The LINNEAN SOCIETY of London ZOOLOGICAL Journal of the Linnean Society

Zoological Journal of the Linnean Society, 2015, **175**, 244–270. With 10 figures

Integrative taxonomy reveals six new species related to the Mediterranean corn stalk borer *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera, Noctuidae, Sesamiina)

GAEL J. KERGOAT[1]*, EMMANUEL F. A. TOUSSAINT[2], CLAIRE CAPDEVIELLE-DULAC[3,4], ANNE-LAURE CLAMENS[1], GEORGE ONG'AMO[5], DESMOND CONLONG[6,7], JOHNNIE VAN DEN BERG[8], DOMINGOS CUGALA[9], BEATRICE PALLANGYO[10], ONESIME MUBENGA[11], GILSON CHIPABIKA[12], ROSE NDEMAH[13], MICHEL SEZONLIN[14], GREGOIRE BANI[15], RICHARD MOLO[16], ABDALLA ALI[17], PAUL-ANDRE CALATAYUD[3,4,18], LAURE KAISER[3,4], JEAN-FRANCOIS SILVAIN[3,4] and BRUNO LE RU[3,4,18]

[1]*INRA – UMR 1062 CBGP (INRA, IRD, CIRAD, Montpellier SupAgro), 755 Avenue du campus Agropolis, 34988 – Montferrier/Lez, France*
[2]*SNSB – Bavarian State Collection of Zoology, Münchhausenstraße 21, 81247 – Munich, Germany*
[3]*IRD/CNRS, Laboratoire Evolution Génomes et Spéciation, Avenue de la terrasse, BP 1, 91198 Gif-sur-Yvette cedex, France*
[4]*Université Paris-Sud 11, 91405 – Orsay, France*
[5]*School of Biological Science, College of Physical and Biological Sciences (Chiromo Campus), University of Nairobi, Nairobi, Kenya*
[6]*South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe, 4300 South Africa*
[7]*School of Biological and Conservation Sciences, University of KwaZulu-Natal, Private Bag X01 – Scottsville, Pietermaritzburg, Republic of South Africa*
[8]*School of Environmental Sciences and Development, North West University (Potchefstroom Campus), Private Bag X6001, Potchefstroom, 2520 – Republic of South Africa*
[9]*Faculty of Agronomy and Forestry Engineering, Eduardo Mondlane University, Av. J. Nyerere, Campus Universitario 1, Maputo, Republic of Mozambique*
[10]*Biocontrol Program, PO Box 30031 – Kibaha, Tanzania*
[11]*Faculté des Sciences agronomiques, Université de Kisangani, Kisangani, Democratic Republic of the Congo*
[12]*Zambia Agriculture Research Institute, Mount Maluku Central Research Station, PO Box 8 – Chilanga, Zambia*
[13]*International Institute of Tropical Agriculture, PO Box 2008 – Messa, Yaoundé, Cameroon*
[14]*Département de Zoologie et de Génétique, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, 01 BP 526 – Cotonou, Bénin*
[15]*Centre de Recherches Agronomiques de Loudima (CRAL), BP 28 – Loudima, Republic of the Congo*
[16]*Namulonge Agricultural and Animal Production Research Institute (NAARI), PO Box 7084 – Kampala, Uganda*
[17]*Plant Protection Division, PO Box 1062, Zanzibar, Tanzania*
[18]*Unité de Recherche IRD 072, African Insect Science for Food and Health (icipe), PO Box 30772 – Nairobi, Kenya*

Received 22 October 2014; revised 18 March 2015; accepted for publication 24 March 2015

*Corresponding author. E-mail: kergoat@supagro.inra.fr

Species in the stem borer noctuid subtribe Sesamiina are notoriously difficult to distinguish because most related species have homogeneous wing patterns and almost indistinguishable genitalia. The latter is potentially problematic because this group includes several important pest species that are usually baregly distinguishable from non-pest species. In this study we focus on the Mediterranean corn stalk borer *Sesamia nonagrioides* (Lefèbvre), an important pest of maize with a wide area of distribution that covers most of Africa and extends to the south of Europe and western Asia. According to a recent study, this pest consists of three allopatric populations that were formerly considered as distinct species or subspecies. Here we rely on recent collections of 5470 specimens (sampled in 17 countries and 175 localities) that putatively belong to *S. nonagrioides*. Integrative taxonomy studies allowed us to unravel the existence of six new species that are closely related to *S. nonagrioides* and described in this paper. In contrast to *S. nonagrioides* these new species have more specific ecological preferences, as they are associated with a limited number of plant species and habitats. Dating and population genetic analyses carried out on 100 *S. nonagrioides* specimens also indicate a more complex population structure than previously thought for *S. nonagrioides*, which can probably be accounted for by late Cenozoic environmental changes.

doi: 10.1111/zoj.12275

ADDITIONAL KEYWORDS: Cenozoic – climatic changes – host plant – integrative taxonomy – molecular dating – molecular species delimitation – population genetics.

INTRODUCTION

In the last decade integrative taxonomy has been presented (Dayrat, 2005; Will, Mishler & Wheeler, 2005) as an elegant way to better delimit species while facing the so-called 'taxonomy crisis' (Godfray, 2002; Wilson, 2004; Riedel *et al*., 2013). The basic principle of integrative taxonomy is to combine several lines of evidence (e.g. behaviour, biochemistry, ecology, genetics, morphology) to better assess or reassess species status (Dayrat, 2005; Wiens, 2007; Padial *et al*., 2010; Schlick-Steiner *et al*., 2010). This approach is especially relevant when dealing with morphologically close species complexes (Padial & La Riva, 2009; Lumley & Sperling, 2010; Hamilton, Formanowicz & Bond, 2011; Gebiola *et al*., 2012). The latter are very common in African noctuid stem borers of the tropical subtribe Sesamiina (Zilli, Ronkay & Fibiger, 2005), in which most related species have homogeneous wing patterns and similar genitalia both in males and in females (Moyal & Le Ru, 2006; Moyal *et al*., 2010, 2011b; Le Ru *et al*., 2014). In this group species are generally oligophagous and usually feed on few species of related host plants belonging to monocotyledon families (Zilli *et al*., 2005). Molecular dating analyses suggest that the subtribe Sesamiina began to diversify about 24 Mya and then radiated in relation to the expansion of grassland biomes and the diversification of C_4 grasses (Toussaint *et al*., 2012). Late Cenozoic climatic cycles and associated environmental changes in tropical Africa, particularly during the Plio-Pleistocene (deMenocal, 1995, 2004; Sepulchre *et al*., 2006; Trauth, Larrasoan?a & Mudelsee 2009; Maslin *et al*., 2012), also probably played an important role in African stem-borer diversification, through habitat fragmentation and niche specialization. Of particular interest is the progressive shift toward cooler and more arid conditions in sub-Saharan Africa between 8.0 and 3.0 Mya (deMenocal, 1995, 2004). Following this period, three major episodes of speciation and extinction apparently occurred at 2.6, 1.8 and 1.0 Mya, coinciding with periods of maximum climate variability on high moisture levels (Trauth *et al*., 2009). In Southern Africa, the climate was apparently more stable in the last 3.5 Myr, with little or no long-term change in the moisture gradient over the last 2.5 Myr (Maslin *et al*., 2012). While vertebrate speciation processes in Africa are well documented (e.g. Fjeldsa & Lovett, 1997; Flagstad *et al*., 2001; Wüster *et al*., 2007; Bryja *et al*., 2010; Zimkus, Rödel & Hillers, 2010; Nicolas *et al*., 2012; Dobigny *et al*., 2013; Guschanski *et al*., 2013; Demos *et al*., 2014), studies on arthropods are far less common despite the fact that they account for most of the Afrotropical diversity (Scholtz & Mansell, 2009). However, recent studies on *Charaxes* butterflies (Aduse-Poku, Vingerhoedt & Wahlberg, 2009), parasitoid fig wasps (McLeisch, Van Noort & Tolley, 2010) and dragonflies (Damm, Schierwater & Hadrys, 2010) tend to support the hypothesis that habitat fragmentation resulting from climatic pulses may be the dominant speciation process.

Among Sesamiina, the genus *Sesamia* Guenée is the second most diversified genus. It includes approximately 50 species mostly found in the tropical area

of Asia and Africa, the latter hosting the richest diversity with about 30 valid species (B. Le Ru, unpubl. data). It comprises four major pests of graminaceous crops (notably maize), *S. calamistis* Hampson, *S. cretica* Lederer, *S. nonagrioides* (Lefèbvre) and *S. poephaga* Tams & Bowden. *Sesamia nonagrioides* is one of the most widespread noctuid stem borer pests, found in sub-Saharan Africa where it extends from Ivory Coast to Kenya and Ethiopia, and in the Palearctic region, where its distribution stretches from Western Europe and North Africa to Iran (Moyal *et al*., 2011c). The Mediterranean corn stalk borer is an important pest of maize in the Mediterranean region (Cordero *et al*., 1998; Eizaguirre & Fantinou, 2012) and in sub-Saharan Africa (Kfir *et al*., 2002). Compared with the majority of stem-borer species it is a quite polyphagous species as it has been reared from plants belonging to three distinct families: Cyperaceae, Poaceae and Typhaceae (Le Ru *et al*., 2006a). In a recent phylogeographical study made in 2011 (Moyal *et al*., 2011c), the authors reported that *S. nonagrioides* consists of three distinct fragmented allopatric populations: a Palearctic population described by Lefèbvre as the true *S. nonagrioides* (Lefèbvre, 1827) and then as *S. nonagrioides nonagrioides* (Nye, 1960), a West African population formerly described as *Sesamia botanephaga* (Tams & Bowden, 1953) and then as *Sesamia nonagrioides botanephaga* (Nye, 1960) and an East African population recorded for the first time from Kenya and Uganda in the 1950s and assigned to *S. nonagrioides botanephaga* by Nye (1960). However, the sampling for this study was quite limited (only four African countries were considered) and biased as Kenya alone represented more than half of the studied specimens.

Extensive surveys carried out since 2006 allowed us to collect several thousand more specimens that appear to belong to *S. nonagrioides* or that are morphologically close. Interestingly, some of this sampling has been conducted in countries where *S. nonagrioides* has never been reported before: Benin and Ghana for West Africa; Cameroon, Democratic Republic of Congo, Republic of Congo and Zambia for Central Africa; Tanzania (including Pemba and Zanzibar Island) in East Africa; and South Africa and Mozambique in Austral Africa. Based on the marked morphological differences (especially in relation to colours and patterns of wings and differences in male and female genitalia), we hypothesize that some of these specimens potentially correspond to new species. To confirm this finding we use an integrative framework – combining morphological, ecological and genetic data – to better delimit species boundaries. Thanks to our comprehensive sampling, we also carry out additional analyses on *S. nonagrioides* alone, to reassess its evolutionary history and population structure.

MATERIAL AND METHODS

Insect sampling and ecological data

Larvae were collected from maize crops in France, Italy and Turkey, and from sugar cane crops in Iran. In sub-Saharan Africa, sampling of monocotyledon plants exhibiting symptoms of stem-borer damage was conducted over eight years (2006–2013) to collect larval stages of stem borers within their host plants (Le Ru *et al*., 2006a,b). The countries surveyed included Benin, Cameroon, Democratic Republic of Congo, Ethiopia, Ghana, Kenya, Mozambique, Republic of Congo, Rwanda, South Africa, Tanzania and Uganda. Larvae were reared on an artificial diet (Onyango & Ochieng'Odero, 1994) until pupation. In addition, several adults were collected with light traps set up in the Democratic Republic of Congo, Republic of Congo and Zambia. All sampling was conducted in agreement with local insect collection permits or local regulations.

Ecological data for this study are based on 5470 specimens collected in 17 countries and 175 localities. We also used additional information from the literature for *S. nonagrioides* (Le Ru *et al*., 2006a; Moyal *et al*., 2011c). For the majority of sampled specimens, reliable host–plant associations were determined by rearing larvae from monocotyledon plants. Habitats were categorized following White (1983).

Adult specimens were kept dry and prepared as vouchers for museum collections. Genitalia of the adults were dissected after quick immersion in a hot 10% KOH aqueous solution to enable species identification.

DNA extraction and sequencing

For this study, 173 adult *Sesamia* specimens were selected for the molecular analyses (see Supporting Information, Table S1). Representatives of ten other genera in the subtribe Sesamiina were included as outgroups based on the results of a recent molecular study (Toussaint *et al*., 2012). DNA was extracted from hind legs using Qiagen DNAeasy tissue kits. The latter procedure allowed us to keep almost complete voucher specimens for all sequenced material. Polymerase chain reaction (PCR) amplifications were conducted for four mitochondrial gene fragments: a 655-bp region of the cytochrome oxidase I (COI), 975 bp of the cytochrome b (Cytb), 338 bp of the ribosomal 12S RNA (12S) and 444 bp of the ribosomal 16S RNA (16S). Two nuclear gene regions were also sequenced: 839 bp of the 28S ribosomal DNA (28S) and 1239 bp of the elongation factor-1a (EF1a). We used the primers and settings detailed by Kergoat *et al*. (2012). Resulting PCR products were processed by the French sequencing centre Genoscope using a BigDye v3.1 sequencing kit and Applied Biosystems 3730xl sequencers. Both strands were sequenced for all specimens to minimize PCR

artefacts and ambiguities. Sequences of complementary strands were edited and reconciled using Geneious v5.1 (available at: http://www.geneious.com/). All sequences generated in this study were deposited in GenBank (accession numbers KP011139 to KP0121264). Unlike the sequences of coding genes (COI, Cytb and EF1a), the sequences of ribosomal genes (12S, 16S and 28S) were variable in length. Their alignment was accomplished using Muscle (Edgar, 2004) with default option settings. For all protein-coding genes, we used Mesquite 3.00 (available at: http://www.mesquiteproject.org) to check the coding frame for possible errors or stop codons. The combination of the six gene fragments resulted in a combined matrix of 183 specimens and 4496 aligned characters.

Phylogenetic analyses

Phylogenetic analyses were conducted using maximum-likelihood (ML) and Bayesian inference (BI). For both methods we carried out partitioned analyses to improve phylogenetic accuracy (Nylander *et al*., 2004). Partitions and substitution models were determined using PartitionFinder v1.1.1 (Lanfear *et al*., 2012), with either the *raxml* or the *beast* set of models, the *greedy* algorithm option and the corrected Akaike information criterion (AICc; Posada & Buckley, 2004) as a metric for model selection.

ML analyses were performed with RAxML v7 (Stamatakis, 2006) using the partitions and substitution models defined by the PartitionFinder analysis. The best ML tree for the combined dataset was obtained using a heuristic search implementing 100 random-addition replicates. Clade support was then assessed using non-parametric bootstrap values (BVs) (1000 replicates were used). Nodes supported by BV ≥ 70% were considered as strongly supported following Hillis & Bull (1993). Supplementary analyses were also carried out for each gene of the dataset, based on the partitions and substitution models defined by the PartitionFinder analysis. For each gene the best ML tree was obtained using a heuristic search implementing 100 random-addition replicates.

The best ML tree from the partitioned analysis was then used to implement Poisson tree process (PTP; Zhang *et al*., 2013) molecular species delimitation analyses. This method does not require an ultrametric tree as input and uses instead branch lengths to estimate the mean expected number of substitutions per site between two branching events. This approach assumes that each substitution has a small probability of generating a speciation event; therefore, the number of substitutions between species is expected to be significantly higher than those within species (Zhang *et al*., 2013). The model then implements two independent classes of Poisson processes (one describing speciation and the other describing within-species branching events) and searches for transition points between inter- and intra-species branching patterns. The latter identifies molecular species clusters, which may be used as a potential line of evidence in an integrative taxonomy framework. Although a recent study suggests that the PTP procedure is quite robust to different phylogenetic methods (Tang *et al*., 2014) it is worth underlining that the PTP procedure is not error-free and that the resulting species clusters are putative (Zhang *et al*., 2013). The corresponding analysis was conducted on the web server for PTP (available at http://species.h-its.org/ptp/).

BI was used to co-estimate phylogenetic relationships and divergence times using BEAST v1.8 (Drummond *et al*., 2012). BEAST uses Bayesian Markov Chain Monte Carlo procedures to approximate phylogenies and simultaneously infer nodes ages. To infer the time-calibrated phylogeny, we used the Bayesian relaxed clock approach (Drummond *et al*., 2006) implemented in BEAST. Analyses were performed on the combined dataset, using partitions and substitution models defined by the PartitionFinder analysis (see Results). For molecular dating analyses, each gene was associated with a specific uncorrelated lognormal relaxed clock model. To calibrate the phylogeny, we used the time-calibrated phylogeny of Toussaint *et al*. (2012) to set a series of secondary calibrations for five nodes shared between the respective datasets. To do so, we used uniform distributions to set minimum and maximum ages corresponding to the 95% of higher posterior probabilities of ages obtained in the study of Toussaint *et al*. (2012). The resulting constraints were as follows: 13.0–30.0 Mya for the most recent common ancestor (MRCA) of *Poeonoma* spp. and *Sesamia* spp.; 14.0–30.0 Mya for the MRCA of *Carelis* spp. and *Sciomesa* spp.; 14.0–32.0 Mya for the MRCA of *Busseola* spp. and *Feraxinia* spp.; 18.0–37.0 Mya for the MRCA of *Acrapex* spp. and *Sesamia* spp.; 20.0–43.0 Mya for the MRCA of *Sesamia* spp. and *Speia* spp. A coalescent model tree prior with a constant population size was then preferentially used to account for the fact that our trees mostly describe intraspecific relationships (Heled & Drummond, 2010). Two distinct runs were carried out with 50 million generations and trees sampled every 5000 generations. In a conservative way, we used a burn-in period of 12.5 million generations per run. Convergence of runs was assessed by examining the effective sample size (ESS) of parameters with Tracer v1.5 (available from http://beast.bio.ed.ac.uk/Tracer). An ESS ≥ 200 was acknowledged as an indicator of convergence for each parameter. Clade support was directly provided by the posterior probability (PP) estimates, with nodes supported by PP ≥ 0.95 considered as strongly supported (Erixon *et al*., 2003).

Population genetic analyses

Analyses at the intraspecific level were performed on datasets encompassing only *S. nonagrioides* individuals. The genetic differentiation between specimens belonging to distinct major geographical regions (Central Africa, East Africa, Palearctic region or West Africa) or reared from distinct host plant families (Cyperaceae, Poaceae or Typhaceae) was assessed for each gene using DnaSP v.5.1.0 (Librado & Rozas, 2009). The fact that DnaSP does not take into account missing data was problematic for several individuals and led to the exclusion of specimens with low genetic coverage in order to maximize the length of fragments to be analysed. To assess the level of genetic differentiation among various groups, we used three distinct statistics (F_{ST}, K_{ST}*, and S_{nn}). F_{ST} (fixation index) is a statistic comparing the level of diversity of randomly chosen alleles in a given population with those found in the entire geographical sample; K_{ST}* is a statistic taking into account the number of nucleotide differences between different haplotypes but does not give much weighting to large numbers of differences (Hudson, Boos & Kaplan, 1992); the nearest-neighbour statistic (S_{nn}) measures how often the nearest neighbours within a matrix of sequences originate from the same population (Hudson, 2000). Because these three indices are known to be more or less sensitive to specific dataset features (such as low level of genetic diversity or low sample size), we used them in combination to ensure a more robust detection of genetic differentiation (Morales-Hojas, Vieira & Vieira, 2008). For each statistic, a permutation test of 1000 replicates was performed to assess the significance of the subdivision parameters. DnaSP was then used to infer the following parameters of genetic diversity: number of segregating sites (S), number of haplotypes (h), and haplotypic (Hd) and nucleotide (π) diversities. We also performed neutrality tests for each gene using Tajima's D (Tajima, 1989). For the latter, values close to zero are expected for historically stable populations, whereas negative values would indicate recent population expansion.

Finally, to obtain a better graphical visualization of relationships between *S. nonagrioides* individuals, we carried out a network analysis under SplitsTree4 (Huson & Bryant, 2006) with the default *Neighbor-Net* algorithm. The corresponding analysis was performed on the combined dataset without outgroups to maximize the amount of phylogenetic information and to offer a direct comparison with the phylogenetic trees resulting from the analyses of the combined dataset.

Morphological studies

Genitalia were dissected after immersion of the extremity of the abdomen in a boiling 10% potash bath for a few minutes, then cleaned, immersed in absolute alcohol for a few minutes and mounted on slides in Euparal (after separating the aedeagus from the rest of the genitalia in the male). Specimens were identified by comparison with types housed in several museums, the Natural History Museum, London (NHM), the Ditsong National Museum of Natural History (DNMH), South Africa, and the Museo Civico di Storia Naturale (MCSN), Milan. Types of new species were deposited in the Muséum National d'Histoire Naturelle (MNHN) in Paris, France. When possible, paratypes were deposited in the Ditsong National Museum of Natural History (DNMH), South Africa, and in the National Museum of Kenya (NMK) in Nairobi, Kenya. The complete addresses of the corresponding collections and institutions are:

CBBLR: BLR Collection, BEI, Unité de Recherche IRD 072, Laboratoire Evolution, Génomes et Spéciation, UPR 9034, 22 CNRS, 91198 – Gif/Yvette, France.

DNMH: Ditsong National Museum of Natural History, Paul Kruger Street, PO Box 413, Pretoria, 0001 South Africa.

MCSN: Museo Civico di Storia Naturale, Corso Venezia, 55, 20121 Milano, Italia.

MNHN: Museum National d'Histoire Naturelle, 57 Rue Cuvier, 75005 Paris, France.

NHM: Natural History Museum, Cromwell Road, London SW7 5BD, UK.

NMK: National Museums of Kenya, PO Box 78420-00500 Ngara Rd, Nairobi, Kenya.

RESULTS

Phylogenetic analyses

Eleven distinct partitions were selected by the AICc (see Table S2 for details on partitions and their substitution models). Both ML and BI partitioned analyses generated similar topologies (see Fig. 1 for the best ML tree and Fig. 2 for the Bayesian maximum clade credibility tree). Overall the corresponding trees are well supported as most interspecific nodes are supported by BV ≥ 70% and PP ≥ 0.95. Within *Sesamia* a large and well-supported (BV of 100% and PP of 1.0) clade encompasses all the specimens that either belong to or are morphologically related to *Sesamia nonagrioides*.

Regarding molecular species delimitation, the PTP analysis revealed seven putative species clusters within the clade of interest (see Fig. 1). The largest cluster (100 individuals) corresponds to the true *S. nonagrioides*. It includes specimens from Central Africa (Cameroon, Democratic Republic of Congo, Republic of Congo, Zambia), East Africa (Ethiopia, Kenya, Rwanda, Tanzania, Uganda), West Africa (Benin, Ghana) and the Palearctic region (France, Iran, Italy, Turkey); it does

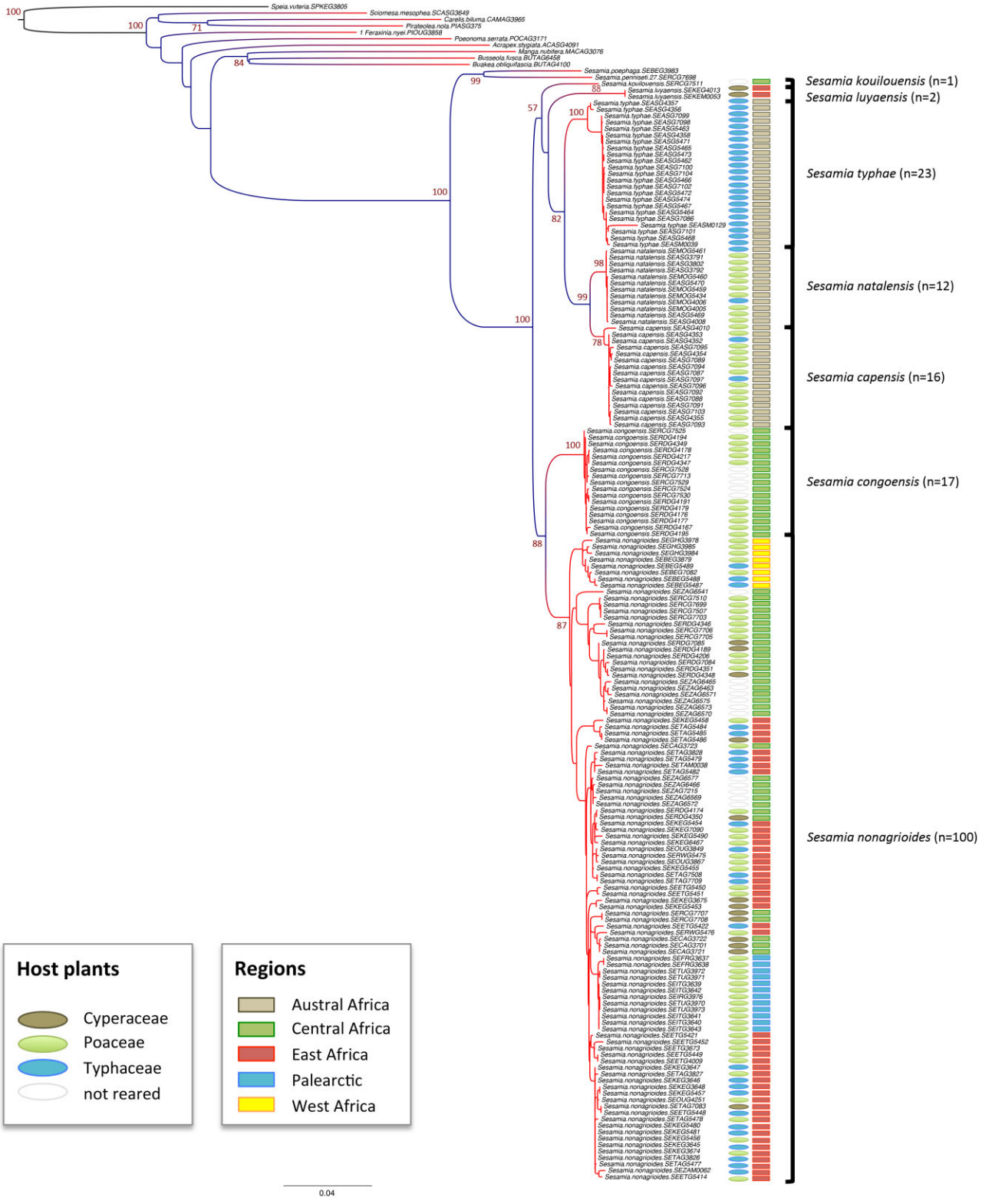

Figure 1. ML tree resulting from analysis of the combined dataset (support of major nodes is provided by BV). Results of the PTP analysis are provided using coloured branches. Putative molecular species are indicated using transitions between blue-coloured branches and red-coloured branches. On the right, brackets are used to distinguish the seven species of the *S. nonagrioides* group (names of species are provided along with the number of sequenced individuals). For each individual of the *S. nonagrioides* group coloured ellipses and rectangles are used to indicate host plant information and the biogeographical region of origin, respectively.

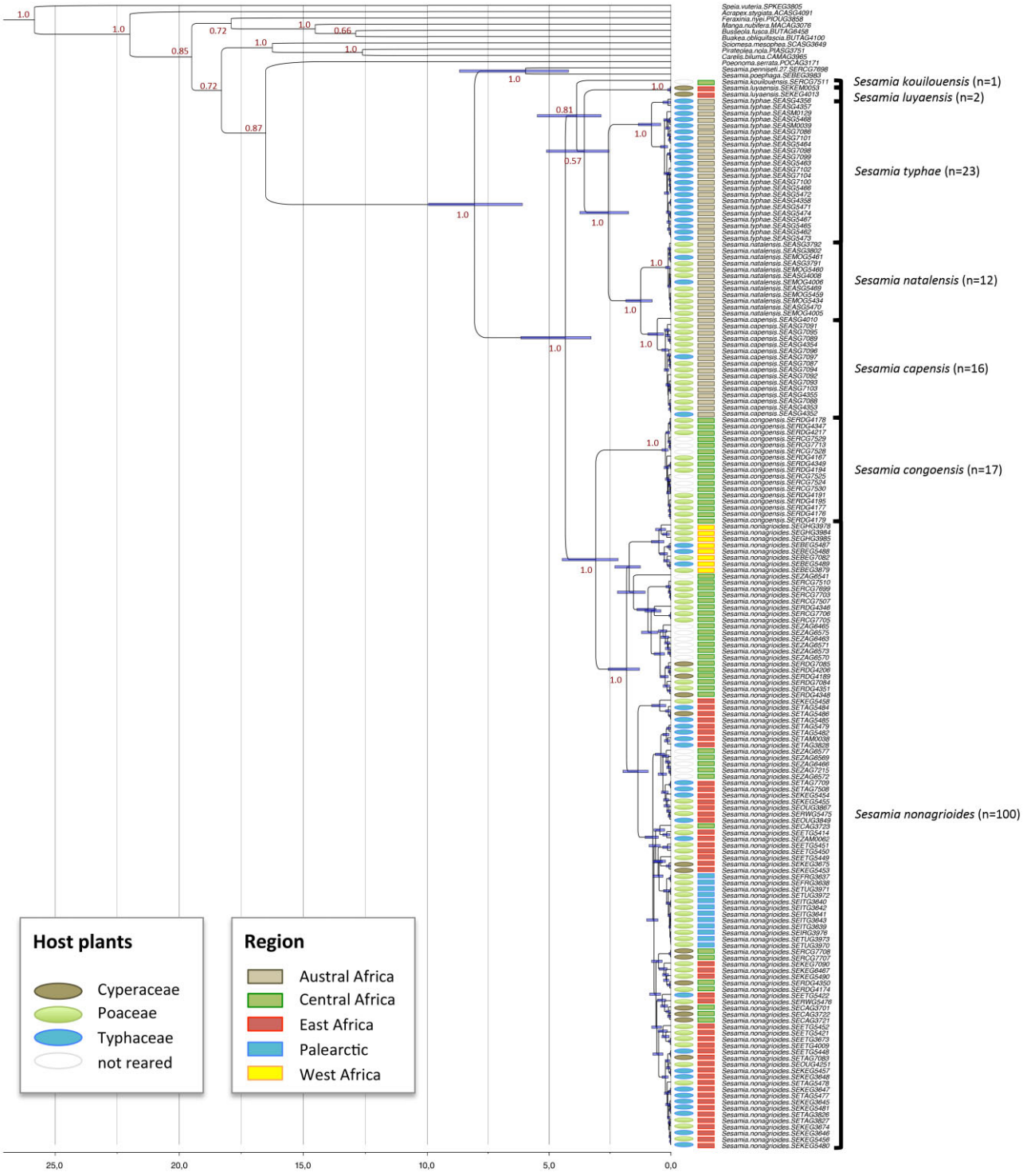

Figure 2. Maximum consensus tree resulting from Bayesian inference analyses of the combined dataset (support of major nodes is provided by PP). On the right, brackets are used to distinguish the seven species of the *S. nonagrioides* group (names of species are provided along with the number of sequenced individuals). For each individual of the *S. nonagrioides* group coloured ellipses and rectangles are used to indicate host plant information and the biogeographical region of origin, respectively.

not include any specimens from Austral Africa. Corresponding rearing information indicates that the sequenced specimens were reared from Cyperaceae, Poaceae and Typhaceae. Sister to this clade is a cluster of 17 individuals, which have only been sampled on Poaceae in the Democratic Republic of Congo and the Republic of Congo. Another large clade encompasses five putative species clusters, and notably a clade of three putative species, which are only distributed in Austral Africa. The putative species clusters are in agreement with the results of morphological studies (see below), which highlight several morphological differences between putative taxa, especially when considering the morphology of genitalia. In comparison with *S. nonagrioides*, these taxa also appear to be more specialized in terms of host plant or habitat preferences (see Discussion for more details on this); three of these species also exclusively occur in Austral Africa, where *S. nonagrioides* has never been confidently recorded. Supplementary analyses conducted for each gene yield topologies that are less well resolved and supported (see Figs S1, S2), especially for the genes exhibiting lower levels of genetic differentiation (12S, EF1a and 28S). Individuals corresponding to the six new putative species clusters highlighted by the PTP analyses are consistently recovered as monophyletic in the trees resulting from the COI, Cytb and 16S analyses.

Dating analyses (see Fig. 2) suggest that the clade containing *S. nonagrioides* and other related species started to diversify about 4.33 Mya (95% HPD: 3.28–6.16), with major splits occurring at 3.87 Ma (95% highest posterior density (HPD): 2.86–5.49; MRCA of *S. kouilouensis*, *S. capensis*, *S. luyaensis*, *S. natalensis* and *S. typhae*), 3.55 Mya (95% HPD: 2.54–5.11; MRCA of *S. luyaensis*, *S. capensis*, *S. natalensis* and *S. typhae*), 3.09 Mya (95% HPD: 2.17–4.47; MRCA of *S. congoensis* and *S. nonagrioides*), 2.57 Mya (95% HPD: 1.74–3.74; MRCA of *S. typhae*, *S. capensis* and *S. natalensis*) and 1.24 Mya (95% HPD: 0.76–1.85; MRCA of *S. capensis* and *S. natalensis*).

Population genetic analyses

The results of *S. nonagrioides* genetic differentiation and genetic diversity analyses are summarized in Tables S3, S4 and S5. For the mitochondrial genes, all statistics (F_{ST}, K_{ST}* and S_{nn}) recovered a significant level of genetic differentiation ($P < 0.01$ for 111 out of 120 comparisons) among the four geographical groups (Table S3). The only non-significant values were observed for the 12S gene for F_{ST} and S_{nn} when looking at the genetic differentiation between individuals from the Palearctic region and individuals collected in other geographical areas (Table S3). By contrast, the level of genetic structure was lower when considering the two nuclear loci: only two values out of 30 were significantly supported for the EF1a gene (East Africa versus Central Africa) whereas a significant structure was recovered only in 15 out of 30 comparisons for the 28S gene (East Africa versus all remaining geographical areas, Central Africa versus all remaining geographical areas, Central Africa versus East Africa, Central Africa versus Palearctic region and Central Africa versus West Africa). When looking at the genetic differentiation between individuals reared from distinct host plant families (Table S4), a lower level of genetic differentiation was found when looking at the mitochondrial genes ($P < 0.01$ for 41 out of 72 comparisons) whereas it was higher when considering the nuclear loci ($P < 0.01$ for 24 out of 36 comparisons). Overall, the number of shared haplotypes was higher when comparing groups based on host plants (on average 1 out 7.13) rather than groups based on geographical areas (on average 1 out of 17.36). A higher number of shared haplotypes was consistently recovered between individuals collected in Central and East Africa. In contrast to the mitochondrial genes (Table S5), genetic diversity was extremely low for the two nuclear loci ($S = 3$, $h = 4$, $Hd = 0.368$ for 28S and $S = 6$, $h = 7$, $Hd = 0.215$ for EF1a). The group gathering individuals from Central Africa generally displayed both the highest haplotype diversity (up to 0.97, depending on genes; Table S5) and the highest nucleotide diversity (up to 0.0023; Table S5). Interestingly, this result was recovered despite the fact that the number of sampled individuals or localities (19 localities versus 34; Table S1) in Central Africa is consistently lower than those from East Africa. The results of the Tajima's *D* neutrality tests for all genes also suggested that *S. nonagrioides* populations were stable over time, as no evidence for expansion of population sizes was found in the majority (45 out of 48) of Tajima's *D* tests (Table S5).

SplitsTree network analyses recovered a well-structured pattern for *S. nonagrioides* (Fig. 3), in which several distinctive geographical groups may be characterized. A first well-delineated cluster comprises three distinct geographical groups from West, Central and East Africa, respectively. The second well-delineated cluster is composed of five distinct groups: one is exclusively composed of individuals from the Palearctic region and is more closely related to a group encompassing three specimens from East Africa (collected in Ethiopia). The three remaining groups consist of one subset including individuals that are only distributed in East Africa whereas the final two subsets include specimens from Central and East Africa.

Morphological studies – species description

As underlined above the results of morphological studies are in agreement with the putative molecular clusters that have been inferred using the molecular species

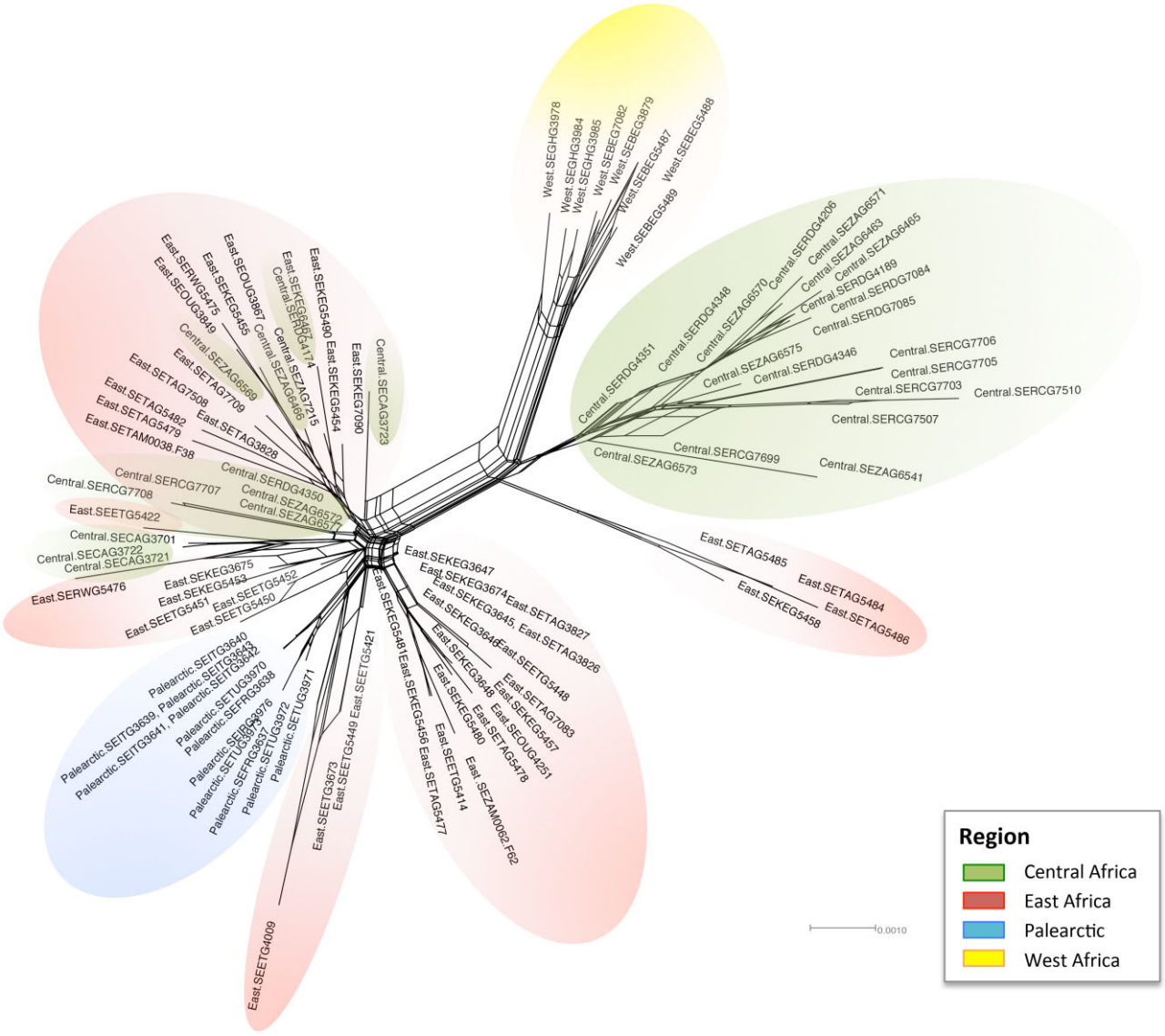

Figure 3. Results of the SplitsTree network analyses (*Neighbor-Net* algorithm) for the sampled *S. nonagrioides* individuals. Coloured ellipses are used to highlight the geographical origin of individuals.

delimitation approach. In the following section we provide the description of the six new species.

Sesamia capensis Le Ru sp. nov.
(Figs 4A–D; 7A,G; 8A; 9A; 10)

Type material examined. Holotype male: **South Africa**: Phamton Pass, Nkysna, Western Cape, 34°01.704′S, 22°59.150′E, 6 m a.s.l., XII 2013, ex larva, in stem of *Phragmites australis* (Cav.) Trin. ex Steud., gen. prep. LERU Bruno/G615, B. Le Ru leg., MNHN, Paris. Paratypes: **South Africa**: 2 females gen. prep. LERU Bruno/G616-G619, 1 male gen. prep. LERU Bruno/G617-G618; 2 males and 2 females, same data as holotype; 1 male, gen. prep. LERU Bruno/G9, 1 male and 2 females, Swartberg, Western Cape, 34°09.038′S, 19°28.662′E, 283 m a.s.l., XII 2009, in stem of *P. australis*, B. Le Ru leg., MNHN, Paris; 1 male and 1 female, Kwanonqubela, Eastern Cape, 33°39.188′S, 26°25.066′E, 158 m a.s.l., XII 2009, in stem of *P. australis*, B. Le Ru leg., MNHN, Paris; 2 males and 2 females same data as holotype, B. Le Ru leg., DMSA, Pretoria.

Description (Fig. 4A–D)

Antennae short, bipectinate, serrate at apex in the male, filiform in the female, flagellum adorned dorsally with ochraceous scales. Body colour and wing pattern similar in both sexes, but slightly darker in male. Palpus

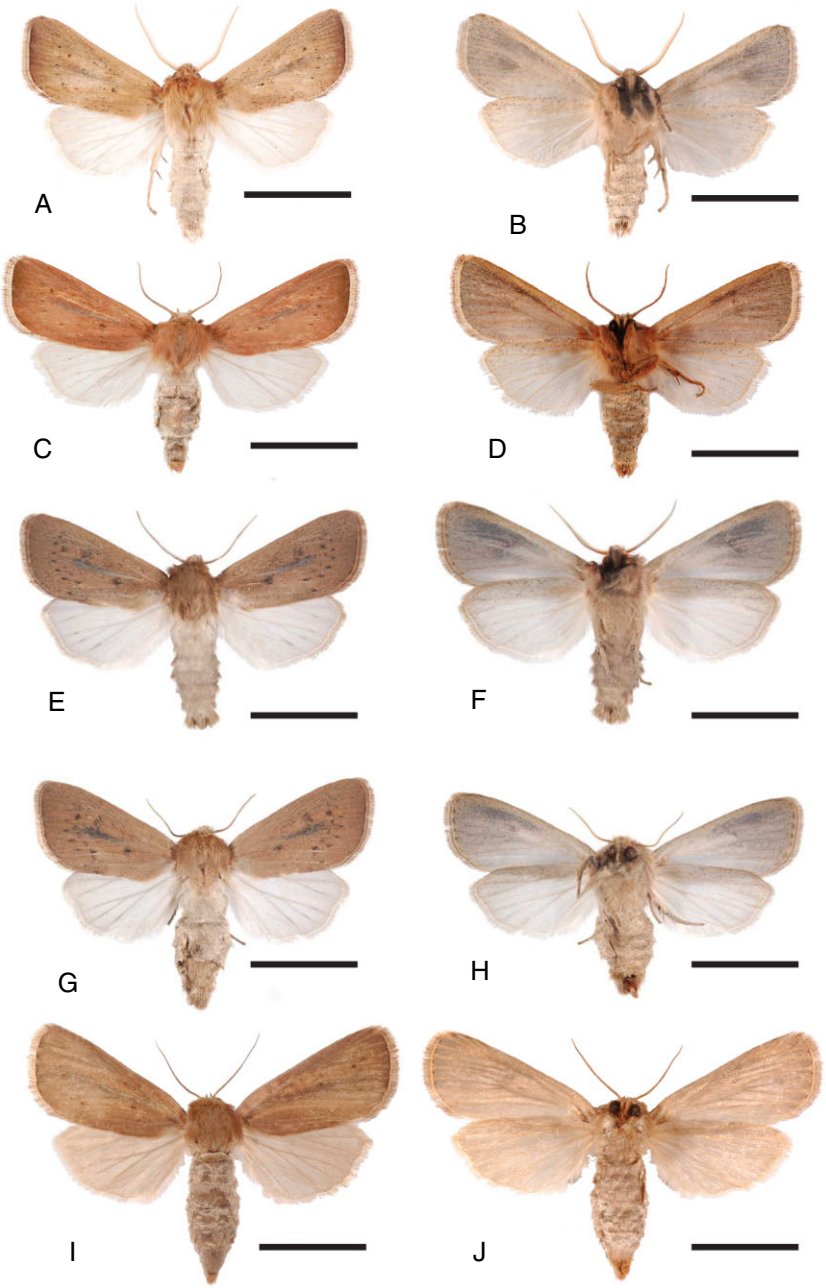

Figure 4. Adults of *Sesamia* species. A–D, *S. capensis*: A, male upper side; B, male under side; C, female upper side; D, female under side. E–H, *S. congoensis*: E, male upper side; F, male under side; G, female upper side; H, female under side. I–J, *S. kouilouensis*: I, female upper side; J, female under side. Scale bars = 10 mm.

ochreous irrorated with fuscous. Head and thorax ochraceous, covered with long hairs. Abdomen ochreous irrorated with fuscous. Legs ochreous, fore femur and tibia strongly infuscated on their inner surfaces. Forewings ochreous suffused with fuscous scales; an antemedial black spot below the cell rather indistinct; an indistinct longitudinal grey fascia from the base along the lower margin of cell, partly within, partly without cell limited to the first two-thirds of the wing and an indistinct postmedial row of spots; in some specimens not distinct at all. Fringe ochreous with a basal fuscous line. Hindwings white, veins with cartridge scales; fringe white iridescent. Underside of forewings pale ochreous at the base, cartridge-buff on costa; apex and terminal area heavily suffused with fuscous, with cartridge-buff scales along the veins. Underside

of hindwings white, costa and apex suffused with fuscous scales; fringe white iridescent, infuscate at the apex, with a basal cartridge-buff line.

Wingspan 27–32 mm (males) (n = 12); 32–36 mm (females) (n = 12).

Male (32, 29, 29, 28, 28, 27, 28, 29, 30, 29, 29, 31)

Female (34, 36, 33, 36, 34, 35, 33, 33, 32, 32, 32, 35)

Male genitalia (Fig. 7A,G) Uncus small, wide, narrowing sharply at about halfway. Tegumen with large peniculi. Vinculum with a large saccus, v-shaped at the bottom margin, w-shaped at the top margin without indentation. Extension of sacculus wide, slightly curved inwards with several rows of short stout spines on the tip. Long slightly spatulated cucullus and long coastal spine, curved inwards, bifid with the inner tooth longer and stronger, slightly dentated at the base. Juxta lampshade-shaped with a short wide neck, shortly bifid. Aedeagus (Fig. 7G) long, slightly dilated basally and curved in the middle, presence of sclerotized elongate carina crest. *Female genitalia* (Fig. 8A) Corpus bursae elongate without signa; ductus bursae short, strongly sclerotized near the ostium; lateral plates of ostial segment small, sclerotized and slightly rounded, 1.2 times longer than wide. Ovipositor lobes less than 2 times longer than wide.

Larvae L5 instar (Fig. 9A): length, 40–45 mm, breadth, 4.0 mm; head smooth, red brown, prothoracic shield pale yellow brown; body with ground colour buff, dorsally suffused with pink, pinnacula pale yellow brown and caudal plate pale brown. Young larvae are very similar in appearance to mature ones.

Etymology: epithet of Cape in Western Cape Province of South Africa where it was discovered.

Bionomics

Sesamia capensis is a markedly hygrophilous species inhabiting lakesides, marshy and boggy areas and stream valleys. Larvae were collected on young stems of common reed *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae: Arundinoideae: Arundineae) and bulrush *Typha latifolia* L. (Typhaceae). Larvae are frequently gregarious (between five and 20 larvae) when young but always solitary when mature. Typically, stems exhibiting signs of infestation by *S. capensis* larvae have dry leaves and shoots (dead hearts); on bulrush the central leaves turn yellow. Pupae were almost never found in stems; therefore, it is likely that most mature larvae leave the stems for pupation.

Distribution

South Africa, below 570 m a.s.l. in coastal areas of Eastern and Western Cape provinces and Coastal areas of South Kwazulu-Natal province, west of Port Shepstone; moths were found in Cape shrubland vegetation mosaic and South East African coastal mosaic ('Coastal mosaics'; White, 1983) belonging to the Southern African bioregion (Linder *et al*., 2012) (Fig. 10).

Sesamia congoensis Le Ru sp. nov.

(Figs 4E–H; 7B,H; 8B; 9B; 10)

Type material examined. Holotype male: **DRC**: Kona Itimbiri, Equateur province, 02°02.424′N, 22°47.270′E, 352 m a.s.l., V 2010, ex larva, in stem of *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase, B. Le Ru leg., gen. prep. LERU Bruno/G244, MNHN, Paris. Paratypes: **DRC**: 1 female gen. prep. LERU Bruno/G243, 1 male gen. prep. LERU Bruno/G437, 1 female gen. prep. LERU Bruno/G245, 2 males, 2 females, same data as holotype; 1 male and 1 female from Lieki, Lomami River, Orientale province, 00°40.756′N, 24°14.034′E, 371 m a.s.l., VI 2010, ex larva, in stem of *Vossia cuspidata* Griff., B. Le Ru leg., MNHN, Paris; **R. Congo**: 2 males, 2 females, Rivière Mpama, Plateaux department, 02°28.079′S, 14°36.897′E, 446 m a.s.l., IV 2013, ex light trap, B. Le Ru leg., MNHN, Paris; 2 males, Rivière de la Léfini, Plateaux department, 02°54.501′S, 15°37.776′E, 320 m a.s.l., IV 2013, ex light trap, B. Le Ru leg., MNHN, Paris.

Description (Fig. 4E–H)

Antennae short, bipectinate, serrate at apex in the male, filiform in the female, flagellum adorned dorsally with grey buff scales. Palpus chestnut; body colour and wing pattern similar in both sexes, slightly darker in male. Head and thorax chestnut covered with long hairs. Abdomen light chestnut suffused with buff. Legs grey, fore femur and tibia slightly infuscated on their inner surfaces. Forewings chestnut suffused with black scales. Some black markings, variable in extent and intensity; an antemedial black spot below the cell; a longitudinal grey fascia from base along lower margin of cell, partly within, partly without cell limited to the first two-thirds of the wing. A curved dentated postmedial fascia split into two postmedial rows of spots, each plots located on the veins; fringe chamoisee with a narrow basal ochre line, infuscated medially and fuscous-white at tips. Hindwings white, veins with brown scales; fringe chestnut iridescent. Underside of forewings tan at the base, camel on costa; apex and terminal area suffused with fuscous, with ochreous scales along the veins. Underside of hindwings white, costa and apex lightly suffused with fuscous scales; fringe chestnut with a basal tan line.

Wingspan 31–32 mm (males) (n = 6); 35–43 mm (females) (n = 10).

Male (31, 32, 32, 33, 33, 32)

Female (36, 36, 43, 37, 43, 39, 36, 35, 37, 35)

Male genitalia (Fig. 7B,H) Uncus very small, narrow, pointed apically. Tegumen with large peniculi. Vinculum with a large and almost quadrangular saccus. Extension of sacculus narrow slightly curved inwards with several rows of short stout spines on the tip. Long slightly spatulated cucullus and short coastal spine bifid with the inner tooth longer and stronger. Juxta oblong pear-shaped with a short wide neck, shortly bifid. Aedeagus (Fig. 7H) long, slightly dilated basally and curved in the middle, presence of sclerotized elongate carina crest. *Female genitalia* (Fig. 8B) Corpus bursae elongate without signa; ductus bursae short, strongly sclerotized near the ostium; lateral plates of ostial segment large, sclerotized and rounded, 1.5 times longer than wide. Ovipositor lobes 2.5 times longer than wide.

Larvae L5 instar (Fig. 9B): length, 40–45 mm, breadth, 4.0 mm; head smooth, dark brown, prothoracic shield pale brown; body with ground colour buff, dorsally suffused with pink, pinnacula pale yellow brown and caudal plate brown. Young larvae are very similar in appearance to mature ones.

Etymology: epithet of Congo where it is distributed (Democratic Republic of Congo and Republic of Congo).

Bionomics

Sesamia congoensis is a markedly hygrophilous species inhabiting floating grasses along banks of rivers. Larvae were collected on young stems of antelope grass (*Echinochloa pyramidalis* (Lam.) Hitchc. & Chase) and hippo grass *Vossia cuspidata* ((Roxb.)Griff.) stems. Larvae are frequently gregarious (between five and 20 larvae) when young but always solitary when mature. Typically, stems exhibiting signs of infestation by *S. congoensis* larvae have dry leaves and shoots (dead hearts). No pupae were found in stems, and therefore it is likely that mature larvae leave the stems and swim to shore for pupation.

Distribution

Democratic Republic of Congo (Equateur and Orientale provinces) and Republic of Congo (Plateaux department); moths were found in Guineo-Congolian rain forests, lowland rain forests and secondary grassland vegetation mosaics ('Moist and swamp forests'; White, 1983) belonging to the Congolian bioregion (Linder *et al*. 2012) (Fig. 10).

Sesamia kouilouensis Le Ru sp. nov.

(Figs 4I–J; 8C; 10)

Type material examined. Holotype female, **R. Congo**, Lac Nanga, Kouilou department, 04°56.090′S, 11°56.713′E, 2 m a.s.l., IV 2013, ex light trap, gen. prep. LERU Bruno/G581, B. Le Ru leg., MNHN, Paris.

Description (Fig. 4I–J)

Antennae filiform, flagellum adorned with buff scales. Palpus ochreous-buff; palpus ochreous-buff irrorated with fuscous. Head and thorax ochreous-buff covered with long hairs. Abdomen uniformly ochreous-buff. Legs ochreous-buff, fore femur and tibia strongly infuscated on their inner surface. Forewings ochreous-buff suffused with fuscous scales; an indistinct longitudinal ochreous fascia from the base along the lower margin of cell, partly within, partly without cell limited to the first two-thirds of the wing and an indistinct postmedial row of spots. Fringe buff with a basal fuscous line. Hindwings pale ochreous, veins with buff scales; fringe white iridescent. Underside of forewings pale ochreous-buff at the base, ochreous-buff on costa; apex and terminal area suffused with fuscous scales, veins irrorated with ochreous scales. Underside of hindwings pale ochreous, costa and apex suffused with fuscous scales; veins irrorated with ochreous scales; fringe white iridescent, infuscated at the apex, with a basal ochreous-buff line.

Wingspan 38 mm (female) (n = 1)

Female genitalia (Fig. 8C) Corpus bursae elongate without signa; ductus bursae about the same length as corpus bursae, without chitinization near the bursae, strongly sclerotized near the ostium; lateral plates of ostial segment medium sized, sclerotized and slightly rounded, less than 1.5 times longer than wide. Ovipositor lobes 4 times longer than wide.

Etymology: epithet of Kouilou, the coastal region of Republic of Congo where it was collected.

Bionomics

Biology unknown. The moth was caught with a light trap in grasslands on a lakeshore.

Distribution

Republic of Congo in Kouilou region, south coast of Pointe Noire. The unique specimen was found in a secondary grassland vegetation mosaic ('Moist and swamp forest'; White, 1983) belonging to the Congolian bioregion (Linder *et al*., 2012) (Fig. 10).

Sesamia luyaensis Le Ru sp. nov.

(Figs 5A–D; 7C,I; 8D; 9C; 10)

Type material examined. Holotype male: **Kenya**: Kakamega Forest, Western province, 00°10.887′N, 34°56.345′E, 1400 m a.s.l., X 2007, ex larva, in stem of *Cyperus papyrus* L, gen. prep. LERU Bruno/G17, B. Le Ru leg., MNHN, Paris. Paratypes: **Kenya**: 1

female gen. prep. LERU Bruno/G443, 1 male gen. prep. LERU Bruno/G445, 1 male gen. prep. LERU Bruno/G446, 1 female gen. prep. LERU Bruno/G444, 2 males, 2 females, same data as holotype, B. Le Ru leg., MNHN, Paris.

Description (Fig. 5A–D)

Antennae short, bipectinate, serrate at apex in the male, filiform in the female, flagellum with ochraceous scales. Palpus camel; body colour and wing pattern similar in both sexes, but slightly darker in male. Head and thorax camel covered with long hairs. Abdomen camel. Legs camel, fore femur and tibia strongly infuscated on their inner surfaces. Forewing camel densely suffused with chocolate scales from the cell towards the termen; costal and basal margin suffused with black scales. Some black markings, variable in extent and intensity; an antemedial black spot below the cell; a postmedial row of spots; a longitudinal grey fascia from base along lower margin of cell, partly within, partly without cell limited to the first two-thirds of the wing. Veins suffused with camel scales; fringe tan with a basal camel line. Hindwings white, veins m3, c1 and c2 adorned with a spot of brown scales, apex and terminal area suffused with black scales; fringe buff iridescent. Underside of forewings bole, chamoisee on the

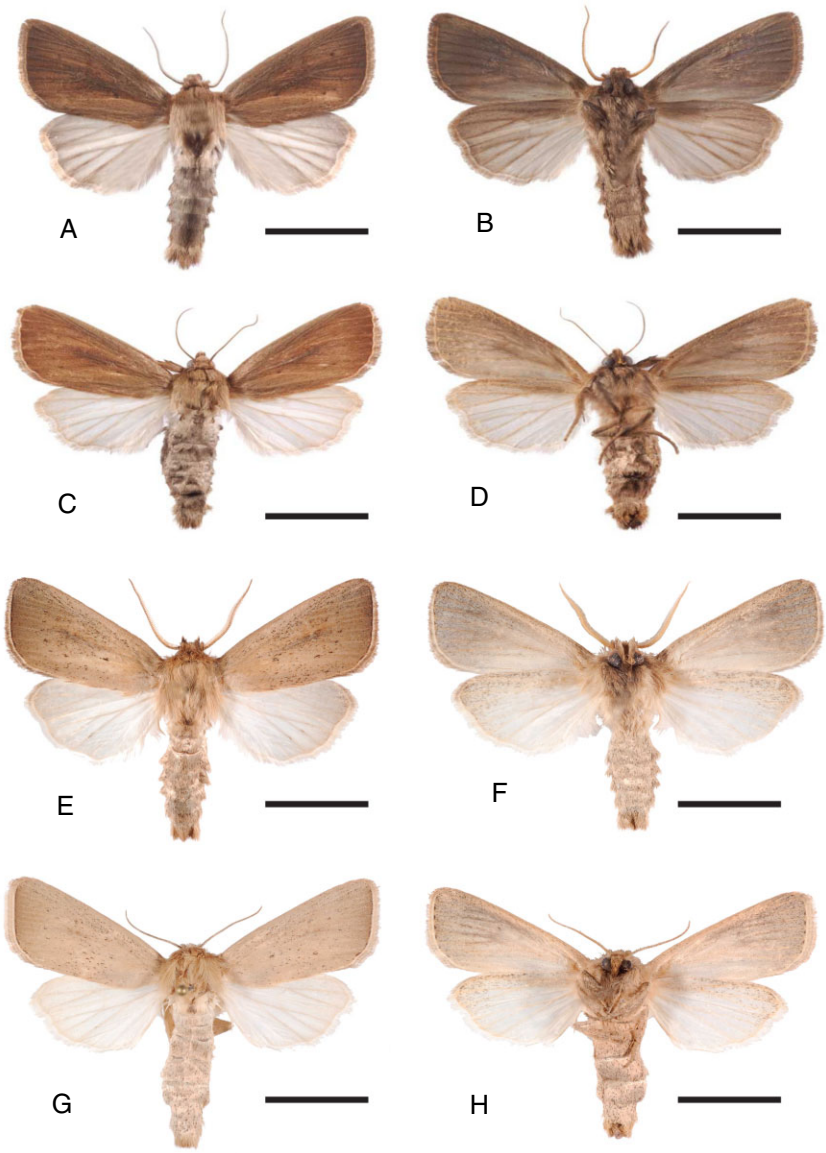

Figure 5. Adults of *Sesamia* species. A–D, *S. luyaensis*: A, male upper side, B, male under side; C, female upper side; D, female under side. E–H, *S. natalensis*: E, male upper side; F, male under side; G, female upper side; H, female under side. Scale bars = 10 mm.

base; fringe bright chamoisee with a basal tan line. Underside of hindwings bright tan, costa and apex densely suffused with chamoisee scales. Veins suffused with chamoisee scales; fringe bright chamoisee with a basal tan line.

Wingspan 32–34 mm (males) (n = 7); 35–44 mm (females) (n = 12).

Male (33, 34, 32, 33, 33, 34, 32, 32)

Female (36, 36, 43, 37, 44, 39, 36, 35, 37, 35, 38, 39)

Male genitalia (Fig. 7C,I) Uncus small, narrow, pointed apically. Tegumen with large peniculi. Vinculum with a large and deep saccus, u-shaped. Extension of sacculus narrow slightly curved inwards with several rows of short stout spines on the tip. Long slightly spatulated cucullus and long coastal spine bifid with the inner tooth longer and stronger, slightly dentated at the base. Juxta pear-shaped with a short narrow neck and long bifid. Aedeagus (Fig. 7I) long, slightly dilated basally and curved in the middle, presence of sclerotized elongate carina crest. *Female genitalia* (Fig. 8D) Corpus bursae elongate without signa; ductus bursae short, strongly sclerotized near the ostium; lateral plates of ostial segment medium sized, slightly sclerotized, not rounded, 2 times longer than wide. Ovipositor lobes 3.5 times longer than wide.

Larvae L5 instar (Fig. 9C): length, 40–45 mm, breadth, 4.0 mm; head smooth, dark brown, prothoracic shield pale brown; body with ground colour pink purple adorned with two lateral, narrow, bright buff stripes, pinnacula pale yellow brown and caudal plate brown. Young larvae are very similar in appearance to mature ones.

Etymology: epithet of Luya, the largest tribe in western Kenya.

Bionomics

Sesamia luyaensis is a markedly hygrophilous species inhabiting floating sedges along banks of rivers. Larvae were collected at the bottom of stems of Nile grass, *Cyperus papyrus* L. (Cyperaceae). Larvae are very gregarious when young (up to 40 larvae per stem). Infested stems did not exhibit signs of infestation compared with other infested grasses and sedges, but only presence of frass. No pupae were found in stems, and therefore it is likely that mature larvae leave the stems and swim to shore to pupate.

Distribution

Kenya, Western province and Uganda, in Mukono province; moths were found in Guineo-Congolian rain forest vegetation mosaic ('Moist and swamp forests'; White, 1983) belonging to the Congolian bioregion (Linder *et al*. 2012) (Fig. 10).

Sesamia natalensis Le Ru sp. nov.

(Figs 5E–H; 7D,J; 8E; 9D; 10)

Type material examined. Holotype male: **South Africa**: SASRI Station, Mount Edgecombe, Kwazulu-Natal province, 29°42.457′S, 31°02.840′E, 45 m a.s.l., II 2006, ex larva, in stem of *P. australis*, gen. prep. LERU Bruno/G75, B. Le Ru leg., MNHN, Paris. Paratypes: **South Africa**: 1 female gen. prep. LERU Bruno/G77, 1 male gen. prep. LERU Bruno/G76, 2 males and 2 females, same data as holotype; 1 male gen. prep. LERU Bruno/G8, 1 male and 2 females, Eston, Richmond, 29°50.685′S, 30°31.084′E, 699 m a.s.l., II 2009, in stem of *P. australis*, B. Le Ru leg., MNHN, Paris; 2 males and 2 females same data as holotype, B. Le Ru leg., DMSA, Pretoria. **Mozambique**: 1 male, 1 female, from Vale de Infulene, Maputo province, 25°55.046′S, 32°02.840′E, 24 m a.s.l., III 2005, in stem of *P. australis*, B. Le Ru leg., MNHN, Paris.

Description (Fig. 5E–H)

Antennae short, bipectinate, serrated at apex in the male, filiform in the female, flagellum with ochraceous scales. Body colour and wing pattern similar in both sexes, but slightly darker in male. Palpus ochreous-buff irrorated with fuscous. Head and thorax ochreous-buff covered with long hairs. Abdomen ochreous-buff irrorated with fuscous. Legs ochreous-buff, fore femur and tibia strongly infuscated on their inner surfaces. Forewings ochreous-buff suffused with fuscous scales; an antemedial black spot below the cell rather indistinct; an indistinct longitudinal ochreous fascia from base along lower margin of cell, partly within, partly without cell limited to the first two-thirds of the wing and an indistinct postmedial row of spots; in some specimens not distinct at all. Fringe ochreous, with a basal fuscous line. Hindwings white, veins with ochreous-buff scales; fringe white iridescent. Underside of forewings pale ochreous-buff at the base, cartridge-buff on costa; apex and terminal area heavily suffused with fuscous, with cartridge-buff scales along the veins. Underside of hindwings white, costa and apex suffused with fuscous scales; fringe white iridescent, infuscated at the apex, with a basal cartridge-buff line.

Wingspan 28–31 mm (males) (n = 13); 32–37 mm (females) (n = 14).

Male (28, 28, 30, 29, 28, 30, 30, 29, 31, 29, 30, 27, 29)

Female (37, 33, 36, 37, 32, 34, 35, 37, 34, 35, 34, 35, 35, 34)

Male genitalia (Fig. 7D,J) Uncus small, wide, narrowing at about halfway. Tegumen with large peniculi. Vinculum with a large saccus, u-shaped at the bottom margin, w-shaped at the top margin without indentation. Extension of sacculus with a long neck, slightly curved inwards with several rows of short stout spines

on the tip. Long slightly spatulated cucullus and long coastal spine bifid with the inner tooth longer and stronger, slightly dentated at the base. Juxta pear-shaped with a long narrow neck, shortly bifid. Aedeagus (Fig. 7J) long, slightly dilated basally and curved in the middle, presence of sclerotized elongate carina crest. *Female genitalia* (Fig. 8E) Corpus bursae elongate without signa; ductus bursae short, strongly sclerotized near the ostium; lateral plates of ostial segment medium sized, slightly sclerotized, not rounded, 1.5 times longer than wide. Ovipositor lobes 2 times longer than wide.

Larvae L5 instar (Fig. 9D): length, 40–45 mm, breadth, 4.0 mm; head smooth, dark brown, prothoracic shield pale brown; body with ground colour buff, dorsally suffused with pink, pinnacula pale yellow brown and caudal plate pale brown. Young larvae are very similar in appearance to mature ones.

Etymology: epithet of Natal, the south-east region of South Africa were the species was discovered.

Bionomics

Sesamia natalensis is a markedly hygrophilous species inhabiting lakesides, marshy and boggy areas, and stream valleys. Larvae were collected on young stems of common reed *P. australis* (Poaceae) and bulrush *Typha latifolia* L. (Typhaceae). Larvae are frequently gregarious (between five and 20 larvae) when young but always solitary when mature. Typically, stems exhibiting signs of infestation by *S. natalensis* larvae have dry leaves and shoots (dead hearts); on bulrush the central leaves turn yellow. Pupae were almost never found in stems; therefore, it is likely that most mature larvae leave the stems for pupation.

Distribution

South Africa, below 837 m a.s.l., in coastal areas of Kwazulu-Natal province, until Port Shepston; Mozambique, coastal areas of Maputo and Ihambane regions; moths were found in East African coastal mosaic ('Coastal mosaics'; White, 1983) belonging to the Southern African and Zambezian bioregion (Linder *et al*. 2012) (Fig. 10).

Sesamia typhae Le Ru sp. nov.
(Figs 6E–H; 7F,L; 8G; 9F; 10)

Type material examined. Holotype male: **South Africa**: Queenstown, Eastern Cape province, 31°55.406′S, 26°49.766′E, 1041 m a.s.l., XI 2009, ex larva, in stem of *T. latifolius*, gen. prep. LERU Bruno/G12, B. Le Ru leg., MNHN, Paris. Paratypes: **South Africa**: 1 female gen. prep. LERU Bruno/G14, 2 males, 2 females, Rustfontein, Kwazulu-Natal province, 30°26.425′S, 29°10.613′E, 1506 m a.s.l., XI 2009, ex larva, in stem of *T. latifolius*; 1 male, 1 female, Roosenekal, Mpumalanga province, 25°05.983′S, 29°51.731′E, 1024 m a.s.l., II 2007, ex larva, in stem of *T. latifolius*; 2 males, 2 females, from Boskop Dam, Potchefstroom, Northwest province, 26°30.904′S, 27°07.417′E, 1506 m a.s.l., II 2006, ex larva, in stem of *T. latifolius*; 1 male gen. prep. LERU Bruno/G13; 1 male, 2 females, same data as holotype; B. Le Ru leg. MNHH, Paris; 1 male, 1 female, same data as holotype, B. Le Ru leg., TMSA, Pretoria.

Description (Fig. 6E–H)

Antennae short-bipectinate, serrated at apex in the male, filiform in the female, flagellum with ochraceous scales. Body colour and wing pattern similar in both sexes, but slightly darker in male. Palpus ochreous-buff irrorated with fuscous. Head and thorax ochreous-buff covered with long hairs. Abdomen cartridge-buff irrorated with fuscous. Legs ochreous, fore femur and tibia strongly infuscated on their inner surfaces. Forewings ochreous suffused with fuscous scales; an antemedial black spot below the cell; a longitudinal ochreous fascia from base along lower margin of cell, partly within, partly without cell limited to the first two-thirds of the wing and a postmedial row of spots. Fringe bright ochreous with a basal black line. Hindwings white, veins with cartridge-buff scales; fringe white iridescent. Underside of forewings pale cartridge-buff at the base, cartridge-buff on costa; apex and terminal area heavily suffused with fuscous, with cartridge-buff scales along the veins. Underside of hindwings white, costa and apex suffused with fuscous scales; fringe white iridescent with a basal cartridge-buff line.

Wingspan 32–34 mm (males) (n = 8); 35–44 mm (females) (n = 12).

Male (33, 34, 32, 33, 33, 34, 32, 32)

Female (36, 36, 43, 37, 44, 39, 36, 35, 37, 35, 38, 39)

Male genitalia (Fig. 7F,L) Uncus small, wide, narrowing sharply at about halfway. Tegumen with small peniculi. Vinculum with a large saccus, u-shaped at the bottom margin, w-shaped at the top margin with an indentation in the middle. Extension of sacculus wide, slightly curved inwards with several rows of short stout spines on the tip. Long slightly spatulated cucullus and long coastal spine bifid. Juxta lampshade-shaped with a long wide neck and shortly bifid. Aedeagus (Fig. 7l) short, slightly dilated basally and curved in the middle, presence of sclerotized elongate carina crest. *Female genitalia* (Fig. 8G) Corpus bursae elongate without signa; ductus bursae short, strongly sclerotized near the ostium; lateral plates of ostial segment medium sized, sclerotized and not rounded, less than 1.5 times longer than wide. Ovipositor lobes 2.5 times longer than wide.

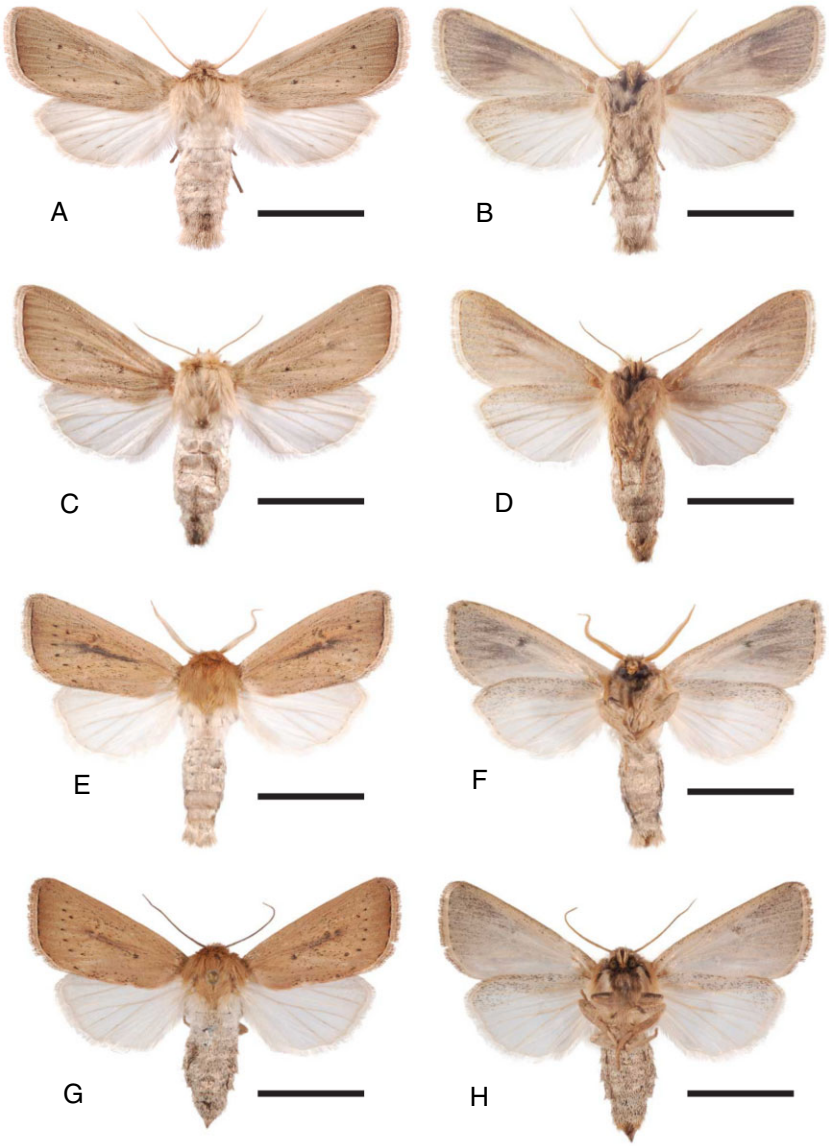

Figure 6. Adults of *Sesamia* species. A–D, *S. nonagrioides*: A, male upper side; B, male under side; C, female upper side; D, female under side. E–H, *S. typhae*: E, male upper side; F, male under side; G, female upper side; H, female under side. Scale bars = 10 mm.

Larvae L5 instar (Fig. 9F): length, 40–45 mm, breadth, 4.0 mm; head smooth, brown, prothoracic shield pink purple; body with ground colour pink purple, dorsally adorned with two lateral, narrow, bright buff stripes, pinnacula and caudal plate pale yellow brown. Young larvae are very similar in appearance to mature ones.

Etymology: epithet of *Typha*, the host plant genus name.

BIONOMICS

Sesamia typhae is a markedly hygrophilous species inhabiting lakesides, marshy and boggy areas, and stream valleys. Larvae were collected on stems of bulrush *Typha latifolia* L. (Typhaceae). Larvae are frequently gregarious (between five and 20 larvae) when young but always solitary when mature. Typically, in bulrush exhibiting signs of infestation by *S. typhae* larvae the central leaves turn yellow. Pupae were almost never found in stems, so it is likely that most mature larvae leave the stems for pupation.

DISTRIBUTION

South Africa, at altitude above 950 m a.s.l in Kwazulu-Natal, Gauteng, Mpumalanga, Free State,

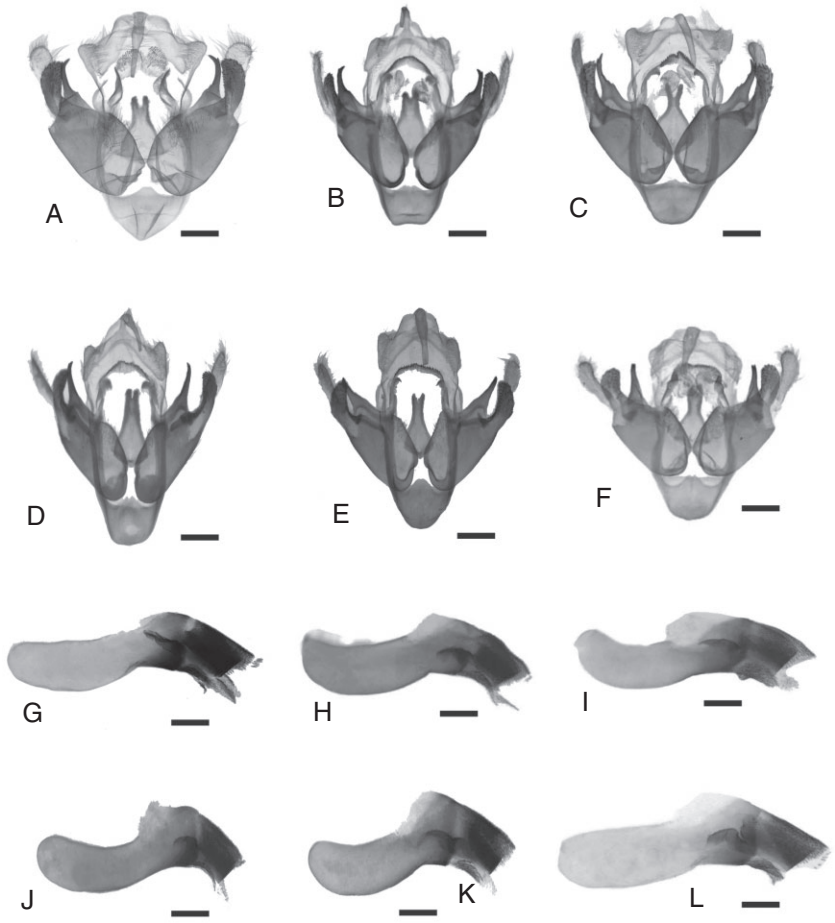

Figure 7. Male genitalia of *Sesamia* species. A, G, *S. capensis*; B, H, *S. congoensis*; C, I, *S. luyaensis*; D, J, *S. natalensis*; E, K, *S. nonagrioides*; F, L, *S. typhae*. Scale bars = 0.5 mm.

Northwest and Eastern part of Eastern Cape and North Cape provinces; moths were found in undifferentiated mountainous and woodland mosaics, transition from Afromontane scrub forest to Highveld grassland and from Karoo shrubland to Highveld and Highveld grassland mosaics ('Coastal mosaics' and 'Highveld grasslands'; White, 1983) belonging to the Southern African bioregion (Linder *et al*., 2012) (Fig. 10).

As reported above, all species of the *S. nonagrioides* group have uniform fore wings. It means identification by wing pattern alone is frequently not possible. To facilitate species identification, we generated two keys of identification of the *Sesamia nonagrioides* species group, one for male genitalia (A) based on two characters only (peniculi and juxta) and the second on female genitalia (B) based also on two characters only (lateral plates of the ostium and length-to-width ratio of valves).

Ecological preferences

Like *S. nonagrioides*, taxa whose biology is known (*S. capensis*, *S. congoensis*, *Sesamia luyaensis*, *S. natalensis* and *S. typhae*) exhibit marked ecological preferences for hygrophilous habitats; however, all new taxa are more specialized than *S. nonagrioides* and feed on a restricted number of host plants from fewer plant families (Table 1). Three of them (*S. capensis*, *S. natalensis* and *S. typhae*) are only distributed in Austral Africa and are therefore completely allopatric with *S. nonagrioides*.

Among the seven species of the group, *S. nonagrioides* presents the wider distributional range and is the most generalist species with 42 known host plants belonging to three families (Le Ru *et al*. 2006a,b; B. Le Ru, unpubl. data). It is also the only species of the group that consumes both C_3 and C_4 plants, although these differ in their nutritional quality and often require specific adaptations to maximize the efficiency of nutrient utilization (Caswell *et al*., 1973; Patterson, 1984; Barbehenn, Karowe & Chen, 2004). All six remaining species were much less generalist with two or three recorded host plants (for *S. capensis*, *S congoensis* and *S. natalensis*) or even specialists with only one recorded host plant (*S. luyaensis* and *S. typhae*). With the

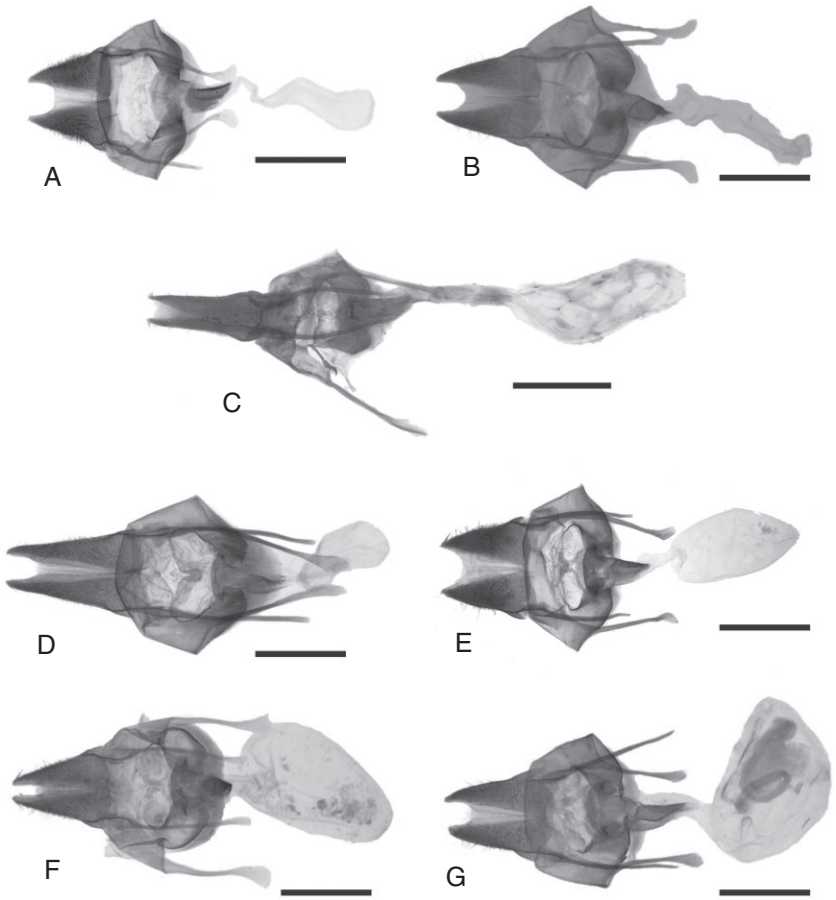

Figure 8. Female genitalia of *Sesamia* species. A, *S. capensis*; B, *S. congoensis*; C, *S. kouilouensis*; D, *S. luyaensis*; E, *S. natalensis*; F, *S. nonagrioides*;8G, *S. typhae.* Scale bars = 1 mm.

exception of *S. congoensis* found on Poaceae only, all other species of the group were found on Cyperaceae, or Typhaceae, or Typhaceae and Poaceae.

With the exception of *S. kouilouensis* (collected on a lake shore inhabited by *C. papyrus* and floating grasses) for which there is no information on host plant association, all six species of the *S. nonagrioides* group are markedly hygrophilous species found along banks of streams, rivers and marshes, a common ecology found in many species of Apameina (Zilli *et al*., 2005), the other major subtribe of Apameini. Nevertheless, this uniform habitat preference corresponds to very different distributions; in sub-Saharan Africa, the most generalist species *S. nonagrioides* has been recorded from five major bioregions (Congolian, Ethiopian, Somalian, Sudanian and Zambezian) as defined by Linder *et al*. (2012) when all the other species have a more restricted distribution range: the Congolian bioregion for *S. congoensis*, *S. kouilouensis* and *S. luyaensis*, the Southern African bioregion for *S. capensis* and *S. typhae*, and the Southern African and the Zambezian bioregion for *S. natalensis*.

DISCUSSION

SESAMIA NONAGRIOIDES SPECIES COMPLEX

This study reveals an unexpected diversity of species related to *S. nonagrioides*, one of the most studied noctuid stem borers in Africa and Europe. It confirms the current lack of knowledge regarding the diversity of the subtribe Sesamiina in sub-Saharan Africa, a consequence of less intensive collection effort in wild habitats both on plants and with light traps, and of the rather unclear systematics of the group. In the past, hundreds of *Sesamia* specimens related to *S. nonagrioides* have been collected and identified as *nonagrioides*. Nye (1960) recorded from Uganda and Kenya and bred out from *Cyperus papyrus*, a form of *S. nonagrioides* 'consistently chocolate brown in colour over the entire fore and hind wing'. Although none of these specimens was found in Sesamiina museum collections, this description could correspond to the newly described species *S. luyaensis*. In the same way, many '*S. nonagrioides*' specimens collected in Austral Africa and preserved in the Ditsong Museum (Pretoria)

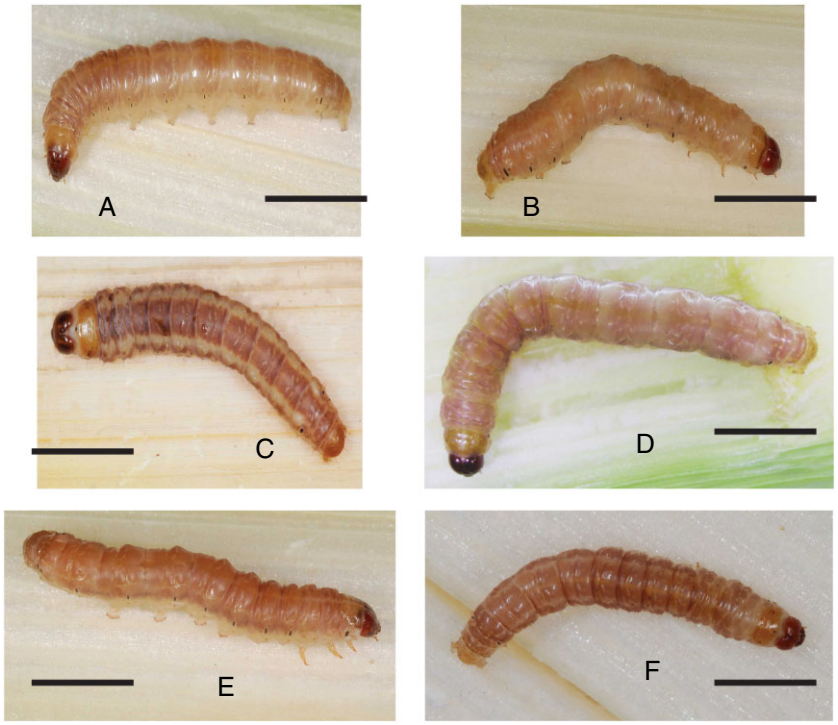

Figure 9. Last instar larvae of *Sesamia* species. A, *S. capensis*; B, *S congoensis*; C, *S. kouilouensis*; D, *S. natalensis*; E, *S. nonagrioides*; F, *S. typhae*. Scale bars = 15 mm.

KEY TO THE *SESAMIA NONAGRIOIDES* SPECIES GROUP BASED ON INTERNAL MORPHOLOGY OF THE ADULT MALES (A) AND FEMALES (B)

(A)

1. tegumen with large peniculi (Fig. 7A–E).....2
tegumen with small peniculi (Fig. 7F).....*typhae*
2. juxta with short neck (Fig. 7A–C).....3
juxta with long neck (Fig. 7D,E).....4
3. juxta with short wide neck, shortly bifid (Fig. 7B) Central Africa.....*congoensis*
juxta with short wide neck, shortly bifid (Fig. 7A) Austral Africa.....*capensis*
juxta with short narrow neck, longly bifid (Fig. 7C) Central Africa.....*luyaensis*
4. juxta with long narrow neck, shortly bifid (Fig. 7D); Austral Africa.....*natalensis*
juxta with long narrow neck, shortly bifid (Fig. 7E); Central, East and West Africa.....*nonagrioides*

(B)

1. valves more than 3 times longer than wide (Fig. 8C,D).....2
valves less than 3 times longer than wide (Fig. 8B,D,E,F).....3
2. valves 4 times longer than wide (Fig. 8C); lateral plates medium sized, rounded.....*kouilouensis*
valves less than 4 times longer than wide (Fig. 8C), lateral plates medium sized, not rounded.....*luyaensis*
3. lateral plates large (Fig. 8B,F).....4
lateral plates small or medium sized (Fig. 8A,E,G).....5
4. lateral plates rounded at the apex (Fig. 8B).....*congoensis*
lateral plates rounded from the bottom (Fig. 8F).....*nonagrioides*
5. lateral plates small (Fig. 8A).....*capensis*
lateral plates medium sized (Fig. 8E,G).....6
6. valves 2 times longer than wide (Fig. 8E).....*natalensis*
valves 2.5 times longer than wide (Fig. 8E).....*typhae*

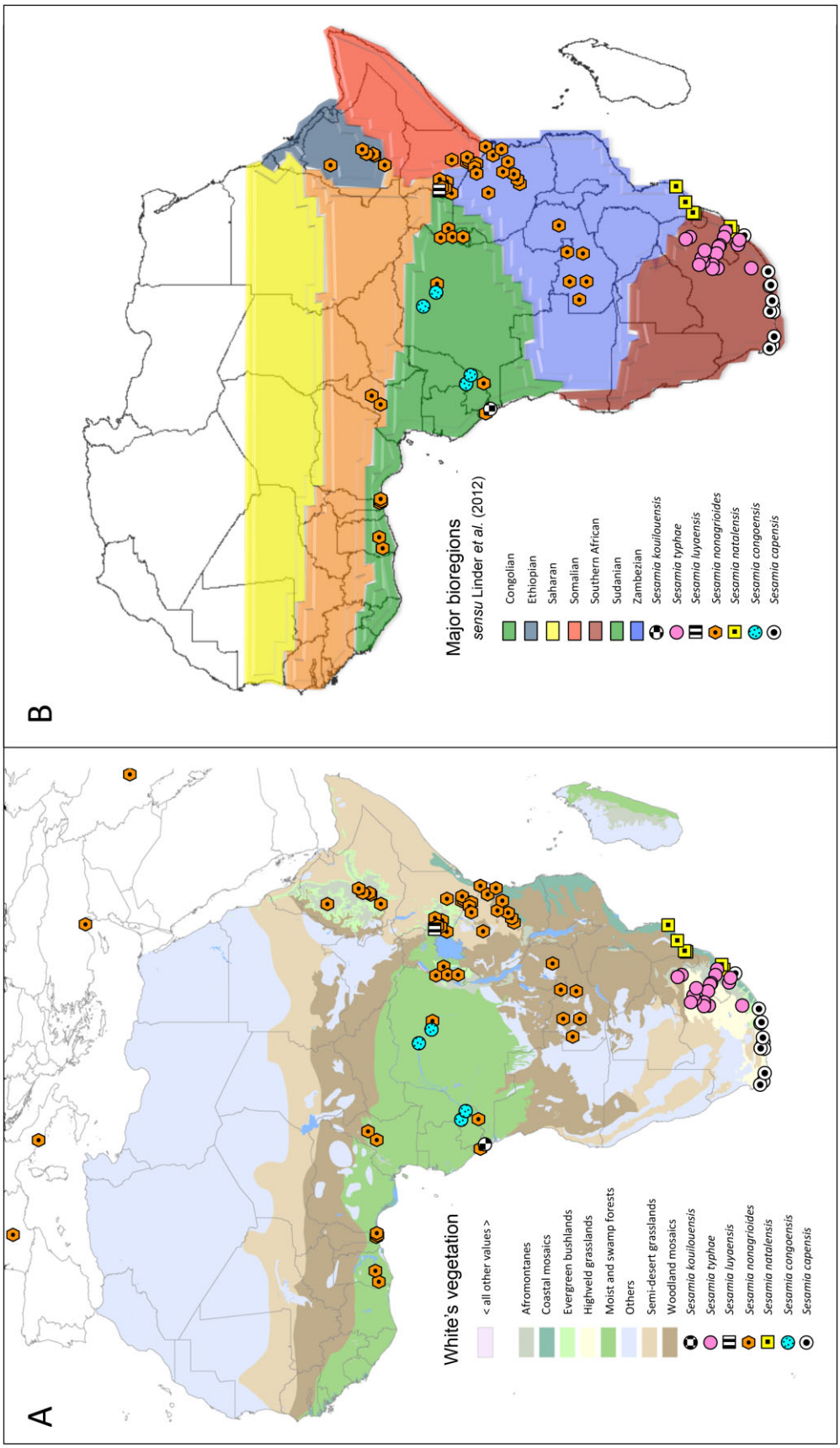

Figure 10. Distribution maps of sampled specimens from *Sesamia nonagrioides group* species. On the left (A) sampled localities are presented on a map that highlights major vegetation formation (White, 1983); on the right (B) sampled localities are presented on a map that highlights major bioregions (Linder *et al.*, 2012).

Table 1. Comparison of ecological preferences between *S. nonagrioides* and morphologically related *Sesamia* species; information on the photosynthetic pathway of host plants (C_3, C_4 or C_3/C_4 type) is also provided

Species	Geographical areas	Type of vegetation (*sensu* White, 1983)	Host-plant range	Host-plant family
S. nonagrioides	Central Africa, East Africa, Palearctic region, West Africa	Coastal mosaics, Evergreen bushlands, Moist and swamp forests, Semi-desert grasslands, Woodland mosaics	Polyphagous species; 42 known host plants (C_3 C_4 and C_3/C_4 plants)	Cyperaceae, Poaceae, Typhaceae
S. capensis	Austral Africa (south-west)	Coastal mosaics	Oligophagous species (C_3 and C_3/C_4 plants)	Poaceae, Typhaceae
S. congoensis	Central Africa	Moist and swamp forests	Oligophagous species (C_4 plants)	Poaceae
S. kouilouensis	Central Africa	Moist and swamp forests	Unknown	Unknown
S. luyaensis	East Africa	Moist and swamp forests	Only known from *Cyperus papyrus* (C_4 plant)	Cyperaceae
S. natalensis	Austral Africa (South East)	Coastal mosaics	Oligophagous species (C_3 and C_3/C_4 plants)	Poaceae, Typhaceae
S. typhae	Austral Africa	Coastal mosaics, Highveld grasslands	Only known from *Typha latifolia* (C_3 plant)	Typhaceae

probably belong to one or several of the new species (*S. capensis*, *S. natalensis* and *S. typhae*) described in this paper. The integrative taxonomic approach developed in this study, combining morphology, ecology and molecular species delimitation analyses, clearly supports the existence of seven species in the *S. nonagrioides* group, including six new species. With the exception of *S. kouilouensis* and *S. luyaensis*, easily separated from the remaining five species by their respective ochraceous hindwing and camel forewing, the remaining five species require the examination of genitalia from both sexes, by comparing the juxta morphology for males and the lateral plates of the ostial segment for females. Interestingly, with the exception of *S. luyaensis* restricted to western Kenya, the five other new species described in this study were all collected from countries where '*Sesamia nonagrioides*' had never been reported before: Democratic Republic of Congo, Republic of Congo for Central Africa and South Africa and Mozambique in Austral Africa.

Diversification of the *Sesamia nonagrioides* species complex

Until now, most studies on the biogeographical impact of major climatic changes during the Pleistocene at the continental scale in Africa were investigated on mammals (e.g. Lorenzen, Heller & Siegismund, 2012), birds (e.g. Fuchs, Crowe & Bowie, 2011) and more recently on one reptile (Barlow *et al*., 2013). Only two studies investigated this question on an arthropod, the maize stalk borer *Busseola fusca* (Sezonlin *et al*., 2006; Dupas *et al*., 2014). Our study based on a Pan-African data set provides a deeper understanding of these macroevolutionary processes from an arthropod point of view. We show that divergence events leading to the different clades of the *S. nonagrioides* group occurred recently between 1.24 and 4.33 Mya. Similar recent divergences have been reported in other genera of the subtribe, such as in *Manga* (Moyal & Le Ru, 2006), *Sciomesa* (Moyal *et al*., 2010), *Buakea* (Moyal *et al*., 2011a) and *Acrapex* (Le Ru *et al*., 2014). Diversification started about 4.33 Mya, followed by splits at 3.87 and 3.55 Mya, the latter date indicating a clear separation between species that are nowadays found in the Congolian bioregion (*S. congoensis*, *S. kouilouensis*, *S. luyaensis* and *S. nonagrioides*) and those found in the South African bioregion (*S. capensis*, *S. natalensis* and *S. typhae*). Another major split at 3.09 Mya (MRCA of *S. congoensis* and *S. nonagrioides*) distinguishes the lineages leading to *S. congoensis* and *S. nonagrioides*. The former became a strict forest species, inhabiting riverbanks of the Congolian basin when the latter probably became a more generalist species colonizing different bioregions in most of Central, East and West Africa. Following their separation from the other lineage of the group, the Southern African lineage progressively diversified with splits at 2.57 and 1.24 Mya. The

allopatric distribution of the resulting three South African species, respectively found along the West South African coastline, the East South African coastline and north-eastern regions of South Africa for *S. capensis*, *S. natalensis* and *S. typhae*, corresponds well to the locations of the South African puff adder refugia inferred from molecular analyses and species distribution models (Barlow *et al*., 2013). Despite the fact that southern Africa appears to have been an area of long-term climate stability over the last 3.5 Myr, with constant moisture levels (Maslin *et al*., 2012), it suggests that similar environmental changes in Austral Africa might be responsible for the distribution of both organisms. Following the view of Barlow *et al*. (2013) we also hypothesize that coastal areas and north-eastern regions worked as refugia during past glacial maxima whereas interior regions of South Africa became largely 'unsuitable' because of lower temperatures (Kulongoski, Hilton & Selaolo, 2004).

Evolutionary history and population structure of *S. nonagrioides*

Both phylogenetic and population genetic analyses provided interesting new insights into the evolutionary history of *S. nonagrioides*. Dating analyses suggest that this species originated during the early Pleistocene about 1.81 Mya (95% HPD 1.28–2.57). We hypothesize that *S. nonagrioides* originated from Central Africa because the highest level of genetic diversity was found for Central African specimens, despite the fact that their number and the number of Central African localities sampled is consistently lower than those from East Africa. Another line of evidence to support this hypothesis is the fact that *S. congoensis*, the sister species of *S. nonagrioides*, is exclusively distributed in Central Africa. Finally, a Central African origin is also consistent with the evolutionary history and population structure of *S. nonagrioides*, with one lineage dispersing westward, and the other eastward (Figs 1–3).

As already underlined in the study of Moyal *et al*. (2011c), the West African lineage appears to be genetically quite well differentiated from the remaining populations, despite the fact that geographical distances between the sampled localities in Benin (West Africa) and Cameroon (Central Africa) are less than those between the sampled localities in Cameroon and other Central African countries (Democratic Republic of Congo, Republic of Congo and Zambia) (Fig. 10). Overall, the West African lineage presents a very low proportion of shared haplotypes (none for the 16S, COI and Cytb fragments and only one for the 12S, 28S and EF1a fragments; Table S3). It is also found as sister to all other lineages under ML (Fig. 1), with high BV support (87%). Finally, when estimating the mean genetic distance (COI, Kimura two-parameter distance (K2P)) between specimens from West Africa and other individuals we recovered a value of 3.02%, which is compatible with expected distances between young species in Lepidoptera (Hebert *et al*., 2003; Hajibabaei *et al*., 2006; Hebert, deWaard & Landry, 2010). However, this hypothesis is not backed up by morphological comparisons of specimens. Both West African populations and other populations also do not appear to have differences in terms of host plant range or ecology. Finally, PTP species delimitation analyses group West African specimens with individuals from other geographical areas in the same putative species cluster. It is worth highlighting that the pattern recovered for *S. nonagrioides* is remarkably similar to the phylogeographical pattern inferred for another sub-Saharan noctuid pest, *Busseola fusca*. In *B. fusca* most of the genetic differentiation was also found between populations from West Africa and populations from Central and East Africa (Sezonlin *et al*., 2006; Dupas *et al*., 2014).

Regarding the Eastern lineage, a more complex phylogeographical pattern is recovered, with at least three East African populations and two East and Central African populations (Fig. 3). Similar complex phylogeographical patterns have been reported in East Africa for mammals (Lorenzen *et al*., 2012) and more recently for the maize stalk borer *B. fusca* (Dupas *et al*., 2014), suggesting the existence of a mosaic of refugia in the region. In fact, if the tectonic activity of this region fuelled a general aridification, it also engendered basins along the East African Rift Valley, probably to become large deep lakes during wet periods (Trauth *et al*., 2010). This is supported by sedimentary records of East African lake deposits suggesting at least eight late Cenozoic lake periods between 4.6 Mya and the present (Maslin *et al*., 2014). As currently recorded in many Rift Valley lakes (e.g. Turkana, Baringo, Bogoria, Elmenteita, Naivasha), lake shores are the most likely suitable habitats for *S. nonagrioides* host plants such as *Cyperus* spp., *Echinochloa* spp., *Typha* spp. *and Vossia* spp. At the same time, the great instability of these lakes could have contributed to the evolution of *S. nonagrioides* either by geographical isolation and/or shift to new host plant. These ephemeral lakes might have worked as a corridor facilitating the colonization of the Ethiopian region. Recent results obtained for *B. fusca* suggest that it may have achieved such dispersal (Dupas *et al*., 2014).

The Eastern lineage also gave rise to the Palearctic lineage, whose origin is estimated at 0.178 Mya (95% HPD: 0.076–0.306). For the latter our temporal framework is broadly consistent with the one inferred by Moyal *et al*. (2011c): in their study they relied on the standard evolution rate of 1.15% per Myr of Brower (1994) to estimate the origin of the Palearctic lineage at 0.108 Mya. Our results do not support their

scenario of multiple independent colonizations of the Palearctic region and instead support the hypothesis of a unique colonization of the Palearctic region from East Africa. During the Late Pleistocene the Arabian Peninsula probably acted as a major biogeographical bridge between East Africa and the Palearctic region when shifts in climatic conditions increased moisture levels in this area (Rosenberg *et al*., 2013). Our age estimate for the Palearctic lineage (0.178 Mya; 95% HPD: 0.076–0.306) is highly congruent with one of the known Late Pleistocene humid periods (*c*. 0.2 Mya; Rosenberg *et al*., 2013) in the Arabian Peninsula. Partial support for this hypothesis is also provided by the results of the BI phylogenetic analysis (Fig. 2) and the SplitsTree network (Fig. 3), which connect the Palearctic lineage with an East African lineage from Ethiopia. An alternative scenario might be related to the present Nile river, which could have played a role as a corridor for the expansion of *S. nonagrioides* during a wet and hot period (Moyal *et al*., 2011c).

Conclusion and perspectives

This study provides a more comprehensive picture of the phylogeography and systematics of the most widespread cereal stem borer, *S. nonagrioides*, found in sub-Saharan Africa and Palearctic region from Western Europe to Western Asia. Our study also highlights the importance of extensive sampling on wild host plants because it provides invaluable biological and ecological information essential when developing integrative taxonomy approaches on phytophagous insect species complexes. The combined use of morphology, ecology and molecular species delimitation analyses on an extensive data set of specimens collected in 17 countries reveals the existence of six unknown species related to *S. nonagrioides*. Despite the very low level of variation exhibited by some of the molecular markers, our integrative approach provides convincing evidence to support the status of the new species, in a way similar to other studies that have highlighted species complexes barely distinguishable with nuclear information alone (e.g. Talavera *et al*., 2013). The diversification of the *S. nonagrioides* group occurred recently between 1.24 and 4.33 Mya, probably in relation to major environmental changes during the Pleistocene. Molecular analyses indicate that *S. nonagrioides* is composed of multiple geographically well-structured populations and support the hypothesis of an eastern Africa origin (at around 0.178 Mya) for the Palearctic lineage. To better advance our understanding of the biogeographical history of the *S. nonagrioides* group, a comprehensive sampling of *Sesamia* species is required. This will allow us to test the hypothesis of a Central African origin for the group. Additional large-scale sample collections combined with species distribution modelling approaches (Guisan & Thuiller, 2005; Elith & Leathwick, 2009) could also help to define the climatic niches of species from the *S. nonagrioides* group, and understanding why *S. nonagrioides* apparently could not spread in Austral Africa.

ACKNOWLEDGEMENTS

We thank Peter Hayward and three anonymous reviewers for their constructive comments on a previous version of the manuscript. We are grateful to the Natural History Museum of London (M. Honey), to the Museo Civico di Storia Naturale (Dr Fabrizio Rigato) and to the Ditsong National Museum of Natural History (Dr M. Krüger) for permission to study and photograph the types and to the *Boyekoli Ebale Congo Expedition* organized in 2010 by a consortium of three Belgian institutions – the Royal Museum of Central Africa, the Royal Belgian Institute of Natural Sciences, and the National Botanical Garden of Belgium – in collaboration with the University of Kisangani (UNIKIS), in the Democratic Republic of Congo who allowed us to participate in the expedition and collect many interesting specimens. We thank Eric Kampan and Férial Kaoula for *S. nonagrioides* specimens collected in France and Gianandrea Salerno (University of Perugia) for *S. nonagrioides* specimens collected in Italy. Financial support was provided by the Institut de Recherche pour le Développement (IRD), by *icipe*, African Insect Science for Food and Health (Kenya) and by the department Santé des Plantes et Environnement of the Institut National de la Recherche Agronomique (INRA, SPE). Laboratory facilities were provided by the Centre de Biologie pour la Gestion des Populations in Montferrier/Lez (France), *icipe*, African Insect Science for Food and Health (Kenya) and the Laboratory Evolution Génomes et Spéciation of the Centre National de la Recherche Scientifique in Gif-Sur-Yvette (France). Part of the sequencing was also supported by the programme 'Bibliothèque du Vivant' (Project Noctuid Stem Borer Biodiversity; NSBB) supported by a joint CNRS, INRA and MNHN consortium. All specimens were collected under appropriate collection permits from the different countries surveyed and the authors delare no conflicts of interest.

REFERENCES

Aduse-Poku KA, Vingerhoedt E, Wahlberg N. 2009. Out-of-Africa again: a phylogenetic hypothesis of the genus *Charaxes* (Lepidoptera: Nymphalidae) based on five gene regions. *Molecular Phylogenetics and Evolution* **53:** 463–478.

Barbehenn RV, Karowe DN, Chen Z. 2004. Performance of a generalist grasshopper on a C_3 and a C_4 grass:

compensation for the effects of elevated CO_2 on plant nutritional quality. *Oecologia* **140:** 96–103.

Barlow A, Baker K, Hendry CR, Peppin L, Phelps T, Tolley KA, Wüster CE, Wüster W. 2013. Phylogeography of the widespread African puff adder (*Bitis arietans*) reveals multiple Pleistocene refugia in southern Africa. *Molecular Ecology* **22:** 1134–1157.

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences of the United States of America* **91:** 6491–6495.

Bryja J, Granjon L, Dobigny G, Patzenhauerova H, Konecny A, Duplantier JM, Gauthier P, Colyn M, Durnez L, Lalis A, Nicolas V. 2010. Plio-Pleistocene history of West African Sudanian savanna and the phylogeography of the *Praomys daltoni* complex (Rodentia): the environment/geography/genetic interplay. *Molecular Ecology* **19:** 4783–4799.

Caswell H, Reed F, Stephenson SN, Werner PA. 1973. Photosynthetic pathways and selective herbivory: a hypothesis. *The American Naturalist* **107:** 465–480.

Cordero A, Malvar MA, Butrón A, Revilla P, Velasco P, Ordás A. 1998. Population dynamics and life-cycle of corn borers in south Atlantic European coast. *Maydica* **43:** 5–12.

Damm S, Schierwater B, Hadrys H. 2010. An integrative approach to species discovery in odonates: from character-based DNA barcoding to ecology. *Molecular Ecology* **19:** 3881–3893.

Dayrat B. 2005. Toward integrative taxonomy. *Biological Journal of the Linnean Society* **85:** 407–415.

Demos TC, Peterhans JCK, Agwanda B, Hickerson MJ. 2014. Uncovering cryptic diversity and refugial persistence among small mammal lineages across the Eastern Afromontane biodiversity hotspot. *Molecular Phylogenetics and Evolution* **71:** 41–54.

Dobigny G, Tatard C, Gauthier P, Ba K, Duplantier JM, Granjon L, Kergoat GJ. 2013. Mitochondrial and nuclear genes-based phylogeography of *Arvicanthis niloticus* (Murinae) and sub-Saharan open habitats Pleistocene history. *PLoS ONE* **8:** e77815.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4:** e88.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Dupas S, Le Ru BP, Branca A, Faure N, Gigot G, Campagne P, Sezonlin M, Ndemah R, Ong'amo G, Calatayud PA, Silvain JF. 2014. Phylogeography in continuous space: coupling species distribution models and circuit theory to assess the effect of contiguous migration at different climatic periods on genetic differentiation in *Busseola fusca* (Lepidoptera: Noctuidae). *Molecular Ecology* **23:** 2313–2325.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acid Research* **35:** 1792–1797.

Eizaguirre M, Fantinou AA. 2012. Abundance of *Sesamia nonagrioides* (Lef.) (Lepidoptera: Noctuidae) on the edges of the Mediterranean Basin. *Psyche* **2012:** 854045.

Elith J, Leathwick JR. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology Evolution and Systematics* **40:** 677–697.

Erixon P, Svennblad B, Britton T, Oxelman B. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology* **52:** 665–673.

Fjeldsa J, Lovett JC. 1997. Geographic patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. *Biodiversity and Conservation* **6:** 325–346.

Flagstad O, Syvertsen PO, Stenseth NC, Jakobsen KS. 2001. Environmental change and rates of evolution: the phylogeographic pattern within the hartebeest complex as related to climatic variation. *Proceedings of the Royal Society B: Biological Sciences* **268:** 667–677.

Fuchs J, Crowe TM, Bowie RCK. 2011. Phylogeography of the Fiscal Shrike (*Lanius collaris*): a novel pattern of genetic structure across the arid zones and savannas of Africa. *Journal of Biogeography* **38:** 2210–2222.

Gebiola M, Gomez-Zurita J, Monti MM, Navones P, Bernardo U. 2012. Integration of molecular, ecological, morphological and endosymbionts data for species delimitation within the *Pnigalio soemius* complex (Hymenoptera: Eupholidae). *Molecular Ecology* **21:** 1190–1208.

Godfray HCJ. 2002. Challenges for taxonomy. *Nature* **417:** 17–19.

Guisan A, Thuiller W. 2005. Predicting species distribution: offering more than simple habitat models. *Ecology Letters* **8:** 993–1009.

Guschanski K, Krause J, Sawyer S, Valente LM, Bailey S, Finstermeier K, Sabin R, Gilissen E, Sonet G, Nagy ZT, Lenglet G, Mayer F, Savolainen V. 2013. Next-generation museomics disentangles one of the largest primate radiations. *Systematic Biology* **62:** 539–554.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* **103:** 968–971.

Hamilton CA, Formanowicz DR, Bond JE. 2011. Species delimitation and phylogeography of *Aphonopelma hentzi* (Araneae, Mygalomorphae, Theraphosidae): cryptic diversity in North American tarantulas. *PLoS ONE* **6:** e26207.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B, Biological Sciences* **270:** 313–321.

Hebert PDN, deWaard JR, Landry JF. 2010. DNA barcodes for 1/1000 of the animal kingdom. *Biology Letters* **6:** 59–362.

Heled J, Drummond AJ. 2010. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* **27:** 570–580.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42:** 182–192.

Hudson RR. 2000. A new statistic for detecting genetic differentiation. *Genetics* **155:** 2011–2014.

Hudson RR, Boos DD, Kaplan NL. 1992. A statistical test for detecting geographic subdivision. *Molecular Biology and Evolution* **9:** 138–151.

Huson DH, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* **23:** 254–267.

Kergoat GJ, Prowell DP, Le Ru BP, Mitchell A, Dumas P, Clamens AL, Condamine FL, Silvain JF. 2012. Disentangling dispersal and vicariance patterns in armyworms: evolution and historical biogeography of the pest genus *Spodoptera* (Lepidoptera: Noctuidae). *Molecular Phylogenetics and Evolution* **65:** 855–870.

Kfir R, Overholt WA, Khan ZR, Polaszek A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology* **47:** 701–731.

Kulongoski JT, Hilton DR, Selaolo ET. 2004. Climate variability in the Botswana Kalahari from the late Pleistocene to the present day. *Geophysical Research Letters* **31:** L10204.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29:** 1695–1701.

Le Ru B, Ong'amo GO, Moyal P, Ngala L, Musyoka B, Abdullah Z, Cugala D, Defabachew B, Haile TA, Kauma Matama T, Lada VY, Negassi B, Pallangyo K, Ravololonandrianina J, Sidumo A, Omwega C, Schulthess F, Calatayud PA, Silvain JF. 2006b. Diversity of lepidopteran stem borers on monocotyledonous plants in eastern Africa and the islands of Madagascar and Zanzibar revisited. *Bulletin of Entomological Research* **96:** 555–563.

Le Ru BP, Capdevielle-Dulac C, Toussaint EFA, Conlong D, Van den Berg J, Pallangyo B, Ong'amo G, Chipabika G, Molo R, Overholt WA, Cuda JP, Kergoat GJ. 2014. Integrative taxonomy of *Acrapex* stem borers (Lepidoptera, Noctuidae, Apameini): combining morphology and Poisson Tree Process analyses. *Invertebrate Systematics* **28:** 451–475.

Le Ru BP, Ong'amo GO, Moyal P, Muchungu E, Ngala L, Musyoka B, Abdullah Z, Kauma Matama T, Lada VY, Pallangyo K, Sidumo A, Omwega C, Schulthess F, Calatayud PA, Silvain JF. 2006a. Major ecological characteristics of East African noctuid stem borers. *Annales de la Société Entomologique de France* **42:** 353–362.

Lefèbvre A. 1827. Cossus nonagrioides. *Annales de la Société Linnéenne de Paris* **6:** 98.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25:** 1451–1452.

Linder HP, de Klerk HM, Born J, Burgess ND, Fjeldsa J, Rahbek C. 2012. The partitioning of Africa: statistically defined biogeographical regions in sub-Saharan Africa. *Journal of Biogeography* **39:** 1189–1205.

Lorenzen ED, Heller R, Siegismund HR. 2012. Comparative phylogeography of African savannah ungulates. *Molecular Ecology* **21:** 3656–3670.

Lumley LM, Sperling FAH. 2010. Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (*Choristoneura fumiferana*) cryptic species complex (Lepidoptera: Tortricidae). *Systematic Entomology* **35:** 416–428.

Maslin MA, Brierley CM, Milner AM, Shultz S, Trauth MH, Wilson KE. 2014. East African climate pulses and early human evolution. *Quaternary Science Reviews* **101:** 1–17.

Maslin MA, Pancost R, Wilson KE, Lewis J, Trauth MH. 2012. Three and half million year history of moisture availability of South West Africa: evidence from ODP site 1085 biomarker records. *Palaeogeography Palaeoclimatology Palaeoecology* **317–318:** 41–47.

McLeisch MJ, Van Noort S, Tolley KA. 2010. Parasitoid figwasp evolutionary diversification and variation in ecological opportunity. *Molecular Ecology* **19:** 1483–1496.

deMenocal PB. 1995. Plio-Pleistocene African climate. *Science* **270:** 53–59.

deMenocal PB. 2004. African climate change and faunal evolution during the Pliocene–Pleistocene. *Earth and Planetary Science Letters* **220:** 3–24.

Morales-Hojas R, Vieira CP, Vieira J. 2008. Inferring the evolutionary history of *Drosophila americana* and *Drosophila novamexicana* using a multilocus approach and the influence of chromosomal rearrangements in single gene analyses. *Molecular Ecology* **17:** 2910–2926.

Moyal P, Le Ru B. 2006. From population to species: morphological and molecular diversity in east African stem borer species of the genus *Manga* Bowden (Lepidoptera: Noctuidae). *Annales de la Société Entomologique de France* **42:** 293–307.

Moyal P, Le Ru B, Conlong D, Cugala D, Defabachew B, Matama-Kauma T, Pallangyo B, Van den Berg J. 2010. Systematics and molecular phylogeny of two African stem borer genera, *Sciomesa* Tams & Bowden and *Carelis* Bowden (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* **100:** 641–659.

Moyal P, Le Ru B, Conlong D, Cugala D, Defabachew B, Matama-Kauma T, Pallangyo B, Van den Berg J. 2011a. *Buakea* (Lepidoptera: Noctuidae), a new genus of African stem borers: morphological, ecological and molecular data. *African Entomology* **19:** 23–35.

Moyal P, Le Ru B, Van den Berg J, Ratnadass A, Cugala D, Matama-Kauma T, Pallangyo B, Conlong D, Defabachew B. 2011b. Morphological reinforcement, ancient introgressive hybridization and species delimitation in African stem-borer species of the genus *Sesamia* Guenée (Lepidoptera: Noctuidae). *Systematic Entomology* **36:** 421–434.

Moyal P, Tokro P, Bayram A, Savopoulou-Soultani M, Conti E, Eizaguirre M, Le Rü B, Avand-Faghih A, Frérot B, Andreadis S. 2011c. Origin and taxonomic status of the Palearctic population of the stem borer *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae). *Biological Journal of the Linnean Society* **103:** 904–922.

Nicolas V, Missoup AD, Colyn M, Cruaud C, Denys C. 2012. West-Central African Pleistocene lowland forest evolution revealed by the phylogeography of Misonne's soft-furred mouse. *African Zoology* **47:** 100–112.

Nye IWB. 1960. *The insect pests of graminaceous crops in East Africa*. London: Her Majesty's Stationery Office.

Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL. 2004. Bayesian phylogenetic analysis of combined data. *Systematic Biology* **53:** 47–67.

Onyango FO, Ochieng'Odero JPR. 1994. Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis et Applicata* **73:** 139–144.

Padial JM, Miralles A, De la Riva I, Vences M. 2010. The integrative future of taxonomy. *Frontiers in Zoology* **7:** 16.

Padial JM, de La Riva I. 2009. Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura: Strabomantidae). *Zoological Journal of the Linnean Society* **155:** 97–122.

Patterson BD. 1984. Correlation between mandibular morphology and specific diet of some desert grassland Acrididae (Orthoptera). *The American Midland Naturalist* **111:** 296–303.

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53:** 793–808.

Riedel A, Sagata K, Suhardjono YR, Tänzler R, Balke M. 2013. Integrative taxonomy on the fast track – towards more sustainability in biodiversity research. *Frontiers in Zoology* **10:** 15.

Rosenberg TM, Preusser F, Risberg J, Plikk A, Kadi KA, Matter A, Fleitmann D. 2013. Middle and Late Pleistocene humid periods recorded in palaeolake deposits of the Nafud desert, Saudi Arabia. *Quaternary Science Reviews* **70:** 109–123.

Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. 2010. Integrative taxonomy: a multisource approach to exploring Biodiversity. *Annual Review of Entomology* **55:** 421–438.

Scholtz CH, Mansell MW. 2009. Insect biodiversity in the Afrotropical region. In: Foottit RG, Adler PH, eds. *Insect biodiversity, science and society*. Oxford: Blackwell Publishing Ltd, 69–82.

Sepulchre P, Ramstein G, Fluteau F, Schuster M, Tiercelin JJ, Brunet M. 2006. Tectonic uplift and Eastern Africa aridification. *Science* **313:** 1419–1423.

Sezonlin M, Dupas S, Le Ru B, Le Gall P, Moyal P, Calatayud PA, Giffard I, Faure N, Silvain JF. 2006. Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (Lepidoptera, Noctuidae) in sub-Saharan Africa. *Molecular Ecology* **15:** 407–420.

Stamatakis A. 2006. RaxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Tajima F. 1989. Statistical-method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123:** 585–595.

Talavera G, Lukhtanov VA, Rieppel L, Pierce NE, Vila R. 2013. In the shadow of phylogenetic uncertainty: the recent diversification of *Lysandra* butterflies through chromosomal change. *Molecular Phylogenetics and Evolution* **69:** 469–478.

Tams WHT, Bowden J. 1953. A revision of the African species of *Sesamia* Guenée and related genera (Agrotidae-Lepidoptera). *Bulletin of Entomological Research* **43:** 645–679.

Tang CQ, Humphreys AM, Fontaneto D, Barraclough TG. 2014. Effects of phylogenetic reconstruction on the robustness of species delimitation methods using single-locus data. *Methods in Ecology and Evolution* **5:** 1086–1094.

Toussaint EFA, Condamine FL, Kergoat GJ, Silvain JF, Capdevielle-Dulac C, Barbut J, Le Ru BP. 2012. Palaeoenvironmental shifts drove the adaptive radiation of a noctuid stemborer tribe (Lepidoptera, Noctuidae, Apameini) in the Miocene. *PLoS One* **7:** e41377.

Trauth MH, Larrasoan?a JC, Mudelsee M. 2009. Trends, rhythms and events in Plio-Pleistocene African climate. *Quaternary Science Reviews* **28:** 399–411.

Trauth MH, Maslin MA, Bergner AGN, Deino AL, Junginger A, Odada E, Olago DO, Olaka L, Strecker MR. 2010. Human evolution and migration in a variable environment: the amplifier lakes of east Africa. *Quaternary Science Reviews* **29:** 2981–2988.

White F. 1983. The vegetation of Africa, a descriptive memoir to accompany the UNESCO/AETFAT/UNSO vegetation map of Africa. *UNESCO, Natural Resources Research* **20:** 1–356.

Wiens JJ. 2007. Species delimitation: new approaches for discovering diversity. *Systematic Biology* **56:** 875–878.

Will KW, Mishler BD, Wheeler QD. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* **54:** 844–851.

Wilson EO. 2004. Taxonomy as a fundamental discipline. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359:** 739.

Wüster W, Crookes S, Ineich I, Mane Y, Pook CE, Trape JF, Broadley DG. 2007. The phylogeny of cobras inferred from mitochondrial DNA sequences: evolution of venom spitting and the phylogeography of the African spitting cobras (Serpentes: Elapidae: *Naja nigricollis* complex). *Molecular Phylogenetics and Evolution* **45:** 437–453.

Zhang J, Kapli P, Pavlidis P, Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **22:** 2869–2876.

Zilli A, Ronkay L, Fibiger M. 2005. *Apameini. Noctuidae Europaea, Vol. 8*. Sorø: Entomological Press.

Zimkus BM, Rödel MO, Hillers A. 2010. Complex patterns of continental speciation: molecular phylogenetics and biogeography of sub-Saharan puddle frogs (*Phrynobatrachus*). *Molecular Phylogenetics and Evolution* **55:** 883–900.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. ML trees resulting from the separate analyses of the COI, Cytb and 12S datasets (support of major nodes is provided by BV). Individuals belonging to the six new species are highlighted using different colour frames.

Figure S2. ML trees resulting from the separate analyses of the 16S, EF1a and 28S datasets (support of major nodes is provided by BV). Individuals belonging to the six new species are highlighted using different colour frames. Results of the PTP analysis are provided using coloured branches. Putative molecular species are indicated using transitions between blue-coloured branches and red-coloured branches. On the right, brackets are used to distinguish the seven species of the *S. nonagrioides* group (names of species are provided along with the number of sequenced individuals). For each individual of the *S. nonagrioides* group coloured ellipses and rectangles are used to indicate host plant information and the biogeographical region of origin, respectively.

Table S1. Taxon sampling (sequenced material). Information on host-plant taxonomy is provided under brackets for each host plant using the following abbreviations: Cyperaceae (C); Poaceae: Arundinoideae (P/Aru); Poaceae: Panicoideae (P/Pan); Typhaceae (T). Information on the photosynthetic pathway of host plants (C_3, C_4 or C_3/C_4 type) is also provided. For some countries we used the following abbreviations: Democratic Republic of Congo (DRC); Republic of Congo (R. of Congo).

Table S2. Model and subset selection based on the AICc. Each column provides information on the way models were approximated under BEAST and RAxML.

Table S3. Results of genetic differentiation analyses for each gene fragment, with groups based on geographical origins of individuals. The following abbreviations were used: Central Africa (C-Afr.), East Africa (E-Afr.), Palearctic region (Pal.), West Africa (W-Afr.); non-significant (ns); significant at $P < 0.01$ (*); significant at $P < 0.001$ (**); significant at $P < 0.0001$ (***).

Table S4. Results of genetic differentiation analyses for each gene fragment, with groups based on rearing host-plant information. The following abbreviations were used: Cyperaceae (C); Poaceae (P); Typhaceae (T); non-significant (ns); significant at $P < 0.01$ (*); significant at $P < 0.001$ (**); significant at $P < 0.0001$ (***).

Table S5. Results of genetic diversity analyses for each gene fragment, with detailed information for the groups that have been defined based on geographical origin or rearing information. For each gene the number of retained sites is indicated in brackets. For each group the corresponding number of individuals is provided in brackets. The following abbreviations were used: number of segregating sites (S); number of haplotypes (h); haplotypic diversity (Hd); nucleotide diversity (π); Tajima's D test value (D); non-significant (ns); significant at $P < 0.01$ (*); significant at $P < 0.001$ (**).