Systematic Entomology

Systematic Entomology (2012), **37**, 635–656 DOI: 10.1111/j.1365-3113.2012.00641.x

A molecular phylogeny for the pyraloid moths (Lepidoptera: Pyraloidea) and its implications for higher-level classification

JEROME C. REGIER[1,2], CHARLES MITTER[1], M. ALMA SOLIS[3], JAMES E. HAYDEN[4], BERNARD LANDRY[5], MATTHIAS NUSS[6], THOMAS J. SIMONSEN[7], SHEN-HORN YEN[8], ANDREAS ZWICK[9] and MICHAEL P. CUMMINGS[10]

[1]Department of Entomology, University of Maryland, College Park, MD, U.S.A., [2]Institute for Bioscience and Biotechnology Research, College Park, MD, U.S.A., [3]Systematic Entomology Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, U.S.A., [4]Florida State Collection of Arthropods, Gainesville, FL, U.S.A., [5]Muséum d'Histoire Naturelle, Geneva, Switzerland, [6]Senckenberg Naturhistorische Sammlungen Dresden, Museum für Tierkunde, Königsbrücker Landstr., Dresden, Germany, [7]Department of Entomology, The Natural History Museum, London, U.K., [8]Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan, [9]Department of Entomology, State Museum of Natural History Stuttgart, Stuttgart, Germany and [10]Laboratory of Molecular Evolution, Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD, U.S.A.

Abstract. Pyraloidea, one of the largest superfamilies of Lepidoptera, comprise more than 15 684 described species worldwide, including important pests, biological control agents and experimental models. Understanding of pyraloid phylogeny, the basis for a predictive classification, is currently provisional. We present the most detailed molecular estimate of relationships to date across the subfamilies of Pyraloidea, and assess its concordance with previous morphology-based hypotheses. We sequenced up to five nuclear genes, totalling 6633 bp, in each of 42 pyraloids spanning both families and 18 of the 21 subfamilies, plus up to 14 additional genes, for a total of 14 826 bp, in 21 of those pyraloids plus all 24 outgroups. Maximum likelihood analyses yield trees that, within Pyraloidea, differ little among datasets and character treatments and are strongly supported at all levels of divergence (83% of nodes with bootstrap ≥80%). Subfamily relationships within Pyralidae, all very strongly supported (>90% bootstrap), differ only slightly from a previous morphological analysis, and can be summarized as Galleriinae + Chrysauginae (Phycitinae (Pyralinae + Epipaschiinae)). The main remaining uncertainty involves Chrysauginae, of which the poorly studied Australian genera may constitute the basal elements of Galleriinae + Chrysauginae or even of Pyralidae. In Crambidae the molecular phylogeny is also strongly supported, but conflicts with most previous hypotheses. Among the newly proposed groupings are a 'wet-habitat clade' comprising Acentropinae + Schoenobiinae + Midilinae, and a provisional 'mustard oil clade' containing Glaphyriinae, Evergestinae and Noordinae, in which the majority of described larvae feed on Brassicales. Within this clade a previous synonymy of Dichogaminae with the Glaphyriinae is supported. Evergestinae **syn. n.** and Noordinae **syn. n.** are here newly synonymized with Glaphyriinae, which appear to be paraphyletic with respect to both. Pyraustinae and Spilomelinae as

Correspondence: Charles Mitter, Department of Entomology, University of Maryland, College Park, MD 20742, U.S.A. E-mail: cmitter@umd.edu

sampled here are each monophyletic but form a sister group pair. Wurthiinae **n. syn.**, comprising the single genus *Niphopyralis* Hampson, which lives in ant nests, are closely related to, apparently subordinate within, and here newly synonymized with, Spilomelinae **syn. n.**

Introduction

The Pyraloidea, comprising the families Pyralidae and Crambidae, are one of the mega-diverse superfamilies of Lepidoptera, trailing only Gelechioidea and Papilionoidea outside the Macroheterocera sensu van Nieukerken *et al*. (2011). The 15 500 + described species (van Nieukerken *et al*., 2011) are distributed worldwide; many more species are undescribed, especially in the tropics. Pyraloidea include numerous major pests of crops, stored foodstuffs, forests and ornamental plants, as well as biological control agents used successfully against invasive plants (e.g. Zhang, 1994; Center *et al*., 2002). They are among the most ecologically diverse lepidopteran superfamilies: in addition to feeding on most major groups of plants, pyraloid larvae collectively exploit a startling range of other resources, with habits including detritivory, coprophagy, predation and parasitism. Pyraloids include one of the largest lepidopteran lineages in which the majority of immature stages are adapted to aquatic habitats (Yen, 2004; Mey & Speidel, 2008; Solis, 2008). Pyraloidea are an ubiquitous element of terrestrial ecosystems, and have served as models in the study of biodiversity and community ecology (e.g. Fiedler & Schulze, 2004; Gounou & Schulthess, 2004; Yamanura *et al*., 2006; Beck *et al*., 2011; Tao *et al*., 2008a, b; Janzen *et al*., 2009), population ecology and management (e.g. Ellsworth *et al*., 1989; Cavalieri & Kocak, 1995; Onstad & Gould, 1998; Small, 2007; Arthur, 2008; Tao *et al*., 2008a, b; Oppert *et al*., 2010), behavioral ecology (e.g. Oliveira, 2005; Lewis & Wedell, 2009; Ingleby *et al*., 2010; Lewis *et al*., 2011), the genetics and evolution of pheromone communication systems (e.g., Roelofs *et al*., 2002; Lassance, 2010; Fuji *et al*., 2011), parasitoid–host co-evolution (e.g. Eliopoulos & Stathas, 2003; Roberts *et al*., 2006; Niogret *et al*., 2009), and physiology and development (e.g. Deniro & Epstein, 1978; Siaussat *et al*., 2008; Ukai *et al*., 2009; Yin *et al*., 2011). The greater wax moth (*Galleria mellonella* (Linnaeus)), long a laboratory model, increasingly has been used recently for study of the infection process, propagation, and population genetics of microbial pathogens (e.g. Cotter *et al*., 2000; Mylonakis *et al*., 2005; Scully & Bidochka, 2006, 2009; Cowen *et al*., 2009; Mukherjee *et al*., 2010).

A reliable classification and phylogeny are indispensable for the organization, communication and prediction of observations about such an economically and scientifically important group of insects, and for understanding how the traits important to their management and exploitation, such as their host-plant ranges, evolve. Whereas much recent progress has been made (see next section), the state of systematics is less well advanced in pyraloids than in some other large superfamilies. A notable gap is the absence of robustly supported estimates of relationships among the subfamilies. Phylogenetic studies based on characters of adult anatomy have been hampered by a dearth of interpretable variation, particularly in Crambidae (Solis & Maes, 2002).

Very recently, molecular data for small numbers of pyraloid taxa, gathered as part of broad phylogenetic surveys across the Lepidoptera, have shown much promise for resolution of relationships within this superfamily (Regier *et al*., 2009; Mutanen *et al*., 2010; Cho *et al*., 2011). The purpose of this paper, building on those preliminary findings, is to present the most detailed molecular estimate of relationships to date across the subfamilies of Pyraloidea. Using up to 19 genes sequenced previously by Cho *et al*. (2011), we expand those authors' taxon sampling from 12 pyraloids to 42, spanning 18 of the 21 subfamilies recognized by Nuss *et al*. (2003–2012). We then review the agreement and disagreement of the molecular phylogeny with traditional morphological data and the subfamily concepts and relationships based on them.

Taxonomic background

The last few decades have seen steady progress in the classification of Pyraloidea, on several fronts (reviews in Minet, 1985; Common, 1990; Munroe & Solis, 1999). The superfamily definition has become more precise and explicitly phylogenetic, due to the exclusion of many taxa which had been placed in this taxon by earlier authors, including Pterophoridae, Thyrididae, Hyblaeidae, Alucitidae, Tineodidae and Dudgeoneidae (Fletcher & Nye, 1984; Minet, 1985; Nielsen, 1989; Common, 1990). The chief synapomorphies of Pyraloidea are now considered to be: ventro-medial tympanal organs on the first two abdominal segments (absent or reduced in a few species) and scales on the base of the proboscis (presumably convergent with Gelechioidea, Tischerioidea and Choreutoidea).

There is now a clearly established basal divergence in the superfamily, supported by multiple differences in construction of the tympanal organs (Börner, 1925) and larval characters (Hasenfuß, 1960). Initially unwilling to elevate the family group name Pyralidae to Pyraloidea, Munroe (1972, 1973, 1976) proposed the informal groups Pyraliformes and Crambiformes to distinguish the two sister clades. Minet (1982), following extensive study of tympanal organs in Lepidoptera, formally raised Munroe's groups to the family level, under the names Pyralidae and Crambidae. However the two-family classification is rejected by some, including one of the present co-authors (BL), on the grounds that use of one versus two family names is subjective, and that to the nonspecialist a crambiforme is not always easily separated from a pyraliforme, whereas a pyraloid can immediately be distinguished from

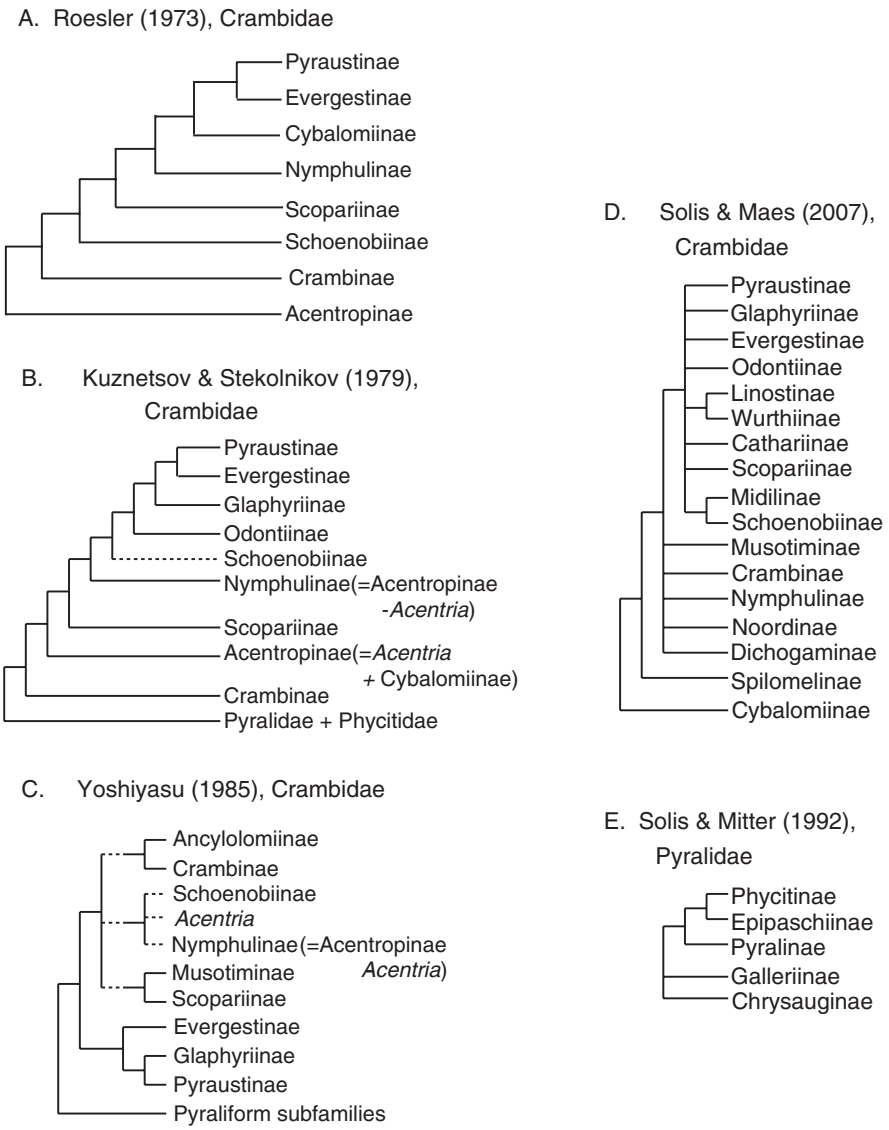

Fig. 1. Previous hypotheses of phylogeny in Pyraloidea. (A) Phylogeny of Crambidae according to Roesler (1973), based on adult and immature characters. (B) Phylogeny of Crambidae according to Kuznetsov & Stekolnikov (1979), based primarily on musculature of male genitalia. (C) Phylogeny of Crambidae according to Yoshiyasu (1985), based on adult and immature characters. (D) Phylogeny of Crambidae according to Solis & Maes (2002), based on phylogenetic analysis of adult characters. (E) Phylogeny of Pyralidae according to Solis & Mitter (1992), based on phylogenetic analysis of adult and larval characters. Tree figures redrawn, but nomenclature follows the original except as noted. In (A–C), Pyraustinae includes Spilomelinae.

other moths by virtue of the scaled proboscis and presence of abdominal tympanal organs.

Recent work has clarified the synapomorphy-based definition and composition of many but not all of the subfamilies. Concomitantly, there has been increasing interest in the phylogenetic relationships among these. The explicit previous phylogenetic hypotheses known to us are summarized in Fig. 1. Relationships within Pyralidae have been addressed only once, in a cladistic analysis based mainly on adult morphology (Solis & Mitter, 1992). The phylogeny is almost completely resolved (Fig. 1E), but most of the nodes are supported by very few characters. Relationships within Crambidae have been addressed several times, but these hypotheses disagree extensively with each other. Roesler (1973) proposed a phylogeny for the crambid subfamilies (Fig. 1A) based on adult morphology, although he did not present a formal analysis. Kuznetsov & Stekolnikov (1979) presented an alternative view of crambid relationships, also without explicit analysis, arguing mainly from male genital musculature (Fig. 1B). Yoshiyasu (1985) combined information from all life stages to arrive at yet another tree for the crambid subfamilies, which differs in most groupings from its two predecessors. The first explicit

Table 1. Species sampled and their distribution across the current pyraloid classification (Nuss *et al*., 2003–2011).

Crambidae (1018 genera, 9666 species; counts are taken from Beccaloni *et al*., 2003; Nuss *et al*., 2003–2011)
Acentropinae (78 genera, 700 species): *Aulacodes* sp. nov.*; Petrophila confusalis*
Crambinae (179 genera, 1987 species): *Chilo suppressalis; Crambus agitatellus; Catoptria oregonica*
Evergestinae (10 genera, 110 species): *Crocidolomia luteolalis; Evergestis subterminalis*
Glaphyriinae (40 genera, ~200 species): *Chalcoela iphitalis; Cosmopterosis spatha; Dicymolomia metalliferalis; Dichogama colotha*
Midilinae (9 genera, 56 species): *Dismidila atoca; Midila daphne; Cacographis osteolalis*
Musotiminae (24 genera, 162 species): *Neurophyseta conantia*
Noordinae (1 genus, 17 species): *Noorda blitealis*
Odontiinae (91 genera, 360 species): *Syntonarcha iriastis; Cliniodes opalalis*
Pyraustinae (190 genera, 1413 species): *Ostrinia furnacalis; Pyrausta nexalis*
Schoenobiinae (29 genera, 201 species): *Rupela albina; Scirpophaga incertulas*
Scopariinae (24 genera, 555 species): *Scoparia isochroali; Eudonia spenceri*
Spilomelinae (317 genera, 3767 species): *Mesocondyla dardusalis; Diaphania indica; Phaeodropsis alitemeralis*
Wurthiinae (1 genus, 8 species): *Niphopyralis chionesis*
Cybalominae (19 genera, 64 species): not sampled
Heliothelinae (5 genera, 49 species): not sampled
Linostinae (1 genus, 4 species): not sampled
Cathariinae (1 genus, 1 species): not sampled
Pyralidae (1056 genera, 5921 species)
Chrysauginae (133 genera, 391 species): *Monoloxis flavicinctalis, Polyterpnes polyrrhoda*
Epipaschiinae (94 genera, 705 species): *Salma pyrastis; Accinctapubes albifasciata*
Galleriinae (65 genera, 259 species): *Macrotheca* sp.*; Galleria melonella*
Phycitinae (635 genera, ~3450 species): *Ambesa laetella;* [unidentified]; *Plodia interpunctella; Dioryctria auranticella*
Pyralinae (129 genera, >1100 species): *Pyralis farinalis; Gauna aegusalis; Hypsopygia (Hypsopygia) olinalis*; *Hypsopygia (Ocrasa) glaucinalis*
Outgroups
Noctuoidea: Doidae: *Doa sp. 'Janzen01'*
Cimelioidea: Cimeliidae: *Axia margarita*
Mimallonoidea: Mimallonidae: *Lacosoma chiridota*
Drepanoidea: Drepanidae: *Cyclidia substigmaria modesta*
Drepanoidea: Epicopeiidae: *Epicopeia hainesii*
Geometroidea: Geometridae: *Dichromodes sp.'7'*
Geometroidea: Uraniidae: *Lyssa zampa*
Noctuoidea: Notodontidae: *Crinodes besckei*
Bombycoidea: Carthaeidae: *Carthaea saturnioides*
Lasiocampoidea: Lasiocampidae: *Chionopsyche montana*
Hyblaeoidea: Hyblaeidae: *Hyblaea ibidias*
Pterophoroidea: Pterophoridae: *Agdistis americana*
Epermenioidea: Epermeniidae: *Epermenia chaerophyllella*
Hesperioidea: Hesperiidae: *Urbanus doryssus*
Papilionoidea: Nymphalidae: *Asterocampa celtis*
Calliduloidea: Callidulidae: *Pterodecta felderi*
Thyridoidea: Thyrididae: *Glanychus insolitus*
Copromorphoidea: Carposinidae: *Sosineura mimica*
Alucitoidea: Alucitidae: *Alucita* sp.
Zygaenoidea: Limacodidae: *Pantoctenia prasina*
Cossoidea: Cossidae: *Archaeoses pentasema*
Gelechioidea: Xyloryctidae: *Leistarcha scitissimella*
Gelechioidea: Gelechiidae: *Pectinophora gossypiella*
Galacticoidea: Galacticidae: *Homadaula anisocentra*

cladistic analysis of crambid relationships (Fig. 1D; Solis & Maes, 2002), based on adult morphology only, resolved only 5 of 15 possible nodes, most supported by a single character change. Despite exhaustive search by the authors, this study yielded only 17 adult characters for the 17 taxa included, not enough to resolve relationships among the subfamilies. In sum, morphological study so far has yielded limited insights on relationships among pyraloid subfamilies.

Materials and methods

Taxon and gene sampling

The central goal of this study was to estimate relationships among the subfamilies of Pyraloidea. Therefore we sought to include diverse representatives of as many of these as possible. The distribution of the 42 sequenced pyraloid species across the current pyraloid classification (Nuss *et al*.,

2003–2012) is shown in Table 1. Our sample encompasses all five subfamilies of Pyralidae and 13 of the 16 subfamilies of Crambidae. We were unable to obtain fresh material for three small crambid subfamilies, namely, the Neotropical Linostinae (1 genus, 4 species) and the Old World Heliothelinae (5 genera, 49 species) and Cybalomiinae (18 genera, 64 species). Fifteen subfamilies were represented by 2 or more divergent genera, and of these, 4 of the 5 largest subfamilies (>1000 species) were represented by 3 or 4 genera each.

Recent molecular studies (Regier *et al*., 2009; Mutanen *et al*., 2010; Cho *et al*., 2011) show that Pyraloidea are closely related to the Macroheterocera sensu van Nieukerken *et al*. (2011), which exclude the butterflies and relatives. However, the exact sister group to pyraloids has not been definitively established. For example, in some molecular analyses (e.g. fig. 2B of Regier *et al*., 2009) the sister group is Macroheterocera; in others (e.g. fig. ESM 1 of Mutanen *et al*., 2010) the pyraloids join first with Hyblaeidae before both join Macroheterocera; and in still others (e.g. figs 2D, E of Regier *et al*., 2009), pyraloids group with a subsection of Macroheterocera (specifically Doidae and Cimeliidae), all without strong support. To take account of this uncertainty, we included 24 diverse outgroup taxa (listed in Table 1), representing all superfamilies which have been identified as possible near relatives to Pyraloidea by previous authors, including published molecular studies as well as preliminary analyses of the Leptree project data set, which is described at http://www.leptree.net. All superfamilies of Macroheterocera are included among the outgroups, and nomenclature for the outgroups follows van Nieukerken *et al*. (2011).

Specimens for this study, obtained with the kind help of collectors around the world (see Acknowledgements), are stored in 100% ethanol at −85°C as part of the ATOLep collection at the University of Maryland (details at http://www.leptree.net/collection). DNA extraction used only the head and thorax for most species, leaving the rest of the body including the genitalia as a voucher, although the entire specimen was used for smaller species (see Table S1). Wing voucher images for most of our exemplars are posted at http://www.leptree.net/voucher_image_list, and DNA 'barcodes' for nearly all specimens have been kindly generated by the All-Leps Barcode of Life project http://www.lepbarcoding.org/, allowing check of our identifications against the BOLD (Barcode of Life Data system) reference library and facilitating future identification of specimens whose identity is still pending (i.e. species listed as 'sp.' or 'unidentified' in this report).

The gene sample for this study, consisting entirely of protein-coding regions of nuclear genes, comprises two components (Table 2). First, all taxa were sequenced for the five gene fragments described by Regier *et al*. (2009), which total 6633 bp, not including 333 bp with uncertain alignments. These genes are: *CAD* (2928 bp; Moulton & Wiegmann, 2003), *DDC* (1281 bp; Fang *et al*., 1997), *enolase* (1134 bp; Farrell *et al*., 2001), *period* (888 bp; Regier *et al*., 1998) and *wingless* (402 bp; Brower & DeSalle, 1998). This set of genes has been used to resolve lepidopteran relationships at a variety of taxonomic levels (Regier *et al*., 2008a, b, 2009; Kawahara *et al*., 2009).

Second, to increase resolving power, all of the outgroups, plus approximately half of the pyraloids (22/42 = 52%), spread over all 18 subfamilies represented, were also sequenced for additional 14 gene regions totaling 8193 bp. The 14 additional gene regions are a subset of the 21 new gene regions first tested across ditrysian Lepidoptera by Zwick *et al*. (2011) and Cho *et al*. (2011). GenBank numbers for these sequences are listed in Table S1.

Generation of DNA sequence data

A detailed protocol of all laboratory procedures is provided by Regier *et al*. (2008c). Further descriptions, including gene amplification strategies, PCR primer sequences, and sequence assembly and alignment methods, can be found in Regier *et al*. (2008a, b, c, 2009). To summarize, total nucleic acids were isolated and specific regions of the cognate mRNAs were amplified by RT-PCR. Specific bands were gel-isolated and re-amplified by PCR using hemi-nested primers, when available. Visible bands that were too faint to sequence were re-amplified using the M13 sequences at the 5′ ends of all primers. PCR amplicons were sequenced directly on a 3730 DNA Analyzer (Applied Biosystems). Sequences were edited and assembled using the TREV, PREGAP4 and GAP4 programs in the STADEN package (Staden, 1999). Multi-sequence alignments were made using the Translation Align program within the Geneious Pro v5.3.4 software package (Protein alignment option: Geneious Alignment; Cost matrix: Blosum62; Gap open penalty: 12; Gap extension penalty: 3; Alignment type: Global alignment with free end gaps; + Build guide tree via alignment; Refinement iterations: 2). A data-exclusion mask of unalignable 1440 characters out of 20 373 total aligned characters (= 7.1% of total) for all 66 species was applied.

Character partitions, taxon × gene dataset design and phylogenetic analyses

Previous studies using these same genes (Regier *et al*., 2009; Cho *et al*., 2011) have shown that in some regions of the phylogeny of Lepidoptera, sites undergoing synonymous substitutions are prone to among-lineage base compositional heterogeneity, thereby obscuring and sometimes misleading phylogeny inference. For this reason, in addition to using all data unpartitioned, we performed analyses in which synonymous change was either potentially down-weighted or excluded. In one such analysis, we partitioned nucleotides into sets undergoing mostly synonymous versus mostly nonsynonymous change following Regier *et al*. (2009).

Second, we used the 'degen-1' coding of Regier *et al*. (2010), which in effect excludes synonymous change (only) entirely. Degen-1 is an extension of the RY coding scheme (Phillips *et al*., 2004). Nucleotides at any codon position that have the potential of directly undergoing synonymous change,

by virtue of the specific codon they are part of, are fully degenerated, using standard IUPAC codenames. For example, CAC and CAT (His) are both coded CAY, whereas TTA, TTG, CTT, CTC, CTA and CTG (Leu) are all coded YTN. As a result, synonymous pairwise differences between species are entirely eliminated. Synonymous change becomes largely invisible to phylogenetic inference methods, and any compositional heterogeneity it produces is eliminated. The substitution model used in all analyses was GTR + gamma + I. This model was applied separately to each character subset in the partitioned analysis.

Our somewhat unconventional sampling plan, in which only about half the ingroup taxa were sequenced for the full set of 19 genes, was designed to maximize efficiency of resource use in resolving the deeper nodes within Pyraloidea. The effectiveness of such deliberately incomplete gene sampling, which in theory might be undercut by phylogenetic artifacts resulting from the large blocks of missing data (Wiens, 1998; Lemmon *et al*., 2009), has been supported by simulations and by a growing body of case studies (Cho *et al*., 2011; Wiens & Morrill, 2011, and references therein). To ensure that our results are not subject to artifacts from blocks of deliberately absent data, and to add to the empirical evidence on this issue, we carried out parallel analyses on the full, deliberately incomplete 19 gene dataset and on a reduced gene sample including only the 5 gene regions sequenced in all 66 taxa.

All phylogenetic analyses were based on the maximum likelihood criterion as implemented in GARLI (Genetic Algorithm for Rapid Likelihood Inference; v1.0; Zwickl, 2006). We used the program default settings, including random stepwise addition starting trees, except that we halved the number of successive generations yielding no improvement in likelihood score that prompts termination (genthreshfortopoterm = 10000), as suggested for bootstrapping in the GARLI manual. Each search for an optimal tree consisted of 970–1000 GARLI runs, whereas bootstrap analyses consisted of 708–750 pseudo-replicates, each based on 15 heuristic search replicates. Optimal-tree searches and bootstrap analyses were parallelized using Grid computing (Cummings & Huskamp, 2005) through The Lattice Project (Bazinet & Cummings, 2009). For consistency in the characterization of results, we will refer to bootstrap support of 70–79% as 'moderate,' 80–89% as 'strong' and ≥90% as 'very strong.'

Results

Molecular analysis

Figure 2 shows the best ML tree found in 1000 GARLI searches using nt123 (unpartitioned) for the 19-gene deliberately incomplete dataset, with bootstrap values for all analyses superimposed on the branches. The nodes are numbered to facilitate presentation. Within Pyraloidea, we find well-resolved topologies, strongly supported at all levels of divergence, that are largely concordant across datasets and character treatments. For the 19-gene deliberately incomplete dataset, the percentages of nodes (of 41 total) with bootstrap support of ≥70, ≥80 and ≥90% were 93, 83 and 71%, respectively, for analysis of nt123. The majority of nodes are also strongly supported by the degen-1 (nonsynonymous-only) dataset (76, 66 and 59% of nodes, respectively, with bootstrap values of ≥70, ≥80 and ≥90%). Only three nodes showed weak bootstrap support in all analyses. Although the degen-1 dataset yielded six nodes that conflict with the nt123 tree, it provided only weak bootstrap support (<50%) for those alternatives. Thus in these data, there is little sign of conflict in apparent signal between synonymous and nonsynonymous change. The 5-gene matrix alone is highly informative about relationships within pyraloids, providing bootstrap support comparable to that from the 19-gene matrix for most nodes. For several deeper nodes within Pyraloidea, however, moderate to strong support is evident only when all 19 genes are included; examples include Pyraloidea (node 9), Crambidae (node 8) and one of the two major lineages within Crambidae (node 7).

In marked contrast to relationships within Pyraloidea, relationships among the outgroups (not shown in Fig. 2; see Fig. 3) are highly unstable to differences among data matrices and character treatments. Strong bootstrap support for relationships among superfamilies is almost entirely lacking. For the most part, the 19-gene and 5-gene analyses give essentially identical topologies within Pyraloidea except for nodes that are very weakly supported in all analyses. However, there are several groupings (nodes 3, 11, 34, 41) to which the 19-gene nt123 analysis gives ≥70% bootstrap support that are contradicted by the corresponding 5-gene analysis, sometimes strongly so. This result is one that might be expected from phylogenetic artifacts due to blocks of missing data in the deliberately incomplete 19-gene matrix (Cho *et al*., 2011). Evidence against that explanation comes from ongoing unpublished analyses (data not shown) of a complete 19-gene matrix of 483 species across the Lepidoptera, including all Pyraloidea sequenced for 19 genes (see Fig. 2). In each case, these trees agree with the results from the deliberately incomplete 19-gene matrix in the present study, suggesting that blocks of missing data have no major effect. A plausible alternative explanation for discrepancies between the 5-gene and 19-gene analyses, further discussed below, is conflicting signal between individual genes in the 5-gene versus 19-gene datasets.

Discussion

In this section we review the agreement and disagreement of our molecular results with previous hypotheses and morphological evidence on the phylogeny of Pyraloidea, including the monophyly and composition of each subfamily. The node numbers referred to below correspond to numbers to the right of nodes in the cladogram of Fig. 2. We begin at the base of Pyraloidea, then treat Pyralidae from base to tips, followed by Crambidae. To help link this discussion to the actual organisms in question, we provide a representative adult habitus image for each subfamily in Fig. 4. In conjunction with this paper we provide an illustrated online synopsis of each subfamily

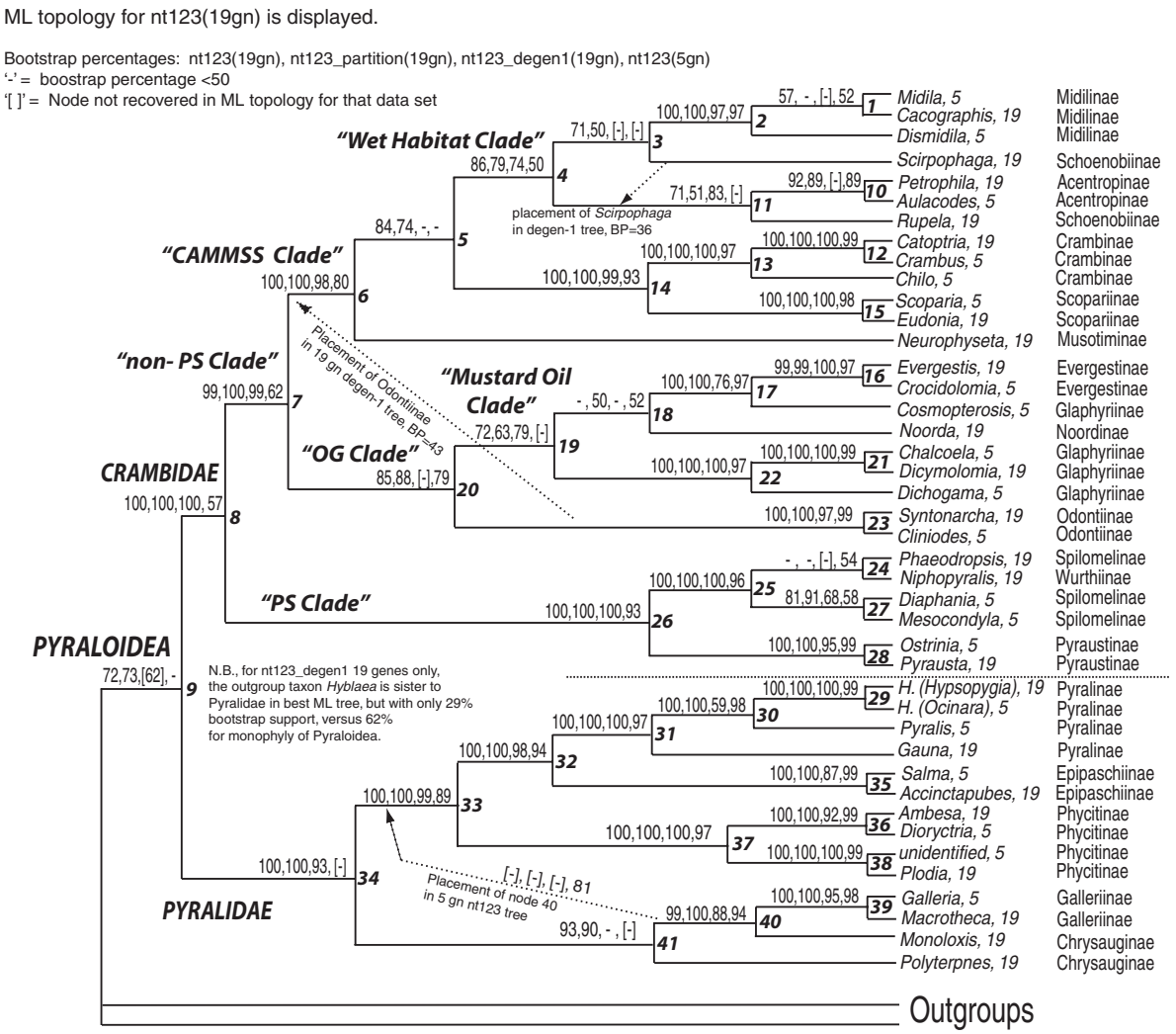

Fig. 2. Maximum likelihood estimate of phylogenetic relationships in Pyraloidea obtained from 500 GARLI searches under a GTR + gamma + I model for all nucleotides (unpartitioned). Bootstrap support values (1000 bootstrap replicates) above branches for: nt123 (19 genes), nt123_partitioned (19 genes), degen-1 (19 genes), and nt123 (5 genes). Hyphen (-) denotes bootstrap value <50%. Square brackets denote node not present in the best ML tree for that analysis. Node numbers (to the right of node) are used to organize text presentation of phylogeny. Dotted lines and associated bootstrap values show alternative placements for selected taxa. The number of genes sequenced (5 vs. 19) is given after each genus name.

as understood currently, at http://www.leptree.net and on the Encyclopedia of Life web site at http://www.EOL.org.

Monophyly and phylogenetic position of Pyraloidea, and basal divergence within the superfamily (node 9)

The molecular data generally favour monophyly for Pyraloidea, with bootstrap support up to 73% (Fig. 2). Our results thus corroborate the strong evidence from pyraloid morphological synapomorphies, which include, among others: a scaled proboscis base; paired tympanal organs situated ventrally on the second abdominal segment; Rs2 and Rs3 stalked in the forewing; and stalking or approximation of veins Sc + R and Rs in the hindwing. Given the abundant morphological evidence, it is somewhat surprising that molecular support for pyraloid monophyly is so modest. Clearly there is some conflicting signal within the molecular dataset. For example, although all nonsynonymous character coding (degen-1) provides 62% bootstrap support for pyraloid monophyly, in the single best ML tree found in that analysis, as well as 29% of the bootstrap replicates, the exemplar of Hyblaeidae groups with Pyralidae, rendering Pyraloidea paraphyletic. No morphological study supports a sister-group relationship between Hyblaeidae and either Pyraloidea or any subgroup thereof. Historically Forbes (1933) associated the two groups based on pupal characters, but Minet (1982) placed Hyblaeidae in their own superfamily because they lack tympanal organs and scales at the base of the proboscis. Conflicting signal also may account in part for the almost complete lack of strong bootstrap

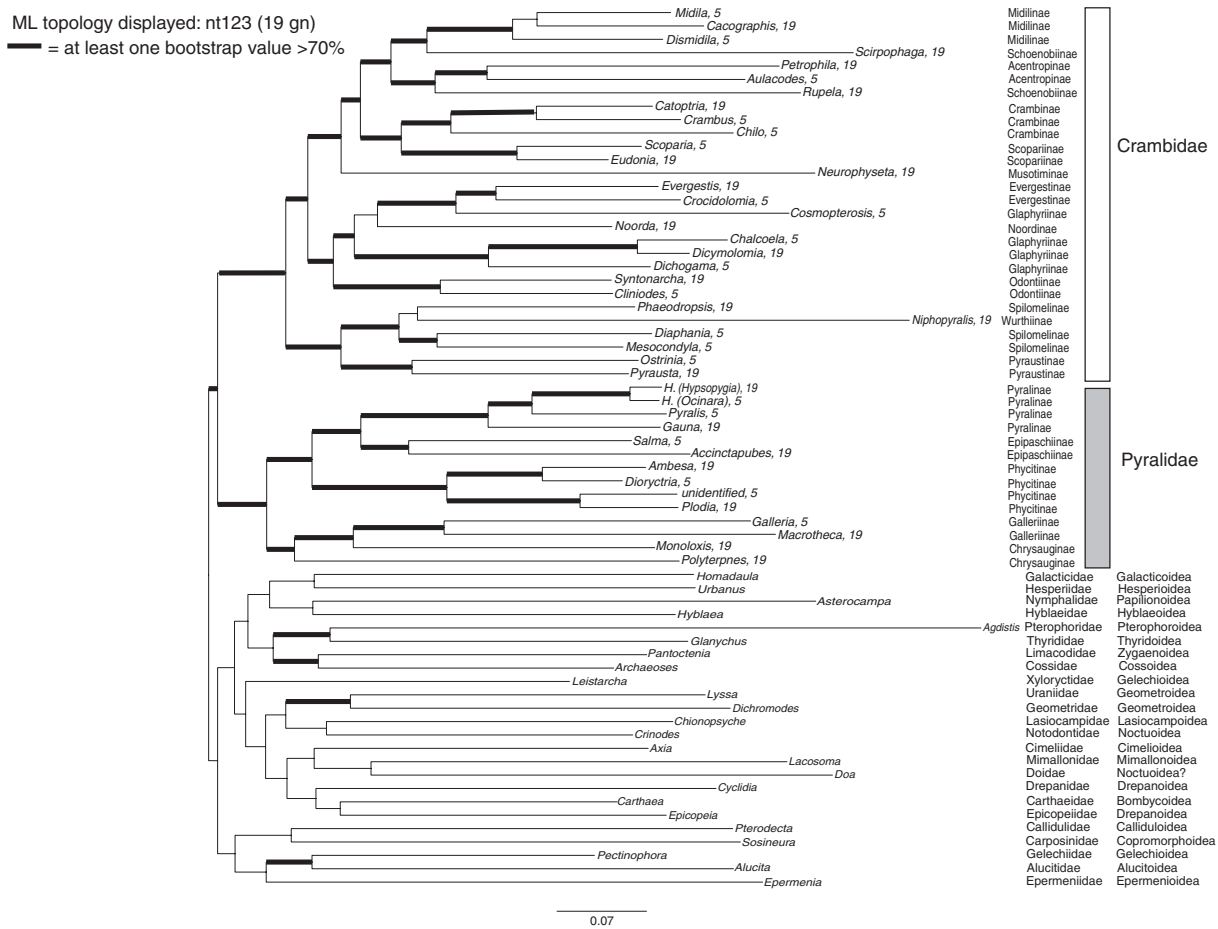

Fig. 3. Phylogram presentation of best tree obtained from 1000 GARLI searches under a GTR + gamma + I model for all nucleotides (unpartitioned). Thickened branches are supported by ≥70% bootstrap in at least one analysis (see Fig. 2).

support for relationships among outgroups in our study, which thus provides no new insight on the sister group to Pyraloidea.

Within Pyraloidea, there is very strong molecular support for the basal divergence between Crambidae (node 8) and Pyralidae (node 34); both with 100% bootstrap. In the remainder of this account we consider relationships within each of these families in turn.

Monophyly of and basal divergence within Pyralidae (node 34)

Pyralidae (node 34; BP = 100) are well supported by morphological synapomorphies, including: closed bulla tympana (Minet, 1982, 1985); forewing Rs4 stalked with Rs2 + 3 (paralleled in some Crambidae) (Minet, 1982, 1985); larva with a sclerotized ring around the base of A8 setae SD1 (Gerasimov, 1947, 1949; Allyson, 1977); and, presence of lateral arms at the base of the uncus (Solis & Mitter, 1992).

The relationships among pyralid subfamilies inferred from the molecular data, which are very strongly supported, largely correspond to those found in the morphological phylogenetic analysis of Solis & Mitter (1992; see Fig. 1E), except that the positions of Phycitinae and Pyralinae are reversed (Fig. 2). Exactly the same relationships found in our current results are evident in the smaller molecular studies of Regier *et al*. (2009) and Mutanen *et al*. (2010). The basal split lies between Chrysauginae + Galleriinae (node 41; BP = 96) and Phycitinae + Epipaschiinae + Pyralinae (node 33; BP = 100). The latter three subfamilies are united by reduction of the secondary venulae of abdominal S2, and by an apparent transformation series in the female frenulum, from more than three bristles in most Pyraloidea, to three bristles in Chrysauginae and Galleriinae, to two bristles in the last common ancestor of Phycitinae + Epipaschiinae + Pyralinae (retained in Epipaschiinae + Pyralinae), to one in Phycitinae (Solis & Mitter, 1992). These transformations may represent fusion rather than loss of bristles.

Phycitinae + Epipaschiinae + Pyralinae (node 33)

Within the lineage comprising Phycitinae + Epipaschiinae + Pyralinae (node 33), there is very strong support for a basal

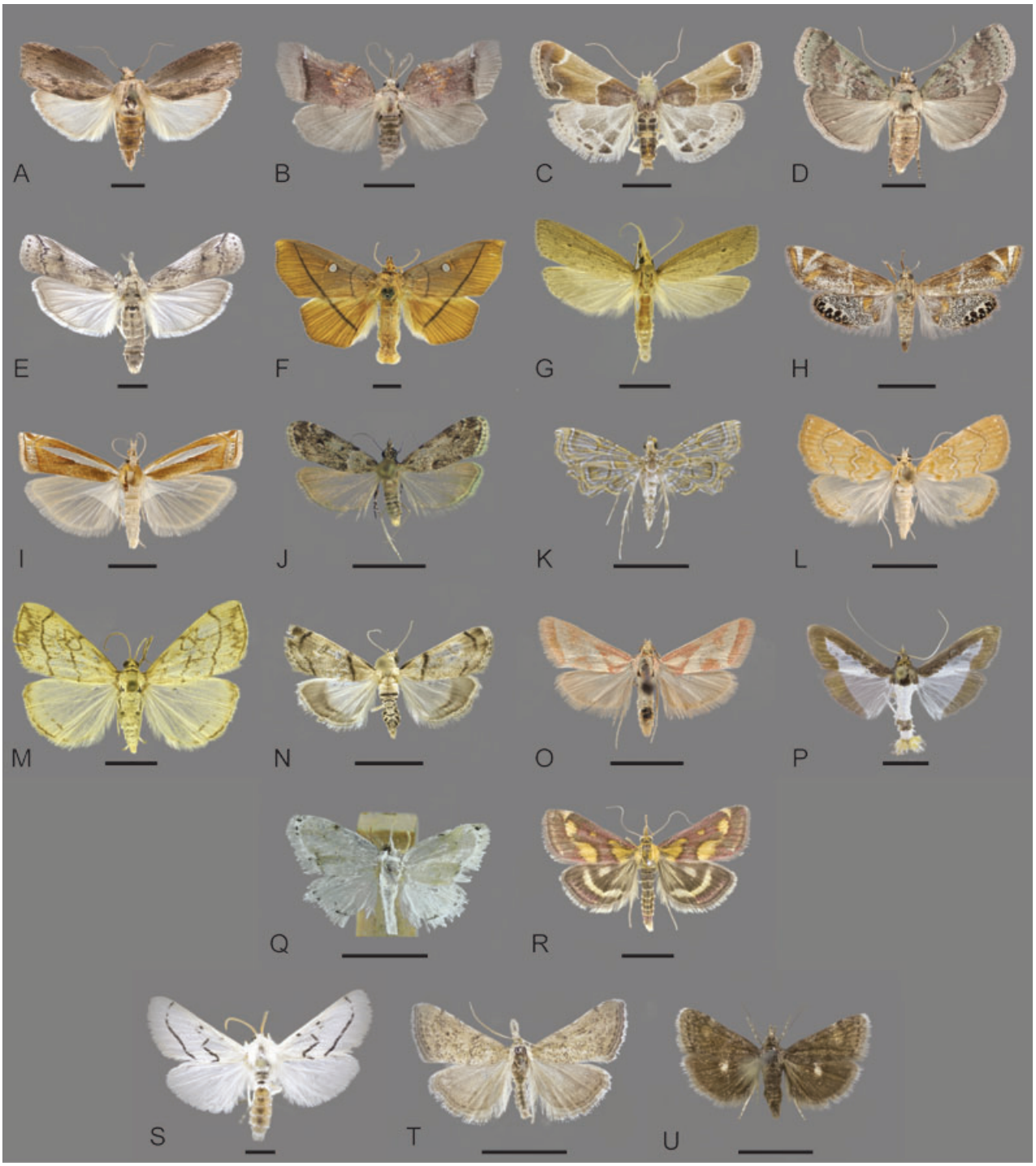

Fig. 4. Representative adult habitus images of Pyraloidea subfamilies. (A) Galleriinae (*Galleria mellonella* (L.)), (B) Chrysauginae (*Clydonopteron sacculana* (Bosc)), (C) Pyralinae (*Pyralis farinalis* (L.)), (D) Epipaschiinae (*Deuterollyta majuscula* Herrich-Schäffer), (E) Phycitinae (*Cactoblastus cactorum* (Berg)), (F) Midilinae (*Midila daphne* (Druce)), (G) Schoenobiinae (*Donacaula sordidellus* (Zincken)), (H) Acentropinae (*Petrophila jaliscalis* (Schaus)), (I) Crambinae (*Crambus praefectellus* (Zincken)), (J) Scopariinae (*Eudonia heterosalis* McDunnough), (K) Musotiminae (*Undulambia polystichalis* Capps), (L) Glaphyriinae (*Glaphyria sesquistrialis* Hübner), (M) Evergestinae **n. syn.** (*Evergestis pallidata* (Hufnagel)), (N) Noordinae **n. syn.** (*Noorda moringae* Tams), (O) Odontiinae (*Noctueliopsis aridalis* (Barnes & Benjamin)), (P) Spilomelinae (*Diaphania indica* (Saunders)), (Q) Wurthiinae **n. syn.** (*Niphopyralis nivalis* Hampson), (R) Pyraustinae (*Pyrausta purpuralis* (L.)), (S) Linostinae (*Linosta sinceralis* Möschler), (T) Cybalomiinae (*Cybalomia lutosalis* Mann), (U) Heliothelinae (*Heliothela wulfeniana* (Scopoli)). Scale bar in each image is 0.5 cm long.

split between Phycitinae (node 37; BP = 100) and Epipaschiinae + Pyralinae (node 32; BP = 100). Solis & Mitter (1992) instead allied Epipaschiinae with Phycitinae on the basis of a single morphological synapomorphy, a unique orientation of the uncus arms. The molecular results, however, suggest that this similarity is either a symplesiomorphy or a parallelism. A probable synapomorphy for Epipaschiinae + Pyralinae is found in the larvae, which lack sclerotized or unsclerotized rings around the base of SD1 on any relatively anterior segment (Allyson, 1977). Larvae in all other pyralid subfamilies have an additional sclerotized ring around seta SD1 on a thoracic segment or on abdominal segment A1.

Monophyly for Epipaschiinae (node 35; BP = 100) is supported by morphological synapomorphies including an upturned and pointed third labial palpal segment, an extended and ventrally curved coecum of the phallus, and a laterobasally subdivided tegumen (Solis & Mitter, 1992; Solis, 1993). Epipaschiinae include 710 species, cosmopolitan except notably absent from the northern Palearctic Region, and as larvae are tiers, rollers or miners of leaves, usually of trees. Morphological support for Pyralinae (node 31; BP = 100), although not as strong, includes a relatively short bursa copulatrix, barely extending beyond segment 7 (Solis & Mitter, 1992; Solis & Shaffer, 1999), and a tegumen fused to the base of the uncus for at least half the width of the uncus base (Solis & Mitter, 1992), a trait paralleled in Chrysauginae + Gallerinae. A recent study (Solis & Metz, 2011) found in addition that muscle IX-X, originating from the tegumen, is apomorphically lacking in Pyralinae. The pyralines comprise 1121 species, mostly in Asia and Africa, that show great variation in larval habits. They include feeders on live plants, seeds, dead vegetation and other nonliving organic matter. The molecular data resolve relationships very strongly among the four genera sampled (nodes 29–31; BP = 100).

Monophyly for the sister group to Epipaschiinae + Pyralinae, the Phycitinae (node 37; BP = 100), is supported by morphological synapomorphies including reduction of the female frenulum to a single composite bristle as noted earlier, and origination of the ductus seminalis from the corpus bursae (Solis & Mitter, 1992). A sclerotized ring around the base of seta SD1 of larval segment T2 was proposed previously as a phycitine apomorphy (Hasenfuß, 1960; Solis & Mitter, 1992). Phycitines are one of the largest and most morphologically diverse pyralid groups, containing 3450 species worldwide. They are chiefly leaf miners but include also a great range of other phytophagous and nonphytophagous habits. Even the few generic and/or tribal groups proposed in Phycitinae so far are under debate (Heinrich, 1956; Horak, 2003; Simonsen, 2008). The molecular results in this study strongly divide the four genera sampled into sister groups containing two genera each (nodes 36, 38; BP = 100).

Chrysauginae and Galleriinae (node 41)

A close affinity between Chrysauginae and Galleriinae, strongly supported by our molecular analysis (nodes 40, 41; BP = 99, 93 respectively), was proposed by Solis & Mitter (1992) on the basis of a single homoplasious synapomorphy, fusion of the tegumen to the base of uncus for at least half the width of the base of the uncus (also found in Pyralinae). A search for additional synapomorphies seems warranted, given the strength of the molecular evidence. Although secondary sexual characters can be homoplasious in Pyraloidea, they have been found to be useful at the genus level (Solis, 1993; Horak, 1997; Simonsen & Roe, 2009). Comparative morphological investigations of features of the highly modified male forewing, including venation, pockets and androconia, may yield additional morphological support for relationships between and within Galleriinae and Chrysauginae.

The Galleriinae (node 39; BP = 100) have historically been defined by the lack of a gnathos (Whalley, 1964; Munroe, 1972; Solis & Mitter, 1992; Munroe & Solis, 1999). This definition needs to be refined in light of a recent study of pyraloid genital musculature (Solis & Metz, 2011) that found the gnathos to be present in two galleriine species, although strongly reduced. We note, however, that two genera of Chrysauginae, *Satole* Dyar and *Clydonopteron* Riley, also have a gnathos that either is absent or greatly reduced (Cashatt, 1968). Monophyly of Galleriinae is supported also by a larval feature, the presence of a sclerotized ring around the base of the SD1 seta on segment A1 (Hasenfuß, 1960; Roesler, 1973; Solis, 2003), although this ring is absent in *Omphalocera* Lederer and *Thyridopyralis* Dyar (Solis & Mitter, 1992). Sclerotization of the ring is lost, although a clear remnant of the ring usually is present, in lab-reared *Galleria mellonella* (MAS, unpublished observations). The Galleriinae include 259 species worldwide. Some are stored product pests, whereas others, including *G. mellonella*, feed on combs in wasp and bee nests (Solis & Metz, 2008).

Monophyly for the remaining pyralid subfamily, Chrysauginae, remains uncertain. Solis & Mitter (1992) proposed only one synapomorphy, a sclerotized ring around the base of the SD1 seta on the larval metathorax, which is missing in some genera. They suggested that the male forewing venation may hold important characters, but further confirmation is lacking. As currently defined, Chrysauginae are a mostly Neotropical group of 391 species. Although larvae mainly feed internally in living plants, as seed, fruit, stem or root borers, or leaf rollers or tiers (Solis *et al*., 2003), a variety of other habits are recorded including inquilinism in hymenopteran nests and symbiosis with sloths.

In our sample, only *Monoloxis* Hampson is a confirmed member of Chrysauginae as evidenced by the larval synapomorphy (Aiello & Solis, 2003). Thus, although our molecular phylogeny appears strongly to demonstrate paraphyly of Chrysauginae with respect to Galleriinae (node 40, BP = 99), this conclusion is warranted only if the problematic Australian genus *Polyterpnes* Turner truly belongs to Chrysauginae. This proposition is uncertain on both molecular and morphological grounds. The molecular evidence concerning *Polyterpnes* is complex (Fig. 2). In the 19-gene analysis, it is placed very strongly at the base of the chrysaugine/galleriine clade (node 41; BP = 93). In the 5-gene analysis, however, it

is strongly excluded from Pyralidae (BP = 81 for monophyly of Pyralidae less *Polyterpnes*) as well as Crambidae, grouping with the latter but only very weakly (BP = 43). As in Schoenobiinae (below), this conflict probably is not an artifact of missing data blocks in the 19-gene matrix, because inclusion of *Polyterpnes* in the chrysaugine/galleriine clade is strongly supported in ongoing analyses (data not shown) of the complete 19 gene × 483 species matrix described earlier. The disagreement with the 5-gene result thus is most likely due to conflicts among one or more individual genes in the 5-gene versus the 14-gene set. Although the 19-gene result is clearly stronger, and accords better with morphology (see below), the existence of such conflict dictates caution in interpretation, as it violates a basic assumption of concatenated gene analysis, namely that all of the individual genes reflect the (single) species phylogeny.

Polyterpnes polyrrhoda Turner has a complex taxonomic history. It was described originally in Crambinae. Bleszynski (1966) transferred it to Pyraustinae without comment, although in LepIndex (Beccaloni *et al*., 2003) it is listed without comment under Odontiinae. Shaffer *et al*. (1996) listed *Polyterpnes* and three Australian genera under Chrysauginae mainly because 'they are brightly colored and have long porrect labial palpi' (M. Shaffer, personal communication to MAS). One of us (MAS, unpublished observations) undertook a preliminary morphological examination of *Polyterpnes* and three other Australian genera (*Hednotodes* Lower, *Anemosa* Walker, and *Anassodes* Turner) assigned to Chrysauginae by Shaffer *et al*. (1996), in an attempt to clarify their placement. These species exhibit enough derived similarity to each other that it would be reasonable to combine them into a single genus, defined by uniquely modified secondary venulae associated with the tympanal organs, and by unique male genitalia. The senior synonym would be *Hednotodes.* All members of *Hednotodes* s.l. possess closed tympanal organs and other pyralid characters, and thus clearly belong in Pyralidae, in accord with the 19-gene molecular result (Fig. 2).

Within Pyralidae, however, *Hednotodes* s.l. as represented by *Polyterpnes polyrrhoda* appears to share only plesiomorphies, and no obvious synapomorphies, with any individual subfamilies or groups thereof. For example, *Polyterpnes* shares with Chrysauginae and Galleriinae the primitive conditions of a female frenulum with three bristles, and the presence of secondary venulae associated with the tympanal organs. A plesiomorphy it shares with Pyralinae is a tegumen that abuts the entire base of the uncus (Solis & Mitter, 1992). *Hednotodes* s.l. as characterized here may represent a new subfamily, part of a poorly known Australian element of Pyralidae that includes genera such as *Macna* Walker, a probable chrysaugine that was misplaced in Pyralinae by Shaffer *et al*. (1996); see Solis & Shaffer (1999). Morphological and molecular study of such uncertainly placed Australian pyralids may help to re-define subfamily limits in the chrysaugine/galleriine clade and possibly at the base of the family more broadly. It would be especially useful to find the immatures of such taxa, currently unknown (M. Horak, personal communication to MAS), because the clearest defining morphological characters for Chrysauginae and Galleriinae are larval.

Monophyly of and basal divergence within Crambidae (node 8)

The observed 100% bootstrap support for Crambidae (node 8) accords well with the multiple synapomorphies known for this family. These include, among others: 1 or 2L setae on A9 of larvae (Gerasimov, 1947, 1949); presence of a praecinctorium (Guenée, 1854) associated with the tympanal organ; tympanal case opening anteromedially (Minet, 1982); and, tympanum and conjunctivum meeting at an angle, not in the same plane (Minet, 1982).

As in Pyralidae, relationships among subfamilies in Crambidae in general are supported strongly by molecular data. Unlike the case in Pyralidae, however, the crambid molecular phylogeny frequently conflicts with previous hypotheses (Fig. 1). An analysis of adult crambid characters used by previous authors showed that these were few, often variable at low taxonomic levels, and frequently homoplasious (Solis & Maes 2002), which may help explain the discrepancy. Whatever the reason, many of the groupings we propose in Crambidae based on the molecular results are new, and hypotheses of morphological synapomorphies for these necessarily are preliminary. To facilitate discussion of the large crambid tree, we have assigned informal names to several clades, some created from the initial letters of the names of the included taxa following Minet (1994).

The 'PS clade': Pyraustinae, Spilomelinae, Wurthiinae (node 26)

The molecular data very strongly divide Crambidae basally into two sister lineages of comparable species diversity, one consisting of Pyraustinae + Spilomelinae + Wurthiinae (node 26; BP = 100; the 'PS Clade'), and the other (the 'non PS Clade') consisting of all other subfamilies (node 7; BP = 99). The close relatedness of Pyraustinae to Spilomelinae (setting Wurthiinae aside for the moment) conflicts with the only previous explicit phylogenetic analysis of Crambidae (Solis & Maes, 2002), based on adult morphology, which identified a monophyletic 'pyraustine group' of subfamilies nested within a paraphyletic 'spilomeline group.' However, very few unambiguous characters were found in that study, and previous recognition of the relatedness between Pyraustinae and Spilomelinae is reflected in the fact that spilomelines were long synonymized with pyraustines. Given the strength of the molecular evidence for the 'PS Clade' (node 26), it would be worthwhile to search further for morphological synapomorphies; at present none are known from any life stage.

Within the PS clade (node 26), the strongly supported basal split is between Spilomelinae + Wurthiinae (node 25; BP = 100) and Pyraustinae (node 28; BP = 100). Many adult synapomorphies support the monophyly of Pyraustinae s.s. (node 28), that is, excluding Spilomelinae (Marion, 1961; Minet, 1982; Maes, 1994, 1995), although larval

synapomorphies are lacking (Allyson, 1981). The Pyraustinae are defined by atrophy of the spinula and venulae, and a narrow fornix tympani in the tympanal organs; male forewings with a subcostal retinaculum; male mesothoracic tibia with a hair pencil in a longitudinal groove; male genitalia with parallel tegumen ridges, and the valva bearing a sella (an outgrowth, clasper, or "poche", of the costa and sacculus; fig. 13 of Marion, 1954) and editum (scales associated with the sella; fig. 14 of Marion, 1954); and, female genitalia with a rhomboidal signum. Pyraustinae are a cosmopolitan group of 1413 species (Solis & Maes, 2002), within which lower-level relationships are poorly understood. A recent study of *Anania* and related genera (Tränkner *et al*., 2009) suggests that rigorous scrutiny of male and female genital morphology often may provide unique characters to synonymize and redefine currently unnatural genera. Pyraustine larvae typically feed on herbaceous plants and crops. Notable members of this subfamily include the notorious *Ostrinia* corn borers and the worldwide, Lamiaceae-feeding genus *Pyrausta*.

In contrast to Pyraustinae, the very large, cosmopolitan subfamily Spilomelinae (3767 species) remains poorly defined (Solis & Maes, 2002). It was subsumed in Pyraustinae (s.l.) in most early treatments (e.g. Munroe, 1976), a combination retained by Munroe & Solis (1999), but was resurrected by Minet (1982) and adopted by Solis & Maes (2002). Minet (1982) provided a combination of apomorphic features to define Spilomelinae: chaetosemata absent, males without subcostal retinaculum, bilobed praecinctorium, fornix tympani projecting, spinula pointed, gnathos absent, and females lacking a rhomboidal signum. However, some of these conditions occur also in the Pyraustinae. Immature-stage characters supporting the monophyly of Spilomelinae are lacking (Allyson, 1984). Spilomeline larvae feed on a wide diversity of both herbaceous and woody plants, and include many pests of cucurbit, solanaceous and other crops.

The Spilomelinae as constituted currently have been hypothesized to be polyphyletic (Minet, 1982; Solis & Maes, 2002), with some genera clearly belonging in other subfamilies including Pyraustinae s.s. However, the three spilomeline genera sampled here are strongly grouped to the exclusion of Pyraustinae s.s. (node 25; BP = 100), and it seems plausible that a large fraction of Spilomelinae will turn out to constitute a monophyletic group. Another possibility is that Spilomelinae could prove to be paraphyletic with respect to Pyraustinae, although no morphological or molecular study has supported this hypothesis. Morphological studies are beginning to clarify the limits of Spilomelinae and relationships therein. For example, modifications of the male patagia, the uncus in the male genitalia and the signa on the female corpus bursae provide solid support for the monophyly of a group of spilomeline genera centred on *Glyphodes* Guenée (Sutrisno, 2002; Sutrisno *et al*., 2006). In an attempt to place the box tree pest *Cydalima perspectalis* (Walker), Mally & Nuss (2010) studied morphological characters of *Glyphodes* and related genera and found unambiguous synapomorphies for groups of genera in features including the length and sclerotization of the ductus bursae, the form of the signa in the female genitalia, and, in the male genitalia, the phallus apodeme, coremata pads, sacculus and uncus, as well as forewing patterns and colour. A possibly important character comprises various modifications of the anepisternal scale organ seen in spilomeline genera including *Diaphania* Hübner, *Palpita* Hübner and *Antigastra* Lederer (Clavijo, 1990); this structure is absent in Pyraustinae s.s. and some genera of Spilomelinae.

The third subfamily belonging to the 'PS clade' (node 26), Wurthiinae, has been problematic because it is so morphologically divergent from all other crambids. The wurthiines comprise just eight Oriental species, in the single genus *Niphopyralis* Hampson, which was described originally in Schoenobiinae. The adults resemble some Bombycoidea in lacking a proboscis, maxillary palpi, ocelli and chaetosemata. These reductions, in turn, may be associated with their unusual life history. The larvae, also highly distinctive, live in silken cases in the nests of arboreal ants (Roepke, 1916; Kemner, 1923; Munroe & Solis, 1999). Solis & Maes (2002) postulated a sister group relationship of Wurthiinae to Linostinae. The latter also comprise a single genus (unsampled here) with similarly reduced adult cephalization but unknown life history.

Molecular data often are valuable especially in placing taxa with such highly modified morphology. Although it has not been proposed previously, the association of *Niphopyralis* with Spilomelinae and secondarily with Pyraustinae, supported by two 100% bootstrap values in our molecular trees (nodes 25, 26), is not obviously contradicted by morphological evidence. It is consistent also with the molecular results of Mutanen *et al*. (2010; see their figure ESM 1). Those authors found *Niphopyralis* to be related most closely, among the other 13 Crambidae sampled (which did not include Spilomelinae), to one of two Pyraustinae sequenced, with 78% bootstrap support. The association with Spilomelinae is unambiguous in our data (node 25; BP = 100), but the grouping of *Niphopyralis* with *Phaedropsis* Warren (node 27; BP $\leq$ 54) is weakly supported, possibly because *Niphopyralis* is one of the longest branches in the entire phylogeny (Fig. 3). Thus, we cannot distinguish confidently between monophyly versus paraphyly of Spilomelinae with respect to Wurthiinae. Morphological synapomorphies of Wurthiinae with Spilomelinae have not been reported. The most likely candidates, based on comparison of the apomorphies cited by Minet (1982) and Munroe & Solis (1999), are reduced maxillary palpi (small in most spilomelines, absent in *Niphopyralis*), upturned labial palpi and a bilobate praecinctorium in the tympanal organs.

Despite some uncertainly about its exact position, our molecular results very strongly place *Niphopyralis* as a member of a clade that otherwise consists only of taxa assigned to Spilomelinae. The affinities of this morphologically aberrant genus now seem firmly established, removing the chief justification for giving it subfamily rank. Moreover, maintaining separate subfamily status for *Niphopyralis* renders Spilomelinae paraphyletic in some of our trees (Fig. 2). For these reasons, we newly synonymize Wurthiinae here within the Spilomelinae **syn.n.,** and recommend that *Niphopyralis* be treated as a genus of spilomelines in future studies.

The 'non PS clade': Musotiminae, Scopariinae, Crambinae, Schoenobiinae, Midilinae, Acentropinae, Evergestinae, Glaphyriinae, Noordinae, Odontiinae (node 7)

The other main lineage of Crambidae as sampled here (node 7; BP = 99) consists of all subfamilies except Pyraustinae, Spilomelinae and Wurthiinae. Like its sister group, this clade, although very strongly supported by molecular evidence, has no close analogue in any previous hypothesis, and no morphological synapomorphies are known. In the nt123 tree, the 'non PS clade' (node 7) is divided basally into a 'CAMMSS clade' consisting of Crambinae, Acentropinae, Midilinae, Musotiminae, Scopariinae and Schoenobiinae (node 6; BP = 100), versus an 'OG' lineage consisting of Odontiinae, Glaphyriinae, Evergestinae and Noordinae (node 20; BP = 85). The latter group is not supported by the degen-1 analysis, which instead allies Odontiinae with the PS clade (node 6; see Fig. 2). This alternative is weakly supported (BP = 43), however, and lacks any known morphological synapomorphy.

The 'CAMMSS' clade: Crambinae, Acentropinae, Musotiminae, Midilinae, Scopariinae and Schoenobiinae (node 6)

For convenience we refer to this large and very strongly supported group (node 6; BP = 100) as the 'CAMMSS clade.' This grouping has not been proposed previously, and no morphological synapomorphies are known. However, Yoshiyasu (1985) did recognize a group comprising all these subfamilies except Midilinae, a New World taxon which he did not examine. He defined this group of subfamilies by a reduced or shortened transtilla. The transtilla in all six subfamilies needs to be re-examined quantitatively, to determine whether this character holds up as a synapomorphy for the CAMMSS group. Reduction of the transtilla is a character that Solis & Maes (1995) decided not to use, because in previous studies the type and degree of reduction appeared to be correlated with the degree of sclerotization, and varied at lower taxonomic levels.

Within the CAMMSS group (node 6), the molecular data support another new proposal, namely, a basal split between Musotiminae and the remaining five subfamilies (node 5, BP = 84). The latter is characterized by the presence of chaetosemata and a gnathos that articulates with the tegument–uncus juncture. This study provides the first strong evidence on the problematic phylogenetic position of Musotiminae. Musotiminae, cosmopolitan but most diverse in the tropics, currently consists of about 170 species whose known larvae feed on ferns and bryophytes. The subfamily was long treated as subordinate within Acentropinae, due to the closely convergent wing patterns of these two groups, and its diversity and limits have only begun to be clarified. Additional genera are being discovered still and/or transferred from other subfamilies (Speidel, 1981; Yoshiyasu, 1985; Phillips & Solis, 1996; Yen, 1996, 1997, 2004; Solis *et al*., 2004, 2005a, b; Yen *et al*., 2004). The morphological evidence supporting monophyly for Musotiminae is ambiguous. All synapomorphies thus far proposed also occur in other subfamilies, or are missing in some presumed musotimines. They include: fern-feeding larval habits (Munroe, 1972); tympanal organs with bullae tympani enlarged and processus tympani located anteriorly (Minet, 1982); parallel line components with termen in both wings; male genitalia with base of gnathos extended anteriorly (Yoshiyasu, 1985); relative position of D1 and D2 to SD1 in the larvae; a pair of lateral horns on both sides of the pupal prothorax; and conical protruding pupal spiracles from A2 to A7 (Yen *et al*., 2004).

Within the sister group to Musotiminae (node 5; BP = 84), the molecular data strongly support a basal split between Crambinae + Scopariinae (node 14; BP = 100) and Schoenobiinae + Midilinae + Acentropinae (node 13; BP = 86). A sister group relationship between Crambinae and Scopariinae, seen also in the molecular study of Mutanen *et al*. (2010), appears to not have been proposed previously, but there are multiple potential synapomorphies needing further investigation. The shared apical scale tuft on the maxillary palpus (found also in Schoenobiinae) is one candidate (Landry, 1995), although Roesler (1973) regarded its presence as primitive. Another possibility is the relatively elongate maxillary palpus observed by Landry (1995) in most Crambinae and in Scopariinae as represented by *Scoparia basalis* Walker and in Schoenobiinae as represented by *Donacaula longirostrella* (Clemens) [incorrectly listed as *longirostris*]. Crambinae and Scopariinae larvae share closely approximated setae on the tenth abdominal segment (Hasenfuß, 1960). Hasenfuß (1960) and Passoa (1988) reported that the larvae of Crambinae, Scopariinae and a few Pyraustinae have extra pinacula on the thorax and abdomen – a derived condition. There are also some life history parallels. Crambinae appear to feed as larvae only on grasses (on roots, or in stems) or on mosses (e.g. *Catoptria* Hübner); some scopariines feed on mosses or lycopods, others feed on ferns and lichens, and some New Zealand scopariines share grass feeding with crambines (Nuss, 1999; Murase, 2005; Heckford, 2009).

The Crambinae (node 13; BP = 100) have long been recognized as a group. Landry (1995), in a study of North American crambines, listed the following synapomorphies: (i) presence of a comb or pecten of hair-like scales on the hindwing cubital stem dorsally (Roesler, 1973); (ii) a unique configuration of the tympanal organs, with the tympanic pockets subconical (Minet, 1985), behind the ridge, and never approximated along the sternal midline; and (iii) median attachment of the phallus to the juxta. Monophyly for Scopariinae (node 15; BP = 100) is supported morphologically by unique wing pattern elements, including an 'X'-like distal discoidal stigma and an associated dentation of the postmedian line (Nuss, 1999). Heliothelinae (2 tribes, 5 genera, 49 species in the Old World), not sampled in this study, were given subfamily rank by Minet (1982). Robinson *et al*. (1994) described Hoploscopini, which were transferred to Heliothelinae by Nuss (1998), who re-defined the subfamily by a sclerotized spine attached to the outer wall of corpus bursae. Monophyly of the entire group needs verification and its sister-group relationship remains unclear.

The sister group to Crambinae + Scopariinae, according to our results, is Acentropinae + Schoenobiinae + Midilinae

(node 4; BP = 86). These subfamilies were also grouped in the molecular analysis of Mutanen *et al*. (2010). The immature stages provide characters supporting this clade. The obtect pupa with exposed metathoracic legs described by Passoa (1988) for Schoenobiinae and Acentropinae is also shared by Midilinae (MAS, unpublished observations). Likewise, the shortened L2 seta on larval abdominal segments, said to be synapomorphic for Schoenobiinae and Acentropinae (Passoa, 1988), also occurs in the midilines *Midila* Walker and *Cacographis* Lederer (MAS, unpublished observations). Yoshiyasu (1985) additionally mentions the loss of seta V1 on the larval thorax in Acentropinae and Schoenobiinae, but Passoa (1988) showed this seta to be present in a species of *Rupela* Walker in Schoenobiinae; in Midilinae it occurs in *Midila*, though not in *Cacographis* (MAS, unpublished observations).

Although the grouping of node 4 has not been proposed formally previously, close relationships between overlapping pairs of these subfamilies have long been recognized, albeit by often-conflicting characters. Hampson (1895) included the then-known midiline genera in Schoenobiinae, whereas Solis & Maes (2002) hypothesized these subfamilies to be sister groups, based on the synapomorphy of a reduced proboscis. In the morphological cladistic analysis of Martinez (2010), whereas Schoenobiinae were grouped most immediately with the exemplar of Spilomelinae (*Lineodes integra* Zeller), these taxa together were sister group to a pair formed by the exemplars of Midilinae and Acentropinae. Other characters instead support grouping of Schoenobiinae with Acentropinae, including loss of the larval thoracic L seta, the very short L2 seta on larval abdominal segments, pupae with exarate appendages including metathoracic legs clearly exposed as outlined by Passoa (1988), and the presence of a tegumeno-ventral (t-v) plate (Yoshiyasu, 1985). The analysis of Landry (1995) further supported relatedness of these two subfamilies based on the presumed apomorphic condition of the cephalic end of the bulla tympani (= tympanic drum) not being concealed in the abdominal cavity in *Donacaula longirostrella* (Schoenobiinae) and *Nymphula ekthlipsis* (Grote) (Acentropinae). Interpretation of this feature is complicated, however, by the observations of Martinez (2010), whose illustrations show it to be variable within Schoenobiinae; the two earliest-branching genera in her cladogram show the bulla tympani to lie entirely inside the abdominal cavity.

There are also life history similarities among these three subfamilies. All are largely restricted to moist habitats, making this one of the largest clades of wetland-associated Lepidoptera. For this reason, we term this lineage informally the 'wet-habitat clade.' Schoenobiinae and Midilinae are restricted to monocots, the former boring in wetland Poaceae, Cyperaceae and Juncaceae, and the latter in Araceae. Many acentropine genera have evolved complex methods of respiration for aquatic life, and feed on a wide variety of plants. In some genera the larvae resemble schoenobiines in living as borers within air-filled stems (e.g. *Elophila* Hübner), at least in the first instar. In other genera (e.g. *Parapoynx* Hübner, *Petrophila* Guilding), the larvae have developed true underwater respiration and live most of their life in water (Solis, 2008).

Relationships within the 'wet-habitat clade' (node 4) remain unclear on the basis of morphology as just discussed, and are only partially resolved by our molecular data. There is strong support for monophyly of Acentropinae (node 10; BP = 92) and of Midilinae (node 2; BP = 100) as sampled here. Probable morphological synapomorphies for Acentropinae are swollen scoloparia supported by their dorsal projections or directly or entirely by the bullae tympanorum of the adult tympanal organs (Minet, 1985); and, protruding spiracles on the second or third to fourth abdominal segments of pupae (Speidel, 1981; Yoshiyasu, 1985). Speidel (1981), Speidel & Stüning (2005) and Passoa (1988) have also suggested: larvae aquatic; stemmatal setae in linear configuration; pupal frontal setae enlarged; and pupal mesothoracic spiracle lost. However, some of these characters are absent in some genera and/or occur in other subfamilies as well. Although most acentropines are aquatic in the larval stage, a few are terrestrial, such as *Nymphicula* Snellen in Japan (Yoshiyasu, 1980; Yen, 2004) and *Paracymoriza* Warren in the Oriental region (SY, unpublished observations). It has been discovered recently that some species of *Aulacodes* Guenée in the Western Hemisphere are also terrestrial (MAS and K. Nishida, unpublished observations).

The Midilinae (node 2; BP = 100), formerly part of the Schoenobiinae of Hampson (1895), were recognized as a subfamily by Munroe (1958). In a subsequent revision, Munroe (1970) associated the midilines with Schoenobiinae and Acentropinae. Apparent synapomorphies of Midilinae include loss of vein 1A in the forewing, and reduction of the proboscis (Munroe, 1970; Solis & Maes, 2002; but note that species with a fully-developed proboscis are known in both Midilinae and Schoenobiinae); antenna thick in both sexes (Munroe, 1970); large, white or hyaline discal spot (Munroe, 1970); praecinctorium broad, transverse and truncate (Minet, 1985); bullae tympani small, not invaginated in S2 (Minet, 1985); and processus tympani transverse (Minet, 1985). Minet (1985) examined only two *Midila* species in his study of tympanal organs, but some of the features listed above are also found in *Dismidila* Dyar and *Cacographis* (JH, unpublished observations). Another probable synapomorphy for Midilinae is the dorsal location and large size of the spiracles on A9, resembling the condition in the aquatic noctuid genus *Bellura* Walker (Noctuidae; MAS, unpublished observations; Solis, 2008, fig. 19.12). Host records of midilines include root boring in *Colocasia* Schott (Munroe, 1970), *Caladium* Vent. (Munroe & Solis, 1999) and *Philodendron corcovadense* Kunth (Pimentel *et al*., 1991), as well as larvae intercepted at U.S. ports on stems, fruits and flowers of *Philodendron* Schott (MAS, unpublished observations). Although there are a few anecdotal reports (e.g. Pimentel *et al*., 1991), the biology of Midilinae is largely unstudied.

Our molecular evidence on Schoenobiinae, in contrast to that on the foregoing two subfamilies, is ambiguous, with clear indications of conflicting signals within the dataset. The two schoenobiine genera, *Scirpophaga* Treitschke and *Rupela*, are never grouped together when all 19 genes are included.

Scirpophaga is grouped with Midilinae (node 3; BP = 71) in all 19-gene all-nucleotide-changes analyses, a position contradicted (albeit weakly) by the all-nonsynonymous (degen-1) analysis, which groups it with *Rupela* + Acentropinae. *Rupela*, however, is grouped, more convincingly, with Acentropinae (node 11), by both nt123 (all changes) and degen-1 (nonsynonymous only) analyses (bootstraps = 71 and 83, respectively).

In contrast, multiple other analyses support schoenobiine monophyly. In the current study, when only five genes are used, the two schoenobiines group, albeit weakly, with each other first (BP = 50) and then Acentropinae (BP = 41). The same two schoenobiines were even more strongly grouped (BP = 80) in the smaller 5-gene study of Regier *et al*. (2009), which however did not include Midilinae. Schoenobiinae (represented by *Schoenobius* Duponchel and *Clepsicosma* Meyrick) are monophyletic also in the molecular analysis of Mutanen *et al*. (2010), and in the morphological phylogeny of Martinez (2010). Multiple morphological synapomorphies have been proposed for Schoenobiinae, including: presence of a prothoracic membranous sac in the larva; a deep, pit-like mesothoracic spiracle, as well as exposed mesothoracic and metathoracic coxae in the pupa (Passoa, 1988; both *Scirpophaga* and *Rupela* examined); and, an anal tuft encircling abdominal segment VII in the adult female (Common, 1960). The presence of a scale tuft medially on the apical margin of sternites VII and VIII also supports the monophyly of Schoenobiinae, although some reversals occur (Martinez, 2010). *Scirpophaga* and *Rupela* also share several other typical adult schoenobiine characters, such as: A2 pleural tubercles on S2 sclerite (Lewvanich, 1981); subteguminal processes (but not universal in Schoenobiinae; Martinez, 2010); squamiform structures present on lateral sides of vinculum (also present in *Noorda* Walker, see below); and, the fornix tympani rounded in a semicircular arc (also in *Midila*). In contrast, we can identify no obvious synapomorphies that would link *Scirpophaga* (alone) with Midilinae, or *Rupela* (alone) with Acentropinae.

Given all the evidence, it seems likely that the nonmonophyly of Schoenobiinae in our 19-gene analysis is an artifact. One might ascribe the observed support for schoenobiine paraphyly stems to missing data blocks in the deliberately incomplete data matrix. Evidence against this explanation comes from our ongoing analyses (data not shown) of a complete 19-gene matrix of 483 species across the Lepidoptera, including all Pyraloidea sequenced for 19 genes (listed in Fig. 2). These trees also consistently fail to group the two schoenobiines together. Moreover, missing data effects were rare in other recent similar studies (Cho *et al*., 2011; Zwick *et al*., 2011). Instead, it seems most likely that the conflict between this study and its predecessor (Regier *et al*., 2009) with respect to Schoenobiinae results from conflicts among individual genes, both within and between the 5-and 14-gene datasets, a possibility to be explored in a subsequent study. In preliminary single-gene analyses of a dataset approximating that of Cho *et al*. (2011; 191 taxa including 9 pyraloids; data not shown), *CAD* strongly favoured polyphyly for the two schoenobiines, whereas *enolase* strongly favoured monophyly. Such conflicts have arisen periodically in previous phylogenetic studies of Lepidoptera (e.g. Regier *et al*., 2008a, b), and can have diverse causes that are difficult to distinguish, including bias in gene-tree construction and species-tree/gene-tree discord.

The 'OG clade': Odontiinae, Glaphyriinae, Evergestinae and Noordinae (node 20)

The remaining major lineage of Crambidae (node 20; BP = 85) consists of Odontiinae, Glaphyriinae, Evergestinae and Noordinae. Historically these subfamilies have been aligned with each other and with Pyraustinae (or combined therein), based partly on one or another morphological character, but mostly on similarity in external appearance. Most recently this relationship was supported by the shared presence of a forewing scale-fringe, absence of chaetosemata, and fusion of the gnathos arms to the tegumen (Solis & Maes, 2002). Our molecular tree strongly implies, however, that these morphological similarities with Pyraustinae probably represent independent origins in pyraustines and in the ancestor of the OG clade (node 20).

Within the OG clade, the molecular data provide moderate support for a basal split between Odontiinae (node 23; BP = 100) and Glaphyriinae + Evergestinae + Noordinae (node 19; BP = 72). Odontiinae were recognized as distinct by Guenée (1854), but subsequently merged with Pyraustinae by Hampson (1898). The full global extent of the Odontiinae was recognized first by Munroe (1961), who grouped together more than fifty existing genera and subsequently proposed many more, but few authors have examined the subfamily broadly since then (Marion, 1961; Martin, 1986). Odontiine monophyly is well supported by unique adult morphological characters including: membranous, pleated valvae and a membranous uncus with lateral flaps (Munroe, 1961, 1972); S8 with paired sets of lamelliform, robust setae (Leraut & Luquet, 1982); squamiform structures attached to the vinculum above the juxta (Minet, 1980); and T8 with posterior scale fringe (Hayden, 2009). Odontiinae include 91 genera and 377 species (Nuss *et al*., 2003–2012), with many species awaiting description. They are most species-rich in the tropics and in arid habitats of the Old World. The known larval habits are diverse, including both internal feeding (leaf mining as well as boring stems, buds, seeds or fruits) and concealed external feeding (e.g. leaf rolling; Hayden, 2011).

The combination of Evergestinae + Glaphyriinae + Noordinae (node 19; BP = 72) has not been proposed previously, and no morphological synapomorphies currently are apparent. The most distinctive features of this clade concern life history. Many of the genera, including all those sampled here except the hymenopteran inquilines *Chalcoela* Zeller + *Dicymolomia* Zeller (see below), feed as larvae on mustard-oil-containing plants in the order Brassicales, where they can be pests of agricultural species. As Solis *et al*. (2009) point out, radiations on Brassicales are rare in Lepidoptera, presumably due to the effectiveness of the mustard oil defence, but insect lineages that

overcome this barrier can sometimes achieve exceptional diversity (Wheat *et al*., 2007). Much further sampling is needed, but node 19 might turn out to represent another large lepidopteran clade that is ancestrally and predominantly specialized on mustard oil plants. For this reason, we will refer to it informally as the 'mustard oil clade.' Possibly this lineage will prove to include Cybalomiinae, an Old World group of 72 species, not sampled here, whose known larvae also feed almost exclusively on Brassicales (Luquet & Minet, 1982).

Subfamily definitions and relationships within the putative 'mustard oil clade' (node 19) remain incompletely understood. The smallest and most clearly defined subfamily is Noordinae, first recognized at this rank by Minet (1980), who removed it from Odontiinae. Minet's suggestion that Noordinae are closely related to Glaphyriinae, Dichogamini and Odontiinae s.s. is consistent with our results. The Noordinae currently consist of 16 primarily Old World tropical species, all in the genus *Noorda* (although many of these species are misplaced in Noordinae; JEH, unpublished observations). The known larvae feed on *Moringa* Adanson (Moringaceae, Brassicales; Amsel, 1965; Matthew Menon, 1984). Noordine synapomorphies include: unusual tympanal organs that are "partially embedded into the thorax" (Minet, 1980) and have a reduced, unilobed and bladelike praecinctorium; and, male genitalia characterized by broadly rounded valvae, a long and slender uncus, and a gnathos with a very short median element (Minet, 1980). Minet assigned secondary importance to apomorphic traits that *Noorda* shares with other groups, particularly the squamiform structures attached to the vinculum that are also found in Odontiinae and Schoenobiinae (see above). The molecular tree, on which *Noorda* is well separated from both Odontiinae and (especially) Schoenobiinae, supports the interpretation that this similarity reflects homoplasy, and suggests that the definition of the squamiform structures in these three clades should be re-evaluated.

The other two subfamilies within the putative 'mustard oil clade' (node 19; BP = 72), Evergestinae and Glaphyriinae, are poorly studied, and their monophyly and limits are not clearly established. Evergestinae as defined currently are a cosmopolitan group of 111 species, feeding mostly on Brassicales. The subfamily was erected for genera that were not Pyraustinae and had a well-sclerotized gnathos attached to the tegumen (Marion, 1952). Munroe (1973) revised the North American Evergestinae and defined them as having a well-developed gnathos, dorsally toothed near the apex. Based on examination of pupae in *Evergestis* Hübner and *Trischistognatha* Warren, Passoa (1985) proposed an additional synapomorphy for Evergestinae, namely, a distinctive cremaster composed of two spheres with setae. The two evergestines in this study are strongly grouped together (node 16; BP = 99).

The most problematic subfamily in this putative clade is Glaphyriinae, which as currently defined includes 199 species, and is most diverse in the New World. The subfamily was erected originally to contain North American genera that were deemed not Pyraustinae and had spatulate scales on the upper side of the hind wing between CuA2 and CuP (Forbes, 1920, 1926). Munroe (1964) expanded the definition and size of the subfamily in a study of Neotropical genera and new species, and then comprehensively redefined the North American Glaphyriinae (Munroe, 1972). Munroe & Solis (1999) further enlarged Glaphyriinae by incorporating Dichogaminae, formerly a tribe in Odontiinae (Munroe, 1961) that was raised to subfamily rank by Minet (1982), on the basis of similarity in genital characters to glaphyriines. This dichogamine transfer is corroborated in our results by the pairing of *Dichogama* Lederer with two undoubted glaphyriines (node 22; BP = 100).

Munroe & Solis (1999) provided a diagnosis for Glaphyriinae, but all characters mentioned either are lacking in some glaphyriines or occur also in other subfamilies; clear synapomorphies are lacking. In a treatment of the Costa Rican glaphyriines, the only recent species-level study, Solis & Adamski (1998) discovered variation in the scale character of Forbes (1920), which they re-described as 'specialized scales' that 'can be piliform, spatulate, or a combination of these types of scales.' More important, they found that a number of glaphyriine genera, including *Eupoca* Warren and *Hellula* Guenée, lacked these modified scales entirely. Glaphyriinae were previously characterized also as having a much reduced or absent gnathos (Munroe, 1972; Munroe & Solis, 1999). Solis & Adamski (1998), however, found that the gnathos varies widely within genera. For example within *Lipocosma* Lederer, the gnathos ranges from absent, in *L. albibasalis* (Hampson), to well developed and apically spinose in *L. fonsecai* Solis & Adamski. The most commonly reported larval host association in Glaphyriinae is with Brassicales, but diverse other habits are also known, including feeding on cactus, feeding on lichens, and predation or parasitism on hymenopteran larvae.

The Glaphyriinae sampled here are not monophyletic on our trees, and fall into two separate, strongly supported groups. One of these (node 22; BP = 100), as noted earlier, pairs *Dichogama* with *Chalcoela* + *Dicymolomia.* No morphological synapomorphies for this clade are known. The three genera share the absence of chaetosema (character three of Solis & Maes, 2002) and the presence of a spinula (character 16 of Solis & Maes, 2002), but these traits occur in other subfamilies as well. Other potential synapomorphies in the male genitalia include a B-shaped vinculum, so far known in *Dichogama, Hellula*, *Aethiophysa* Munroe and *Glaphyria* Hübner, and a setose base of the valval sacculus, observed so far in *Dichogama*, *Hellula*, *Chilozela* Munroe and *Scybalistodes* Munroe, and the distally cleft valvae in *Dichogama* and *Hellula* (JH, unpublished observations). A further possible synapomorphy, potentially more conserved than the adult features just cited, is the absence of maxillary palpi in the pupa, found so far in *Hellula*, *Dicymolomia* and *Chalcoela* (Passoa, 1985). Lack of pupal maxillary palpi is otherwise known only in Schoenobiinae. The subordinate position of *Chalcoela* + *Dicymolomia* (node 21; BP = 100) within the otherwise plant-feeding 'mustard oil clade' strongly suggests that their inquilinism in hymenopteran nests is a derived habit.

The other strongly supported group that includes Glaphyriinae allies *Cosmopterosis* Amsel with the two exemplars of

Evergestinae (node 17; BP = 100). [The association of *Noorda* with this lineage (node 18; BP ≤ 52) is weakly supported.] *Cosmopterosis*, currently placed in Glaphyriinae (Munroe, 1964; Solis *et al*., 2009), exemplifies the difficulty of separating these two subfamilies on morphological grounds. It shares potential synapomorphies with both Glaphyriinae (modified scales on the hind wing) and Evergestinae (elongate, apically toothed gnathos) as currently defined. It shares also with most Evergestinae a ductus seminalis originating from the ductus bursae and a corpus bursae bearing two oval signa (Munroe, 1976; Minet, 1982; Goater, 2005). In contrast, in most Glaphyriinae, including Dichogamini, as well as the evergestine *Trischistognatha*, the ductus seminalis emerges from a large diverticulum of the posterior corpus bursae, which is more or less lined with massive, irregular, striose and spinulose sclerotization.

Given the difficulty of separating Glaphyriinae and Evergestinae, and the molecular evidence for paraphyly of Glaphyriinae, as currently delimited, with respect to Evergestinae (node 17; BP = 100), we propose to combine these entities into a single subfamily. We also include *Noorda* in this group, as the evidence suggests (albeit weakly; node 18; BP ≤ 52) that it is phylogenetically subordinate within the putative 'mustard oil clade' (node 19; BP = 72). Thus, hereby we synonymize both Evergestinae **syn. n**. and Noordinae **syn. n**. under the oldest name, Glaphyriinae. The mustard oil clade itself is supported only moderately by the molecular data, but the concordance between these and larval host use is striking. No morphological diagnosis can yet be given, but feeding on Brassicales appears to be a synapomorphy for the Glaphyriinae in the new sense. Our goal in making this change is to increase the proportion of crambid genera and species that can be confidently assigned to a monophyletic subfamily.

Summary and conclusions

Our results, from the first molecular-phylogenetic analysis directed specifically at Pyraloidea, offer substantial clarification of deeper-level relationships. The monophyly of both families is very strongly confirmed. Our molecular analyses yield a new working hypothesis of relationships among the subfamilies (Fig. 2) in which the great majority of inferred groupings (83%) are strongly supported (≥80% bootstrap). Its major features are given below.

Pyralidae:

1 Within Pyralidae, all relationships among subfamilies are very strongly resolved (≥90% bootstrap).
2 The phylogeny can be summarized as: Galleriinae + Chrysauginae (Phycitinae + (Pyralinae + Epipaschiinae)).
3 The molecular phylogeny for Pyralidae agrees entirely with a previous numerical-phylogenetic analysis based on morphology, except that the positions of Phycitinae and Pyralinae are switched.
4 The chief remaining uncertainty about higher-level relationships in Pyralidae concerns Chrysauginae, for which monophyly has not been convincingly established. The little-studied Australian genera currently assigned to Chrysauginae, here represented by *Polyterpnes*, may constitute the basal lineages of the Chrysauginae + Galleriinae clade, or possibly Pyralidae as a whole. They merit close investigation.

Crambidae:

1 The molecular phylogeny shows much less concordance with previous hypotheses in Crambidae than in Pyralidae, possibly because traditional morphological characters provide less phylogenetic information in crambids. Therefore, most of the proposed groupings are new, including two which increase the correspondence of crambid phylogeny with life history features.
2 Crambidae divide basally into a 'PS clade' containing Pyraustinae + (Spilomelinae + Wurthiinae) and a 'non PS' clade containing the remaining subfamilies.
3 The 'non PS' clade divides in turn into a 'CAMMSS' clade that includes Crambinae, Acentropinae, Musotiminae, Midilinae, Scopariinae and Schoenobiinae, and an 'OG' clade consisting of Odontiinae plus a putative 'mustard oil clade.' The latter includes Glaphyriinae, Noordinae and Evergestinae, all of which as larvae feed most commonly on Brassicales. Much further work is needed to determine the limits and internal divisions of the 'mustard oil clade,' which is only moderately well supported by the molecular data (BP ≤ 79), but this is potentially one of the largest lineages of Lepidoptera associated with mustard oil plants.
4 The basal split in the CAMMSS clade separates the fern-feeding Musotiminae from all other subfamilies. The remaining subfamilies in turn divide into a clade consisting of Crambinae + Scopariinae, and a 'wet-habitat' clade containing Schoenobiinae + Acentropinae + Midilinae. The latter constitutes one of the largest wetland-associated lineages of Lepidoptera.

Based on these results, we propose three changes in the subfamily classification of Crambidae (Table 2). Two of these involve reduction in rank of single genera, *Noorda* and *Niphopyralis*, which had previously been raised to the subfamily level (Noordinae, Wurthiinae) because each is highly apomorphic and seemed, on morphological grounds, not to fit into any existing subfamily. In both cases, the molecular data now provide clear evidence for close association with a single larger subfamily, and suggest that the latter would be rendered paraphyletic if subfamily status were maintained for the aberrant genus. Synonymization of these monogeneric subfamilies should thus increase the naturalness of crambid classification. The same holds true for our synonymization of Evergestinae (as well as Noordinae) with Glaphyriinae, as the latter currently lack a clear definition and, according to the molecular phylogeny, are paraphyletic with respect to the former.

The well-supported hypothesis of among-subfamily relationships presented here will, we hope, facilitate progress on the

Table 2. Revised Pyraloidea classification resulting from this study.

Pyralidae
Galleriinae Zeller, 1848
Chrysauginae Lederer, 1863
Pyralinae Latreille, 1809
Epipaschiinae Meyrick, 1884
Phycitinae Zeller, 1839
Crambidae
Midilinae Munroe, 1958
Schoenobiinae Duponchel, 1846
Acentropinae Stephens, 1836
Crambinae Latreille, 1810
Scopariinae Guenée, 1854
Musotiminae Meyrick, 1884
Glaphyriinae Forbes, 1923
Evergestinae Marion, 1952, **n. syn.**
Noordinae Minet, 1980, **n. syn.**
Odontiinae Guenée, 1854
Spilomelinae Guenée, 1854
Wurthiinae Roepke, 1916, **n. syn.**
Pyraustinae Meyrick, 1890
Unplaced subfamilies (not included in this study)
Linostinae Amsel, 1956
Cybalomiinae Marion, 1955
Heliothelinae Amsel, 1961

systematics of individual subfamilies. For several of these, particularly Spilomelinae, Acentropinae and Chrysauginae, much work is still needed to firmly establish subfamily limits and definitions. In all subfamilies, an enormous effort will be required, drawing on all life stages and character sets, to establish generic and tribal level relationships.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-3113.2012.00641.x

Table S1. Species sampled, collecting localities and Genbank numbers.

Please note: Neither the Editors nor Wiley-Blackwell are responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Acknowledgements

We are greatly indebted to the many generous colleagues who provided specimens for this study, including James K. Adams, Joaquin Baixeras, Norris Bloomfield, John W. Brown, Tom Burbidge, Soowon Cho, Robert F. Denno, E.D. Edwards, Janet Farr, Michael Fibiger, Timothy P. Friedlander, Winnie Hallwachs, R.J.B.Hoare, Marianne Horak, Daniel H. Janzen, Akito Y. Kawahara, David C. Lees, Marcus J. Matthews, Wolfram Mey, Andrew Mitchell, Kenji Nishida, Ron Robertson, B. Spinosa, J. Bolling Sullivan, Bruce Tabashnik and Allan Willis. Suwei Zhao and Kongyi Jiang provided technical assistance, K. Mitter made essential contributions as specimen collection and database manager, and M. Metz (SEL, USDA) made contributions to technical support and literature search. The grid-based phylogenetic analyses were made possible by expert support from Adam Bazinet. We thank Paul Goldstein and Michael Pogue for helpful comments on the manuscript. Financial support was provided by the U.S. National Science Foundation's Assembling the Tree of Life program, award numbers 0531626 and 0531769, the Maryland Agricultural Experiment Station, and US. National Science Foundation grant DEB 0515699 to D. H. Janzen. JH's studies were supported by a Rea Postdoctoral Fellowship (Carnegie MNH). This publication was made possible by the Leptree project (www.Leptree.net) and the efforts of the entire Leptree team. We especially thank our co-P.I.s Don Davis, Cynthia Parr and Susan Weller. Construction of the web pages associated with this paper was supported by Cynthia Parr, Dana Campbell and John Park. Author Contributions: JCR, CM, MAS and MPC designed the study. JCR directed the generation, assembly and alignment of the sequence data, and, with support from MPC and AZ, performed the phylogenetic analyses. All authors participated in drafting, error-checking and revising the manuscript, with MAS and CM serving as overall coordinators and editors. The accompanying web pages were constructed by MAS, JH, TJS, BL, MN and SY.

No conflicts of interest were discovered.

References

Aiello, A. & Solis, M.A. (2003) Defense mechanisms in Pyralidae and Choreutidae: fecal stalactites and escape holes, with remarks about cocoons, camouflage and aposematism. *Journal of Lepidopterists' Society*, **57**, 168–175.

Allyson, S. (1977) A study of some North American larvae of the genus *Tetralopha* Zeller (Lepidoptera: Pyralidae: Epipaschiiinae). *Canadian Entomologist*, **109**, 329–336.

Allyson, S. (1981) Last instar larvae of Pyraustini of America North of Mexico (Lepidoptera; Pyralidae). *Canadian Entomologist*, **113**, 463–518.

Allyson, S. (1984) Description of last-instar larvae of 22 species of North American Spilomelini (Lepidoptera: Pyralidae: Pyraustinae) with a key to species. *Canadian Entomologist*, **116**, 1301–1334.

Amsel, H.G. (1965) Die *Noorda blitealis* Wlk.-Gruppe (Lep.: Pyralidae). Ergebnisse der entomologischen Reise Willi Richter, Stuttgart, in Äthiopien 1959/60. *Stuttgarter Beiträge zur Naturkunde*, **144**, 1–7.

Arthur, F.H. (2008) Aerosol distribution and efficacy in a commercial food warehouse. *Insect Science*, **15**, 133–140.

Bazinet, A.L. & Cummings, M.P. (2009) The lattice project: a Grid research and production environment combining multiple Grid computing models. *Distributed & Grid Computing – Science Made Transparent for Everyone. Principles, Applications and Supporting Communities* (ed. by M.H.W. Weber), pp. 2–13. Tectum Publishing House, Marburg.

Beccaloni, G.W., Scoble, M.J., Robinson, G.S. & Pitkin, B. (2003) *The Global Lepidoptera Names Index (LepIndex)* [WWW document]. URL http://www.nhm.ac.uk/entomology/lepindex [accessed on 13 March 2012].

Beck, J., Brehm, G. & Fiedler, K. (2011) Links between the environment, abundance and diversity of Andean moths. *Biotropica*, **43**, 208–217.

Bleszynski, S. (1966) Studies on the Crambinae (Lepidoptera). Part 43. Further taxonomic notes on some tropical species. *Acta Zoologica Cracoviensia*, **11**, 451–497.

Börner, C. (1925) Lepidoptera, Schmetterlinge. *Fauna von Deutschland* (ed. by P. Brohmer), 561 pp. Quelle & Meyer, Berlin.

Brower, A.V.Z. & DeSalle, R. (1998) Mitochondrial vs. nuclear DNA sequence evolution among nymphalid butterflies: the utility of Wingless as a source of characters for phylogenetic inference. *Insect Molecular Biology*, **7**, 1–10.

Cashatt, E.D. (1968) *Revision of the Chrysauginae of North America*. PhD Dissertation, Catholic University of America, Washington, DC.

Cavalieri, L.F. & Kocak, H. (1995) Chaos: a potential problem in the biological control of insect pests. *Mathematical Biosciences*, **127**, 1–17.

Center, T.D., Dray, F.A. Jr., Jubinsky, G.P. & Grodowitz, M.J. (2002) Insects and other arthropods that feed on aquatic and wetland plants. *USDA, ARS Technical Bulletin*, **1870**, 1–200.

Cho, S., Zwick, A., Regier, J.C. *et al*. (2011) Can deliberately incomplete gene sample augmentation improve a phylogeny estimate for the advanced moths and butterflies (Hexapoda: Lepidoptera)? *Systematic Biology*, **60**, 782–796.

Clavijo, A.J.A. (1990) *Systematics of black and white species of the genus* Diaphania *Hübner (1818) (Lepidoptera: Pyralidae: Pyraustinae)*. PhD Dissertation, McGill University, Montreal.

Common, I.F.B. (1960) A revision of the Australian stem borers hitherto referred to *Schoenobius* and *Scirpophaga* (Lepidoptera: Pyralidae, Schoenobiinae). *Australian Journal of Zoology*, **8**, 307–347.

Common, I.F.B. (1990) Superfamily Pyraloidea. *Moths of Australia*, pp. 343–358, 535 pp. Melbourne University Press, Carlton.

Cotter, G., Doyle, S. & Kavanagh, K. (2000) Development of an insect model for the *in vivo* pathogenicity testing of yeasts. *FEMS Immunology & Medical Microbiology*, **27**, 163–169.

Cowen, L.E., Singh, S.D., Koehler, J.R. *et al*. (2009) Harnessing Hsp90 function as a powerful, broadly effective therapeutic strategy for fungal infections disease. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 2818–2823.

Cummings, M.P. & Huskamp, J.C. (2005) Grid computing. *EDUCAUSE Review*, **40**, 116–117.

Deniro, M.J. & Epstein, S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495–506.

Eliopoulos, P.A. & Stathas, G.J. (2003) Temperature-dependent development of the koinobiont endoparasitoid *Ventura canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae): effect of host instar. *Environmental Entomology*, **32**, 1049–1055.

Ellsworth, P.C., Umeozor, O.C., Kennedy, G.G., Bradley, J.R. Jr & Van Duyn, J.W. (1989) Population consequences of diapause in a model system, the European corn borer. *Entomologia Experimentalis et Applicata*, **53**, 45–56.

Fang, Q., Cho, S., Regier, J. *et al*. (1997) A new nuclear gene for insect phylogenetics: Dopa decarboxylase is informative of relationships within Heliothinae (Lepidoptera: Noctuidae). *Systematic Biology*, **46**, 269–283.

Farrell, B.D., Sequeira, A.S., O'Meara, B., Normark, B.B., Chung, J.H. & Jordal, B.H. (2001) The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). *Evolution*, **55**, 2011–2027.

Fiedler, K. & Schulze, C. (2004) Forest modification affects diversity (but not dynamics) of speciose tropical pyraloid moth communities. *Biotropica*, **36**, 615–627.

Fletcher, D.S. & Nye, I.W.B. (1984) The generic names of the Moths of the World. London: trustees of the British Museum. *Natural History*, **5**, 1–185.

Forbes, W.T.M. (1920) Notes on the Crambinae (Lepidoptera). *Journal of the New York Entomological Society*, **28**, 24–217.

Forbes, W.T.M. (1926) The relationships of some aberrant pyralids (Lep.). *Journal of the New York Entomological Society*, **34**, 333–338.

Forbes, W.T.M. (1933) The pupa of *Hyblaea* (Lepidoptera, Hyblaeidae). *Annals of the Entomological Society of America*, **26**, 490.

Fuji, T., Ito, K., Tatematsu, M., Shimada, T., Katsuma, S. & Ishikawa, Y. (2011) Sex pheromone desaturase functioning in a primitive *Ostrinia* moth is cryptically conserved in congeners' genomes. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 7102–7106.

Gerasimov, A.M. (1947) Larvae and pupae of pyralids (Pyralididae, Lepidoptera). *Entomologicheskoe Obozrenie*, **29**, 165–183 (in Russian).

Gerasimov, A.M. (1949) Larvae and pupae of pyralids (Pyralididae, Lepidoptera). *Entomologicheskoe Obozrenie*, **30**, 365–369 (in Russian).

Goater, B. (2005) Subfamily Evergestinae. *Pyraloidea I (Crambidae: Acentropinae, Evergestinae, Heliothelinae, Schoenobiinae, Scopariinae)* (ed. by B. Goater, M. Nuss and W. Speidel), *Microlepidoptera of Europe* (ed. by P. Huemer and O. Karsholt), Vol. 4, pp. 69–17, 304 pp. Apollo Books, Stenstrup.

Gounou, S. & Schulthess, F. (2004) Spatial distribution of lepidopterous stem borers on indigenous host plants in West Africa and its implications for sampling schemes. *African Entomology*, **12**, 171–178.

Guenée, A. (1854) Species général des Lépidoptères. Tome huitième. *Deltoïdes et pyralites*, 448 pp. Librairie Encyclopédique Roret, Paris.

Hampson, G.F. (1895) On the classification of the Schoenobiinae and Crambinae, two subfamilies of moths, of the family Pyralidae. *Proceedings of the Zoological Society of London*, **1895**, 897–974.

Hampson, G.F. (1898) A revision of the moths of the subfamily Pyraustinae and Family Pyralidae. *Proceedings of the Zoological Society of London*, **1898**, 590–761.

Hasenfuß, I. (1960) *Die Larvalsystematik der Zünsler.* Academie Verlag, Berlin.

Hayden, J.E. (2009) A new genus of Caribbean Odontiinae with Palaeotropical affinities (Lepidoptera: Crambidae). *Journal of the Lepidopterists' Society*, **63**, 185–208.

Hayden, J.E. (2011) Revision of C*liniodes* Guenée (Lepidoptera: Crambidae: Odontiinae). *Annals of the Carnegie Museum*, **79**, 231–347.

Heckford, R.J. (2009) Notes on the early stages of *Scoparia ambigualis* (Treitschke, 1829) and *Eudonia pallida* (Curtis, 1827) (Lepidoptera: Pyralidae). *Entomologist's Gazette*, **60**, 221–231.

Heinrich, C. (1956) American moths of the subfamily Phycitinae. *Bulletin of the United States National Museum*, **207**, 1–581.

Horak, M. (1997) The Phycitinae Genera *Faveria* Walker, *Morosaphycita*, gen. nov., *Epicrocis* Zeller, *Ptyobathra* Turner and *Vinicia* Ragonot in Australia (Pyralidae: Phycitinae). *Invertebrate Taxonomy*, **11**, 333–421.

Horak, M. (2003) Reassessment of the Anerastiini and their status in the Phycitinae (Pyralidae): a century long controversy. *Invertebrate Systematics*, **17**, 89–98.

Ingleby, F.C., Lewis, Z. & Wedell, N. (2010) Level of sperm competition promotes evolution of male ejaculate allocation patterns in a moth. *Animal Behaviour*, **80**, 37–43.

Janzen, D., Hallwachs, W., Blandin, P. *et al*. (2009) Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Molecular Ecology Resources*, **9**, 1–26.

Kawahara, A.Y., Mignault, A.A., Regier, J.C., Kitching, I.J. & Mitter, C. (2009) Phylogeny and biogeography of hawkmoths (Lepidoptera: Sphingidae): evidence from five nuclear genes. *PLoS One*, **4**, e5719. DOI: 10.1371/journal.pone.0005719.

Kemner, N.A. (1923) Hyphaenosymphylie, eine neue, merkwürdige Art von Myrmekophilie bei einem neuen myrmekophilen Schemetterling (*Wurthia aurivillii* n. sp.) aus Java beobachtet. *Arkiv für Zoologi*, **15**, 1–28.

Kuznetzov, V.I. & Stekolnikov, A.A. (1979) Classification and phylogenetic relationships of the families and subfamilies of the Pyraloidea (Lepidoptera) of the palearctic fauna with regard to functional morphology of the male genitalia. *Trudy Institute Zoological Leningrad*, **82**, 43–74 (In Russian).

Landry, B. (1995) A phylogenetic analysis of the major lineages of the Crambinae and of the genera of Crambini of North America (Lepidoptera: Pyralidae). *Memoirs on Entomology, International*, **1**, 1–242.

Lassance, J.-M. (2010) Journey in the *Ostrinia* world: from pest to model in chemical ecology. *Journal of Chemical Ecology*, **36**, 1155–1169.

Lemmon, A.R., Brown, J.M., Stanger-Hall, K. & Lemmon, E.M. (2009) The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. *Systematic Biology*, **58**, 130–145.

Leraut, P. & Luquet, G.C. (1982) Statut de quelques genres et espèces d'Odontiinae paléarctiques et description de quatre nouveaux taxa (Lep. Crambidae). *Linneana Belgica*, **8**, 527–555.

Lewanich, A. (1981) A revision of the Old World species of *Scirpophaga* (Lepidoptera: Pyralidae). *Bulletin of the British Museum, Natural History, Entomology*, **42**, 185–298.

Lewis, Z. & Wedell, N. (2009) Male moths reduce sperm investment in relatives. *Animal Behaviour*, **77**, 1547–1550.

Lewis, Z., Sasaki, H. & Miyatake, T. (2011) Sex-starved: do resource-limited males ensure fertilization success at the expense of precopulatory mating success? *Animal Behaviour*, **81**, 579–583.

Luquet, G.C. & Minet, J. (1982) Découverte dans le Vaucluse d'une nouvelle espèce de pyrale et désignation de l'espèce-type du genre *Krombia* Chrétien (Lepidoptera, Crambidae, Cybalomiinae). *Alexanor*, **12**, 317–326.

Maes, K.V.N. (1994) Some notes on the taxonomic status of the Pyraustinae (sensu Minet 1981[1982]) and a check list of the Palearctic Pyraustinae (Lepidoptera, Pyraloidea, Crambidae). *Annales de la Société Royale Entomologique de Belgique*, **130**, 159–198.

Maes, K.V.N. (1995) A comparative morphological study of the adult Crambidae (Lepidoptera: Pyraloidea). *Annales de la Société Royale Entomologique de Belgique*, **131**, 383–434.

Mally, R. & Nuss, M. (2010) Phylogeny and nomenclature of the box tree moth, *Cydalima perspectalis* (Walker, 1859) comb. n., which was recently introduced into Europe (Lepidoptera: Pyraloidea: Crambidae: Spilomelinae). *European Journal of Entomology*, **107**, 393–400.

Mally, R. & Nuss, M. (2011) Molecular and morphological phylogeny of European *Udea* moths (Insecta: Lepidoptera: Pyraloidea). *Arthropod Systematics & Phylogeny*, **69**, 55–71.

Marion, H. (1952) Ébauche d'une classification nouvelle des Pyraustidae. *Revue Française de Lépidoptérologie*, **13**, 260–270.

Marion, H. (1961) Révision des Pyraustidae de France. *Alexanor*, **2**, 83–90.

Martin, M.O. (1986) A guide to the insects of the European part of the U.S.S.R. Lepidoptera. Family Pyraustinae. *Opredeliteli Faune SSSR, No. 144*, **4**, 340–429 (in Russian).

Martinez, E.L. (2010) *A revision of the New World species of* Donacaula *Meyrick and a phylogenetic analysis of related Schoenobiinae (Lepidoptera: Crambidae)*. PhD Dissertation, Mississippi State University, Starkville, MS.

Matthew, G. & Menon, R. (1984) The pyralid fauna (Lepidoptera: Pyraloidea: Pyralidina) of Kerala (India). *Journal of Entomological Research*, **8**, 5–13.

Mey, W. & Speidel, W. (2008) Global diversity of butterflies (Lepidoptera) in freshwater. *Hydrobiologia*, **595**, 521–528.

Minet, J. (1980) Création d'une sous-famille particulière pour *Noorda* Walker, 1859, et définition d'un nouveau genre parmi les Odontiinae. *Bulletin de la Société entomologique de France*, **85**, 79–87.

Minet, J. (1982) Les Pyraloidea et leurs principales divisions systématiques. *Bulletin de la Société Entomologique de France*, **86**, 262–280.

Minet, J. (1985) Étude morphologique et phylogénétique des organes tympaniques des Pyraloidea. 2 - Pyralidae, Crambidae, première partie (Lepidoptera Glossata). *Annales de la Société Entomologique de France*, **21**, 69–86.

Minet, J. (1994) The Bombycoidea: phylogeny and higher classification (Lepidoptera: Glossata). *Entomologica Scandinavica*, **25**, 63–88.

Moulton, J.K. & Wiegmann, B.M. (2003) Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged eremoneuran Diptera (Insecta). *Molecular Phylogenetics and Evolution*, **31**, 363–378.

Mukherjee, K., Altincicek, B., Hain, T., Domann, E. & Vilcinskas, A. (2010) *Galleria mellonella* as a model system for studying *Listeria* pathogenesis. *Applied & Environmental Microbiology*, **76**, 310–317.

Munroe, E.G. (1958) Hampson's Schoenobiinae (Lepidoptera: Pyralidae). *Proceedings of the Tenth International Congress of Entomology*, **1**, 301–302.

Munroe, E.G. (1961) Synopsis of the North American Odontiinae, with descriptions of new genera and species (Lepidoptera: Pyralidae). *Memoirs of the Entomological Society Canada*, **24**, 1–93.

Munroe, E.G. (1964) New genera and species of Glaphyriinae (Lepidoptera: Pyralidae). *Canadian Entomologist*, **96**, 1261–1307.

Munroe, E.G. (1970) Revision of the subfamily Midilinae, (Lepidoptera: Pyralidae). *Memoirs of the Entomological Society of Canada*, **74**, 1–94.

Munroe, E.G. (1972) Pyraloidea. *The Moths of America North of Mexico. Fasc. 13.1 (A, B). Subfamilies Scopariinae, Nymphulinae, Odontiinae, Glaphyriinae* (ed. by R.B. Dominick, C.R. Edwards, D.C. Ferguson, J.G. Franclemont, R.W. Hodges, E.G. Munroe and E.W. Classey), pp. 1–250. E. W. Classey, London.

Munroe, E.G. (1973) Pyraloidea. *The Moths of America North of Mexico. Fasc. 13.1(C). Subfamily Evergestinae* (ed. by R.B. Dominick, C.R. Edwards, D.C. Ferguson, J.G. Franclemont, R.W. Hodges, E.G. and Munroe), pp. 251–370. E. W. Classey, London.

Munroe, E.G. (1976) Pyraloidea. *The Moths of America North of Mexico. Fasc. 13.2 (A, B). Subfamily Pyraustinae* (ed. by R.B. Dominick, T. Dominick, D.C. Ferguson, J.G. Franclemont, R.W. Hodges, and E.G. Munroe), pp. 1–150. E. W. Classey, London.

Munroe, E. & Solis, M.A. (1999) Pyraloidea. *Lepidoptera, Moths and Butterflies*, Vol. 1, *Arthropoda, Insecta, Vol. 4*, Part 35, *Handbook of Zoology* (ed. by N. Kristensen), pp. 233–256, 491 pp. Walter de Gruyter & Co, Berlin.

Murase, M. (2005) The larva of *Micraglossa aureata* Inoue (Pyralidae: Scopariinae). *Japan Heterocerists' Journal*, 203–204.

Mutanen, M., Wahlberg, K. & Kaila, L. (2010) Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. *Proceedings of the Royal Society, B*, **277**, 2839–2849.

Mylonakis, E., Moreno, R., El Khoury, J.B. *et al*. A. (2005) *Galleria mellonella* as a model system to study *Cryptococcus neoformans* pathogenesis. *Infection & Immunity*, **73**, 3842–3850.

Nielsen, E.S. (1989) Phylogeny of major lepidopteran groups. *The Hierarchy of Life* (ed. by B. Fernholm, K. Bremer and H. Jörnvall), pp. 281–294. Elsevier Science Publishers, Copenhagen.

van Nieukerken, E.J., Kaila, L., Kitching, I.J. *et al*. (2011) Order Lepidoptera Linnaeus, 1758. (ed. by Z.-Q. Zhang) Animal Biodiversity: An Outline of Higher-Level Classification and Survey of Taxonomic Richness. *Zootaxa*, **3148**, 212–221.

Niogret, J., Sait, S.M. & Rohani, P. (2009) Parasitism and constitutive defence costs to host life-history traits in a parasitoid-host interaction. *Ecological Entomology*, **34**, 763–771.

Nuss, M. (1998) The Scopariinae and Heliothelinae stat. rev. (Lepidoptera: Pyraloidea, Crambidae) of the Oriental Region – a revisional synopsis with descriptions of new species from the Philippines and Sumatra. *Nachrichten des Entomologischen Vereins Apollo*, (**Suppl. 17**), 475–528.

Nuss, M. (1999) Revision der Gattungen der Scopariinae, Lepidoptera, Pyraloidea, Crambidae. *Nova Supplementa Entomologica*, **13**, 1–151.

Nuss, M., Landry, B., Vegliante, F. *et al*. (2003–2012) *Global Information System on Pyraloidea* [WWW document]. URL http://www.pyraloidea.org [accessed on 13 March 2012].

Oliveira, F.B. (2005) Body size influence on defensive behavior of Amazonian moths: an ecophysiological approach. *Brazilian Journal of Biology*, **65**, 101–106.

Onstad, D.W. & Gould, F. (1998) Do dynamics of crop maturation and herbivorous insect life cycle influence the risk of adaptation in transgenic host plants? *Environmental Entomology*, **27**, 517–522.

Oppert, B., Ellis, R.T. & Babcock, J. (2010) Effects of Cry1F and Cry34Ab1/35Ab1 on storage pests. *Journal of Stored Products Research*, **46**, 143–148.

Passoa, S. (1985) *Taxonomy of the larvae and pupae of economically important Pyralidae in Honduras*. Master's Thesis, University of Florida, Gainesville, FL.

Passoa, S. (1988) Systematic positions of *Acentria ephemerella* (Denis and Schiffermůller), Nymphulinae, and Schoenobiinae based on morphology of immature stages (Pyralidae). *Journal of the Lepidopterists' Society*, **42**, 247–262.

Phillips, E. & Solis, M.A. (1996) *Neurophyseta* (Lepidoptera: Crambidae) de Costa Rica. *Revista de Biología Tropical*, **44**, 693–717.

Phillips, M.J., Delsuc, F. & Penny, D. (2004) Genome-scale phylogeny and the detection of systematic biases. *Molecular Biology and Evolution*, **21**, 1455–1458.

Pimentel, M.C.P., Macedo, M.V. & Monteiro, R.F. (1991) Notes on the biology and behavior of *Eupastraia fenestrata* Ménétriés (Lepidoptera: Pyralidae: Midilinae). *Journal of the New York Entomological Society*, **99**, 699–700.

Regier, J.C., Fang, Q.Q., Mitter, C., Peigler, R.S., Friedlander, T.P. & Solis, M.A. (1998) Evolution and phylogenetic utility of the *period* gene in Lepidoptera. *Molecular Biology and Evolution*, **15**, 1172–1182.

Regier, J.C., Cook, C.P., Mitter, C. & Hussey, A. (2008a) A phylogenetic study of the "bombycoid complex" (Lepidoptera) using five protein-coding nuclear genes, with comments on the problem of macrolepidopteran phylogeny. *Systematic Entomology*, **33**, 175–189.

Regier, J.C., Grant, M.C., Peigler, R.S., Mitter, C., Cook, C.P. & Rougerie, R. (2008b) Phylogenetic relationships of wild silkmoths (Lepidoptera: Saturniidae) inferred from four protein-coding nuclear genes. *Systematic Entomology*, **33**, 219–228.

Regier, J.C., Shultz, J.W., Ganley, A.R.D. *et al*. (2008c) Resolving arthropod phylogeny: Exploring phylogenetic signal within 41 kb of protein-coding nuclear gene sequence. *Systematic Biology*, **57**, 920–938.

Regier, J., Zwick, A., Cummings, M. *et al*. C. (2009) Toward reconstructing the evolution of advanced moths and butterflies (Lepidoptera: Ditrysia): an initial molecular study. *BioMed Central Evolutionary Biology*, **9**, 280–301.

Regier, J.C., Shultz, J.W., Zwick, A. *et al*. (2010) Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. *Nature*, **463**, 1079–1083.

Roberts, Harry, L.S., Keller, M. & Schmidt, O. (2006) An empirical model of sympatric coexistence of two strains of the endo parasitoid wasp *Ventura canescens*. *Archives of Insect Biochemistry & Physiology*, **61**, 184–194.

Robinson, G., Tuck, K. & Shaffer, M. (1994) *A Field Guide to the Smaller Moths of South-East Asia*. The Malaysian Nature Society, Kuala Lumpur.

Roelofs, W., Liu, W., Hao, G., Jiao, H., Rooney, A.P. & Linn, C.E. (2002) Evolution of moth sex pheromones via ancestral genes. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 13621–13626.

Roepke, F.W. (1916) Eine neue myrmekophile Lepidoptere aus Java (*Wurthia myrmecophila* n. g. n. sp.). *Zoologische Mededelingen Leiden*, **2**, 141–146.

Roesler, R.U. (1973) Phycitinae. *Microlepidoptera Palaearctica* (ed. by H.G. Amsel, F. Gregor, H. Reisser and G. Fromme), 752 pp, G. Fromme, Vienna.

Scully, L.R. & Bidochka, M.J. (2006) The host acts as a genetic bottleneck during serial infections: an insect-fungal model system. *Current Genetics*, **50**, 335–345.

Scully, L.R. & Bidochka, M.J. (2009) An alternative insect pathogenic strategy in an *Aspergillus flavus* auxotroph. *Mycological Research*, **113**, 230–239.

Shaffer, M., Nielsen, E.S. & Horak, M. (1996) Pyraloidea. *Checklist of the Lepidoptera of Australia*, *Monographs on Australian Lepidoptera, Vol. 4* (ed. by E.S. Nielsen, E.D. Edwards and T.V. Rangsi), pp. 164–199. CSIRO Publishing, Collingwood.

Siaussat, D., Bozzolan, F., Porcheron, P. & Debernard, S. (2008) The 20-hydroxyecydone-induced signaling pathway in G2/M arrest of *Plodia interpunctella* imaginal wing cells. *Insect Biochemistry & Molecular Biology*, **38**, 529–539.

Simonsen, T. (2008) Phylogeny of the cactus-feeding phycitines and their relatives (Lepidoptera: Pyralidae) based on adult morphology: evaluation of adult character-systems in phycitine systematics and evidence for a single origin of Cactaceae-feeding larvae. *Insect Systematics and Evolution*, **39**, 303–325.

Simonsen, T. & Roe, A.D. (2009) Phylogenetic utility and comparative morphology of the composite scale brushes in male phycitine moths (Lepidoptera: Pyralidae). *Zoologischer Anzeiger*, **248**, 119–136.

Small, G.J. (2007) A comparison between the impact of sulfuryl fluoride and methyl bromide fumigations on stored product insect populations in UK flour mills. *Journal of Stored Products Research*, **43**, 410–416.

Solis, M.A. (1993) A phylogenetic analysis and reclassification of the genera of the *Pococera*-complex (Lepidoptera: Pyralidae: Epipaschiinae). *Journal of the New York Entomological Society*, **101**, 1–83.

Solis, M.A. (2003) A new species of *Epimorius* Zeller feeding on Bromeliaceae in Costa Rica (Lepidoptera: Pyralidae: Galleriinae). *Tropical Lepidoptera*, **11**, 28–32.

Solis, M.A. (2008) Aquatic and semiaquatic Lepidoptera. *Aquatic Insects of North America* (ed. by R.W. Merritt, K.W. Cummins and M.B. Berg), pp. 553–569, 1158 pp. Kendall/Hunt Publishing Company, Dubuque, IA.

Solis, M.A. & Adamski, D. (1998) Review of the Costa Rican Glaphyriinae (Lepidoptera: Pyraloidea: Crambidae). *Journal of the New York Entomological Society*, **106**, 1–55.

Solis, M.A. & Maes, K.V.N. (2002) Preliminary phylogenetic analysis of the subfamilies of Crambidae (Pyraloidea: Lepidoptera). *Belgian Journal of Entomology*, **4**, 53–95.

Solis, M.A. & Metz, M.A. (2008) Species of *Aphomia* Hübner and *Paralipsa* Butler (Pyralidae: Galleriinae) known to occur in the United States and Canada and their associations with stored products and social Hymenoptera. *Proceedings of Entomological Society of Washington*, **110**, 592–601.

Solis, M.A. & Metz, M. (2011) Male genital homology based on musculature originating from the tegumen in snout moths (Lepidoptera: Pyraloidea). *Arthropod Structure and Development*, **40**, 435–488.

Solis, M.A. & Mitter, C. (1992) Review and preliminary phylogenetic analysis of the subfamilies of the Pyralidae (sensu stricto) (Lepidoptera: Pyraloidea). *Systematic Entomology*, **17**, 79–90.

Solis, M.A. & Shaffer, M. (1999) Contribution towards the study of the Pyralinae (Pyralidae): historical review, morphology, and nomenclature. *Journal of the Lepidopterists' Society*, **53**, 1–10.

Solis, M.A., Sullivan, J.J. & Janzen, D.H. (2003) *Cromarcha stroudagnesia*, a new chrysaugine species boring in shoots of *Tabebuia ochracea* (Bignoniaceae) in a Costa Rica dry forest (Lepidoptera: Pyralidae: Chrysauginae). *Tropical Lepidoptera*, **11**, 33–39.

Solis, M.A., Yen, S.-H. & Goolsby, J.R. (2004) Description and life history of *Lygomusotima* New Genus, and *Neomusotima conspurcatalis* (Lepidoptera: Crambidae) from Australia and Southeast Asia feeding on *Lygodium microphyllum*. *Annals of the Entomological Society of America*, **97**, 64–76.

Solis, M.A., Davis, D.R. & Nishida, K. (2005a) Biology and systematics of *Albusambia elaphaglossumae*, a new genus and species of Crambidae (Lepidoptera: Pyraloidea: Crambidae) mining the fronds of *Elaphoglossum conspersum* (Pteridophyta: Lomariopsidaceae) in Costa Rica. *International Journal of Tropical Biology*, **53**, 487–501.

Solis, M.A., Yen, S.-H., Goolsby, J.H. *et al*. (2005b) *Siamusotima aranea*, a new stem-boring musotimine (Lepidoptera: Crambidae) from Thailand feeding on *Lygodium flexuosum* (L.) Sw. (Schizaeaceae). *Annals of the Entomological Society of America*, **98**, 887–895.

Solis, M.A., Metz, M.A. & Janzen, D.H. (2009) Phylogenetic analysis of *Cosmopterosis* Amsel (Lepidoptera: Crambidae: Glaphyriinae) with discussions on male secondary sexual characters and larval feeding on *Capparis* L. (Capparaceae) in the Pyraloidea and Lepidoptera (Insecta). *Annals of the Entomological Society of America*, **102**, 766–784.

Speidel, W. (1981) Die Abgrenzung der Unterfamilie Acentropinae (Lepidoptera: Pyraloidea). *Atalanta*, **12**, 117–128.

Speidel, W. & Stüning, D. (2005) *Ambia naumanni* sp. n., a new species of Musotiminae from Yunnan (Lepidoptera: Crambidae). *Bonner Zoologische Beiträge*, **53**, 221–225.

Staden, R. (1999) *Staden Package.* MRC Laboratory of Molecular Biology, Cambridge, U.K. [www document]. URL http://www.mrc-lmb.cam.ac.uk/pubseq/ [accessed on 19 September 2007].

Sutrisno, H. (2002) A preliminary study on relationships among selected Australian members of the tribe Spilomelini (Lepidoptera: Crambidae: Pyraustinae). *Zoological Science*, **19**, 915–929.

Sutrisno, H., Azuma, N. & Higashi, S. (2006) Molecular phylogeny of the Indo-Australian *Glyphodes* and its allied genera (Insecta: Lepidoptera: Crambidae: Spilomelinae) inferred from mitochondrial COI and COII and nuclear EF-1α gene sequences. *Species Diversity*, **11**, 57–69.

Tao, F.-L., Min, S.-F., Wu, W.-J., Liang, G.-W. & Zeng, L. (2008a) Estimating index of population trend by re-sampling techniques (jackknife and bootstrap) and its application to the life table study of the rice leaf roller, *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). *Insect Science*, **15**, 153–161.

Tao, F.-L., Min, S.-F., Liang, G.-W. & Zeng, L. (2008b) An approximate variance estimator for index of population trend developed with delta-method and its application. *Acta Entomologica Sinica*, **51**, 521–525.

Tränkner, A., Li, H. & Nuss, M. (2009) On the systematics of *Anania* Hübner, 1823 (Pyraloidea: Crambidae: Pyraustinae). *Nota lepidopterologica*, **32**, 63–80.

Ukai, M., Kameya, H., Imamura, T., Miyanoshita, A., Todoriki, S. & Shimoyama, Y. (2009) ESR signals of irradiated insects. *Radioisotopes*, **58**, 799–806.

Whalley, P.E.S. (1964) Catalogue of the Galleriinae (Lepidoptera, Pyralidae) with descriptions of new genera and species. *Acta Zoologica Cracoviensia*, **9**, 561–617.

Wheat, C.W., Vogel, H., Wittstock, U., Braby, M.F., Underwood, D. & Mitchell-Olds, T. (2007) The genetic basis of a plant-insect coevolutionary key innovation. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 20427–20431.

Wiens, J.J. (1998) Does adding characters with missing data increase or decrease phylogenetic accuracy? *Systematic Biology*, **47**, 625–640.

Wiens, J.J. & Morrill, M.C. (2011) Missing data in phylogenetic analysis: reconciling results from simulations and empirical data. *Systematic Biology*, **60**, 719–731.

Yamamura, K., Yokozawa, M., Nishimori, M., Ueda, Y. & Yokosuka, T. (2006) How to analyze long-term insect population dynamics under climate change: 50-year data of three-year insect pests in paddy fields. *Population Ecology*, **48**, 31–48.

Yen, S.-H. (1996) *Aeolopetra lanyuensis* Yen, a new pyralid moth (Pyralidae: Musotiminae) from Taiwan. *Tinea*, **14**, 293–299.

Yen, S.-H. (1997) *A phylogenetic analysis of the major lineages of Nymphulinae and Musotiminae (Lepidoptera*, *Pyralidae sensu lato)*, *with taxonomic revision on the Taiwanese fauna*. Master's Thesis, National Sun Yat-Sen University, Kaohsiung.

Yen, S.-H. (2004) Insecta: Lepidoptera, Crambidae, Acentropinae. *Freshwater Invertebrates of the Malaysian Region* (ed. by C.M. Yule and Y.H. Sen), pp. 545–554, 861 pp. Academy of Sciences Malaysia, Kuala Lumpur.

Yen, S.-H., Solis, M.A. & Goolsby, J.A. (2004) *Austromusotima*, a new musotimine genus (Lepidoptera: Crambidae) feeding on Old World climbing fern. *Annals of the Entomological Society of America*, **97**, 397–410.

Yin, J., Zhong, T., Wei, Z., Li, K.-B. & Cao, Y.-Z. (2011) Molecular characters and recombinant expression of the carboxylesterase gene of the meadow moth *Loxostege stictalis* L. (Lepidoptera: Pyralidae). *African Journal of Biotechnology*, **10,** 1794–1801.

Yoshiyasu, Y. (1980) A systematic study of the genus *Nymphicula* Snellen from Japan (Lepidoptera: Pyralidae). *Tyô to Ga*, **31**, 1–28.

Yoshiyasu, Y. (1985) A systematic study of the Nymphulinae and the Musotiminae of Japan (Lepidoptera: Pyralidae). *Scientific Report of Kyoto Prefectural University, Agriculture*, **37**, 1–162.

Zhang, B.-C. (1994) *Index of Economically Important Lepidoptera.* CAB International, Wallingford, Oxon.

Zwick, A., Regier, J.C., Mitter, C. & Cummings, M.P. (2011) Increased gene sampling yields robust support for higher-level clades within Bombycoidea (Lepidoptera). *Systematic Entomology*, **36**, 31–43.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Dissertation, The University of Texas, Austin.

Accepted 20 April 2012
First published online 6 July 2012