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Molecular phylogeny, divergence time, biogeography and trends in host plant usage in the agriculturally important tortricid tribe Grapholitini (Lepidoptera: Tortricidae: Olethreutinae)

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Abstract

The leaf-roller moth tribe Grapholitini comprises about 1200 described species and contains numerous notorious pests of fruits and seeds. The phylogeny of the tribe has been little studied using contemporary methods, and the monophyly of several genera remains questionable. In order to provide a more robust phylogenetic framework for the group, we conducted a multiple-gene phylogenetic analysis of 104 species representing 27 genera of Grapholitini and 29 outgroup species. Divergence time, ancestral area, and host plant usage were also inferred to explore evolutionary trends in the tribe. Our analyses indicate that *Larisa* and *Corticivora*, traditionally assigned to Grapholitini, are best excluded from the tribe. After removal of these two genera, the tribe is found to be monophyletic, represented by two major lineages—a *Dichrorampha* clade and a *Cydia* clade, the latter of which can be divided into seven generic groups. The genus *Grapholita* was found to be polyphyletic, comprising three different clades, and we propose three genera to accommodate these groups: *Grapholita* (sensu stricto), *Aspila* (formerly a subgenus of *Grapholita*) and *Ephippiphora* (formerly considered a synonym of *Grapholita*). We summarize each generic group, including related genera not included in our analysis, providing morphological, pheromone and food plant characters that support particular branches within the molecular hypotheses. Biogeographical analyses indicate that Grapholitini probably originated in the Nearctic, Afrotropical and Neotropical regions in the Lutetian of the middle Eocene (ca. 44.3 Ma). Our results also indicate that most groups in Grapholitini originated from Fabaceae-feeding monophagous or oligophagous ancestors, and that host plant shifts probably promoted species diversification within the tribe.

Introduction

The phylogeny of the superdiverse insect order Lepidoptera (butterflies and moths), and many groups within the order, has received dramatically increased attention over the last 15 years, resulting in significantly improved insight into the backbone phylogeny of the order and that of many major lineages (Mutanen et al., 2010; Zahiri et al., 2011; Zwick et al., 2011; Heikkilä et al., 2012, 2014; Regier et al., 2012a, b, 2013, 2015a, b; Sohn et al., 2013, 2016; Kawahara and Breinholt, 2014; Bazinet et al., 2017; Kawahara et al., 2017; Breinholt et al., 2018; Murillo-Ramos et al., 2019). These phylogenetic studies have resulted in considerable re-classification of several lepidopteran superfamilies, families, and subfamilies, leading to more stable and robust classifications. Although among the most species-rich groups of the so-called "microlepidoptera", the leaf-roller moths (Tortricidae) have received relatively limited attention. While Regier et al. (2012a) and Fagua et al. (2017) together provide a fairly robust molecular backbone hypothesis of

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*Corresponding author: *E-mail address:* huguilin@zzu.edu.cn relationships among the tribes of Tortricidae, most taxa below the family level have not been subjected to intensive phylogenetic sampling, an exception being the recent analysis of the subtribe Cochylina of Tortricinae (Brown et al., 2020).

Members of the tortricid tribe Grapholitini, of the subfamily Olethreutinae, are among the economically most important phytophagous insect species on the planet, with larvae that are primarily internal feeders in fruit, pods, seeds and nuts, and less frequently in shoots, stems and roots. The tribe is worldwide in distribution, encompassing about 1200 described species assigned to 83 genera (Gilligan et al., 2018), with the greatest species richness in the Holarctic Region. Owing to increased globalization (trade and travel), at least two important pests, codling moth (*Cydia pomonella* (Linnaeus)) and Oriental fruit moth (*Grapholita molesta* (Busck)), are now nearly cosmopolitan in distribution, causing damage that results in significant loss in crop production of apple, pear, peach, apricot, quince and many other rosaceous fruits. The Manchurian fruit moth (*Grapholita inopinata* Heinrich) has recently expanded its range across the Palaearctic and is becoming a serious pest of quince, apple, plum and pear in Europe. The false codling moth, *Thaumatotibia leucotreta* (Meyrick), native to the Afrotropical region, is among the most polyphagous species of Tortricidae and a notorious pest of citrus, eggplant, peppers and many other crop plants. Like the codling moth and Oriental fruit moth, the false codling moth is frequently intercepted at ports of entry around the world on a wide range of plant commodities, representing an important threat to biosecurity (Madden et al., 2019). Countless other grapholitines are economically important pests of conifers, legumes and various fruit and vegetable crops.

The systematics of Grapholitini have received considerable attention in the past, including Heinrich's (1926) revision of the North American fauna, Obraztsov's (1959) review of the European fauna, and Danilevsky and Kuznetsov's (1968) review of the Russia fauna. More recently, Komai (1999) presented a contemporary review of the Palaearctic members of "*Grapholita* and allied genera" based on morphology. Razowski (2003) provided excellent colour illustrations of adults and line drawings of genitalia of the European species, along with brief synopses; Komai and Horak (2006) did the same for the Australian members of the tribe; and Razowski (2019) presented brief diagnoses and remarks on the genera of the tribe worldwide. Brown (2022) recently reviewed larval host plants, including species reared from fruit in disparate localities around the globe, including Kenya, Papua New Guinea, Thailand and Panama. Despite the economic significance of the group and the preponderance of morphological, distributional and host data, no comprehensive, worldwide, contemporary analysis of the phylogeny of the genera of Grapholitini has been conducted or a robust classification proposed.

The goals of this contribution are four-fold: (i) to define generic groups and expose paraphyly within Grapholitini based on molecular data, and propose a classification; (ii) to estimate divergence time within the tribe; (iii) to assess patterns of biogeographic distribution; and (iv) to examine evolutionary patterns of host usage within and among genera and generic groups.

Historical background

Grapholitini (as Grapholithidi) was first recognized by Guenée (1845) as one of eight higher groups in his broad concept of Tortricoidea. The category was adopted by virtually all subsequent workers (Stainton, 1859; Meyrick, 1895; Diakonoff, 1961; Clarke, 1963), with different authors treating the group at various taxonomic levels within Tortricidae. For example, whereas Meyrick (1895) assigned grapholitine genera to Eucosmidae, Heinrich (1926) and McDunnough (1946) treated the group as a subfamily (Laspeyresiinae). Some workers in the 1950s (Pastrana, 1950, 1952; da Costa Lima, 1952) considered the group to represent a distinct family (Grapholitidae), while others, such as Obraztsov (1950, 1959), Hannemann (1961) and Danilevsky and Kuznetsov (1968), treated the group as a tribe (Laspeyresiini) of Olethreutinae.

Razowski (1976) recognized that the tribal name "Laspeyresiini" is invalid because its type genus, *Laspeyresia* Hübner, is a homonym of *Laspeyresia* R.L., the latter an unjustified emendation of *Laspeyria* Germar. Hence, he proposed a new tribal name, derived from the oldest family group name, Grapholithidi of Guenée (1845), resulting in the tribal name "Grapholitini." Brown (1979) describes the situation in detail.

The phylogenetic position and taxonomic level of the group within Tortricidae were stabilized by Kuznetsov and Stekolnikov (1973, 1977), with Grapholitini recognized as sister to Eucosmini, within the supertribe Eucosmidii. Razowski (1976) followed the same basic arrangement, but considered Eucosmini as a tribe, treating Grapholitini as a subtribe (Grapholitina) within Eucosmini. Since the late 1970s, nearly all workers have treated the group as a tribe (Bradley et al., 1979; Powell, 1983; Diakonoff, 1986; Horak and Brown, 1991; Razowski, 1992, 2003; Horak, 1998). Contemporary molecular studies (Regier et al., 2012a; Fagua et al., 2017) further confirmed that the tribe is most likely sister to Eucosmini.

The first hypothesis of phylogenetic relationships within Grapholitini was presented by Heinrich (1926) for the North American fauna (Fig. 1), which included 16 genera at the time. Danilevsky and Kuznetsov (1968) were the next to propose a phylogeny of the group

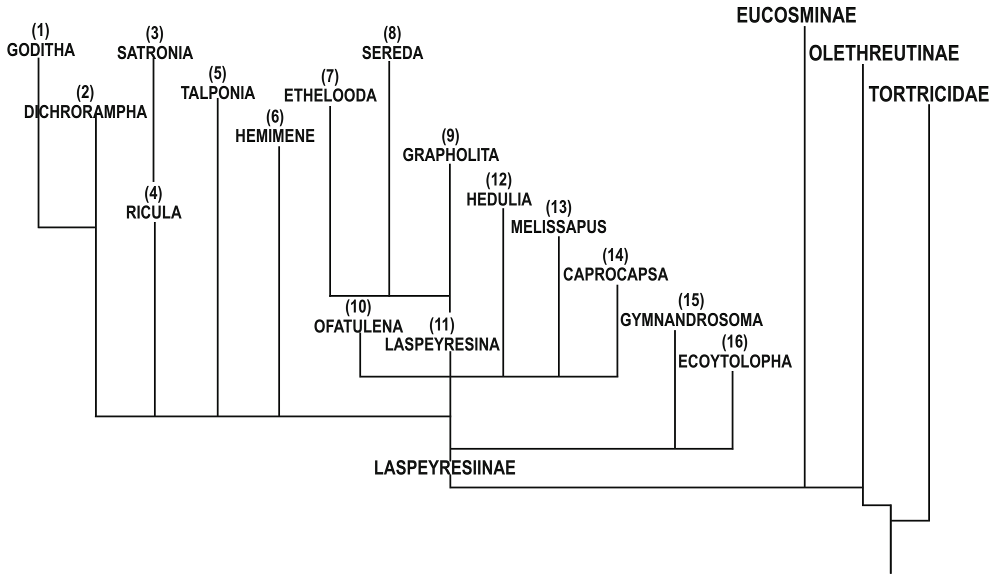

Fig. 1. Phylogenetic tree of the Laspeyresiinae from Heinrich (1926).

(Fig. 2), treating the 19 genera known to occur in Russia. The two faunas have exceedingly few genera in common, so together the two studies present a fragmented picture with few similarities of putative relationships. In a more thorough and contemporary analysis of Palaearctic Grapholitini, based on morphological characters, Komai (1999) recognized three lineages within the tribe—a *Dichrorampha* genus group, a *Cydia* genus group, and a *Grapholita* genus group—and proposed a phylogeny for the 11 genera included in the *Grapholita* group (Fig. 3). He also recognized species groups in the genus *Grapholita*, many of which were initially proposed by Danilevsky and Kuznetsov (1968). Komai and Horak (2006) followed the arrangement of Komai (1999) and added a fourth (most basal) lineage—the *Loranthacydia* group.

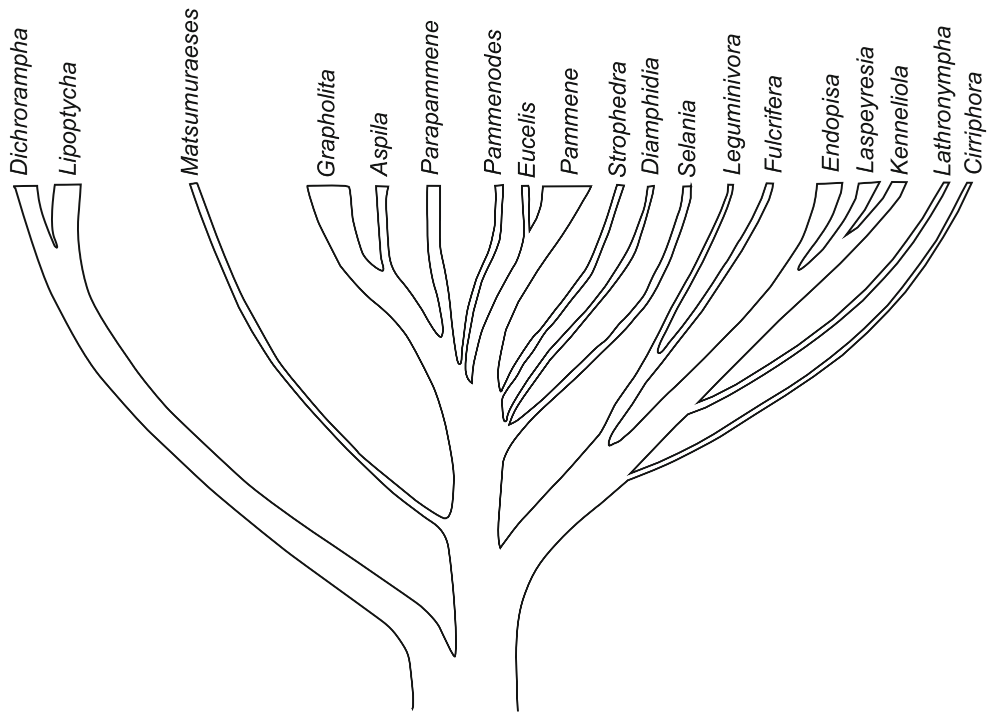

Fig. 2. Phylogeny of the tribe Grapholitini proposed from Danilevsky and Kuznetsov (1968).

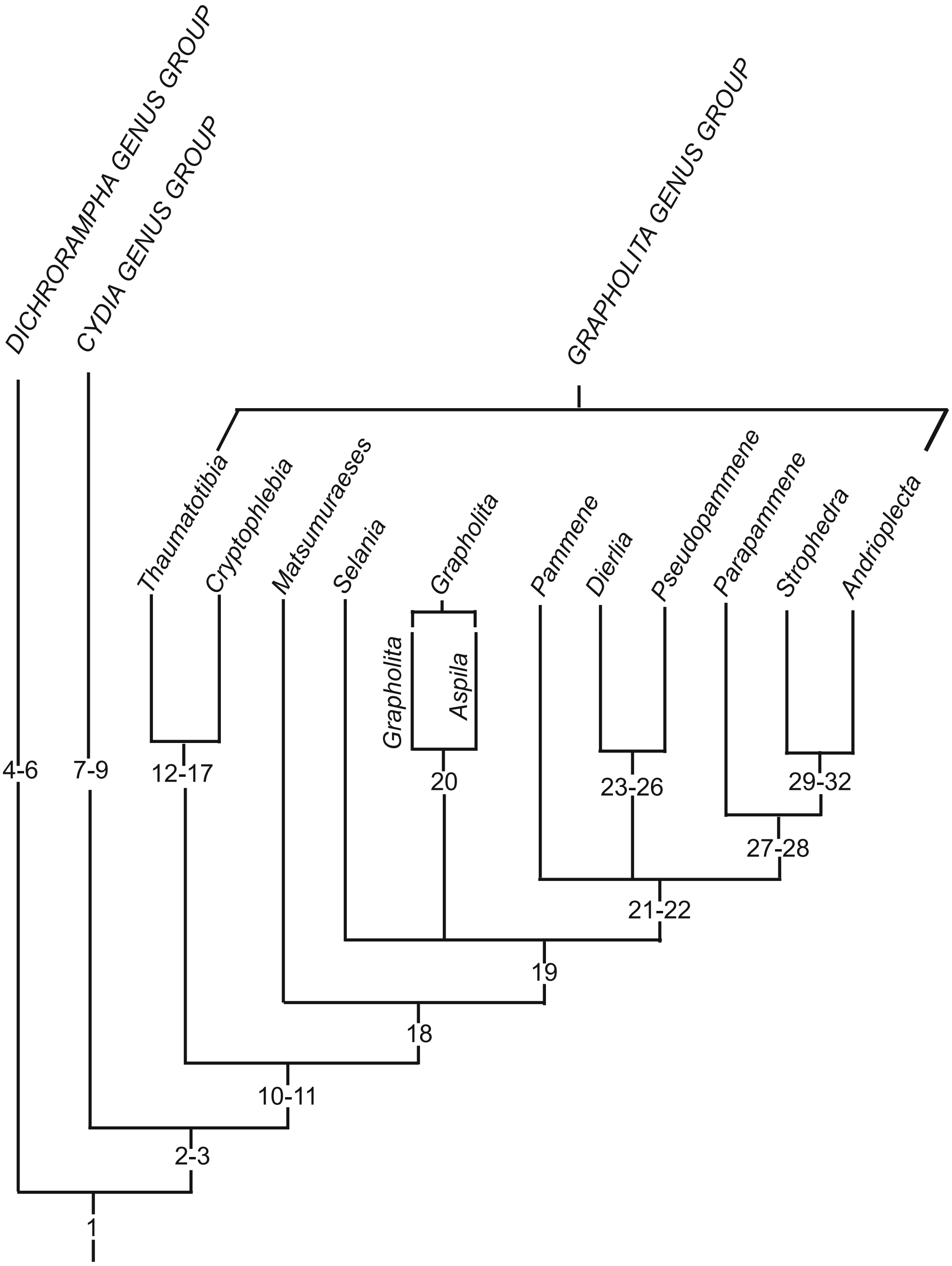

Fig. 3. Cladogram of the tribe Grapholitini in the Palaearctic region from Komai (1999).

The precise circumscription of Grapholitini has always been, and continues to be, somewhat elusive. Brown (1984) and Horak and Brown (1991) proposed that the tribe was para- or polyphyletic, composed of similarly derived members of other Olethreutinae tribes; i.e. it was merely an assemblage of genera in which the uncus and socii were secondarily and independently reduced and/or lost. Subsequently, Horak (2006) suggested that the relatively distant origin and parallel course of hindwing veins M_2 and M_3 and the loss of the uncus and socii in the male genitalia, recognized by Heinrich (1926), may be synapomorphies for the tribe. However, hindwing venation in the grapholitine genera *Acanthoclita*, *Thaumatotibia* and *Cryptophlebia* is considerably more similar to that of Eucosmini; the grapholitine genera *Talponia* and *Ricula* possess exceedingly long slender socii; and many species of Grapholitini possess at least a rudimentary uncus. Hence, none of these characters is consistent or uniform throughout the tribe.

Rota et al. (2009) found that the number of spines in the female frenulum was reduced from three to two in many Grapholitini, suggesting that "this character may be of phylogenetic significance, but an overall pattern is not immediately obvious". Komai (1999) had previously recognized this feature as a synapomorphy for *Andrioplecta* + *Strophedra*, but the character state is widespread in many species of *Grapholita*, *Pammene* and others. Although variable throughout the tribe, there is a preponderance of two-bristled frenula in all but the *Ecdytolopha* group and the *Cydia* group + *Lathronympha* group, where the three-bristled condition is the norm. Again, we find significant trends in the character, but with little uniformity.

Komai (1999) recognized the absence of a compelling synapomorphy for the tribe and proposed that Grapholitini might be defined by a feature of the male abdomen: sternum 8 short and the posterior margin nearly straight, or sternum 8 modified into an element of the hairpencil. In contrast, in all other Olethreutinae, sternum 8 is excavated along its posterior margin. However, like the previously mentioned characters, there is some variation in this rather qualitative feature.

Komai (1999) also proposed that the presence of paired hairpencils, which he referred to as "coremata", from segment seven of the male was a synapomorphy for the *Grapholita* group of genera. However, similar hairpencils occur throughout the tribe, being absent primarily from the *Ecdytolopha* group and the *Cydia* + *Lathronympha* group.

Given the mosaic distribution of potential synapomorphies, it is no wonder that Horak and Brown (1991) considered the tribe a para- or polyphyletic assemblage. However, it is possible that the size of the animal may have an effect on at least some of these features. For example, within Grapholitini, it appears that the distant origin and parallel course of M_2 and M_3 are strongly associated with smaller species. That is, most species of Grapholitini are comparatively small, whereas those of the *Ecdytolopha* group are typically larger animals and possess the Eucosmini condition of these two veins. This also may explain, in part, the occurrence of this "Grapholitini character" in two putative Eucosmini—*Corticivora* and *Larisa*, both of which are exceedingly small animals. However, there are many deviations from this general pattern.

A similar pattern is exhibited by the number of spines in the female frenulum—although an oversimplification, smaller species tend to have two bristles, whereas larger species tend to have three. Within the family, we find a parallel pattern in Cochylina (Brown et al., 2020), where *Phtheochroa* and several genera of the *Phtheochroa* group, which includes the larger species of the subtribe, have a three-bristled frenulum and the more advanced, smaller species (e.g. *Cochylis* group) usually have two (Pérez Santa-Rita et al., 2022). This character reaches its pinnacle in members of Ceracini, the largest species in the family, in which females may have four, five or more spines in the frenulum (Monsalve et al., 2011). Species of the *Ecdytolopha*, *Cydia* and *Lathronympha* groups are mostly larger animals and typically have three spines, whereas other Grapholitini have two. Again, there are many deviations from this general pattern, and "large" and "small" are highly qualitative and depend on the group in question.

Materials and methods

Taxon sampling

A total of 134 samples (i.e. 133 species) of Tortricidae were used in this study (Table S1). The ingroup consisted of 104 species (105 individuals) of Grapholitini, and the outgroup included 22 species of non-Grapholitini Olethreutinae and seven species of Tortricinae. Almost all voucher specimens are deposited in the Unit of Ecology and Genetics at the University of Oulu (Oulu, Finland) and the National Museum of Natural History, Smithsonian Institution, USA.

Molecular dataset

One mitochondrial and five nuclear genetic fragments were amplified (Table S2), resulting in a potential total of 4013 bp: cytochrome oxidase subunit I (*COI*) (658 bp), carbamoyl-phosphate synthetase II (*CAD*) (849 bp), elongation factor 1 alpha begin and end (*EF1α*-begin and -end) (1008 bp), glyceraldhyde-3-phosphate dehydrogenase (*GAPDH*) (691 bp), cytosolic malate dehydrogenase (*MDH*) (407 bp) and wingless (*WG*) (400 bp). Unfortunately, not all genes were available for all taxa; hence, the dataset includes a considerable amount of missing data. The number of basepairs per taxon ranged from 368 to 3966. Table S2 presents the genes and the number of basepairs available for each species. Dividing *EF-1α* into two non-overlapping fragments (a begin portion and an end portion) results in a dataset of seven gene fragments that allows for a more meaningful analyses of missing data. Table S2 shows that eight taxa are represented by the full complement of seven gene fragments; 47 taxa by six; 22 taxa by five; 18 taxa by four; 21 taxa by three; eight taxa by two; and 10 taxa by one, primarily *COI*.

DNA extraction, PCR amplification, sequencing and alignment

Total genomic DNA was extracted from legs of adult specimens following the protocol described in the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The primers of polymerase chain reaction (PCR) largely followed those of previous studies of Lepidoptera (Wahlberg and Wheat, 2008; Mutanen et al., 2010; Heikkilä et al., 2015; Brown et al., 2020). The PCR conditions were as follows: 12.5 μL dH_2O, 2.0 μL 10× buffer, 2.0 μL $MgCl_2$, 1 μL forward and reverse primer, 0.4 μL dNTP, 0.1 μL Taq DNA polymerase and 1 μL DNA template. PCR products were checked through 1.5% agarose electrophoretic runs. PCR products with bright target bands were cleaned up with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and Sephadex columns (Sigma-Aldrich, St Louis, MO, USA), and sequenced using an ABI 3730 DNA Analyser

(Applied Biosystems, Foster City, CA, USA). The PCR primer sets, annealing temperatures and product lengths are listed in Table S3.

Multiple sequence alignments were implemented using MUSCLE (Edgar, 2004) in the software MEGA v11.0.11 (Tamura et al., 2021). Sequences were manually edited using BioEdit v7.0.9.0 (Hall, 1999). *COI* barcode sequences, along with collection and taxonomic data, were uploaded to BOLD (http://v4.boldsystems.org/) (Ratnasingham and Hebert, 2007), and can be retrieved from the BOLD dataset DS-GRAPHOLI. The full genetic dataset was assembled from the database VoSeq (https://lepidoptera.lepdb.net/) (Peña and Malm, 2012). Detailed information, including geographical distribution, host plant data and GenBank accession numbers, are listed in Table S1.

Phylogenetic analyses

Phylogenetic analyses were performed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) based on the total dataset and on the 77-taxon dataset. The best scheme of partitions and substitution models for the combined dataset were selected using PartitionFinder v2.1.1 (Lanfear et al., 2017) with codon position as input. A greedy search and the Bayesian information criterion were applied with branch lengths linked across partitions. Nine partitions and their best substitution models were generated from PartitionFinder (Table S4) and used in the subsequent ML and BI reconstruction.

Maximum parsimony analysis was executed using the program TNT v1.5 (Goloboff and Catalano, 2016). A New Technology search was performed using the following search options: tree fuse, ratchet, random drift and sectorial search; find minimum length 100 times; initial addseq 35; random seed five. Characters were weighted equally. Bremer support values (BR) were obtained by finding trees suboptimal by 10 steps, then performing a traditional search with trees from RAM and calculating support values on the consensus tree.

Maximum likelihood reconstruction was implemented in the IQ-TREE v.2.1.2 web server (http://iqtree.cibiv. univie.ac.at/) using ultrafast bootstrap analysis (Trifinopoulos et al., 2016). Bootstrap support values (BS) were assessed with 1000 replicates.

Bayesian inference was conducted using MrBayes v3.2.6 (Ronquist and Huelsenbeck, 2003) through the CIPRES Science Gateway (Miller et al., 2010). Two independent runs were performed, each with four Markov chain Monte Carlo (MCMC) chains, with 50 million generations for the total dataset and 20 million generations for 77-taxa dataset, and sampled every 1000 generations. The sampling of posterior distribution was adequate when the average standard deviation of the split frequency approached 0.01 and the potential scale reduction factor approached 1.0. Convergence was assessed by plotting log-likelihood values vs. generation number in Tracer v1.6 (http://beast.bio.ed.ac.uk/software/tracer/). The first 25% of the samples were discarded as burn-in. The remaining trees were used to construct a majority rule consensus tree and estimate posterior probabilities (PP). FigTree 1.3.1 was used to visualize the tree (http://tree.bio.ed.ac.uk/software/figtree/).

In order to evaluate the effect of missing data on tree topology and stability, we conducted another analysis based on taxa represented by five or more gene fragments, i.e. 2395–3966 bp, which included 77 of the 134 taxa (Table S2). We then compared this 77-taxa tree with those derived from the total dataset. We also calculated maximum likelihood (ML) trees for each gene to briefly examine the contribution of each to the overall tree topology of the ML 134-taxon tree.

Based on simulation models using DNA sequences from members of the subtribe Polyommatina (Lepidoptera: Lycaenidae), Talavera et al. (2022) demonstrated that “when a strategic and representative selection of species for higher-level categories [e.g. representatives of genera and generic groups] has been made for multigene sequencing . . . DNA data for as few as 5–10% of the specimens in the total data set can produce high-quality phylogenies, comparable to those resulting from 100% multigene sampling.” They found that the combination of DNA barcodes and multilocus genes were capable of producing highly reliable phylogenies that included considerably larger taxon sampling. Hence, it is likely that our ML tree based on all taxa surveyed (134) is as robust as our 77-taxon tree (of five or more genes).

Divergence time estimation

A time-calibrated tree was inferred from the full genetic dataset for Grapholitini using BEAST v2.5.2 (Bouckaert et al., 2014) with the Yule branching process and an uncorrelated lognormal distribution clock model. The substitution models were unlinked, while the time-tree and the clock model were linked across partitions. Three independent MCMC chains were run for 200 million generations with sampling every 1000 generations. The resulting trees were resampled with a frequency of 10 000, and combined with LogCombiner 2.5.0 (BEAST package). The first 25% of samples were discarded as burn-in. The remaining subsampled trees were used to generate a maximum clade credibility tree using TreeAnnotator v2.5.0, with the annotation of mean height and 95% highest posterior density (HPD) intervals. BEAST analyses were implemented using CIPRES Science Gateway v3.3 (Miller et al., 2010). The convergence of the Bayesian runs was ensured by checking that the effective sample size value was higher than or nearly equal to 150 for all parameters in Tracer v1.7.1. The maximum clade credibility tree was visualized in FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

The molecular clock was calibrated using fossil records of Tortricidae with a uniform distribution. The current known fossil record of Tortricidae is extremely sparse; all available specimens are relatively young and can be identified reliably only to family level (Heikkilä et al., 2018). Heikkilä et al. (2018) concluded that the Baltic amber *Tortricites skalskii* Kozlov 1988 possesses labial palpi typical of Grapholitini (Olethreutinae), although in the absence of additional evidence, this placement is equivocal. The most recent common ancestor (MRCA) of Grapholitini was calibrated based on the fossil record of *Tortricites skalskii* from the Lutetian of the middle Eocene, 41.3–47.8 Myr. The upper limit of the uniform distribution at 41.3 Myr was defined for the minimum root age prior, while the lower limit was set by the estimated divergence time of 120 Myr for Tortricidae (Fagua et al., 2017, 2018).

Ancestral range reconstruction

The ancestral range of Grapholitini was inferred using the BioGeoBEARS package (Matzke, 2014) in the program RASP 4.2 build 20 211 014 (Yu et al., 2020). The time-calibrated tree from BEAST analysis was used as input of RASP, with the outgroups trimmed. The geographical distributions of Grapholitini species (Table S1) were obtained from the GBIF database (https://www.gbif.org/) and the Tortricidae website (http://www.tortricidae.com/). The samples were categorized into eight zoogeographic areas: (i) West Palaearctic; (ii) East Palaearctic; (iii) West Nearctic; (iv) East Nearctic; (v) Neotropical; (vi) Afrotropical; (vii) Oriental (including Hawaii); and (viii) Australia. The Ural Mountains were used to divide the Palaearctic, and the Mississippi River to divide the Nearctic.

Three different models were tested in BioGeoBEARS with or without the “jump dispersal” parameter J, i.e. DEC (+ J), DIVALIKE (+ J), and BAYAREALIKE (+ J). The parameter J weights founder-event speciation in the evolutionary estimation (Matzke, 2014). The most optimal model was selected using the lowest corrected Akaike information criterion. First, we performed an unconstrained (M0) test allowing all area combinations with equal probability of dispersal between them. Second, we ran a constrained (M1) analysis with a time-stratified palaeographic model taken into account according to Fagua et al. (2017). M1 analysis was performed considering changes in continental plate distribution for each of the following four time slices: 0–5.33, 5.33–23.03, 23.03–33.90 and 33.90–44.31 Myr. A connectivity matrix for each time interval was built to explain whether the aforementioned areas were connected to each other over time (Appendix S3). The maximum number of ancestral areas was limited to six.

Evolution of host plants

The program Mesquite version 3.70 (build 940) was used to reconstruct evolutionary history of host plant use using the "Trace character history" function (Maddison and Maddison, 2021). We selected the best ML tree from the total dataset as input, and pruned the outgroups and species lacking host information. Finally, a total of 93 ingroup species were used to evaluate the evolution of host plants using the MP reconstruction with unordered states.

The larval host plants and food ranges for Grapholitini were determined from records found primarily in the literature (http://www.tortricidae.com/foodplants.asp; Brown, 2022), and the host details are listed in Table S1. Among the records are a few possible misidentifications of larvae and/or hosts. Species without host plant data are denoted with a question mark. Each species was coded for host plant family (i.e. Asteraceae, Betulaceae, Fabaceae, Pinaceae, Rosaceae, etc.) and larval food range (i.e. monophagous, oligophagous and polyphagous). The categories of host range were distinguished as follows: monophagous, species that feed on plants within a single genus; oligophagous; species that feed on more than one genus in a single plant family; and polyphagous, species that feed on plants in more than one family without a conspicuous preference. A few species recorded from more than one plant family were categorized as oligophagous because the vast majority of rearings are from a single plant family. For example, during a survey in Thailand, Brown et al. (2019) reported rearing 149 specimens of *Cryptophlebia rhynchias*, all but nine of which (94%) were from Fabaceae (Brown, 2022).

Results and discussion

Phylogeny

Our three different methods (i.e. MP, ML, BI) of analysing the total sequence data resulted in trees with extremely similar topologies (Fig. 4, Figs. S1 and S2); however, minor deviations can be found in each. For example, in the MP tree, a member of the outgroup (i.e. *Spilonota eremitana*; Eucosmini) represented by two genes, is placed near the base of Clade I, within Grapholitini (Fig. S1). We applied different parameters in the MP analysis, and the results indicate that the phylogenetic position of *S. eremitana* is unstable. Evidence from morphology (Horak, 2006) and a previous molecular phylogenetic analysis (i.e. Regier et al., 2012a) both convincingly assign *Spilonota* to Eucosmini. Plus, in the two other trees (based on 134 taxa) *Spilonota* falls convincingly in the outgroup (Fig. 4, Fig. S2). Hence, the placement of *Spilonota* within Grapholitini is untenable. Of the three trees based on the total sequence dataset, the BI tree was slightly less resolved with several polytomies (Fig. S2), each of which involves a taxon represented by only one gene (e.g. *Dichrorampha banana*, *Multiquaestia purana*, *Andrioplecta* sp.). Of the three trees, the ML tree appears to conform best with morphological data and previous molecular phylogenetic hypotheses (Fig. 4).

The three trees (i.e. MP, BI, ML) based on taxa represented by five or more gene fragments (Figs S3–S5), i.e. our limited taxon sample (77 species with more complete gene sampling), have topologies that are extremely similar to each other and to the trees based on the larger taxon sample. However, support values for these trees are generally higher, particularly for the generic groups proposed below. In these trees, the monophyly of the proposed clades and generic groups, putative relationships among nearly all genera and the positions of taxa whose current generic assignments have been questioned, are consistent, with few exceptions, with trees based on the total dataset. Taxa represented by few (or even one) genes are invariably linked with congeners represented by more complete gene sampling. Hence, the placements of taxa with limited gene sampling in trees of the large taxon set (i.e. 134 taxa) are suspect only when they lack congeners represented by more complete gene sampling (e.g. *Leguminivora*, *Multiquaestia*). For example, although all three species of *Cryptophlebia* were represented by only *COI*, they link together as sister to *Pseudogalleria*, represented by four genes, the latter of which is almost certainly the senior synonym of the former as indicated by morphological similarity (Brown, 2022). To maximize the number of taxa included in our discussion below, we selected the ML tree (Fig. 4) as the basis for that discussion.

Based on our larger taxon dataset, the ML and BI results (Fig. 4, Fig. S2) provide support for a monophyletic Eucosmini (BS = 87, PP = 0.64) and its position as sister to Grapholitini (BS = 98, PP = 0.92), consistent with virtually all previous studies based on morphological and/or molecular data. However, the monophyly of Grapholitini is achieved only if *Corticivora* and *Larisa* are excluded from the tribe. Both possess many grapholitine features but are aberrant in others. Plus, their assignments to the tribe have historically been somewhat provisional.

When Clarke (1951) described *Corticivora*, he compared it with *Gypsonoma* (Eucosmini), perhaps the first indication of the enigmatic placement of the genus. Although Clarke (1951) considered the genus to be structurally similar to *Gypsonoma*, he indicated that it is "clearly laspeyresiine" and "most nearly related to *Laspeyresia*" (now known as *Cydia*). Clarke (1951) mentioned that hindwing veins M_2 and M_3 of

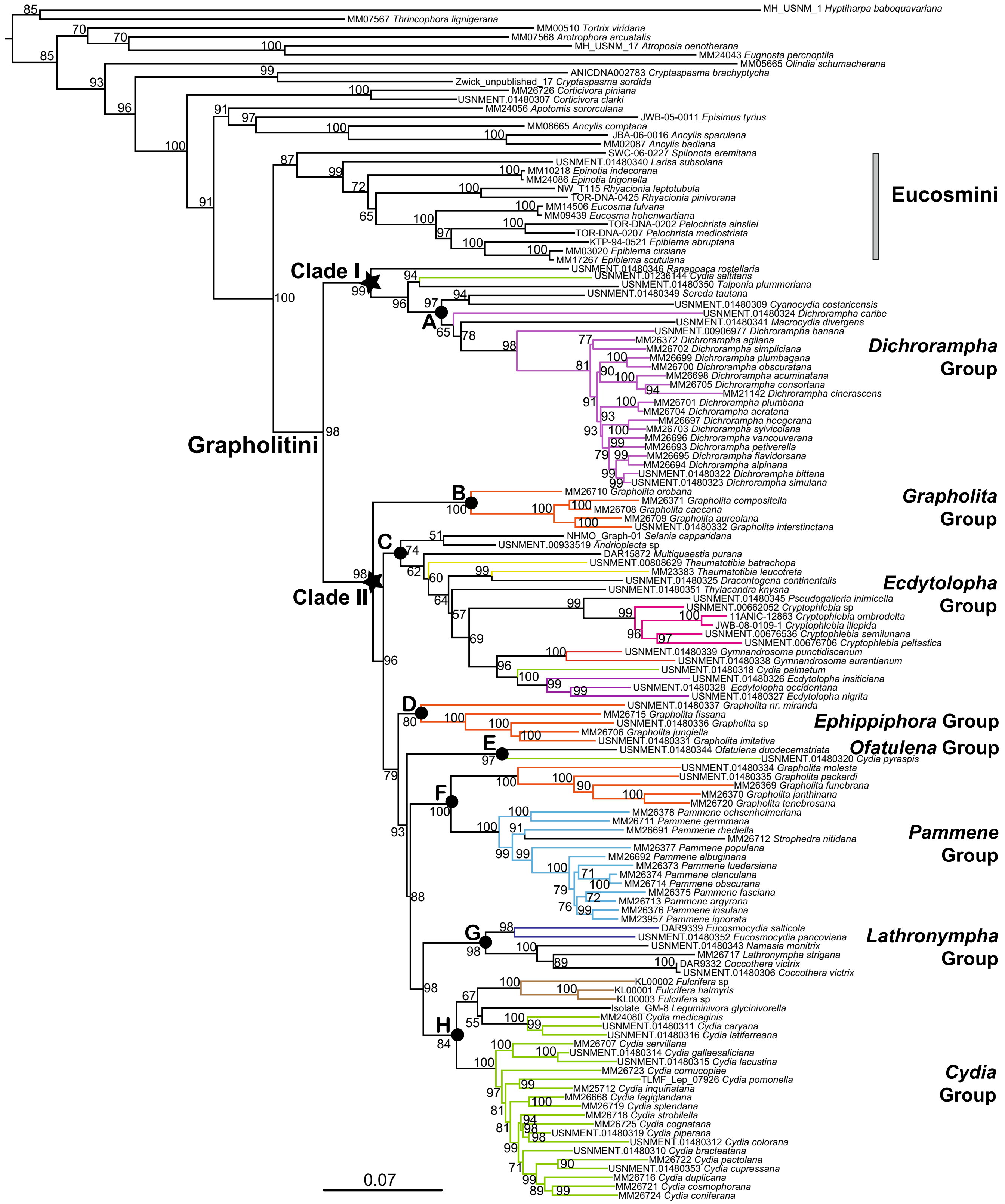

Fig. 4. Phylogenetic tree of Grapholitini from maximum likelihood analysis based on the total dataset of 134 taxon samples. The tree was conducted under nine partitions and best substitution models from PartitionFinder. Numbers at nodes represent bootstrap support values (BS) from 1000 ultrafast bootstrap analysis with IQ-Tree. Species from the same genus are represented by branches of the same colour, while outgroups and the other genera with only one species are in black. The letters a–h correspond to the generic groups on the right-most side.

Corticivora are well separated basally, as in other Grapholitini. He differentiated *Corticivora* from *Cydia* by the presence of socii (lacking in *Cydia*), veins R_s and M_1 stalked in the hindwing (separate in *Cydia*) and the signa of the female genitalia scobinate-dentate, the latter of which are typically paired pointed thorns or horns in nearly all Grapholitini. The single European species currently placed in *Corticivora* was long treated as *Rhyacionia piniana* (Eucosmini), further suggesting its similarity to Eucosmini. The larvae of *Corticivora* feed in the bark of Pinaceae, and although this is a highly unusual behaviour for a member of Grapholitini, a similar larval niche is known for several species of *Cydia* (Brown, 2022). The combined molecular, morphological and biological data provide evidence that *Corticivora* belongs outside of Grapholitini. Although it appears to fit best in Eucosmini, based on the molecular data, its tribal assignment remains uncertain.

In Miller's (1978) description of *Larisa*, he stated "*Larisa* keys to Laspeyresiinae or Eucosminae (Heinrich, 1923; Obraztsov, 1959) depending on character variability and interpretation. It is an intermediate genus but is tentatively placed in Laspeyresiinae". The male genitalia of *Larisa* (Miller, 1978: fig. 3) differ from those of most other Grapholitini in the presence of a well-developed uncus and a dense patch of setae from the sacculus. Although it shares long, slender socii with *Talponia* and *Ricula* (Grapholitini), this feature also occurs elsewhere in the subfamily Olethreutinae. Based on morphological features of the male genitalia and its position in the molecular tree, we transfer *Larisa* to Eucosmini. The exclusion of *Corticivora* and *Larisa* results in a monophyletic Grapholitini that is sister to Eucosmini.

Our analyses reveal two major lineages within Grapholitini—a *Dichrorampha* clade (Clade I) and a *Cydia* clade (Clade II). The *Cydia* clade can be divided into seven generic groups. Although Horak and Brown (1991) initially stated that "The tribe has been divided into two artificial subtribes, one including *Dichrorampha* and the other including *Grapholita*, *Cydia*, *Pammene*, and related genera", our molecular data appear to recognize these two major clades as natural groups. Furthermore, Witzgall et al. (1996) indicate that the division of the tribe into the two clades, "Dichroramphae" (including *Dichrorampha*) and "Laspeyresiae" (including *Cydia*, *Grapholita*, *Pammene*, *Lathronympha*, and *Strophedra*), as proposed by Danilevsky and Kuznetsov (1968), is well supported by the chemical structure of pheromones. Based on the Palaearctic fauna, Razowski (2003) likewise recognized two subtribes within Grapholitini, referring to them as Lipoptychina (including *Dichrorampha*) and Grapholitina (including the remaining genera). Hence, there appears to be considerable support for this two-clade arrangement.

Clade I is represented by a single genus group (i.e. the *Dichrorampha* group—"Dichroramphae" *sensu* Danilevsky and Kuznetsov, 1968, and "Lipoptychina" *sensu* Razowski, 2003) that comprises *Dichrorampha*, *Sereda*, *Cyanocydia*, "*Dichrorampha*" *caribe* (which requires a new genus), *Macrocydia*, "*Cydia*" *saltitans* (which may belong in *Ethelgoda*), *Talponia* and *Ranapoaca*. *Dichrorampha* is primarily Holarctic, and the other genera are primarily Neotropical, reaching their northern limits in southern North America (Razowski, 2011).

Node A, which includes *Dichrorampha*, *Sereda*, *Cyanocydia*, "*Dichrorampha*" *caribe* and *Macrocydia*, is supported by the loss of one of the two signa in the female genitalia; however, *Sereda* is the exception, retaining two. At least four genera not included in our analyses (i.e. *Ricula*, *Riculorampha*, *Phloerampha* and *Goditha*) share this morphological feature (i.e. the loss of a signum) and possess a forewing pattern element that is shared with *Dichrorampha*—a row of tiny dots along the termen. Hence, they probably belong in this clade as well, as hypothesized by Heinrich (1926) (Fig. 1). Although *Satronia* has two signa and lacks the forewing termen dots, its male genitalia bear a strong resemblance to those of *Ricula*, and it probably belongs here as well. In his review of Neotropical Grapholitini, Razowski (2011) also included *Balbis* in his *Dichrorampha* group, along with all of the genera mentioned above. *Ethelgoda* Heinrich (not included in our analyses) was considered monotypic for over a century until Razowski (2011) and Razowski and Becker (2012) added five new species. Based on morphology of the male genitalia, "*Cydia*" *saltitans*, along with "*C.*" *motrix* (Berg), may belong in *Ethelgoda*. This action (not proposed herein) would result in a primarily Neotropical genus in which three of the included species feed exclusively on Euphorbiaceae; hosts are unknown for the remaining species.

Within Clade I, two genera, *Talponia* and *Ricula* (the latter not included in our analyses), share similar facies and several features of the male genitalia, including the presence of extremely long slender socii. The latter feature apparently has evolved multiple times within the subfamily (e.g. *Larisa*, *Sycacantha*). Also, within Clade I, we find the only occurrence of a male costal fold in all of Grapholitini, with the structure present in several species of *Dichrorampha* (e.g. *D. bittana*, *D. simulana*, *D. azteca*, *D. odorata*) and *Ranapoaca*, and absent in other congeners (e.g. *D. radicolana*, *D. banana*, *D. piperana*, *D. sedatana*). A male costal fold is widespread among members of the sister tribe Eucosmini.

Whereas host plants are rather diverse within Clade I, including Annonaceae (for *Talponia*), Euphorbiaceae (for *Ethelgoda*), Sapotaceae (for "*Dichrorampha*" *caribe*), Pinaceae (for *Satronia*), Fagaceae (for *Sereda*) and Lauraceae (for *Riculorampha*), with few exceptions, species of *Dichrorampha* appear to feed on Asteraceae (Horak and Brown, 1991; Brown, 2022). Two species, *D. odorata* Brown and Zachariades and *D. aeratana* (Pierce and Metcalfe), have been identified as potential biocontrol agents against weedy Asteraceae, and no species of *Dichrorampha* is considered a pest of cultivated plants.

Clade II is represented by seven generic groups: a *Grapholita* group (B), an *Ecdytolopha* group (C), an *Ephippiphora* group (D), an *Ofatulena* group (E), a *Pammene* group (F), a *Cydia* group (G) and a *Lathronympha* group (H). Based on the Palaearctic fauna, Razowski (2003) recognized this clade as "Grapholitina."

The *Grapholita* group (Clade II-B) is composed nearly entirely of species that feed on Fabaceae: *G. compositella*, *G. caecana*, *G. aureolana*, *G. interstinctana* and *G. orobana*. Other Fabaceae-feeding *Grapholita* with forewing patterns extremely similar to the aforementioned are *G. conversana*, *G. coronillana*, *G. fana*, *G. lunulana* (the type species of *Grapholita*), *G. tristrigulana* and several others, and these are all suspected to belong to this exclusively Holarctic clade. This group of species was treated as the subgenus *Euspila* by Obraztsov (1959: 207) and as *Grapholita* (sensu stricto) section *compositellae* by Danilevsky and Kuznetsov (1968: 354). Komai (1999) noted that members of the *compositella* group are characterized superficially by a well-defined forewing dorsal blotch (although absent in *G. caecana*), the speculum (= ocellar patch) indicated by a single vertical leaden line, and the costa of the forewing with six (sometimes paired) costal strigulae. The valvae of the male genitalia usually lack a scale patch on the outer surface, and the male hairpencils are represented by a pair of tufts of filiform scales. The larvae, which lack an anal fork, are Fabaceae-feeders, except for *G. delineana*, which is a gall inducer on Cannabaceae, and a few other deviations (Brown, 2022).

The *Ecdytolopha* group (Clade II-C) was originally defined by Adamski and Brown (2001) to include *Ecdytolopha*, *Gymnandrosoma*, *Pseudogalleria*, *Thaumatotibia* and *Cryptophlebia*, which are at the base of Komai's (1999) "*Grapholita* genus group". Most members are large species, many with a distinct pretornal patch on the forewing, a female frenulum with three spines (Rota et al., 2009), the accessory cell of the forewing small or absent and a short discal cell in the male hindwing (Komai, 1999); the larvae usually lacking an anal fork, and the L pinaculum on T1 elongate, extending below the spiracle (Adamski and Brown, 2001). Members of the group are distributed nearly worldwide, occurring on every continent except Antarctica. Based on our molecular analyses, we add to the *Ecdytolopha* group *Thylacandra* (and its synonym *Celsumaria*, new synonymy), *Dracontogena*, *Multiquaestia*, *Selania* and *Andrioplecta*. *Thylacandra*, *Multiquaestia* and *Dracontogena* appear to fit exceedingly well into the group; *Thylacandra* and the *niphodonta* species group of *Dracontogena* share an usual orbicular sclerotized process near the base of the valva in the male genitalia that may be a synapomorphy for the two (Brown and Timm, 2017), and male genitalia in both genera have the typical "inflated" valva, characteristic of *Cryptophlebia*. However, *Selania* and *Andrioplecta* appear somewhat out of place here. Komai (1999) placed *Andrioplecta* as sister to *Strophedra*, the latter of which sits within *Pammene* in our analyses. Hence, the position of *Selania* and *Andrioplecta* is not particularly compelling in the total taxon ML tree (Fig. 4). In the total taxon MP tree (Fig. S1), the two genera form a polytomy with *Grapholita* near *miranda*, outside the *Ecdytolopha* group, and this position seems more compelling.

Foodplants are variable within the group, but there is a preference for Fabaceae and a tendency for oligophagy and/or polyphagy (Brown, 2022). The group includes many notorious pest species, mostly in the genera *Cryptophlebia* and *Thaumatotibia*. Hosts of *Selania* and *Andrioplecta* are among the most divergent in the tribe, with the larvae of *Selania* feeding mostly on Brassicales and those of *Andrioplecta* mostly on Dipterocarpaceae, but with a couple of species on Fabaceae and Fagaceae, and a small group of entomophagous species (Komai, 1999; Brown, 2022).

The female genitalia of *Selania* have a short, broad ductus bursae with a sclerotized ring (colliculum) in the anterior end, and the corpus bursae has an accessory bursa (probably homologous with a bulla seminalis) (Komai, 1999). The presence of a sclerotized ring is shared with *Coniostola* and *Age*, which are both Fabaceae feeders (Agassiz and Aarvik, 2014). These three genera appear to form a monophyletic group. One of the traits defining *Andrioplecta* is a "corpus bursae with a large bulla seminalis directly originating from its posteroventral wall" (Komai, 1999). From the ML tree it appears that the large bulla seminalis of *Andrioplecta* may be homologous with the accessory bursa of *Selania*. However, a similar configuration of the corpus bursae and bulla seminalis is also present in some species of *Pammene* (Komai, 1999) of clade II-F.

The *Ephippiphora* group (Clade II-D) is another assemblage of Holarctic, Fabaceae-feeding *Grapholita*, plus members of the Afrotropical *G. mesoscia* complex, whose species all feed on Ochnaceae (Brown et al., 2014). The group also probably includes *Commoneria* Komai and Horak, with species similar to those of the *G. mesoscia* complex on the basis of fascies and male genital morphology. The group includes species assigned to two different groups by Danilevsky and Kuznetsov (1968) and referred to as *Grapholita* (sensu stricto) section *jungiellae* and *Grapholita* (sensu stricto) section *fissanae*, or the *jungiella* group and the *fissana* group of Komai (1999). Komai (1999) noted that the forewing has a dorsal blotch composed of four weak whitish lines (in *G. pavonana*) or two weak whitish lines (in *G. jungiella*, *G. fimana*), or lacks lines altogether (in *G. gemmiferana*, *G. larseni*, *G. semifusca*, *G. nebritana* and *G. lathyrana*), and that in the male genitalia, the outer surface of the valvae bears longitudinal wrinkles and a patch of scale tufts. The larvae, which possess an anal fork, feed in spun leaves, buds or seed pods of Fabaceae. We can hypothesize that the North American "*Grapholita* sp.", an undescribed species from Arizona included in our analysis, will be found to feed on Fabaceae. According to Witzgall et al. (1996), the three members of this group for which data are available are attracted to pheromones based on single compounds and binary blends of Δ8, Δ10–12Ac, which is different from that of other *Grapholita* (*sensu lato*).

The *Ofatulena* group (Clade II-E) includes two genera: *Ofatulena* and an undescribed genus for the "*Cydia*" *pyraspis* group of species. The group is restricted to Central America and southern North America, and all recorded hosts are in Fabaceae.

The *Pammene* group (Clade II-F) is a strongly supported (BR = 1, BS = 100, PP = 0.97) Holarctic clade that includes *Pammene*, *Strophedra* and the Rosaceae-feeding members of *Grapholita* (Fig. 4, Figs. S1 and S2), for which the genus name *Aspila* (type species: *janthinana*), formerly considered a subgenus, is available. According to Witzgall et al. (1996), all species of *Aspila* ($n = 3$), *Pammene* ($n = 7$) and *Strophedra* ($n = 1$) that they evaluated are attracted to pheromones based on single compounds and binary blends of Δ8–12Ac and Δ10–12Ac, which is different from that of all other Grapholitini, except for a group of Pinaceae-feeding *Cydia*.

Rosaceae-feeding *Grapholita* were assigned to two closely related groups by Danilevsky and Kuznetsov (1968) (i.e. *Grapholita* (*Aspila*) section *molesta* and *Grapholita* (*Aspila*) section *funebranae*) and Komai (1999) followed this arrangement with his *molesta* group and *funebrana* group. Komai (1999) recognized 17 described species as belonging to *Aspila* (see Appendix S4): 11 from the Palaearctic, three from the Oriental region and three from the Nearctic. Five of these species form a monophyletic group in our multi-gene tree; six others are associated with these species by DNA barcodes; and six additional species were included based on morphology. For 13 of the 17 species, Rosaceae are the dominant or only hosts; Ericaceae serve as the host for one (i.e. *Grapholita libertina*) and hosts are unknown for the remaining three, but are assumed to be Rosaceae. The only other species of *Grapholita* recorded from Rosaceae is the North American *G. angleseana*, and its repeated host record (Miller, 1987; Brown, 2022) originates from a 120-year-old rearing note from Fernald: "feeds on strawberry" cited by Heinrich (1926) (USNM). However, five specimens in the USNM collection were reared from *Amphicarpaea* [probably *bracteata*] (Fabaceae), and because NJ trees (based on DNA barcodes) place *G. angleseana* near Fabaceae-feeding *Grapholita*, the old Fernald record may be in error, with Fabaceae the true host of *G. angleseana*.

According to Brown (2022), "Larval hosts have been reported for 44 species [of *Pammene*], and encompass a wide range of plant families, the most common of which are Fagaceae (supporting 12 species), Rosaceae (supporting six species), Pinaceae (supporting four species), and Cupressaceae (supporting four species). Seven *Quercus*-feeding species are also recorded from cynipid galls on their Fagaceae hosts." Hence, although there may be patterns within the genus, there is no over-arching host preference for the entire genus.

Strophedra (represented by four genes) is embedded within the genus *Pammene* in the three trees based on the larger taxon sample, suggesting that is a synonym of the latter. However, further taxon and gene sampling are necessary to confirm this synonymy. Based on the morphological phylogeny of Komai (1999), it is likely that the *Pammene* group also includes the Oriental genera *Dierlia*, *Pseudopammene* (on Fagaceae), *Parapammene* (on Fagaceae, Sapindaceae and Tiliaceae) and *Matsumuraeses* (based on barcodes) (on Fabaceae). Komai (1999) indicated that the "Male hindwing with a narrow androconial field consisting of modified scales along Sc + R1 and Rs on the upper surface" represents a synapomorphy for this group of genera (excluding *Aspila* and *Matsumuraeses*).

The *Lathronympha* group (Clade II-G) includes four genera (*Coccothera*, *Lathronympha*, *Namasia* and *Eucosmocydia*). The group appears to be primarily Afrotropical with a handful of species from the Palaearctic (i.e. *Lathronympha*). The monophyly of the group is well supported (BR = 1, BS = 98, PP = 0.96; Fig. 4, Figs. S1 and S2), and it is sister to the *Cydia* group. Although not included in our analysis, *Neonamasia* (almost certainly the sister of *Namasia*) and a species group of *Grapholita* that includes *G. chytranthusi* Razowski, *G. taocosma* (Meyrick) and two apparently undescribed species from Kenya (Brown, 2022), also belong in the *Lathronympha* group. Hosts are highly variable from one genus to the next, with *Lathronympha* on Clusiaceae, *Eucosmocydia* primarily on Sapindaceae (but with two species recorded from Fabaceae; Agassiz and Aarvik, 2014), *Namasia* and *Neonamasia* on Anacardiaceae, and *Coccothera* primarily on Fabaceae (Brown, 2022). Owing to the similarity of its genitalia to those of *Namasia*, it appears that the Afrotropical *Camptrodoxa* Meyrick also belongs here. *Camptrodoxa sorindeiae* (Razowski and Brown) was bred from Anacardiaceae and Loganiaceae (Razowski and Brown, 2012). The

Lathronympha group and *Cydia* group combined are roughly equivalent to Komai's (1999) *Cydia* genus group.

The *Cydia* group (Clade II-H) is another well-supported lineage (BR = 1, BS = 84, PP = 0.72; Fig. 4, Figs. S1 and S2) that comprises Holarctic species of *Cydia*, Afrotropical species of *Fulcrifera* (although the latter includes several Palaearctic species not included in our analysis) and *Leguminivora* (from Asia, Australia, and Africa). It is likely that *Notocydia* and *Apocydia* (both from Australia) also belong to this group. Within the *Cydia* group, species form three lineages: (i) a well-supported *Cydia* lineage; (ii) a well-supported *Fulcrifera* lineage; and (iii) a lineage that includes three species of *Cydia* and one of *Leguminivora*. Because *Leguminivora* is represented by only *COI*, its position in the tree is not particularly compelling. Nonetheless, even if it is removed, *Cydia* still appears to be paraphyletic in relation to *Fulcrifera*, but these relationships are not well supported in our tree. *Fulcrifera* and *Leguminivora* are both Fabaceae feeders, as is a group of *Cydia* species, placed by Danilevsky and Kuznetsov (1968) (as "*Laspeyresia*") in the sections *nigricanae* and *succedanae*. Members of the latter group possess an anteriorly directed process on the phallus. The position and size of this process varies among species, and in some cases resembles the typical "fulcrum" that characterizes *Fulcrifera*. A similar process is also present in the Nearctic *C. caryana*. This suggests that the phallic process in species of *Cydia* may be homologous with the fulcrum in *Fulcrifera*, providing a morphological feature in support of the *Cydia* group (Clade II-H), although not consistent throughout the group.

Within the larger lineage of *Cydia*, host plants are rather diverse; however, embedded within the genus is a large radiation of species on conifers (Pinaceae and Cupressaceae), a smaller group of species on Fagaceae (*C. fagiglandana* and *C. splendana*) and a group of species on Betulaceae and Salicaceae (*C. cornucopiae*, *C. servillana*, *C. gallaesaliciana* and *C. lacustina*—host unknown). The bark-mining species *C. coniferana* and *C. cosmophorana* are attracted to pheromones based on E8- and E10-12Ac (Witzgall et al., 1996), suggesting a close phylogenetic relationship of the two. As mentioned above, the *Cydia* group also encompasses a lineage of Fabaceae feeders, including *Fulcrifera* and *Leguminivora*. Like the *Ecdytolopha* group, there are many pest species in the *Cydia* group—coincidentally, the two groups also share a three-spined female frenulum.

The fate of Grapholita

Komai (1999) stated "The monophyly of the genus [*Grapholita*], however, has not been well established. Diagnostic characters which previous authors have used to unite them (e.g. the presence of coremata) are plesiomorphic". Horak (2006) echoed a similar opinion, indicating that "The genus *Grapholita* comprises at least two possible monophyletic groups, the subgenera *Grapholita* with the majority of Australian species and *Aspila* Stephens that includes *Grapholita* (*Aspila*) *molesta*". The first convincing evidence of the para- or polyphyletic nature of the genus came from Regier et al. (2012a). Six representatives of the tribe Grapholitini were included by Regier et al. (2012a) in their backbone phylogeny of Tortricidae, and the two species of *Grapholita* they used did not form a monophyletic group. Fagua et al. (2018) recovered a similar anomaly for the same two species of *Grapholita* in their phylogeny. Hence, molecular studies finally confirmed the suspicions of previous workers based on morphology, pheromones and food plants.

Our molecular studies revealed that members of the genus *Grapholita* are found in three separate clades (II-B, II-D and II-F; Appendix S4). Komai (1999) and Komai and Horak (2006) both recognized two subgenera within the genus, i.e. *Grapholita* s. str. and the subgenus *Aspila*. The latter is undoubtedly monophyletic, and in our phylogeny it falls within the *Pammene* group. It is a Rosaceae-feeding lineage found primarily in the Holarctic. Komai (1999) suggested that *Aspila* is supported by the following characters: (i) a ductus bursae with a polygonal, ovate, or conical, strongly sclerotized concave sclerite; (ii) a phallus connecting dorso-anteriorly with the bulbus ejaculatoris; (iii) a stout valva that is pincer-shaped in lateral view; and (iv) reduced hairpencils.

Following Danilevsky and Kuznetsov (1968), Komai (1999) divided the subgenus *Grapholita* into eight species groups, of which the *fissana* group, the *compositella* group, the *lunulana* group and the *jungiella* group are represented in our sequenced specimens. The *Grapholita* group (II-B) in our phylogeny comprises Komai's *compositella* group and *lunulana* group. The type species of *Grapholita* Treitschke, 1829 is *G. lunulana*, and thus the genus name *Grapholita* should be restricted to this group. The single member of the *discretana* group, the Palaearctic *G. discretana* (not included in our analyses), which feeds in the stems of *Humulus* (Cannabaceae), is a phylogenetically isolated species, but probably falls within the *Grapholita* group as well. One member of Komai's *compositella* group also feeds on Cannabaceae, i.e. *G. delineana*, and the similar biology of the two suggests a closer relationship than previously realized.

For the *Ephippiphora* group (II-D), discussed above, the name *Ephippiphora* Duponchel, 1834 (type species: *G. jungiella*) is available. This group is represented by four species in our analyses, including members of both the *jungiella* group and the *fissana* group. We also place in *Ephippiphora* members of the *jesonica* group, which share with the *jungiella* group a mandible with a distinct transverse ridge on the inner surface (Komai, 1999). Several members of the latter species group share a similar white hindwing, including the Asian *E. jesonica* and *E. dilectabilis* and the North American *E. eclipsana*.

The following genus groups mentioned by Komai (1999) are not represented in our study: the *hyalitis* group, the *discretana* group (included in *Grapholita* senso stricto above), the *scintillana* group and the *jesonica* group. Based on larval and adult characters mentioned by Komai (1999), the *scintillana* group and the *jesonica* group have much in common with the *jungiella* group and most likely also belong in the *Ephippiphora* group.

Based on forewing maculation, Fabaceae-feeding larvae and the presence of a sclerotized ring bearing minute thorns in the basal area of the ductus bursae, Harrison et al. (2014) recognized seven North American species as belonging to Komai's *jungiella* group, the latter of which was based solely on the Palearctic fauna. Harrison et al. (2014) added the following North American species to the group: *G. orbexilana* Harrison, *G. eclipsana*, *G. lunatana* (Walsingham), *G. conversana* (Walsingham), *G. vitrana* (Walsingham), *G. caeruleana* (Walsingham) and *G. imitativa* (Heinrich). The assignment of these species to *Ephippiphora* is highly consistent with the results of our molecular analyses.

Although the division of *Grapholita* into three genera provides a more stable classification for well-known species, there are many species whose assignment to one of the three remains unknown. Although it is likely that many of these belong to one of the three genera, others almost certainly belong in other genera and some probably require the description of new genera. In Appendix S4 we list all species currently recognized as members of our three newly circumscribed genera, along with a list of those species that require additional scrutiny for confident generic assignment.

The fate of Cydia

Like *Grapholita*, the genus *Cydia* has long been the repository for a wide array of Grapholitini species that do not fit convincingly elsewhere; hence, it is almost certainly polyphyletic. However, to our surprise the vast majority of *Cydia* species included in our analyses fall into the *Cydia* group (Clade H). Nonetheless, the closely related genera *Leguminivora* and *Fulcrifera* also fall within the group, creating a paraphyletic *Cydia*. Because our sampling of *Cydia* species was not as thorough as that for *Grapholita*, we refrain from proposing changes in generic compositions or assigning the species to two or more "new" genera. However, it is clear that a species group that includes *saltitans* and *motrix* and another that includes *ninana*, *rhodaspis* and *pyraspis*, fall well outside the *Cydia* group and almost certainly require new genera. Also, *Cydia palmetum* is almost certainly a member of the *Ecdytolopha* group. Other *Cydia* species that probably belong elsewhere include *C. connara* (an Afrotropical species that feeds on Connaraceae), which in *COI* trees links convincingly with the superficially similar *Eriosocia guttifera* (a Neotropical species that feeds on Clusiaceae) and *Thylacogaster garcinivora* (an Afrotropical species that also feeds on Clusiaceae).

A brief comparison of gene trees

Here we provide brief qualitative assessments of the six individual gene trees in comparison with the ML 134-taxon tree, identifying their contributions to that tree. In particular, we focus on the ability of each gene to recover the monophyly of Grapholitini and the two major clades within the tribe, exclude the two genera *Corticivora* and *Larisa*, and recover generic groups.

COI ($n = 131$ taxa) (Fig. S6). *COI* is unable to recover the monophyly of Grapholitini or the two major clades. Although it successfully excludes *Corticivora* from the tribe, *Larisa* is placed within Grapholitini. In genera for which we included numerous congeners (e.g. *Dichrorampha*, *Cydia*, *Pammene*, *Grapholita*), *COI* successfully links most of them with each other, leaving outliers of questionable generic assignment (e.g. especially in the genera *Grapholita* and *Cydia*). However, many species that lack congeners occupy positions that seem incongruous with their positions in the ML 134-taxon tree. *COI* also successfully clusters a few genera previously recognized as genus groups (i.e. four of six genera in the *Ecdytolopha* group, and the *Pammene* group to include *Strophedra*), but many of the relationships among genera outside these groups are untenable, often in conflict with morphology, foodplants and pheromones. One of the most conspicuous areas of discordance between the COI tree and our ML 134-taxon tree is found in the *Ecdytolopha* group, where three outgroup taxa, *Atroposia* (Cochylini), *Tortrix* (Tortricini) and *Arotrophora arcuatalis* ("Arotrophorini"), are embedded within the genus group, and two other genera that belong in the genus group (i.e. *Pseudogalleria* and *Cryptophlebia*) form a clade quite distant from the *Ecdytolopha* group. Two other outgroup genera, *Apotomis* (Olethreutini) and *Hyptiharpa* (Cochylini), also fall within Grapholitini in the *COI* tree. Hence, as expected, the major contribution of *COI* is at the terminal branches, bringing together closely related species, primarily congeners.

CAD ($n = 78$ taxa) (Fig. S7). *CAD* likewise is unable to recover the monophyly of Grapholitini, and although it recognizes two major clades within the tribe, one of the clades includes the six outgroup genera of the sister tribe Eucosmini. This gene successfully excludes *Corticivora* from the tribe, but *CAD* was not sequenced for *Larisa*. Like the *COI* tree, the *CAD* tree consistently links the majority of congeners, in particular those in the genera identified above for the *COI* tree. With the exception of the single lineage that includes all members of Eucomsini, *CAD* successfully eliminates all other outgroup genera that are scattered throughout Grapholitini in the *COI* tree. It also provides support for several of the genus groups (e.g. *Pammene* group, *Cydia* group, *Lathronympha* group, *Ofatulena* group and *Ecdytolopha* group) and the separation of the genus *Grapholita* into three distinct groups.

EF1α ($n = 113$ taxa with at least partial sequences) (Fig. S8). This gene is unable to recover the monophyly of Grapholitini: the single Eucosmini genus *Epinotia* (represented by two species) falls within the tribe, and the Grapholitini genus *Sereda* falls outside the tribe. It also fails to recover the two major clades within Grapholitini. However, it successfully excludes both *Corticivora* and *Larisa* from Grapholitini. Like the *CAD* and *COI* trees, the gene tree for *EF1α* successfully links nearly all congeners, although sometimes as unresolved polytomies (e.g. in *Pammene* and *Dichrorampha*). The *Cydia* group and the *Ecdytolopha* group are consistent with those captured by *COI* and *CAD*; however, many genera included in the *Dichrorampha* clade in the ML 134-taxon tree, especially those that lack congeners (e.g. *Macrocydia*, *Sereda*, and *Cyanocydia*), are scattered throughout the tree.

GAPDH ($n = 86$) (Fig. S9). *GAPDH* is unable to recover a monophyletic Grapholitini, instead recognizing a monophyletic Eucosmini (the sister of Grapholitini), a monophyletic *Dichrorampha* clade and a monophyletic *Cydia* Clade as an unresolved trichotomy. It successfully excludes *Corticivora* and *Larisa* from Grapholitini; and it recovers the *Lathronympha* group, the *Ecdytolopha* group and the *Cydia* group. It also delineates the three distinct groups of *Grapholita* (i.e. *Grapholita*, *Aspila*, and *Ephippiphora*), but with *Aspila* embedded within the *Pammene* group. As in most other gene trees, nearly all congeners (except for *Grapholita*) are grouped together as monophyletic groups; and as in other trees, *Strophedra* falls within the genus *Pammene*.

MDH ($n = 85$ taxa) (Fig. S10). *MDH* failed to capture the monophyly of Grapholitini because of the outgroup species *Hyptiharpa baboquavariana*. With the exception of *H. baboquavariana*, MDH recovered the two major clades (I and II), and most of the generic groups (i.e. *Dichrorampha* group, *Ecdytolopha* group, *Pammene* group and *Cydia* group). It also recognizes three distinct groups of *Grapholita* (i.e. *Grapholita*, *Aspila* and *Ephippiphora*). This gene also supports the exclusion of *Larisa* and *Corticivora* from Grapholitini. Overall, it is the primary contributor to the backbone structure of the phylogeny of the tribe as illustrated in the ML 134-taxon tree. As in the *EF1α* tree, there are a few unresolved polytomies at the species level within some genera (i.e. in *Dichrorampha*, *Pammene* and *Cydia*). In this tree, *Cydia* (with the exceptions of the divergent and misplaced *pyraspis*, *palmetum* and *saltitans*) is monophyletic.

Wingless ($n = 44$) (Fig. S11). With representatives of only about one-third of the total taxon sample, the wingless tree is unable to capture many of the groups identified by the ML 134-taxon tree. Nonetheless, this gene successfully recovers a monophyletic Grapholitini, recognizes the two major clades identified by the ML 134-taxon tree and excludes *Larisa* from the tribe (*Corticivora* was not sequenced for this gene). It also recovers a monophyletic, albeit weakly supported, *Ecdytolopha* group and a monophyletic *Cydia* (minus *C. saltitans*, which is certainly not congeneric with other species of *Cydia*). Because only a single species of *Grapholita* was sequenced for this gene, the three genera formerly included in *Grapholita* (i.e. *Grapholita*, *Aspila* and *Ephippiphora*) are not distinguished. Overall, the topology of the *wingless* tree is similar to that of *MDH*, but with only about half as many taxa.

In regards to the question of whether more taxa or more genes would increase tree resolution (Rokas and Carroll, 2005), for our particular dataset, it seems likely that additional gene sampling in genera unrepresented by congeners would make the most significant contribution to tree support and reliability. For example, we lack confidence in the position of *Andrioplecta*, represented by only *COI*, *Selania*, represented by only *COI* and *EF1α*, and *Leguminivora*, represented by only *COI*, although the relative position of the last in the ML 134-taxon tree is compelling based on morphology and hosts. Also, the outgroup genus *Spilonota*, which falls within Grapholitini in one or more trees, is represented by only *COI* and *CAD*. Additional gene sampling in these and a few other taxa would undoubtedly increase the reliability of the multigene tree.

On the other hand, the addition of taxa represented by only *COI*, which are already represented by congeners with more complete gene sampling, would provide a much broader picture of relationships within those genera, and perhaps even improve relationships within the generic groups to which they belong. Our five included species of *Cryptophlebia* provide evidence in support of this proposal. Although four are represented by only *COI* and the fifth by *COI* and *CAD*, they link together as a monophyletic group, and are positioned convincingly as sister to *Pseudogalleria*, represented by four genes, which is suspected to be a synonym of *Cryptophlebia*. Hence, the position of *Pseudogalleria* within the *Ecdytolopha* group is well supported, and the position of the five species of *Cryptophlebia* is compelling.

Perhaps the most significant improvement to the tree could be achieved by the addition of several genera (with complete gene sampling) that we were unable to include—the Australian *Loranthacydia*, the Afrotropical *Camptrodoxa* and the Neotropical *Ricula* to name a few.

Molecular dating

Divergence of the family Tortricidae from its sister group, the latter of which has been somewhat elusive to determine, is estimated to be between 122 (Wahlberg et al., 2013) and 133 Myr (Fagua et al., 2017), in the early Cretaceous, which is within the estimated time of diversification of angiosperms, 90–135 Myr (Endress, 2001; Magallon et al., 2015; Silvestro et al., 2015; Fagua et al., 2017). Although Fagua et al. (2017) initially estimated the time of divergence of Olethreutinae from Tortricinae, the two subfamilies that comprise about 95% of the species richness of the family, to be about 72 Myr, they subsequently revised it to be about 53 Myr (Fagua et al., 2018). Fagua et al. (2017) further estimated that the divergence of most tribes occurred before 40 Myr, during the Eocene.

Our divergence time chronogram (Fig. 5) indicates that the MRCA of Grapholitini originated approximately 44.3 Myr (95% HPD, 41.3–47.4 Myr), later than the divergence of *Corticivora* from other groups at 59.6 Myr (95% HPD, 52.3–67.2 Myr). *Larisa subsolana* diverged from other species of Eucosmini at 33.2 Myr (95% HPD, 26.2–40.7 Myr). Within Grapholitini, Clade I is inferred to have arisen around 38.4 Myr (95% HPD, 33.3–43.4 Myr), whereas Clade II is estimated to date from 38.4 Myr (95% HPD, 34.5–42.6 Myr) (Table 1, Fig. 5). The MRCAs of the eight groups (A–H) were estimated to date from the Oligocene and the lower Miocene (20.8–31.5 Myr, Table 1, Fig. 5).

Within the *Dichrorampha* group (Clade I-A), the genus *Dichrorampha* is assumed to be a monophyletic lineage (with the exclusion of *Dichrorampha caribe*). *Dichrorampha* species originated about 20.0 Myr (95% HPD, 14.3–25.6 Myr), and are among the most recently evolved Grapholitini. *Multiquaestia purana* represents a sister species to the *Grapholita* group (Clade II-B), and the divergence is estimated to be around 30.8 Myr (95% HPD, 22.2–39.0 Myr). Diversification of many genera in Grapholitini, such as *Cryptophlebia*, *Ecdytolopha*, *Pammene* and *Fulcrifera*, is inferred to have occurred in the Miocene.

Ancestral area reconstruction

Based on the current distribution of tortricid species, Fagua et al. (2017) concluded that ancestral area analysis supports the hypothesis of a Gondwanan origin of Tortricidae in the South American plate. They further concluded that the South American plate is the most likely ancestral area of Chlidanotinae, the most basal subfamily, and its tribes, and for the Tortricinae tribes Sparganothini and Euliini. In all of these taxa, greatest species richness is found in the Neotropical Region. However, no genera have yet been recognized as exhibiting a convincingly southern continental distribution. Fagua et al. (2017) also hypothesized that Australia is the most likely ancestral area of Tortricinae and Olethreutinae and several of their tribes, and that the Palaearctic is the ancestral area for Grapholitini and Eucosmini (Olethreutinae).

In our study, we found that the optimal models of ancestral area reconstruction for the tribe Grapholitini are the DEC + J and the BAYAREALIKE under M0 and M1 analysis, respectively (Table S5). Generally, the DEC + J model (Fig. 6) produced a more confident and clearer estimation than the BAYAREALIKE model (Fig. S12). Under the DEC + J model (Fig. 6) the Nearctic + Afrotropical + Neotropical represents the ancestral range of Grapholitini, whereas under the BAYAREALIKE model, the Holarctic + Afrotropical + Neotropical represents the ancestral range (Fig. S12). Based on DEC + J, the MRCA of Clade I most likely originated in the Neotropical region and subsequently dispersed to the Nearctic and Palaearctic (Table 1; Fig. 6). However, based on BAYAREALIKE, the common ancestor of Clade I was widely distributed in the Holarctic + Neotropical (Fig. S12). In Clade II, the DEC + J model supports an Afrotropical ancestral area of the *Lathronympha* group, and *Ecdytolopha* group, and the west Palaearctic as the origin of the *Ephippiphora* group, *Grapholita* group, *Pammene* group and *Cydia* group (Table 1; Fig. 6). Multiple dispersals are hypothesized from the Afrotropical to the Palaearctic, from the Palaearctic to the Nearctic, from the Neotropical to the Afrotropical, etc. In contrast, the BAYAREALIKE model exhibits high uncertainties in the estimation of the ancestral areas of the seven generic groups in Clade II (Fig. S12).

Although these findings are inconsistent with those of Fagua et al. (2017), they are based on a much larger data set of Grapholitini, both taxonomic and molecular, than that of Fagua et al. (2017).

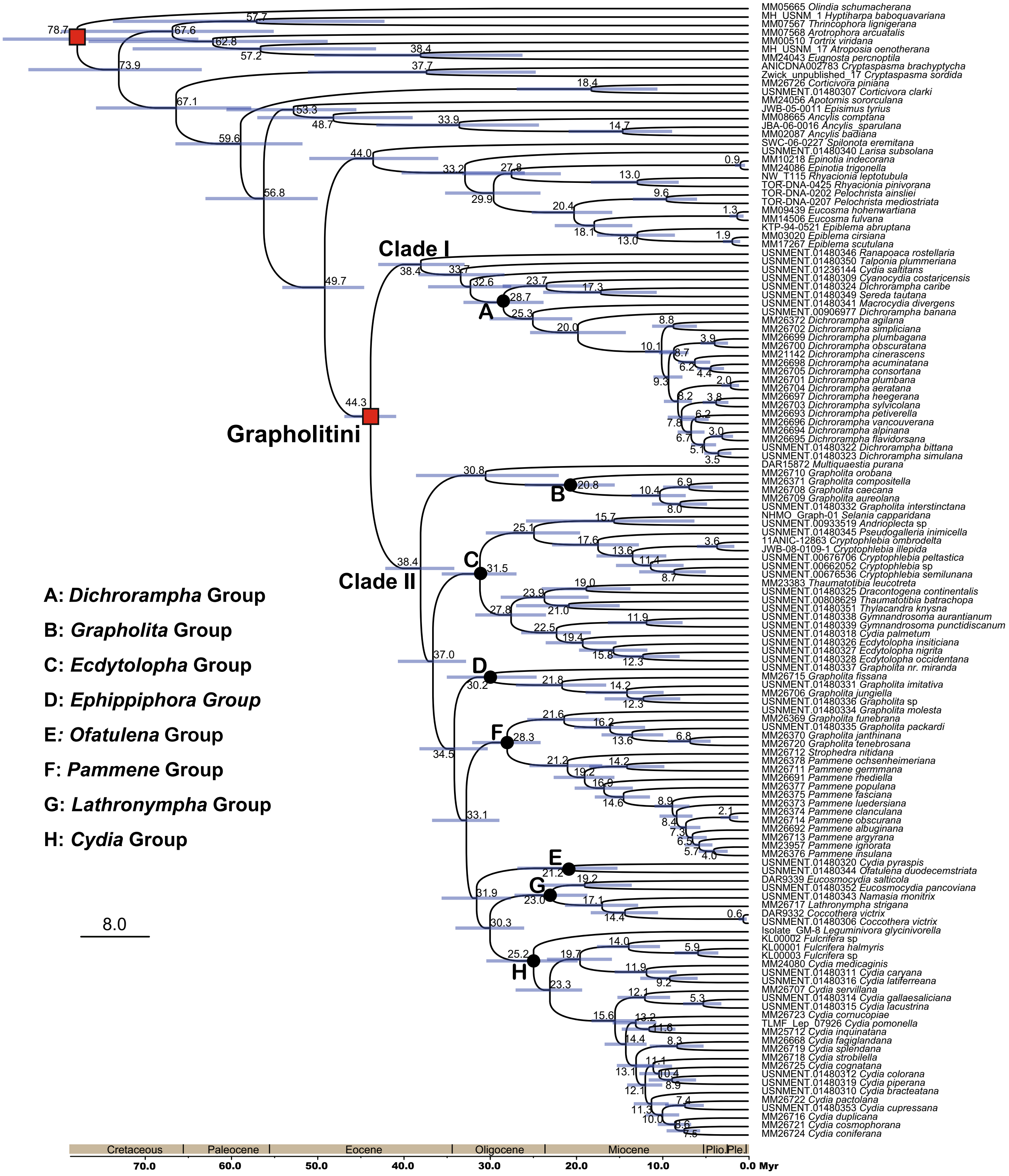

Fig. 5. Maximum clade credibility tree showing divergence time of Grapholitini inferred from the total dataset using BEAST. Red-filled square shows the calibration node. Number at node represents node age in million years ago (Myr). The bar at node represents 95% highest posterior density interval for each node age. Ple., Pleistocene; Plio., Pliocene.

Table 1
The divergence time, ancestral area and host data from molecular dating, ancestral area estimation and character evolution analyses

Group	Median age 95% HPD (Myr) (Fig. 5)	Ancestral area (Fig. 6)	Ancestral host family (Fig. 7a)	Ancestral host range (Fig. 7b)
Clade I	38.4 (33.3–43.4)	Neotropical	Annonaceae, Asteraceae, Euphorbiaceae, Fabaceae, Fagaceae, Sapotaceae	Monophagous, oligophagous
Clade II	38.4 (34.5–42.6)	Afrotropical	Fabaceae	Monophagous, oligophagous
Dichrorampha Group (I-A)	28.7 (24.0–33.4)	Neotropical	Annonaceae, Asteraceae, Euphorbiaceae, Fabaceae, Fagaceae, Sapotaceae	Monophagous, oligophagous
Grapholita Group (II-B)	20.8 (15.6–26.2)	West Palearctic	Fabaceae	Oligophagous
Ecdytolopha Group (II-C)	31.5 (27.2–36.0)	Afrotropical	Fabaceae	Monophagous
Ephippiphora Group (II-D)	30.2 (24.8–35.4)	West Palearctic	Fabaceae	Monophagous
Ofatulena Group (II-E)	21.2 (15.3–27.1)	Neotropical	Fabaceae	Monophagous
Pammene Group (II-F)	28.3 (24.3–32.4)	West Palearctic	Rosaceae	Monophagous, polyphagous
Lathronympha Group (II-G)	23.0 (18.9–27.4)	Afrotropical	Fabaceae	Monophagous
Cydia Group (II-H)	25.2 (20.0–30.7)	West Palearctic	Fabaceae	Monophagous

Patterns of host plant utilization

Within the family Tortricidae, there is a general pattern of polyphagy among members of most tribes of the subfamily Tortricinae (e.g. Archipini, Sparganothini, Atteriini), but, of course, with many exceptions. In contrast, there is a considerably higher level of host fidelity in the subfamily Olethreutinae (Regier et al., 2012a), with Grapholitini among the most specialized herbivores in the subfamily (Brown, 2022).

Brown (2022) reported that 97 different plant families "have been reported at least once for a species of Grapholitini, with the greatest number of grapholitines recorded from Fabaceae (168 species), followed by Fagaceae (43 species), Pinaceae (43), Sapindaceae (36), Rosaceae (32), Asteraceae (30), Euphorbiaceae (15), Rutaceae (12), Annonaceae (12), Salicaceae (11), and Cupressaceae (11)". "Thirty-two genera appear to be restricted, or nearly so, to specific host families, but many of these are either monotypic or are represented by exceedingly few records".

In Eucosmini, the putative sister group of Grapholitini, host plants are varied, but a number of "core" eucosmine genera (e.g. *Eucosma*, *Phaneta*, *Epiblema*, *Sonia*) are predominantly crown- and stem-borers in Asteraceae. Hence, Asteraceae is likely to be among the putative ancestral larval host families for Grapholitini.

Based on our analysis, the ancestral host families for the Grapholitini clade were inferred to be Annonaceae, Asteraceae, Euphorbiaceae, Fabaceae, Fagaceae and Sapotaceae (Fig. 7a), and the ancestors of the clade were estimated to be monophagous and oligophagous (Fig. 7b). Clade I has the same ancestral host family and larval host range as the Grapholitini, as does the *Dichrorampha* group (Clade I-A) (Table 1, Fig. 7b). Within the *Dichrorampha* group, species of *Dichrorampha* feed almost exclusively on Asteraceae. In contrast, the MRCA of Clade II is assumed to have fed on Fabaceae and have originated from monophagous and oligophagous ancestors. Fabaceae is also assumed to be the ancestral larval host family of the *Grapholita* group (Clade II-B), *Ecdytolopha* group (Clade II-C), *Ephippiphora* group (Clade II-D), *Ofatulena* group (Clade II-E), *Lathronympha* group (Clade II-G) and *Cydia* group (Clade II-H) (Table 1, Fig. 7a). The *Ecdytolopha* group (Clade II-C), *Ephippiphora* group (Clade II-D), *Ofatulena* group (Clade II-E), *Lathronympha* group (Clade II-G) and *Cydia* group (Clade II-H) are presumed to have originated from monophagous ancestors, whereas the *Grapholita* group (Clade II-B) is presumed to have an oligophagous ancestor (Table 1, Fig. 7b). The *Pammene* group (Clade II-F) shows a clear host plant transition to Rosaceae and polyphagy, suggesting a pivotal event in the evolutionary history of Grapholitini. Also, the host plant shift to other plant families and polyphagy occurs in some genera of the *Ecdytolopha* group (Clade II-C), such as *Thaumatotibia*, and in the *Cydia* group (Clade II-H), such as *Cydia*. In *Cydia* we found a large clade of Pinaceae-feeding species, and smaller groups of Fabaceae feeders and Fagaceae feeders (Brown, 2022).

According to Brown (2022), at least three genera of Grapholitini "include species whose larvae are entomophagous: *Andrioplecta*, with two species that feed on aphids and one on the larvae of cynipid wasps; *Coccothera*, with one species that is predaceous on *Ceroplastes* (*Waxellia*) *egbara* (Coccidae); and *Parapammene*, with one species that feeds on lecanium scales (Coccidae: *Parthenolecanium*)". This life style may be opportunistic, with predaceous grapholitines

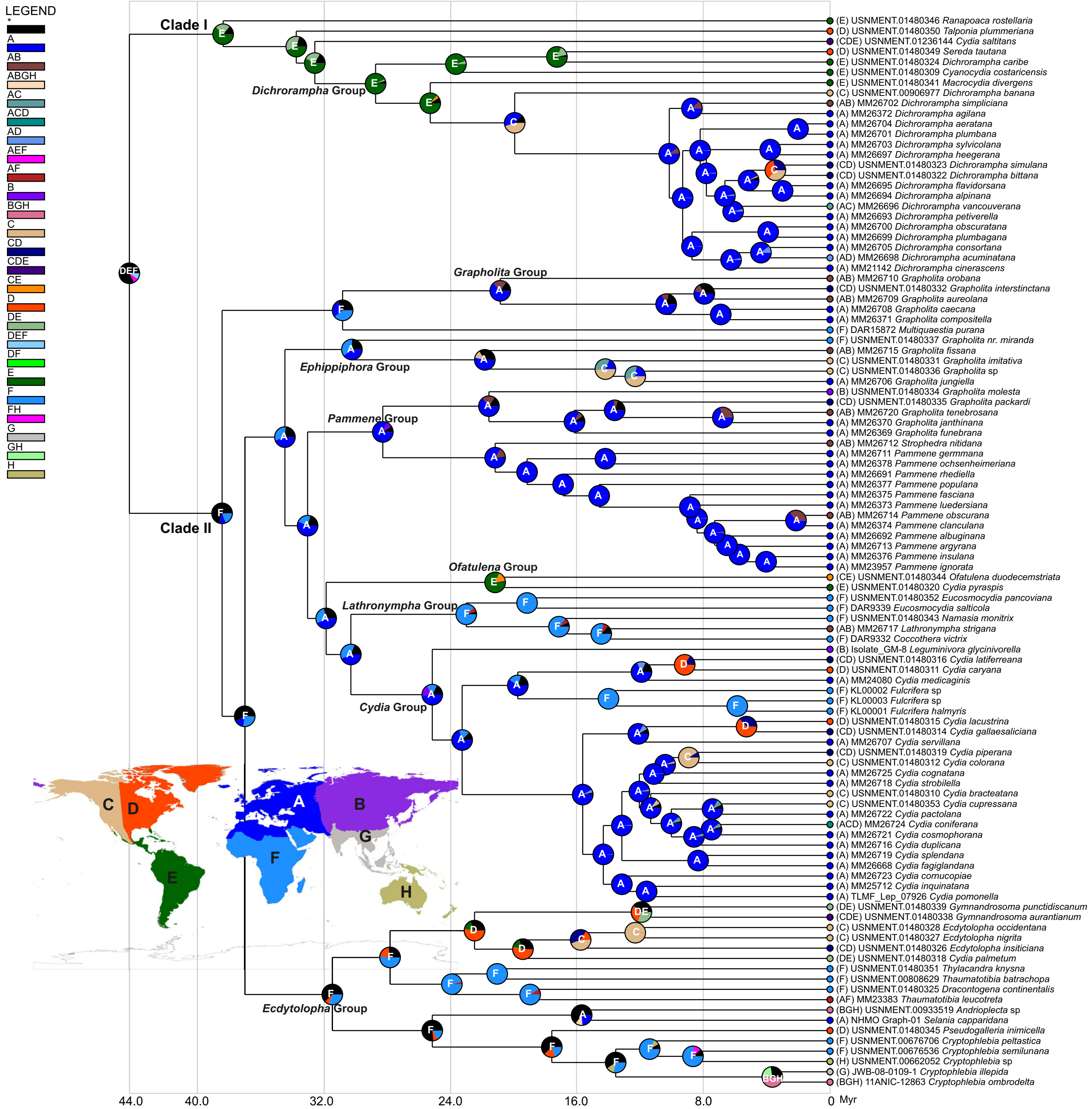

Fig. 6. Ancestral distribution range of Grapholitini reconstructed under DEC + J model using BioGeoBEARS in RASP without palaeographical model (M0). Eight biogeographical regions were identified with different colours in the map at bottom left: (a) Western Palaearctic; (b) Eastern Palaearctic; (c) Western Nearctic; (d) Eastern Nearctic; (e) Neotropical; (f) Afrotropical; (g) Oriental (including Hawaii); and (h) Australia. The capital letter in the circle indicates the most likely distribution range. The coloured pie chart at node shows relative probability of estimated ancestral range. Black pie chart means uncertain estimation (*).

feeding on prey encountered on their host plants, often associated with galls. Hence, a number of other Grapholitini that are associated with galls, sometimes as inquilines, may occasionally feed on the gall-inducing insect. These species are scattered throughout the tribe, suggesting multiple origins of entomophagy.

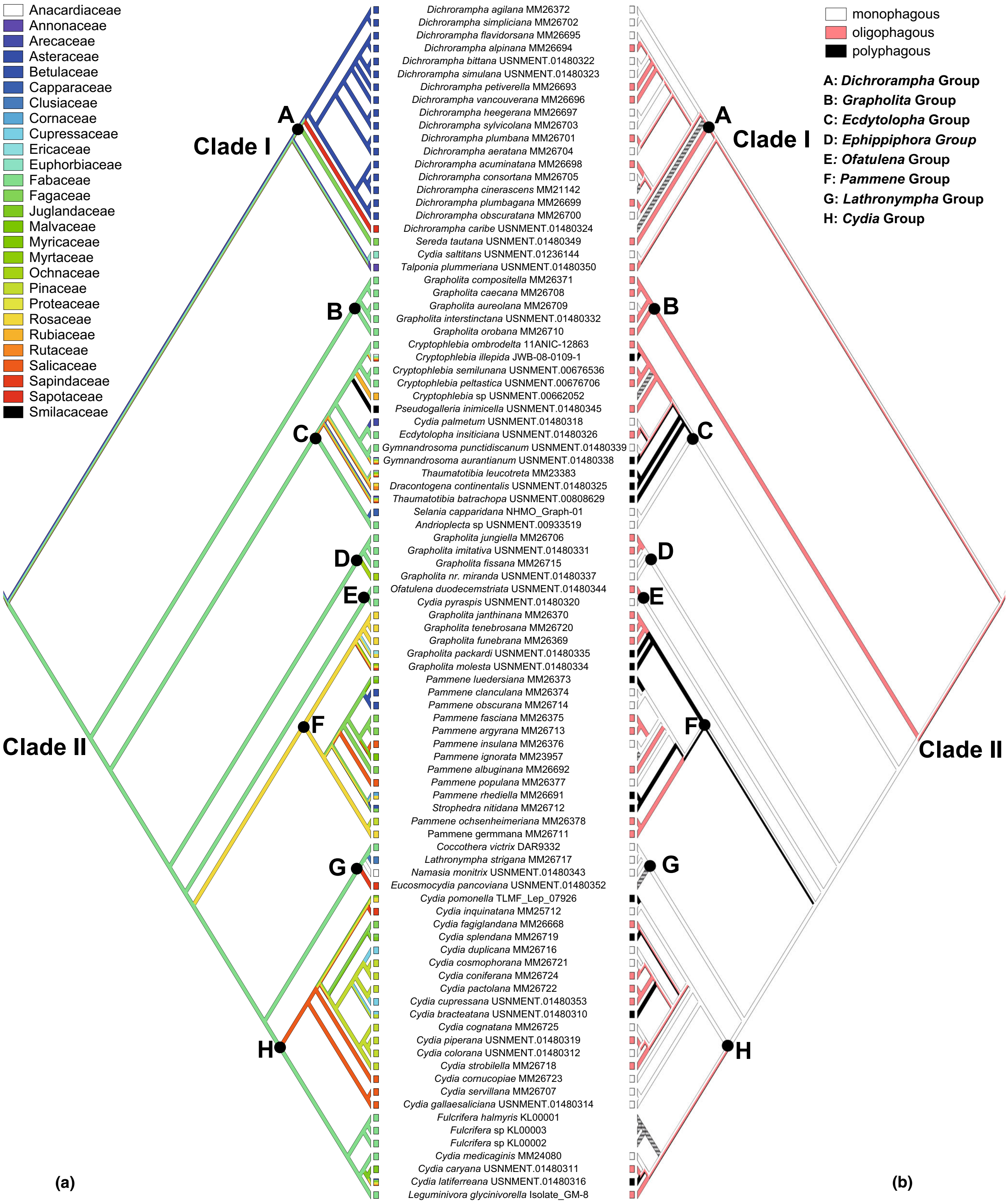

Fig. 7. Ancestral state reconstruction of the host plant family (a) and larval host range (b) of Grapholitini using Mesquite. The coloured square next to tip shows the presence of a host plant record for this particular taxon. Branches are coloured based on the most likely state. Dashed lines on branches indicate the tip taxa without host range data.

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Conflict of interest

None declared.

Data availability statement

The DNA sequence data has been deposited in Voseq and accession numbers are provided in Table S1. COI barcode data can be retrieved from the BOLD dataset DS-GRAPHOLI. DNA Sequence alignments used for phylogenetic analyses are available upon request.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Strict consensus tree of 255 equally most-parsimonious trees (length 12 288 steps) of Grapholitini inferred from the total dataset of 134 taxon samples using TNT.

Fig. S2. The majority-rule consensus tree of Grapholitini based on the total dataset of 134 taxon samples using MrBayes.

Fig. S3. Most parsimonious tree (length 9006 steps) of Grapholitini inferred from dataset of 77 taxon samples using TNT.

Fig. S4. Maximum likelihood tree of Grapholitini based on a 77-taxa dataset.

Fig. S5. The majority-rule consensus tree of Grapholitini based on the 77-taxa dataset using MrBayes.

Fig. S6. Maximum likelihood tree based on *COI* gene sequences.

Fig. S7. Maximum likelihood tree based on *CAD* gene sequences.

Fig. S8. Maximum likelihood tree based on *EF1α* gene sequences.

Fig. S9. Maximum likelihood tree based on *GAPDH* gene sequences.

Fig. S10. Maximum likelihood tree based on *MDH* gene sequences.

Fig. S11. Maximum likelihood tree based on *wingless* gene sequences.

Fig. S12. Ancestral area reconstruction of Grapholitini under BAYAREALIKE model in BioGeoBEARS with constrained condition (M1).

Table S1. The detailed information of samples used in this study, including collection localities, GenBank accession numbers, geographic distribution and host plant associations.

Table S2. The forward (F) and reverse (R) primers for the genetic markers used in this study.

Table S3. The genes and the number of basepairs available for each taxon sample.

Table S4. The best partition schemes and substitution models for the total and 77-taxa dataset inferred from PartitionFinder.

Table S5. Best fit model for unstrained (M0) and constrained (M1) models inferred from BioGeoBEARS in RASP.

Appendix S1. Alignment file in phylip format with all genetic markers concatenated for the total dataset.

Appendix S2. Alignment file in phylip format with five or more genetic markers concatenated for the 77-taxa dataset.

Appendix S3. Connectivity matrix used for constrained analysis with a time-stratified palaeographic model, based on Fagua et al. (2017).

Appendix S4. Revised generic assignments for species of *Grapholita* and other proposed nomenclatural changes.