nov. wird bewertet und als distinkte Art eingestuft. Dieses ursprünglich undetailliert als Unterart von *Poecilocampa alpina* (Frey & Wullschlegel, 1874) beschriebene Taxon basiert auf lediglich zwei Männchen. Ihre andalusischen Populationen werden hier als *Poecilocampa navalagamellae turdetana* ssp. nov. beschrieben. Imagines, Genitalien, die Chorionstruktur des Eies, Habitat, Wirtspflanzen und die ab-ovo-Zuchtergebnisse von *P. n. turdetana* werden dokumentiert. Eine aktualisierte Verbreitungskarte wird beigefügt. Das Ergebnis unserer molekularen Studie mit DNA barcoding bestätigt, dass *P. navalagamellae* einer genetischen Linie angehört, die sie von den restlichen europäischen Taxa von *Poecilocampa* Stephens, 1828 klar unterscheidet. Zudem bestehen Unterschiede in der Flügelzeichnung, der männlichen Genitalstruktur, der Chorionstruktur des Eies und in den Charakteristiken der Raupe.

Key words. Lasiocampidae, Poecilocampinae, *Poecilocampa*, phylogeny, barcoding, taxonomy, Spain, Madrid, Cádiz, Palaearctic Region, new status, new subspecies.

Introduction

Poecilocampa Stephens, 1828 is a group of medium-sized moths typical of Eurasian temperate broadleaved and mixed forests. The genus comprises eight species (excluding the taxa *P. coluchei* and *P. canensis*) distributed from the Iberian Peninsula in the West to the Pacific coast in the East (Saldaitis & Pekarsky 2015, Zolotuhin et al. 2010).

The species present in Europe are highly variable in morphology. As a result, several taxa have been described without convincing morphological support, and the absence of molecular data has led to taxonomic disputes and caused confusion in the systematics of the group (e.g. Gomez Bustillo & Fernandez Rubio 1976, de Freina & Witt 1987, Jost et al. 2000, Expósito Hermosa 2004, Leraut 2006). We tentatively consider, based on the continuous study that has been carried out in recent years (see Huemer & Hebert 2016, Zolotuhin et al. 2010), that this genus is represented in the non-Iberian part of Europe by *Poecilocampa populi* Linnaeus, 1758 and *Poecilocampa alpina* (Frey & Wullschlegel, 1874). The European taxa *P. coluchei* (Varenne & Billi, 2002) and *P. canensis* (Millière, 1875) have been treated at either specific or subspecific (subspecies of *P. populi* or *P. alpina*) level (see Leraut 2006, Zolotuhin et al. 2010). It is clear that the European taxa form a species complex for which a detailed revision is needed.

With respect to the Iberian Peninsula, the entomological literature contains numer-

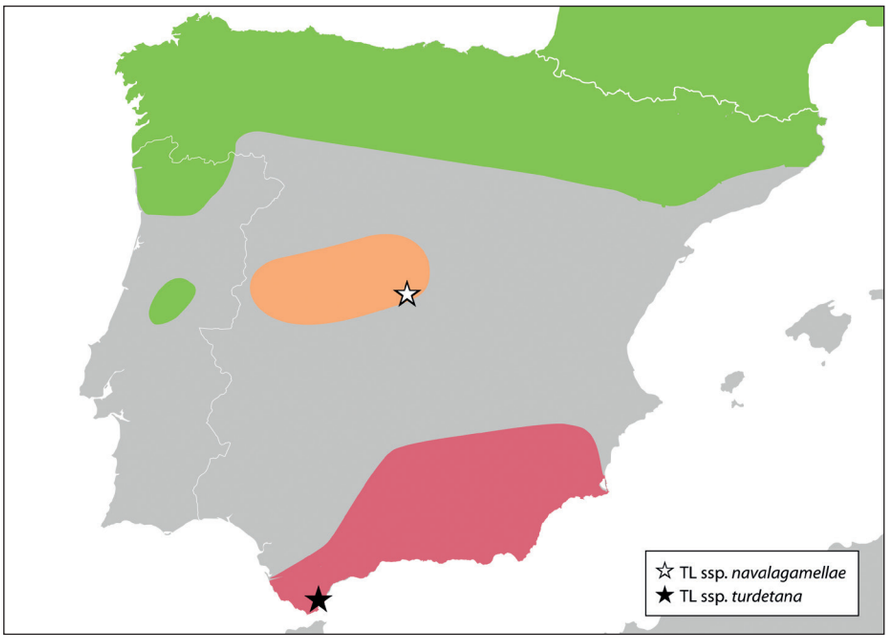


Fig. 1. Probable distribution of *Poecilocampa* Stephens, 1828 in the Iberian Peninsula in accordance with the available records.
Green. *Poecilocampa populi.*
Orange. *Poecilocampa n. navalagamellae.*
Red. *Poecilocampa n. turdetana.*
White star. Type locality of *P. n. navalagamellae* stat. nov.
Black star. Type locality of *P. n. turdetana* ssp. nov. The distribution areas given for the two *P. navalagamellae* subspecies are not yet adequately defined by the small number of specimens examined to date. However, as genitalia of the examined specimens from Cádiz and Granada were identical, it is likely that the populations of Albacete and Murcia can also be assigned to *P. n. turdetana*.

ous inconsistent taxonomic assessments of the Iberian populations, which are generally distributed across various mountainous regions. *Poecilocampa populi* inhabits the northern third of the Iberian Peninsula, including Portugal, where all the records have been assigned to this species (Monteiro 1956; Corley et al. 2011, 2012, 2014). In Spain, its presence is documented in Cataluña (see Cervelló et al. 1992, Pérez De-Gregorio et al. 2009), northern Aragón (Grustán 1995, Murria Beltrán 2012), the País Vasco and Navarra (Gómez de Aizpúrua 1974, 1985, 1988; Cifuentes et al. 1993, Cifuentes, 1996), La Rioja (Latasa et al. 2001), the northern provinces of Castilla y León (Magro & Jambrina 2013) and Galicia (Fernández Vidal 2017).

The populations of central and southern Spain (Fig. 1) have received a very heterogeneous treatment, partly as a consequence of the existing taxonomic confusion in relation to this genus in Europe. Captures from Murcia, Albacete, Jaén, and Almería have been assigned to *P. populi* (Notario & Castresana 2002, Ortíz et al. 2010, Garre et al. 2020). The population from Granada was assigned first to *P. canensis* (Pérez López 1989) and later to *P. populi* (Pérez López 1993). Captures from Málaga and Cádiz were identified as *P. canensis* (Agenjo 1973, Gómez Bustillo & Fernández Rubio 1976; Mateo Lozano 1990, 1991). The specimens from Madrid were first identified as *P. populi* (Gómez de Aizpúrua 1974), but later were treated as *P. canensis* (Expósito Hermosa & Gómez Bustillo 1974, Gómez Bustillo & Fernández Rubio 1976), subsequently as *Poecilocampa "alpicola"* (sic) (Expósito Hermosa 1997) and finally as *P. alpina navalagamellae* (Expósito Hermosa 2004). Populations from Cáceres have been determined as *P. alpina* (Blázquez et al. 1997, Hernández-Roldán et al. 1999, Blázquez et al. 2000, García Santano et al. 2002, Blázquez-Caselles 2014).

In this work, we assessed the taxonomic status of the populations of *Poecilocampa* from the Iberian Peninsula through the study of morphology (of the eggs, caterpillars and adults) and the use of molecular data (DNA barcoding).

Abbreviations

DF – Collection de Freina; GP – Genitalia preparation; IBE – Instituto de Biología Evolutiva (CSIC-UPF), Barcelona; RE – collection/leg. Jiménez; TF – leg. Farino; AIB – collection/leg. Iglesias Baquero; YML – collection/leg. Monasterio León.

Material and methods

Collection. We collected *Poecilocampa* individuals from northern (Cantabria and Navarra), central (Autonomous Community of Madrid) and southern (Cádiz, Andalucía) Spain. Specimens were captured between 2018 and 2020 through the use of ultraviolet light attractants. We declare that all samples analysed in this study were collected in compliance with institutional, national, and international guidelines. The morphological and phenological analysis of the *Poecilocampa* populations of southern Spain is based on adults found in the Parque Natural Los Alcornocales, Cádiz (Andalucía). *Ab ovo* larvae were subsequently reared to adults in captivity under outdoor conditions.

Preparation. From the above material, kept in the collections of YML and DF, a total of 20 genitalia dissections were made using standard techniques (Tab. 1). Genitalia were dissected, macerated, cleaned and either mounted in Euparal on glass slides or preserved in vials containing 70 % alcohol (nine parts) and glycerol (one part). Illustrations are all based on Euparal mounted preparations and photographed under magnification using an Olympus Camedia C-750 camera with a Soligor Adapter Tube for Olympus. The photographs of the egg, taken with a Scanning Electron Microscope (SEM), were taken with a Fei Inspect Quanta 200 microscope. The samples were previously mounted on graphite adhesive and metallized with gold in an Aname Q150V Plus metallizer.

DNA extraction. Individuals collected from the field and used for the genetic analyses were dried as soon as possible and legs were taken and stored in 99 % ethanol at –20 °C. The barcode region of the cytochrome c oxidase subunit I (*COI*) was extracted from nine individuals, all from Spain (Tab. 2). We added all the *Poecilocampa* sequences (48) available on BOLD; they corresponded to *P. populi*, *P. alpina* and the taxa *canensis* and *coluchei*. Three European species of the sister genus *Trichiura* Stephens, 1828 (three individuals of *Trichiura verenae* Witt, 1981, three of *Trichiura crataegi* (Linnaeus, 1758) and two of *Trichiura castiliana* Spuler, 1908) and a sequence of *Artace cribraria* Ljungh, 1825 were used as outgroups. Total

Tab. 1. *Poecilocampa* Stephens, 1828 samples examined.

Species	Region, Locality	Altitude	UTM (10 × 10 mm)	Note	Date	Collector/ observer	Stage
P. n. navalagamellae	Madrid, Robledo de Chavela	856	30TUK98	GP–DF: 2016/34, 2016/35	8.X.2019	YML, REJ	9 ♂
P. n. navalagamellae	Madrid, Fresnedillas de la Oliva	880	30TVK08	GP–YM: YMG23.2021, YMG21.2021, YMG20.2021, YMG22.2021, YMG19.2021	12.X.2020	YML, REJ	19 ♂, 3 ♀
P. n. turdetana	Cádiz, Bosque de Niebla, Tarifa	750	30STE79	Paratype	1.I.2019	Dave Grundy	1 ♂
P. n. turdetana	Dto.	700	30STE79	Paratypes	30.XII.2019	AIB	1 ♂, 1 ♀
P. n. turdetana	Cádiz, Embalse de Charco Redondo, Los Barrios	96	30STF61	Paratype	23.XII.2019	AIB	1 ♂
P. n. turdetana	Cádiz, Arroyo del Tiradero, Los Barrios	186	30STF60	Holotype, Paratypes GP–DF: 2016/36, 2016/37; GP–YM: YMG29.2021, YMG30.2021, YMG31.2021, YMG33.2021	5.–7.I.2019	TF	15 ♂, 4 ♀
P. populi	Navarra, Valle de Aranguren, Ilundáin	656	30TXN23		19.XII.2019	Kike Vergara	1 ♂
P. populi	Cantabria, Tudanca, Embalse de La Cohilla	700	30TUN87	GP–YM: YMG26.2021	1.XII.2018	TF	2 ♂
P. populi	Cantabria, La Gloria, Pesaguero	550	30TUN77	GP–YM: YMG28.2021	9.XII.2018	TF	1 ♂, 1♀
P. populi	Russia, Penza, Akhuny Vill.	--	--	GP–YM: YMG24.2021 YMG25.2021	8.X.2004	S. Shibaev	1 ♂, 1♀
P. populi	Thuringia, Jena, Remderoda	285	32UPB74	--	3.XI.2011	E. Friedrich	1 ♂, 1♀
P. populi	Bavaria, Wildbad Kreuth, Schweiger Alm	900	--	GP–DF 2016/40	8.XI.2020	H. Fischer	1 ♂
P. alpina	Tirol, Matrei am Brenner	1100	--	GP–DF 2016/41	1.XI.1999	R. Mayrhofer	1 ♂
P. alpina	Tirol, St. Sigmund im Sellraintal	1700	--	GP–DF 2016/39	5.XI.2014	H. Fischer	1 ♂

genomic DNA was extracted using Chelex 100 resin, 100–200 mesh, sodium form (Biorad), under the following protocol: one leg was removed and introduced into 100 µl of Chelex 10 % and 5 µl of Proteinase K (20 mg/ml) were added. The samples were incubated overnight at 55 °C in a shaker and were subsequently incubated at 100°C for 15 minutes.

DNA sequencing. LepF1 and LepR1 primers (Hebert *et. al*. 2004), 5'-ATTCAACCAATCATAAAGATATTGG-3' and 5'-TAAACTTCTGGATGTCCAAAAAATCA-3' respectively, were employed for the amplification of the barcode fragment. Double-stranded DNA was amplified in 25 µl volume reactions: 13.2 µl ultra pure (HPLC quality) water, 5 µl 5X Green GoTaq Flexi Buffer (Promega), 3.2 µl 25 mM $MgCl_2$, 0.5 µl 10 mM dNTP, 0.5 µl of each primer (10 mM), 0.1 µl GoTaq G2 Flexi Polymerase (Promega) and 2 µl of extracted DNA. Reaction conditions comprised a first denaturation at 92 °C for 60 s, then 92 °C for 15 s, 48 °C for 45 s and 62 °C for 150 s in five cycles, and other 30 cycles changing the annealing temperature to 52 °C, with the final extension step at 62 °C for seven minutes. PCR products were purified and Sanger sequenced by Macrogen Inc. Europe (Amsterdam, the Netherlands). All sequences have been deposited in BOLD and GenBank (Tab. 2).

Phylogenetic reconstruction. Sequences were visualized, edited and aligned in Geneious Prime 2019.0.3 (https://www.geneious.com). Minimum genetic distances (dXY) were calculated using Geneious v11.0.5. The best fitting substitution model (GTR+G) was calculated in jModelTest v2.1.7 (Darriba *et al*., 2012) under the AIC. The *COI* alignment was then used for both Bayesian and maximum likelihood phylogenetic inferences. The Bayesian phylogeny was reconstructed using BEAST v2.5.0 (Bouckaert et al. 2014). Distinct partitions were selected for each gene using the best model indicated by jModelTest and four rate categories if gamma and base frequencies were estimated. Estimates of node ages were obtained by applying a strict clock and a normal prior distribution centred on the mean between two substitution rates for invertebrates: 1.5 % and 2.3 % uncorrected pairwise distance per million years (Quek et al. 2004, Brower 1994), respectively. These substitution rates provide only very rough divergence estimates. The standard deviation was tuned so that the 95 % confidence interval of the posterior density coincided with the 1.5 % and 2.3 % rates. Parameters were retrieved using two independent runs of 20 million generations each and convergence was checked with TRACER 1.7.1 (Rambaut 2018). A 10 % burn-in was applied and results from both runs was

merged. Maximum likelihood (ML) inferences were obtained in CIPRES (Miller et al. 2010) using RAxML-HPC2 v8.2.12 (Stamatakis 2014). The ML + thorough bootstrap workflow was selected with a GTR+G model and 1,000 bootstrap replicates.

General results and discussion

Genetic and morphological (male genitalia, egg and larval morphology) evidence highlighted differences, explained below, between the central and southern Spanish populations and the other European *Poecilocampa*, comparable to those expected at interspecific level. In consequence, we raise these populations from subspecies to species level, *P. navalagamellae* stat. nov. We also found genetic and morphological (in the *COI*, male genitalia and wing pattern) differences between the central and southern Spanish populations (which are geographically isolated), hence we divided them in two subspecies: *P. n. navalagamellae*, from central Spain, and *P. n. turdetana* ssp. nov., from southern Spain.

The separation at species or subspecies level of allopatric populations underlies considerable subjectivity among taxonomists. Mutanen et al. (2021) recommended at least two independent characters as minimum support for separating species, e.g. morphology and genetic distances, as found within *P. navalagamellae*. Thus, it would even be possible to consider treating the two allopatric Iberian lineages as separate species, which would agree with the comparatively minor diagnostic characters of the widely accepted species *P. alpina* and *P. populi*. However, we here take a conservative approach and propose a split of *P. navalagamellae* into two subspecies.

Genetic results

The *Poecilocampa* individuals from central and southern Spain form a well supported clade (posterior probability, pp = 1; bootstrap = 91), sister to that including the specimens of *P. populi* + *P. alpina* + taxon *coluchei* (Figs 2, 3). This last clade is here referred as *P. populi* complex. The Spanish clade and the *P. populi* complex presumably diverged ca. 3.15 (1.95-4.44) million years ago (mya). The minimum genetic p-distance between the two clades is 4.3 %. In Lepidoptera, these values typically imply interspecificity, e.g. in the European Papilionoidea the median of the

Tab. 2. Samples used in the analyses of the barcode region of the *cytochrome c oxidase subunit I* (*COI*).

IBE ID	BOLD ID	Species	GenBank code	Locality
	ASARD5901-12	*Artace cribraria*	-	Coasta Rica, La Cruz, Santa Elena, P.N. Santa Rosa, P.N. Sta. Rosa, Estación Murciélago
	GWOSD765-10	*P. alpina*	JF848992	Germany, Trischübelhütte
	GWOSD766-10	*P. alpina*	JF848993	Germany, Torrener Joch, Hagengeb. bei Stahlhaus
	PHLAG387-12	*P. alpina*	-	Liechtenstein, Saminatal/Alp Valuena
	PHLAG388-12	*P. alpina*	-	Austria, Reith bei Seefeld
	PHLAG391-12	*P. alpina*	-	Austria, Reith bei Seefeld
	PHLAG392-12	*P. alpina*	-	Austria, Igleralm
	PHLAG398-12	*P. alpina*	-	Austria, Igleralm
	PHLAG399-12	*P. alpina*	-	Austria, Reith bei Seefeld
	PHLAG400-12	*P. alpina*	-	Austria, Igleralm
	PHLAG402-12	*P. alpina*	KP253640	Austria, Rona Alpe W Bürserberg
	PHLAG403-12	*P. alpina*	-	Austria, St. Sigmund, Sellraintal
	PHLAG432-12	*P. alpina*	-	Italy
	PHLAG433-12	*P. alpina*	-	Italy
RVcoll19E036	BDE917-21	*P. n. naval.*	MZ315159	Spain, Robledo de Chavela
RVcoll19E038	BDE918-21	*P. n. naval.*	MZ315160	Spain, Robledo de Chavela
RVcoll18O439	BDE892-21	*P. n. turdet.*	MZ315152	Spain, Arroyo del Tiradero, P.N. Alcornocales
RVcoll18O441	BDE893-21	*P. n. turdet.*	MZ315153	Spain, Arroyo del Tiradero, P.N. Alcornocales
RVcoll18O442	BDE894-21	*P. n. turdet.*	MZ315154	Spain, Arroyo del Tiradero, P.N. Alcornocales
RVcoll18O443	BDE895-21	*P. n. turdet.*	MZ315155	Spain, Arroyo del Tiradero, P.N. Alcornocales
RVcoll18O446	BDE896-21	*P. populi*	MZ315156	Spain, Pesaguero
RVcoll18O449	BDE898-21	*P. populi*	MZ315157	Spain, Polaciones, Embalse de La Cohilla
RVcoll18O923	BDE901-21	*P. populi*	MZ315158	Spain, Valle de Aranguren, Ilundáin
	BLUTI004-16	*P. populi*	-	UK
	CGUKB113-09	*P. populi*	-	UK, Lightfoot Green
	CGUKB316-09	*P. populi*	-	UK, Oldmeldrum
	CGUKC032-09	*P. populi*	-	UK
	CGUKC664-09	*P. populi*	-	UK, Woodford Green
	GBLAA1270-15	*P. populi*	-	Germany, Plön / Stadtheide
	GBLAB034-13	*P. populi*	-	Germany, Schönwalde-Glien, Pausin
	GBLAC009-13	*P. populi*	-	Germany, Hüttersdorf, Saarland
	GBLAC010-13	*P. populi*	-	Germany, Hüttersdorf, Saarland
	GBLAC978-13	*P. populi*	-	Germany, Bad Blankenburg, Schieferbrueche

Tab. 2.
Continuation

IBE ID	BOLD ID	Species	GenBank code	Locality
	GBLAC985-13	*P. populi*	-	Germany, Lichtenstein
	GBLAF237-14	*P. populi*	-	Germany, Zerpenschleuse, Wandlitz
	GBMIN23136-13	*P. populi*	AB731750	Denmark
	LEFIA1308-10	*P. populi*	GU828686	Finland, Imatra
	LEFIB801-10	*P. populi*	HM871678	Finland
	LEFIE074-10	*P. populi*	HM873821	Finland
	LEFIE126-10	*P. populi*	HM873874	Finland
	LON3422-16	*P. populi*	-	Norway, Nedre Timenes
	LON3437-16	*P. populi*	-	Norway, Nedre Timenes
	LON718-09	*P. populi*	HM387279	Norway, Skogfoss
	LON719-09	*P. populi*	HQ989558	Norway, Bjorkaren, Sand
	ODOPE727-11	*P. populi*	KX040482	Germany, Teisendorf
	PHLAD067-11	*P. populi*	JN276729	Italy, Montiggl/ Kleiner Priol
	PHLAG393-12	*P. populi*	-	Austria, Igleralm
	PHLAG395-12	*P. populi*	-	Italy, Taufers NE
	PHLAG396-12	*P. populi*	-	Austria, Innsbruck, Hechenberg SW
	PHLAG397-12	*P. populi*	-	Austria, Innsbruck, Hechenberg SW
	PHLAG401-12	*P. populi*	KP253636	Austria, Rona Alpe W Bürserberg
	PHLAG431-12	*P. populi*	-	Austria, Kreith/ Patsch W
	PHLSA760-11	*P. populi*	KM572544	Austria, Marul, Marulbach linksseitig
	GWORZ157-10	Taxon *canensis*	HM909939	Italy, Donnici, 10 km S CS
	PHLAG381-12	Taxon *canensis*	-	France, St. Michel l`Observatoire
	PHLAG382-12	Taxon *canensis*	-	France, L`Escarene
	PHLAG384-12	Taxon *coluchei*	-	France, Luceram
	PHLAG385-12	Taxon *coluchei*	-	France, Luceram
	GWOTI462-12	*Trichiura castiliana*	KX047486	Spain, Sierra de Albarracin
	GWOTI463-12	*T. castiliana*	KX046811	Spain, Sierra de Albarracin
	GWOTI461-12	*T. crataegi*	KX047036	Austria, Grossglockner Fensterbach
	LEALT305-16	*T. crataegi*	MG522211	Russia, 17 km NNE Kokorya vill., Chikhacheva Mts. Range, T
	LEFIE032-10	*T. crataegi*	HM873781	Finland, Kolari
	GWOTI456-12	*T. verenae*	KX046214	Greece, between Leukimi and Itea
	GWOTI457-12	*T. verenae*	KX046929	Greece, between Lefkimi and Itea
	GWOTI459-12	*T. verenae*	KX046962	Greece, between Lefkimi and Itea

minimum p-distances between neighbouring taxa is 2.1% (Dincă et al. 2021). Furthermore, the individuals from central (pp = 1; bootstrap = 94) and southern Spain (pp = 1; bootstrap = 84) formed sister clades and they were separated by minimum p-distance of 1.4%. Finally, the *Poecilocampa* specimens from northern Iberia placed within *P. populi*. In this area, only this species seems to be present. Genetic analyses based on the *COI* gene show that taxa *P. coluchei* and *P. canensis* cluster together with *P. populi* and *P. alpina*. However, it is out of the scope of our work to revise the status of these taxa, a task for which further reassessment of morphology and additional molecular markers will be necessary.

Taxonomy

Poecilocampa Stephens, 1828

Type species. *Phalaena populi* Linnaeus, 1758

Poecilocampa navalagamellae Expósito, 2004 stat. nov.

Poecilocampa n. navalagamellae Expósito, 2004

(Figs 4–7, 14, 15, 73, 74)

Poecilocampa alpina navalagamellae Expósito, 2004: 263.

Holotype. ♂, and paratype ♂ in A. Expósito coll., Móstoles (Madrid).

Zoobank registration. urn:lsid:zoobank.org:act:CA4A5B1A-33A2-476B-B8A6-D028D076F337.

Type locality. Navalagamella, Madrid province.

Further material examined. 5 ♂, Madrid province, Robledo de Chavela, 8.X.2019 (YML coll.); 4 ♂, same data (DF coll.); 19 ♂, 3 ♀, Madrid province, Fresnedillas de la Oliva, 12.X.2020 (YML coll.).

Remark. The original description was based on a pair of males in a very brief, uninformative note that reads (in translation from spanish) "It has been drawn to our attention that the external morphology of the specimens from Navalagamella is more similar to that of *alpina* than to *canensis*, despite the proximity to the *canensis* from the south of France. Indeed, the specimens from Madrid have a darker

Fig. 2. Phylogeny of the barcode region of the cytochrome c oxidase subunit I *(COI)* obtained through Bayesian inference. The X-axis indicates time in million years and the bar show the 95 % HPD range for the posterior distribution of node ages; only posterior probabilities > 0.60 are indicated. Provinces are indicated for the Spanish specimens.

white background and more marked bands, as it can be seen in the photo. Therefore, and based on these characters, it is considered that the aforementioned specimens from Madrid belong to a new subspecies that we name *Poecilocampa alpina navalagamellae* Expósito, n. ssp. and which is described from the preceding data, being the holotypes and paratype as they appear in the caption of the photograph that Expósito has kindly provided and whom I thank for his collaboration." We supplement the previous description as follows.

Redescription. *Male.* Alar expanse 33–37 mm. Antenna bipectinate, head, frons and thorax blackish brown; collar and tegulae brown with some bright whitish hairs, abdomen entirely dark brown; forewing ground colour of the upper surface blackish in centre, basal area blackish brown, distally whitish tinged light grey-brown, somewhat contrasting to the rest of the wing; black postmedial line narrow, slightly sinuous, fringes on both pairs of wings with less pronounced alternative colouration. Hind wings brownish black, medial transverse line less distinct, light brown, slightly roundish, the terminal

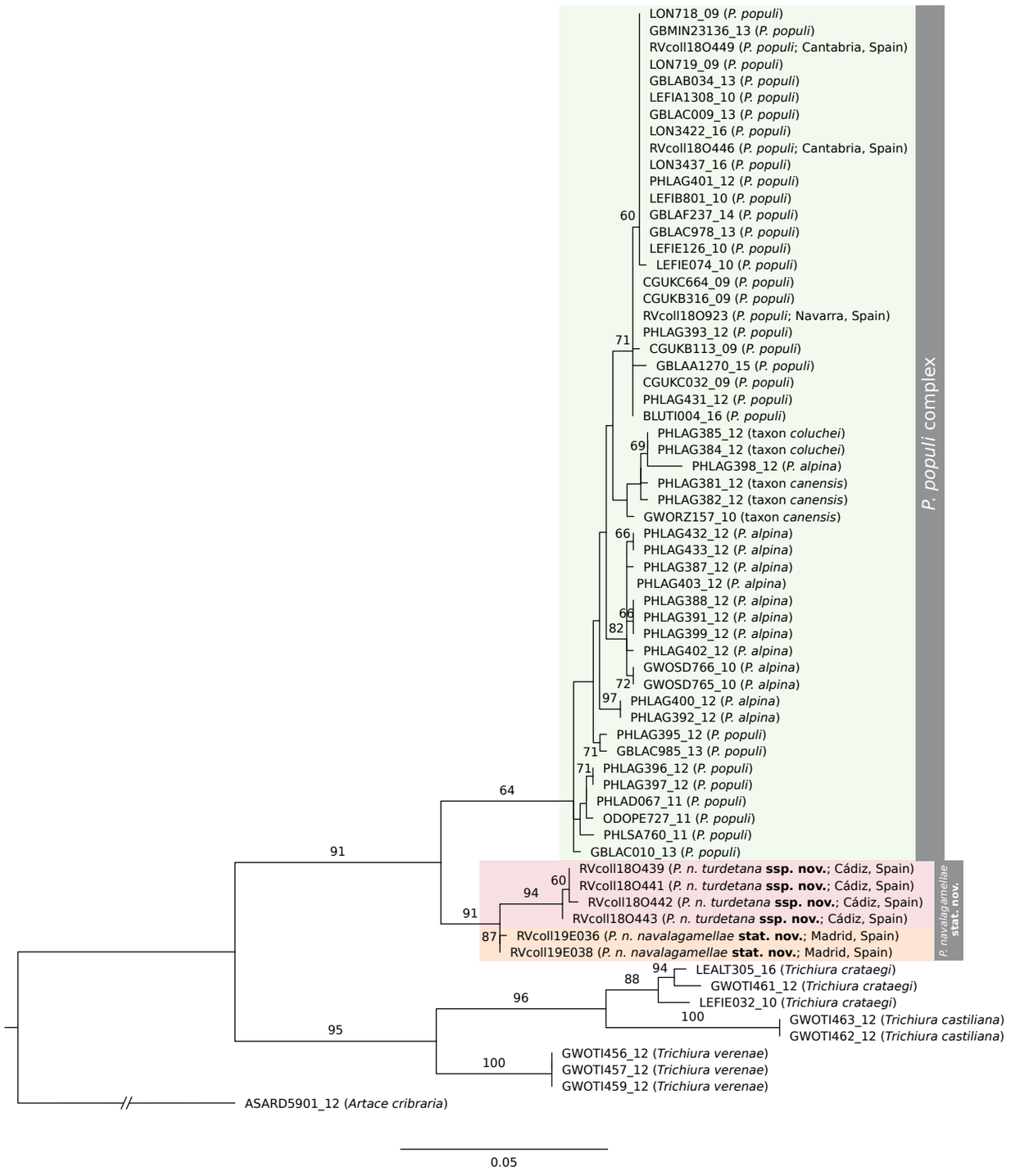


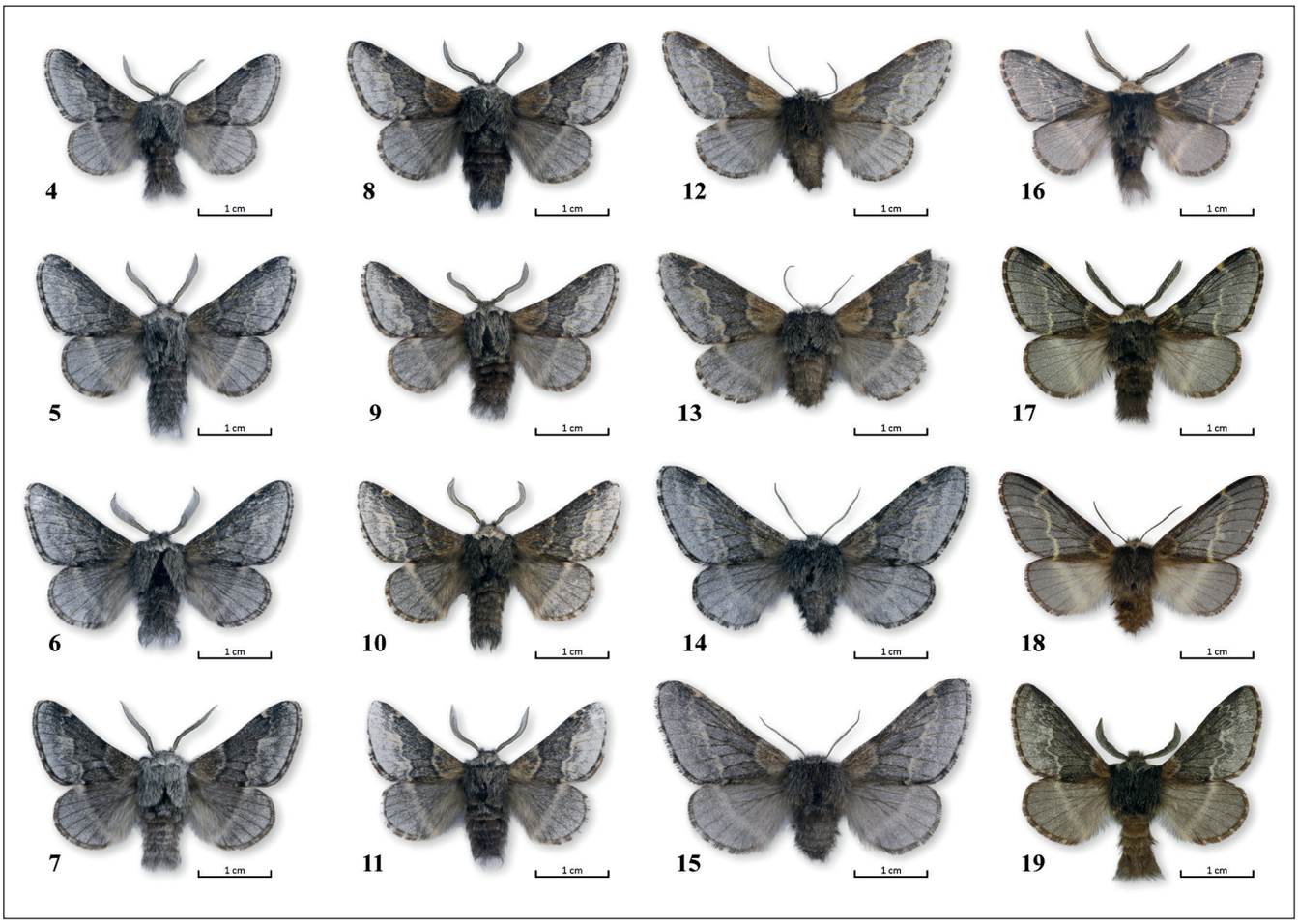
Fig. 3. Phylogeny of the barcode region of the cytochrome c oxidase subunit I *(COI)* obtained through maximum likelihood inference. Scale units are presented in substitutions per site and only bootstrap values > 60 are indicated. Provinces are indicated for the Spanish specimens.

area somewhat lighter.

Male genitalia **(F**igs 25–30, 42). The following preparations were made GP–DF ♂ 2016/34 and 2016/35 (Figs 20, 21), Madrid province, Robledo de Chavela, 8.X.2019, in coll. DF. GP–YML ♂ YMG18/21, G19/21, G20/21, G21/21 and G22/21 (Figs 22–24), Madrid province, Fresnedillas de la Oliva, 12.X.2020, in coll. YML. Uncus and gnathos well developed, uncus flattened with short bilobate top; juxta shield, valvae short; wide and slightly curved distally; sacculus lobe large and wide; phallus relatively slender, distally widened, strongly chitinized, pronouncedly curved; vesica tubular with basal swelling and a few inconspicuous spicules.

Female. Alar expanse 43–44 mm. Antenna filiform, inconspicuous shortly serrated; wing shape somewhat more elongated than in male, wing pattern less pronounced. In all other aspects not different from the male.

Female genitalia **(F**ig. 36). The following preparations were made: GP–YML ♀ G23/21 (Fig. 36): Madrid province, Fresnedillas de la Oliva, 12/X/2020, in coll. YML. The female genitalia are similar to



Figs 4–19. *Poecilocampa*. Imagines upper side**. 4–7.** *P. n. navalagamellae* ♂, Spain, Madrid province. **8–13.** *P. n. turdetana* (8. Holotype ♂, 9–11. Paratypes ♂, 12, 13. Paratypes ♀), Spain, Cádiz province. **14, 15.** *P. n. navalagamellae* ♀; same data as figs 4–7. **16–18.** *P. populi* (16. ♂, Cantabria, 17 ♂, 18 ♀. Germany. **19.** *P. alpina* ♂, Austria. (4–16 in coll. YML, 17–19 in coll. Friedrich).

those of *P. navalagamellae turdetana* described below, but the appendix bursae is more rounded posteriorly and only lightly sclerotized.

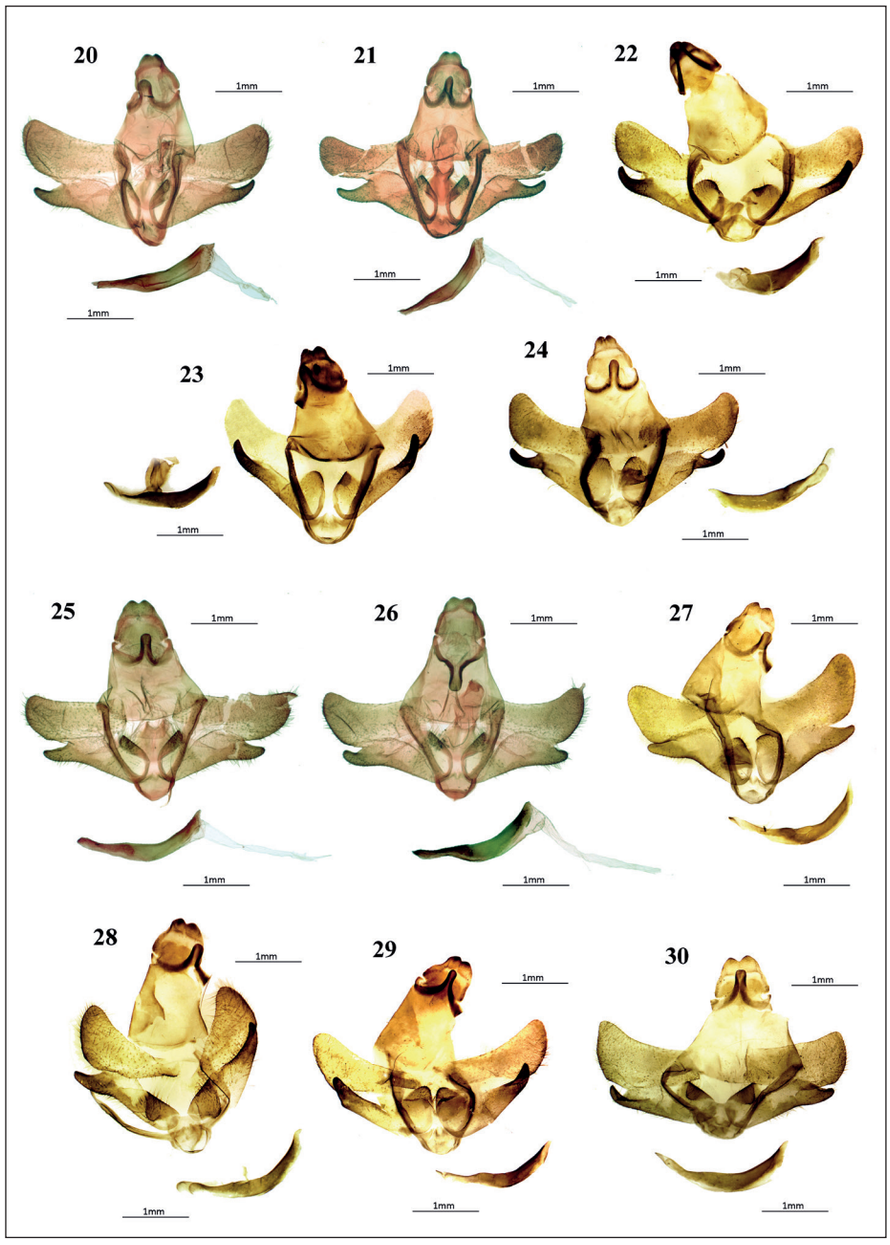
Variability. The specimens examined display very little variation.

Structure of the eggshell (Figs 43–52). Dierl (1984) provides information on the egg structure of *P. populi* and *P. alpina*, which he considers to be different species. Eitschberger & Thiele (2008) offer electronic microscope images of eggs from *P. populi*, *P. alpina* and the taxon *canensis*. Optical photography with high magnification and SEM photography is very useful, since macroscopically the size and pigmentation of the *P. navalagamellae* egg resembles that of other species, such as *P. populi, P. n. navalagamellae* and *P. n. turdetana* egg structure is identical. The egg has a size of 1.65 mm (n=10). It has an elliptical shape, flattened dorsally and ventrally. In the chorion of both faces it presents a central zone with semi-spherical or irregular micro pits, isolated or in small groups. The rest of the chorion presents a reticular pattern of irregular polygons, with the pores in each reticulum arranged geometrically. At one of the poles is the micropylar rosette formed by petals in the shape of rounded wedges. At the other pole, the chorion presents the same polygonal lattice as the part of the rest of the egg that does not have pits. As for the colour, it is greyish brown on the dorsal and ventral faces, dotted with darker spots. The area occupied by the polygonal grid is white dotted with brown spots of different intensities. The pole of the micropyle is dark surrounded by a white crown, while the opposite pole presents a spotted whitish circle.

Larva. Unknown. Morphology probably similar to the *turdetana* subspecies (Figs 55–64).

Pupa. Pupation occurs close to the ground inside a very robust, dark gray-brown cocoon or in a cocoon that is camouflaged in appearance, resembling the colour and texture of the ground. The dark red-brown pupae remain in the cocoon throughout the summer and autumn.

Diagnosis. In the barcode region of the COI, *P. navalagamellae* (both subspecies) can be distinguished from the *P. populi* complex in the positions 11 (T), 55 (T), 59 (T), 88 (C), 145 (T), 259 (C), 265 (G), 287 (T), 334 (G), 337 (C), 376 (T), 499 (C), 505 (C), 517 (T), 532 (C). Externally, *P. navalagamellae* adults display a more contrasting forewing pattern than *P. populi*. The wing pattern may be similar to some *P. alpina* specimens and no diagnostic characters have been found to differentiate them. Both *P. navalagamellae* male and female genitalia are diagnostic and clearly distinguishable from those of *P. populi* and *P. alpina*. In the male genitalia the bilobed valve shows a shorter and less-unciform shaped saccular lobe, while the less massive and slender shape of the phallus with its slightly less prominent spicule group in the vesica is also diagnostic.



Figs 20–30. *Poecilocampa navalagamellae* male genitalia (ventral, phallus lateral).
20–24. *P. n. turdetana*. Cádiz province (20, 21. GP–DF 2016/36, GP–DF 2016/37; 22–24. GP–YM YMG29.2021, YMG30.2021, YMG31.2021). **25–30.** *P. n. navalagamellae*. Madrid province (25, 26. GP–DF 2016/34, GP–DF 2016/35; 27–30. GP–YM YMG21.2021, YMG20.2021, YMG22.2021, YMG19.2021).

The structure of the egg is another differential characteristic between *P. navalagamellae*, *P. populi* and *P. alpina*. The main difference that we find in the *P. navalagamellae* egg structure with respect to the eggs of the rest of European populations is in the roughness of the chorion (Figs 45–54). The egg of *P. navalagamellae* resembles macroscopically those of other species, such as *P. populi*, in size and pigmentation. However, they can be distinguished with ease by the presence of a central zone in both faces of the chorion with irregular micro pits forming a reticular pattern of polygons, absent in the egg chorions of *P. populi* (Figs 65, 66) or *P. alpina*, which are completely smooth.

Distribution (Fig. 1). At present this species is known only from Madrid province. We suggest that the Cáceres populations (previously assigned to *P. alpina* in the literature) also belong to this species, although, unfortunately, it has not been possible to study specimens. It should also be present in other geographically intermediate regions, such as the province of Ávila. The occurrence of adults in late autumn to early winter in a period comparatively neglected by lepidopterologists is certainly the reason why relatively few records have been reported to date.

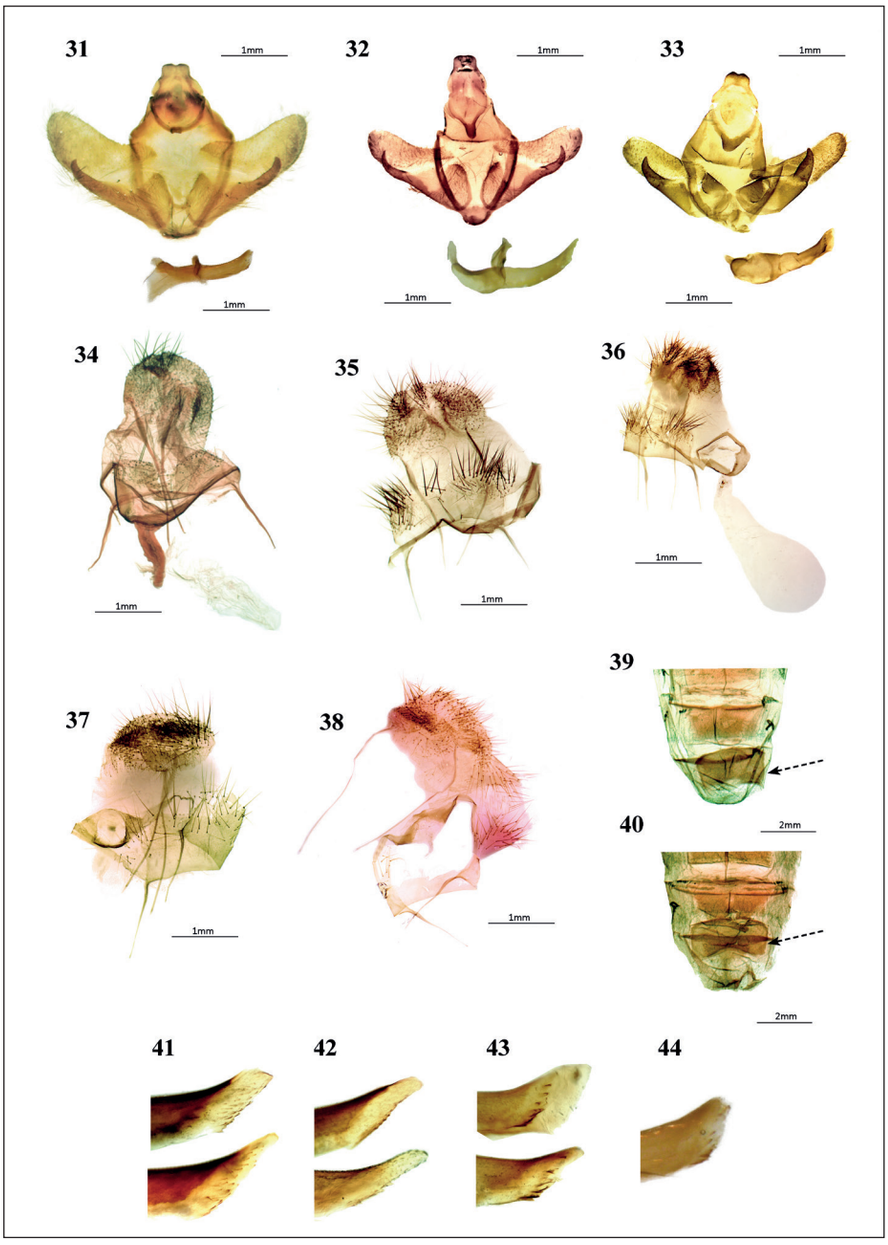
Habitat, host plants and life history (Fig. 70). Little is known about the ecology of *P. navalagamellae*. In the Madrid region, *P. n. navalagamellae* is found in a mountain environment at around 850 meters above sea level, where the semi-natural pastures of *Quercus ilex* dominate the landscape (Fig. 70). *Quercus pyrenaica* formations also appear in the habitat and other trees such as *Fraxinus angustifolia* are abundant. Other locations known in the literature that probably correspond to this species occur in Cáceres, between 375 and 600 meters above sea level. According to the collecting data and as typical for *Poecilocampa*, moths hatch late in the year, usually after the first night frosts. Based on current data, they are on the wing between early October and January, depending on the location.

We know, both for *P. n. turdetana* and *P. n. navalagamellae*, that females lays gray-brown and lightly speckled eggs individually or in small untidy groups on the branches of the host plants. The egg is the hibernation phase. Caterpillars of both subspecies hatch in the spring when temperatures become milder and their host plants start to produce leaves. They develop throughout the spring, and can probably be found in the wild until June. They pupate close to the ground in a very rigid, dark gray-brown cocoon or one that is the same colour as the ground. The dark red-brown pupae remain in the cocoon throughout the summer and autumn.

Poecilocampa navalagamellae turdetana ssp. nov.
(Figs 8–13, 20-24, 34, 35, 41, 55–64, 71, 72).

Holotype. ♂, Spain, Cádiz province, Los Barrios, Arroyo del Tiradero, Parque Natural Los Alcornocales, 186 m, 5.I.2019, TF leg. Deposited in the tissue collection of the Institute of Evolutionary Biology (CSIC-UPF). Tagged with the code ZEcoll-19CA1.

Paratypes. 10 ♂, 3 ♀, same data as holotype. Deposited in the tissue collection of the Institute of Evolutionary Biology (CSIC-UPF) (3 ♂, 1 ♀), and the private collections of YML (7 ♂, 2 ♀), tagged with the codes ZEcoll19CA3, ZEcoll19CA4, ZEcoll-19CA5, ZEcoll19CA6, ZEcoll19CA7,



Figs. 31–33. Male genitalia (ventral, phallus lateral). **31.** *Poecilocampa alpina* from Tirol, Austria (GP–DF 2016/39). **32.** *P. populi* from Bayern, Germany (GP–DF 2016/40). **33.** *P. populi* from Cantabria. GP–YM YMG26.2021. **Figs 34–38.** Female genitalia (ventral). **34, 35.** *P. navalagamellae turdetana* from Cádiz (34. GP–DF 2016/38; 35. GPYM YMG33.2021). **36.** *P. n. navalagamellae* from Madrid (GP–YM YMG23.2021). **37.** *Poecilocampa populi* from Cantabria. GP–YM YMG28.2021.). **38.** *Poecilocampa populi* from Russia (GP–YM YMG24.2021). **Figs 39, 40.** Shape of the distal tergites/sternites. **39.** *P. n. turdetana* from Cádiz. **40.** *P. n. navalagamellae* from Madrid. **Figs 41–44.** Aegeagus, detail of vesica, spiculae group. **41.** *P. navalagamellae turdetana* from Cádiz. **42.** *P. n. navalagamellae* from Madrid. **43:** *P. populi*, Cantabria and Russia (GP–YM YMG25.2021). **44:** *P. alpina*, Austria, Tirol.

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Derivatio nominis. The name *turdetana* refers to the Turdetani, an ancient pre-Roman people of the Iberian Peninsula, considered the successors to the people of Tartessos, living in the valley of the Guadalquivir, which is nowadays part of Andalucía. The species name *turdetana* is used as a noun in apposition.

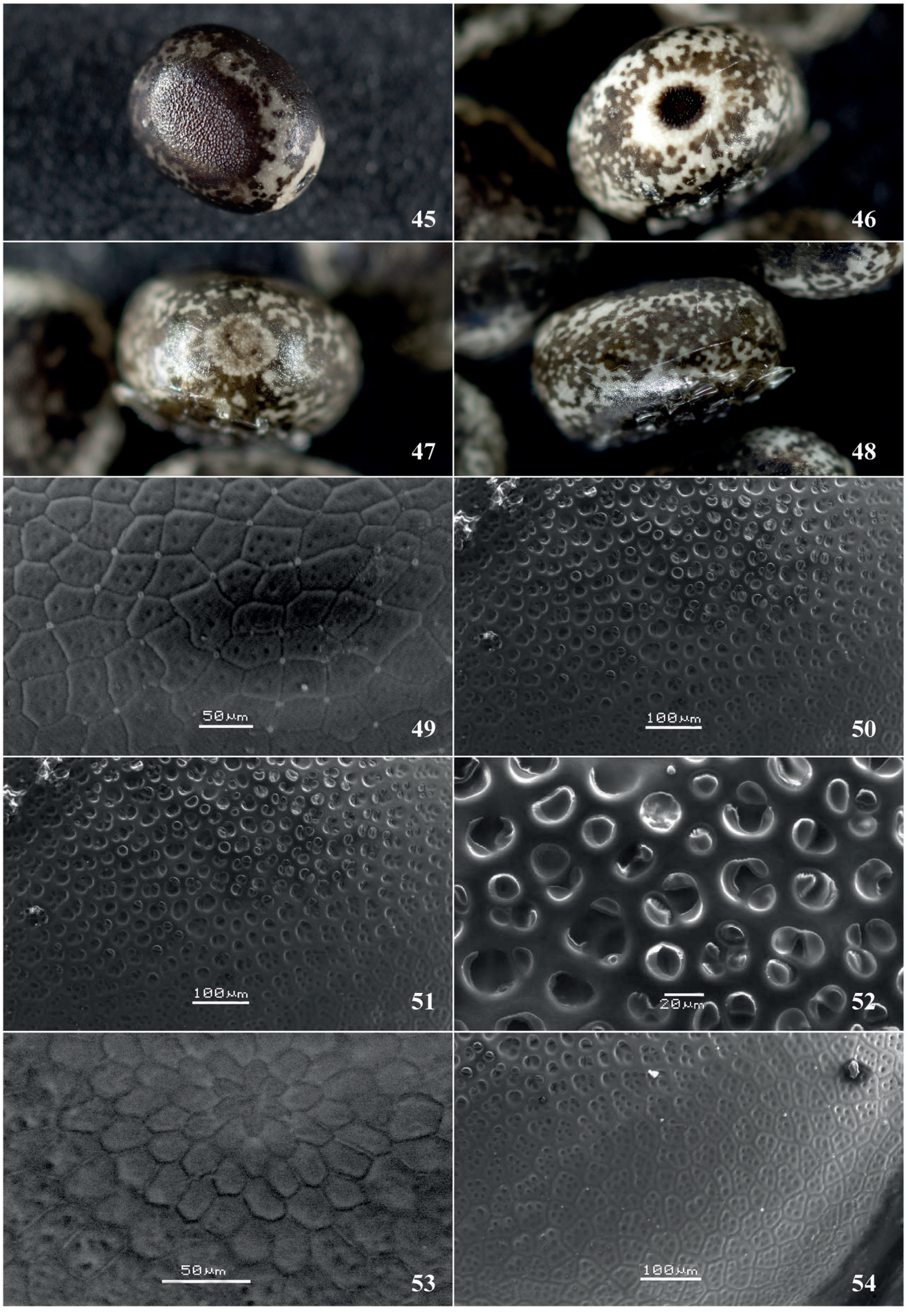
Description. *Male.* Alar expanse of holotype 38 mm, wingspan of paratypes 29–38 mm (n=16). Antenna bipectinate; head, frons, vertex, thorax, tegulae and abdomen blackish-brown with some light brown hair-like scales; patagia whitish, interspersed with a few light brown hair-like scale and with an admixture of brown hairs; forewing ground colour blackish brown with lighter postmedial area; base red-brown, discal area whitish grey, contrasting with the rest of the wing; antemedial line pronounced, well traceable; postmedial line blackish, protruding, clearly sinuous, sharply demarcated from the whitish-grey distal area; fringe alternately spotted light grey and black. Hindwing brownish, medial transverse line distinct, light brown, almost straight.

Male genitalia (Figs 20–24, 41). The following preparations were made: GP–DF ♂ Paratypes 2016/36 and 2016/37: Cádiz province, Los Barrios, Arroyo del Tiradero, Parque Natural Los Alcornocales, 7.I.2019. GP–YML ♂ Paratypes G29/21, G30/21, G31/21 and G32/21, same locality, in coll. YML. Uncus and gnathos well-developed, uncus distally flattened bilobed; juxta in the shape of a shield with a notch on the posterior margin. Valvae short and wide; sacculus large, broad at its base, digitiform and curved distally, with a rounded tip, strongly sclerotized in its upper third; phallus relatively slender, distally widened, strongly chitinised, only slightly curved; vesica tubular with basal swelling

ZEcoll19CA8, ZEcoll19CA9, ZEcoll-19CA10, ZEcoll19CA11, ZEcoll19CA12, ZEcoll19CA13, ZEcoll19CA14, ZEcoll-19CA15; 5 ♂, same data as holotype (GP–DF ♂ 2016/34 (Fig. 25) and 2016/35 (Fig. 26), 1 ♀ (GP–DF 2016/38, Fig. 34) in coll. DF. Deposited in the Bavarian State Collection of Zoology, Munich, tagged with the codes DF#ZSM1, DF#ZSM2, DF#ZSM3, DF#ZSM4, DF#ZSM5; 1 ♂, Spain, Cádiz province, Bosque de Niebla, Tarifa, Dave Grundy leg. Deposited in the tissue collection of the Institute of Evolutionary Biology (CSIC-UPF). Tagged with the code ZEcoll19CA2; 1 ♂, Spain, Cádiz province, Los Barrios, Embalse de Charco Redondo, Parque Natural Los Alcornocales, AIB leg. Deposited in the tissue private collection of AIB, tagged with the code, LEP-LAS-0002#0003.

Zoobank registration. urn:lsid:zoobank.

Figs 45-54. *Poecilocampa navalagamellae turdetana* egg structure (photographed with SEM). **45.** Dorsal view of the egg. **46.** View of the pole that houses the micropyle. **47.** View of pole opposite the micropyle. **48.** Lateral view. **49.** Chorion reticulation. **50–52.** Pits zone in the central part of the dorsal and ventral faces. **53.** Micropylar area. **54.** Transition zone between the grid and the pits.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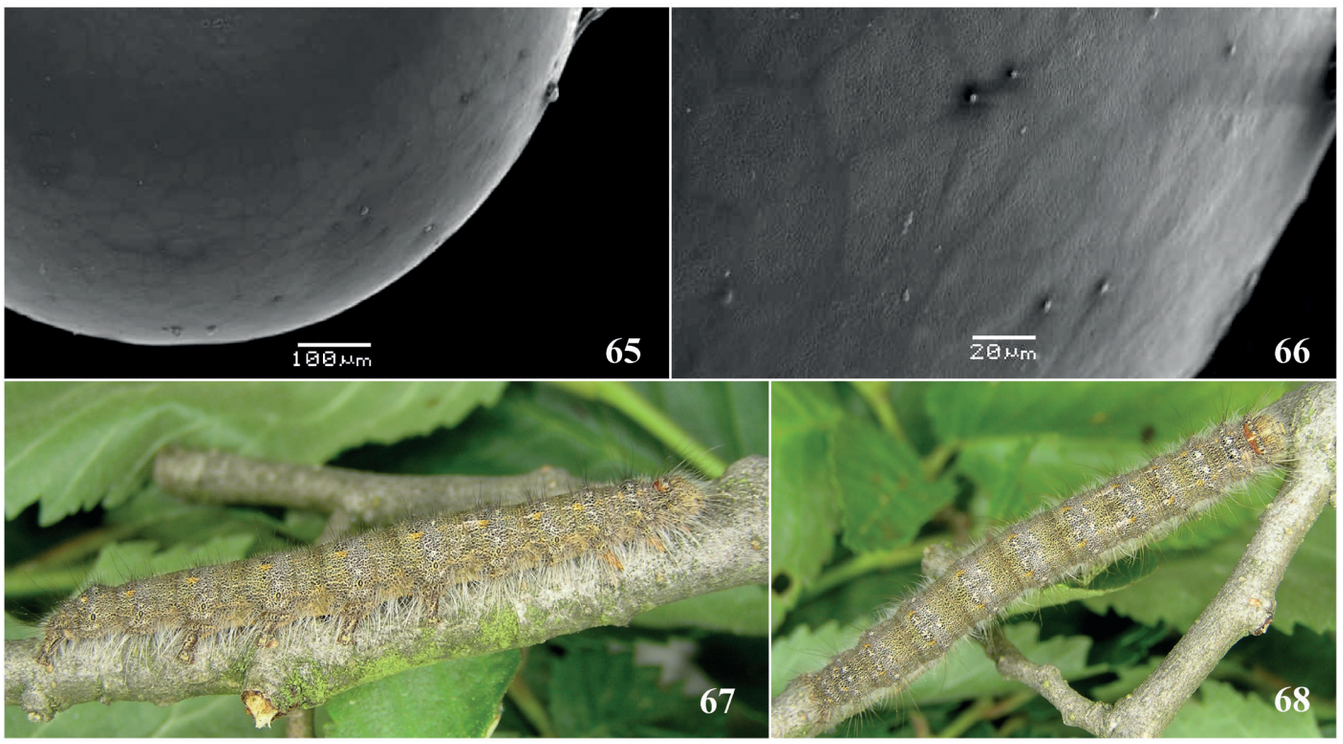

Figs 65, 66. *Poecilocampa populi*. Eggs from Cantabria, Spain (photographed with SEM). **65.** Chorion surface. **66.** Detail of the sculpture of the chorion.
Figs 67, 68. *Poecilocampa populi*. Caterpillar, final instar, Germania central, Thuringia, vic. Jena, Remderoda (50°56'24'' N 11°32'08'' E, 285 m, leg. et cult. Friedrich. 65. Dorsal view. 66. Lateral view. (Photos: Figs 67, 68 E. Friedrich).

and a group of smaller but sclerotized spicules. The examined male genitalia show high homogeneity; no significant differences were found.

Female. Wingspan paratypes 39–42 mm (n=4). Antenna filiform, inconspicuously shortly serrated; wing shape somewhat elongated, patagia light grey, tegulae monochrome dark brown with some light brown hair-like scales, otherwise hardly different from male.

Female genitalia (Figs 34, 35). The following preparations were made: GP–DF ♀ Paratype 2016/38: Cádiz province, Los Barrios, Arroyo del Tiradero, Parque Natural Los Alcornocales. 7.I.2019, in coll. DF; GP–YML ♀ Paratype YMG33.2021, same locality, in coll. YML.

Figs 55–64. *Poecilocampa navalagamellae turdetana*, caterpillars ab ovo breeding (cult. YML). – **55–57.** First instars. **58–64.** Final instar. **58.** Dorsal view. **59.** Lateral view. **60.** Detail of the thoracic segments ornamentation. **61.** Detail of the final segments, false legs and anal plate. **62.** Dorsoventral view with thoracic legs. **63.** Detail of hairs in lateral view. **64.** Detail of the head.

Abdominal segments slightly sclerotized; anal papillae ovate with a coarsely structured surface and densely hairy with numerous setae; apophyses of equal length; post-vaginal plate present as a narrow sclerite; antevaginalis plate broadly semicircular; antrum conical, with no structure merging into a long and wide membranous ductus bursae; ostium bursae broadly funnel-shaped, heavily sclerotized; corpus bursae elongated with two tiny, inconspicuous round signa.

Variability. The males examined exhibit no significant variation in the ground colour and the intensity of the ochraceous suffusion of the forewing upper side.

Larva (Figs 55–64). With a total length of approx. five centimeters, the somewhat flattened adult caterpillars are largely similar to those of *P. populi* or *P. alpina* but there are visible differences. There are two colour morphs known of *P. populi*. Caterpillars of the light morph are light greybrown in colour, have dense, dark, very fine speckles and a light spot dorsolaterally on each segment (Figs 67, 68). The dark morph is deep grey in colour and has diamond-shaped spots dorsally with pairs of light spots next to them. In *ab ovo* caterpillars of the *P. n. turdetana* population from Cádiz the markings on the dorsal area are barely discernible, with a dark mottling predominating on a whitish background and without a visible dorsal line. They have six small dorsal protrusions and two larger protrusions on the sides. The whole body is covered with hairs, on the flanks with long light hairs, on the dorsal section with short white, medium-length reddish and longer, almost black hairs. The head is cream-coloured with dark spots. The dorsal part of the thoracic segments is ornamented with a black spot interrupted by some light spots. In the first segment there is a broad orange three-pointed spot. The thoracic legs are orange. The false legs have a colouring similar to that of the rest of the body. A faint central reddish spot appears on the anal plate. The spiracles are grey with a black outline. The ventral part of the abdominal segments shows dark spots. In the intermediate stages of development, the caterpillars show two stripes of yellow lines and dots on their dorsal part that become less visible as they successively shed their skin.

Egg and pupa. Same as detailed in the redescription of the nominotypical subspecies.

Diagnosis. *Poecilocampa n. turdetana* adults differ noticeably to those of *P. n. navalagamellae*, mainly in the pattern of


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the fore wings, which present a more contrasting dark brown base colour and a more strongly dentate postmedial line. The male genitalia of *P. n. turdetana* are similar to those of *P. n. navalagamellae*, but are characterized by having wider valvae and a remarkably elongated and wider sacculus. The phallus is less curved, the spicule field of the vesica is more pronounced, and the cornuti are somewhat larger (Figs 41–44). The highly characteristic degree of sclerotization of the abdominal segments (n = 4) shows moderate but typical differences between the two subspecies (Figs 39, 40). The eggs of both *P. navalagamellae* subspecies resemble macroscopically those of other species, such as *P. populi*, in size and pigmentation. However, they can be distinguished with ease by the presence of a central zone in both faces of the chorion with irregular micro pits forming a reticular pattern of polygons, absent in the *P. populi* (Figs 65, 66) or *P. alpina* chorion, which is completely smooth.

Habitat, host plants and life history (Fig. 69). In Cádiz *P. navalagamellae turdetana* is found between 180 and 750 m. Other locations known from the literature that we provisionally attribute to this species suggest a high montane habitat preference: Murcia (1250 m), Albacete (1130 m), Granada (1550 m), Jaén (590 m). Pérez López (1989) describes the habitat in the province of Granada as forests dominated by *Quercus pyrenaica* and *Quercus faginea*. In the province of Cádiz, the habitat is a mature, open and humid forest of the Afro-Iberian endemic Algerian Oak (*Quercus canariensis*) accompanied by other trees such as *Fraxinus angustifolia* and *Alnus glutinosa* (Fig. 69).

Distribution (Fig. 1). *Poecilocampa n. turdetana* is known to occur in the province of Cádiz. However, its distribution area is likely to include most of Andalucía (Cádiz, Málaga, Jaén, Granada, Almería); records from Castilla La Mancha (Albacete) and Murcia probably refer also to this subspecies. The genitalia from Granada shown in Pérez López (1993) correspond to those of the new subspecies which allows us to provisionally assume that its distribution occupies a wide area in the south and southeast of Spain.

Figs 69, 70. Characteristic habitats. **69.** Habitat of *Poecilocampa navalagamellae turdetana* at Bosque de Niebla, Tarifa, Cádiz. Humid forest of the Afro-Iberian endemic Algerian Oak (*Quercus canariensis*) **70.** Habitat of *Poecilocampa navalagamellae navalagamellae* at Fresnedillas de la Oliva, Madrid. Open pasture with stands of Holm Oak (*Quercus ilex*). **Figs 71–76. Iberian** *Poecilocampa*, adult males. **71, 72.** *Poecilocampa navalagamellae turdetana,* Cádiz. **73, 74.** *Poecilocampa navalagamellae navalagamellae,* Madrid. **75, 76.** *Poecilocampa populi*, Cantabria. (Photo: Fig. 69 Arturo Iglesias).

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