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A new species of *Phyllonorycter* (Lepidoptera: Gracillariidae) from Kenya discovered by using the sex attractant Z8-tetradecen-1-yl acetate

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Abstract

Phyllonorycter obandai, new species, was discovered in Kenya using traps baited with the synthetic sex attractant Z8-tetradecen-1-yl acetate. This is the first species of *Phyllonorycter* recorded from Kenya. External morphological features of adult males, along with their genitalia are described and illustrated from specimens attracted to the traps. A distribution map for *P. obandai* is also presented.

Key words: *Phyllonorycter obandai*, new species, Kenya, *Phyllonorycter*, Lithocolletinae, Gracillariidae, morphology, sex attractant, pheromone, Afrotropical.

Introduction

Gracillariidae is one of the largest families of plant-mining Lepidoptera with 1818 species currently recognized (Scoble 1992; Davis & Robinson 1998; De Prins & De Prins 2005; World Gracillariidae Database of the Royal Museum for Central Africa). However, the Afrotropical Gracillariidae fauna still remains poorly explored. Only 235 species of Gracillariidae are recorded from the Afrotropical region (Dall'Asta *et al*. 2001; De Prins 2002; De Prins & De Prins 2005). The genus *Phyllonorycter* Hübner, 1822 (= *Lithocolletis* Hübner, 1825) is undisputedly assigned to the subfamily Lithocolletinae in all main systematic works on the Gracillariidae (Davis 1983, 1987; Common 1990; Davis & Robinson 1998; Kumata 1998; Kuznetzov & Baryshnikova 2001, 2004; De Prins & De

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Prins 2005). It is the most species-rich genus within Gracillariidae and one of the most species-rich genera of all Lepidoptera. Currently it includes 386 described species from all zoogeographical regions except Antarctica (De Prins & De Prins 2005). The vast majority of these are found in the temperate regions with 244 species in the Palaearctic and 80 in the Nearctic (Davis 1983; Buszko 1996; De Prins & De Prins 2005). The African *Phyllonorycter* fauna, in comparison with that of the other zoogeographical regions, remains poorly known, with only 19 recognized species. Besides the classical work of Vári (1961) on South African Gracillariidae, very few entomologists have augmented the list of Afrotropical *Phyllonorycter* with descriptions of new species. Meyrick described *P. encaeria* (1911) and *P. melanosparta* (1912), both from South Africa, and *P. loxozona* (1936) from Uganda; Viette described *P. madagascariensis* (1949) and *P. lemarchandi* (1951) from Madagascar; Bland (1980) described *P. caudasimplex* from Nigeria; and Triberti (2004) described *P. leucaspis* from Namibia. In such a huge and diverse region as continental Eastern Africa, the recorded *Phyllonorycter* fauna is represented by only one species: *P. loxozona* (Meyrick, 1936).

Such poor sampling of *Phyllonorycter* in East Africa cannot adequately represent the true situation and is probably related to the biological peculiarities of *Phyllonorycter.* Most *Phyllonorycter* species are oligophagous and in many cases even restricted to a single plant species. Even if the mines are found, up to 70% of the larvae are parasitized by Braconidae, Eulophidae, Ichneumonidae, or Pteromalidae (Fulmek 1962; Vidal & Buszko 1990; Davis & Deschka 2001; Noyes 2003). In addition to these biological difficulties, the Afrotropical *Phyllonorycter* species are not strongly attracted to light. This may be due to a variety of reasons: a) poor flight abilities, b) the species develop in hard to reach or even inaccessible places, or c) the lack of studies on the Microlepidoptera of East Africa.

Sex attractants have been shown to be a valuable tool in zoogeographical, applied, and a number of other studies where the collection of certain insect species is an intricate and labour-consuming process, due to specific biological features of the species or difficult natural characteristics of the test areas. The sex attractants and pheromones of the genus *Phyllonorycter* can be characterized as alifatic C_{12}–C_{14} straight chain, saturated, mono- or di-unsaturated acetates or alcohols with *Z*- or *E*- double bonds occurring in positions 4, 8, 10 and 12 (Arn *et al.* 1992; El-Sayed 2005). *E*10-12:OAc and *Z*10-14:OAc are the compounds most often used in sex communication by *Phyllonorycter* species.

Little is known about the diurnal calling periodicity or pheromone communication in moths of the genus *Phyllonorycter.* As far as we know, such data are available for nine species: *P. ulmifoliella* (Hübner, 1817) (Mozūraitis *et al*. 1997), *P. ringoniella* (Matsumura, 1931) (Boo & Jung 1998), *P. blancardella* (Fabricius, 1781) (Mozūraitis *et al.* 1999; El-Sayed *et al.* 2005), *P. acerifoliella* (Zeller, 1839) [=*P. sylvella* (Haworth, 1828)], *P. heegeriella* (Zeller, 1846) (Mozūraitis *et al*. 2000), *P. strigulatella* (Zeller, 1846), *P. sorbi* (Frey, 1855) (Mozūraitis 2000), *P. emberizaepenella* (Bouché, 1834) (Mozūraitis *et al*. 2002), and *P. junoniella* (Zeller, 1846) (Mozūraitis & Būda 2006 in

press). In most of these species a high level of sex pheromone release behaviour was registered 0.5–1 hour after the beginning of day light and lasted for about 2–3 hours; *P. junoniella* females exhibited two peaks of the signalling activity.

The purpose of this study is to present a description and illustrations of a new taxon discovered using alternative means of attracting *Phyllonorycter* specimens in tropical areas.

Material and methods

Lures and field tests

Seven of the most distributed sex attractant compounds in the *Phyllonorycter* species [(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 1:0, as well as *Z*8-14:OAc and *Z*10-14:OAc in the same ratios mentioned above] were used to screen for possible *Phyllonorycter* species in Kenya. All the compounds used in the field tests were synthesized in Tartu, Estonia, and purified by preparative liquid chromatography, as described by Mozūraitis *et al*. (1998). The diastereomeric and chemical purities of the compounds exceed 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” traps and Pestifix glue were obtained from Flora Co., Tartu, Estonia. The attractiveness tests were carried out in two localities: Kakamega Forest (00°21’N 34°51’E) and Gatamaiyu Forest (00°58’S 36°41’E), in West and Central Kenya from 25 March to 3 April 2003 and from 4 to 6 April 2003, respectively. Each trap was fixed on a tree branch 1.5–2.2 m above the ground and was inspected and moved to the next trap location (within each replication) every 3 days. The distance between the traps was approximately 50 m. Five replicates of each compound and mixture were used.

Identification and description procedures

The moths captured were identified by analysis of their external morphology, colour pattern, and genitalia. When both body and wings were covered by sticky material, the moth was rinsed in hexane before identification. Descriptions of adults are based on the three best preserved specimens and one specimen attracted to light. The measurements given for wingspan are to the nearest millimetre. The descriptive terminology follows Vári (1961), Bradley *et al*. (1969), and Davis & Deschka (2001). Genitalia were prepared

according to standard methods (Robinson 1976). The genital parts were stained in Eosin B Acid Red ($C_{20}H_6N_2O_9Br_2Na_2$ SIGMA Ec No 208-943-1) and mounted on slides in Euparal. Genitalia slides were customarily examined under a standard research microscope. The drawings of the genitalia were made after photographing the slides with a 3CCD Toshiba Camera connected to a Leica DMLB light microscope and processed with the Image-Pro Plus image analysing programme. The Auto-Montage Syncroscopy software was used to produce composite results from several separate photographs in planes of different depths.

The distribution map was prepared with the DMAP 7.2a programme (Morton 2003) linked with the Faunistic Module of the World Gracillariidae Database of the RMCA (Microsoft Access 2002).

Abbreviations:

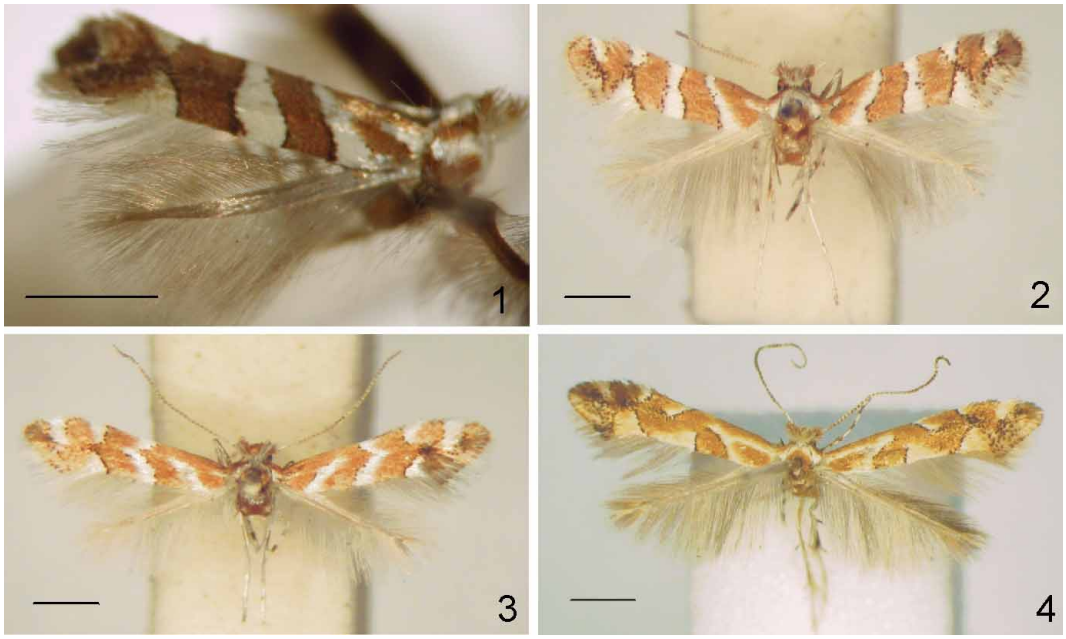
BMNH	The Natural History Museum, London, UK.
JDP	Jurate De Prins
NMK	National Museums of Kenya, Nairobi, Kenya
RMCA	Royal Museum for Central Africa, Tervuren, Belgium
TMSA	Transvaal Museum, Pretoria (Tshwane), Republic of South Africa.

***Phyllonorycter obandai*, new species**
Figs. 4, 8, 9

Diagnosis. This new species is clearly distinguishable by its wing pattern and male genitalia from *P. loxozona* (Meyrick, 1936), described from Uganda, but which could occur in Kenya also. Its wing pattern is comparable to that of *P. rhynchosiae* (Vári, 1961) and its male genitalia to those of *P. brachylaenae* (Vári, 1961). The last two species are known from South Africa. *P. obandai* is easily distinguishable from *P. loxozona* (Fig. 1) by the following differences in wing pattern:

- The forewing of *P. obandai* has a long basal streak reaching 7 ¼ of wing; in *P. loxozona* the streak is short and oblique.
- The forewing of *P. loxozona* has two very clearly defined white fascia; in *P. obandai* only strigulae are present.
- The forewing of *P. loxozona* possesses two opposite triangular spots at 3/4 finely edged with blackish interiorly; in *P. obandai* the 3rd costal is situated just before the apex and not edged.

This species is distinguishable from *P. brachylaenae* (Fig. 2) by the longer basal streak and the absence of two transverse fasciae, which are clearly defined in the forewing of *P. brachylaenae*. The new species is distinguished from the superficially similar *P. rhynchosiae* (Fig. 3) by its smaller size, the brighter golden ground coloration of the forewing, the longer basal white streak reaching to 1/4 of the forewing, the second dorsal strigula that reaches only the middle of the wing, and the absence of an ochreous-fuscous patch on the outer side of the tibia.



FIGURES 1–4. Adults of *Phyllonorycter* species. 1. *Phyllonorycter loxozona* (Meyrick, 1936); female holotype, Uganda, Busunju, 02.x.1935, leg. H.O. Taylor, coll. BMNH, Imp.Inst.Ent. B.M. 1936-552; 2. *Phyllonorycter brachylaenae* (Vári, 1961); male holotype No. 6414, South Africa, Gauteng, Pretoria, 20.x.1949, leg. L. Vári, coll. TMSA, Ac. No. 184; 3. *Phyllonorycter rhynchosiae* (Vári, 1961); male holotype No. 6379, South Africa, Gauteng, Pretoria, 14.iii.1949, leg. L. Vári, coll. TMSA, Ac. No. 130; 4. *Phyllonorycter obandai* sp. n.; male paratype, Kenya, Rift Valley, Turi, 8000ft, 27.ii.2000, leg. D. J. L. Agassiz, coll. BMNH. Scale bar=1 mm.

In this group it is advisable to examine the genitalia for a correct identification of the species. *P. obandai* (Fig. 8, 9) is distinguishable from *P. loxozona* (Fig. 5) by clear differences in the form of the valva, the saccus length, and the form of the 8th sternite.

•The valva of *P. loxozona* is long, slender and curved; in *P. obandai* it is enlarged in the apical third and rounded.

•The saccus of *P. loxozona* is short, pointed; in *P. obandai* it is almost twice as long as valva, slender bearing a small apical process.

•The 8th sternite of *P. loxozona* is triangular and bifurcated at its apex; in *P. obandai* it is semicircular.

The following are differences between the male genitalia of *P. obandai* (Fig. 8, 9) and *P. brachylaenae* (Fig. 6):

•The costal and dorsal sides of the valva of *P. brachylaenae* are more or less parallel throughout; the valva of *P. obandai* is enlarged in the apical third and much more rounded.

•The valva of *P. brachylaenae* has a conspicuous ridge from the centre of the costa

towards the centre of the outer margin; this ridge is missing or only very weekly indicated in *P. obandai*.

- The width/length of the valva in *P. brachylaenae* = 0.225; *P. obandai* = 0.294.
- The valval length/aedeagus length in *P. brachylaenae* = 0.8; in *P. obandai* = 0.47 (the aedoeagus in *P. obandai* is much longer in comparison to the valval length).

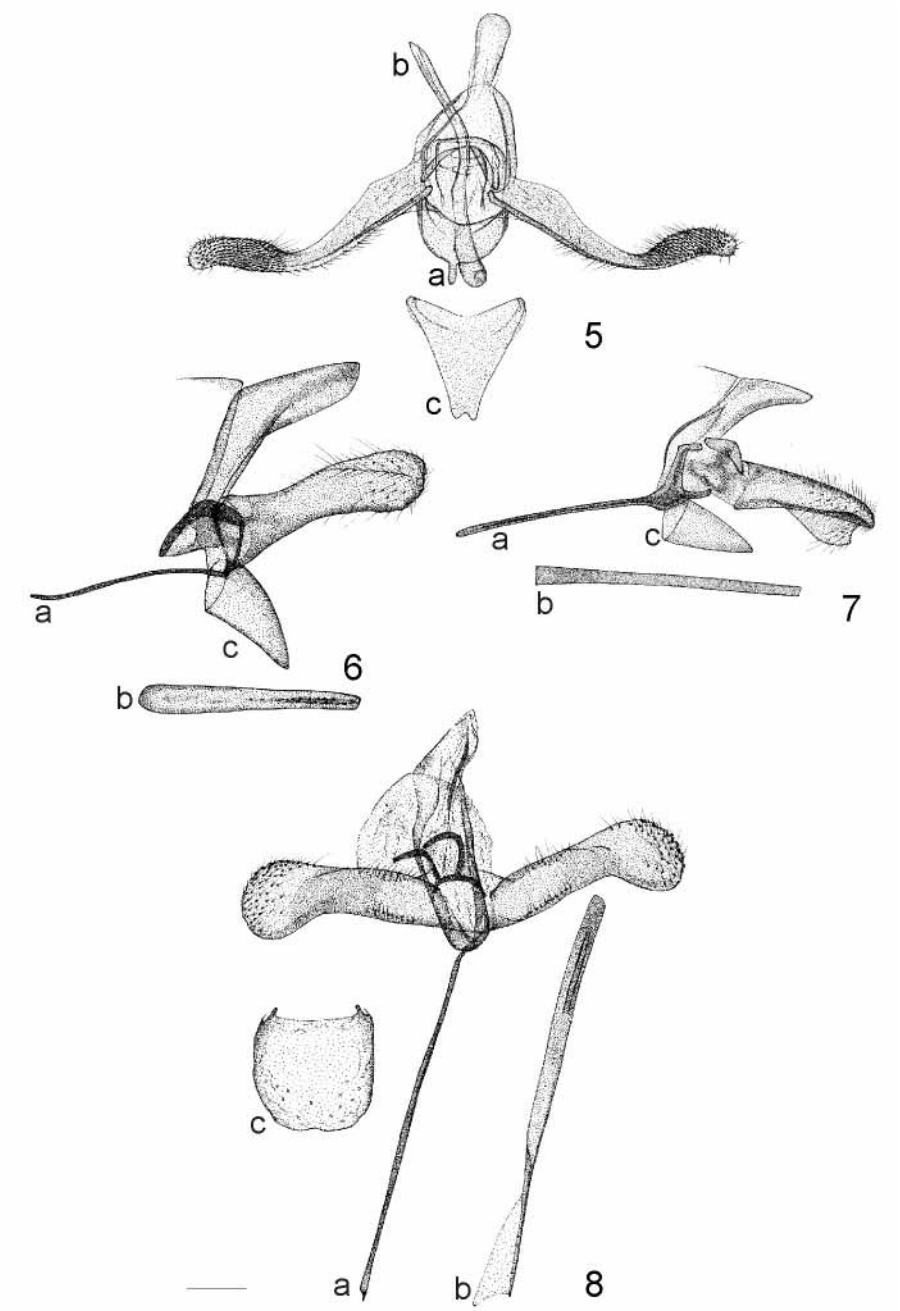
The new species is distinguishable from *P. rhynchosiae* (Fig. 7) by the characteristic rounded caudal margin of the valvae lacking any defined projection.

Description

Adult (Fig. 4, 8, 9). Length of forewing: 3.0–3.3 mm. Forewing ground colour golden brown, with white markings consisting of basal streak, three costal, and three dorsal strigulae. Valvae of male genitalia symmetrical, rounded apically, vinculum slender with very long and slender saccus, aedoeagus very long with two needlelike cornuti.

Head: Vertex tufted with golden brown intermixed with white piliform scales; frons smooth, shining white, with pale brown suffusion apically. Antenna as long as forewing or slightly shorter, whitish brown, segments also with brownish scales in their posterior half, but not clearly ringed; scape white above, yellowish brown beneath, pecten brown basally and whitish distally, with scales as long as one flagellomere length. Maxillary and labial palpi light fuscous, whitish distally.

Thorax: Dorsum golden brown, whitish laterally, tegulae whitish with brownish suffusion posteriorly. Forewing elongate, ground colour golden brown marked with white; with slender, straight basal streak reaching almost 1/4 of forewing, not edged; with three costal and three dorsal strigulae; 1st dorsal strigula at 1/3, narrow, reaching 3/4 of forewing width, obliquely curved towards apex, finely edged with blackish posteriorly and prolonged with whitish towards base along dorsum, sometimes connected with basal streak; 2nd dorsal strigula triangular, at 1/2, with slight suffusion of golden scales posteriorly and sharply edged with row of black scales basally; 3rd dorsal strigula at 7/10, subtriangular, blackish edged basally; 1st costal strigula at 1/2, narrow, directed dorsally, edged basally with two rows of black scales; 2nd costal strigula at 2/3, small, subtriangular, situated opposite 3rd dorsal strigula, oblique, blackish edged basally, with suffusion of black scales separating it from next strigula; 3rd costal strigula just before apex, comma shaped, directed basally, not edged with black; with suffusion of black dispersed scales in apical part of forewing; dark brown fringe line preceded by ochreous shade, fringe pale golden shining brown. Hindwing pale fuscous, fringe pale golden ochreous. Legs pale golden brown dorsally, apices of tibia and tarsal segments faintly marked with dark brown scales. Hind legs almost uniformly pale brown with some brownish scales at apex of tibia.



FIGURES 5–8. Male genitalia of *Phyllonorycter* species. 5. *Phyllonorycter loxozona* (Meyrick, 1936) (drawing made from the paratype slide No. 3924, coll. BMNH); 6. *Phyllonorycter brachylaenae* (Vári, 1961) (drawing made from holotype slide No. 7126, coll. TMSA); 7. *Phyllonorycter rhynchosiae* (Vári, 1961) (drawing made from paratype slide No. 7498, coll. TMSA); 8. *Phyllonorycter obandai* sp. n. (drawing made from holotype slide No. 00252, coll. RMCA). Figs. 6–7 in lateral view. Figs. 5, 8 in ventral view; a - saccus, b - aedoeagus, c - 8th sternite. Scale bar=0.1 mm.

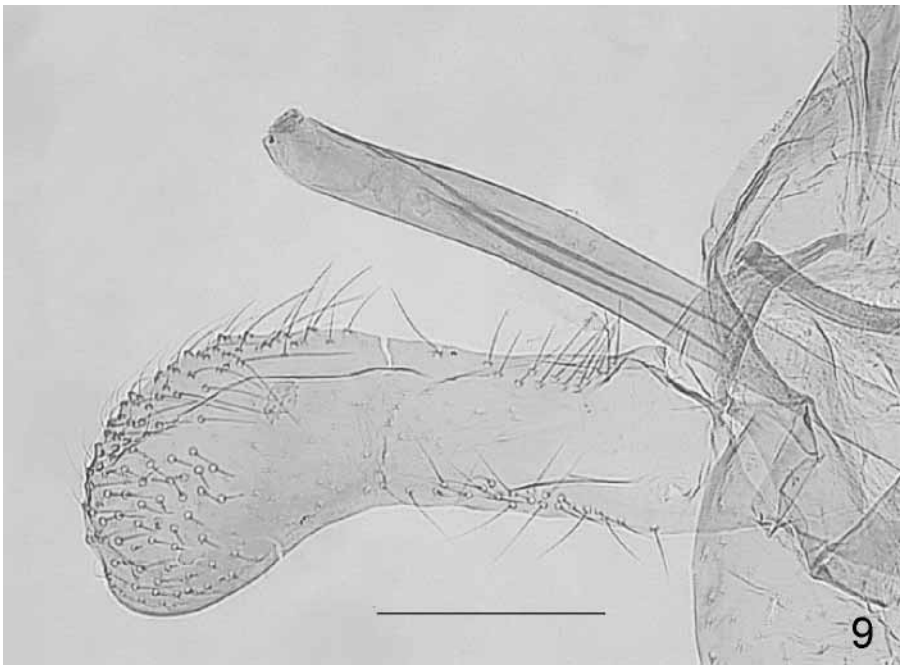


FIGURE 9. Left valva and tip of aedoeagus of *Phyllonorycter obandai* sp. n.; male paratype, Kenya, Gatamaiyu Forest, 00°58'S, 036°41'E, 2280 m, 04.iv.2003, leg. J. & W. De Prins; slide RMCA 00252. Scale bar=0.1 mm.

Abdomen: Brown dorsally, lustrous white ventrally; conspicuous sex-scaling absent. Eighth sternum of male rounded, approximately as long as vinculum, rounded caudally, apex slightly concave.

Male Genitalia (Figs. 8, 9). Tegumen short, membranous, apex evenly rounded, tuba analis slightly protruding, truncate apically, naked. Valvae symmetrical, moderate, 1.5× longer than eighth sternite, straight in basal half and then gradually broadened and rounded distally, slightly curved ventrally, bearing longitudinal ridges in apical section and line of subcostal and subdorsal setae stretching from subbasal section to middle; basal section without hairs. Transtilla well developed and sclerotized. Vinculum narrow, short, rounded; saccus very long, 1.87 times as long as valva and slender, bearing small apical process. Aedoeagus very long, approximately 2.5 as long as valva, slender, almost straight with parallel sides; vesica with 2 narrow, needlelike cornuti, parallel to one another, about 1/5 of aedoeagus length.

Female unknown.

Variation. There is little variation other than forewing length and shape of the third strigulae. The colour intensity varies slightly from golden ochreous to golden brown.

Holotype: ♂: KENYA, Gatamaiyu Forest, 00°58’S, 036°41’E, 2280 m, 04.iv.2003, leg. J. & W. De Prins. Specimen ID: RMCA ENT 000002420. Gen. prep. 3664♂ De Prins (RMCA 00251), in RMCA.

Paratypes: 21♂: 1♂, KENYA, Rift Valley, Turi, 8000ft, 27.ii.2000, leg. D.J.L. Agassiz, gen. prep. 3502♂ De Prins, in BMNH. 5♂, KENYA, Gatamaiyu Forest, 00°58'S, 036°41'E, 2280 m, 04.iv.2003, leg. J. & W. De Prins, 4 specimens and gen. prep. 3640♂ De Prins (rmca 00252) in RMCA (specimen IDs: rmca ent 000002421, 000002931–000002933), one specimen and gen.prep. 3641♂ De Prins in BMNH. 15♂ KENYA, Kakamega Forest, 00°21'N 034°51'E, 1590 m, 28.iii.2003, leg. J. & W. De Prins. Gen. prep. 3662–3663♂ De Prins (rmca 00253–00254); 12 specimens in RMCA, (specimen IDs: rmca ENT 000002422-000002432, 000002934), 2 specimens in NMK, 1 specimen in BMNH.

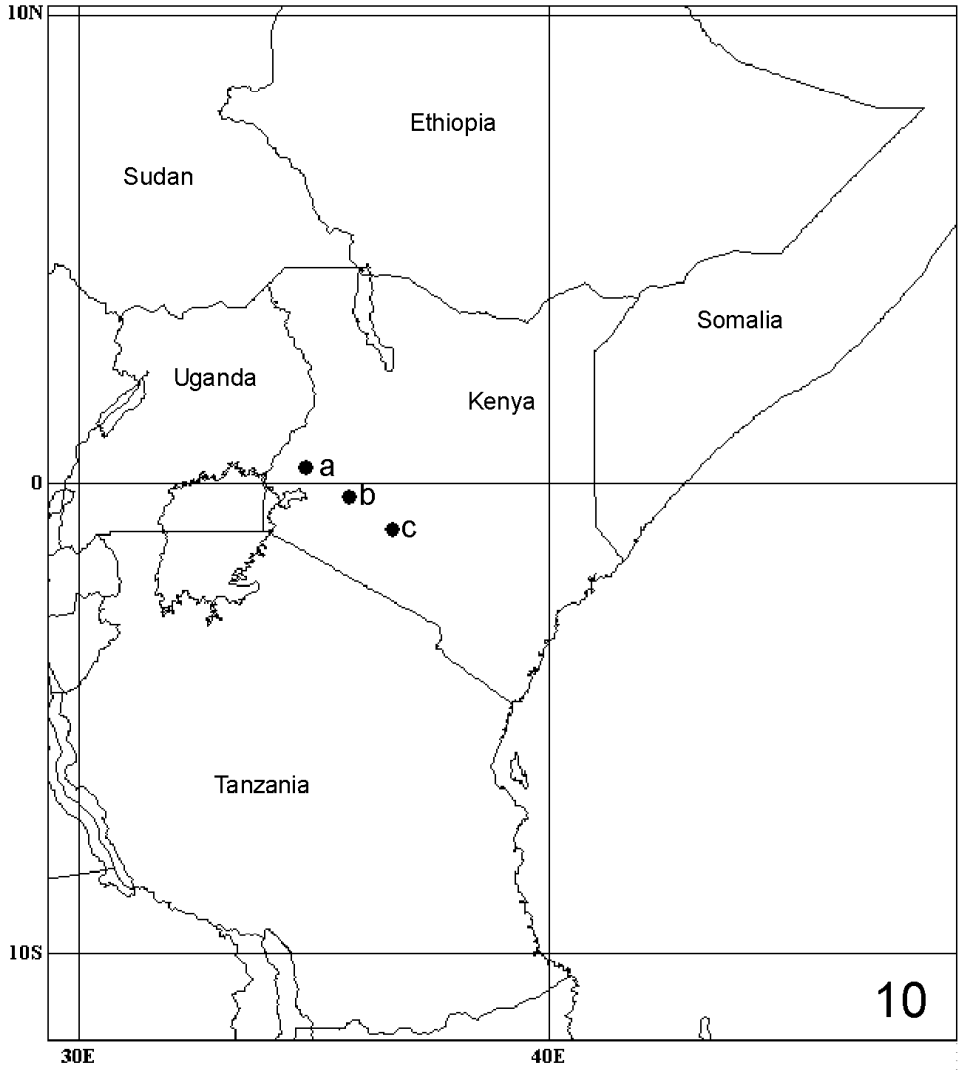
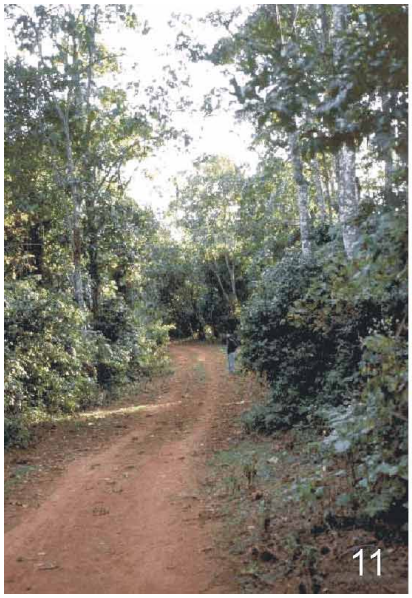


FIGURE 10. Distribution map of *Phyllonorycter obandai* sp. n.; a - Kakamega Forest, b - Turi, Rift Valley, c - Gatamaiyu Forest.



FIGURES 11–12. Collecting localities for *Phyllonorycter obandai* sp. n. during the flight period; 11. Type locality on 4 April 2003; 12. Kakamega Forest on 28 March 2003 (Photographs by JDP).

Etymology. The species name is formed from the family name of the tragically deceased Kenyan biologist Ernest Obanda, who was a devoted companion to JDP in his native Kakamega Forest.

Biology. Unknown.

The new species was discovered at three different sites: the Rift Valley, the montane Gatamaiyu Forest and the Kakamega tropical rainforest where Guineo-Congolian flora intermixes with savanna plant species (Kokwaro 1988).

The type locality, Gatamaiyu Forest (Fig. 11), is located within the Kikuyu escarpment and is considered an important indigenous forest. The forest lies in the Central province of Kenya. It is found at about 48 km from Nairobi at an altitude of 2240–2400 m. The forest covers an area of 4,600 ha of which 3,768 ha is indigenous forest and the rest is plantation forest.

The Kakamega Forest (Fig. 12) located in the Western province of Kenya is the easternmost relict of the equatorial forests stretching from the Atlantic coast westward across the Congo basin. It is related to the equatorial rain forests of West Africa, but it has a cooler and less humid climate (Wass 1995).

Flight period. Based upon the specimens available, adults fly from 27 February to 4 April.

Distribution. This species is presently known from a few localities in the Rift Valley in the Central and Western provinces of Kenya (Fig. 10).

Sex attractant. Nine specimens were attracted to lures baited with *Z*8-14:OAc and another 3 moths were caught by traps baited with a binary mixture of *Z*8-14:OAc and *Z*10-14:OAc at 10:1.

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