

Revised status of *Chloridea* Duncan and (Westwood), 1841, for the *Heliothis virescens* species group (Lepidoptera: Noctuidae: Heliothinae) based on morphology and three genes

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Abstract. The Heliothinae comprise some of the world's most injurious agricultural pests. This study reanalyses a subsample of the *Heliothis* group to determine the monophyly of *Chloridea* (*Heliothis virescens* and *H. subflexa*). Two nuclear gene regions, *elongation factor-1 α* (*EF-1 α* ; 1240 bp) and *dopa decarboxylase* (*DDC*; 687 bp), and the barcoding region of mitochondrial *cytochrome oxidase I* (*COI*; 708 bp) were used in this analysis for a total of 2635 bp and a morphological dataset of 20 characters and 62 character states. Sixteen species representing five genera plus two outgroup species were used in the analysis. Analyses used were Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). The revised status for the monophyletic genus *Chloridea* Duncan and (Westwood) was supported by a very strong bootstrap support (BP = 98–100). Larval host-plant usage is discussed within the *Heliothis* clade. Polyphagy is most likely the ancestral condition with a host shift to monophagy and oligophagy. Based on known larval hosts, *Heliocheilus* is oligophagous on Poaceae. Traits of host plant use in *Helicoverpa* and *Chloridea* where both polyphagy and oligophagy occur in closely related species are discussed.

Introduction

Heliothinae (Noctuidae) currently comprises 381 named species worldwide with several unnamed species awaiting description. The heliothines include some of the most destructive agricultural pests in the world with some of the most significant being species in the genera *Helicoverpa* Hardwick and *Heliothis* Ochseneimer. Larvae are generally highly polyphagous and feed on both fruits and flowers, thus resulting in more economic damage than if feeding on the leaves only. Important crops attacked by these species include corn, cotton, wheat, tomatoes, alfalfa, quinoa, tobacco, soybean, various vegetable crops and many native weeds (Sudbrink & Grant, 1995). Damage by heliothine pests results in billions of dollars of losses annually to agricultural crops throughout

the world (Fitt, 1989). Cho *et al.* (2008) emphasized the importance of a reliable classification and phylogeny for workers to communicate, organize and predict how traits important to their management, especially polyphagy, evolve.

Hardwick (1965) was the first to recognize that the species related to the Old World bollworm, *Helicoverpa armigera* (Hübner), and the corn earworm, *Helicoverpa zea* (Boddie), did not belong in *Heliothis* Ochseneimer, but constituted a separate monophyletic group. He described a new genus, *Helicoverpa* Hardwick, to include these species. Other morphology-based studies have increased our knowledge of heliothine systematics (Hardwick, 1970a, 1996; Matthews, 1991, 1999; Poole *et al.*, 1993; Pogue & Harp, 2003a, 2003b, 2003c, 2004, 2005; Pogue, 2006, 2007). Heliothinae genera are generally defined based on structures of the male and female genitalia (Hardwick, 1970a). These structures – especially the male genitalia – are simplified and thus make informative phylogenetic characters more difficult to recognize. An example of this difficulty can be found in Matthews (1991), who could not resolve the 'Pyrrhia-group'

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Table 1. Specimens examined with locality and gene Genbank accession numbers.

Species	Locality	<i>EF-1α</i>	<i>DDC</i>	<i>COI</i>
<i>Helicoverpa armigera armigera</i>	Thailand	U20129	U71411	EU768935
<i>Helicoverpa assulta</i>	Thailand	EU769062	EU769006	EU768937
<i>Helicoverpa gelotopoeon</i>	Argentina, lab colony	U20132	U71418	EU768938
<i>Helicoverpa zea</i>	USA, Mississippi, Maryland	U20136	U71429	EU768942
<i>Heliocheilus paradoxus</i>	USA, Texas	EU769046	EU768987	EU768915
<i>Heliocheilus toralis</i>	USA, Texas	EU769048	EU768989	EU768917
<i>Heliothis virescens</i>	USA, Mississippi, lab colony	U20135	U71428	EU768933
<i>Heliothis subflexa</i>	USA, Florida, lab colony	U20134	EU769003	EU768932
<i>Heliothis oregonica</i>	USA, California	EU769056	EU768998	EU768927
<i>Heliothis viriplaca</i>	Finland, Uusimaa	EU769061	EU769004	EU768934
<i>Heliothis phloxiphaga</i>	USA, California	EU769058	EU769000	EU768929
<i>Heliothis proruptionis</i>	USA, California	EU769059	EU769001	EU768930
<i>Masalia decorata</i>	Mali, Mourdiah	EU769053	EU768995	EU768923
<i>Masalia terracottoides</i>	Mali, Mourdiah	AF151631	U71427	EU768926
<i>Masalia galathea</i>	Mali, Mourdiah	EU769054	EU768996	EU768924
<i>Masalia nubila</i>	Mali, Mourdiah	EU769055	EU768997	EU768925
<i>Adisura bella</i>	Mali, Mourdiah	U20123	U71407	EU768891
<i>Adisura purgata</i>	Australia, Queensland	EU769031	EU768972	EU768895

Table 2. Morphology data set.

Species	Characters																			
	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	2
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
<i>Adisura purgata</i>	0	0	1	3	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
<i>Adisura bella</i>	0	0	0	3	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
<i>Heliothis proruptionis</i>	1	2	0	1	0	1	1	1	0	0	1	1	1	0	0	1	0	0	1	2
<i>Heliothis phloxiphaga</i>	1	2	0	3	0	1	1	1	0	0	1	1	1	0	0	1	0	0	1	2
<i>Heliothis viriplaca</i>	1	2	0	3	0	1	1	1	0	0	1	1	1	0	0	1	0	0	1	2
<i>Heliothis oregonica</i>	1	2	0	3	0	1	1	1	0	0	1	1	0	0	0	1	0	0	1	2
<i>Heliocheilus toralis</i>	0	1	1	3	0	0	0	0	1	0	2	3	1	0	0	1	0	1	1	0
<i>Heliocheilus paradoxus</i>	0	1	0	3	0	0	1	1	1	0	2	3	0	0	0	1	0	1	1	0
<i>Masalia decorata</i>	0	1	1	3	0	0	0	0	0	0	3	1	1	2	1	2	0	0	2	0
<i>Masalia galathea</i>	0	1	3	3	0	0	0	0	0	0	3	1	1	2	1	2	0	0	2	0
<i>Masalia nubila</i>	0	1	1	3	0	0	0	0	0	0	3	1	1	2	1	2	0	0	2	0
<i>Masalia terracottoides</i>	0	1	0	3	0	0	0	0	0	0	3	1	1	2	1	2	0	0	2	0
<i>Chloridea subflexa</i>	0	2	2	2	1	0	1	0	0	0	4	1	1	0	0	1	0	0	1	1
<i>Chloridea virescens</i>	0	2	2	2	1	0	1	0	0	1	4	1	1	0	0	1	0	0	1	1
<i>Helicoverpa gelotopoeon</i>	1	3	0	0	2	1	1	1	0	0	5	2	0	3	3	1	2	2	3	1
<i>Helicoverpa assulta</i>	1	2	1	0	2	1	1	1	0	0	5	2	0	3	3	1	2	2	3	1
<i>Helicoverpa zea</i>	1	2	4	0	2	1	1	1	0	0	5	2	0	3	3	1	2	2	3	1
<i>Helicoverpa armigera</i>	1	2	4	0	2	1	1	1	0	0	5	2	0	3	3	1	2	2	3	1

of genera based on morphology; he recognized several species pairs and clusters that corresponded to existing genera or parts of them, yet could find no autapomorphies to define these groups. Pogue (2007) found a synapomorphy in the male genitalia to unite two of the 'Pyrrhia-group' genera under *Psectrotarsia* Dognin. Because of these problems resulting from simplified morphology, molecular analyses of the Heliothinae have generally been more useful for defining monophyletic groups within the subfamily (Cho *et al.*, 1995, 2008; Fang *et al.*, 1997).

Larval host-plant usage in the Heliothinae is host specific or oligophagous in the ancestral lineages with host plant shifts to polyphagy in some species. In the ancestral *Pyrrhia*, *P. aurantiago* (Guenée) is oligophagous and *P. exprimens* (Walker) is polyphagous on a wide variety of plants including

deciduous trees (Hardwick, 1970b, 1996). *Schinia* is host specific or oligophagous and accounts for approximately 38% of the heliothine species (Poole, 1989). In the *Heliothis* clade there was a host shift to polyphagy, which is most likely the ancestral condition (Cho *et al.*, 2008). Within *Heliothis* there have been host shifts to monophagy in *H. proruptionis* Grote and *H. belladonna* (Hy. Edwards) in North America (Hardwick, 1996) and in *H. scutigerana* Guenée in South Africa (Matthews, 1991). In *Helicoverpa* and *Chloridea* there have also been host shifts from polyphagy to oligophagy in a few species.

The purpose of this paper is to combine an analysis of the genes used in Cho *et al.* (2008) with morphological characters to test the monophyly of the genera in the 'Heliothis group' as presented in Cho *et al.* (2008) and make appropriate changes in classification based on the results.

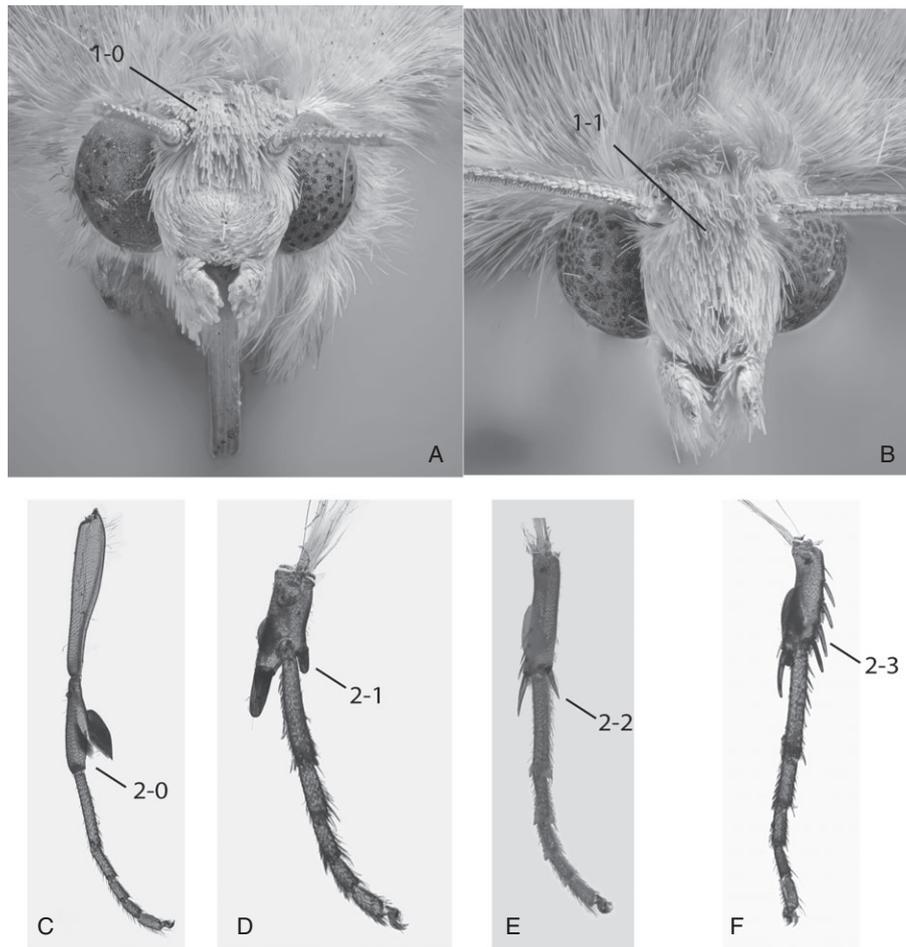


Fig. 1. Morphological characters. Head. (A) *Heliocheilus paradoxus*; (B) *Heliothis phloxiphaga*. Protibial spiniform setae. (C) *Adisura purgata*. (D) *Heliocheilus toralis*. (E) *Chloridea virescens*. (F) *Helicoverpa gelotopoeon*. Hyphenated numbers refer to the character and character state, respectively.

Materials and methods

Taxon sampling

The main objective of this study is to test the monophyly of *Heliothis* as presented in Cho *et al.* (2008), with the addition of a morphological data partition. A subset of taxa was sampled from Cho *et al.* (2008) based on the species available for morphological study and the goal of this analysis (Tables 1–2). The analysis of the ‘*Heliothis* group’ included 16 ingroup taxa and 2 outgroup taxa, *Adisura bella* Gaede, and *A. purgata* Warren (Table 1). *Adisura* Moore formed the sister group of the ‘*Heliothis* group’ in Cho *et al.* (2008). Species names follow Poole (1989) DNA sequences used in this study were from the genes *EF1 α* , *DDC* and *COI* acquired from GENBANK and corresponded to those used by Cho *et al.* (2008, Table 1). Details regarding sampling techniques are presented in Cho *et al.* (2008).

Morphological characters

Morphological characters 2, 3 and 10 below were adapted from Poole *et al.* (1993) but the remaining characters are new. A total of 20 characters used in the analysis were scored; 8 were binary and 12 multistate with up to six character states. All characters were unordered. The characters emphasized generic placement. The character matrix is given in Table 2.

Head. Character 1: Vertex scales: narrow (0; Fig. 1A); hairlike (1; Fig. 1B).

Thorax. Character 2: Foretibia spiniform setae: absent (0; Fig. 1C); a pair of heavily sclerotized, apical spiniform setae; longest on inner margin, shortest on outer margin (1; Fig. 1D); series of three to six fine spiniform setae on inner margin; one fine apical spiniform seta on outer margin (2; Fig. 1E); one large apical inner spiniform seta and one to three finer progressively smaller spiniform setae; outer margin with two to four robust spiniform setae that get progressively smaller distally (3; Fig. 1F).

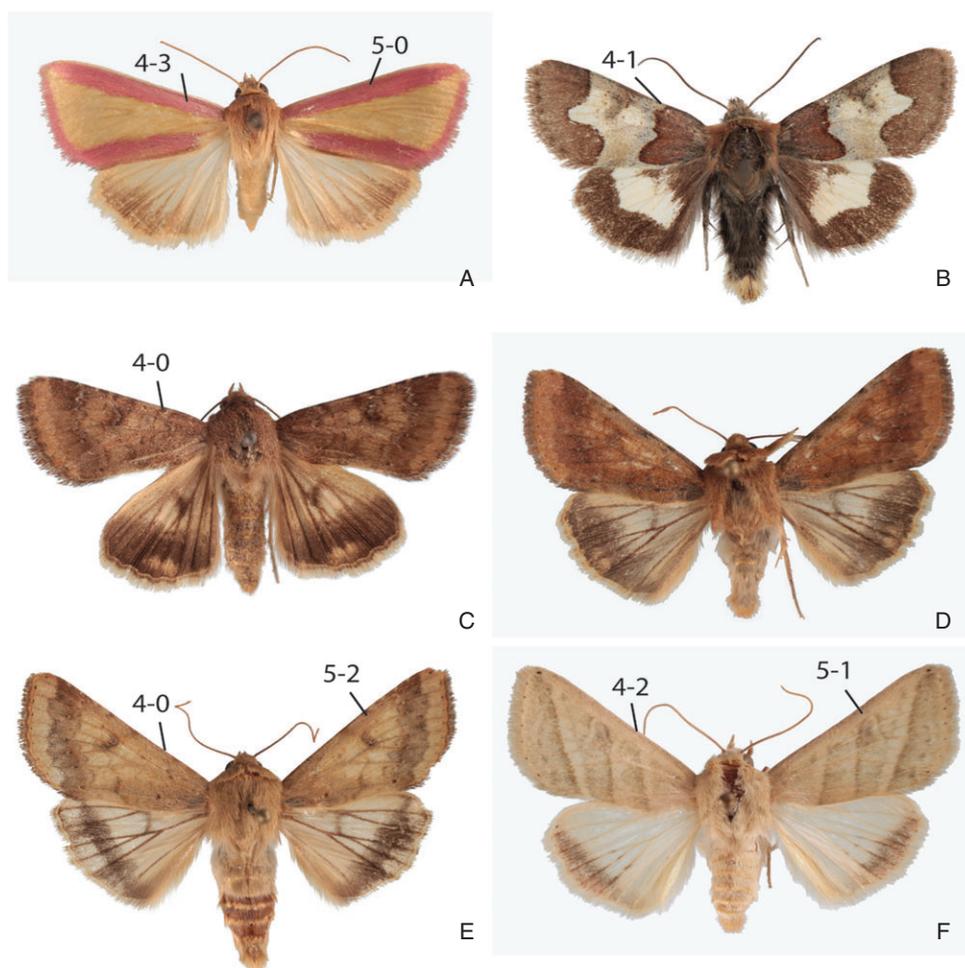


Fig. 2. Forewing ground colour and pattern characters. Adults. (A) *Adisura purgata*; (B) *Heliothis proruptionis*; (C) *Helicoverpa gelotopoeon*; (D) *Helicoverpa zea*; (E) *Helicoverpa zea*; (F) *Chloridea virescens*. Hyphenated numbers refer to the character and character state, respectively.

Forewing. Character 3: Ground colour: brown (all shades) (0; Fig. 2C and Fig. 3A, B); yellow (all shades) (1; Figs 2A, 3C); green (2; Fig. 2F); white (3; Fig. 3D); males yellowish-olive, females ferruginous (4; Fig. 2D, E).

Character 4: Antemedial line shape. crenulate (0; Fig. 3C, E); sinuate (1; Fig. 2B); straight (2; Fig. 2F); absent (3; Fig. 2A).

Character 5: Medial line shape. absent (0; Fig. 2A); straight (1; Fig. 2F); crenulate (2; Fig. 2E).

Character 6: Orbicular spot on underside: absent (0; Fig. 3E); present (1; Fig. 3F).

Character 7: Reniform spot on underside. absent (0; Fig. 3E); present (1; Fig. 3F).

Character 8: Postmedial band on underside. absent (0; Fig. 3E); present (1; Fig. 3F).

Character 9: Modified male forewing vein. absent (0; Fig. 3C); present (1; Fig. 3A).

Abdomen. Character 10: Eighth sternite arms. present (0; Fig. 4B); absent (1; Fig. 4A).

Male genitalia. Character 11. Valve shape. narrow basally, expanded cucullus, costa gently curved near apex (0; Fig. 5A); base slightly expanding to cucullus, curved near apex (1; Fig. 5B); overall narrow, becoming wider toward cucullus, overall slightly curved, becoming more curved toward apex (2; Fig. 5C); valve shaft approximately same width to four fifths length, then expanded and abruptly curved toward apex (3; Fig. 5D); valve shaft approximately same width to two thirds length, shaft slightly sinuate to straight, curved toward apex (4; Fig. 5E); valve slightly sinuate, wide, cucullus not much wider than valve (5; Fig. 5F).

Character 12. Vesica shape. short, loosely coiled (0; Fig. 6A); elongate, weakly twisted tube (1; Fig. 6B); elongate, tightly coiled (2; Fig. 6C).

Character 13. Aedeagus scobinate bar. absent (0; Fig. 6D); present (1; Fig. 6E).

Character 14. Cornuti form. absent (0; Fig. 6A); a pair of long spine-like cornuti at base of vesica (1; Fig. 6D); scale-like (2; Fig. 6F); spine-like (3; Fig. 6C).

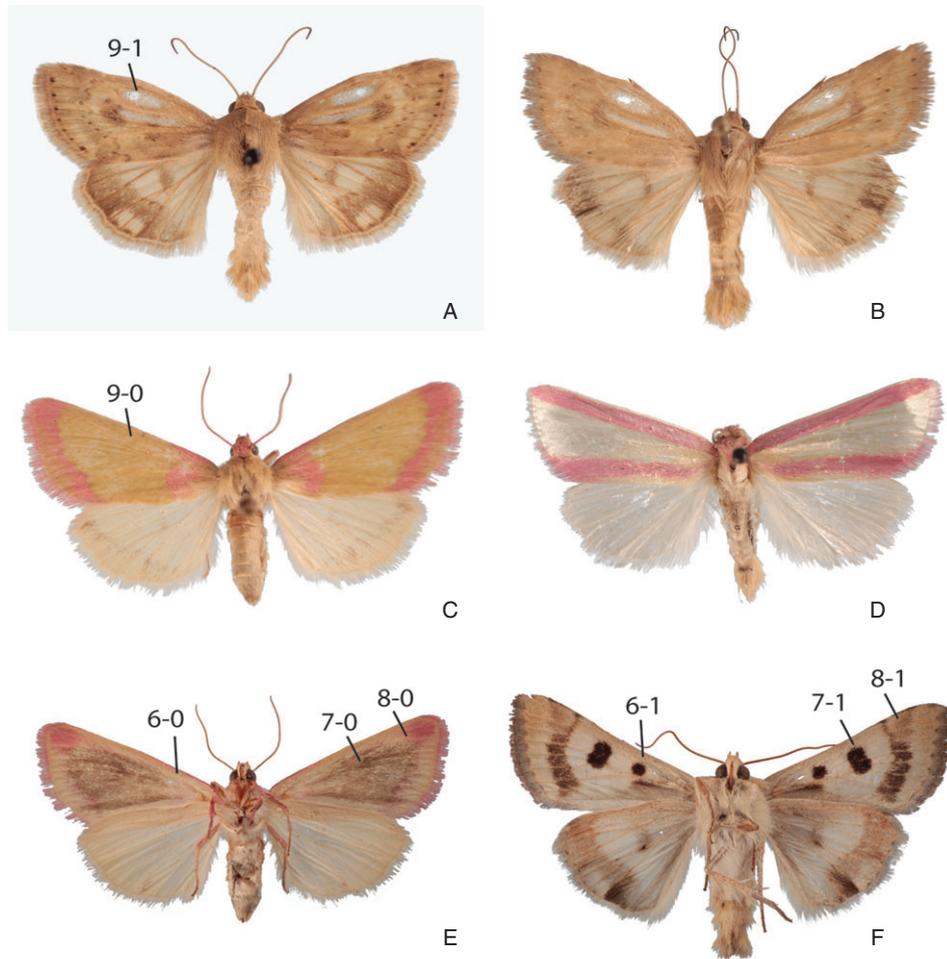


Fig. 3. Forewing ground colour and pattern characters. Adults. (A) *Heliocheilus paradoxus*; (B) *Heliocheilus paradoxus*; (C) *Heliocheilus toralis*; (D) *Heliocheilus galathea*; (E) *Heliocheilus toralis*; (F) *Heliothis phloxiphaga*. Hyphenated numbers refer to the character and character state, respectively.

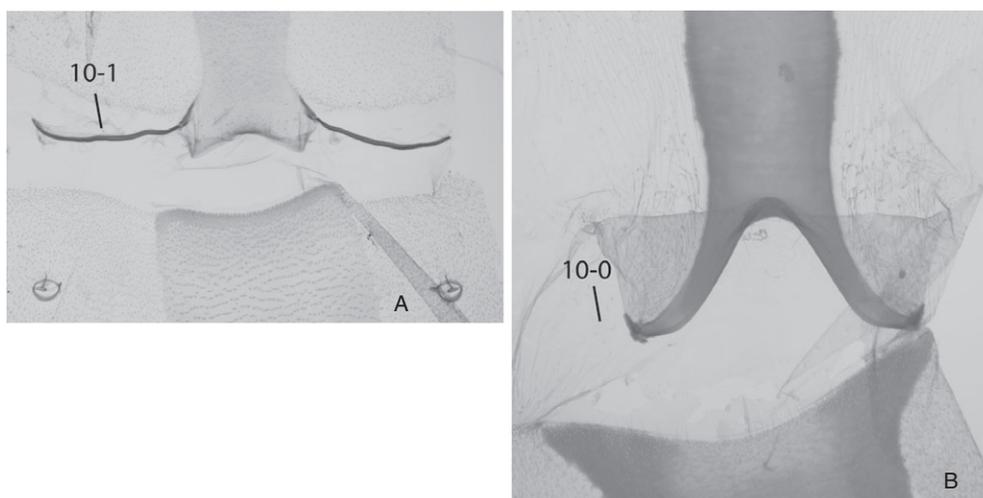


Fig. 4. Morphological character. Male abdominal tergite 8. (A) *Chloridea virescens*; (B) *Heliocheilus paradoxus*. Hyphenated numbers refer to the character and character state, respectively.

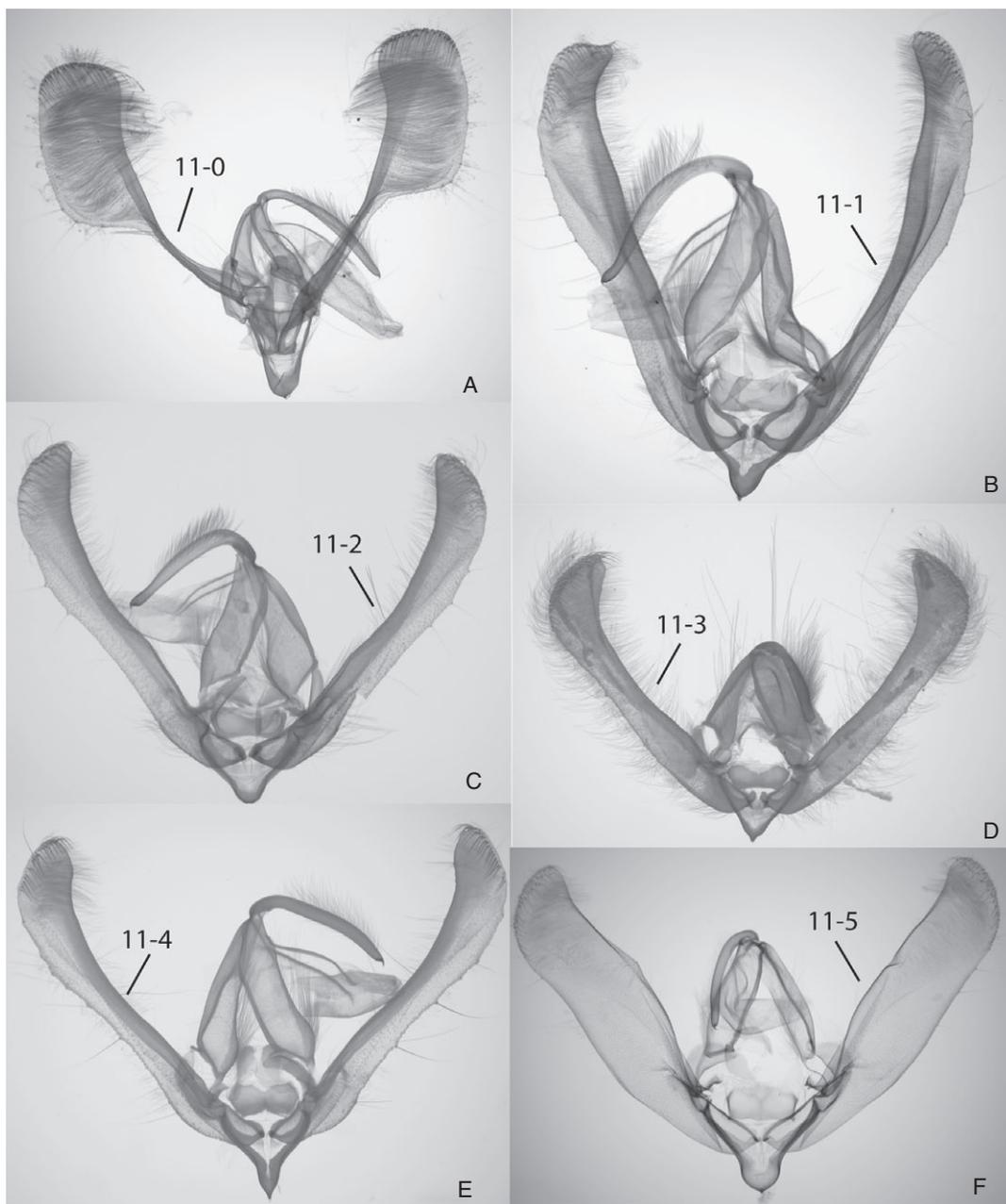


Fig. 5. Morphological characters. Male genitalia. Valve shape. (A) *Adisura purgata*; (B) *Heliothis phloxiphaga*; (C) *Heliocheilus toralis*; (D) *Heliocheilus toralis*; (E) *Chloridea subflexa*; (F) *Helicoverpa zea*. Hyphenated numbers refer to the character and character state, respectively.

Female genitalia. Character 15. Papillae anales. membranous (0; Fig. 7A); sclerotized (1; Fig. 7B).

Character 16. Papillae anales shape. apex round, width greater than height (0; Fig. 7C); apex tapered, height greater than width (1; Fig. 7A); apex produced, height greater than width (2; Fig. 7B).

Character 17. Appendix bursae shape. straight (0; Fig. 8A); coiled (1; Fig. 8B); twisted (2; Fig. 8C).

Character 18. Appendix bursae length. shorter than corpus bursae (0; Fig. 8B); approximately equal to or slightly longer or shorter than corpus bursae (1; Fig. 8A); much longer than corpus bursae (2; Fig. 8C).

Character 19. Ostium bursae shape. lamella antevaginalis elongate, ostium bursae height greater than width (0; Fig. 9A); lamella antevaginalis short, ostium bursae width greater than height (1; Fig. 9B); lamella antevaginalis absent, ostium bursae a sclerotized rectangle with width greater than height

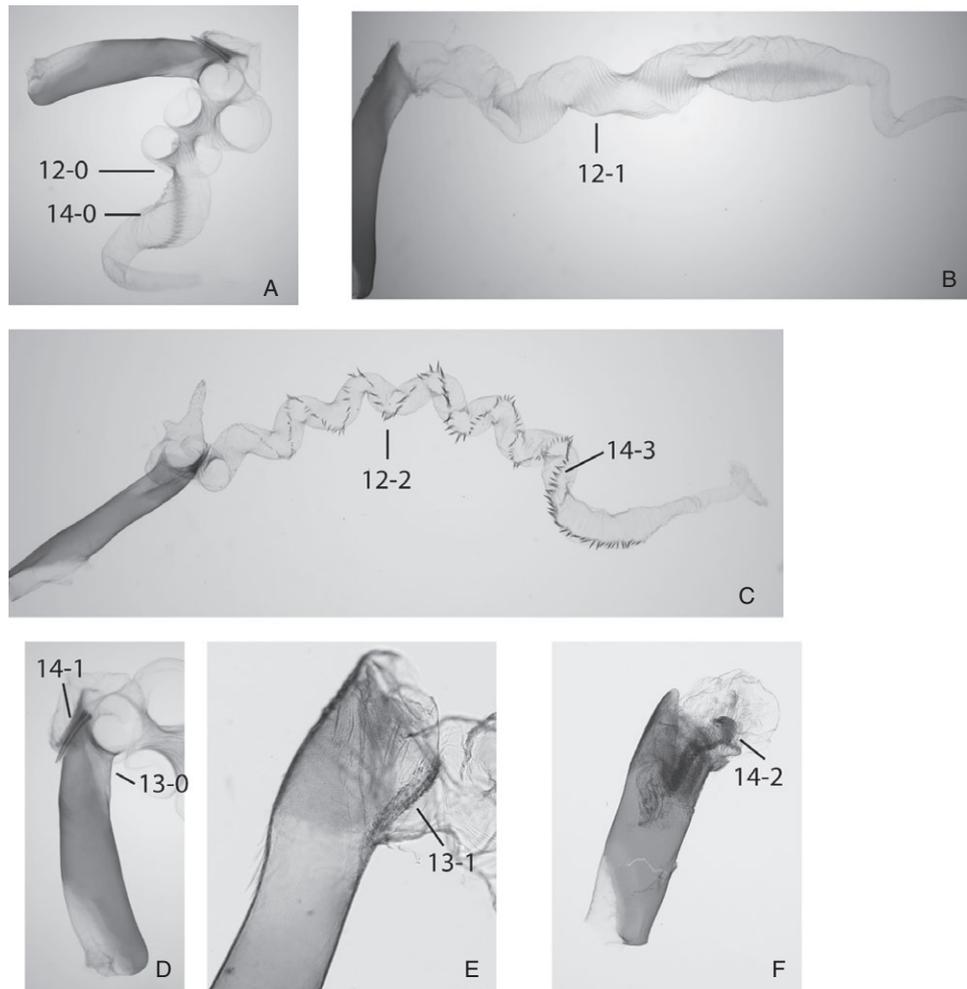


Fig. 6. Morphological characters. Male aedeagus. (A) *Adisura purgata*; (B) *Chloridea subflexa*; (C) *Helicoverpa gelotopoeon*; (D) *Adisura purgata*; (E) *Heliothis phloxiphaga*; (F) *Heliocheilus galathea*. Hyphenated numbers refer to the character and character state, respectively.

(2; Fig. 9C); lamella antevaginalis moderate, ostium bursae width slightly greater than height, more quadrate than in 1 (3; Fig. 9D).

Character 20. Ductus bursae sclerotization. sclerotization absent (0; Fig. 9E); elongate sclerotized ribbon (1; Fig. 9F); sclerotized area around base of appendix bursae (2; Fig. 9G).

Phylogenetic analysis

DNA sequences from the genes *EF1 α* , *DDC* and *COI* were initially aligned using the program Clustal X2 (Larkin *et al.*, 2007), using the default settings, and further adjusted by eye using the program BioEdit (Hall, 1999). Phylogenetic relationships were reconstructed using Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Likelihood (BI). MP and ML analyses were carried out in PAUP v4.0b10 (Swofford, 2002) and BI analyses were carried out in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). Heuristic MP analysis

was run with equally weighted characters, (TBR) branch swapping and minimum 1000 random replicates to reduce the chance of missing the most optimal solution due to being isolated within a tree island (Page, 1993). If analyses produced more than one most parsimonious tree, a strict consensus was performed. Clade support was assessed using the nonparametric bootstrap (Felsenstein, 1985) under the same search conditions described above for MP. MP analysis was carried out for: (i) *All Genes* dataset, (ii) morphology dataset and (iii) combined molecular and morphology dataset with the morphological matrix included.

For both the ML and BI phylogenetic analysis, nucleotide substitution model parameters were inferred using MrModeltest v 2.3 (Nylander, 2004). The Akaike Information Criterion (AIC) was used to select the best-fit nucleotide substitution model (Posada & Buckley, 2004). ML analysis was carried out for the combined gene dataset. ML analysis was performed using the (GTR+I+G) model for the *All Genes* dataset, which as was used in Cho *et al.* (2008).

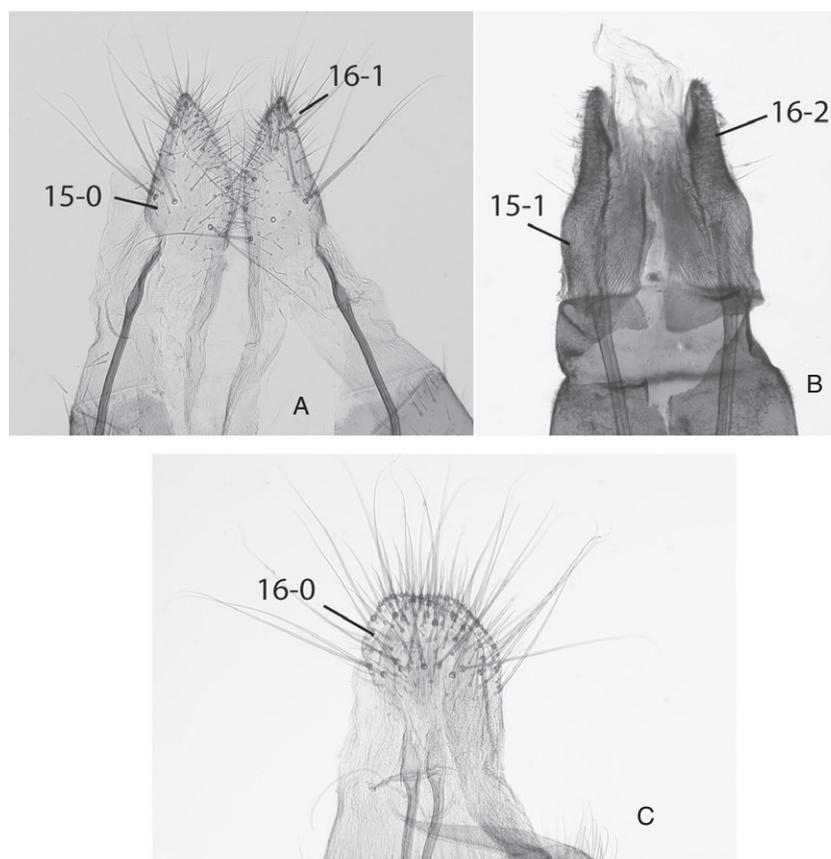


Fig. 7. Morphological characters. Female genitalia. Papillae anales. (A) *Helicoverpa titicacae*; (B) *Heliocheilus galathea*; (C) *Adisura atkinsoni*. Hyphenated numbers refer to the character and character state, respectively.

MrModeltest (Nylander, 2004) selected the GTR+I+G (lnL = 8221.3799; K = 10; AIC = 16462.7598) model with estimated base frequencies: A = 0.2619, C = 0.2407, G = 0.2266, T = 0.2709. The rate matrix parameters estimated were: R(a) [A–C] = 2.6361, R(b) [A–G] = 10.2477, R(c) [A–T] = 10.1331, R(d) [C–G] = 1.0133, R(e) [C–T] = 23.9690, R(f) [G–T] = 1.00. The proportion of invariable sites (PINVAR) was 0.6787 and the alpha shape parameter (α) of the gamma (Γ) distribution was 0.9586. Clade support was assessed for the combined *All Genes* dataset only using the nonparametric bootstrap (Felsenstein, 1985) under the same search conditions described above for MP.

BI analysis was performed for: (i) the combined gene dataset and (ii) combined molecular and morphology dataset with the morphological matrix included. A mixed models approach was used for all BI analyses (Ronquist & Huelsenbeck, 2003). MrModeltest (Nylander, 2004) selected the following models for each respective gene: *EFl α* = GTR + I + G; *DDC* = SYM + I + G; *COI* = GTR + I + G. The MCMC was run for 10 000 000 generations with sampling every 100 generations. The morphological partition in the Total Evidence analysis was analysed as unordered using the Mk model (Lewis, 2001) with the following settings: coding = variable

and rates = gamma. In the BI analysis, a random start tree was generated and two independent simulations of four simultaneous chains (three hot and one cold) were used under default heating values. To be conservative, burn-in was set at 250 000 generations in an analysis of 10 000 000 generations with sampling every 100 generations. The equilibrium-sampled trees were used to generate a 50% majority rule consensus tree with bipartition frequencies equal to posterior probability values (Huelsenbeck & Ronquist, 2001).

Bootstrap values (BP) for the Maximum Parsimony and Maximum Likelihood trees and posterior probability (PP) for the Bayesian Inference trees are used to quantify the level of support at particular nodes and are expressed as a percentage. Values below 60% are considered to have ‘weak’ support; 61–74%, ‘moderate’; 75–89%, ‘strong’; and 90–100%, ‘very strong’. Values for all analyses are superimposed on the trees.

Bremer support (BS) (Bremer, 1988, 1994) was calculated in TNT v1.1 (Goloboff *et al.*, 2003, 2008). Heuristic MP analysis was run with equally weighted characters, (TBR) branch swapping and minimum 1000 random replicates. To evaluate the individual contributions of each data partition to the overall BS values, a partitioned Bremer support analysis (Gatesy *et al.*, 1999; Simonsen *et al.*, 2006) was carried out using the methods of Peña *et al.* (2006).

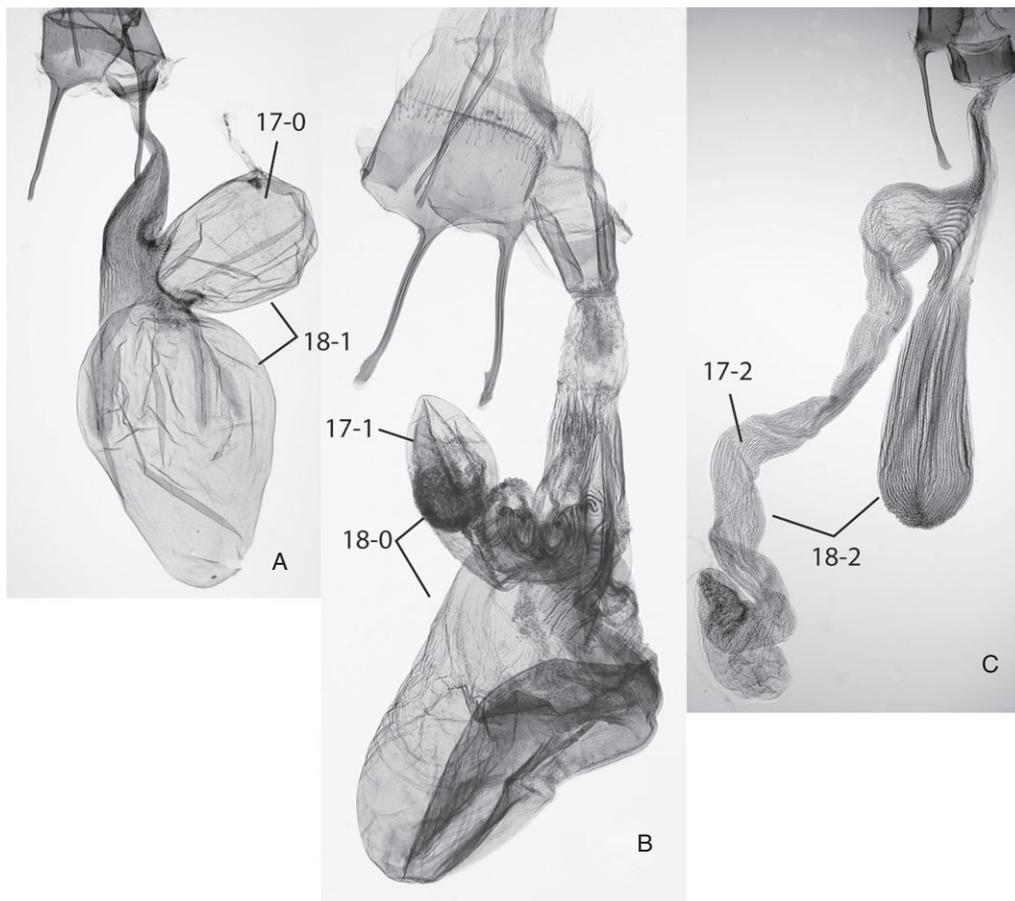


Fig. 8. Morphological characters. Female genitalia. Appendix bursae. (A) *Heliocheilus toralis*; (B) *Adisura atkinsoni*; (C) *Helicoverpa titicacae*. Hyphenated numbers refer to the character and character state, respectively.

Results

Maximum parsimony

Maximum Parsimony trees were produced from the morphological dataset, *All genes* dataset (*EF1 α* + *DDC* + *COI*), and molecular and morphology dataset (*EF1 α* + *DDC* + *COI* + morphology). The morphology MP tree (Fig. 10) showed strong support for *Heliothis* s.s. (BP = 89); strong support for a monophyletic *Heliocheilus* (BP = 87); strong support for a monophyletic *Masalia* (BP = 87); very strong support for a monophyletic *Chloridea* (BP = 98); and very strong support for a monophyletic *Helicoverpa* (BP = 100). The *All genes* MP tree (Fig. 11) showed strong support for a monophyletic *Heliothis* s.s. (BP = 82), strong support for a monophyletic *Heliocheilus* (BP = 88), moderate support for a monophyletic *Masalia* (BP = 61); very strong support for a monophyletic *Chloridea* (BP = 100), and very strong support for a monophyletic *Helicoverpa* (BP = 100). By adding the morphology dataset (Table 2) to the *All genes* dataset, the molecular and morphology tree (Fig. 12) showed stronger support for all monophyletic genera.

Masalia had strong support (BP = 88), *Heliocheilus* had very strong support (BP = 92), *Helicoverpa* had very strong support (BP = 100) and *Chloridea* with very strong support (BP = 100) and *Heliothis* with very strong support (BP = 96). In the molecular and morphology analysis, the type species of each genus was included. Even though the type species of *Masalia*, *M. radiata* Moore, lacked the *All genes* data, support was still higher for a monophyletic *Masalia* using the molecular and morphology dataset than in the *All genes* analysis.

Maximum likelihood and Bayesian inference

A ML tree for the *All gene* dataset (Fig. 13) showed similar results to the MP *All gene* tree (Fig. 11). Monophyly of *Heliothis*, *Heliocheilus*, *Chloridea* and *Helicoverpa* was strongly supported, with BP values of 97, 95, 100 and 100, respectively, whereas support for *Masalia* was weak (BP = 58) (Fig. 13). The Bayesian analysis using the *All gene* dataset (Fig. 14) gave similar results but with increased support for *Masalia* (PP = 89), hence giving very strong support for all other genera (PP = 100). The BI tree for the *molecular and*

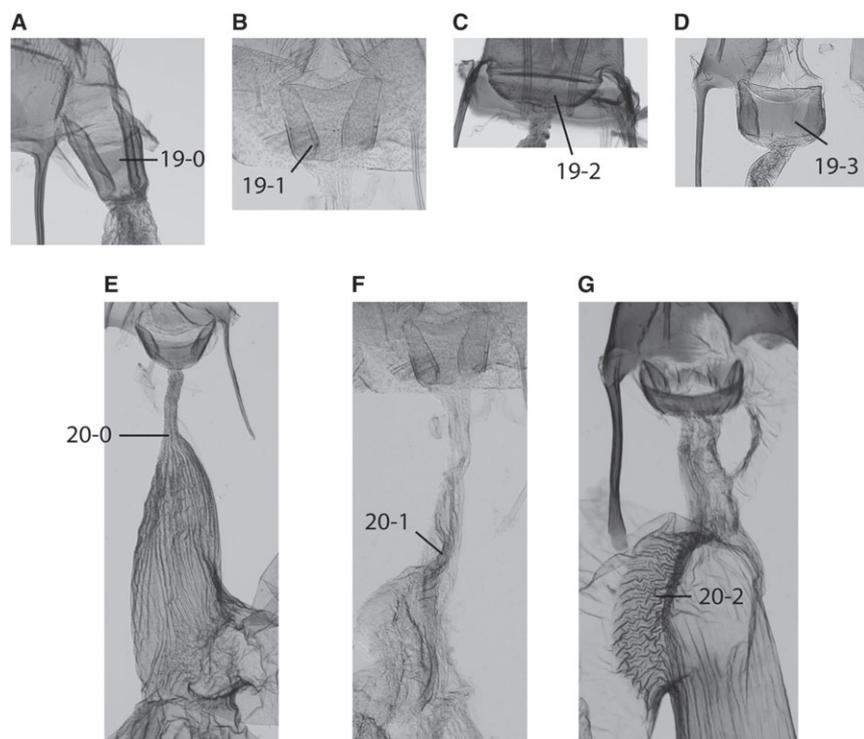


Fig. 9. Morphological characters. Female genitalia. Ostium bursae and ductus bursae. (A) *Adisura atkinsoni*; (B) *Heliocheilus toralis*; (C) *Heliocheilus galathea*; (D) *Helicoverpa titicacae*; (E) *Heliocheilus paradoxus*; (F) *Helicoverpa zea*; (G) *Heliothis proruptionis*. Hyphenated numbers refer to the character and character state, respectively.

morphology dataset (Fig. 15) recovered the monophyly of *Heliothis*, *Heliocheilus*, *Masalia*, *Chloridea* and *Helicoverpa* with very strong support (PP = 100).

Bremer support tree

The MP tree with Bremer support (BS) values is shown in Fig. 16. The *Heliothis* group had a high BS (9). *Heliothis* s.s. was moderately supported (BS = 5), but *H. proruptionis* and *H. phloxiphaga* Grote and Robinson had very high support BS (17); whereas *H. viriplaca* (Hufnagel) and *H. oregonica* (Hy. Edwards) were fairly well supported (BS = 7). The *Chloridea* + *Helicoverpa* clade was modestly supported (BS = 5), but *Chloridea* was highly supported (BS = 11) and *Helicoverpa* very highly supported (BS = 17). *Heliocheilus* + *Masalia* had weak support (BS = 3), but *Heliocheilus* was fairly well supported (BS = 8) and *Masalia* with moderate support (BS = 4).

Of 13 nodes, only one was unanimously supported by all datasets – the *H. proruptionis* + *H. phloxiphaga* node. Three nodes were supported only by morphology and included the entire *Heliothis* group, *Chloridea* clade and *Helicoverpa* clade. The partitioned Bremer support (PBS) values were summed across all branches of the combined molecular and morphology tree for a given partition to show total support of that partition in the resulting tree (Fig. 16). Morphology gave the most

support for the combined tree with a PBS value of 46.3; *COI* gave the least (PBS = 1.6), followed by *DDC* (PBS = 16.4) and *EF-1 α* (PBS = 30.2).

Discussion

Using both molecular and morphological data in a total evidence approach frequently results in a better supported phylogeny (Nylin *et al.*, 2001; Astruc *et al.*, 2004; Cabrero-Sañudo & Zardoya, 2004; Mattern & McLennan, 2004; Ylla *et al.*, 2005; Bond & Hedin, 2006; Simonsen *et al.*, 2006; Specht & Stevenson, 2006; Miller *et al.*, 2007; Lee & Brown, 2008; Pilgrim & von Dohlen, 2008; Warren *et al.*, 2009; Zrzavý *et al.*, 2009; Brower *et al.*, 2010; Price *et al.*, 2011). This study and that of Cho *et al.* (2008) produced similar results.

The total PBS values summed across all nodes as given in the results above indicated that morphology (total PBS = 46.3) was the main contributor to tree topology. *COI* contributed the least to tree topology (PBS = 1.6). Strong morphological characters can accurately predict phylogenies and in this study morphology was not overpowered by molecular characters. In a study on diving beetles (Dytiscidae), *COI* contributed the most to tree topology based on partitioned Bremer support and morphology contributed the least (Miller *et al.*, 2007).

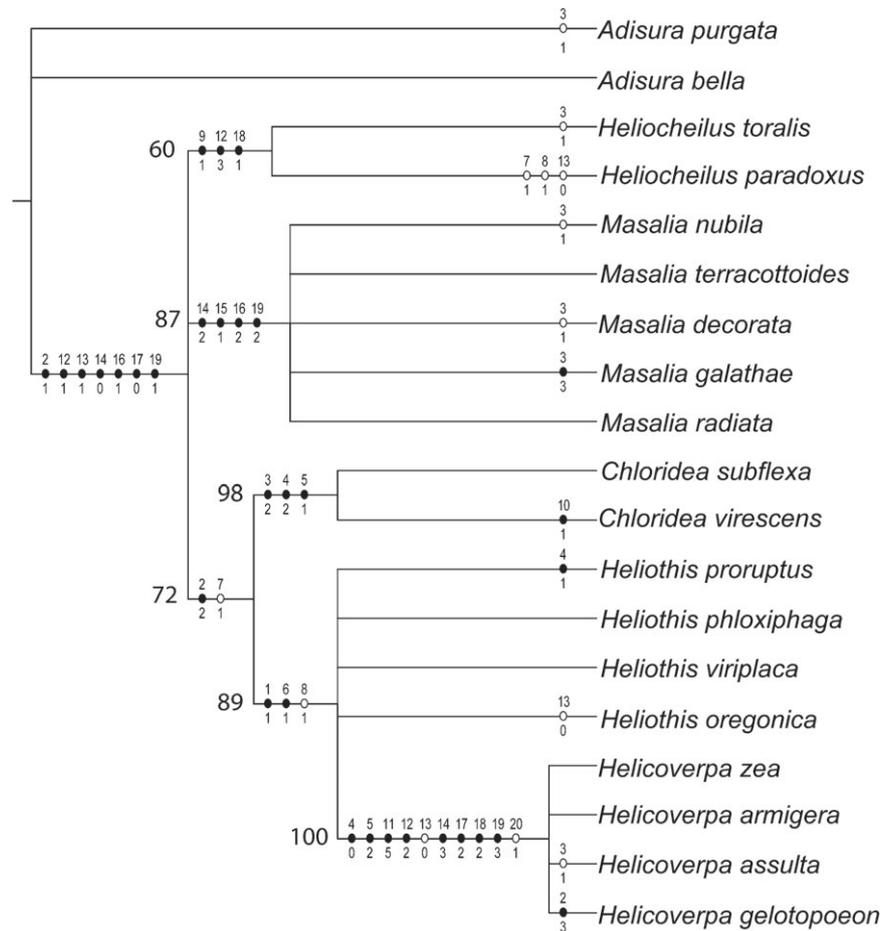


Fig. 10. The strict consensus of 108 trees from the morphology dataset inferred by Maximum Parsimony using equally weighted characters. Solid circles denote apomorphies and open circles homoplasies with the character above and character state below. Values above nodes represent bootstrap support. Length = 50; CI = 0.840; RI = 0.929; RC = 0.781.

In both Cho *et al.* (2008) and the analyses presented here, *Masalia* was shown to be monophyletic. Matthews (1991) synonymized *Masalia* with *Heliiothis* because he could not find any synapomorphies to support *Masalia*. Fibiger *et al.* (2009) synonymized *Masalia* with *Heliocheilus* based on the conclusions that the supposed morphological synapomorphies of both *Masalia* and *Heliocheilus* can occur in species assigned to both genera. However, findings presented here and supported in Cho *et al.* (2008) can be interpreted in two different ways. Recent papers have treated *Masalia* as a valid genus (Ebert & Hacker, 2002; Vári *et al.*, 2002; Kravchenko *et al.*, 2005; Matov *et al.*, 2008). A second way to interpret these results is that *Heliocheilus* and *Masalia* be combined into *Heliocheilus*. Both the results presented here and in Cho *et al.* (2008) support the inclusive group of *Masalia* and *Heliocheilus*. Therefore, the interpretation of how these genera should be treated ultimately is decided by morphological data. I am following the interpretation presented by Fibiger *et al.* (2009) and treating the all-inclusive group of *Heliocheilus* + *Masalia* as *Heliocheilus*. This interpretation is most conservative and

it will be easier to assign all of the species involved in these groups to a single genus. Future research to remove uncertainty in the classification would be to add genetic data for those species that show a combination of characters of both *Masalia* and *Heliocheilus* as mentioned in Fibiger *et al.* (2009).

Larval host-plant usage is defined as those groups feeding on a single host family as oligophagous and those feeding on more than one plant family as polyphagous. Ancestral lineages of heliothines are typically host specific or oligophagous and polyphagy has evolved multiple times (Mitter *et al.*, 1993; Cho *et al.*, 2008). One of these origins of polyphagy occurred in the *Heliiothis* clade. All of the *Heliiothis* species in Fig. 12, except *H. proruptions*, are polyphagous, which is most likely the ancestral condition of the *Heliiothis* clade. In North America there may have been a host shift to monophagy in *Heliiothis proruptions* and *H. belladonna*. Larvae of both of these species have only been found on *Castilleja* sp. (Scrophulariaceae) (Hardwick, 1996). In South Africa, *H. scutiligera* Guenée is host specific on *Helichrysum* sp. (Asteraceae) (Matthews, 1991).

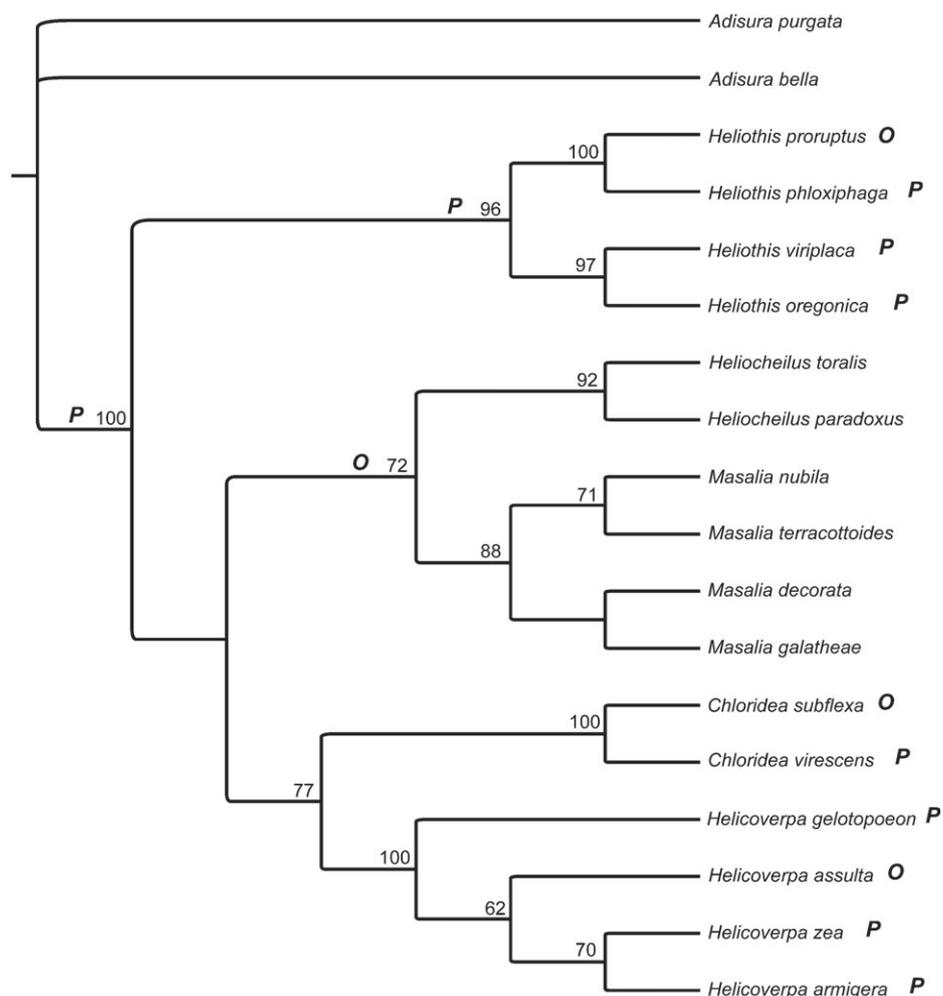


Fig. 11. The strict consensus of one tree from the ALL-Genes (*EF1- α* sequence + *DDC* sequence + *COI* sequence) dataset inferred by Maximum Parsimony using equally weighted characters. Values above nodes represent bootstrap support. Length = 844; CI = 0.618; RI = 0.563; RC = 0.348.

Heliocheilus is a large genus with close to 80 species that has its centre of diversity in Australia (Seymour, 1972; Matthews, 1999). Of these 80 species, there are only seven species in which the host plant is known and all seem to be host specific on various species of Poaceae. The Bayesian Inference trees (Figs 14, 15) show very strong support for *Heliocheilus*, which could indicate a major evolutionary host shift to oligophagy.

In the *Chloridea* clade there are three species in which one is polyphagous and the other two are oligophagous. *Chloridea tergemina* (Felder and Rogenhofer) and *C. subflexa* (Guenée) are both oligophagous on Solanaceae and are sister species in a separate clade from *C. virescens* (Fabricius) (Poole *et al.*, 1993). Both of these species are considered minor pests and feed on Solanaceae; *C. tergemina* is a pest on tobacco in Venezuela and Colombia and *C. subflexa* is an occasional pest of tomatillo (*Physalis* spp.) from Mexico. *Chloridea virescens* is a pest on many agricultural crops including cotton, tomato, soybean, tobacco, garbanzo bean and alfalfa (Graham &

Robertson, 1970; Waldvogel & Gould, 1990; Blanco *et al.*, 2007).

Host-plant use is complex and can involve different traits such as neurosensory, physiological, morphological, and behavioural (Oppenheim *et al.*, 2012). It appears that *C. subflexa* has evolved behavioural and physiological traits enabling it to become a specialist on *Physalis* spp. (Oppenheim & Gould, 2002a, b). They showed that the larva of *C. subflexa* could escape parasitism by the adaptive behaviour of feeding inside the inflated calyx of the *Physalis* fruit. Although *C. virescens* could also feed on *Physalis*, it had not fully adapted the ability to completely feed inside the calyx, and therefore was subject to increased parasitism (Oppenheim & Gould, 2002a, b). The use of *Physalis* by *C. subflexa* is genetically based (Oppenheim *et al.*, 2012).

The *Helicoverpa* clade shows a similar relationship between the oligophagous *H. assulta* (Guenée) and the polyphagous *H. armigera* (Hübner) in the Palaearctic. Feeding preference in *H. assulta* is also genetically based (Tang *et al.*, 2006).

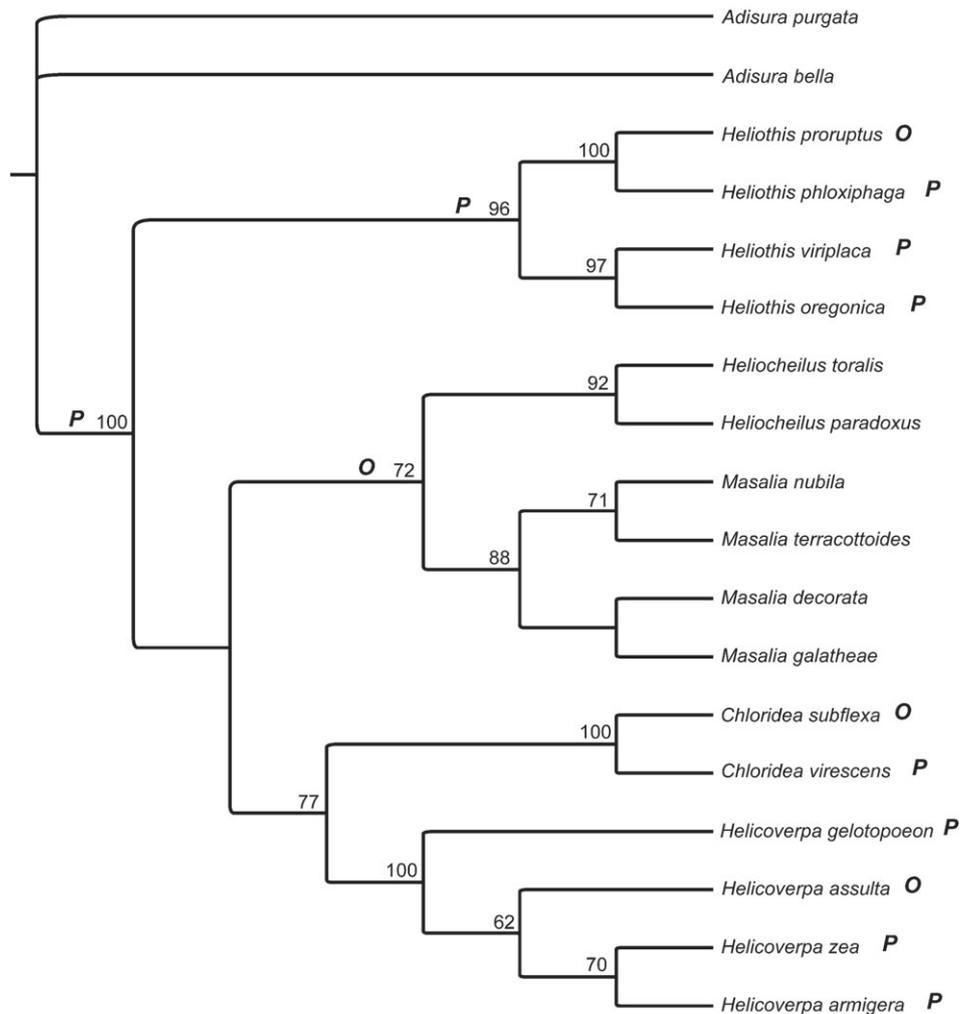


Fig. 12. The strict consensus of one tree from the molecular and morphology dataset (*EF1- α* sequence + *DDC* sequence + *COI* sequence + morphology) inferred by Maximum Parsimony using equally weighted characters. ‘O’ and ‘P’ on tree refer to Oligophagous and Polyphagous and represent the most parsimonious assignments of ancestral condition when the host range is mapped as a binary character on tree next to the species name. Numbers above branches indicate bootstrap support using 1000 replicates. Length = 907; CI = 0.626; RI = 0.604; RC = 0.379.

An example of an evolutionary model that might explain the origins of the genera in the *Heliothis* group is one in which a single evolutionary origin might initiate an entire clade (genus) within a domain (continent or geographical realm). Each new species originates only within or adjacent to the geographical range of its ancestor (Gotelli *et al.*, 2009). Using this hypothesis, the geographical areas with the highest species richness would indicate the origin of the genera within the *Heliothis* group.

In Cho *et al.* (2008), *Heliothis* was paraphyletic with respect to the *Heliothis virescens* group. By resurrecting the genus *Chloridea* for the *H. virescens* group, *Heliothis* becomes monophyletic (Figs 10–14). However, this only includes four of the 47 species currently placed in *Heliothis*. One problem that was pointed out in the phylogenetic tree

in Cho *et al.* (2008) was the placement of the Australian *H. punctifera* Walker as sister to the entire *Heliothis* group and not being placed within ‘typical’ *Heliothis*. In examining the genitalia of *H. punctifera* in Matthews (1999), the vesica has numerous basal diverticula apically adjacent to a tight coil. This does not conform to the slightly twisted vesica of the type species, *H. virescens* (Hufnagel). *Heliothis punctifera* could possibly represent a distinct genus from *Heliothis* based on its placement as a sister species to the *Heliothis* group as shown in Cho *et al.* (2008). The Australian species *H. hoare* Matthews and *H. roseivena* (Walker) also have vesicae that more resemble species of *Heliocheilus* than *Heliothis* and their forewing patterns are typical of many species of *Heliocheilus*. The 18 species of *Heliothis* in the Palaearctic, Nearctic and Neotropical regions are well understood and all are similar,

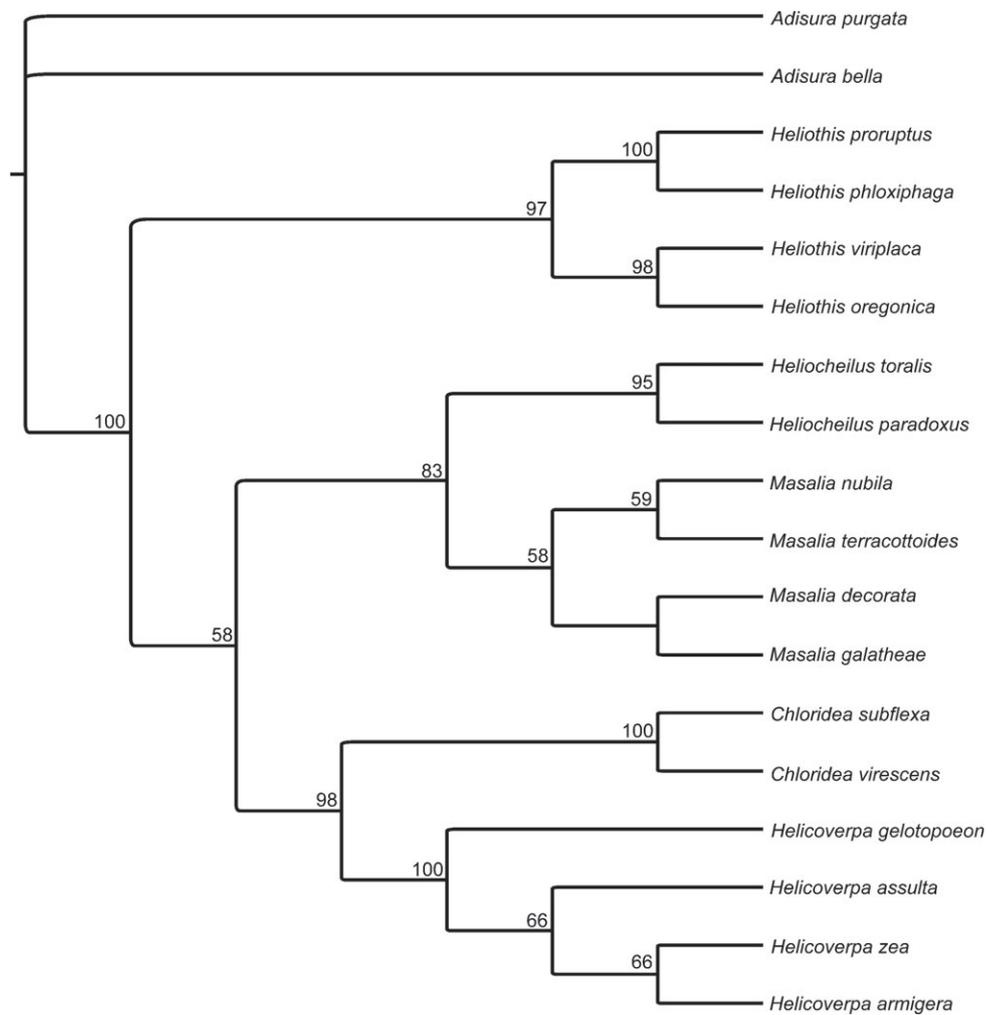


Fig. 13. Phylogenetic relationships from All-Genes (*EF1- α* sequence + *DDC* sequence + *COI* sequence) sequence data inferred by Maximum Likelihood. Values above nodes represent bootstrap support 500 replicates.

both molecularly (*EF-1 α* , *DDC*, *COI*) and morphologically to *H. viriplaca*. The remaining 29 species, mostly from Africa and India, need to be revised to better understand *Heliiothis*. Without understanding the world fauna of *Heliiothis*, it is not possible to predict an area of origin for the genus.

Chloridea is undoubtedly Neotropical in origin as all 13 species occur in the neotropics and *C. virescens* and *C. subflexa* also extend their ranges into the Nearctic. Within South America none of the *Chloridea* species occur in the Amazon basin. All of the species occur along both coasts, along the western Andes, in the cerrado of Brazil, Matto Grosso of Brazil, southeastern highlands of Brazil, and Chocó of Bolivia and Argentina (Poole *et al.*, 1993).

Based on species richness, *Helicoverpa* possibly originated in the Neotropics where six species (including *H. zea*) occur. Other species include five in Sub-Saharan Africa (including *H. assulta*), five in Australia (including *H. armigera* and *H. assulta*), two in the Palearctic and one in the Nearctic.

Helicoverpa also displays adaptive radiation with five endemic species of *Helicoverpa* in Hawaii plus the introduced *H. zea*.

Heliiocheilus includes 84 species of which 27 are from Australia, 26 from Africa and 17 from India. The Palearctic, Nearctic and Neotropical regions all have four species. It is likely that there have been several independent dispersal events followed by radiations in Australia, Africa and India.

Future work in the Heliothinae, besides the needed revision of *Heliiothis*, should focus on the African (six species) and Indian (one species) genus *Timora*. *Timora* has not been revised and Seymour (1972) resurrected *Masalia* from synonymy with *Timora* in his revision, but that is all that he mentions about the remaining species in *Timora*.

Phylogenies presented here and elsewhere (Cho *et al.*, 2008) can help to understand the relationship between pest groups within the Heliothinae. These phylogenies have demonstrated that the major lineages of pest species, *Chloridea*, *Helicoverpa*

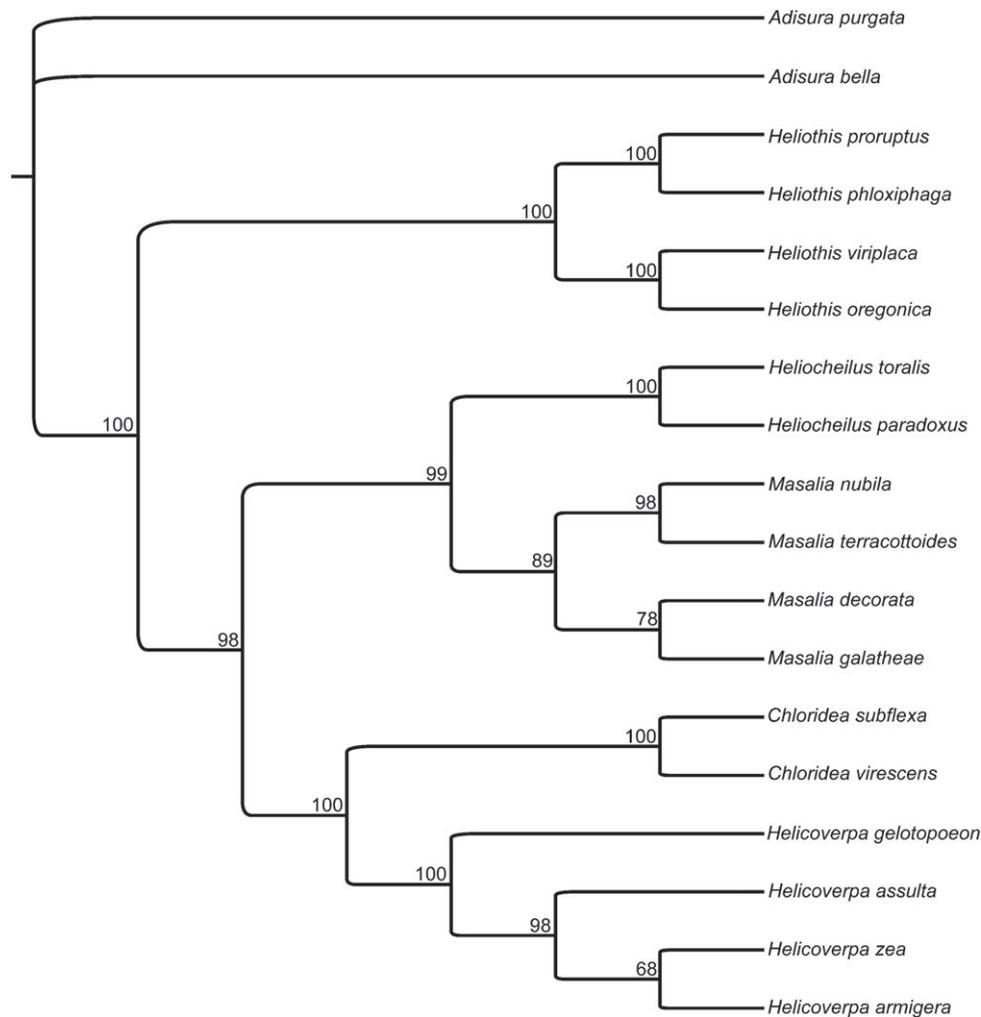


Fig. 14. The majority rule consensus tree inferred by Bayesian inference analysis, from All-Genes (*COI* sequence + *EFI- α* sequence + *DDC* sequence) data, using a mixed model approach. Numbers at the interior branches indicate the posterior probability ($\times 100$) values.

and *Heliothis* belong to different evolutionary lineages. *Heliothis* appears to be ancestral to the *Heliothis* group and *Chloridea* and *Helicoverpa* are more closely related to each other than to typical *Heliothis*.

Chloridea Duncan and (Westwood), 1841 revised status

Chloridea Duncan and Westwood, 1841, in Jardine, W. The Naturalist's Library, 33: 198.

Type species: *Phalaena rhexiae* J. E. Smith, 1797. Synonym of *Noctua virescens* Fabricius, 1777.

Aspila Guenée, 1852, Histoire Naturelle des Insectes. Species Général des Lépidoptères, 6: 174. A junior homonym of *Aspila* Stephens, 1834.

Type species: *Noctua virescens* Fabricius, 1777.

Included species:

Chloridea beckeri (Poole and Mitter), **new comb.**

Chloridea citrea (Poole and Mitter), **new comb.**

Chloridea distincta Schaus, **comb. rev.**

Chloridea ebenicor (Poole and Mitter), **new comb.**

Chloridea enigma (Poole and Mitter), **new comb.**

Chloridea mirabilis (Poole and Mitter), **new comb.**

Chloridea molochitina Berg, **comb. rev.**

Chloridea parana (Poole and Mitter), **new comb.**

Chloridea planaltina (Poole and Mitter), **new comb.**

Chloridea puno (Poole and Mitter), **new comb.**

Chloridea subflexa (Guenée), **comb. rev.**

Chloridea tergemina (Felder and Rogenhofer), **comb. rev.**

Chloridea virescens (Fabricius), **comb. rev.**

Diagnosis

Chloridea has three distinct autapomorphies. The ground colour of the forewing and abdomen are green, except in *C. planaltina*, in which the forewing is rusty-red, but the apex of the female abdomen bears some green scales and the antemedial and medial lines are straight. In other species

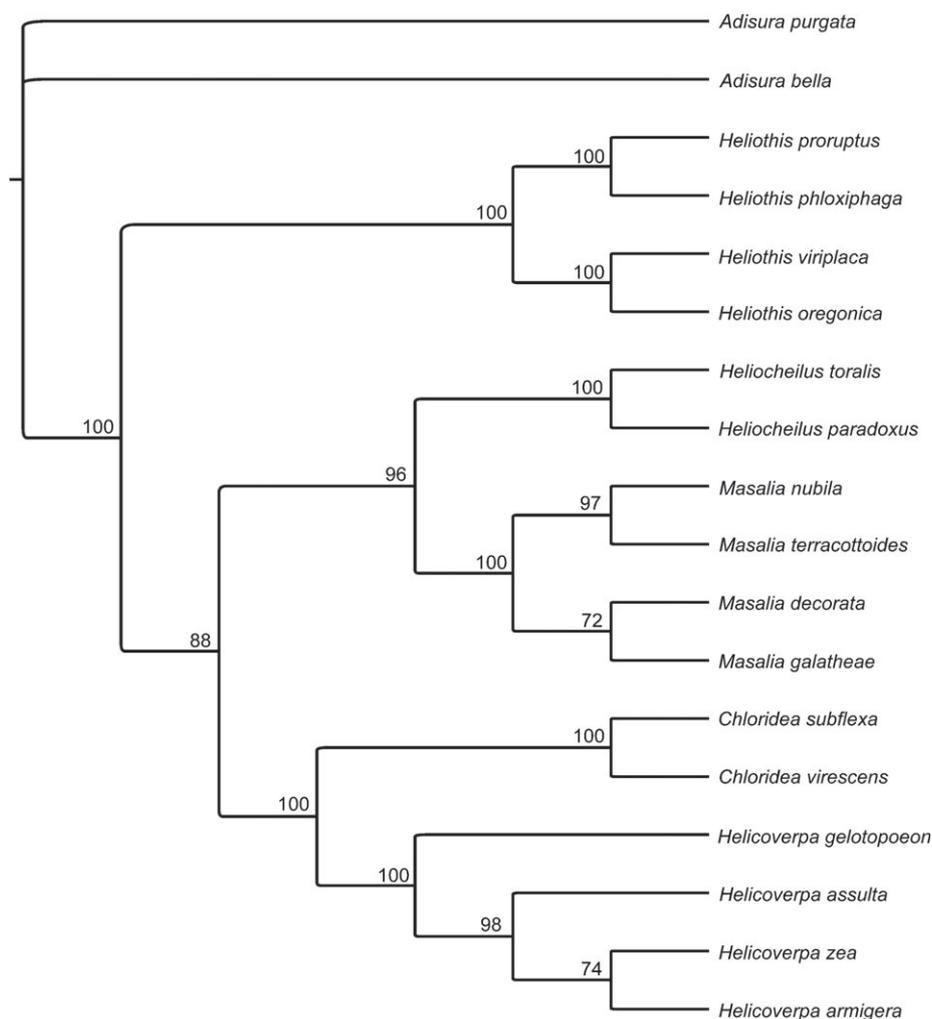


Fig. 15. The majority rule consensus tree inferred by Bayesian inference analysis, from molecular and morphology (*COI* sequence + *EF-1 α* sequence + *DDC* sequence + morphology) data, using a mixed model approach. Numbers at the interior branches indicate the posterior probability ($\times 100$) values.

of heliothines these lines are scalloped, sinuate, or dentate and curved. Another character is the presence of a patch of sex scales on the fore femur of the male in all species of *Chloridea*. This is also shared with males of *Helicoverpa*. In the female genitalia there is a unique sclerotization in the form of an elongate, ridged ribbon that extends from approximately the middle of the ductus bursae down and around the opening of the accessory bursae.

Larva

The D1 pinacula on A1, A2 and A8 are without spinules and the mandible either lacks or has a very small inner tooth in *Helicoverpa*. In *Chloridea* the D1 pinacula on A1, A2 and A8 have minute spinules and the mandible has a distinct inner tooth.

Distribution

Chloridea virescens and *C. subflexa* are widely distributed in the Western Hemisphere from the prairie provinces to the east coast of southern Canada south to South America, except in Chile and southern Argentina. The genus is also absent from the Pacific Northwest. Both species occur throughout the Caribbean islands. *Chloridea virescens* was introduced to the Hawaiian Islands, on Oahu in 1956 and also occurs on Lanai (Zimmerman, 1958). It is also found on the Galapagos Islands and is superficially very distinct from other *C. virescens* populations (Poole *et al.*, 1993).

Discussion

Early workers who relied almost exclusively on colour and pattern of the wings of Lepidoptera for classification used *Chloridea* for the genus name of *C. subflexa* (Guenée) and

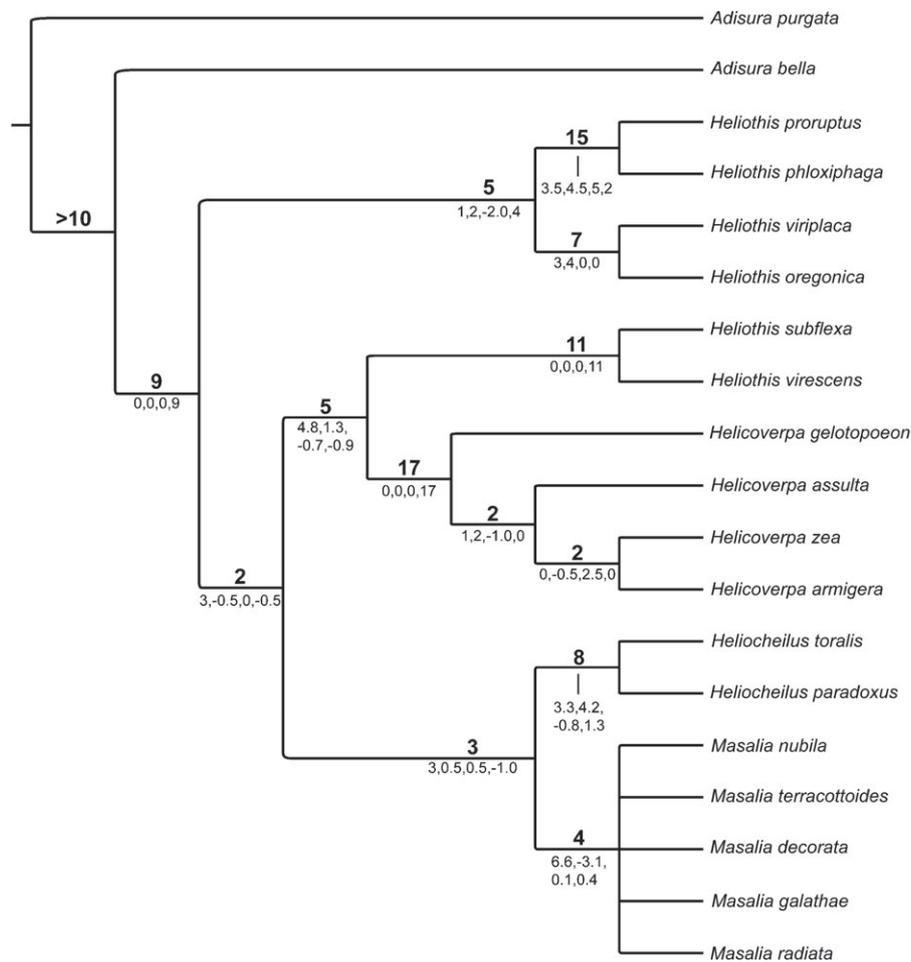


Fig. 16. The strict consensus of five most parsimonious trees from the molecular and morphology datasets (3484 steps, CI = 0.904, RI = 0.605). Numbers above the nodes are Bremer support values, numbers below the nodes are partitioned Bremer support values yielded by *EFI-α*, *DDC*, *COI* and morphology, respectively.

C. virescens (Fabricius) (Grote, 1874, 1882, 1890, 1895; Smith, 1893; Dyar, 1903 [1902]). In his list of the Lepidoptera of boreal America, Smith (1891) listed both *virescens* and *subflexa* in *Heliothis*, but then put them back in *Chloridea* (Smith, 1893). He justified placing them back in *Chloridea* because ‘they can be separated and an overloading of the genus [*Heliothis*] prevented’ (Smith, 1893: 268). In the checklists of Barnes & McDunnough (1917) and McDunnough (1938) both *virescens* and *subflexa* were listed in *Heliothis*. Neither author indicated who synonymized *Chloridea* with *Heliothis*.

Hardwick (1965) was the first modern systematist to remove a group of species in *Heliothis* to a monophyletic group when he described *Helicoverpa*. Matthews (1991) similarly described *Australothis* that includes four species occurring in Indo-Australasia in which two were originally placed in *Heliothis*. Both of these generic concepts are supported here and by Cho *et al.* (2008). There is similar morphological and genetic data to support the revised status of *Chloridea*.

Fibiger *et al.* (2009) alludes to the fact that *Chloridea* is monophyletic but does not officially revise the status to a valid genus. Here the revised status of *Chloridea* is established. Poole *et al.* (1993) revised the ‘*virescens* group’ of species and demonstrated the monophyly of the group based on morphological characters. The results of Cho *et al.* (2008) and those presented here support the monophyly of *Chloridea*.

Cho *et al.* (2008) presented a phylogeny of most of the genera known to be included in the Heliiothinae. Because the synapomorphies that define the Heliiothinae are in the larvae, some genera in which the larvae are not known are included in the Heliiothinae based on adult characters. These genera have never been included in any phylogeny of the Heliiothinae. Until fresh specimens are collected and subjected to molecular phylogenetic analysis, they will remain tentatively assigned to the Heliiothinae. An analysis using morphological characters and *COI* barcoding data, which includes the *Schinia* group s.s., *Psectrotarsia* and the *Pyrrhia* group s.s. is in progress.

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