Molecular Phylogenetics and Evolution 161 (2021) 107161

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

A novel reference dated phylogeny for the genus *Spodoptera* Guenée (Lepidoptera: Noctuidae: Noctuinae): new insights into the evolution of a pest-rich genus

Gael J. Kergoat [a,*], Paul Z. Goldstein [b], Bruno Le Ru [c,d], Robert L. Meagher Jr. [e], Alberto Zilli [f], Andrew Mitchell [g], Anne-Laure Clamens [a], Sylvie Gimenez [h], Jérôme Barbut [i], Nicolas Nègre [h], Emmanuelle d'Alençon [h], Kiwoong Nam [h,*]

[a] CBGP, INRAE, IRD, CIRAD, Institut Agro, Univ Montpellier, Montpellier, France
[b] Systematic Entomology Laboratory, USDA, Smithsonian Institution, National Museum of Natural History, PO Box 37012, Washington DC, USA
[c] Unité de Recherche UMR 247, African Insect Science for Food and Health (icipe), PO Box 30772 – Nairobi, Kenya
[d] IRD/CNRS, UMR IRD 247 EGCE, Laboratoire Evolution Génomes Comportement et Ecologie, Avenue de la terrasse, BP1, 91198, Gif-sur-Yvette, France and Université Paris-Sud 11, 91405 Orsay, France
[e] United States Department of Agriculture, Agricultural Research Service, Gainesville, Florida, USA
[f] Natural History Museum, Life Sciences, DC2-2N, Cromwell Road, SW7 5BD, London, UK
[g] Australian Museum Research Institute, 1 William Street, Sydney, NSW 2010, Australia
[h] DGIMI, Univ Montpellier, INRAE, Montpellier, France
[i] Institut de Systématique, Evolution, Biodiversité, Muséum national d'Histoire naturelle, Direction des collections, Paris cedex 05, France

ARTICLE INFO

Keywords:
Armyworm
Insect pest
Molecular dating
Multiple species coalescent
Phylogenetics
Spodoptera

ABSTRACT

The noctuid genus *Spodoptera* currently consists of 31 species with varied host plant breadths, ranging from monophagous and oligophagous non-pest species to polyphagous pests of economic importance. Several of these pest species have become major invaders, colonizing multiple continents outside their native range. Such is the case of the infamous fall armyworm, *Spodoptera frugiperda* (J.E. Smith), which includes two recognized host strains that have not been treated as separate species. Following its accidental introduction to Africa in 2016, it quickly spread through Africa and Asia to Australia. Given that half the described *Spodoptera* species cause major crop losses, comparative genomics studies of several *Spodoptera* species have highlighted major adaptive changes in genetic architecture, possibly relating to their pest status. Several recent population genomics studies conducted on two species enable a more refined understanding of their population structures, migration patterns and invasion processes. Despite growing interest in the genus, the taxonomic status of several *Spodoptera* species remains unstable and evolutionary studies suffer from the absence of a robust and comprehensive dated phylogenetic framework. We generated mitogenomic data for 14 *Spodoptera* taxa, which are combined with data from 15 noctuoid outgroups to generate a resolved mitogenomic backbone phylogeny using both concatenation and multi-species coalescent approaches. We combine this backbone with additional mitochondrial and nuclear data to improve our understanding of the evolutionary history of the genus. We also carry out comprehensive dating analyses, which implement three distinct calibration strategies based on either primary or secondary fossil calibrations. Our results provide an updated phylogenetic framework for 28 *Spodoptera* species, identifying two well-supported ecologically diverse clades that are recovered for the first time. Well-studied larvae in each of these clades are characterized by differences in mandibular shape, with one clade's being more specialized on silica-rich C_4 grasses. Interestingly, the inferred timeframe for the genus suggests an earlier origin than previously thought for the genus: about 17-18 million years ago.

* Corresponding authors.
E-mail addresses: gael.kergoat@inrae.fr (G.J. Kergoat), ki-woong.nam@inrae.fr (K. Nam).

https://doi.org/10.1016/j.ympev.2021.107161
Received 20 October 2020; Received in revised form 16 March 2021; Accepted 23 March 2021
Available online 29 March 2021

1. Introduction

With over 42,407 described species and 3,772 genera distributed among six families (Goldstein 2017), the Noctuoidea are the most diverse superfamily of Lepidoptera, accounting for more than a quarter of the known diversity of Lepidoptera. The group is known for encompassing more than one thousand agricultural species of economic importance (Goldstein 2017) that are mostly found in a large clade of Noctuidae, coined the 'pest clade' (Mitchell et al. 2006). Bollworms in the genus *Helicoverpa* Hardwick have long been considered the most widespread and problematic of these pests, but are now rivalled by *Spodoptera* armyworms. Specifically, the recent invasion of the polyphagous fall armyworm (FAW), *Spodoptera frugiperda*, through Africa, Asia and Australia has put the genus and its role as a group of major crop pests in the spotlight. The FAW comprises two recognized ecological strains ('corn strain' and 'rice strain': Pashley 1986; Prowell et al. 2004; Meagher et al. 2004; Nagoshi et al. 2020; also referred to as 'corn form' and 'rice form' by Juárez et al. 2014), which may correspond to sibling species as evidenced by a high level of genetic differentiation (Dumas et al. 2015a; Gouin et al. 2017; Le Ru et al. 2018) and both pre-zygotic (Pashley and Martin 1987; Pashley et al. 1992; Schöfl et al. 2011) and post-zygotic (Quisenberry 1991; Velásquez-Vélez et al. 2011; Dumas et al. 2015b) reproductive isolation mechanisms (see also the review of Groot et al. 2010 and the detailed thesis of Hänniger 2015). However, they have proven difficult to differentiate sufficiently, whether morphologically or by other means, to warrant nomenclatural change. In its native range in the Western hemisphere the FAW is well-known for its migratory habits (Nagoshi et al. 2012a, 2012b) and occasional outbreaks (Sparks 1979). Since their accidental introduction in West Africa in early 2016 (Goergen et al. 2016), the two FAW strains quickly spread from Africa to Asia, becoming established in India in 2018 and China in 2019, and in Australia in February 2020 (Nagoshi et al. 2017, 2019; Tay et al. 2020; Yainna et al. 2020; Zhang et al. 2020), causing billions of dollars in damage (Abrahams et al. 2017; FAO 2020). With more than 353 known host plants belonging to 76 plant families (Montezano et al. 2018), the FAW is likely one of the most polyphagous species of *Spodoptera*. The comprehensive revision of the genus by Pogue (2002) lists 30 species, of which half are polyphagous pests of economic importance (see also van der Gaag and van der Straten 2017). In addition to the FAW, pest species of note include the southern armyworm *S. eridania* (Stoll), the African armyworm *S. exempta* (Walker), the beet armyworm *S. exigua* (Hübner), the African cotton leafworm *S. littoralis* (Boisduval), the tobacco cutworm *S. litura* (Fabricius) and the lawn armyworm *S. mauritia* (Boisduval). There is also evidence that some of these are expanding their natural range due to the ongoing global climate change; such is the case of the grasslawn armyworm *S. cilium* Guenée, which in Europe was previously known only from Mediterranean countries and which has increased in abundance in more northern and temperate areas, with recent records reported as far north as the English coast (Sparks et al. 2007). Non-pest *Spodoptera* species are considered monophagous or oligophagous (Pogue 2002), but this may simply reflect our limited understanding of the ecology of species without obvious economic importance. *Spodoptera* species are referred to as armyworms because of the well-known gregarious behavior of larvae of several outbreak species, which sometimes aggregate in high densities as they travel from one feeding source to another. Not all *Spodoptera* species exhibit this behavior, and similar behaviors are also found in unrelated noctuid genera (Brown and Dewhurst 1975) also referred to as armyworms, particularly within Mythimnini (*sensu* Hacker et al. 2002). *Spodoptera* species typically present disjunct geographic distributions associated exclusively with either the Western or the Eastern Hemisphere (Brown and Dewhurst 1975; Todd and Poole 1980; Pogue 2002). In addition to the FAW, at least two species have expanded their ranges across multiple continents and hemispheres. One of these is the beet armyworm *S. exigua*, which colonized the Western Hemisphere as a result of an accidental introduction in North America in 1876 (Wilson 1932). More recently, in 2016 the southern armyworm *S. eridania,* which is native to the Americas, was discovered in four countries of West and Central Africa (Goergen 2018), where it now appears to be well-established as evidenced by recurring outbreaks.

The taxonomic position of *Spodoptera* within the Noctuidae has long been unstable. The genus was first assigned to the subfamily Acronictinae by Hampson (1909), and over the years assigned either to the subfamily Amphipyrinae (in tribe Amphipyrini) or to the former subfamily Xyleninae (now tribe Xylenini of the Noctuinae). Not only has the tribal/subfamily placement of *Spodoptera* shifted, but also the positions of the tribes themselves. In the most recent taxonomic treatments the genus assigned to the tribe Prodeniini of the Noctuinae (*e.g.,* see the catalogue of Lafontaine and Schmidt 2010), a placement supported by molecular phylogenetics studies (*e.g.,* Mitchell et al. 2006; Kawahara et al. 2019). Since Pogue's (2002) revision, one species, *Leucochlaena hipparis* (Druce), was transferred back to *Spodoptera* (Pogue 2011; see also Todd and Poole 1980; Pogue 2002) after having been removed, and another (*S. teferii* Laporte *in* Rougeot) resurrected from synonymy (Le Ru et al. 2018) on the basis of both morphological and molecular evidence. In addition, *S. marima* (Schaus) was recently synonymized with *S. ornithogalli* (Guenée) by Brito et al. (2019), also based on a combination of morphological and molecular data. This puts the number of valid *Spodoptera* species at 31 (Table 1), a number that will likely increase given the results of several recent molecular studies (Kergoat et al. 2012; Dumas et al. 2015a; Le Ru et al. 2018) which suggest the existence of at least three potential new *Spodoptera* species. In the case of the FAW, morphological evidence remains lacking (Nagoshi et al. 2020) and the occurrence of hybrids in the field indicates that both strains may represent incipient stages of ecological speciation (Groot et al. 2010). Additional uncertainty surrounds the status of Australasian *S. exigua* populations (also known as *S. exigua antipodea* (Warren)) and the subspecies *S. mauritia acronyctoides* Guenée.

Our understanding of the evolution and diversification of the genus has benefited from a wealth of recent comparative genomic studies. Among the more significant findings are suggestions that major expansions of several gene families may account for the polyphagous nature of *S. frugiperda*, *S. litura*, and *S. exigua* (see Gouin et al. 2017; Cheng et al. 2017; Zhang et al. 2019, respectively). Population genomic studies pinpointed adaptive changes and migratory ability in *S. litura* (Cheng

Table 1
Checklist of currently recognized *Spodoptera* species. Species native to the Western hemisphere are listed in the left column while species native to the Eastern hemisphere are listed in the right column. Pest species are highlighted using asterisks.

Western hemisphere species	Eastern hemisphere species
Spodoptera albula (Walker, 1857)*	*Spodoptera apertura* (Walker, 1865)
Spodoptera androgea (Stoll *in* Cramer, 1782)	*Spodoptera cilium* Guenée, 1852*
Spodoptera compta (Walker, 1869)	*Spodoptera depravata* (Butler, 1879)*
Spodoptera cosmioides (Walker, 1858)*	*Spodoptera exempta* (Walker, 1857)*
Spodoptera descoinsi Lalanne-Cassou & Silvain, 1994	*Spodoptera exigua* (Hübner, 1808)*
Spodoptera dolichos (Fabricius, 1794)	*Spodoptera littoralis* (Boisduval, 1833)*
Spodoptera eridania (Stoll *in* Cramer, 1782)*	*Spodoptera litura* (Fabricius, 1775)*
Spodoptera evanida Schaus, 1914	*Spodoptera malagasy* Viette, 1967
Spodoptera frugiperda (J.E. Smith, 1797)*	*Spodoptera mauritia* (Boisduval, 1833)*
Spodoptera hipparis (Druce, 1889)*	*Spodoptera pecten* Guenée, 1852*
Spodoptera latifascia (Walker, 1856)*	*Spodoptera pectinicornis* (Hampson, 1895)
Spodoptera ochrea (Hampson, 1909)*	*Spodoptera picta* (Guérin-Méneville, 1838)
Spodoptera ornithogalli (Guenée, 1852)*	*Spodoptera teferii* Laporte *in* Rougeot, 1984
Spodoptera praefica (Grote, 1875)*	*Spodoptera triturata* (Walker, 1857)
Spodoptera pulchella (Herrich-Schäffer, 1868)	*Spodoptera umbraculata* (Walker, 1858)
Spodoptera roseae (Schaus, 1923)	

et al. 2017), and characterized multiple introductions and genomic features unique to invasive populations of the FAW (Tay et al. 2020; Yainna et al. 2020; Zhang et al. 2020). The increased interest in *Spodoptera* highlights the importance of comprehensive and robust phylogenetic frameworks to contextualize changes in genomic architecture. As underlined by Gloss et al. (2019) comparative genomics of diet shifts should focus on densely-sampled and phylogenetically-resolved clades (*e.g.,* see Yassin et al. 2016 for a study on a clade of drosophilid flies). The latter is particularly important to assess whether specific genomic signatures (*e.g.,* the expansion of gene families) are associated with resource shifts and changes in host-breadth, or simply reflect lineage-specific evolutionary change.

Phylogenetic analyses of *Spodoptera* were first attempted by Pogue (2002), who analyzed a morphological dataset of 24 parsimony-informative (PI) characters for 30 *Spodoptera* species and two outgroups. The resulting phylogenetic hypotheses were neither well-supported nor fully resolved but they did recover relevant groupings. The first molecular phylogenetic study of the genus was that of Kergoat et al. (2012), who analyzed a molecular dataset of six genes (four mitochondrial and two nuclear gene fragments; 5,080 aligned nucleotide characters, of which 832 were PI) from 135 individuals representing 24 *Spodoptera* species and six outgroup species. The resulting topologies were fairly well-supported, highlighting the existence of several major lineages, each distributed in either the Western or the Eastern hemisphere. The inferred phylogenetic framework also enabled the investigation of the evolution of host-use in the genus, providing support for the existence of a clade of specialist grass-feeders (Poales) with chisel-like mandibles in larvae. Additional analyses made by Gouin et al. (2017) also highlighted a dynamic pattern in terms of host-breadth evolution, with distinct lineages independently experiencing drastic contractions or expansions of host-range. Molecular dating analyses carried out by Kergoat et al. (2012) recovered mean age estimates for the most recent common ancestor (MRCA) of *Spodoptera* between 22 to 30.7 Million years ago (Ma), and age estimates ranging from 14.7 to 23.2 Ma for the clade of species that feed preferentially on Poales. Further phylogenetic analyses were also performed by Le Ru et al. (2018), who analyzed a molecular dataset of eight genes (four mitochondrial and four nuclear gene fragments; 6,580 aligned nucleotide characters) encompassing 28 *Spodoptera* species and eight outgroup taxa. Interestingly, Bayesian inference analyses in this study suggested for the first time a more derived placement of *S. exigua* which had previously and consistently been recovered as sister to all other *Spodoptera* species (Pogue 2002; Kergoat et al. 2012; Dumas et al. 2015a).

To improve our understanding of the diversification dynamics of *Spodoptera*, a more robust, comprehensive, and resolved dated phylogenetic framework is needed. One way to achieve this objective is to capitalize on extant collections and to sequence old material (up to 150 years old) using modern museomics approaches such as genome skimming (Cameron 2014; Dodsworth 2015), which allows the recovery of mitogenomes from specimens older than one hundred years (*e.g.,* see the study of Jin et al. 2020); such approaches are also more cost-effective than other high throughput sequencing methods (Matos-Maraví et al. 2019; Young and Gillung 2020). In this study, we implemented a genome skimming approach to generate mitogenome data to further our understanding of *Spodoptera* evolution, akin to recent studies of various insect groups, *e.g.,* Timmermans et al. (2014) on Lepidoptera, Timmermans et al. (2016) on Coleoptera, Wang et al. (2017) on Neuropterida, Condamine et al. (2018) on Papilionoidea, Song et al. (2019) on Palaeopteran insects, and Nie et al. (2020) on Chrysomelidae. Our aim here is to infer a robust mitogenomic backbone that can be used in further analyses, using an extended dataset with more taxa but reduced gene coverage. To infer this backbone we used both concatenation and multiple species coalescent (MSC) approaches. MSC approaches are commonly used in nuclear phylogenomics and have recently been shown to be powerful inference methods that complement concatenation approaches when analyzing insect mitogenomic data (Kim et al. 2020). In addition we expanded on recent reviews of the insect fossil record (Sohn et al. 2012; Sohn and Lamas 2013; Sohn et al. 2015) and on the results of two comprehensive molecular dating studies on Lepidoptera (Wahlberg et al. 2013; Kawahara et al. 2019) to reassess the timing of diversification of the genus *Spodoptera*.

2. Material and Methods

2.1. Taxon sampling

For this study we generated mitogenomic data for the following 13 *Spodoptera* species (14 if one consider the FAW strains as distinct): *S. depravata* (Butler), *S. dolichos* (Fabricius), *S. exempta* (Walker), *S. exigua* (Hübner), *S. frugiperda* (J.E. Smith) (corn strain and rice strain), *S. latifascia* (Walker), *S. littoralis* (Boisduval), *S. litura* (Fabricius), *S. mauritia* (Boisduval), *S. ochrea* (Hampson), *S. ornithogalli* (Guenée), *S. pectinicornis* (Hampson) and *S. picta* (Guérin-Méneville). To complete this mitogenomic sampling we drew on GenBank data, for 15 noctuoid outgroups with available mitogenome data (see online supplementary Table S1). The rationale was to favor either closely related taxa (from the same subfamily) and species that could be used to implement both primary and secondary calibrations. Three Erebidae species, including a representative of the Arctiinae subfamily (*Hyphantria cunea* (Drury)), were selected in order to further enforce a primary fossil calibration based on the oldest known arctiine fossil (see below the corresponding section on molecular dating for further information). For secondary calibrations, outgroup selection was guided by the results of the two most comprehensive studies on Lepidoptera diversification: *(i)* that of Wahlberg et al. (2013), which reanalyzed a dataset of 350 representative lepidopteran taxa sequenced for one mitochondrial and seven nuclear genes (see Mutanen et al. 2010 for the original dataset), and *(ii)* the phylogenomic study of Kawahara et al. (2019), in which 186 representative lepidopteran taxa were sequenced for 2,098 nuclear genes.

In addition to the sampling of specimens with mitogenomic data, we included species for which more limited mitochondrial data were available (between one and four genes) (see online supplementary Table S2). This allowed us to include 15 additional *Spodoptera* species and another outgroup, *Galgula partita* Guenée (Noctuinae: Elaphriini), believed to be closely related to *Spodoptera* based on morphological and molecular analyses (Pogue 2002; Mitchell et al. 2006, respectively). We also included a representative of the subspecies *S. mauritia acronyctoides* and a representative of the Australian subspecies of *S. exigua* (*S. exigua antipodea*; hereby referred to as *S.* sp. nr. *exigua*), each of which could represent distinct biological species (Dumas et al. 2015a). Following Le Ru et al. (2018), whenever possible we also added sequence data for the following three nuclear genes: 28S ribosomal DNA (*28S*), elongation factor-1a (*Ef1a*) and dopa decarboxylase (*ddc*). These additional sequences were downloaded from GenBank, and most correspond to sequences previously generated by our research group.

2.2. DNA extraction, sequencing of new mitogenome data

For most specimens we relied on old DNA extracts from experiments we conducted between 1998 and 2010 (with most specimens collected between 1990 and 2000), where total DNA was extracted from hind legs and thoracic muscles following the phenol–chloroform protocol of Kocher et al. (1989). DNA from recently collected specimens of *S. littoralis* and *S. frugiperda* (both strains) was extracted using Bio Basic DNA Miniprep kits (BioBasic Inc., Ontario, Canada). All DNA extracts were further quantified using a Qubit fluorometer (Thermo Fisher Scientific, Renfrew, UK). Libraries for whole genome sequencing were constructed from 1.0μg DNA per sample using NEBNext DNA Library Prep kits (New England Biolabs, Ipswich, USA). The Illumina NovaSeq 6000 system was then used to perform whole genome sequencing for one individual of each species (with *c.* 20X coverage) with 150bp read length and 300bp insert size. Adapter sequences were removed using

AdapterRemoval v2 (Schubert et al. 2016). Bam files were generated by mapping the reads against a publicly available mitochondrial genome of *S. frugiperda* (NCBI_ID: KM362176) using Bowtie 2 (Langmead and Salzberg 2012) with the '*-very-sensitive-local*' preset. Mitochondrial reads were further extracted from the .bam files using SAMtools v1.3 (Li et al. 2009). Mitochondrial genomes were assembled, and gene annotation was performed on these mitochondrial reads using MitoZ (Meng et al. 2019) with default options. In a complementary way we also used NOVOPlasty v2.7.0 (Dierckxsens et al. 2017) for mitochondrial genome assembling with a k-mer size of 33 and *S. exigua* mitochondrial genome as a guide (NCBI_ID: JX316220); gene annotation was then performed using MEGA4 (Tamura et al. 2007) by aligning each mitochondrial gene from the *S. exigua* mitochondrial genome with genes from the newly assembled mitochondrial genomes. For the purpose of this study, we used sequences from all 13 mitochondrial protein coding genes (PCGs): NADH dehydrogenase subunit 2 (*nd2*), cytochrome oxidase *c* subunit (*cox1*), cytochrome oxidase *c* subunit 2 (*cox2*), ATP synthase subunit 8 (*atp8*), ATP synthase subunit 6 (*atp6*), cytochrome oxidase *c* subunit 3 (*cox3*), NADH dehydrogenase subunit 3 (*nd3*), NADH dehydrogenase subunit 5 (*nd5*), NADH dehydrogenase subunit 4 (*nd4*), NADH dehydrogenase subunit 4L (*nd4L*), NADH dehydrogenase subunit 6 (*nd6*), cytochrome oxidase *b* (*cob*), and NADH dehydrogenase subunit 1 (*nd1*) (in that order). All corresponding sequences were deposited on GenBank (under accession numbers MW665864-MW666021).

2.3. Concatenated molecular datasets

For the specimens from which we had complete mitogenomic data (14 *Spodoptera* and 15 outgroups), we designed three distinct sets of molecular concatenated datasets.

The first concatenated dataset consists of the 13 PCGs plus the large and small mitochondrial ribosomal RNA (rRNA) genes (*rrnL* and *rrnS*). For the two rRNA sequences we relied on data already available on GenBank. The sequences of the two rRNA and of several PCGs varied in length; these were aligned using MAFFT v7 (Katoh and Standley 2013) with default option settings. For all protein-coding genes, we used Mesquite v3.61 (Maddison and Maddison 2019) to check the reading frame for possible errors and stop codons. We also used DAMBE v7 (Xia 2018) to conduct two-tailed tests of substitution saturation (Xia et al. 2003) for each codon position of the 13 PCGs; the proportion of invariant sites was taken into account following Xia and Lemey (2009). According to the observed index of substitution saturation (I_{SS}), third codon positions show little saturation ($I_{SS} < I_{SS}cSym.$ and $I_{SS} < I_{SS}cAsym.$; see online supplementary Table S3). Nucleotide saturation for each codon position of the 13 PCGs was also visually assessed with DAMBE by plotting transitions and transversions against K2P (Kimura 1980) distances; again, little saturation was found for third codon positions as indicated by the nonlinear growth of the best-fit lines of the plot (see online supplementary Fig. S1).

The combination of the 15 genes resulted in a concatenated dataset (referred to as the *13PCGs_rRNAs_nt* dataset) of 29 specimens and 13,729 aligned nucleotide characters. To build the second concatenated dataset, we used Mesquite to remove all third codon positions from the *13PCGs_rRNAs_nt* dataset. The resulting dataset (referred to as the *13PCGs_rRNAs_pos1&2* dataset) consists of 9,967 aligned nucleotide characters for each of the 29 specimens. For the third concatenated dataset, we first used Mesquite to remove the two rRNA genes from the *13PCGs_rRNAs_nt* dataset. We then used MEGA X (Kumar et al. 2018) to translate nucleotides to amino-acids on the basis of the invertebrate mitochondrial DNA genetic code. The resulting dataset (referred to as the *13PCGs_AA* dataset) is composed of 3,762 aligned amino-acid characters for each of the 29 specimens.

A fourth molecular concatenated dataset was assembled using Mesquite. This dataset includes 18 additional taxa (17 of which belong to *Spodoptera*), for which we only have mitochondrial data for as many as four gene fragments. As underlined in the *Taxon sampling* section above, this dataset also encompasses three nuclear gene fragments for two coding (*Ef1a* and *ddc*) and one non-coding (*28S*) genes (see online supplementary Table S2 for details). The resulting dataset (referred to as the *extended* dataset) is composed of 47 specimens and 17,046 aligned nucleotide characters.

2.4. Phylogenetic analyses of concatenated mitogenomic datasets

Phylogenetic analyses were conducted using both Bayesian inference (BI) and maximum likelihood (ML). For both analytical approaches, we carried out partitioned analyses to improve phylogenetic accuracy (Nylander et al. 2004); we also followed Timmermans et al. (2016), who advocate the use of complex partitioning schemes (partitioning by gene and codon) to analyze insect mitogenomes. The first mitogenomic dataset (*13PCGs_rRNAs_nt* dataset) was divided a priori into 41 partitions: we used three partitions (one per codon position) for each of the 13 PCGs and one partition for each of the rRNA genes (*rrnL* and *rrnS*). The second mitogenomic dataset (*13PCGs_rRNAs_pos1&2* dataset) was divided a priori into 28 partitions: two partitions (one for first-codon positions and one for second-codon positions) were used for each of the 13 PCGs and one partition for each of the rRNA genes (*rrnL* and *rrnS*). The third mitogenomic dataset (*13PCGs_AA* dataset) was divided a priori into 13 partitions, one for each of the 13 PCGs. The best partitioning schemes and substitution models were further determined using PartitionFinder v2.1.1 (Lanfear et al. 2017) using the default search algorithm ('*greedy*' option) and the most complete set of models ('*model=all*' option); we also used the 'linked branch lengths' option, where each subset has its own 'rate multiplier' parameter but only one underlying set of branch lengths. For both partition and model selection we relied on the corrected Akaike information criterion (AICc; Hurvich and Tsai 1989).

Bayesian inference analyses were performed with MrBayes 3.2.6 (Ronquist et al. 2012) and ML analyses were performed with RAxML 8.2.8 (Stamatakis 2014). All analyses were performed on the online computer cluster CIPRES Science Gateway 3.3 (Miller et al. 2015; www.phylo.org). For ML partitioned analyses, the best-scoring tree from each dataset was obtained using a heuristic search implementing 100 random-addition replicates. Clade support was assessed first using standard non-parametric bootstrap support (BS) values, with 1,000 replicates; nodes supported by BV $\geq$ 70% were considered strongly supported following Hillis and Bull (1993). In addition, we implemented the transfer bootstrap expectation (TBE) method, which is intended to provide a better measure of branch repeatability, or robustness (*sensu* Lemoine et al. 2018); TBE is also less sensitive to individual misplaced taxa in replicate trees (Kozlov et al. 2019). For TBE values we also used a 70% threshold, which is considered conservative by Lemoine et al. (2018). For BI partitioned analyses, instead of relying on a single substitution model per partition, we used the '*mixed*' option of MrBayes to sample across substitution models with reversible-jump Markov Chains Monte Carlo (rj-MCMC; Huelsenbeck et al. 2004). Two independent runs with eight MCMC (one cold and seven incrementally heated chains) were conducted: they ran for 50 million generations, with trees sampled every 5,000 generations. A conservative 25% burn-in was applied after checking for stability on the log-likelihood curves and the split-frequencies of the runs in Tracer v.1.7 (Rambaut et al. 2018). Support of nodes for MrBayes analyses was provided by clade posterior probabilities (PP) directly estimated from the majority-rule consensus topology. Following Erixon et al. (2003), nodes supported by PP $\geq$ 0.95 were considered well-supported.

2.5. Multi-species coalescent analyses

In addition to analyses carried out on the concatenated mitogenomic datasets, we conducted multi-species coalescent (MSC) analyses with the most recent implementation (ASTRAL-III; Zhang et al. 2018) of the gene tree summary method ASTRAL (Mirarab et al. 2014; Mirarab and

Warnow 2015). ASTRAL allows the estimation of an unrooted species tree given a set of unrooted gene trees while accounting for gene tree heterogeneity. Gene trees were generated under ML for each of the 15 genes from the *13PCGs_rRNAs_nt* dataset. ML analyses were performed with RAxML 8.2.8 with general time-reversible (GTR; Tavaré 1986) models allowing for gamma-distributed rate variation across sites (+G; Yang 1994). For each gene the best-scoring ML tree was obtained using a heuristic search implementing 100 random-addition replicates, with clade support assessed using 200 BS replicates. The ASTRAL MSC analysis was further carried out with default parameters; the 200 BS replicates inferred for all gene trees were used as input, allowing the measure of local posterior probability support values (Sayyari and Mirarab 2016) for all nodes.

2.6. Phylogenetic analyses with a backbone enforced

A final set of phylogenetic analyses was conducted on the *extended* dataset, which included 18 additional taxa and three additional nuclear genes at the cost of a significant amount of missing data (*ca.* 40% of missing data). Although we consider it undesirable, such a high degree of missing data is not uncommon in concatenation phylogenetic analyses, and several studies have even suggested that adding even incomplete taxa may benefit phylogenetic accuracy if certain conditions are met (Wiens 2005; Cho et al. 2011; Crête-Lafrenière et al. 2012; Wiens and Tiu 2012; see also the review of Xi et al. 2016). In this study, we build upon the results of the mitogenomic analyses to constrain a mitogenomic backbone which we enforced in the phylogenetic analyses of *extended* dataset. Phylogenetic analyses were conducted using both Bayesian inference (BI) and maximum likelihood (ML). The *extended* dataset was divided a priori into 48 partitions: we used three partitions (one per codon position) for each of the 15 coding genes (13 PCGs and the two coding nuclear genes) and one partition for each of the rRNA genes (mitochondrial *rrnL* and *rrnS*, nuclear *28S*). The best partitioning schemes and substitution models were determined using PartitionFinder v2.1.1 following the procedure described above.

For ML analyses, we enforced a binary backbone constraint specification (option *-r* in RAxML) based on the results of the analyses of the mitogenome data. The best-scoring tree was obtained using a heuristic search implementing 100 random-addition replicates. Clade support was assessed using standard BS (1,000 replicates) and TBE values. For BI analyses, we implemented the same backbone constraint with the '*constraint*=' and '*constraint partial*=' options. Two independent runs with eight rj-MCMC (one cold and seven incrementally heated chains) were conducted; they ran for 50 million generations, with trees sampled every 5,000 generations. A standard 25% burn-in was applied after checking for stability on the log-likelihood curves and the split-frequencies of the runs in Tracer v.1.7. Support for nodes in MrBayes analyses was provided by PP as directly estimated from the majority-rule consensus topology.

2.7. Dating analyses

Divergence times were estimated using Bayesian relaxed clocks as implemented in BEAST v1.10.4 (Suchard et al. 2018). For this study, we implemented a node-dating approach with three calibration strategies. The first relied on primary fossil calibrations. The fossil record of Lepidoptera is poor, and biased in terms of preservation type, age, and taxonomic composition (Sohn et al. 2015). The Noctuidae are no exception, and only two fossils are considered verifiable noctuids (Sohn et al. 2012). But these fossils cannot be reliably assigned to any known subfamily, and more importantly they are too young (late Pleistocene) to be useful for calibrating molecular dating procedures. Within the Noctuoidea, however, several reliable fossils are found in the Erebidae (Sohn et al. 2012). Out of the 14 known fossils reliably assigned to the superfamily (Sohn et al. 2012) the oldest is an undescribed species of Arctiinae. This fossil (specimen UWBM 66000) is a relatively complete (whole body) compression fossil described in detail by Douglas (1991), and several morphological traits support its inclusion within the Arctiinae (Douglas and Stockey 1996). This fossil was found embedded in Early Lutetian tuffs from the Klondike Mountain Formation (city of Republic, Ferry County, Washington State, USA, which were radiometrically dated at approximately 48–49 Ma (Wolfe and Wehr 1987); this corresponds to the boundary between the Lutetian and Ypresian age, estimated at 47.8 Ma (Walker et al. 2018). For this study we used this fossil as a stem calibration for the Arctiinae. The prior for this fossil constraint was set with uniform statistical distributions, with a minimum age of 47.8 Ma and a conservative maximum age of 78.79 Ma based on the upper bound (from the 95% highest posterior densities, HPD) of the estimated age of the MRCA of [Noctuidae+Erebidae] in the study of Kawahara et al. (2019) (see Fig. S12 of their study).

The second calibration strategy relied on secondary calibrations derived from the study of Wahlberg et al. (2013). In this study six fossil calibrations and one secondary calibration were used to infer divergence time estimates within Lepidoptera based on the phylogenetic dataset developed by Mutanen et al. (2010). Based on the taxa shared between our dataset and theirs, we were able to constrain the MRCA's of following five taxon pairs: *(i) Sesamia* Guenée (representing the Apameini, which also contains *Apamea* Ochsenheimer) and *Noctua* L. (minimum age of 14.57 Ma and maximum age of 24.02 Ma); *(ii) Acronicta* Ochsenheimer and *Noctua* (minimum age of 35.07 Ma and maximum age of 49.2 Ma); *(iii) Catocala* Schrank and *Lymantria* Hübner (minimum age of 48.01 Ma and maximum age of 63.0 Ma); *(iv) Eutelia* Hübner and *Noctua* (minimum age of 52.33 Ma and maximum age of 70.73 Ma); and *(v) Catocala* and *Noctua* (minimum age of 59.51 Ma and maximum age of 76.27 Ma).

The third calibration strategy relied on secondary calibrations derived from the study of Kawahara et al. (2019). In that study, the authors used 16 fossil calibrations to infer divergence time estimates within Lepidoptera. As a result of the taxa shared between our dataset and theirs, we were able to constrain MRCA's of the following six taxon pairs: *(i) Heliothis* Ochsenheimer and *Helicoverpa* (minimum age of 5.34 Ma and maximum age of 14.9 Ma); *(ii) Spodoptera exigua* and *Spodoptera frugiperda* (minimum age of 6.24 Ma and maximum age of 16.34 Ma); *(iii) Noctua* (representing the Noctuini, containing *Agrotis* Ochsenheimer) and *Sesamia* (minimum age of 16.2 Ma and maximum age of 29.57 Ma); *(iv) Noctua* and *Heliothis* (minimum age of 30.28 Ma and maximum age of 46.77 Ma); *(v) Hyphantria* Harris (representing the Arctiinae, containing *Arctia* Schrank) and *Lymantria* (minimum age of 45.38 Ma and maximum age of 64.85 Ma); and *(vi) Lymantria* and *Noctua* (minimum age of 58.57 Ma and maximum age of 78.79 Ma).

Partitions, clocks and substitution models were selected under PartitionFinder v2.1.1 with the '*beast*' set of models. To provide substitution rates for every gene, while limiting the number of potential parameters to estimate, the *extended* dataset was divided a priori into 18 partitions (one partition per gene). The Tree Model was set to a birth-death speciation process (Gernhard 2008) to account more accurately for extinct and missing lineages. To limit the risk of over-parameterization, we enforced a fixed topology corresponding to the tree with the greatest support, corresponding to the best-score tree obtained under ML (see the 'Results' section). Further BEAST analyses were performed on the CIPRES Science Gateway. All analyses consisted of 50 million generations of MCMC with the parameters and trees sampled every 5,000 generations. A burn-in of 25% was applied after checking the log-likelihood curves. The maximum credibility tree, median ages and their 95% highest posterior density (HPD) were generated with TreeAnnotator v1.10.4, which is part of the BEAST software package. Convergence of runs was assessed graphically and by examining the ESS of parameters under Tracer v.1.7, using the recommended threshold of 200 (Drummond et al. 2006).

3. Results

3.1. Mitogenomics analyses

Best-fit partitioning schemes recovered by PartitionFinder2 analyses of the three concatenated datasets (*13PCGs_rRNAs_nt*, *13PCGs_rRNAs_pos1&2*, and *13PCGs_AA* datasets) are reported in online Supplementary Table S4. All analyses provide the highest level of support (for all analyses, TBE and BS of 100%; PP of 1.0) for the monophyly of the genus *Spodoptera* (see Fig. 1A and online supplementary Figs. S2-S4). The family Noctuidae is also always recovered as monophyletic with the highest support and sister group to the only sampled representative of the Euteliidae, *Eutelia adulatricoides* Mell. When considering the sampled Noctuidae, the placement of the five representatives of the Heliothinae (*Heliothis* and *Helicoverpa* species) varies. Heliothinae are always recovered as monophyletic; they are generally found in a derived position among Noctuinae representatives (BI and ML analyses of the *13PCGs_rRNAs_nt* dataset, BI analyses of the *13PCGs_AA* dataset, ML analyses of the *13PCGs_rRNAs_pos1&2* dataset) or as sister group to them (BI analyses of the *13PCGs_rRNAs_pos1&2* dataset and ML analyses of the *13PCGs_AA* dataset). The support for a more derived placement of Heliothinae is lower (TBE of 72-76%, BS of 47-57%, PP of 0.56-0.66; to be compared with TBE of 90%, BS of 62%, PP of 1.0).

Partitioned phylogenetic analyses of the *13PCGs_rRNAs_nt* and *13PCGs_AA* datasets yield almost identical topologies under BI and ML (see online supplementary Figs. S2 and S3); the only exceptions are: *(i)* the position of Heliothinae, which is placed as sister to all sampled Noctuinae when analyzing the *13PCGs_AA* dataset under ML (TBE of 90% and BS of 62%; see online supplementary Fig. S2); and *(ii)* the placement of the outgroup *Catocala* sp. (Erebidae: Erebinae), which is

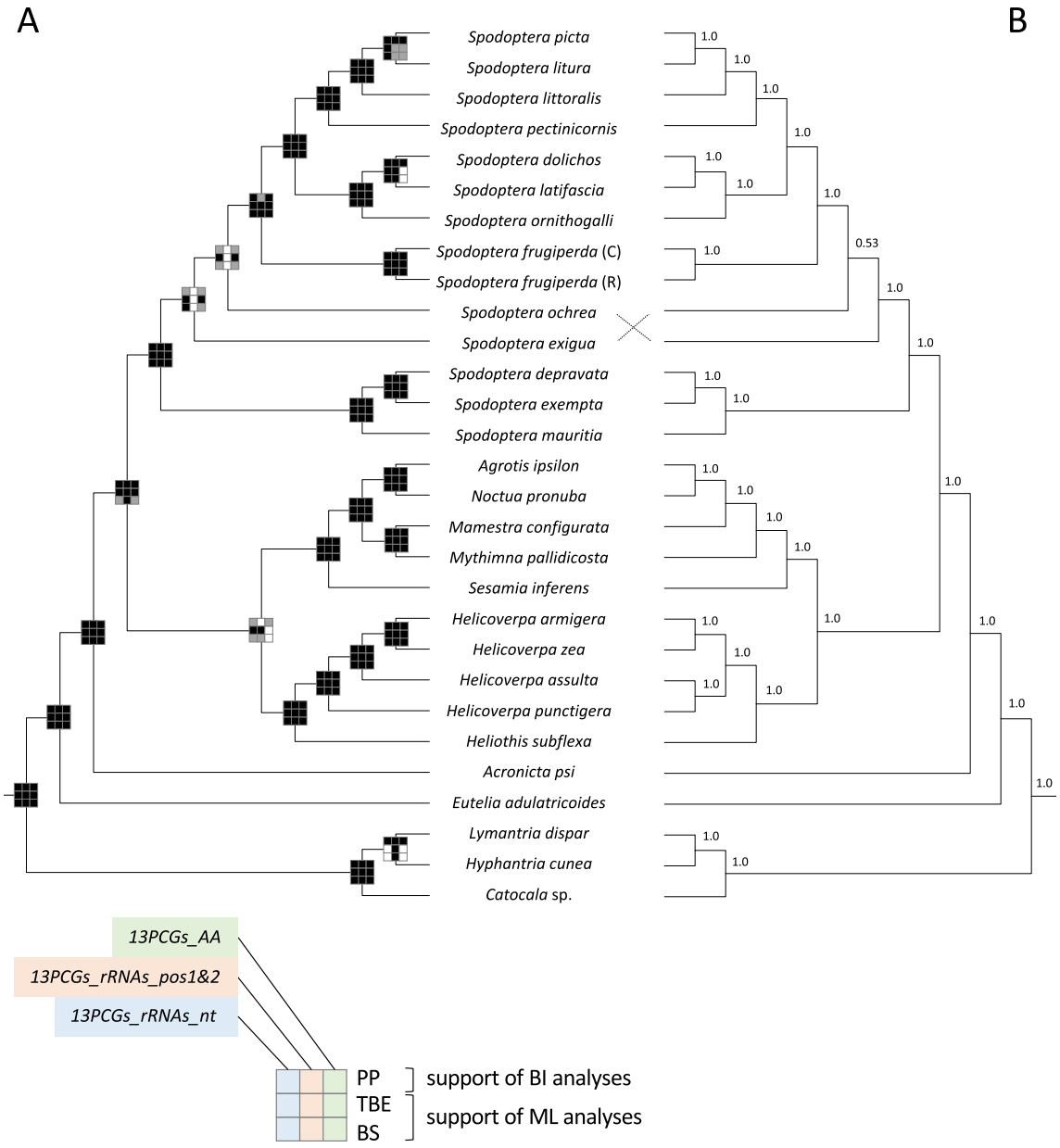

Fig. 1. 1A. Results of the partitioned analyses of the mitogenomic dataset. The topology presented on the left corresponds to the tree resulting from the BI analyses of the *13PCGs_rRNAs_nt* dataset. Support values for all partitioned analyses of the mitogenomic dataset are presented on nodes using nine colored boxes (see the corresponding legend on the bottom left); black squares indicate well-supported nodes, grey squares indicate values < 0.95 for the BI analyses or $< 70\%$ for ML analyses), and white squares indicate nodes not recovered by a specific analysis. 1B. Results of the MSC analyses. Support values (local posterior probabilities) are provided on nodes.

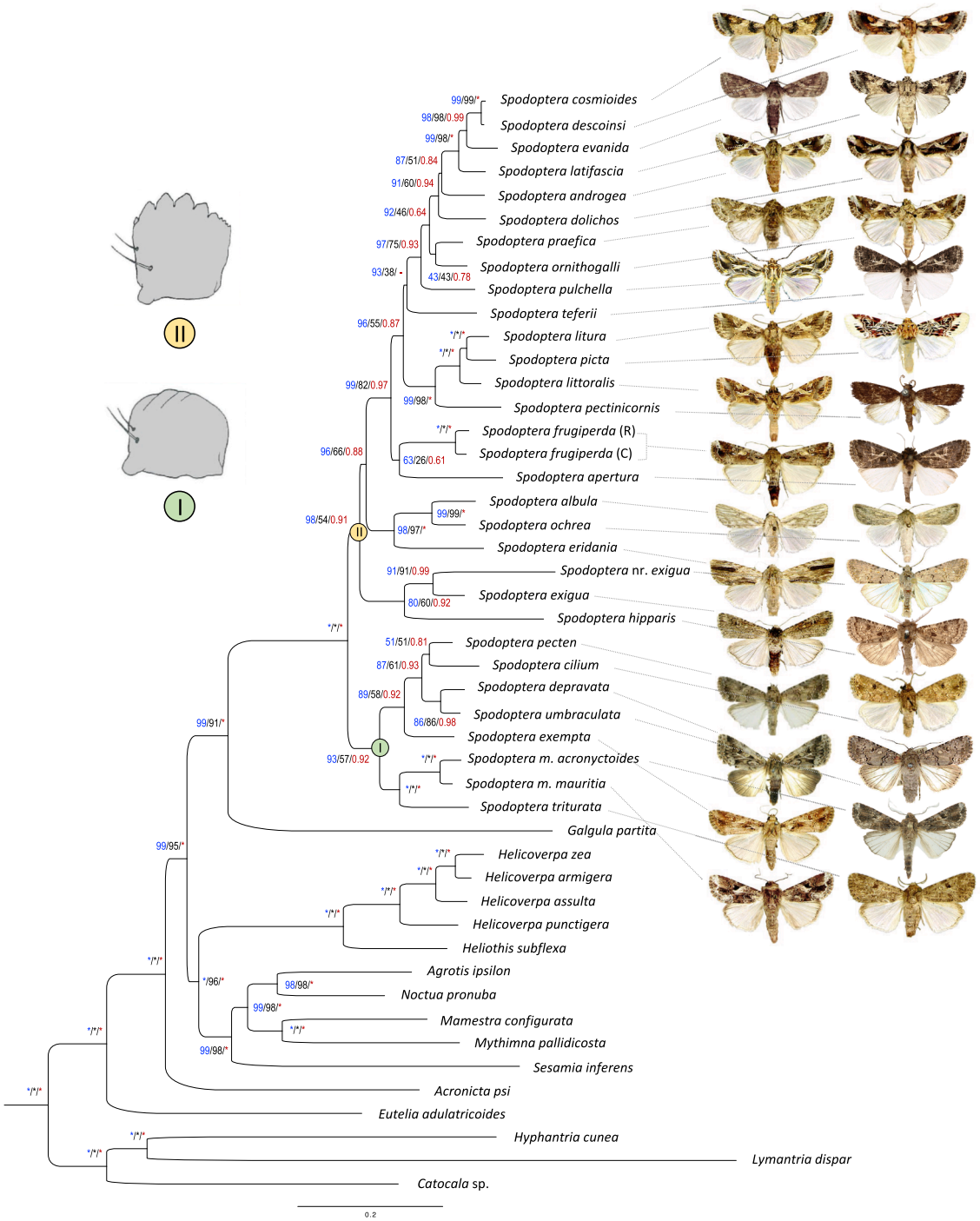

Fig. 2. Results of the ML analyses of the *extended* dataset. Support values are provided on nodes (TBE values in dark blue and BS values in dark; PP are also provided for nodes shared with the tree resulting from the BI analyses), asterisks are used to underline maximum support values. The two main *Spodoptera* clades (labelled I and II in the figure) are highlighted; on the left drawings showing the two distinct mandible type are presented (chisel-like mandibles for Clade I and serrate mandibles for Clade II). On the right pictures of adult *Spodoptera* species are also displayed for illustrative purpose (most pictures were taken by Gael J. Kergoat and Bruno Le Ru; pictures of *S. hipparis* (male syntype) and *S. pectinicornis* (male lectotype) were taken by Alberto Zilli, the picture of *S. pulchella* is courtesy of James T. Troubridge, the pictures of *S.* sp. nr. *exigua* and *S. umbraculata* are courtesy of the CSIRO/BIO photography group (Centre for Biodiversity Genomics, University of Guelph), and the picture of *S. m. acronyctoides* is courtesy of Hsu Hong Lin).

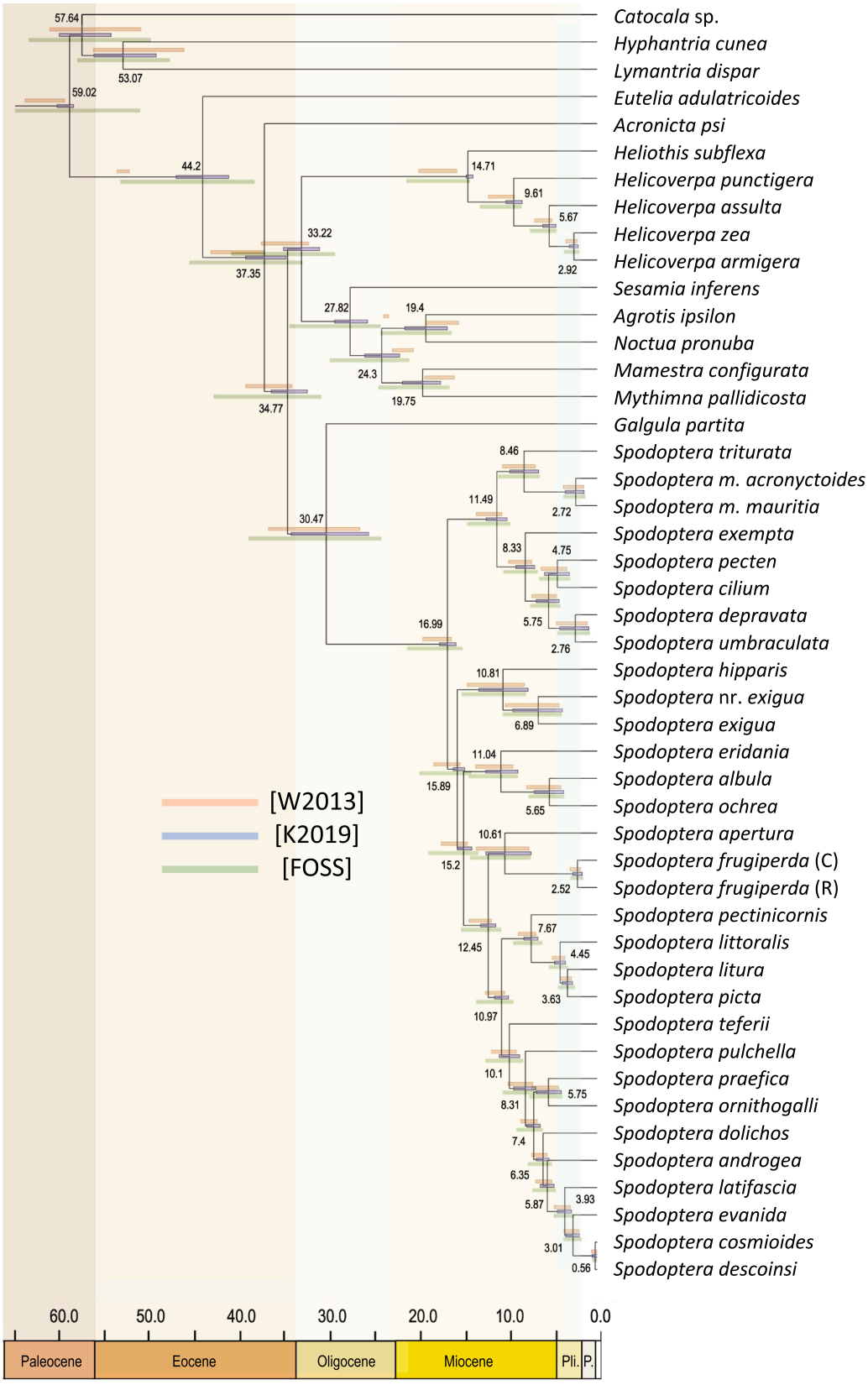

Fig. 3. Results of the molecular dating analysis relying on secondary calibrations based on Kawahara et al. 2019 [K2019]. Median ages are provided on nodes, along with coloured bars showing the 95% HPD of estimated ages (in blue). For comparison purpose we also added the coloured bars showing the 95% HPD of estimated ages for: *(i)* the analysis relying on secondary calibrations based on Wahlberg et al. 2013 [W2013] (orange bars), and *(ii)* the analysis relying on a primary fossil calibration [FOSS] (green bars).

sister to *Lymantria dispar* (L.) (Erebidae: Lymantriinae) under ML, but with weak support (TBE and BS of 21-45%; see online supplementary Figs. S2 and S3). Partitioned phylogenetic analyses of the *13PCGs_rRNAs_pos1&2* dataset yield slightly different topologies (see online supplementary Fig. S3), mostly with respect to the placement of *S. exigua* and *S. ochrea*; otherwise, the relationships among species in both *Spodoptera* clades match, one comprising *S. depravata*, *S. exempta* and *S. mauritia* and the other *S. dolichos*, *S. frugiperda* corn strain, *S. frugiperda* rice strain, *S. latifascia*, *S. littoralis*, *S. litura*, *S. ornithogalli*, *S. pectinicornis* and *S. picta*.

For all partitioned analyses, clade support is generally very high, with TBE values $\geq$ 70% for up to 96% of nodes, BS values $\geq$ 70% for up to 92% of nodes, and PP values $\geq$ 0.95 for up to 92% of nodes. All analyses also unambiguously support a nested position of *S. exigua*, instead of its placement as sister to all remaining *Spodoptera* species.

Bootstrapped gene trees generated for each of the 15 mitochondrial genes are presented using DensiTree v2.2.7 (Bouckaert and Heled, 2014) as supplementary material (online supplementary Figs. S5-S12). The tree resulting from the MSC ASTRAL analysis is almost identical to the results of the partitioned phylogenetic analyses of the *13PCGs_rRNAs_nt* and *13PCGs_AA* datasets (see Fig. 1B). Branch supports for the ASTRAL tree are maximal (local posterior probabilities of 1.0) for all nodes but one, which bears on the respective placement of *S. exigua* and *S. ochrea*. The MSC ASTRAL analysis also recovers the Heliothinae embedded within the Noctuinae.

Comparing the results of the MSC ASTRAL analysis with the results from the partitioned phylogenetic analyses of the *13PCGs_rRNAs_nt* and *13PCGs_AA* datasets allows for the generation of a consensus tree, which is used as the mitogenomic backbone in subsequent analyses (see online supplementary Fig. S13).

3.2. Phylogenetic analyses with the backbone enforced

The best-fitting partitioning scheme recovered by the PartitionFinder2 analysis of the final dataset ('*extended*') is given in online supplementary Table S5. The partitioned phylogenetic analyses of this dataset yield almost identical topologies under BI and ML (see Fig. 2 for the ML tree and online supplementary Fig. S14 for the BI tree). Overall, clade support is higher for the ML tree (91% of nodes with TBE $\geq$ 70%; 68% of nodes with BS $\geq$ 70%; 66% of nodes are supported by PP $\geq$ 0.95 in the BI analyses). The topologies differ only in the placement of *S. teferii*; under ML, this species is recovered as sister (with TBE of 93% and BS of 38%) to a clade of nine species from the Western hemisphere (*S. androgea* Stoll, *S. cosmioides* (Walker), *S. descoinsi* Lalanne-Cassou & Silvain, *S. dolichos, S. evanida* Schaus, *S. latifascia, S. ornithogalli*, *S. praefica* (Grote) and *S. pulchella* (Herrich-Schäffer)) whereas under BI it is placed as sister (with a PP of 0.52) to a clade of four species from the Eastern hemisphere (*S. littoralis*, *S. litura, S. pectinicornis* and *S. picta*). Two primary *Spodoptera* clades (hereafter referred to as Clade I and Clade II) are inferred with both analyses: *(i)* Clade I (supported by TBE of 93%, BS of 57%, and PP of 0.92) is composed of seven species (*S. cilium, S. depravata, S. exempta, S. mauritia, S. pecten, S. triturata* and *S. umbraculata*) originally confined to the Eastern hemisphere (although *S. exempta* is invasive in Hawaii; Haggis 1986), all of which have larvae with chisel-like mandibles (see Fig. 2); *(ii)* Clade II (supported by TBE of 98%, BS of 54%, and PP of 0.91) is composed of 21 species distributed in both hemispheres; all larvae with described morphology in clade II have serrate mandibles (see Fig. 2).

3.3. Dating analyses

The best-fit partitioning scheme recovered by PartitionFinder2 for the BEAST analyses is given in online supplementary Table S6. All parameters show ESS values $\geq$ 200 for all BEAST analyses. There is a high degree of overlap among the results of the three distinct dating analyses (see Fig. 3 for the analysis relying on secondary calibrations based on Kawahara et al. 2019 [K2019], online Fig. S15 for the analysis relying on secondary calibrations based on Wahlberg et al. 2013 [W2013], and online Fig. S16 for the analysis relying on a primary fossil calibration [FOSS]). The primary difference is the magnitudes of the confidence intervals, which are smaller for the two dating analyses relying on secondary calibrations ([K2019] and [W2013]). Based on the results of the BEAST analyses, we also provide estimates of substitution rates for each analysis (see online supplementary Table S7).

Age estimates for the genus *Spodoptera* suggest an origin in the Early Miocene, *ca.* 17-18 Ma: median age 16.99 Ma, 95% HPD: 16.04-17.87 Ma [K2019]; median age 17.8 Ma, 95% HPD: 15.42-21.46 Ma [FOSS]; median age 18.18 Ma, 95% HPD: 16.48-19.68 Ma [W2013]. Within *Spodoptera*, Clade II (larvae with serrate mandibles) diversified first, with an origin at the boundary of the Early and Middle Miocene about 15-17 Ma (median age 15.89 Ma, 95% HPD: 15.08-16.34 Ma [K2019]; median age 15.94 Ma, 95% HPD: 14.39-20.08 Ma [FOSS], median age 17 Ma; 95% HPD: 15.51-18.47 Ma [W2013]). Clade I (larvae with chisel-like mandibles) diversified more recently at the boundary of the Middle and Late Miocene, about 11-12 Ma (median age 11.49 Ma, 95% HPD: 10.36-12.67 Ma [K2019]; median age 12.03 Ma, 95% HPD: 10.14-14.8 Ma [FOSS]; median age 12.22 Ma, 95% HPD: 10.87-13.72 Ma [W2013]). Interestingly, age estimates for *Spodoptera* species pairs are almost always older than 2 million years (Myrs), including the two FAW strains and the species pairs [*S. m. mauritia* + *S. m. acronyctoides*] and [*S. exigua* + *S.* sp. nr. *exigua*]; only in the case of the species-pair [*S. cosmioides* + *S. descoinsi*] is a younger age recovered, at 0.56-0.57 Ma.

Outside the genus *Spodoptera*, the age of the MRCA of the Noctuoidea is estimated at *ca.* 56-61 Ma (median age 56.33 Ma, 95% HPD: 51.11-66.1 Ma [FOSS]; median age 59.02 Ma, 95% HPD: 58.57-60.41 Ma [K2019]; median age 61.03 Ma, 95% HPD: 59.51-63.93 Ma [W2013]). For the MRCA of the Noctuidae, dating estimates suggest an age of about 37-40 Ma (median age 37.35 Ma, 95% HPD: 34.93-39.44 Ma [K2019]; median age 38.16 Ma, 95% HPD: 33.1-45.59 Ma [FOSS]; median age 40.31 Ma, 95% HPD: 37.3-43.28 Ma [W2013]).

4. Discussion

4.1. Mitogenomics analyses

Based on the results of partitioned analyses of the concatenated datasets (Fig. 1A), the choice of inference method had little effect on the phylogenetic reconstructions as conflicting branching orders were only recovered for three of the 28 nodes when comparing the results of BI and ML (see also Figs. S2-S4). For the ML analyses there were few discrepancies between TBE and BS values; in only seven instances (out of 84 possible combinations; see Fig. 1) was a node supported only by TBE. Topological incongruences were likewise trivial when comparing the results of analyses using all the nucleotides with results of analyses relying on AA. However, differences were more pronounced when comparing the former with the results of analyses where third codon positions were excluded (see Figs. S2-S4). Additional support for the topology associated with the partitioned analyses of all nucleotides and those relying on AA was provided by the MSC analysis, which yielded an almost identical phylogenetic tree (Fig. 1B). This consistency between concatenation analyses and the MSC approach echoes the conclusion of Kim et al. (2020) on the utility of using both methods in a complementary way.

Within *Spodoptera*, the only topological incongruences attributable to differences in data handling were the alternate placements of *S. exigua* and *S. ochrea*, which changed when third codon positions are excluded. Several studies (Yang et al. 2013; Zahiri et al. 2013; Yang et al. 2015) have explored the phylogenetic impact of removing third codon positions on analyses of diverse insect groups and found that it could be detrimental, especially in cases of recent diversifications. This may be the case for our data as well. In our study, the resolution of *Spodoptera*

relationships is likely impacted by the loss of phylogenetically informative data associated with the removal of third codon positions, leading to the rearrangement of *S. exigua* and *S. ochrea*. Although third codon positions exhibit significant levels of saturation in our dataset (see Fig. S1), excluding them incurs an adverse trade-off associated with the loss of 2,145 PI characters (out of 3,155 in total for the 13 PCGs); this suggests that the additional homoplasy incurred by the inclusion of third codon positions is outweighed by their information content (see Källersjö et al. 1999). One of the six distinct partitioned analyses (ML analyses of the *13PCGs_AA* dataset) also recovered an alternative placement for *S. dolichos* as sister to *S. ornithogalli*; however this placement is weakly supported (TBE and BS of 52%) and likely artifactual.

Regarding outgroup relationships, we stress that our sampling was not designed to investigate the phylogenetic relationships of other noctuoid lineages, but to include a relevant set of benchmarks to investigate the evolutionary history of *Spodoptera* while providing primary and secondary calibrations for the dating analyses. With that in mind, we discuss only briefly two specific groupings within the two alternative topologies consistently recovered by our analyses. The first of these relates to the placement of Heliothinae within the Noctuinae (hence rendering the Noctuinae paraphyletic) that we recover in two analyses out of six (BI analyses of the *13PCGs_rRNAs_pos1&2* dataset and ML analyses of the *13PCGs_AA* dataset). It is tempting to discard this result as artifactual, especially since several molecular studies have recovered Heliothinae sister to Noctuinae (Mitchell et al. 2006; Cho et al. 2008; Kawahara et al. 2019; Keegan et al. 2019). However, we note that a similar arrangement was recovered in the studies of Regier et al. (2017) and Keegan et al. (2021). All these studies are potentially sensitive to sampling biases, and a proper test of the monophyly of both subfamilies requires much denser sampling of genera and type species as well as additional genomic data. Our interpretation of this result is also complicated by the fact that the composition of and relationships within the Noctuinae and among other noctuid subfamilies, including the Heliothinae (see Fibiger et al. 2009), are very much in flux (*e.g.,* see Mitchell et al. 2006; Keegan et al. 2019). We do not purport to have brought any novel insight to bear on the placement of Heliothinae, which should be considered provisional. Another inconsistency inferred from the analyses of mitogenomic data is the placement of *Hyphantria* (representative of Arctiinae). An alternative placement of *Hyphantria* as sister to [*Lymantria* + *Catocala*] (loosely, the exemplars for Lymantriinae and Erebinae, respectively) was inferred only twice, and was always poorly supported (TBE and BS of 21% or 45% for the two ML analyses suggesting this relationship). We suspect that this result is artifactual, as a result of minimal sampling for the Erebidae (given that over 24,569 erebid species are described; Goldstein 2017), and of the potential impact of the long branch leading to *Lymantria dispar*.

4.2. New insights in the phylogenetic relationships of Spodoptera.

In comparison to previous studies relying on fewer molecular markers (Kergoat et al. 2012; Dumas et al. 2015a; Le Ru et al. 2018), our analyses of the *extended* dataset with the mitogenomic backbone enforced yielded more consistent and better-supported phylogenetic reconstructions, with less incongruence between BI and ML analyses (with the position of only one species in conflict). Our results also provide for the first time strong support for the existence of two major *Spodoptera* clades, each with distinct larval morphologies (see Fig. 2) and ecology. Clade I comprises *S. cilium, S. depravata, S. exempta, S. mauritia, S. pecten, S. triturata* and *S. umbraculata*, of which the first four are pest species on grasses and one of which one (*S. exempta*) is known for its regular outbreaks (Haggis 1986; Cheke and Tucker 1995). This group was not recovered as monophyletic in previous studies based on the analysis of the *cox1* gene alone (Dumas et al. 2015a; Le Ru et al. 2018) but was found to be monophyletic when analyzing multilocus datasets (Kergoat et al. 2012; Le Ru et al. 2018). Clade II encompasses most of the known *Spodoptera* pest species and is composed of several species groups distributed in both hemispheres. This group was not recovered in previous studies (Kergoat et al. 2012; Dumas et al. 2015a; Le Ru et al. 2018), due to the placement of *S. exigua* and allied species *S. hipparis* and *S.* sp. nr. *exigua,* when they were included. It also likely includes the three species for which we do not have any molecular data: *S. compta* (Walker), *S. malagasy* Viette, and *S. roseae* (Schaus). According to Pogue and Passoa (2000), *S. compta* belongs to the morphologically homogeneous *eridania*-group comprising *S. albula, S. compta, S. eridania* and *S. ochrea*; in our study, these three sampled species form a triad, and we expect *S. compta* falls within it as well. *Spodoptera malagasy* was initially described as a subspecies of *S. apertura* (under *S. leucophlebia malagasy*; syn. *S. apertura*) before being elevated to species by Brown and Dewhurst (1975); it is morphologically similar to *S. apertura* (Pogue 2002; Le Ru et al. 2018), and we can reasonably hypothesize that the two are sister species. Finally, although *S. roseae* is morphologically quite peculiar (Todd and Poole 1980), the morphological analyses of Pogue (2002) consistently associate it with a group of nine other species from the Western hemisphere.

Overall, the branch support for relationships among *Spodoptera* species is high but there remains uncertainty in the placement of taxa for which we have few molecular data. This is especially so for *S. apertura*, for which only *cox1* data was available. In our study its placement as sister to the two FAW strains is only weakly supported (TBE of 63%, BS of 26%, and PP of 0.61) and potentially artifactual; this grouping was not recovered in other studies based on the analysis of the *cox1* gene alone (Dumas et al. 2015a; Le Ru et al. 2018) and it is not supported by morphological evidence (Pogue 2002; Le Ru et al. 2018). *Spodoptera apertura* is widely distributed in the Afrotropical, Oriental and Australasian regions and is also morphologically quite variable (especially in the coloration of forewings; Brown and Dewhurst 1975). A re-examination of male and female genitalia of Asian and African *S. apertura* specimens (Le Ru et al. 2018) also revealed no uniquely shared features. Male genitalia of Asian specimens we examined have a straight aedeagus (versus curved), a cucullus broadly rounded apically (versus evenly rounded apically), and a shorter ampulla, longer valvula, and longer costal process than African specimens. The female genitalia of Asian specimens have a shorter signum, spatulate apophyses anteriores, and a longer corpus bursae than African specimens. *Spodoptera apertura* had been considered two distinct species, with *S. leucophlebia* (Hampson) in the Afrotropics (mostly in southern Africa and Madagascar) and *S. apertura* in the Oriental and Australasian regions; since the only specimens that have been sequenced originate from Australia, we cannot evaluate these taxa without additional sequences from Asia and Africa. In contrast, we obtained moderate (for *S. hipparis* sister to [*S. exigua* + *S.* sp. nr. *exigua*]) to strong support (for *S.* sp. nr. *exigua* sister to *S. exigua* and *S. umbraculata* sister to *S. depravata*) for the phylogenetic placement of three other taxa for which only *cox1* data were available. The position of *S.* sp. nr. *exigua* was expected because it is generally treated as a subspecies, *S. exigua antipodea,* which was described by Warren on the basis of subtle differences in forewing coloration patterns. Interestingly, Warren (1914: 323) stated that 'this form from N.S. Wales is probably a good species'. The position of *S. umbraculata* is also strongly supported by morphological evidence, including larval and male genitalic characters (Pogue 2002). Little morphological evidence supports the placement of *S. hipparis* as sister to *S. exigua* and *S.* sp. nr. *exigua*; it was thought that both the latter species share the absence of a large scale tuft associated with the 8th abdominal segment in the female (Todd and Poole 1980), but this character was actually overlooked, as pointed out by Pogue (2011). Our study also provides for the first time strong support (TBE of 97%, BS of 75%, and PP of 0.93) for the placement of *S. pulchella* (only sequenced for *cob* and *cox1*) as sister to a clade of eight species also originating from the Western hemisphere. Given its morphological similarity to *S. ornithogalli* (Todd and Poole 1980; Pogue 2002), this placement seems plausible. The placement of *S. teferii* is also still unclear despite the fact that it was successfully sequenced for six molecular markers (*cob, cox1, rrnL, rrnS,*

28S and *Ef1a*); the species is recovered under ML as sister to a clade of nine species from the Western hemisphere (placement supported by a TBE of 93% and BS of 38%) and under BI (placement supported by a PP of 0.52; see Fig. S14) as sister to a clade of four species from the Eastern hemisphere, an instance of lack of robustness to analysis. Interestingly, in the study of Le Ru et al. (2018), *S. teferii* was consistently recovered as sister to the same clade of four species, albeit with low support (ultrafast bootstrap value of 41% and PP of 0.53). Because *S. teferii* is only known from Ethiopia, its placement as sister to the Eastern hemisphere clade composed of *S. littoralis, S. litura, S. pectinicornis* and *S. picta* is plausible but it needs to be reassessed. The instability in the placement of a few taxa, most accounted for by the impact of missing data, can also be inferred from the higher level of discrepancy between TBE and BS values. In contrast to the results of the mitogenome-based datasets, in which there were only seven instances where a node was supported by TBE $\geq$ 70% and BS <70%, analyses of the *extended* dataset recovered 11 instances (out of 46; see Fig. 2) where a node was only supported by TBE). This would seem to corroborate the utility of TBE in dealing with unstable taxa.

4.3. Implications for the ecology and evolution of the genus

We have provided a novel and robust dating framework for the genus *Spodoptera* than was previously available, reducing the estimated age of the genus by about 10 Ma from that of Kergoat et al. (2012). Our three calibration strategies consistently inferred similar median age estimates for the origin of *Spodoptera* with narrow confidence intervals (see Fig. 2). This more recent timeframe for the genus is consistent with the hypothesis that long range dispersal events (instead of vicariance events or dispersal through old land bridges) took place during the diversification of the genus (Kergoat et al. 2012). With reference to the two main clades recovered in our study, the older one (Clade II) is more speciose and includes species that exhibit the more extreme range in diet breadths, including the nearly monophagous species (*S. pectinicornis*) to highly polyphagous species such as *S. exigua, S. littoralis, S. litura* and the two FAW host strains. In this group, species for which information on larval morphology is available (i.e., all sampled species except *S. apertura, S. evanida* and *S. hipparis*) possess the common noctuid serrate mandible type (Bernays 1981), and we postulate this to be the case for all the species belonging to this clade (including the three unsampled species *S. compta, S. malagasy* and *S. roseae*, whose larvae are unknown).

The timeframe inferred for the diversification of *Spodoptera* also suggests a more recent origin - about 11-12 Ma - for the clade (Clade I) comprising species whose larvae have chisel-like mandibles. This stands in contrast to older estimates of between 17 and 24 Ma for this clade (Kergoat et al. 2012). This clade exhibits a higher level of specialization in that all its species feed almost exclusively on silica-rich C_4 grasses (Poaceae) and sedges (Cyperaceae) (see Kergoat et al. 2012 for a compilation of host-records). On the basis of their shared and highly specialized mandible type (Brown and Dewhurst 1975; Pogue 2002), we suspect species in this group are adapted to the consumption of silica-rich grass leaves. The evolution of such a feature is common in multiple lepidopteran groups that have become grass-specialists and may represent another example of what appears increasingly to be a classic case of adaptive convergence in insects (see the review of Bernays 1981). Most interestingly, the younger age inferred in this study is more consistent with our understanding of the emergence and spread of C_4 grasslands during the Miocene and Pliocene (Keeley and Rundel 2005; Edwards et al. 2010; Strömberg 2011; Estep et al. 2014); it also parallels other known radiations of lepidopteran grass specialists in relation to the extension of C_4 grasslands in the Eastern hemisphere (Toussaint et al. 2012; Kergoat et al. 2018; Toussaint et al. 2019; Halali et al. 2020).

5. Conclusion and perspectives

In this study, we generated a novel mitogenomic backbone for 14 *Spodoptera* species that we used to provide an updated phylogenetic framework for 28 of the 31 known species in the genus, identifying two ecologically-diverse clades that are recovered for the first time. Our divergence time estimates indicate a more recent origin than previously thought for *Spodoptera*. Even if the placement of a few taxa for which few molecular markers were available remain unresolved, this study represents a valuable step towards a comprehensive understanding of *Spodoptera* systematics and evolution. To further hone our understanding of the evolutionary history of the genus, additional sampling and information on species' ecology and morphology are required. The genome skimming approach implemented here may be the most cost-effective and relevant solution to completing the sampling of *Spodoptera*, especially when it comes to the sequencing of rare species that only known by a few old museum specimens (*e.g., S. compta*, only known by three specimens; Pogue 2002). Imperfect as it is, the evolutionary framework we present here will also be of use in guiding and prioritizing whole genome sequencing of new *Spodoptera* species for future comparative genomic studies. Finally, we generated detailed sets of substitution rates for the 18 genes used in our molecular dating analyses (Table S7). Due to the high degree of overlap among the results of our dating analyses, we conclude that these rate estimates will be of interest for researchers conducting molecular dating studies based on mitogenomic datasets for other lepidopteran groups.

CRediT authorship contribution statement

Gael J. Kergoat: Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Paul Z. Goldstein:** Writing - original draft, Writing - review & editing. **Bruno Le Ru:** Resources, Writing - review & editing, Visualization. **Robert L. Meagher:** Resources, Writing - review & editing. **Alberto Zilli:** Resources, Writing - review & editing, Visualization. **Andrew Mitchell:** Writing - original draft, Writing - review & editing. **Anne-Laure Clamens:** Investigation, Writing - review & editing. **Sylvie Gimenez:** Investigation, Writing - review & editing. **Jérôme Barbut:** Resources, Writing - review & editing. **Nicolas Nègre:** Resources, Writing - review & editing, Visualization. **Emmanuelle d'Alençon:** Writing - review & editing. **Kiwoong Nam:** Conceptualization, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Funding acquisition.

Acknowledgments

We thank two anonymous reviewers for their comments and suggestions on a previous version of the manuscript. We also want to thank J.L. Abbate for comments and suggestions. This work (ID 1702-018) was publicly funded through ANR (the French National Research Agency) under the "Investissements d'avenir" programme with the reference ANR-10-LABX-001-01 Labex Agro and coordinated by Agropolis Fondation under the frame of I-SITE MUSE (ANR-16-IDEX-0006). Additional funding and support was provided by the 'Plant Health and Environment' Division of INRAE (project 'Geno_Army'). The specimen of *S. littoralis* used in this study was collected in France; the use of the corresponding genetic resources (under the Nagoya protocol) was authorized thanks to the internationally recognized certificate of compliance TREL2002508S/280 delivered to the CBGP. A certificate of compliance was not requested for the specimens of *S. frugiperda* because they were collected in the USA (non-signatory of the Nagoya protocol). All other specimens used for this study were collected a long time before October 2014 and are thus not regulated by legislations of the Nagoya protocol. No conflicts of interest were discovered. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA; USDA is an equal opportunity provider and employer.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107161.

References

Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Day, R., Early, R., Godwin, J., Gomez, J., Moreno, P.G., Murphy, S.T., Oppong-Mensah, B., Phiri, N., Pratt, C., Richards, G., Silvestri, S., Witt, A., 2017. Fall armyworm: impacts and implications for Africa. Evidence note (2), September 2017. Report to DFID. Wallingford: CABI.

Bernays, E.A., 1981. Evolution of insect morphology in relation to plants. Phil. Trans. R. Soc. Lond. B. 333, 257–264. https://doi.org/10.1098/rstb.1991.0075.

Bouckaert, R.R., Heled, J., 2014. DensiTree 2: seeing trees through the forest. bioRxiv https://doi.org/10.1101/012401.

Brito, R., Specht, A., Gonçalves, G.L., Moreira, G.R.P., Carneiro, E., Santos, F.L., Roque-Specht, V.F., Mielke, O.H.H., Casagrande, M.M., 2019. *Spodoptera marima*: a new synonym of *Spodoptera ornithogalli* (Lepidoptera: Noctuidae), with notes on adult morphology, host plant use and genetic variation along its geographic range. Neotrop. Entomol. 48, 433–449. https://doi.org/10.1007/s13744-018-0654-z.

Brown, E.S., Dewhurst, C.F., 1975. The genus *Spodoptera* (Lepidoptera: Noctuidae) in Africa and the Near East. Bull Entomol. Res. 65, 221–262. https://doi.org/10.1017/S0007485300005939.

Cameron, S.L., 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. Annu. Rev. Entomol. 59, 95–117. https://doi.org/10.1146/annurev-ento-011613-162007.

Cheke, R.A., Tucker, M.R., 1995. An evaluation of potential economic returns from the strategic control approach to the management of African armyworm *Spodoptera exempta* (Lepidoptera: Noctuidae) populations in eastern Africa. Crop Prot. 14, 91–103. https://doi.org/10.1016/0261-2194(95)92862-H.

Cheng, T., Wu, J., Wu, Y., Chilukuri, R.V., Huang, L., Yamamoto, K., Feng, L., Li, W., Chen, Z., Guo, H., Liu, J., Li, S., Wang, X., Peng, L., Liu, D., Guo, Y., Fu, B., Li, Z., Liu, C., Chen, Y., Tomar, A., Hilliou, F., Montagné, N., Jacquin-Joly, E., d'Alençon, E., Seth, Bhatnagar, R.K., Jouraku, A., Shiotsuki, T., Kadono-Okuda, K., Promboon, A., Smagghe, G., Arunkumar, K.P., Kishino, H., Goldsmith, M.R., Feng, Q., Xia, Q., Mita, K., 2017. Genomic adaptation to polyphagy and insecticides in a major East Asian noctuid pest. Nat. Ecol. Evol. 1, 1747-1756. doi: 10.1038/s41559-017-0314-4.

Cho, S., Mitchell, A., Mitter, C., Regier, J., Matthews, M., Robertson, R., 2008. Molecular phylogenetics of heliothine moths (Lepidoptera: Noctuidae: Heliothinae), with comments on the evolution of host range and pest status. Syst. Entomol. 33, 581–594. https://doi.org/10.1111/j.1365-3113.2008.00427.x.

Cho, S., Zwick, A., Regier, J.,C., Mitter, C., Cummings, M.P., Yao, J.X., Du, Z.L., Zhao, H., Kawahara, A.Y., Weller, S., Davis, D.R., Baixeras, J., Brown, J.W., Parr, C., 2011. Can deliberately incomplete gene sample augmentation improve a phylogeny estimate for the advanced moths and butterflies (Hexapoda: Lepidoptera)? Syst Biol. 60, 782–796. doi: 10.1093/sysbio/syr079.

Condamine, F.L., Nabholz, B., Clamens, A.-L., Dupuis, J.R., Sperling, F.A.H., 2018. Mitochondrial phylogenomics, the origin of swallowtail butterflies, and the impact of the number of clocks in Bayesian molecular dating. Syst. Entomol. 43, 460–480. https://doi.org/10.1111/syen.12284.

Crête-Lafrenière, A., Weir, L.K., Bernatchez, L., 2012. Framing the Salmonidae family phylogenetic portrait: a more complete picture from increased taxon sampling. PLoS One 7, e46662. https://doi.org/10.1371/journal.pone.0046662.

Dierckxsens, N., Mardulyn, P., Smits, G., 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45, e18 https://doi.org/10.1093/nar/gkw955.

Dodsworth, S., 2015. Genome skimming for next-generation biodiversity analysis. Trends Plant. Sci. 20, 525–527. https://doi.org/10.1016/j.tplants.2015.06.012.

Douglas, S.D., 1991. Flower Form and Pollinator Diversity in the Middle Eocene of British Columbia and Washington. Thesis, University of Alberta, Edmonton, M. Sc.

Douglas, S.D., Stockey, R.A., 1996. Insect fossils in middle Eocene deposits from British Columbia and Washington State: faunal diversity and geological range extensions. Rev. Canad. Zool. 74, 1140–1157. https://doi.org/10.1139/z96-126.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88 https://doi.org/10.1371/journal.pbio.0040088.

Dumas, P., Barbut, J., Le Ru, B.P., Silvain, J.-F., Clamens, A.-L., d'Alençon, E., Kergoat, G. J., 2015a. Phylogenetic molecular species delimitations unravel potential new species in the pest genus *Spodoptera* Guenée, 1852 (Lepidoptera, Noctuidae). PLoS One 10, e0122407. https://doi.org/10.1371/journal.pone.0122407.

Dumas, P., Legeai, F., Lemaitre, C., Scaon, E., Orsucci, M., Labadie, K., Gimenez, S., Clamens, A.-L., Henri, H., Vavre, F., Aury, J.-M., Fournier, P., Kergoat, G.J., d'Alençon, E., 2015b. *Spodoptera frugiperda* host plant variants: two strains or two distinct species? Genetica 143, 305–316. https://doi.org/10.1007/s10709-015-9829-2.

Edwards, E.J., Osborne, C.P., Strömberg, C.A.E., Smith, S.A., C4 Grasses Consortium, 2010. The origins of C4 grasslands: integrating evolutionary and ecosystem science. Science 328, 587–591. doi: 10.1126/science.1177216.

Erixon, P., Svennblad, B., Britton, T., Oxelman, B., 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. Syst. Biol. 52, 665–673. https://doi.org/10.1080/10635150390235485.

Estep, M.C., McKain, M.R., Diaz, D.V., Zhong, J., Hodge, J.G., Hodkinson, T.R., Layton, D.J., Malcomber, S.T., Pasquet, R., Kellogg, E.A., 2014. Allopolyploidy, diversification, and the Miocene grassland expansion. Proc. Natl Acad. Sci. USA 111, 15149–15154. https://doi.org/10.1073/pnas.1404177111.

FAO, 2020. The global action for fall armyworm control: action framework 2020–2022. Working together to tame the global threat - Rome. https://doi.org/10.4060/ca9252en.

Fibiger, M., Ronkay, L., Steiner, A., Zilli, A., 2009. Noctuidae Europaeae. Vol. 11, Pantheinae, Dilobinae, Acronictinae, Eustrotiinae, Nolinae, Bagisarinae, Acontiinae, Metoponiinae, Heliothinae, and Bryophilinae. Entomological Press, Sorø.

Gernhard, T., 2008. The conditioned reconstructed process. J. Theor. Biol. 253, 769–778. https://doi.org/10.1016/j.jtbi.2008.04.005.

Gloss, A.D., Abbot, P., Whiteman, N.K., 2019. How interactions with plant chemicals shape insect genomes. 36, 149–156. Curr. Opin. Insect Sci. doi: 10.1016/j.cois.2019.09.005.

Goergen, G., 2018. New alien invasive pest identified in West and Central Africa! IITA Factsheet, Cotonou, Benin.

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A., Tamò, M., 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11, e0165632. https://doi.org/10.1371/journal.pone.0165632.

Goldstein, P.Z., 2017. Diversity and significance of Lepidoptera: a phylogenetic perspective. In: Foottit, R.G., Adler, P.H. (Eds.), Insect biodiversity: Science and society. Volume 1, second edition. John Wiley & Sons Ltd., Hoboken, New Jersey, pp. 463–495. doi: 10.1002/9781118945568.ch13.

Gouin, A., Bretaudeau, A., Nam, K., Gimenez, S., Aury, J.-M., Duvic, B., Hilliou, F., Durand, N., Montagné, N., Darboux, I., Kuwar, S., Chertemps, T., Siaussat, D., Bretschneider, A., Moné, Y., Ahn, S.-J., Hänniger, S., Gosselin Grenet, A.-S., Neunemann, D., Maumus, F., Luyten, I., Labadie, K., Xu, W., Koutroumpa, F., Escoubas, J.-M., Llopis, A., Maïbèche, M., Salasc, F., Tomar, A., Anderson, A., Khan, S.A., Dumas, P., Orsucci, M., Guy, J., Belser, C., Alberti, A., Noel, B., Couloux, A., Mercier, J., Nidelet, S., Dubois, E., Liu, N.-Y., Boulogne, I., Mirabeau, O., Le Goff, G., Gordon, K., Oakeshott, J., Consoli, F., Volkoff, A.-N., Fescemyer, H., Marden, J., Luthe, D., Herrero, S., Heckel, D., Wincker, P., Kergoat, G. J., Amselem, J., Quesneville, H., Groot, A., Jacquin-Joly, E., Nègre, N., Lemaitre, C., Legeai, F., d'Alençon, E., Fournier, P., 2017. Two genomes of highly polyphagous lepidopteran pests (*Spodoptera frugiperda*, Noctuidae) with different host-plant ranges. Sci. Rep. 7, 11816. https://doi.org/10.1038/s41598-017-10461-4.

Groot, A.T., Marr, M., Heckel, D.G., Schöfl, G., 2010. The roles and interactions of reproductive isolation mechanisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ecol. Entomol. 35, 105–118. https://doi.org/10.1111/j.1365-2311.2009.01138.x.

Hacker, H., Ronkay, L., Hreblay, M., 2002. Noctuidae Europaeae. 4. Hadeninae I. Entomological Press, Sorø.

Haggis, M.J., 1986. Distribution of the African armyworm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae), and the frequency of larval outbreaks in Africa and Arabia. Bull. Entomol. Res. 76, 151–170. https://doi.org/10.1017/S0007485300015376.

Halali, S., Brakefield, P.M., Collins, S.C., Brattström, O., 2020. To mate, or not to mate: The evolution of reproductive diapause facilitates insect radiation into African savannahs in the Late Miocene. J. Anim. Ecol. 89, 1230–1241. https://doi.org/10.1111/1365-2656.13178.

Hampson, G.F., 1909. Catalogue of the Lepidoptera Phalaenae in the British Museum, Volume 8. Taylor and Francis, London.

Hänniger, S., 2015. Chasing sympatric speciation - The relative importance and genetic basis of prezygotic isolation barriers in diverging populations of *Spodoptera frugiperda*. PhD thesis. University of Amsterdam, The Netherlands.

Hillis, D.M., Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42, 182–192. https://doi.org/10.1093/sysbio/42.2.182.

Huelsenbeck, J.P., Larget, B., Alfaro, M.E., 2004. Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. Mol. Biol. Evol. 21, 1123–1133. https://doi.org/10.1093/molbev/msh123.

Hurvich, C.M., Tsai, C.-L., 1989. Regression and time series model selection in small samples. Biometrika 76, 297–307. https://doi.org/10.1093/biomet/76.2.297.

Jin, M., Zwick, A., Slipinski, A., Dekeyzer, R., Pang, H., 2020. Museomics reveals extensive cryptic diversity of Australian prionine longhorn beetles with implications for their classification and conservation. Syst. Entomol. 45, 745–770. https://doi.org/10.1111/syen.12424.

Juárez, M.L., Schhöfl, G., Vera, M.T., Vilardi, J.C., Murua, M.G., Willink, E., Hänniger, S., Heckel, D.G., Groot, A.T., 2014. Population structure of *Spodoptera frugiperda* maize and rice host forms in South America: are they host strains? Entomol Exp Appl. 152, 182–199. https://doi.org/10.1111/eea.12215.

Källersjö, M., Albert, V.A., Farris, J.S., 1999. Homoplasy increases phylogenetic structure. Cladistics 15, 91–93. https://doi.org/10.1111/j.1096-0031.1999.tb00400.x.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Kawahara, A.Y., Plotkin, D., Espeland, M., Meusemannd, K., Toussaint, E.F.A., Donathe, A., Gimniche, F., Frandsen, P.B., Zwick, A., dos Reis, M., Barber, J.R., Peters, R.S., Liu, S., Zhoum, X., Mayer, C., Podsiadlowski, L., Storer, C., Yack, J.E., Misof, B., Breinholt, J.W., 2019. Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. Proc. Natl. Acad. Sci. USA 116, 22657–22663. https://doi.org/10.1073/pnas.1907847116.

Keegan, K.L., Lafontaine, J.D., Wahlberg, N., Wagner, D.L., 2019. Towards resolving and redefining Amphipyrinae (Lepidoptera, Noctuoidea, Noctuidae): a massively

polyphyletic taxon. Syst. Entomol. 44, 451–464. https://doi.org/10.1111/syen.12336.
Keegan, K.L., Rota, J., Zahiri, R., Zilli, A., Wahlberg, N., Schmidt, B.C., Lafontaine, J.D., Goldstein, P.Z., Wagner, D.L., 2021. Toward a stable global Noctuidae (Lepidoptera) taxonomy. Insect Syst. Divers. in press. doi: 10.1093/isd/ixab005.
Keeley, J.E., Rundel, P.W., 2005. Fire and the Miocene expansion of C_4 grasslands. Ecol. Lett. 8, 683–690. https://doi.org/10.1111/j.1461-0248.2005.00767.x.
Kergoat, G.J., Condamine, F.L., Toussaint, E.F.A., Capdevielle-Dulac, C., Clamens, A.-L., Barbut, J., Goldstein, P.Z., Le Ru, B., 2018. Opposite macroevolutionary responses to environmental changes in grasses and insects during the Neogene grassland expansion. Nat. Commun. 9, 5089. https://doi.org/10.1038/s41467-018-07537-8.
Kergoat, G.J., Prowell, D.P., Le Ru, B.P., Mitchell, A., Dumas, P., Clamens, A.-L., Condamine, F.L., Silvain, J.-F., 2012. Disentangling dispersal and vicariance patterns in armyworms: evolution and historical biogeography of the pest genus *Spodoptera* (Lepidoptera: Noctuidae). Mol. Phylogenet. Evol. 65, 855–870. https://doi.org/10.1016/j.ympev.2012.08.006.
Kim, M.J., Kim, I., Cameron, S.L., 2020. How well do multispecies coalescent methods perform with mitochondrial genomic data? A case study of butterflies and moths (Insecta: Lepidoptera). Syst. Entomol. 45, 857–873. https://doi.org/10.1111/syen.12431.
Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120. https://doi.org/10.1007/BF01731581.
Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200. https://doi.org/10.1073/pnas.86.16.6196.
Kozlov, A.M., Darriba, D., Flouri, T., Morel, B., Stamatakis, A., 2019. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35, 4453–4455. https://doi.org/10.1093/bioinformatics/btz305.
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549. https://doi.org/10.1093/molbev/msy096.
Lafontaine, J.D., Schmidt, B.C., 2010. Annotated check list of the Noctuoidea (Insecta, Lepidoptera) of North America north of Mexico. ZooKeys 40, 1–239. https://doi.org/10.3897/zookeys.40.414.
Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34, 772–773. https://doi.org/10.1093/molbev/msw260.
Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359. https://doi.org/10.1038/nmeth.1923.
Lemoine, F., Domelevo-Entfellner, J.B., Wilkinson, E., Correia, D., Felipe, M.D., De Oliveira, T., Gascuel, O., 2018. Renewing Felsenstein's phylogenetic bootstrap in the era of big data. Nature 556, 452–456. https://doi.org/10.1038/s41586-018-0043-0.
Le Ru, B., Barbut, J., Capdevielle-Dulac, C., Muluken, G., Kergoat, G.J., 2018. Re-establishment of *Spodoptera teferii* Laporte in Rougeot (Lepidoptera, Noctuidae, Noctuinae), with an updated molecular phylogeny for the genus *Spodoptera* Guenée. Ann. Soc. Entomol. Fr. (N.S.) 54, 497–510. https://doi.org/10.1080/00379271.2018.1528886.
Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 1000 genome project data processing subgroup, 2009. The sequence alignment/map format and SAMtools. Bioinformatics, 25, 2078–2079. doi: 10.1093/bioinformatics/btp352.
Maddison, W.P., Maddison, D.R., 2019. Mesquite: a modular system for evolutionary analysis. Version 3.61 <http://www.mesquiteproject.org>.
Matos-Maraví, P., Ritter, C.D., Barnes, C.J., Nielsen, M., Olsson, U., Wahlberg, N., Marquina, D., Sääksjärvi, I., Antonelli, A., 2019. Biodiversity seen through the perspective of insects: 10 simple rules on methodological choices and experimental design for genomic studies. PeerJ 7, e6727. https://doi.org/10.7717/peerj.6727.
Meagher, R.L., Nagoshi, R.N., Stuhl, C., Mitchell, E.R., 2004. Larval development of fall armyworm (Lepidoptera: Noctuidae) on different cover crop plants. Fla. Entomol. 87, 454–460. https://doi.org/10.1653/0015-4040(2004)087[0454:LDOFAL]2.0.CO;2.
Meng, G., Li, Y., Yang, C., Liu, S., 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Res. 47, e63. https://doi.org/10.1093/nar/gkz173.
Miller, M.A., Schwartz, T., Pickett, B.E., He, S., Klem, E.B., Scheuermann, R.H., Passarotti, M., Kaufman, S., O'Leary, M.A., 2015. A RESTful API for access to phylogenetic tools via the CIPRES Science Gateway. Evol. Bioinform. 11, 43–48. https://doi.org/10.4137/EBO.S21501.
Mirarab, S., Reaz, R., Bayzid, M.S., Zimmermann, T., Swenson, M.S., Warnow, T., 2014. ASTRAL: genome-scale coalescent-based species tree estimation. Bioinformatics 30, 541–548. https://doi.org/10.1093/bioinformatics/btu462.
Mirarab, S., Warnow, T., 2015. ASTRAL-II: coalescent-based species tree estimation with many hundreds of taxa and thousands of genes. Bioinformatics 31, 44–52. https://doi.org/10.1093/bioinformatics/btv234.
Mitchell, A., Mitter, C., Regier, J., 2006. Systematics and evolution of the cutworm moths (Lepidoptera: Noctuidae): evidence from two protein-coding nuclear genes. Syst. Entomol. 31, 21–46. https://doi.org/10.1111/j.1365-3113.2005.00306.x.
Montezano, D.G., Specht, A., Sosa-Gómez, D.R., Roque-Specht, V.F., Sousa-Silva, J.C., Paula-Moraes, S.V., Peterson, J.A., Hunt, T.E., 2018. Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. Afr. Entomol. 26, 286–300. https://doi.org/10.4001/003.026.0286.
Mutanen, M., Wahlberg, N., Kaila, L., 2010. Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. Proc. Biol. Sci. B 277, 2839–2848. https://doi.org/10.1098/rspb.2010.0392.
Nagoshi, K.L., Allan, S.A., Meagher, R.L., 2020. Assessing the use of wing morphometrics to identify fall armyworm (Lepidoptera: Noctuidae) host strains in field collections. J. Econ. Entomol. 113, 800–807. https://doi.org/10.1093/jee/toz344.
Nagoshi, R.N., Koffi, D., Agboka, K., Tounou, K.A., Banerjee, R., Jurat-Fuentes, J.L., Meagher, R.L., 2017. Comparative molecular analyses of invasive fall armyworm in Togo reveal strong similarities to populations from the eastern United States and the Greater Antilles. PLoS One 12, e0181982. https://doi.org/10.1371/journal.pone.0181982.
Nagoshi, R.N., Goergen, G., Plessis, H.D., van den Berg, J., Meagher, R., 2019. Genetic comparisons of fall armyworm populations from 11 countries spanning sub-Saharan Africa provide insights into strain composition and migratory behaviors. Sci. Rep. 9, 8311. https://doi.org/10.1038/s41598-019-44744-9.
Nagoshi, R.N., Meagher, R.L., Hay-Roe, M., 2012a. Inferring the annual migration patterns of fall armyworm (Lepidoptera: Noctuidae) in the United States from mitochondrial haplotypes. Ecol. Evol. 2, 1458–1467. https://doi.org/10.1002/ece3.268.
Nagoshi, R.N., Murúa, M.G., Hay-Roe, M., Juárez, M.L., Willink, E., Meagher, R.L., 2012b. Genetic characterization of fall armyworm (Lepidoptera: Noctuidae) host strains in Argentina. J. Econ. Entomol. 105, 418–428. https://doi.org/10.1603/EC11332.
Nie, R.-E., Andújar, C., Gómez-Rodriguez, C., Bai, M., Xue, X.-J., Tang, M., Yang, C.-T., Tang, P., Yang, X.-K., Vogler, A.P., 2020. The phylogeny of leaf beetles (Chrysomelidae) inferred from mitochondrial genomes. Syst. Entomol. 45, 188–204. https://doi.org/10.1111/syen.12387.
Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian Phylogenetic Analysis of Combined Data. Syst. Biol. 53, 47–67. https://doi.org/10.1080/10635150490264699.
Pashley, D.P., 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? Entomol. Soc. Am. 79, 898–904.
Pashley, D.P., Martin, J.A., 1987. Reproductive incompatibility between host strains of fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 80, 731–733. https://doi.org/10.1093/aesa/80.6.731.
Pashley, D.P., Hammond, A.M., Hardy, T.N., 1992. Reproductive isolating mechanisms in fall armyworm host strains (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 85, 400–405. https://doi.org/10.1093/aesa/85.4.400.
Pogue, M.G., Passoa, S., 2000. *Spodoptera ochrea* (Lepidoptera: Noctuidae): a new host record (*Asparagus*) from Peru and description of the female genitalia. Ann. Entomol. Soc. Am. 93, 1019–1021. https://doi.org/10.1603/0013-8746(2000)093[1019:SOLNAN]2.0.CO;2.
Pogue, M.G., 2002. A world revision of the genus *Spodoptera* Guenée (Lepidoptera: Noctuidae). Mem. Am. Entomol. Soc. 43, 1–202.
Pogue, M.G., 2011. Using genitalia characters and mitochondrial COI sequences to place "*Leucochlaena*" *hipparis* (Druce) in *Spodoptera* Guenée (Lepidoptera: Noctuidae). Proc. Entomol. Soc. Wash. 113, 497–507. https://doi.org/10.4289/0013-8797.113.4.497.
Prowell, D.P., McMichael, M., Silvain, J.-F., 2004. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 97, 1034–1044. https://doi.org/10.1603/0013-8746(2004)097[1034: MGAOHU]2.0.CO;2.
Quisenberry, S.S., 1991. Fall armyworm (Lepidoptera: Noctuidae) host strain reproductive compatibility. Fla. Entomol. 74, 194–199. https://doi.org/10.2307/3495297.
Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67, 901–904. https://doi.org/10.1093/sysbio/syy032.
Regier, J.C., Mitter, C., Mitter, K., Cummings, M.P., Bazinet, A.L., Hallwachs, W., Janzen, D.H., Zwich, A., 2017. Further progress on the phylogeny of Noctuoidea (Insecta: Lepidoptera) using an expanded gene sample. Syst. Entomol. 42, 82–93. https://doi.org/10.1111/syen.12199.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. https://doi.org/10.1093/sysbio/sys029.
Sayyari, E., Mirarab, S., 2016. Fast coalescent-based computation of local branch support from quartet frequencies. Mol. Biol. Evol. 33, 1654–1668. https://doi.org/10.1093/molbev/msw079.
Schöfl, G., Dill, A., Heckel, D.G., Groot, A.T., 2011. Allochronic separation versus mate choice: nonrandom patterns of mating between fall armyworm host strains. Am. Nat. 177, 470–485. https://doi.org/10.1086/658904.
Schubert, M., Lindgreen, S., Orlando, L., 2016. AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Res. Notes 9, 88. https://doi.org/10.1186/s13104-016-1900-2.
Sohn, J.C., Labandeira, C., Davis, D., Mitter, C., 2012. An annotated catalog of fossil and subfossil Lepidoptera (Insecta: Holometabola) of the world. Zootaxa 3286, 1–132. https://doi.org/10.11646/zootaxa.3286.1.1.
Sohn, J.C., Labandeira, C., Davis, D., Mitter, C., 2015. The fossil record and taphonomy of butterflies and moths (Insecta, Lepidoptera): implications for evolutionary diversity and divergence-time estimates. BMC Evol. Biol. 15, 12. https://doi.org/10.1186/s12862-015-0290-8.
Sohn, J.C., Lamas, G., 2013. Corrections, additions, and nomenclatural notes to the recently published World catalog of fossil and subfossil Lepidoptera. Zootaxa 3599, 395–399. https://doi.org/10.11646/zootaxa.3599.4.8.

Song, N., Li, X., Li, X., Yin, X., Pan, P., 2019. The mitochondrial genomes of palaeopteran insects and insights into the early insect relationships. Sci. Rep. 9, 17765. https://doi.org/10.1038/s41598-019-54391-9.
Sparks, A.N., 1979. A review of the biology of the fall armyworm. Fla. Entomol. 62, 82–87. https://doi.org/10.2307/3494083.
Sparks, T.H., Dennis, R.L.H., Croxton, P.J., Cade, M., 2007. Increased migration of Lepidoptera linked to climate change. Eur. J. Entomol. 104, 139–143. https://doi.org/10.14411/eje.2007.019.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
Strömberg, C.A.E., 2011. Evolution of grasses and grassland ecosystems. Annu. Rev. Earth Planet. Sci. 39, 517–544. https://doi.org/10.1146/annurev-earth-040809-152402.
Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A., 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus. Evolution 4, vey016. https://doi.org/10.1093/ve/vey016.
Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599. https://doi.org/10.1093/molbev/msm092.
Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Am. Math. Soc. Lect. Math. Life Sci. 17, 57–86.
Tay, W.T., Rane, R., Padovan, A., Walsh, T., Elfekih, S., Downes, S., Nam, K., d'Alençon, E., Zhang, J., Wu, Y., Nègre, N., Kunz, D., Kriticos, D.J., Czepak, C., Otim, M., Gordon, K.H.J., 2020. Whole genome sequencing of global Spodoptera frugiperda populations: evidence for complex, multiple introductions across the Old World. bioRxiv 2020.06.12.147660 doi: 10.1101/2020.06.12.147660.
Timmermans, M.J.T.N., Barton, C., Haran, J., Ahrens, D., Culverwell, C.L., Ollikainen, A., Dodsworth, S., Foster, P.F., Bocak, L., Vogler, A.P., 2016. Family-level sampling of mitochondrial genomes in Coleoptera: compositional heterogeneity and phylogenetics. Genome Biol. Evol. 8, 161–175. https://doi.org/10.1093/gbe/evv241.
Timmermans, M.J.T.N., Lees, D.C., Simonsen, T.J., 2014. Towards a mitogenomic phylogeny of Lepidoptera. Mol. Phylogenet. Evol. 79, 169–178. https://doi.org/10.1016/j.ympev.2014.05.031.
Todd, E.L., Poole, R.W., 1980. Keys and illustrations for the armyworm moths of the noctuid genus *Spodoptera* Guenée from the Western Hemisphere. Ann. Entomol. Soc. Am. 73, 722–738. https://doi.org/10.1093/aesa/73.6.722.
Toussaint, E.F.A., Condamine, F.L., Kergoat, G.J., Silvain, J.-F., Capdevielle-Dulac, C., Barbut, J., Le Ru, B.P., 2012. Palaeoenvironmental shifts drove the adaptive radiation of a noctuid stemborer tribe (Lepidoptera, Noctuidae, Apameini) in the Miocene. PLoS One 7, e41377. https://doi.org/10.1371/journal.pone.0041377.
Toussaint, E.F.A., Vila, R., Yago, M., Chiba, H., Warren, A.D., Aduse-Poku, K., Storer, C., Dexter, K.M., Maruyama, K., Lohman, D.J., Kawahara, A.Y., 2019. Out of the Orient: Post-Tethyan transoceanic and trans-Arabian routes fostered the spread of Baorini skippers in the Afrotropics. Syst. Entomol. 44, 926–938. https://doi.org/10.1111/syen.12365.
van der Gaag, D., van der Straten, M., 2017. Assessment of the impact of American Spodoptera species for the European Union. Netherlands Food and Consumer Product Safety Authority, Utrecht, the Netherlands.
Velásquez-Vélez, M.I., Saldamando-Benjumea, C.I., Ríos-Diez, J.D., 2011. Reproductive isolation between two populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected in corn and rice fields from Central Colombia. Ann Entomol. Soc. Am. 104, 826–833. https://doi.org/10.1603/AN10164.
Wahlberg, N., Wheat, C.W., Peña, C., 2013. Timing and patterns in the taxonomic diversification of Lepidoptera (butterflies and moths). PLoS One 8, e80875. https://doi.org/10.1371/journal.pone.0080875.
Walker, J.D., Geissman, J.W., Bowring, S.A., Babcock, L.E., 2018. Geologic Time Scale v. Geological Society of America. <https://www.geosociety.org/documents/gsa/timescale/timescl.pdf>, 5.0.
Wang, Y., Li, X., Garzon-Ordun, I.J., Winterton, S.L., Yan, Y., Aspöck, U., Aspöck, H., Yang, D., 2017. Mitochondrial phylogenomics illuminates the evolutionary history of Neuropterida. Cladistics 33, 617–636. https://doi.org/10.1111/cla.12186.
Warren, W., 1914. 2. Family: Noctuidae [part]. In: Seitz, A. (Ed.), The Macrolepidoptera of the World. XI. Volume: Noctuiform Phalaenae. Verlag des Seitz'schen Werkes (Alfred Kernen), Stuttgart, pp. 321–344.
Wiens, J.J., 2005. Can incomplete taxa rescue phylogenetic analyses from long-branch attraction? Syst. Biol. 54, 731–742. https://doi.org/10.1371/journal.pone.0042925.
Wiens, J.J., Tiu, J., 2012. Highly incomplete taxa can rescue phylogenetic analyses from the negative impacts of limited taxon sampling. PLoS One 7, 42925. https://doi.org/10.1371/journal.pone.0042925.
Wilson, J.W., 1932. Notes on the biology of *Laphygma exigua* Huebner. Fla. Entomol. 16, 33–39. https://doi.org/10.2307/3492536.
Wolfe, J.A., Wehr, W., 1987. Middle Eocene dicotyledonous plants from Republic. Northeastern Washington. US Geol. Surv. Bull, No, p. 1597.
Yainna, S., Tay, W.T., Fiteni, E., Legeai, F., Clamens, A.-L., Gimenez, S., Frayssinet, M., Asokan, R., Kalleshwaraswamy, C.M., Meagher, Jr., R.L., Blanco, C.A., Silvie, P., Brévault, T., Dassou, A., Kergoat, G.J., Walsh, T., Gordon, K., Nègre, N., d'Alençon, E., Nam, K., 2020. Genomic balancing selection is key to the invasive success of the fall armyworm. bioRxiv 2020.06.17.154880 doi: 10.1101/2020.06.17.154880.
Yang, X., Cameron, S.L., Lees, D.C., Xue, D., Han, H., 2015. A mitochondrial genome phylogeny of owlet moths (Lepidoptera: Noctuoidea), and examination of the utility of mitochondrial genomes for lepidopteran phylogenetics. Mol. Phylogenet. Evol. 85, 230–237. https://doi.org/10.1016/j.ympev.2015.02.005.
Yang, X.S., Xue, D.Y., Han, H.X., 2013. The complete mitochondrial genome of *Biston panterinaria* (Lepidoptera: Geometridae), with phylogenetic utility of mitochondrial genome in the Lepidoptera. Gene 515, 349–358. https://doi.org/10.1016/j.gene.2012.11.031.
Yang, Z., 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J. Mol. Evol. 39, 306–314. https://doi.org/10.1007/BF00160154.
Yassin, A., Debat, V., Bastide, H., Gidaszewski, N., David, J.R., Pool, J.E., 2016. Recurrent specialization on a toxic fruit in an island *Drosophila* population. Proc. Natl. Acad. Sci. USA 113, 4771–4776. https://doi.org/10.1073/pnas.1522559113.
Young, A.D., Gillung, J.P., 2020. Phylogenomics - principles, opportunities and pitfalls of big-data phylogenetics. Syst. Entomol. 45, 225–247. https://doi.org/10.1111/syen.12406.
Xi, Z., Liu, L., Davis, C.C., 2016. The impact of missing data on species tree estimation. Mol. Biol. Evol. 33, 838–860. https://doi.org/10.1093/molbev/msv266.
Xia, X., 2018. DAMBE7: New and improved tools for data analysis in molecular biology and evolution. Mol. Biol. Evol. 35, 1550–1552. https://doi.org/10.1093/molbev/msy073.
Xia, X., Lemey, P., 2009. Assessing substitution saturation with DAMBE. In: Lemey, P., Salemi, M., Vandamme, A.M. (Eds.), The phylogenetic handbook. Cambridge University Press, Cambridge, pp. 615–630.
Xia, X., Xie, Z., Salemi, M., Chen, L., Wang, Y., 2003. An index of substitution saturation and its application. Mol. Phylogenet. Evol. 26, 1–7. https://doi.org/10.1016/S1055-7903(02)00326-3.
Zahiri, R., Lafontaine, J.D., Holloway, J.D., Kitching, I.J., Schmidt, B.C., Kaila, L., Wahlberg, N., 2013. Major lineages of Nolidae (Lepidoptera, Noctuoidea) elucidated by molecular phylogenetics. Cladistics 29, 337–359. https://doi.org/10.1111/cla.12001.
Zhang, C., Rabiee, M., Sayyari, E., Mirarab, S., 2018. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. BMC Bioinformatics 19, 153. https://doi.org/10.1186/s12859-018-2129-y.
Zhang, F., Zhang, J., Yang, Y., Wu, Y., 2019. A chromosome-level genome assembly for the beet armyworm (Spodoptera exigua) using PacBio and Hi-C sequencing. bioRxiv 2019.12.26.889121 doi: 10.1101/2019.12.26.889121.
Zhang, L., Liu, B., Zheng, W., Liu, C., Zhang, D., Zhao, S., Li, Z., Xu, P., Wilson, K., Withers, A., Jones, C.M., Smith, J.A., Chipabika, G., Kachigamba, D.L., Nam, K., d'Alençon, E., Liu, B., Linag, X., Jin, M., Wu, C., Chakrabarty, S., Yang, X., Jiang, Y., Liu, J., Liu, X., Quan, W., Wang, G., Fan, W., Qian, W., Wu, K., Xiao, Y., 2020. Genetic structure and insecticide resistance characteristics of fall armyworm populations invading China. Mol. Ecol. Res. 20, 1682–1696. https://doi.org/10.1111/1755-0998.13219.