Zootaxa 2281: 53–67 (2009)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Sex attractant, distribution and DNA barcodes for the Afrotropical leaf-mining moth *Phyllonorycter melanosparta* (Lepidoptera: Gracillariidae)

JURATE DE PRINS[1,6], RAIMONDAS MOZŪRAITIS[2, 5], CARLOS LOPEZ-VAAMONDE[3] & RODOLPHE ROUGERIE [4]

[1]*Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium. E-mail: jurate.de.prins@africamuseum.be*
[2]*School of Chemistry and Engineering, Royal Institute of Technology, Teknikringen 30 SE-10044, Stockholm, Sweden. E-mail: raimis@kth.se*
[3]*Institut National de la Recherche Agronomique, UR0633 Zoologie Forestière, F-45075 Orléans, France. E-mail: Carlos.Lopez-Vaamonde@orleans.inra.fr*
[4]*Biodiversity Institute of Ontario, University of Guelph, 579 Gordon Street, Guelph, Ontario, N1G 2W1, Canada. E-mail: rrougeri@uoguelph.ca*
[5]*Institute of Ecology, Vilnius University, Akademijos 2, LT-08412, Vilnius, Lithuania*
[6]*Corresponding author*

Abstract

The sex attractant for *Phyllonorycter melanosparta* (Meyrick, 1912) has been determined as (10*E*)-dodec-10-en-1-yl acetate and (10*E*)-dodec-10-en-1-ol combined in a ratio 10:1. The distribution of this species in Eastern Africa is updated and its presence in Kenya is recorded for the first time. We discuss the taxonomic status of *P. melanosparta* with reference to three character sets: semiochemicals, morphological and molecular characters (DNA barcodes). This combination of characters is also proposed as a new approach to study the diversity and phylogeny of *Phyllonorycter* in the Afrotropical region.

Key words: Kenya, molecular systematics, morphology, semiochemicals

Introduction

The family Gracillariidae is one of the most species-rich families of leaf-mining Lepidoptera with 1901 species (De Prins & De Prins 2009). Much of its species diversity resides in a number of super-rich genera. Among these is the genus *Phyllonorycter* Hübner, 1822, currently comprised of 401 species (De Prins & De Prins 2009), with a worldwide distribution. The vast majority of species are found in the temperate regions, with about 257 species described from the Palaearctic region and 81 from the Nearctic. In the tropics, *Phyllonorycter* is species-poor, with 36 species described from Indoaustralia, 13 from the Neotropics and 22 from the Afrotropical region (De Prins & De Prins 2009).

The host ecology of these micro-moths is quite well known, at least for the Holarctic region. As all other Gracillariidae, larvae of *Phyllonorycter* moths feed internally on living plant tissues where the instars initiate a supra- or infra-tentiform mine by devouring mainly the parenchyma cells. All preimaginal stages of *Phyllonorycter*, including the pupa, develop within a tentiform mine (Emmet *et al*. 1985; Davis & Robinson 1998). Overall, the genus *Phyllonorycter* has been recorded feeding on 112 plant genera from 31 different families, 15 orders, and six subclasses (Lopez-Vaamonde *et al*. 2003; De Prins & De Prins 2009).

The alpha taxonomy, ecology and host range of tropical *Phyllonorycter* is less well known. For instance, host plants of 11 species of *Phyllonorycter* in Africa are known from the rearing efforts of L. Vári in the middle of last century (Vári 1961), while the host plants and feeding habits are still unknown for the

Accepted by J.Rota: 29 Sept. 2009; published: 4 Nov. 2009

remaining 10 Afrotropical species. With such gaps in the knowledge of Afrotropical leaf-mining moths, more studies are needed to improve our understanding of their faunistics, systematics and ecology.

In this paper we used recently developed sex attractants for European species of *Phyllonorycter* (Table 1) and tested the attractiveness of a set of formulations in Central and Eastern Africa, specifically targeting representatives of the family Gracillariidae. The main aims of this contribution are the following: 1) to illustrate how sex attractants — a method rarely used for biodiversity studies (but see De Prins & Mozūraitis 2006) — represent a promising original approach for collecting rare taxa and better documenting their geographic distribution; 2) to identify the chemical formulation of attractants for Afrotropical *Phyllonorycter* species; 3) to obtain new data on the distribution of *Phyllonorycter* species in the Afrotropical area; and 4) to provide DNA barcodes as identification tags and an additional dataset for documenting the diversity of gracillariid micro-moths, especially in the Afrotropical region.

Abbreviations of institutions:

BMNH	The Natural History Museum, London, U. K.
CCDB	Canadian Centre for DNA Barcoding, University of Guelph, Canada
INRA	Institut National de la Recherche Agronomique, Orléans, France
MNHN	Muséum national d'Histoire naturelle, Paris, France
MSNV	Museo Civico di Storia Naturale, Verona, Italy
NMK	National Museums of Kenya, Nairobi
RMCA	Royal Museum for Central Africa, Tervuren, Belgium
RMNH	Nationaal Natuurhistorisch Museum "Naturalis", Leiden, the Netherlands
TMSA	Transvaal Museum, Pretoria, Republic of South Africa
ZFMK	Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany
ZMHB	Zoologisches Museum der Humboldt Universität, Berlin, Germany.

Methods

Attractiveness tests

To screen the attractiveness of different compounds to *Phyllonorycter* species in Central and East Africa, we used seven of the most distributed sex attractants in the genus *Phyllonorycter* (10*E*)-dodec-10-en-1-yl acetate (*E*10-12:OAc), (10*E*)-dodec-10-en-1-ol (*E*10-12:OH) (10*Z*)-dodec-10-en-1-yl acetate (*Z*10-12:OAc), (8*E*)-tetradec-8-en-1-yl acetate (*E*8-14:OAc), (8*Z*)-tetradec-8-en-1-yl acetate (*Z*8-14:OAc), (10*E*)-tetradec-10-en-1-yl acetate (*E*10-14:OAc), (10*Z*)-tetradec-10-en-1-yl acetate (*Z*10-14:OAc), and the binary mixtures of *E*10-12:OAc and *E*10-12:OH in ratios of 10:1, 1:1, and 1:10, as well as *Z*8-14:OAc and *Z*10-14:OAc in the same ratios mentioned above. We used a dispenser without any ingredients as control.

All the compounds used in the field tests were synthesized at Tartu University, Estonia, and purified by preparative liquid chromatography, as described by Mozūraitis *et al*. (1998). The isomeric and chemical purities of the compounds exceeded 99%. A dosage of 0.2 mg per dispenser was used both with single compounds and mixtures. The synthetic sex attractant components were dissolved in hexane (Merck) and soaked from the inside into the walls of red rubber tube dispensers (8 × 15 mm). Each lure was placed in an opaque white delta trap (trapping window sides of 10 × 11 × 10 cm and trap length of 18 cm) that had an exchangeable bottom (11 × 18 cm) coated with sticky material. "Atracon A" and Attract®Delta traps and sticky inserts were obtained from Flora Co., Tartu, Estonia and Biobest Belgium N.V., Westerlo, Belgium. Each trap was fixed on a tree branch 1.5–2.2 m above ground level and was inspected and moved to the next trap location (within each replication) every 3 days. In the West African primary rain forest an additional set of seven traps was suspended in the canopy about 20–25 m high and another set at 1.5–2 m beneath it. The traps were placed in a semi round circle and the distance between the traps was approximately 50 m. Five

TABLE 1. List of the chemical compounds determined as sex pheromones or sex attractants in the genus *Phyllonorycter*. [(1)] The compounds which function only as sex pheromones (P) or sex attractants (A) are included in the list; [(2)] only the author(s) who first reported on the compounds are listed.

Species name	Compound name	Ratio	Status[(1)]	Reference[(2)]
P. acerifoliella	*Z*10-14:OAc	94	P	Mozūraitis *et al*. 2000
	*Z*8-14:OAc	6	P	
	*E*9-14:OAc	10	A	Mozūraitis *et al*. 1998
	*Z*10-14:OAc	1	A	
P. blancardella	*E*10-12:OAc		P	Mozūraitis *et al*. 1999
	*E*4*E*10-12:OAc		A	Gries *et al*. 1993
P. coryli	*E*9-14:OAc	10	A	Mozūraitis *et al*. 1998
	*Z*10-14:OAc	1	A	
P. corylifoliella	*E*4*Z*7-13:OAc		A	Voerman & Herrebout 1978
	*E*4-12:OAc		A	Voerman 1991
P. crataegella	*Z*10*Z*12-14:OAc	100	P	Ferrao *et al*. 1998
	*E*10*E*12-14:OAc	1	P	
P. cydoniella	*E*10-12:OAc		A	Mozūraitis *et al*. 1998
P. dubitella	*E*9-14:OAc	10	A	Mozūraitis *et al*. 1998
	*Z*10-14:OAc	1	A	
P. elmaella	*E*10-12:OAc		A	Shearer & Riedl 1994
P. heegeriella	*Z*8-14:OAc	44	P	Mozūraitis *et al*. 2000
	14:OAc	1	P	
	*E*9-14:OAc	10	A	Mozūraitis *et al*. 1998
	*Z*10-14:OAc	1	A	
P. junoniella	*Z*10-12:OAc		A	Mozūraitis *et al*. 1998
P. insignitella	*Z*8*E*10-14:OAc	96	P	Mozūraitis *et al*. 2008
	*Z*8*E*10-14:OH	4	P	
	*E*8*Z*10-14:OAc	traces	P	
P. klemannella	*Z*10-14:OAc	1	A	Booij & Voerman 1984
	*E*10-14:OAc	3	A	
P. melanosparta	*E*10-12:OAc	10	A	This publication
	*E*10-12:OH	1	A	
P. mespilella	*E*4*E*10-12:OAc		P	Gries *et al*. 1993
	*E*10-12:OAc		A	Hrdy *et al*. 1989
P. nigrescentella	*Z*8*E*10-14:OAc		P	Mozūraitis *et al*. 2008
P. obandai	*Z*8-14:OAc		A	De Prins & Mozūraitis 2006
P. orientalis	*Z*10-14:OAc		A	Ando *et al*. 1977
P. oxyacanthae	*E*10-12:OAc	10	A	Mozūraitis *et al*. 1998
	*E*10-12:OH	1	A	
P. platani	*Z*10-14:OAc		P	Subchev *et al*. 2003
P. pulchra	*E*8*E*10-14:OAc		A	Ando *et al*. 1987
P. pygmaea	*Z*8-14:OH		A	Ando *et al*. 1977
P. pyrifoliella	*E*10-12:OAc		A	Laanmaa 1990

continued next page

TABLE 1. (continued)

Species name	Compound name	Ratio	Status[(1)]	Reference[(2)]
P. ringoniella	*Z*10-14:OAc	10	P	Sugie *et al.* 1986
	*E*4*Z*10-14:OAc	1	P	
P. kuhlweiniella	*E*8-14:OAc	1	A	Voerman 1988
	*E*10-14:OAc	1	A	
P. sorbi	*E*10-12:OH		A	Mozūraitis *et al.* 1998
P. ulmifoliella	*Z*10-14:OAc		P	Mozūraitis *et al.* 1997
P. watanabei	*Z*10-13:OAc		A	Ando *et al.* 1977

replicates of each compound and mixture were used. The attractiveness tests were carried out in three African countries: 25 March–6 April 2003 and 13 March–11 April 2004 in Kenya; 21 November–8 December 2003 and 26 April–12 May 2005 in Cameroon; 13 March–15 April 2006 and 13 May–8 June 2007 in the Democratic Republic of Congo. The test areas were situated in different habitats: savannah and the eastern edge of the remnants of the Guineo-Congolian forests in East Africa (Kenya 2003–2004), secondary forest intermixed with bushland in Central African areas (Northern Cameroon 2005–2006) and the primary rain forest in the Congo basin (the Democratic Republic of Congo 2006–2007). Data from field tests were analyzed by nonparametric Kruskal-Wallis analyses of variance followed by Mann-Whitney U-test (Sokal & Rohlf 1995).

Sample collection

In addition to attractant traps, adult specimens were attracted to a mercury vapour light placed in front of a white vertical screen. Captured specimens of micro-moths were spread in the field under a stereomicroscope on small plastozote rectangles which were attached to microscope slides and dry-preserved in a hermetic plastic microscope slide box along with moisture absorbing silica gel crystals. This enabled us to keep the forewing pattern of Lithocolletinae specimens intact for further identification despite rough travelling and hard weather conditions often experienced in tropical Africa. The adults were examined externally using a stereomicroscope. Genitalia were prepared following Robinson (1976) with some modifications. After maceration of the abdomen in 10% KOH for 15 hours and subsequent cleaning and deionization, the male genitalia were stained with 2% eosine B, a mixture of 2% azophloxine and 2% acid fuchsin; the female genitalia were stained with a 1% chlorazol black E solution. The genital morphology was examined using a Leica DMLB microscope under magnifications of 150×, 200×, and 400×. The image of the genitalia was made with a Q imaging Micropublisher 5.0 RTV camera connected to a Leica DMLB light microscope and processing the images by Auto-Montage Syncroscopy to produce composite results from several separate photographs in planes of different depths. Sternum 8 is removed to show the tips of valvae which are diagnostically important for this species. The terminology of the anatomy follows Vári (1961), Klots (1970), Kumata (1993, 1995), and Kristensen (2003). The specimens caught by automatic traps in Kakamega forest, Kenya within the project BIOTA-East, supervised by the ZMHB and ZFMK (Kühne 2008), were briefly examined by JDP. JDP examined the primary types of *P. melanosparta* (Meyrick, 1912) deposited at the TMSA and the primary types of all of the remaining 21 Afrotropical *Phyllonorycter* species deposited at the BMNH, RMCA, TMSA, MNHN, and ZMHB. Additional material, deposited at the TMSA and RMCA, was studied as well.

Molecular protocols and analysis

DNA was extracted from adult specimens (dry hind legs) using the routine protocol of the CCDB (See Ivanova *et al.* 2006 and the CCDB website: www.dnabarcoding.ca/pa/ge/research/protocols). The 'DNA barcode' region of COI was amplified with the primer set LepF1/LepR1 (Hebert *et al.* 2004). Failed samples

went through a second PCR attempt with primer pairs LepF1/MLepR1 and MLepF1/LepR1 (Hajibabaei *et al.* 2006) targeting 2 shorter fragments with a 55 bp overlap. All PCR amplifications were performed according to the standard PCR reaction protocol used in CCDB (Hajibabaei *et al.* 2005). PCR products were checked on a 2% E-gel® 96 Agarose (Invitrogen). Unpurified PCR fragments obtained from the LepF1/ LepR1 primer pair were sequenced in both directions, while shorter fragments obtained using the internal primers MLepR1 and MLepF1 were sequenced in one direction only using LepF1 and LepR1 primers. The sequencing reactions followed CCDB protocols (Hajibabaei *et al.* 2005), with products subsequently purified using Agencourt® CleanSEQ protocol (Agencourt, Beverly, MA, USA). The sequences were managed in SeqScape version 2.1.1 (Applied Biosystems, Foster City, CA, USA) and Sequencher 4.5 (Gene Code Corporation, Ann Arbor, MI, USA) and aligned using Bioedit version 7.0.5.3 (Hall 1999) and MEGA4 (Tamura 2007).

In total, 19 individuals were barcoded (see Table 2), encompassing 4 species of Lithocolletinae (*Phyllonorycter melanosparta, P. salictella* (Zeller, 1846), *P. obandai* De Prins & Mozūraitis, 2006 and *Cameraria ohridella* Deschka & Dimić, 1986) and 2 Gracillariinae (*Gibbovalva quadrifasciata* (Stainton, 1862) and *Eucalybites aureola* Kumata, 1982); the latter two were used as outgroups for the analysis.

Records for those specimens are gathered within the project 'Gracillariidae — PUBLIC records' (code GRPUB) in the Published Projects section of the Barcode of Life Data systems (BOLD; www.barcodinglife.org) (Ratnasingham & Hebert 2007). Information on specimen vouchers (field data and GPS coordinates) and sequences (nucleotide composition, trace files) can be found in this project by following the 'view all records' link and clicking on the 'specimen page' or 'sequence page' links for each individual record. Sequences are also available on GenBank with accession numbers GQ144154 and GU073228 to GU073245 (see also Table 2).

TABLE 2. Samples used for the DNA barcoding analysis. The Sample ID code is a unique identifier linking the record in the BOLD database and the voucher specimen from which the sequence is derived. Additional collecting and specimen data are accessible in BOLD's public project GRPUB, as well as all sequence data.

Sample ID	Species	Country	Process ID (BOLD)	Accession number (GENBANK)
RV337	*Cameraria ohridella*	France	CAMER355-07	GQ144154
CLV21407	*Eucalybites aureola*	Japan	GRACI102-07	GU073228
CLV21707	*Gibbovalva quadrifasciata*	Japan	GRACI105-07	GU073229
CLV11107	*Phyllonorycter melanosparta*	Kenya	GRACI001-07	GU073239
CLV11307	*Phyllonorycter melanosparta*	Kenya	GRACI003-07	GU073238
CLV13907	*Phyllonorycter melanosparta*	Kenya	GRACI029-07	GU073237
CLV15507	*Phyllonorycter melanosparta*	Kenya	GRACI045-07	GU073240
CLV24107	*Phyllonorycter melanosparta*	Kenya	GRACI129-07	GU073235
CLV24207	*Phyllonorycter melanosparta*	Kenya	GRACI130-07	GU073234
CLV24807	*Phyllonorycter obandai*	Kenya	GRACI136-07	GU073245
CLV23307	*Phyllonorycter salictella*	Sweden	GRACI121-07	GU073244
CLV23407	*Phyllonorycter salictella*	Sweden	GRACI122-07	GU073233
CLV23507	*Phyllonorycter salictella*	Sweden	GRACI123-07	GU073243
CLV23807	*Phyllonorycter salictella*	Sweden	GRACI126-07	GU073232
CLV23907	*Phyllonorycter salictella*	Sweden	GRACI127-07	GU073231

Aligned sequences were analyzed with NONA 2.0 (Goloboff 1999) as implemented in Winclada 1.00.08 (Nixon 2002) with the following settings: hold 100000, hold/100, mult*500 followed by max*. The Gracillariinae *Gibbovalva quadrifasciata* was used as the primary outgroup for the analysis. Bootstrap support values were calculated after 1000 pseudo-replications using the parameters hold/100, mult*100 in Winclada. The genetic distances were calculated with MEGA4 with the pairwise deletion option, considering all 3 codon positions, both transitions and transversions, and a homogeneous pattern of variation among lineages as well as uniform rates among sites. Kimura's two parameter (K2P) correction method was chosen.

Results

Sex attractant

Eight specimens of *Phyllonorycter melanosparta* were attracted to the lures of *E*10-12:OAc and *E*10-12:OH in the ratio 10:1 in the Kakamega Forest (West Kenya) (Fig. 4) and one male was caught in the trap baited with *E*10-12:OAc and *E*10-12:OH in the ratio 1:1. Other lures and control were not active. The attractiveness of the binary mixture composed of *E*10-12:OAc and *E*10-12:OH in the ratio 10:1 differ significantly from the other lures tested (Kruskal-Wallis analyses of variance followed by Mann-Whitney U-test at $P < 0.05$) which suggests that it is a sex attractant for *P. melanosparta* (Fig. 1A, B). This is the first report of a mixture of semiochemicals to which males of *P. melanosparta* react. Along with these gracillariid specimens, the lures attracted six specimens of a *Cydia* species (Tortricidae).

Two characteristic features of the molecular structure of the sex attractant of *P. melanosparta* are the double-bonded carbons at even positions in both sex attractant components and the functional group of acetate in *E*10-12:OAc. This composition is known to be functional for other species of *Phyllonorycter* as well (Table 1, Fig. 1).

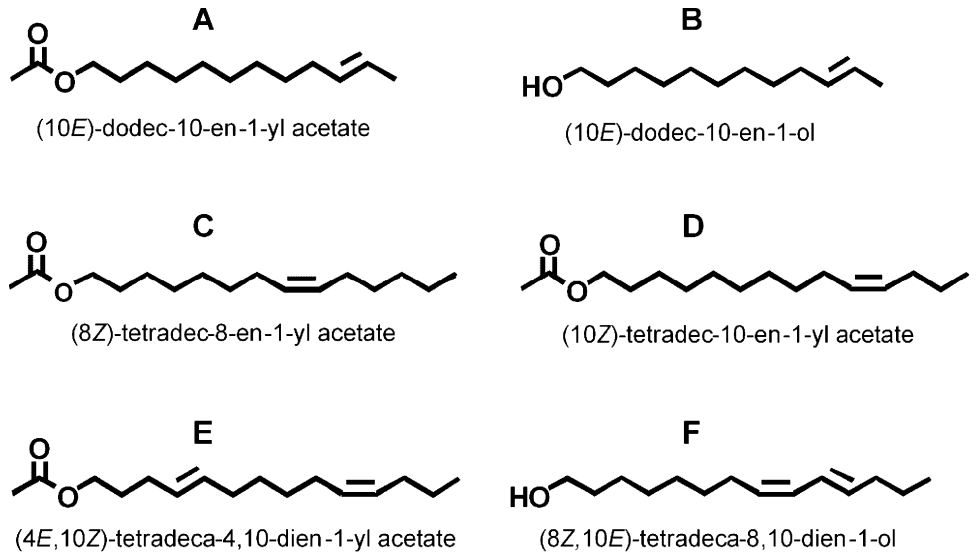

FIGURE 1. Structures of the representative compounds determined as sex pheromones and/or sex attractants in the genus *Phyllonorycter*: A, (10*E*)-dodec-10-en-1-yl acetate is a major sex attractant component of *P. melanosparta* and is used in sex communication by 6 other *Phyllonorycter* species; B, (10*E*)-dodec-10-en-1-ol is a minor sex attractant component of *P. melanosparta* species and is used in sex communication by 2 other *Phyllonorycter* species; C, (8*Z*)-tetradec-8-en-1-yl acetate is known as sex attractant for the African species *P. obandai* and is used in sex communication by 2 other *Phyllonorycter* species; D, (10*Z*)-tetradec-10-en-1-yl acetate is the most abundant compound used in sex communication by 9 *Phyllonorycter* species; E, (4*E*,10*Z*)-tetradeca-4,10-dien-1-yl acetate represents non-conjugated dienic acetate type structure known as sex pheromone component of *P. ringoniella*; F, represents conjugated dienic alcohol type structure known as sex pheromone component of *P. insignitella*.

FIGURE 2. *Phyllonorycter melanosparta* male: Pretoria, 26 Apr 1952, L. Vári, "*Lithocolletis melanosparta* Meyrick, Metallotype 6347", Gen. prep. 6925♂, Ac. no. 514, in TMSA. Wing span 6.5 mm.

FIGURE 3. Male genitalia of *Phyllonorycter melanosparta* of the specimen ID: RMCA ENT 000004148, Kenya, Kakamega Forest, Primary Forest, 1600m, 00°20'N 34°52'E, 27 Mar 2003, leg. J. De Prins, pheromone trap baited with *E*10-12:OAc, genitalia preparation De Prins 3691♂ (RMCA 00377), in RMCA. Scale bar 500 µm.

Taxonomy and faunistics

***Phyllonorycter melanosparta* (**Meyrick, 1912)
(Figs. 2, 3)

Lithocolletis melanosparta — Meyrick (1912: 21); Vári (1961: 208–210).
Phyllonorycter melanosparta — Vári & Kroon (1986: 54, 136, 157; Kroon (1999: 50, 104, 114, 120); Dall'Asta *et al*. (2001: 34); Vári *et al*. (2002: 26); De Prins & De Prins (2005: 319–320).

Type material: *Holotype*: ♀, [South Africa], Barberton, 31 Dec 1910, A. J. T. Janse. '47/41'. *L. melanosparta* 4741. G[enitalia] 7142. *Lithocolletis melanosparta* M.[eyrick], Type No 360, in TMSA (studied).

Additional material: 73 adults of *P. melanosparta* were studied (nine individuals were attracted to attractant traps (see Table 3), 59 were reared from mines and five were attracted to light traps). *P. melanosparta* specimens are recorded from nine localities from three different African countries: Kenya (one locality, new record), South Africa (seven localities: (Meyrick 1912: 21; Vári 1961: 210), and Zimbabwe (one locality: (Vári 1961: 210)). Three species of Fabaceae are recorded as host plants: *Flemingia grahamiana* Wight & Arn., *Rhynchosiae caribea* (Jacq.) DC, and *Vigna* sp. (Vári 1961: 210; Kroon 1999: 50; Dall'Asta *et al*. 2001: 34; De Prins & De Prins 2005: 320).

For a detailed description of the adult morphology please refer to Vári (1961: 208–210; pl. 22, fig. 4; pl. 65, fig. 1; pl. 104, fig. 1).

Remarks: We have studied all available material of *P. melanosparta*, which was either reared, caught or attracted to lures.

TABLE 3. Collection data on *P. melanosparta* individuals attracted to lures and used for morphological analysis.

Country	Collection date	Collector	Original slide number	Museum slide number	Specimen ID
Kenya	27/03/2003	De Prins J. & W.			RMCA ENT 000003180
Kenya	27/03/2003	De Prins J. & W.	DP 3691	RMCA 00377	RMCA ENT 000004148
Kenya	27/03/2003	De Prins J. & W.	DP 3690	RMCA 00378	RMCA ENT 000004149
Kenya	30/03/2003	De Prins J. & W.			RMCA ENT 000003272
Kenya	30/03/2003	De Prins J. & W.	DP 3642	RMCA 00375	RMCA ENT 000004147
Kenya	01/04/2003	De Prins J. & W.	DP 3701	RMCA 00403	RMCA ENT 000003288
Kenya	03/04/2003	De Prins J. & W.			RMCA ENT 000003179
Kenya	03/04/2003	De Prins J. & W.	DP 3639	RMCA 00376	RMCA ENT 000004150
Kenya	03/04/2003	De Prins J. & W.			RMCA ENT 000004146

DNA barcoding

DNA barcodes were obtained from six specimens of *Phyllonorycter melanosparta*; three sequences are complete barcodes of 658 bp whereas the other three are 291 to 293 bp long. Reducing all sequences to the 291 bp shared by all terminals has no effect on the results (complete deletion option in MEGA4, results not shown). There are four haplotypes, all originating from the same locality in Kenya, which differ by a single nucleotide substitution. The mean genetic variation (K2P distances) within *P. melanosparta* is 0.2%, with a maximum distance of 0.35% between individuals. On the other hand, three haplotypes are observed in the European species *P. salictella*, with a maximum intraspecific distance of 1.93%. Interspecific distances are high: within the genus *Phyllonorycter*, their range extends from 10.6% between *P. melanosparta* and *P. salictella* to 19.6% between the latter and *P. obandai*. Interestingly, the phylogenetic analysis of the barcode

sequences results in paraphyly of the genus *Phyllonorycter* (Fig. 6), with the species *P. obandai* resolved as sister to *Cameraria ohridella* + other *Phyllonorycter* species.

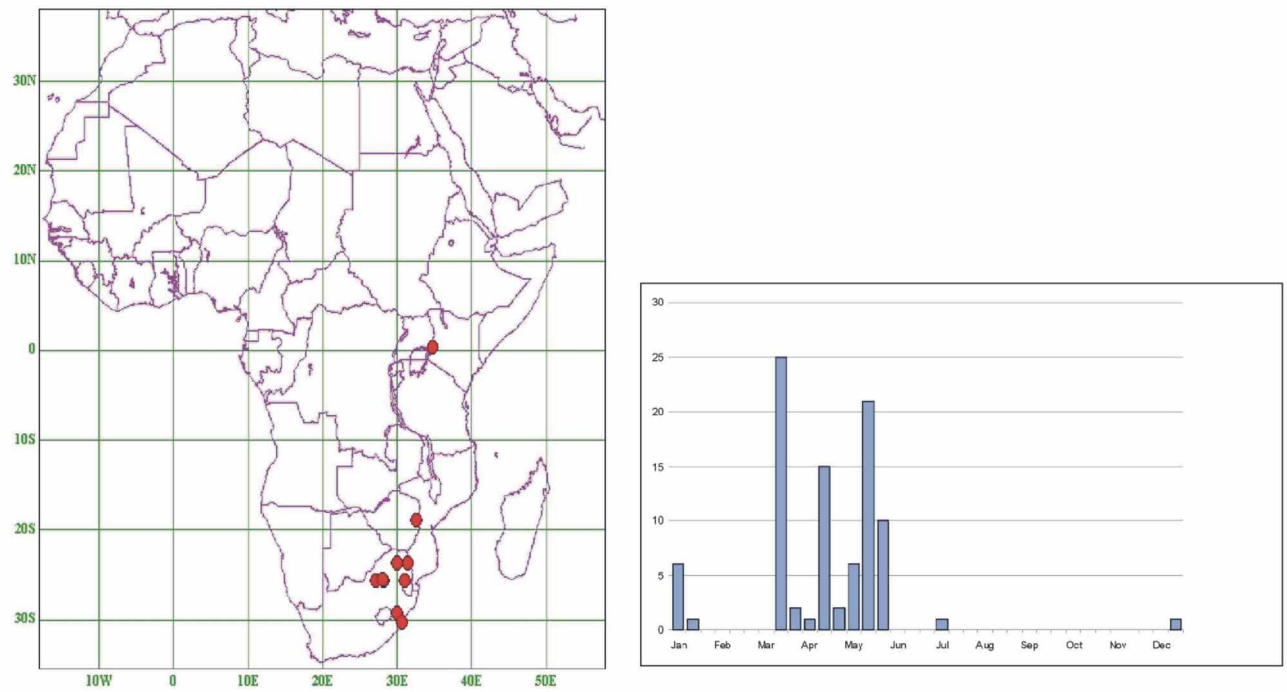

FIGURE 4. Map showing the distribution of *Phyllonorycter melanosparta*.
FIGURE 5. Flight histogram per decade of *Phyllonorycter melanosparta.*

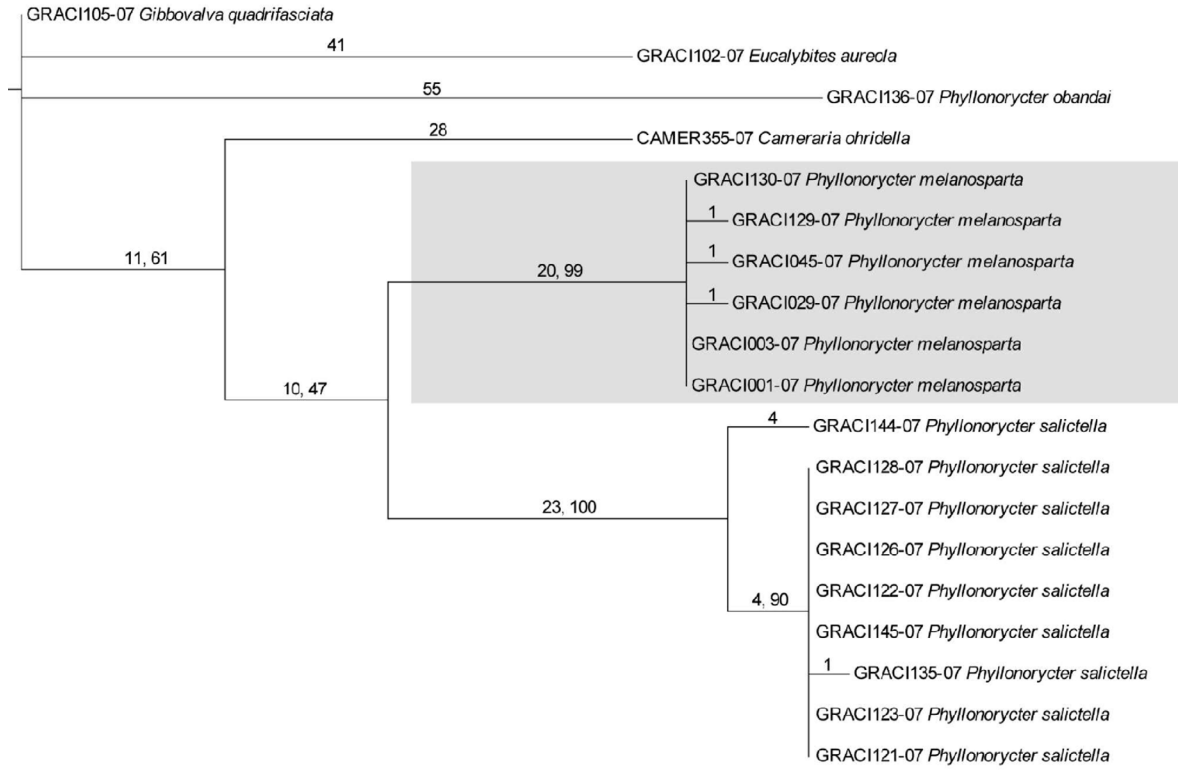

FIGURE 6. Strict consensus of the six most-parsimonious trees (Length = 352, CI=0.74, RI=0.79) obtained after a phylogenetic analysis of DNA barcodes for 19 terminals. The branch lengths are proportional to the number of changes, which are indicated above branches and followed by the bootstrap support value at each node.

Discussion

Sex attractants, faunistics and flight periods. The application of sex attractants resulted in a recent discovery of a new species in the Afrotropical region, *Phyllonorycter obandai* (De Prins & Mozūraitis 2006), and until now it was the only Afrotropical Gracillariidae species for which the sex attractant was recorded (De Prins & Mozūraitis 2006). Here we present the sex attractant for a second Afrotropical *Phyllonorycter* species. The application of the sex attractant *E*10-12:OAc and *E*10-12:OH in the ratio 10:1 made it possible to record *P. melanosparta* in a new Afrotropical locality. Prior to applying attractant, *P. melanosparta* was known from the holotype specimen collected by Meyrick in 1910 in South Africa and from the specimens reared by Vári in South Africa and Zimbabwe during the middle of the last century; the last collecting record of *P. melanosparta* is 1 Jan 1981 (unpublished diary notes of L. Vári). The use of sex attractants allowed us to find this species without the time-consuming effort of searching for larvae and rearing them under difficult tropical conditions.

*E*10-12:OAc is a commonly-used compound in sex communication of *Phyllonorycter* moths. It has also been identified as a sex pheromone and its biological activity was confirmed in two other European *Phyllonorycter* species: *P. blancardella* (Fabricius, 1781) (Mozūratis *et al*. 1999) and *P. mespilella* (Hübner, 1805) (Gries *et al.* 1993). Additionally, *E*10-12:OAc has been reported as a sex attractant for males of four more *Phyllonorycter* species: *P. cydoniella* ([Denis & Schiffermüller], 1775), *P. elmaella* Doğanlar & Mutuura, 1980, *P. oxyacanthae* (Frey, 1855), and *P. pyrifoliella* (Gerasimov, 1933) (Table 1). It is worth noting that males of *P. oxyacanthae* are attracted to the binary mixture comprised of *E*10-12:OAc and corresponding alcohol in the ratio 10:1 (Mozūraitis *et al*. 1998), which is exactly the same mixture as it was determined for *P. melanosparta*. In *Phyllonorycter* moths, alcohols are rarely used as sex attractants compared to acetates. In this regard *P. melanosparta* belongs to a small group of *Phyllonorycter* species that differs from the remainder of the genus. *E*10-12:OH alone was reported as sex attractant for *Phyllonorycter sorbi* (Frey, 1855) (Mozūraitis *et al*. 1998). The low catch of males of *P. melanosparta* to lures baited with *E*10-14:OAc and *E*10-14:OH in the ratio 10:1 could be caused by the different optimal composition of sex attractant in tropical areas. In addition, the diverse biotopes and the low density of moths during the trapping period could contribute to this low number of captures as well (Beck & Kitching 2007). Furthermore, low density of *Phyllonorycter* species might be common in the central parts of the Afrotropical region. Emergence peaks of *Phyllonorycter* are typical for the temperate climate zone including South Africa (Le Marchand 1936; Vári 1961; Bradley *et al.* 1969; Davis & Deschka 2001), but sufficient data on *Phyllonorycter* emergence periods in tropical areas are lacking. JDP has searched for *Phyllonorycter* specimens in the collected material of 17 automatic traps which were running every night in the period of April–September 2001–2003 in the Kakamega Forest (Kenya) in the framework of the Biota programme (Lepidoptera collecting was supervised by the ZMHB staff) and no emergence peaks of leaf-mining moths were documented.

The capture of several specimens of *Cydia* was likely due to the similarity in the chemical structure and composition of sex pheromones and sex attractants between moths from genera *Cydia* and *Phyllonorycter* (El-Sayed 2009). However, *Cydia* females release their pheromones at night (Castrovillo & Cardè 1979) while *Phyllonorycter* females call in the morning during the light part of the day (Mozūratis 2006), thus their periods of sex pheromone communication do not overlap.

The *melanosparta* species complex. Vári, in his review of the South African Gracillariidae (1961), diagnosed three African *Phyllonorycter* species: *anchistea* (Vári, 1961), *melanosparta* and *hexalobina* (Vári, 1961) as a separate group having forewings with fuscous instead of white markings. The species *anchistea* and *melanosparta*, showing only minor external and internal morphological differences, are considered by Vári (1961) as "very similar" species [cryptic]. 'Cryptic', 'allied', 'hidden' species and their complexes are known to be especially common among phytophagous insects (Bickford *et al*. 2007). Gracillariidae moths, and particularly those assigned to the subfamily Lithocolletinae, serve as a classic example of phytophagous internal leaf-tissue feeders showing the most intimate relationship with their host plant (Lopez-Vaamonde *et al*. 2003, 2006). The possible correlation between the phytophagous feeding mode of insects and the presence

of morphologically allied species complexes is not unusual (Berlocher & Feder 2002; Martin *et al.* 2003; De Prins & De Prins 2007; Triberti 2007; Condon *et al.* 2008; Lozier *et al.* 2008; Ohshima 2008; Schmitz *et al.* 2008; Trewick 2008). Morphological similarity among species of tropical Lithocolletinae complexes raises taxonomic problems for generic assignment of these species. Kumata (1993, 1995), describing the Lithocolletinae species from India, Malaysia and the Philippines, stated that *Phyllonorycter* is very similar to *Cameraria* Chapman, 1902 in adult features and particularly in wing venation, but distinguishable from the latter by the simple tegumen lacking apical setae in the male genitalia (Fig. 3) and by larval characters. The atypical forewing pattern (the lack of white markings) of both *melanosparta* and *anchistea* recently raised some doubts on the justification of their assignment to the genus *Phyllonorycter* (van Nieukerken & De Prins 2007). Larval characters were not available for our study. Hence, the tegumen is the only morphological feature that could be used for generic assignment of this species complex. *P. melanosparta* has a simple tegumen, lacking setae, which is typical for the genus *Phyllonorycter* (Kumata 1967, 1993, 1995). However, we do not consider that placement as definitive, and additional characters, shared either with *Phyllonorycter* or with *Cameraria* species, are still under investigation to address the generic assignment of this Afrotropical species.

Molecular systematics. DNA barcoding represents a useful and practical means of species identification, as well as a valuable tool for revealing or investigating cases of cryptic diversity (Burns *et al.* 2008; Vaglia *et al.* 2008). This work represents the first attempt to address the ability of COI to be used as a DNA barcode for species identification in the large genus *Phyllonorycter.* The DNA barcodes obtained for the target species, *Phyllonorycter melanosparta*, are highly conserved (maximum intraspecific distance = 0.35%) and as such they unequivocally characterize the species. Although taxon sampling should obviously be more comprehensive to better assess COI variation within the genus *Phyllonorycter*, it is nevertheless worth noting that the interspecific distances observed within the genus are significantly higher (as high as 19.6% between *P. salictella* and *P. obandai*) than the ones reported in other families of Lepidoptera (Hebert *et al.* 2004; Hajibabaei *et al.* 2006). Furthermore, it would be interesting to screen the remainder of the family for a relatively high level of intraspecific variation, such as observed here for *P. salictella* (1.93%). A high level of genetic divergence among species within the genus may be correlated with a high level of genetic divergence within species. For example, samples of *P. melanosparta* from other parts of its distribution (e.g., South Africa) are likely to reveal a high level of genetic variation within this species. However, these findings do not seriously challenge the use of DNA barcodes as diagnostic markers, as documented in other Lepidoptera when geographical sampling is extended (Lukhtanov *et al.* 2009). Preliminary analyses of a larger number of samples and species (unpublished data) converge toward such deep interspecific divergences within the family; intraspecific variation still needs to be further investigated, though none of the results obtained so far (see also Shapiro *et al.* 2008) challenge the observed "gap" between intra and interspecific variation. This observed gap indicates that DNA barcoding is a highly promising approach for a group of micro-moths whose identification and systematics is particularly difficult. It is also expected that, combined with morphological data and possibly other genetic markers, DNA barcodes will bring valuable information to address phylogenetic questions at the supraspecific level, for example the distinction and definition of the genera *Phyllonorycter* and *Cameraria*. The paraphyly of *Phyllonorycter* as revealed by our analysis (Fig. 6), though not strongly supported by bootstrap values and based on a small sample of taxa, represents a nice example of how additional data can stimulate and enhance systematic studies in difficult and poorly known groups of moths.

Concluding notes

Studies on tropical insects have always been most challenging and in most cases no single approach can satisfactorily be applied to answer ecological and/or evolutionary questions in Africa. We have attempted to present combined evidence of several aspects of the natural history of the Afrotropical leaf-mining moth *P.*

melanosparta: 1) chemical communication, 2) taxonomy & faunistics, and 3) molecular systematics. Our main goals are to advance our understanding of the delimitation of gracillariid species in the Afrotropics and discover characters that are suitable for the distinction between newly and previously recognized *Phyllonorycter* species. A multidisciplinary study seems to be the best approach. We intend to extend this work creating a synergy from morphological, molecular and ecological characters of all Afrotropical *Phyllonorycter* species. We hope in this way to increase our understanding of the diversity and phylogeny of this genus in the Afrotropical region.

Acknowledgments

JDP is grateful to Wanja Kinuthia (NMK) for her kind assistance in arranging the permits to access the natural biotopes in Kenya and the late Ernest Obanda for his invaluable practical assistance in the field. Barbara Dombrowsky (TMSA) is kindly thanked for providing the possibility to consult the unpublished collecting diaries of L. Vári. JDP also expresses many thanks to Torben Larsen (London) for his inspiring and fruitful correspondence on the Lepidoptera biodiversity studies in Africa. Erik van Nieukerken (RMNH) is gratefully acknowledged for his inspiring ideas. We are grateful to Paul Hebert for barcoding Afrotropical Gracillariidae. This work was supported by a grant from the Belgian Science Policy Office (contract MO/37/011). The sequencing of specimens at CCDB was supported by a grant from Genome Canada. Authors sincerely thank David Lees (BMNH) and Paolo Triberti (MSNV), who provided valuable criticism, comments and suggestions on an earlier version of the manuscript. We gratefully acknowledge an anonymous referee whose well-founded critical remarks significantly improved the content of this paper.

References

Ando, T., Yoshida, S., Tatsuki, S., & Takahashi, N. (1977) Sex attractants for male Lepidoptera. *Agricultural Biology and Chemistry*, 41, 1485–1492.

Ando, T., Koike, M., Uchiyama, M., & Kuroko, H. (1987) Lepidopterous sex attractants with a conjugated diene system. *Agricultural Biology and Chemistry*, 51, 2691–2694.

Beck, J. & Kitching, I.J. (2007) Estimating regional species richness of tropical insects from museum data: a comparison of a geography-based and sample-based methods. *Journal of Applied Ecology*, 44, 672–681.

Berlocher, S.H. & Feder, J.L. (2002) Sympatric speciation in phytophagous insects: moving beyond controversary? *Annual Review of Entomology*, 47, 773–815.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. & Das, I. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, 22(3), 148–155.

Booij, C.J.H. & Voerman, S. (1984) New sex attractants for 35 tortricid and 4 other lepidopterous species, found by systematic field screening in the Netherlands. *Journal of Chemical Ecology*, 10, 135–144.

Bradley, J.D., Jacobs, S.N.A. & Tremewan, W.G. (1969) A key to the British and French species of *Phyllonorycter* Hübner (*Lithocolletis* Hübner) (Lep. Gracillariidae). *Entomologist's Gazette*, 20, 3–33.

Burns, J.M., Janzen, D.H., Hajibabaei, M., Hallwachs, W. & Hebert, P.D.N. (2008) DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservacion Guanacaste, Costa Rica. *Proceedings of the National Academy of Sciences*, 105, 6350–6355.

Castrovillo P.J. & Cardè R.T. (1979) Environmental regulation of female calling and male pheromone response periodicities in the codling moth (*Laspeyresia pomonella*). *Journal of Insect Physiology*, 25, 659–661.

Chapman, T. A. (1902) The classification of *Gracilaria* [sic] and allied genera. *Entomologist, an Illustrated Journal of British Entomology*, 1902, 81–88, 138–142, 159–164.

Condon, M., Adams, D.C., Bann, D., Flaherty, K., Gammons, J., Johnson, J., Lewis, M.L., Marsteller, S., Scheffer, S.J., Serna, F. & Swensen, S. (2008) Uncovering tropical diversity: six sympatric cryptic species of *Blepharoneura* (Diptera: Tephritidae) in flowers of *Gurania spinulosa* (Cucurbitaceae) in eastern Ecuador. *Biological Journal of the Linnean Society*, 93, 779–797.

Dall'Asta, U., De Prins, J. & De Prins, W. (2001) Preliminary checklist of Gracillariidae of the Afrotropical Region. *Documentation zoologique. Musée royal de l'Afrique centrale*, 25, 1–48.

Davis, D.R. & Deschka, G. (2001) Biology and systematics of the North American *Phyllonorycter* leafminers on

Salicaceae, with a synoptic catalog of the Palearctic species (Lepidoptera: Gracillariidae). *Smithsonian Contributions to Zoology*, 614, 1–89.

Davis, D.R. & Robinson, G. S. (1998) The Tineoidea and Gracillarioidea. *In:* Kristensen, N.P. (Ed.), *Handbook of Zoology* IV/35, *Lepidoptera, Moths and Butterflies*. Vol. 1. *Evolution, Systematics, and Biogeography.* Walter de Gruyter, Berlin, New York, pp. 91–117.

Denis, M. & Schiffermüller, I. (1775) *Ankündung eines systematischen Werkes von den Schmetterlingen der Wienergegend herausgegeben von einigen Lehrern am k. k. Theresianum*. Augustin Bernardi, Wien, 322 pp, pls. frontispiece, 1–1b.

De Prins, J. & De Prins, W. (2007). Two new *Phyllonorycter* species (Lepidoptera: Gracillariidae) from West Africa. *Acta Zoologica Academiae Scientiarium Hungariae*, 53 (suppl. 1), 47–60.

De Prins, J. & De Prins, W. (2009) Global Taxonomic Database of Gracillariidae (Lepidoptera). Royal Museum for Central Africa, Belgian Biodiversity Platform, Tervuren, Brussels, Belgium. Available from http://gc.bebif.be (accessed 25 August 2009).

De Prins, J. & Mozūraitis, R. (2006) A new species of *Phyllonorycter* (Lepidoptera: Gracillariidae) from Kenya discovered by using the sex attractant Z8-tetradecen-1yl acetate. *Zootaxa*, 1124, 55–68.

De Prins, W. & De Prins, J. (2005) *Gracillariidae. In:* Landry, B. (Ed.), *World catalogue of insects. Volume 6.* Apollo Books, Stenstrup, 502 pp.

Deschka, G. & Dimić, N. (1986) *Cameraria ohridella* sp. n. (Lep., Lithocolletidae) aus Mazedonien, Jugoslawien. *Acta entomologica Jugoslavica*, 22(1–2), 11–23.

Doğanlar, M. & Mutuura, A. (1980) A new species of *Phyllonorycter* Hbn. (=*Lithocolletis* Hbn.) (Lepidoptera: Gracilariidae [sic]) from western North America. *Canadian Entomologist*, 112**,** 311–314.

El-Sayed, A.M. (2009) The Pherobase: Database of Insect Pheromones and Semiochemicals. Available from http://www.pherobase.com (accessed 18 September, 2009).

Emmet, A.M., Watkinson, I.A. & Wilson, M.R. (1985) Gracillariidae. *In:* Heath, J. & Emmet, A.M. (Eds.), *The moths and butterflies of Great Britain and Ireland. Volume 2 Cossidae–Heliodinidae*. Harley Books, Colchester, Essex, pp. 244–363.

Fabricius, J.C. (1781–[1782]) *Species insectorum exhibentes eorum differentias specificas, synonyma autorum, loca natalia, metamorphosin adiectis observationibus, descriptionibus. Tom II*. Impensis Carol. Ernest, Bohnii, Hamburgi et Kilonii, pp. 1–494 (1781), Appendix pp. 495–514 [1782], Index pp. 515–517 [1782].

Ferrao, P., Gries, G., Wimalaratne, P.D.C., Maier, C.T., Gries, R., Slessor, K.N., & Li, J.X. (1998) Sex pheromone of apple blotch leafminer, *Phyllonorycter crataegella*, and its effect on *P. mespilella* pheromone communication. *Journal of Chemical Ecology*, 24, 2059–2078.

Frey, H. (1855) Ueber die in der Schweiz beobachteten Arten des Genus *Lithocolletis*. Zell. *Mittheilungen der Naturforschenden Gesellschaft in Zürich*, 3, 600–635.

Gerasimov, A.M. (1933). Minierende Motten. III. Neue auf Rosaceen lebende *Lithocolletis*-Arten (Lep. Gracil.). *Deutsche Entomologische Zeitschrift "Iris"*, 47, 119–122.

Goloboff, P.A. (1999) NONA (NO NAME). Program and Documentation, Version 2.0. Published by the author, Tucumán, Argentina.

Gries, G., McBrien, H.L., Gries, R., Li, J.X., Judd, G.J.R., Slessor, K.N., Borden, J.H., Smith, R.F., Christie, M., Troubridge, J.T., Wimalaratne, P.D.C. & Underhill, E.W. (1993) (*E*4,*E*10)-dodecadienyl acetate: novel sex pheromone component of tentiform leafminer, *Phyllonorycter mespilella* (Hübner) (Lepidoptera: Gracillaridae). *Journal of Chemical Ecology*, 19, 1789–1798.

Hajibabaei, M., deWard, J.R., Ivanova, N.V., Ratnasingham, S., Door, R.T., Kirk, S.L., Mackie, P.M. & Hebert, P.D.N. (2005) Critical factors for assembling a high volume of DNA barcodes. *Proceedings of the Royal Society of London* B, 272, 1525–1534.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America*, 103(4), 968–971.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.

Hebert, P.D.N., Penton, E.H., Burns, J., Janzen, D.J. & Hallwachs, W. (2004) Ten species in one: DNA barcoding revealed cryptic species in the neotropical skipper butterfly, *Astraptes fulgerator. Proceedings of the National Academy of Sciences of the United States of America*, 101(41), 14812–14817.

Hrdy, I., Liska, J., Vrkoc, J., & Hochmut, R. (1989) New records on sex attractants for males and faunistic comments on moth (Lepidoptera) from Czechoslovakia. *Acta Entomologica Bohemoslovaca*, 86, 252–268.

Hübner, J. (1796–[1838]) *Sammlung europäischer Schmetterlinge. Achte Horde. Tineae Die Schaben; nach der Natur geordnet, beschrieben und vorgestellt.* J. Hübner Verlag, Augsburg, 78 pp, 71 pls.

Hübner, J. (1822) *Systematisches-alphabetisches Verzeichniss aller bisher bey den Fürbildungen zur Sammlung europäischer Schmetterlinge angegebenen Gattungsbennungen; mit Vorbemerkung auch augsburgischer Gattungen*.

J. Hübner Verlag, Augsburg, vi+81 pp.
Ivanova, N.V., deWaard, J.R. & Hebert, P.D.N. (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, 6, 998–1002.
Klots, A.B. 1970. Lepidoptera. *In:* Tuxen, S.L. (Ed.), *Taxonomist's glossary of genitalia in insects*. Munksgaard, Copenhagen, pp. 115–130.
Kristensen, N.P. 2003. Skeleton and muscles: adults. *In*: Kristensen, N.P. (Ed.), *Lepidoptera, Moths and Butterflies*. *Vol. 2. Morphology, Physiology, and Development.* Walter de Gruyter, Berlin, New York, pp. 39–122.
Kroon, D.M. (1999) *Lepidoptera of Southern Africa. Host–plants & other associations. A catalogue*. Lepidopterists' Society of Africa, Sasolburg, xi+159 pp.
Kühne, L. (Ed.) (2008) *Butterflies and moth diversity of the Kakamega forest (Kenya)*. Branderburgische Universitätsdruckerei und Verlagsgesellschaft, Berlin, 203 pp.
Kumata, T. (1967) New or little-known species of the genus *Lithocolletis* occurring in Japan (Lepidoptera: Gracillariidae). *Insecta Matsumurana*, 29, 59–72.
Kumata, T. (1982) A taxonomic revision of the *Gracillaria* group occurring in Japan (Lepidoptera: Gracillariidae). *Insecta Matsumurana*, N. S., 26, 1–186.
Kumata, T. (1993) A contribution to the knowledge of the Malaysian Lithocolletinae (Gracillariidae, Lepidoptera), with a revision of Indian *Cameraria* associated with Leguminosae. *Insecta Matsumurana*, N. S., 48, 1–85.
Kumata, T. (1995) Some species of the subfamily Lithocolletinae (Gracillariidae, Lepidoptera) collected in the Philippines. *Insecta Matsumurana*, N. S., 52, 105–131.
Laanmaa, M.K., Ivanova V.T., Mottus, E.R., & Kalinkin, V.M. (1990) Sex attractant for leaf miner moth, *Lithocolletis pyrifoliella* Grsm. (Lepidoptera, Gracillariidae). *Proceedings of Conference, Insect Chemical Ecology*, Tabor, pp. 125–128
Le Marchand, S. (1936) Clé ou table analytique pour le détermination des espèces françaises de *Lithocolletis* (Famille des Gracilariidae [sic]). *L'Amateur de Papillons*, 8(6–8), 83–118.
Lopez-Vaamonde, C., Godfray, H.C.J. & Cook, J.M. (2003) Evolutionary dynamics of host-plant use in a genus of leaf-mining moths. *Evolution*, 57, 1804–1821.
Lopez-Vaamonde, C. Wikström, N., Labandeira, C., Godfray, C.J., Goodman, S.J. & Cook, J.M. (2006) Fossil-calibrated molecular phylogenies reveal that leaf-mining moths radiated millions of years after their host plants. *Journal of Evolutionary Biology*, 19(4), 1314–1326.
Lozier, J.D., Fottit, R.G., Miller, G.L., Mills, N. J. & Roderick, G. K. (2008) Molecular and morphological evaluation of the aphid genus *Hyalopterus* Koch (Insecta: Hemiptera: Aphidae), with a description of a new species. *Zootaxa*, 1688, 1–19.
Lukhtanov, V.A., Sourakov, A., Zakharov E.V. & Hebert, P.D.N. (2009) DNA barcoding Central Asian butterflies: increasing geographical dimension does not significantly reduce the success of species identification. Molecular Ecology Resources, 9(5), 1302–1310.
Martin, J.-F., Gilles, A. & Descimon, H. (2003) Species concepts and sibling species: the case of *Leptidea sinapis* and *Leptidea reali*. *In*: Boggs, C.L., Watt, W.B. & Ehrlich, P.R. (Eds.), *Butterflies: ecology and evolution taking flight.* The University Chicago press, Chicago, London, pp. 459–476.
Meyrick, E. (1912) *Exotic Microlepidoptera*. Marlborough 1(1), 1–32.
Mozūraitis, R. (2006) Pheromone release behaviour in females of *Phyllonorycter strigulatella* (Lien. & Z.) and *Ph. sorbi* (Frr.) (Lepidoptera, Gracillariidae) under daily cycling temperature regime. *Ekologija*, (2006) 4, 7–11.
Mozūraitis, R., Būda, V., Borg-Karlson, A.-K. & Ivinskis, P. (1997) Sex pheromone in *Phyllonorycter ulmifoliella* (Lepidoptera: Gracillariidae): identification and field tests. Calling rhythm of females. *Journal of Chemical Ecology*, 23, 175–189.
Mozūraitis, R., Būda, V., Borg-Karlson, A.-K., Ivinskis, P., Karalius V., Laanmaa, M. & Plepys, D. (1998) New sex attractants and inhibitors for 17 moth species from the families Gracillariidae, Tortricidae, Yponomeutidae, Oecophoridae, Pyralidae and Gelechiidae. *Journal of Applied Entomology*, 122, 441–452.
Mozūraitis, R., Borg-Karlson, A.-K., Būda, V. & Ivinskis P. (1999) Sex pheromone of the spotted tentiform leafminer moth *Phyllonorycter blancardella* (Fabr.) (Lep., Gracillariidae). *Journal of Applied Entomology*, 123, 603–606.
Mozūraitis, R., Būda, V., Jonušaitė, V., Borg-Karlson, A.-K. & Noreika, R. (2000) Sex pheromones of *Phyllonorycter acerifoliella* and *Ph. heegerella* [sic] and communication peculiarities in three species of leafmining moths. *Entomologia Experimentalis et Applicata*, 94, 15–23.
Mozūraitis, R., Liblikas, I. & Noreika, R. (2008) Sex pheromone communication of tentiform leaf-miners *Phyllonorycter insignitella* and *Ph. nigrescentella* from two related species groups. *Chemoecology*, 18, 171–176.
Nixon, K.C. (2002) WinClada, Version 1.00.08. Published by the author, Ithaca, New York.
Ohshima, I. (2008) Host race formation in the leaf-mining moth *Acrocercops transecta* (Lepidoptera: Gracillariidae). *Biological Journal of the Linnean Society*, 93, 135–145.
Ratnasingham S. & Hebert P.D.N. (2007) BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* 7, 355–364.

Robinson, G.S. (1976) The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. *Entomologist's Gazette*, 27, 127–132.
Schmitz, P., Cibois, A. & Landry, B. (2008) Cryptic differenciation in the endemic micromoth *Galagete darwini* (Lepidoptera, Autostichidae) on Galápagos volcanoes. *Philosophical Transactions of the Royal Society* B, 363, 3453–3458.
Shapiro, L.H., Scheffer, S.J., Maisin, N., Lambert, S., Purung, H.B., Sulistyowati, E., Vega, F.E., Gende, P., Laup, S., Rosmana, A., Djam, S. & Hebbar, P.K. (2008). *Conopomorpha cramerella* (Lepidoptera, Gracillariidae) in the Malay archipelago: genetic signature of a bottlenecked population? *Annals of the Entomological Society of America*, 101, 930–938.
Shearer, P. & Riedl, H. (1994) Comparison of pheromone trap bioassays for monitoring insecticide resistance of *Phyllonorycter elmaella* (Lepidoptera: Gracillariidae). *Journal of Economic Entomology*, 87, 1450–1454.
Sokal, R.R. & Rohlf, F.J. (1995) Biometry. Freeman and Co., New York, 887 pp.
Stainton, H.T. 1862. Descriptions of nine exotic species of the genus *Gracilaria* [sic]. *Transactions of the Entomological Society of London*, (series 3) 1(3), 291–300.
Subchev, M., Mircheva, A., Pickett, J., Wadhams, L., Woodcock, C., Dos Santos, A., Franke, S., & Francke, W. (2003) Sex pheromone of the leaf-miner *Phyllonorycter platani*: (Z10)-tetradecenyl acetate. *Journal of Chemical Ecology*, 29, 2391–2396.
Sugie, H., Tamaki, Y., Kawasaki, K., Wakou, M., Oku, T., Hirano, C. & Horiike, M. (1986) Sex pheromone of the apple leafminer moth, *Phyllonorycter ringoniella* (Matsumura) (Lepidoptera: Gracillariidae): activity of geometrical isomers of tetradecadienyl acetates. *Applied Entomology and Zoology*, 21, 578–581.
Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599.
Trewick, S. A. (2008) DNA barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae) *Cladistics*, 24, 240–254.
Triberti, P. (2007) The *Phyllonorycter* species from Palaearctic Region feeding on Rosaceae (Lepidoptera, Gracillariidae). *Bollettino del Museo Civico di Storia Naturale di Verona, Botanica Zoologia*, 31, 147–221.
Vaglia, T., Haxaire, J., Kitching, I.J., Meusnier, I. & Rougerie, R. (2008) Morphology and DNA barcoding reveal three cryptic species within the *Xylophanes neoptolemus* and *loelia* species-groups (Lepidoptera: Sphingidae). *Zootaxa*, 1923, 18–36.
van Nieukerken, E. & De Prins, J. (2007) A new *Cameraria* on Annonaceae in Vietnam and the genus in the Afrotropical region. *Abstracts of presentations at the 15th European Congress of Lepidopterology*, Berlin, without pagination.
Vári, L. (1961) South African Lepidoptera. Vol. 1. Lithocolletidae. *Transvaal Museum Memoir*, 12, i–xix, 1–238, 112 pls.
Vári, L. & Kroon, D.M. (1986) *South African Lepidoptera. A series of cross-referenced indices*. Lepidopterists' Society of Southern Africa & the Transvaal Museum, Pretoria, x+198 pp.
Vári, L., Kroon,D. M. & Krüger, M. (2002) *Classification and checklist of the species of Lepidoptera recorded in Southern Africa*. Simple Solutions, Chastwood, xxi+385 pp.
Voerman, S. (1988) The pheromone bank: a collection of unsaturated compounds indispensible for discovery of sex attractants for Lepidoptera. *Agriculture, Ecosystems & Environment*, 21, 31–41.
Voerman, S. (1991) A mono-unsaturated acetate as sex attractant for the leaf miner moth *Lithocolletis corylifoliella* (Hübner) (Lep., Gracillariidae). *Journal of Applied Entomology*, 112, 207–210.
Voerman, S. & Herrebout, W. M. (1978) A sex attractant for the leaf miner moth *Lithocolletis corylifoliella* and its influence on that of *L. blancardella* (Lep., Gracillariidae). *Entomologia Experimentalis et Applicata*, 23, 96–98.
Zeller, P.C. (1846) Die Arten der Blattminierergattung *Lithocolletis*. *Linnaea Entomologica*, 1, 166–261, pl. 1.