A Molecular Phylogeny for the Leaf-Roller Moths (Lepidoptera: Tortricidae) and Its Implications for Classification and Life History Evolution

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Abstract

Background: Tortricidae, one of the largest families of microlepidopterans, comprise about 10,000 described species worldwide, including important pests, biological control agents and experimental models. Understanding of tortricid phylogeny, the basis for a predictive classification, is currently provisional. We present the first detailed molecular estimate of relationships across the tribes and subfamilies of Tortricidae, assess its concordance with previous morphological evidence, and re-examine postulated evolutionary trends in host plant use and biogeography.

Methodology/Principal Findings: We sequenced up to five nuclear genes (6,633 bp) in each of 52 tortricids spanning all three subfamilies and 19 of the 22 tribes, plus up to 14 additional genes, for a total of 14,826 bp, in 29 of those taxa plus all 14 outgroup taxa. Maximum likelihood analyses yield trees that, within Tortricidae, differ little among data sets and character treatments and are nearly always strongly supported at all levels of divergence. Support for several nodes was greatly increased by the additional 14 genes sequenced in just 29 of 52 tortricids, with no evidence of phylogenetic artifacts from deliberately incomplete gene sampling. There is strong support for the monophyly of Tortricinae and of Olethreutinae, and for grouping of these to the exclusion of Chlidanotinae. Relationships among tribes are robustly resolved in Tortricinae and mostly so in Olethreutinae. Feeding habit (internal versus external) is strongly conserved on the phylogeny. Within Tortricinae, a clade characterized by eggs being deposited in large clusters, in contrast to singly or in small batches, has markedly elevated incidence of polyphagous species. The five earliest-branching tortricid lineages are all species-poor tribes with mainly southern/tropical distributions, consistent with a hypothesized Gondwanan origin for the family.

Conclusions/Significance: We present the first robustly supported phylogeny for Tortricidae, and a revised classification in which all of the sampled tribes are now monophyletic.

Introduction

Tortricoidea, currently comprised of the single family Tortricidae, constitute one of the largest superfamilies of Lepidoptera, second only to Gelechioidea among the non-Obiectomeridae [1]. The nearly 10,000 described species [2] are distributed worldwide, with greatest species richness in the New World tropics. Tortricidae include numerous major pests of crops, forests, and ornamental plants [3–5], as well as biological control agents used successfully against invasive weeds [6–10]. Several tortricids have also become model organisms for the study of lepidopteran genetics, insect pheromones, and evolution [11]. A reliable classification and phylogeny are indispensable for the organization, communication and prediction of facts about such an economically important group of insects and for understanding how the traits important to their management and exploitation, particularly their host-plant ranges, evolve.

With minor exceptions, i.e., the exclusion of Carposinidae (Walsingham 1907) and the inclusion of several small groups from Glyphipterigidae sensu lato (e.g., Hilarographa and relatives [12,13]), the circumscription/definition of Tortricoidea has remained constant for over a century. The ranks of the included taxa, however, and our understanding of relationships among the many well-defined lineages have changed extensively, particularly...
in the last 50 years. Many groups treated as separate families by earlier authors are now recognized as tribes or subfamilies within Tortricidae (summary in Table 1), most recently Olethreutinae [14], Chlidanotinae [15,16], and Cochylini [17]. Although hypotheses on the phylogeny of Tortricoida were proposed historically by Meyrick [18] and Kennel [19], the first modern morphological and biological characters in a treatment of the North American tribes of Tortricinae (see Fig. 1A). Based on morphology of the male and female genitalia, with an emphasis on musculature of the male genitalia, Kuznetsov and Stekolnikov [17,21,22] and Razowski [23] provided phylogenies for the Paleartic Tortricidae, examining a broader range of taxa than Powell, but focusing on a narrower range of characters (Figs. 1B–1E). These authors, however, do not identify the characters that support specific branches, nor do they differentiate between synapomorphic and symplesiomorphic states, and many of their taxa are defined by hypothesized shared losses. Horak and R. Brown [24], in a review of the morphology, biology, and biogeography of tortricid tribes, divided the family into the three currently recognized subfamilies, and presented a detailed working hypothesis of phylogeny for Olethreutinae ([24] their figure 1.2.5). Saфонkin [25], in a review of the phylogenetic distribution of tortricid pheromones, presented the first phylogenetic tree to include three subfamilies (Fig. 1F), with relationships among tribes following Kuznetsov and Stekolnikov [22].

Our current classification stems from Horak and R. Brown ([24]; see Table 2), and has undergone only a few minor changes in the past two decades, including proposed relegation of Enodotheniini to Bactrini [26], and of Gatesclarkeanini to Olethreutini [27]. Although the classification has been stable, it has become increasingly clear over the past 20 years that our understanding of tortricid phylogeny remains highly provisional (review in Powell and J. Brown [28]). While many tribes include a core monophyletic group of genera, the tribal assignments of “orphan” genera renders some of the tribes paraphyletic. The monophyly of the largest subfamily, Tortricinae, has been repeatedly doubted [24,29]. Evidence on phylogenetic relationships among tribes within subfamilies remains very scarce, and morphological analyses, thus far, have not yielded compelling clarification. For example, although Horak [27] presented a thorough cladistic analysis of the genera of Olethreutinae of Australia, she declined to propose a revised classification based on those results because they showed little similarity to traditional and intuitive relationships that could be easily supported by synapomorphies.

Very recently, molecular data for small samples of Tortricidae, gathered as part of broad phylogenetic surveys across the Lepidoptera, have shown much promise for resolving relationships within tortricids [30–32]. The purpose of this paper, building on those preliminary findings, is to present the first detailed molecular estimate of relationships across the tribes and subfamilies of Tortricidae. Using 19 genes previously sequenced by Cho et al. [32], we expand those authors’ taxon sampling from nine tortricids to 32, spanning all three subfamilies and 19 of the 22 tribes recognized by Horak and R. Brown [24]. We then review the agreement and disagreement of the molecular phylogeny with traditional morphological data and the tribal and subfamily concepts based on them. Finally, we use the new phylogeny to reconsider previous hypotheses about the evolution of host plant use in Tortricidae [33].

Materials and Methods

Taxon and gene sampling

The central goal of this study was to estimate relationships among the tribes and subfamilies of Tortricidae. We therefore sought to include representatives of as many of these as possible. The distribution of the 52 tortricid species we sequenced across the classification of Horak and R. Brown [24] is depicted in Table 3. We use this classification as the hypothesis to be tested because it is the most finely subdivided among recent schemes, permitting independent assessment of subsequent proposals for merging of tribes. Our sample encompasses all three subfamilies and 19 of the 22 tribes recognized by Horak and R. Brown [24]. Adult habitus images for representative species of all tribes are shown in Figure 2. We were unable to obtain fresh material for two of the eleven tribes of Tortricinae, the Oriental/Australian Schoenotenini (17 genera, 204 species) and Epitymbiini (13 genera, 103 species), and for the small Oriental tribe Gatesclarkeanini (4 genera, 23 species) of Olethreutinae. Eleven tribes were represented by two or more genera, and the six largest tribes (670 to 1650+ species) were represented by three to six genera each. Outgroup choice was not straightforward because the phylogenetic position of Tortricidae among the lower ditrysians is quite unclear. We therefore included fourteen diverse outgroup taxa, listed in Table 4, representing all superfamilies which have been proposed as near relatives to Tortricidae by previous authors and/or previous molecular phylogenetic studies of ditrysian relationships [30–32], including preliminary analyses of the 800+ taxon LepTree project data set, which is described at http://www.leptree.net/status_matrix. The root of the entire tree, ingroups plus outgroups, was provisionally placed at Urodidae+Millieriidae, also on the basis of those preliminary analyses. Nomenclature for the outgroup taxa follows van Nieukerken et al. [34].

The specimens used in this study, listed in Table S1, were obtained with the kind help of collectors around the world (see Acknowledgments). They 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). No permits were required for collection of these specimens, and no endangered species were used. 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. 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.

Table 1. Groups previously considered families by one or more tortricid workers.

| Historical family name | Current name (Horak & Brown [24]) |
|------------------------|----------------------------------|
| Olethreutidæ            | Olethreutinae                     |
| Chlidanotidæ           | Chlidanotinae                    |
| Sparganothidæ          | Sparganothini                    |
| Schoenotenidæ          | Schoenotenini                    |
| Ceracidæ               | Ceracini                         |
| Phalonidæ (=Cochylidæ) | Cochylini                        |
| Atteridæ               | Atterini                         |
| Eucosmidæ              | Eucosmini                        |
| Grapholithidæ          | Grapholithini                    |
| Melanolophidæ          | Olethreutinae                    |

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These data allow checks of our identifications against the BOLD (Barcode of Life Data system) reference library, and will facilitate future identifications of specimens whose identities are 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. First, all taxa were sequenced for the five gene fragments described by Regier et al. [30], which total 6633 base pairs (bp), not including 333 with uncertain alignments. These genes are: CAD (2928 bp) [35], DDC (1281 bp) [36], enolase (1134 bp) [37], period (888 bp) [38] and wingless (402 bp) [39]. This set of genes has been used to resolve lepidopteran relationships at a variety of levels [30,40–42].

To increase resolving power, all of the outgroup taxa, plus approximately half of the tortricids (29/52 = 56%), spread over all 19 tribes represented, were also sequenced for an 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. [43] and Cho et al. [32]. Based on the results of those studies, we selected these 14 gene regions as especially useful for further studies of Lepidoptera, as judged from their frequency of amplification and sequencing success and their total contribution to branch lengths.

To summarize, total nucleic acids were isolated and specific regions of the cognate mRNAs were amplified by RT-PCR.

Table 2. Three recent classifications of Tortricidae and their agreement with molecular evidence. Number of exemplars in current study is given.

|                | Powell [96] | Horak & Brown [24] | Horak [27] | Evidence from current study |
|----------------|-------------|--------------------|------------|-----------------------------|
| **CHLIDANOTINAE** |             |                    |            |                             |
| (not included)  | Polyorthini | Polyorthini        | Polychlorini (2, monophyletic) |
| (not included)  | Chlidanotini| Chlidanotini       | Chlidanotini (2, monophyletic) |
| Hilarographini  | Hilarographini| Hilarographini     | Hilarographini (1)          |
| **TORTRICINAE** |             |                    |            |                             |
| (not included)  | Phricanthini| Phricanthini       | Phricanthini (1)            |
| Tortricini      | Tortricini  | Tortricini         | Tortricini (1)              |
| Cochlidae       | Cochlyni    | Cochlyni           | Cochlyni (3, monophyletic)  |
| Cnephasiini (in part) | Cnephasiini | Cnephasiini     | Cnephasiini (2, monophyletic) |
| Cnephasiini (in part) | Eullini | Eullini          | Eullini (4, paraphyletic). Here synonymized with Cochlyni |
| (not included)  | Schoenotenini| Schoenotenini    | [not sampled]              |
| (not included)  | Atterini    | Atterini           | Atterini (1)               |
| Sparganothini/Niasomini | Sparganothini | Sparganothini | Sparganothini (3, monophyletic) |
| (not included)  | Epitymbiini | Epitymbiini       | [not sampled]              |
| Archipini       | Archipini   | Archipini          | Archipini (6, monophyletic) |
| (not included)  | Ceracini    | Ceracini           | Ceracini (1)               |
| **OLETHREUTINAE** |             |                    |            |                             |
| (not included)  | Microcorsini| Microcorsini      | Microcorsini (1)           |
| Olethreutini    | Bactrini    | Bactrini           | Bactrini (1). Here synonymized with Olethreutini |
| Olethreutini    | Endotheni   | Bactrini           | Endotheni (1). Here synonymized with Olethreutini |
| (not included)  | Gatesclarkeanini| Olethreutini | [not sampled] |
| Olethreutini    | Olethreutini| Olethreutini       | Olethreutini (6, paraphyletic). Here broadened to include Bactrini and Endotheni |
| Eucosmini       | Enarmoniini | Enarmoniini        | Enarmoniini (1)            |
| Grapholitini    | Grapholitini| Grapholitini      | Grapholitini (6, monophyletic) |
| Eucosmini       | Eucosmini  | Eucosmini          | Eucosmini (6, monophyletic) |

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Figure 1. Previous hypotheses of phylogenetic relationships in Tortricidae. A. Powell (1964; [20]), B. Kuznetsov and Stekolnikov (1973; [21]), C. Razowski (1976; [23]), D. Kuznetsov and Stekolnikov (1977; [17]), E. Kuznetsov and Stekolnikov (1984; [22]), F. Safonkin (2007; [25]). Tree figures redrawn, but nomenclature in each case follows the original.

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Table 3. Species sampled and their distribution across the current classification. Diversity numbers based on Baixeras et al. [2], distributions largely based on Horak [27,29].

| CHLIDANOTINAE (44 genera, 288 species) |
|----------------------------------------|
| **Polyorthini** (21 genera, 144 species; mainly Neotropical and Oriental/Australian): *Pseudatteria volcanica* (Butler), *Histura perseavora* Brown |
| **Chlidanotini** (18 genera, 76 species; Neotropical, Oriental/Australian): *Auratanota dispersa* Brown, *Heppnerographa tricesimana* (Zeller) |
| **Hilarographini** (5 genera, 68 species; mainly pantropical): *Hilarographa* sp. |

| TROTRICINAE (439 genera, 4,176 species) |
|----------------------------------------|
| **Phricanthini** (3 genera, 21 species; Australia, Southeast Asia, Madagascar): *Phricanthes asperana* Meyrick |
| **Atteriini** (8 genera, 45 species; mainly Neotropical): *Anacrasis nephodes* (Walsingham) |
| **Sparganothini** (17 genera, 219 species; mainly New World): *Amorbia humerosa* Clemens, *Sparganothis reticulatana* (Clemens), *Platynota idaeusalis* (Walker) |
| **Ceracini** (4 genera, 29 species; eastern Paleartic, Oriental): *Cerace* sp. |
| **Archipini** (160 genera, 1,623 species; cosmopolitan): *Dichelia cosmopis* (Lower), *Ateletora* sp., *Clephis melaleuca* (Walker), *Argyrotaenia alisellana* (Robinson), *Choristoneura rosaceana* (Harris), *Pandemis limitata* (Robinson) |
| **Cnephasinini** (19 genera, 261 species; mainly Himalayan; Oriental, Paleartic): *Decodes asapheus* Powell, *Cnephasia alacarana* Razowski |
| **Tortricini** (41 genera, 406 species; nearly world-wide): *Acleris sempipurpurea* (Kearfott), *Acleris affinatana* (Snellen) |
| **Eulini** (87 genera, 670 species; mainly Neotropical): *Bonogota* sp., *Eulia ministrana* L., *Pseudometastis* sp., *Netechma* sp. |
| **Cochylini** (75 genera, 1,028 species; cosmopolitan): *Aethes promptana* (Robinson), *Eugnosta busckana* (Comstock), *Carolella sartana* (Hübner) |
| **OLETHREUTINAE (355 genera, 4,417 species) |
| **Microcosini** (2 genera, 36 species; southern Hemisphere and Oriental): *Cryptaspasma queraula* (Meyrick), *Cryptaspasma* sp. |
| **Bactrini** (9 genera, 113 species; cosmopolitan, mainly Old World): *Bactra furfaranata* (Haworth), *Bactra maiorana* Heinrich |
| **Endotheniini** (5 genera, 52 species; cosmopolitan, mostly Paleartic): *Endothenia hebesana* (W.) |
| **Olethreutini** (133 genera, 1,077 species; cosmopolitan): *Oxysphara* sp., *Euprostys tyrius* Heinrich, *Lobesa aelapopa* Meyrick, *Hedy dimidiana* (Clerck), *Olethreutes fasciata* (Clemens), *Atropoe karsholti* Aarkv |
| **Enarmonini** (38 genera, 298 species; nearly cosmopolitan, especially Oriental and Australian): *Angyia sporulana* (Staudinger) |
| **Eucosmini** (106 genera, 1,651 species; cosmopolitan, predominantly Paleartic): *Spilonota eremitana* Moriuti, *Gypsonoma paradoxala* (Meyrick), *Epinota* sp., *Pelochrista zimonana* (Kearfott), *Epipteryx abruptana* Walsingham, *Epipteryx foenella* L. |
| **Grapholitori** (62 genera, 898 species; cosmopolitan): *Dichroaraphra cancellatana* (Kernen) *Cryptophlebia illepida* (Butler), *Grapholita packardi* Zeller, *Grapholita delineana* Walker, *Cydia paminella* (L.), *Multiquaestia purana* Karisch |

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 [45]. Multi-sequence alignments were made using the Translation Align program within the Geneious Pro 5.3.4 software package [46], which eliminates the contribution of any potential synonymous change. For this reason, we also used “degen1” coding [46], which eliminates the contribution of any synonymous change to pairwise distances between extant taxa (see http://www.phylotools.com for Perl script). Degen1 is an extension of the RY coding scheme [47]. Nucleotide sites 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, while TTA, TTG, CTT, CTC, CTA and CTG (Leu) are all coded YTN. 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 both deeper and shallower nodes within Tortricidae. 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 [48,49], has been supported by simulations [50] and by a growing body of case studies ([32,51,52] and references therein). To ensure that our results are not subject to artifacts from deliberate blocks of missing data, and to add to the empirical evidence on this issue, we carried out parallel analyses on the full, deliberately incomplete 19 gene data set and on a reduced gene sample, the “five-gene complete matrix,” comprising only the five gene regions sequenced in all 66
taxa. If the large blocks of missing data created by our design result in artifactual groupings, we might expect to see strong support, in trees from the expanded, deliberately incomplete matrix, for groups which do not occur in trees from the five-genes-only matrix [32]. Conversely, finding the same topology from the two matrices would imply that large missing data blocks in the full deliberately incomplete matrix do not themselves mislead phylogenetic inference. Even if it did not induce artifacts, however, deliberately incomplete augmentation of gene sampling would be an ineffective strategy if it failed to strengthen phylogenetic signal, or worse, obscured it. Therefore, we also asked whether bootstrap support was increased or decreased, on average and for which and how many nodes, by the deliberately incomplete 19-gene matrix as this matrix was increased or decreased, on average and for which and how much of the phylogenetic artifacts arising from the large blocks of missing data in the 19-gene deliberately incomplete matrix, as this matrix...
and the five-gene complete matrix give essentially identical topologies except for nodes which are very weakly supported in all analyses. The five-gene matrix alone is highly informative about relationships within tortricids, providing bootstrap support comparable to that from the 19-gene matrix for most nodes (even higher in a few cases, e.g., node 2). For three nodes embodying deeper divergences within Tortricinae, however, substantial support is evident only when all 19 genes are included. Support for several nodes was greatly increased by the additional 14 genes, despite the fact that these were sequenced in only about half of the taxa (29/52), and there was no indication of phylogenetic artifacts from the deliberately incomplete gene sampling design. Our results thus provide further support for the effectiveness of augmenting the gene sample in only a subset of taxa, as a resource-efficient approach to improving node support [32].

In contrast to relationships within Tortricidae, relationships among the outgroup taxa are highly unstable to differences among data matrices and character treatments, and strong bootstrap support is almost entirely lacking.

**Discussion**

In this section we first review the agreement and disagreement of our molecular results with previous hypotheses on the phylogeny of Tortricocidae. Finding strong evidence for paraphyly of two tribes, Euliini and Olethreutini, we propose formal taxonomic changes for both. A summary of our findings regarding monophyly or lack thereof for each of the tribes and subfamilies of Horak and R. Brown [24] is provided in Table 2. The information on diversity, distribution and life history given in the accounts for individual tribes is summarized in Figure 5. We end with an overview of the new phylogeny’s bearing on previous postulates about evolutionary trends in tortricid larval feeding habits. In addition, we provide an illustrated on-line synopsis of each tribe as currently understood at http://www.leptree.net/lep_taxon_page/Tortricidae/view.

**Phylogenetic position of Tortricidae and basal divergences within the family**

Like previous molecular studies [30–32], the present results provide essentially no credible support for any hypothesis about
the sister group to Tortricoidea, despite the availability of 19 genes of sequence (up to 14.8 kb) for all 14 outgroup taxa (see Fig. 4). For example, the tortricid-like genus *Heliocosma*, sometimes included in Tortricoidea but excluded by Horak et al. [60], never groups next to Tortricidae. Relationships among the superfamilies of lower apoditrysian Lepidoptera appear to be a very difficult phylogenetic problem [32].

Our results do however provide very clear resolution for a majority of relationships within Tortricidae, as evidenced in Figures 3 and 4. Monophyly for the family (Fig. 3, node 1) receives 100% bootstrap support in all analyses, consistent with traditional morphological concepts as well as recent molecular analyses [30–32]. Basal divergences within the family are also mostly strongly supported. The monophyly of Olethreutinae [24], strongly corroborated here (node 7; bootstrap percentage [BP] = 100%), is supported by morphological characters including a single ring of scales on the antennal flagellomeres and fusion of the phallosome to the juxta through the anellus.

Somewhat more surprisingly, monophyly for Tortricinae (22 genera, 9 of 11 tribes sampled) is supported almost as strongly (node 8; 88% bootstrap for nt123, 19 genes). The ML analysis of Mutanen et al. [31] also recovered a monophyletic Tortricinae, based on a smaller, mostly non-overlapping sample (nine genera), but with <50% bootstrap support, probably because they excluded n3. For the past two decades, Tortricinae have been thought by most tortricid workers to be para- or polyphyletic [24,29], because convincing morphological synapomorphies have not been found, although no one proposed constituent taxa whose removal would render it monophyletic. Our results strongly suggest that the search for tortricine synapomorphies deserves more effort.

Our analyses unambiguously support a sister group relationship between Tortricinae and Olethreutinae (node 6; BP = 100), confirming the prevailing view of Chlidanotinae as the earliest-diverging subfamily [24,61]. The same hypothesis is strongly supported by the results of Mutanen et al. [31], based on a smaller taxon sample.

The more problematic question about basal tortricid relationships is the monophyly of Chlidanotinae. In all the analyses for this study, Chlidanotinae were inferred to be paraphyletic, with Chlidanotiini+Hilarographini allied to the remaining subfamilies to the exclusion of Polyorthini (node 2; BP = 79 for nt123).
Table 5. Synopsis of species diversity, distribution and larval host-plant use for the tribes of Tortricidae studied, mapped onto a phylogeny condensed from Fig. 3. The multiple branches leading to the names Eucommini and Oletreutini s.s. denote the paraphyly of these tribes discovered here. Col. 1 = number of described species, taken from Table 3. Col. 2 = summary of geographic distributions, following Horak [29].

| Tribe/Synonym | Col. 1 | Col. 2 |
|---------------|-------|-------|
| Eucommini | >400 | cosmopolitan |
| Oletreutini s.s. | >400 | cosmopolitan |
| Tortricini s.s. | >400 | cosmopolitan |
| Cerininae | >400 | cosmopolitan |
| Phricanthini | >400 | cosmopolitan |
| Chlidanotini | >400 | cosmopolitan |
| Polyorthini | >400 | cosmopolitan |

* Abbreviation "nt123" refers to mitochondrial gene partitioning. However, in ongoing studies of much larger data sets (>400 outgroups to Tortricidae, across all Lepidoptera; data not shown), analyses that included synonymous change (nt123, nt123 partitioned) favored monophyly for Chlidanotini with bootstrap support up to 77%, while paraphyly was almost always favored when synonymous change was excluded (i.e., degen-1 analysis), albeit with bootstrap <50%. Seeking the reasons for this unusual conflict, we experimented with widely differing outgroup samples. We found, surprisingly, that the divergent results from nt123 can be ascribed entirely to base composition heterogeneity, among the outgroups and between these and Tortricidae. That is, outgroups with some patterns of composition yield paraphyly for Chlidanotini, while those with other patterns, no more or less closely related to Tortricidae, yield monophyly. Thus, synonymous change seems to provide no reliable information regarding chlidanotine monophyly. Non-synonymous change, in contrast, consistently favors paraphyly, which we therefore take as our working hypothesis, although bootstrap support is not strong. However, this conclusion contradicts the widely accepted view that Chlidanotini are monophyletic, which is supported most clearly by a complex morphological synapomorphy: in all chlidanotines and in no other Lepidoptera, the valve of the male genitalia bears a deep longitudinal invagination that holds distinctive hairpencils arising from the eighth abdominal segment [12,16,24]. On the molecular tree, one must interpret this suite of traits as either arising at the base of Tortricidae and undergoing subsequent loss, or as arising independently in Polyorthini and in Chlidanotini.+Hilarographini.

The question of subfamily monophyly aside, the relative positions of the three tribes of Chlidanotini conform to those proposed by previous authors based on morphology [16,24,62–64], including Horak [29], who suggested that Hilarographini may be subordinate within Chlidanotini. Our two exemplar genera of Polyorthini (Fig. 2A) group strongly with each other, as do the two exemplars of Chlidanotini (Auratonota [Fig. 2C] and Heparographa); and, the latter in turn group strongly with the representative of Hilarographini (Fig. 2B), to the exclusion of Polyorthini. In our trees, therefore, Polyorthini are the earliest-branching group of Tortricidae. All three tribes of Chlidanotini are primarily tropical. Polyorthini comprise about 144 species found mainly in the Neotropical and Oriental/Australian regions, a distribution similar to that of Chlidanotini, which include 76 species, whereas the 68 species of Hilarographini are pantropical. Life history observations on all three tribes are few, but suggest that all three are internal feeders or leaf rollers on living plants [29].

Relationships within Tortricinae

When all 19 genes are included, our data provide especially clear resolution of relationships among and within the nine (of 11) sampled tribes of Tortricidae. There is very strong support for placement of Phricanthini (Fig. 2D) as sister group to the remaining Tortricinae (node 9; BP = 99). The subfamilial assignment of Phricanthini has been in question owing to the many distinctive features that this tribe shares with few or no other tortricids, such as an upright (lemon-shaped) egg, abdominal tergal spines, presence of a well-developed saccus in the male genitalia (in some but not all species), and enlarged SD pinacula on the larva [65–68]. Phricanthini are also unusual in that the larvae are restricted to the early-diverging eudicot family Dilleniaceae, where they feed either in shelters on foliage or on living bark [29]. While these features have been used to argue for placement of Phricanthini at the base of the entire family, they now appear to represent autapomorphies rather than synapomorphies with outgroup families. Phricanthini comprise 21 species found in
Molecular Phylogeny for Tortricidae (Lepidoptera)

Australia, Southeast Asia and Madagascar. Their presence in continental Africa and in the Neotropics is almost certainly the result of inadvertent introductions [69].

The remaining Tortricinae diverge basally into two clades, one of which consists of Archipini+Ceracini and Sparganothini+Atteriini (node 10; BP = 95). Members of this clade are characterized by slender, spine-like, deciduous cornuti on the vesica of the phallus. The clade is further corroborated by a multi-step transformation series in egg-laying behavior proposed by Powell [20] (see our Fig. 1A). The condition of laying single eggs or small clusters, shared by all other Tortricinae in our sample, was considered primitive by Powell [20]. In contrast, females in Archipini, Ceracini, Sparganothini, and Atteriini lay their eggs in large clusters, over which the female deposits a layer of colleterial secretion. In an additional step, females of Atteriini have have specialized scales on the venter of the abdomen that are used to build a “fence” around the large egg mass covered by colleterial secretion [68,70]. The grouping of Atteriini with Sparganothini, strongly supported here (node 11; BP = 99), is further suggested by morphological traits that have often been interpreted as synapomorphies rather than synapomorphies, such as the male genitalia having large, rectangular, simple valva and densely scaled soci. Atteriini (Fig. 2E) include 45 species, mainly Neotropical, while Sparganothini (Fig. 2F), also mainly New World, comprise 219 species. The larvae in both tribes are mostly polyphagous leaf rollers [29,33,57].

The strongly supported grouping (node 14; BP = 100) of Archipini with Ceracini (Fig. 2J; considered a distinct family by Diakonoff [71]) was suggested by several previous authors [22–24] based on many morphological similarities, in particular, the overall configuration of the male genitalia. Monophyly for Archipini as sampled here is also strongly supported, as in previous molecular analyses [30,31], but no definite synapomorphies are known. A large number of genera are unambiguously assigned to the tribe based on a brush of hairs from the venter of the uncus in the male genitalia and a curved, spine-shaped signum with a capitulum in the female genitalia, and this core of genera includes Choristoneura (Fig. 2H), Archips, Pandemis, Argotaenia, Clepsis, Epiphyas and many others. However, extensive additional taxon sampling might reveal a slightly different picture, as the tribe also consists of Tortricini with large, day-flying adults with brightly colored forewings, female genitalia with a complex sclerotized costa than is typical in Archipini. According to the morphology-based analysis of Jinbo [74], Ramapesiini comprise an assemblage of plesiomorphic genera that may or not be monophyletic and sister group to the remaining Archipini. In contrast, there is very strong evidence against the monophyly of Eulini: as currently defined the tribe is paraphyletic with respect to Cochylini (node 24; BP = 100). The definition of Eulini has long been problematic. The group was proposed as a subtribe of Cochylini by Kuznetsov and Stekolnikov [17] based on the Holarctic genus Eulia, but was subsequently elevated to tribal status by Powell [78] and redefined to consist almost entirely of Neotropical genera. Many of the included genera were initially described in Archipini by Razowski [79]. The tribe has at times been considered an assemblage of similarly plesiomorphic tortricines [80,81]. The presence of a unique male foreleg hairpencil was hypothesized to represent a synapomorphy for Eulini by J. Brown [80], but this structure subsequently was found in at least one cochlionine genus as well (Aethes; JWB, unpublished observations). Hence, although the character is highly variable (perhaps easily lost or suppressed), as are many male secondary sexual structures, it may represent a synapomorphy for Eulini+Cochylini. The same structure is widespread among males of Schoenotenini, which were not included in our analysis. As currently defined, the Eulini contain 670 species. The larvae are ecologically diverse, including polyphagous leafrollers [82], stem-borers [83], and leaf litter feeders [84].

The strong evidence for paraphyly of Eulini dictates that the tribe should either be subdivided into multiple monophyletic tribes, or combined with Cochylini to eliminate paraphyly. As an interim solution we choose the latter, and hereby formally synonymize Eulini with Cochylini. Our justification is as follows. The taxonomic sampling of the present study, designed to estimate relationships among tribes, provides very limited insight into the structure within them. Current understanding of relationships of the genera now assigned to Eulini is so incomplete that attempting to assign them to monophyletic tribes would leave many incertae sedis. Conversely, given the strong support for the clade Eulini+Cochylini, a broadened definition of Cochylini allows confident assignment of all current euliine genera to a monophyletic tribe. Continued studies may reveal an increasing number of monophyletic subgroups of former Eulini, for example one centered on Eulina+Bonagota (node 22; BP = 100), eventually allowing reclassification and resurrection of a family-group name.
based on *Eulia*. In the meantime, the clearly monophyletic Cochylini s.s. may be provisionally referred to as the subtribe Cochylina.

The strongly-supported grouping (node 27; BP = 100) of Cnephasiini (Fig. 2I) with Tortricini (Fig. 2G) contradicts the hypotheses of Kuznetsov and Stekolnikov [17,21,22] (see Figs. 1B, 1D, 1F) and Razowski [23], but conforms to the view of Powell [20] (Fig. 1A), who cited loss of the costal fold in the male forewing as a shared character change. The proposed relationship is also supported by other characters not previously recognized as synapomorphies, such as the distinctive stellate signum seen in the female genitalia of both tribes, and a “loricaceous ovipositor” that is universal in Cnephasiini and also occurs sporadically in Tortricini. Powell & Common [68] suggested that these specialized setae of the papillae anales were “analogous” between the two tribes. The two tribes also share the condition of laying eggs singly or in small clusters and the lack of a male forewing costal fold (as mentioned above), but these are probably plesiomorphies. Contrary to Powell [20], the tortricid ground plan is now thought to lack the costal fold, which is also missing in most outgroups and in basal tortricids (Chilidianotini and Phricanthisi). Moreover, the phylogenetic interpretation of male secondary sexual characters in Tortricidae has long been controversial [85,86], and a male costal fold occurs sporadically throughout Olethreutinae and Tortricinae.

Monophyly for Cnephasiini as sampled here is strongly supported (node 29; BP = 100) bootstrap), but corresponding morphological synapomorphies are not obvious, because, as discussed above, some of the characters previously thought to support this tribe are now seen as linking them with Tortricini. The distinctive shape of the papillae anales, the finely spined transstilla, and the absence of cornuti are possible synapomorphies. However, circumscription of a monophyletic Cnephasiini is still a work in progress, as some taxa traditionally assigned there seem clearly not to fit, lacking even the characters uniting the tribe with Tortricini. An example is the Australian *Arotaphora* group (sensu Common [87]), restricted to Proteaceae, included in “Cnephasiini sensu lato” by Horak et al. [60] but later “excluded from the Cnephasiini” by Horak [29]. The tribe as currently delimited contains 261 species and is most diverse in the Oriental and Palaearctic regions. The larvae typically feed on tied leaves or tunnel in flowers.

Monophyly of Tortricini was previously supported by the molecular study of Razowski et al. [88], who sampled 23 species in four genera. Morphological synapomorphies for Tortricini include the loss of the uncus, the development of a subscaphium, the development of a brachiola (a unique membranous digitate projection from the valva), raised scales on the forewing, and development of a brachiola (a unique membranous digitate projection from the valva), raised scales on the forewing, and hindwing venation with M2 distant and approximated to the male genitalia. This is however a plastic character that may disappear or be strongly modified secondarily (as in Dichrorampha).

Two large subclades of Olethreutinae apart from Microcorsini are strongly supported. One of these consists of Eucosmini—Grapholitini (node 31; BP = 100%), a grouping proposed by previous authors including Razowski [23] (Fig. 1C) and Kuznetsov & Stekolnikov [17,21,22] (see Figs. 1B, 1D, 1E). A possible synapomorphy is that in the females of both tribes the sternigma is derived from a smooth peristomial sclerite. Both tribes also bear a distinct speculum (ocellar area) on the upper side of the forewing, only faintly visible in a few Olethreutini. This is however a plastic character that may disappear or be strongly modified secondarily (as in Dichrorampha).

The molecular data strongly support monophyly of Grapholitini (node 38; BP = 100%), for which Komai [90] proposed as a possible apomorphy a shortened male sternum 8 with a straight posterior margin. However, the generic groupings within Grapholitini proposed by Danilevsky and Kuznetsov [91] and refined by Komai [90] are not exactly recovered by our data. Komai recognized a Grapholitha group, a Cydia group, and a Dichrorampha group for the Palaearctic fauna; Komai & Horak [92] added a fourth group from Australia (*Laranthycidia* group, not represented in our sampling). The basal position of Dichrorampha is in agreement with Komai’s view. Grapholitha itself as currently defined, however, appears not to be monophyletic. One of the two species sampled here, *G. (Aspila) packardi*, groups strongly (node 40; BP = 95) with *Cydia* (Fig. 2R; in the “Cydia Genus Group” of Komai) and the newly-discovered *Multiquesta*, as predicted by Aarvik and Karish [93], whereas the other, *G. (s. str.) delineana*, groups with *Cryptopheina* (node 41; BP = 70); both are in the “Grapholitha Genus Group” of Komai. Horak and R. Brown [24]: their figure 1.2.5 hypothesized Grapholithi to be a polyphyletic group, derived from within Olethreutini and Eucosmini and defined by convergent reductions in the male genitalia, i.e., loss of the uncus and socius, and hindwing venation with M2 distant and parallel to M3, a hypothesis later rejected by Horak [27]. Our analyses provide no support for this hypothesis. Grapholithini are a cosmopolitan group of 898 described species. The larvae are typically oligophagous borers in fruits, shoots or roots, collectively spanning a very wide range of host plant taxa and growth forms [29,56].

Monophyly for Eucosmini as sampled here is also well supported by our data (node 33; BP = 94) in agreement with preexisting views [17,21–23]. However, no morphological synapomorphy is known for the entire tribe, one of the largest in Tortricidae, and its precise limits are unclear. The classical character, hind wing vein M3 curved and approximated to the stalk of M3 and CuA1 [92,94], is far from universal [27]. Other characters common in but not exclusive to Eucosmini were supported (node 27; BP = 100). The relatively basal position of Microcorsini as sister group to the remaining Olethreutinae as sampled here (node 30; BP = 100). The relatively basal position of Microcorsini is supported by their retention of some apparently plesiomorphic traits shared with Tortricinae. In the female genitalia the sternigma is connected to the apophyses anteriores, and in the male genitalia the coecum of the phallus is large (absent in the rest of Olethreutinae); in the pupa there is an alar furrow with raised margins [29]. Microcorsini (Fig. 2M) are a small, possibly relict group consisting of 36 species in two genera, found on all the southern continents with northward extensions in the Oriental region to Japan and in the Neotropical region to Mexico and the southeastern United States. The larvae bore in nuts, seeds and fruits [29,99].

Relationships within Olethreutinae

Our data strongly support placement of Microcorsini as sister group to the remaining Olethreutinae as sampled here (node 30; BP = 100). The relatively basal position of Microcorsini is supported by their retention of some apparently plesiomorphic traits shared with Tortricinae. In the female genitalia the sternigma is connected to the apophyses anteriores, and in the male genitalia the coecum of the phallus is large (absent in the rest of Olethreutinae); in the pupa there is an alar furrow with raised
Kuznetsov and Stekolnikov’s proposal [22] of a ‘supertribe Eucosmidii’ including Enarmonini, Eucosmini and Grapholitini (Figs. 1B, 1D, 1E) reflects the widespread view that Enarmonini are closely related to Ecosmini in some of our analyses, but its position is weakly supported and varies among analyses (node 13; BP<50). However, Our data do strongly support removal of Enarmonini from Ecosmini, where they were assigned historically [24,95,96], as well as from Ecosmini+Grapholitini. The tribe was recently redefined by Horak [27] to include taxa previously in Ecosmini, but no convincing evidence of its monophyly was found. Enarmonini as presently delineated include 298 species, cosmopolitan in distribution, but especially diverse in the Oriental and Australian regions. The larvae include both leaf rollers and borers [29], typically with restricted host ranges [56].

The remaining subclade of Olethreutinae minus Microcosminini in our results consists of Bactrini (Bactri), Enadotheniini (Endothenia) and Olethreutini (node 43). Apart from Episimus and Oxysemaphora, whose placements are weakly established (nodes 43, 44; BP<50), this clade is strongly supported (node 45; BP = 84). It corresponds approximately to the supra-tribal group “Oletheureutidii” of Kuznetsov and Stekolnikov [17,22] (Fig. 1D, 1E), adopted by Saфонkin [25] (Fig. 1F), equivalent in turn to the broad concept of Olethreutini of Diakonoff [97]. Diakonoff divided Olethreutini s.l. into 12 subtribes, of which five are represented in our sampling: “Bactrae” (by Bactri); “Lobesiae” (by Lobesia), “Endotheniae” (by Endothenia), “Neoptamiae” (by Afrophora); and “Oletheureutae” (by Oxysemaphora, Hedya and Olethreutae). Subsequent authors, including Kuznetsov and Stekolnikov [see Fig. 1], treated some of these groups as tribes.

Within the “Oletheureutidii” clade (node 43), the tribes Bactri and Endothenii, each represented by its type genus, are strongly supported as sister groups (node 49; BP = 100). The monophyly of Bactri, unquestioned since the tribe was proposed by Falkovitsch [84], is supported by multiple synapomorphies, including a spined globular saccus in the male genitalia, an anteriorly invaginated sterigma in the female, and a characteristic cryptic forewing pattern (Fig. 2O). The close relationship of Endothenii to Bactri was recognized by Dang [26], based on Endothenia. He suggested two synapomorphies in the male genitalia: a convex, decurved tegumen, and an unusual condition in which the dorsal edge of the basal opening (or basal cavity) and the basal portion of the costa of the valva are confluent... Dang also argued that the set of secondary sexual characters used by Falkovitsch [84] to define the tribe Olethreutini, as well as the typical distal setae of the tarsi in both sexes of that tribe, had been secondarily lost in Endothenia and Bactra. He therefore transferred Endothenia to Bactri. Although Horak & R. Brown [24] and J. Brown [1] maintained the previous definition of Endothenii, Horak [27,29] accepted the synonymy with Bactri. Bactri s.s. are adapted to grasslands associated with mesic habitats, where their hosts are dominant; the larvae are stem borers in Poaceae, Juncaceae and Cyperaceae (94% of records; [56]). The 52 species in Endothenii are typically borers in stems, roots, flowers or seeds of herbaceous plants, especially in the clade Asteriidae. Bactri s.s. are a cosmopolitan but mainly Old World group of 113 species.

While in some respects our results agree with previous ideas on the “Oletheureutidii clade,” the placement of Bactri+Endothenii is a point of conflict. Prior phylogenies (Fig. 1) that include Bactri as a terminal taxon portray that tribe (and sometimes Endothenii) as branching off early from the remainder of “Oletheureutidii”, leaving Olethreutini s.s. in a relatively derived position. Horak [29] stated that “Obvious similarities in the genitalia and shared reduction of tarsal setae suggest a close relationship between Gatesclarekania [formerly considered to be at the base of Olethreutinae but included by Horak [29] within Olethreutini following the Neoptamia group], Endothenia, and Bactri.” Our results strongly place Bactri+Endothenia as deeply nested within Olethreutini as currently defined (nodes 46, 45; BP = 100, 84), rendering the latter paraphyletic. This finding is consistent with the lack of clear synapomorphies for Olethreutini. Olethreutini as recently delimited are a cosmopolitan group of 1,077 species whose larvae are typically leaf rollers.

The strong evidence for paraphyly of Olethreutini requires either that the tribe be subdivided into multiple monophyletic tribes, or that its definition be broadened to eliminate the source of paraphyly. For several reasons, we take the latter course, and hereby formally synonymize Bactri and Endothenii with Olethreutini. Horak [27] foreshadowed this conclusion. The present limited taxon sample was designed to estimate relationships among tribes, and provides little new information on structure within them. Current understanding of the taxa belonging to “Oletheureutidii” is so incomplete that an attempt to break it into monophyletic tribes would leave many genera incertae sedis. Conversely, a broadened definition of Olethreutini would immediately place most genera (though not all; see below) confidently in a monophyletic tribe. With further research, an increasing number of clearly-defined subtribes [97] or generic groupings (sensu Horak [27]) should emerge, along with better understanding of their relationships. At that point it might be desirable to (re-) elevate the rank of those sub-clades, and to break up, or elevate in rank, the Olethreutini sensu novo proposed here.

The Diakonoff subtribe Neoptamiae exemplifies recent progress in this direction. Recent study of African and related taxa has clarified the content of this group and yielded a striking synapomorphy supporting its monophyly, a characteristic sigma in the female genitalia consisting of a single plate with 1–3 anteriorly directed projections [98,99]. Furthermore, the present study, in which Neoptamiae are represented by Afrophora (Fig. 2N), provides strong evidence that this subtribe is related to Bactrii+Enadothenii (node 47; BP = 100), and is possibly even the sister group of these (node 48; BP = 62). Several shared morphological characters of Bactrii and the Gatesclarekania and Neoptamiae groups are discussed by Horak [27]. These related clades differ markedly in the prevalence of male secondary sexual characteristics such as ancillary scent organs, which are widespread in Neoptamiae but nearly absent in Bactrii+Endothe-

The exact limits of Olethreutini sensu novo remain tentative, even in our own results. The grouping of Episimus and Oxysemaphora with the other Olethreutini sampled is very weakly supported (nodes 43, 44; BP<50), and these taxa fall elsewhere on the tree, also with little support, when synonymous change is excluded. Episimus is an enigmatic genus which Diakonoff [97] did not try to place in his classification, but Oxysemaphora was placed in his “Oletheureutae.” Horak [27] tentatively includes both genera in the Oxysemaphora group at the base of Olethreutini, acknowledging the numerous plesiomorphic characters. Thus, further instances of paraphyly of Olethreutini, with respect to Enarmonini+Eucosmini+Grapholitini [24,29] or other taxa, cannot be confidently ruled out, and further adjustments may be necessary.

Molecular Phylogeny: Summary and Conclusions

The molecular analyses presented here yield a new working hypothesis of relationships among the tribes and subfamily of Tortricidae (Fig. 3) in which the great majority of inferred groupings (82%) are strongly supported (≥80% bootstrap). The
molecular phylogeny mostly upholds previous hypotheses, but not always. Its major features are as follows:

1. The subfamilies Tortricinae and Olethreutinae are each monophyletic, and are sister groups. Previously, Tortricinae had been hypothesized to be para- or polyphyletic.

2. Chlidanotinae are confirmed to comprise the earliest-diverging tortricid lineages. In contrast to morphology, the present molecular data moderately favor paraphyly for this subfamily, with Polyorthini as sister group to all other tortricids. This result varies with outgroup and character choice, however, and no strong conclusion can be drawn.

3. Within Tortricinae, Phricanthini diverge first, while the remaining tribes form two strongly supported sister groups: (Sparganothini+Atterini)+ (Ceracini+Archipini), and (Cneosphaeriini+Tortricini)+(Eulini+Cochylini). Eulini are paraphyletic with respect to, and are here synonymized with, Cochylini.

4. Within Olethreutinae, Microcorsini diverge first, while the remaining tribes fall into two lineages. One of these contains the strongly supported group (Eucosmini+Grapholitini), joined with very weak support by Enarmonini. The other consists of Olethreutini+(Bactrini+Endotheniini), with the latter group nested inside, and here synonymized with, the former. The limits of Olethreutini as thus redefined are tentative, as the association of two early-diverging genera with the remainder is very weakly supported (<50% bootstrap).

Evolutionary trends in life history and distribution

The new phylogenetic framework permits re-examination of several conjectures about life history evolution and biogeography in Tortricidae. To facilitate such a review, we present in Figure 5 a provisional synopsis of species diversity, prevailing larval feeding habits and geographic distribution for the tribes included in this study, synthesized from Brown et al. [56], Janzen and Hallwachs [57], and other sources. These are superimposed on a simplified version of the molecular phylogeny, in the manner of Powell et al. [33], with tribes as the terminal taxa. This overview has multiple limitations, among which are the paucity of observations for some tribes, the absence of three tribes from our sampling, and ambiguity in the interpretation of some host records (see Methods). For these reasons we do not attempt any formal comparative analyses, and aim mainly to help to identify the most promising directions, and most critical observations to be made, for future more detailed studies.

There have been multiple conjectures on the ancestral larval feeding habits of Tortricidae [33]. The idea that the most basal tortricids belong to Tortricinae, which include several saprophagous groups, led to the suggestion the family initially fed on detritus or fungi [24]. In the new phylogeny, however, the basal lineages of the family as a whole (i.e., Chlidanotinae), as well as of Tortricinae (i.e., Phricanthini) and Olethreutinae (i.e., Microcorsini), are all phytophagous so far as is known, very strongly suggesting that this was the ancestral tortricid condition. Subsequent shifts to saprophagy or mycophagy have apparently occurred multiple times, e.g., in Tortricinae: Epitymbiini (not included here) and some Eulini.

For phytophagous tortricids, it has previously been postulated that the ancestral habit was external feeding, albeit from within a rolled leaf or other shelter, as is typical for external feeders outside the Macroheterocera [24,33]. The new phylogeny, in contrast, suggests that the ancestor is equally likely to have been an internal feeder; the data available do not support a confident choice between these alternatives. Observations on Chlidanotini and Hilarographini are few, but suggest that both tribes are borers as larvae, in twigs, fruits or seeds. On the other hand, both internal feeding and leaf rolling have been reported from the most basal lineage, Polyorthini, and there is no information as to which is more primitive. However, an anal comb is present in all known polyorthine larvae, suggesting external feeding as this structure serves to eject feces from the larval feeding shelter. Appeal to outgroups does not help, as no other lepidopteran lineage is obviously closely related to Tortricidae.

Limited life history evidence, long branches and repeated transitions between internal and external feeding may preclude us from ever confidently deciding whether or not Tortricidae were ancestrally internal feeders. However, there is clear evidence within the family for broad-scale evolutionary trends in this character, as well as extensive homoplasy (Fig. 5). External feeding was probably ancestral for Tortricinae (node 8), in which nearly all the tribes, including the earliest-branching (Phricanthini), consist entirely or mainly of external feeders. The only major internal-feeding tortricine lineage is Cochylini sensu stricto, which probably evolved from an externally-feeding ancestor, given its highly derived cladistic position (nodes 21–24). In contrast, Olethreutinae (node 7) show much more even representation of, and more frequent apparent transition between, internal and external feeding. Although the oldest lineage, Microcorsini, feeds internally, the ancestral state for the subfamily is not obvious, as the earliest-branching lineages within both major subdivisions of the remaining olethreutines (nodes 31, 43) are partially or entirely external feeding. Repeated shift in habit is evidenced by the fact that two independent tribes (Enarmonini, Eucosmini) show substantial proportions of both internal and external feeders. Moreover, both major subdivisions of olethreutines (nodes 31, 43) include a predominantly internally-feeding lineage (Grapholitini; Bactrini+Endotheniini) which is sister-group to and/or nested within an externally-feeding lineage. Two recent studies suggest that external-feeding phytophagous insect lineages may consistently undergo greater diversification than their internal-feeding relatives [100,101]. Given the patterns documented here, it appears that Tortricidae can potentially contribute important evidence on this hypothesis. For Olethreutinae, internal feeding appears to have favored diversification, at least in the Eucosmino-Epithelima group of genera, all with known larva being internal feeders, as well as in Dichrorampha and many other Grapholitini.

Previous authors have also addressed the evolution of host range and its correlation with other life history traits. As is evident from our compilation (Fig. 5), host range varies widely within many tribes and genera of Tortricidae, but also shows broader phylogenetic trends. In Chlidanotinae and Olethreutinae, oligophagy as defined and measured here (see Methods) is the predominant habit in nearly all tribes. In Tortricinae, on the other hand, polyphagy predominates in seven of the nine tribes studied. Within Tortricinae, relative prevalence of polyphagy is consistent with Powell's hypothesis [20] of an association with oviposition in large clusters. Deposition of eggs singly or in small scattered groups, found in most tortricid tribes, is probably the ancestral condition both in Tortricinae and in the family as a whole. Oviposition in large clusters is restricted to, and may have arisen in the ancestor of, the clade consisting of Atteriini+Sparganothini and Ceracini+Archipini (node 10). All four tribes in that clade also have higher estimated proportions of polyphagous species (69–100%) than all other tortricine tribes, and indeed all other tribes in Tortricidae except Microcorisini. There is also an apparent correlation of host range with feeding mode. Both across Tortricidae as a whole, and within the two largest subfamilies, tribes which are entirely or mainly internal feeders almost invariably have lower...
fractious of polyphagous species than any of the tribes which feed outside the plant, mostly as leaf rollers or tiers. The only exceptions to this trend are the earliest-diverging (and relatively little-studied) tribes in both Olethreutinae and Tortricinae, namely the Microcosini, whose larval feed inside seeds or fruits, and the Phricanthini, which appear to live on bark or leaves. This ostensible pattern merits detailed examination in future studies, as a linkage between internal feeding and restricted host range has been repeatedly postulated but rarely tested empirically [100,101].

Previous authors have also remarked, finally, on the mainly southern geographic distribution of several putatively plesiomorphic tortricid tribes or genera, suggesting that these taxa represent relics from an ancestral Gondwanan radiation of Tortricidae [29,102,103]. The new phylogeny (Fig. 5) appears at least consistent with this idea. The earliest-branched tribes in the family as a whole, as well as within the two largest subfamilies – Polychrotini, Chilidanotini, Hilarographini, Phricanthini and Microcosini – are all relatively species-poor groups with mainly southern/tropical distributions. The cosmopolitan distributions typical of the other tribes, especially the larger ones, with substantial representation in temperate regions, appear to be derived. Additional evidence, including fossil-calibrated divergence dating, is needed to further test the Gondwanan-origin hypothesis.

Supporting Information

Table S1 List of specimens sampled, including collection localities, LepTree voucher identification numbers and codes, and GenBank accession numbers.

(XLS)

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