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Phylogenetic Analysis of the New World Family Heterothripidae (Thysanoptera, Terebrantia) based on Morphological and Molecular Evidence

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Abstract
The New World family Heterothripidae (~90 spp., four genera) comprises flower-feeding and ectoparasitic thrips. The monophyly of the group has remained untested and species-level relationships were unknown. Morphological (123 characters) and molecular (28S rDNA D2 and D3-D5, H3, and partial COI) data were compiled to reconstruct phylogenetic relationships of this group. The ingroup was represented by 65 species of the four recognized Heterothripidae genera (*Aulacothrips* Hood, *Heterothrips* Hood, *Lenkothrips* De Santis & Sureda, and *Scutothrips* Stannard). The monophyly of Heterothripidae was recovered in the total evidence and molecular data only analyses with the ectoparasitic *Aulacothrips* placed as the sister group of the remaining Heterothripidae. The large genus *Heterothrips* (>80% of the species-level diversity), which was thoroughly sampled in our analyses (56 species), was recovered as paraphyletic with respect to *Scutothrips* and *Lenkothrips*. We conclude that additional morphological and molecular data would be desirable before revising the classification of Heterothripidae

Keywords
cladistics; ectoparasitic thrips; flower feeding thrips; morphology

Introduction

Of the nine extant families of Thysanoptera, only Heterothripidae is restricted to the New World with members ranging from Argentina to the Northeastern USA. About 90 species are currently recognized in this family and are classified into four genera: *Aulacothrips* Hood (5 spp.); *Heterothrips* Hood (76 spp.); *Lenkothrips* De Santis & Sureda (5 spp.); and *Scutothrips* Stannard (4 spp.) (ThripsWiki 2018). Remarkably,

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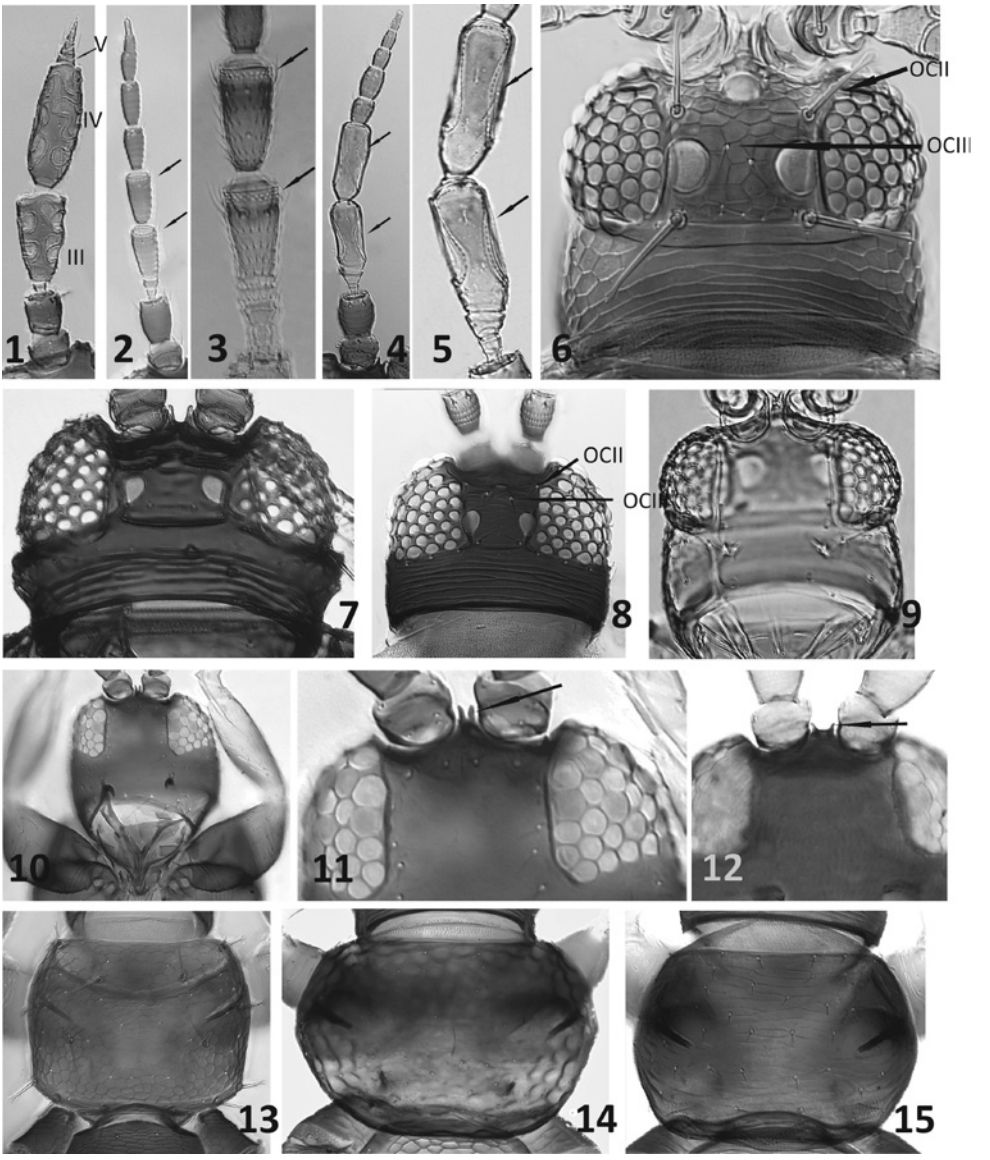
two very distinct life styles occur in this group. Species of *Heterothrips*, *Lenkothrips* and *Scutothrips* are exclusively flower-feeders, with many exhibiting some degree of host specificity and a few potentially acting as pollinators (Rust 1980; Cavalleri & Mound 2014). *Aulacothrips* species, in contrast, are the only Thysanoptera that exhibit an ectoparasitic life style, attacking ant-tended hemipterans in South America (Cavalleri et al. 2010, 2012, 2014). Despite their restricted distribution and considerable diversity in the Neotropics, no targeted phylogenetic analysis and widely accepted modern classification are available for Heterothripidae. The monophyly of this family was never tested but cladistic studies for the order using molecular and morphological data suggest that it is closely related to the extant Stenurothripidae, a family that is found in western North America and from the Mediterranean region to India (Mound & Morris 2007; Buckman et al. 2013). However, these studies included only few *Heterothrips* species as representatives of the entire family, leaving its monophyly and relationships within the group largely untested and unknown.

The family Heterothripidae was erected by Bagnall (1912) and diagnosed based on "the structure and segmentation of the antennae, the characters of the sensoria, and the tarsal appendages" (Bagnall 1912: 222). The tarsal appendage mentioned by Bagnall possibly refers to a claw-like thickening on the apex of the fore tarsus, although this feature is also found in other thrips including aeolothripids and the closely related stenurothripids (Mound et al. 1980). The most recent diagnosis of Heterothripidae is based on the nine-segmented antennae and a sensorial area that is formed by a continuous porous band (=circumpolar) on antennal segments III and IV (Mound & Marullo 1996) (Figs. 1–5). Heterothripidae appears to share a fairly conservative, and likely plesiomorphic, morphology although *Aulacothrips* shows some modifications in its external anatomy (Mound & Morris 2007; Pereyra & Cavalleri 2012; Cavalleri et al. 2014) (Figs. 1, 6, 9, 13, 16, 20, 23).

Although useful for grouping Heterothripidae into distinct genera, most characters used in generic diagnoses are poorly defined and possibly prone to convergence. Because they are not defined by uniquely derived characters, the currently recognized genera may not be monophyletic and the family classification may therefore not reflect phylogenetic relationships. For instance, *Scutothrips* species have a triangular metanotum with a strongly reticulate sculpture and a pronounced transverse ridge posterior to the eyes (Figs. 7, 17). However, the heads of *Heterothrips flavicornis* Hood (1915) and *H. pubescens* Hood (1934) also carry a projection behind the eyes. Additionally, the triangular pattern on the metanotum is similar to that of *Scutothrips* (Mound & Marullo 1996). *Lenkothrips* was erected by De Santis & Sureda (1970) as a subgenus of *Heterothrips* and recognized at genus level by Mound & Marullo (1996) to include one Brazilian species with a distinctive sensorium on antennal segments III–IV, which have one lateral loop which extends to the midpoint of these segments (Fig. 4, 5). This character may be unusual, but it is also found in *Aulacothrips*. In addition, all *Lenkothrips* species are quite similar in external morphology to several species of *Heterothrips* (Mound & Marullo 1996; Cavalleri & Mound 2014). Finally, members of *Heterothrips* are not characterized by any unique feature but by a combination of characters shared with other members of the family. This genus currently includes



Figs. 1–15. Antennae: **1.** *Aulacothrips minor* fem. **2.** *Heterothrips stellae* fem. **4.** *Lenkothrips guaraniticus* fem. Sensoria on antennal segments III–IV: **3.** *Heterothrips paulistarum* fem. **5**. *Lenkothrips kaminskii* fem. Head dorsal view: **6.** *Aulacothrips amazonicus* fem. **7.** *Scutothrips nudus* fem. **8.** *Heterothrips albipennis* fem. Head ventral view: **9.** *Aulacothrips amazonicus* fem. **10.** *Heterothrips pectinifer* fem. Interantennal projection: **11.** *Heterothrips pectinifer* fem. **12.** *Heterothrips obscurus* fem. Pronotum dorsal view: **13.** *Aulacothrips minor* fem. **14.** *Scutothrips nudus* fem. **15.** *Heterothrips pedicellatus* fem.
Abbreviations: OCII, ocellar setae II; OCIII, ocellar setae III.

those species that exhibit circumpolar sensoria being restricted to the apex of antennal segments III–IV (Figs. 3–4) and a metanotum with concentric lines of sculpture bearing microtrichia (Figs. 18–19). However, this type of sensorium is also found in *Scutothrips*, and as mentioned above, certain species of *Heterothrips* show the metanotal sculpture arranged in a triangular pattern. Moulton (1932) divided *Heterothrips* into two groups using characters present on the abdominal tergites. His 'group I' shows a posteromarginal fringe of microtrichia (Fig. 25), whereas the species of 'group II' have microtrichia that arise on the posterior margin of the abdominal tergites from a well-developed craspedum (Fig. 24). This classification has never been tested and these informal groups are only used by a limited number of taxonomists.

An alternative classification for heterothripids was proposed by Bhatti (2006), who considered Heterothripidae as a superfamily, Heterothripoidea, and erected the family Aulacothripidae to include the genus *Aulacothrips*. This decision was based on the greatly enlarged antennal segments III and IV and the presence of sensorial areas that curve multiple times and extend across the entire antennal segments (Fig. 1). Bhatti treated Aulacothripidae as the sister taxon of the rest of the Heterothripidae, but like the previous studies on these thrips, this classification lacked explicit analyses of phylogenetic relationships. Consequently, there is no consensus on the relationships among genera of Heterothripidae, which limits the understanding on character and life style evolution as well as the temporal diversification of the group.

Based on extensive field and museum work, we have assembled a collection of Heterothripidae that includes species representing all four genera and an extensive sample of the described and undescribed species-level diversity of *Heterothrips*. Based on this comprehensive taxon sample, we here perform the first phylogenetic analysis of Heterothripidae using morphological and molecular characters to (i) test its monophyly, (ii) analyze phylogenetic relationships among the four currently recognized genera, and (iii) analyze relationships within the large genus *Heterothrips*.

Materials and methods

Taxon sampling

A total of 101 terminals belonging to 97 species of four of the eight Terebrantia families were included in this analysis, although molecular data were not available for all of the terminals. The ingroup was represented by 65 species of the four recognized Heterothripidae genera: three species of *Aulacothrips*, 56 species of *Heterothrips*, four species of *Lenkothrips*, and two species of *Scutothrips*. The outgroup included 32 taxa of Aeolothripidae, Melanthripidae, and representatives of the four subfamilies of Thripidae: Dendrothripinae, Panchaetothripinae, Sericothripinae, and Thripinae. Supplementary material 1 summarizes voucher information including the material studied, depositories, GenBank accession numbers, collecting locality, and other data.

Specimens used for the molecular analyses were collected in Australia, Southwestern USA, Costa Rica, Brazil, and Argentina and were stored in 95% ethanol

(Supplementary material 1). A unique number code was associated with each voucher specimen. Molecular data were obtained for a total of 57 taxa, including all four genera of Heterothripidae (Supplementary material 1). Voucher specimens were slide mounted in Canada balsam for species-level identification following DNA extraction. Identification was performed using comparisons with type specimens and other authoritatively identified material representing the different families (Supplementary material 1); species descriptions, and identifications keys (Bailey & Cott 1954; Mound & Marullo 1996; de Borbón 2010; Pereyra & Cavalleri 2012). Undescribed new species are listed as 'sp.1, sp.2, etc.' and the material that could not be identified to species level is referred to as 'sp.' In the event that two specimens of the same species from different localities were included, they were distinguished by adding the locality name next to the species name.

Molecular Markers and Primers

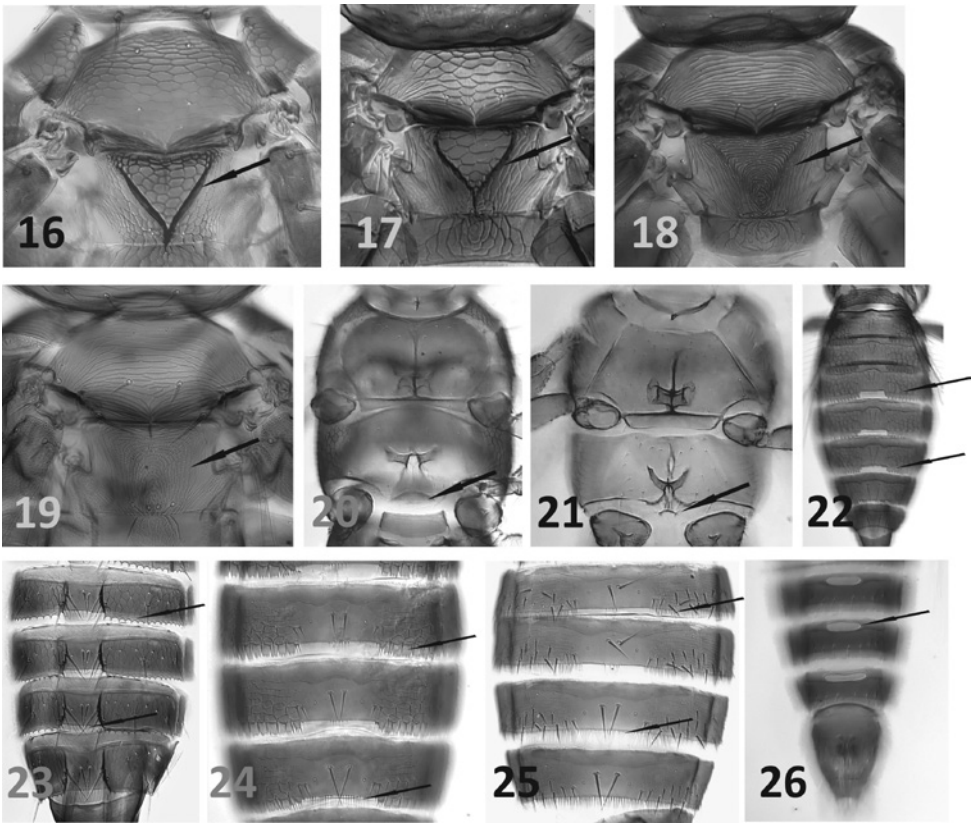
Four molecular markers were amplified comprising two regions of the ribosomal 28S rDNA gene (D2 and D3-D5), the nuclear gene *Histone 3* (H3), and part of the mitochondrial *cytochrome oxidase c subunit I* (COI) gene. These markers were successfully used in previous phylogenetic studies for the order and related groups (Johnson & Clayton 2000; Weirauch & Munro 2009; Buckman et al., 2013). For reference on primer information and PCR thermocycling regimes see Weirauch & Munro (2009), Buckman et al. (2013) and Johnson & Clayton (2000).

DNA Extraction, Amplification, Purification, and Sequencing

Entire specimens were used for DNA extraction, clearing specimens as part of the process and therefore preparing them for slide mounting. Standard protocols for DNA extraction were performed, following protocols provided with each of the kits. The Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) was used for DNA extraction. PCR was amplified using Roche Taq for most of the material. For samples that were difficult to amplify, we used Illustra PuReTaq Ready-To-Go PCR beads. PCR products were checked on an agarose gel and purified using SureClean (Bioline) for the ribosomal genes and Exosap IT for the mitochondrial and nuclear genes. DNA sequencing was conducted at the Genomics Core facility of the Institute for Integrative Genome Biology at the University of California, Riverside (UCR) for the ribosomal genes and the Pritzker laboratory at the Field Museum for the mitochondrial and nuclear genes. Sequences are deposited in GenBank (Supplementary material 1).

Morphological data set

A total of 123 characters were included in the analysis: 14 were continuous characters, 37 binary, and 72 multistate; 16 discrete characters were treated as additive. Morphological characters were coded from voucher specimens and compared with information from the literature. The continuous characters were scaled from 0–1.



Figs. 16–26. Mesonotum–metanotum: **16.** *Aulacothrips minor* fem. **17.** *Scutothrips nudus* fem. **18.** *Heterothrips pedicellatus* fem. **19.** *Heterothrips pectinifer* fem. Mesosternum–Metatasternum: **20.** *Aulacothrips minor* fem. **21.** *Heterothrips gillettei* fem. Abdominal tergites: **22.** *Scutothrips nudus* fem. **23.** *Aulacothrips minor* fem. **24.** *Heterothrips pedicellatus* fem. **25.** *Heterothrips paulistarum* fem. Male pore plates on abdominal sternites: **26.** *Scutothrips nudus* fem.

For additional comparative analysis continuous characters were also log-transformed; and log-transformed and scaled from 0–1. The list of characters and the description of the most remarkable morphological characters (e.g. Ch 33–35, Fig. 8; Ch 38, 47, Figs. 10–12; Ch 78, Fig. 21; Ch 120, Fig. 26) are included as supplementary material 2.

Sequence Alignment and Phylogenetic Analysis

Sequences were edited and concatenated using Sequencher 4.8 or Geneious R7.1.2. Sequences were aligned individually with MAFFT (L-INS-i and Q-INS-i strategies) (Katoh & Standley 2013) to compare effects of alignment on phylogenetic analyses. Mesquite 3.1 (Maddison & Maddison 2016) was used to check the alignments and to concatenate the aligned gene regions. The lengths of the combined aligned datasets ranged from 2332 bp (L-INS-i) to 2376 bp (Q-INS-i). Internal gaps were treated as fifth character state and terminal gaps converted to missing data.

Parsimony analyses were carried out for the morphological and molecular data sets (Supplementary material 3); both data sets were analyzed separately and in combination. The combined analysis was performed in TNT (Goloboff et al. 2008; Goloboff & Catalano 2016). The searches were conducted using New Technology Search with tree-drifting, sectorial search, and tree-fusing. The matrix was analyzed under three different concavity values (K 8, K10, and K12) using extended implied weighting (Goloboff 2013). The default K value is 3 which weights strongly against homoplasy. Therefore, a variety of less strict concavity functions (K8, K10, and K12) were employed to determine the effect of character weighting on hypotheses of relationships. The use of the extended implied weighting method is more adequate for analyses of combined morphological and molecular data sets, as well as data sets with a large number of missing entries (Goloboff 2013). Since some of the taxa were scored only for morphological data, entire gene regions are missing for specific taxa in the molecular data set (see supplementary material 3). Using extended implied weighting (Goloboff 2013); we are aiming on minimizing the effect of these missing entries. Each data partition was weighted according to its average homoplasy, i.e. we determined homoplasy in the entire partition and then gave each character in the partition the same weight. Using this approach, the inclusion of uninformative (i.e. missing) characters may influence the results by inflating implied weights for a given partition (Goloboff 2013). For the purpose of weight calculation, we therefore assume that these missing entries have 50% of the homoplasy of the observed entries. Homoplasy in missing entries could not increase beyond 5 times the observed homoplasy.

Symmetric resampling (Goloboff et al. 2003) and relative Bremer support (Goloboff & Farris 2001) were calculated as support measures (Table 1). Relative Bremer support was calculated from 2500 suboptimal trees, found in 10 rounds, with a fit of up to 0.5 units lower than the previous trees. Symmetric resampling values were calculated using tree-drifting and sectorial search for each replication (1000 altogether) and keeping 1 optimal tree per replication. Symmetric resampling values are shown as GC values (Goloboff, et al. 2003) (or GC for group present/contradicted). GC has the advantage over standard group frequencies of reporting the support of groups retrieved after Jackknife with low resample frequency (with less than 50%), which are otherwise collapsed.

Optimal trees obtained from the analyses of the molecular, morphological and combined data sets under the three concavity values, and the two alignments strategies, were compared. The optimal trees obtained from the analysis of the combined data set under K10, Q-INS-i, alignment and continuous characters scaled from 0–1 will be referred as the reference trees. These trees were selected because they were the most resolved. The comparison measures employed were: 1. SPR Distance and Similarity: a. SPR Distance: The number or SPR-swaps necessary to transform one tree to another. b. Similarity: The number of SPR-swaps divided by the number of taxa. 2. Distortion Coefficient: The RI of the MRP of one tree mapped onto the other matrix. 3. Number of nodes of the strict consensus between the trees under comparison. Comparison measures were calculated as implemented in TNT (Goloboff & Catalano 2016).

Results

Parsimony analyses of the two combined datasets (morphological and molecular L-INS-i and Q-INS-i alignments) resulted in 4 optimal trees for each of the three concavity values employed (K8, K10, and K12; length: L-INS-i 357.25–357.28; 357.55–357.61; Q-INS-i 357.71–357.73). Morphological synapomorphies are provided in supplementary material 4 and support values in Table 1. The strict consensus of these 12 fundamental trees is shown for the L-INS-i (Fig. 27a) and Q-INS-i (Fig. 27a) alignments. The monophyly of Heterothripidae was recovered in all analyses under all concavity values and both of the alignment strategies we employed (Fig. 27). The hypothesis based on molecular data also strongly supports the monophyly of Heterothripidae (Fig. 28), although Heterothripidae is rendered polyphyletic – with *Aulacothrips* as being only distantly related to the remaining Heterothripidae (Fig. 29) – in analyses of the morphological data set alone.

Heterothripidae was supported by numerous morphological synapomorphies in the combined analyses (both datasets; Fig. 27, Supplementary material 4). In all trees, *Heterothrips* was rendered paraphyletic by *Lenkothrips* and *Scutothrips*, with *Aulacothrips* being recovered as the sister group to the remainder of the family (*Heterothrips* + *Scutothrips* + *Lenkothrips*). Within the *Aulacothrips* clade, *A. amazonicus* forms the sister taxon to *A. minor* + *A. dictyotus*. The genus was strongly supported in the analyses with 20 morphological synaphomorphies (Supplementary material 4).

The *Heterothrips* + *Scutothrips* + *Lenkothrips* clade is subdivided into two large groups (Fig. 27). One clade is comprised of *Lenkothrips* and *Heterothrips* species with a posteromarginal comb of microtrichia on the abdominal tergites (ch 100). This clade is recovered under both analyses (L-INS-i and Q-INS-i alignments, Fig. 27) with the same topology. The second clade comprises *Scutothrips*, (*H. auranticornis* + *H. gillettei*), and most *Heterothrips* species with a posteromarginal craspeda (Ch 100) on the abdominal tergites (Figs. 22–24) – although this second clade is only recovered under Q-INS-i alignment strategy (Fig. 27). We here therefore refer to these clades as groups A (comb of microtrichia) and B (craspeda). Note that these groups are not sensu Moulton because they now include *Lenkothrips* and *Scutothrips.*

Panchaetothripinae species + *Bradinothrips williamsi* were recovered as the sister group of Heterothripidae (Fig. 27b) in the combined analyses under Q-INS-i alignment although the support values were low or negative. Aeolothripidae was recovered as the sister group of all of them (Fig. 27b). This result contradicts the most recent phylogenetic hypotheses of the entire order (Buckman et al. 2013), where Heterothripidae was placed as the sister group of Stenurothripidae, both sister to Melanthripidae. However, Stenurothripidae was not included in our analyses. Within Aeolothripidae, taxa of *Dactuliothrips* were recovered as the sister group to the remaining Aeolothripidae. *Desmothrips* was the sister group of *Stomatothrips* – both being the sister group of *Erythrothrips*. The Thripidae subfamilies Dendrothripinae, Sericothripinae, and Panchaetothripinae were recovered as monophyletic groups (Fig. 27). Thripinae was always recovered as a polyphyletic assemblage.

Table 1. Relative Bremer support (RBS) and symmetric resampling (SR) values for major groups for the total evidence analysis under the three concavity values and the two alignment strategies employed for the analyses.

	L-INS-i alignment						Q-INS-i alignment					
	RBS			SR			RBS			SR		
Group / Concavity value	**K8**	**K10**	**K12**	**K8**	**K10**	**K12**	**K8**	**K10**	**K12**	**K8**	**K10**	**K12**
Heterothripidae	3	4	100	95	87	81	100	100	47	86	96	90
Aulacothrips	100	100	100	100	100	100	100	100	100	100	100	100
Heterothrips + *Scutothrips* + *Lenkothrips*	3	4	17	63	67	65	30	32	34	75	71	74
Group A (Abdomen with posteromarginal microtrichia)	17	18	19	–25	–17	–21	16	17	18	–23	–24	–20
Group B (Abdomen with posteromarginal craspeda)	12	12	33	–28	–24	–30	14	15	16	–21	–23	–22
Panchaetothripinae + Heterothripidae	7	14	–	–28	–41	–	12	13	13	–43	–36	–34
Aeolothripidae + Heterothripidae	–	–	100	–	–	36	–	–	–	–	–	–
Aeolothripidae	100	100	100	90	87	87	100	100	100	75	88	79
Dendrothripinae	100	100	100	98	100	100	100	100	100	96	100	100
Panchaetothripinae	11	22	22	13	16	12	12	13	13	30	46	40
Sericothripinae	100	100	100	100	100	100	100	100	100	100	100	100

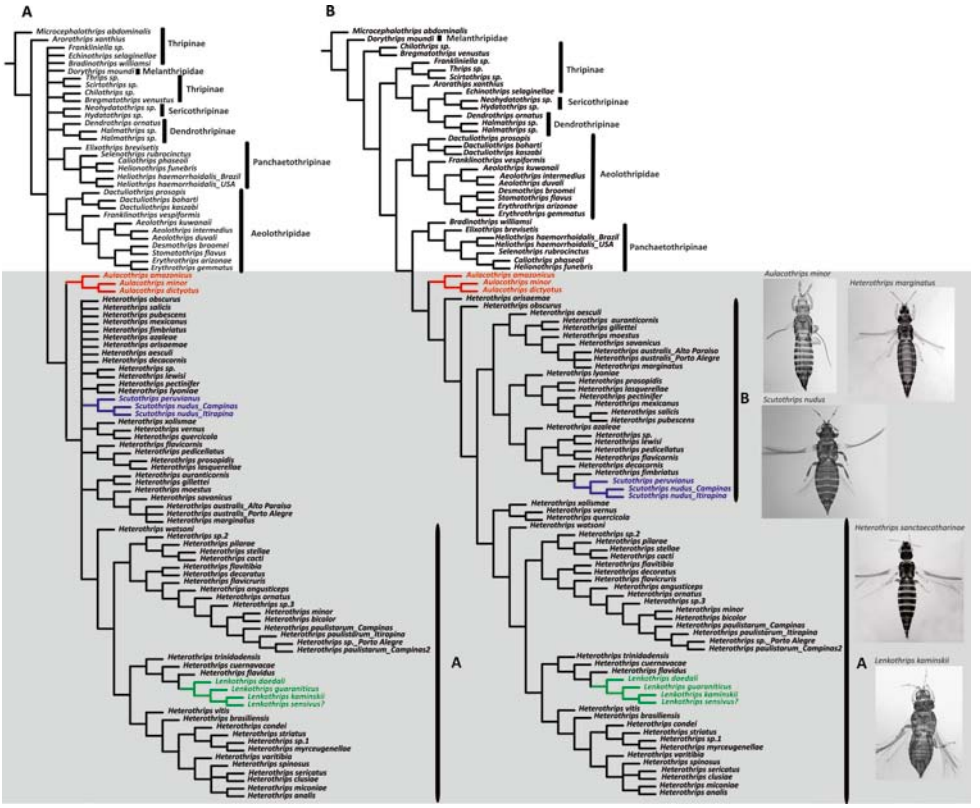


Fig. 27. Consensus of the 12 optimal trees obtained from the analysis of the morphological and molecular data set under K8, K10, K12 **(a)** L-INS-i alignment strategy, **(b)** Q-INS-i alignment strategy.

Heterothripidae, *Aulacothrips*, and the *Heterothrips* + *Scutothrips* + *Lenkothrips* clade showed high support values for symmetric resampling and medium to low values for relative Bremer support (Table 1). Relationships within the *Heterothrips* + *Scutothrips* + *Lenkothrips* clade had low or negative values for symmetric resampling. Groups A and B showed negative-to-low support values for both support measures (Table 1).

In the molecular data-only analyses, the strict consensus of the optimal trees recovered using the L-INS-i alignment under all three concavity values (K8, K10, K12) supported a monophyletic Heterothripidae (Fig. 28a). Relationships between the Heterothripidae genera were similar to those recovered in the combined molecular and morphological analyses. Again, the two groups within the *Heterothrips* + *Scutothrips* + *Lenkothrips* clade were recovered with *Lenkothrips* nested inside group A and *Scutothrips* inside group B (Fig. 28a). The analysis based on the Q-INS-i alignment also recovered a monophyletic Heterothripidae with *Aulacothrips* as the sister group of the rest of the members of the family (Fig. 28b). Again, the *Heterothrips* + *Scutothrips* + *Lenkothrips* clade was divided into two groups. Aeolothripidae resulted in being the sister group of Heterothripidae in this analysis.

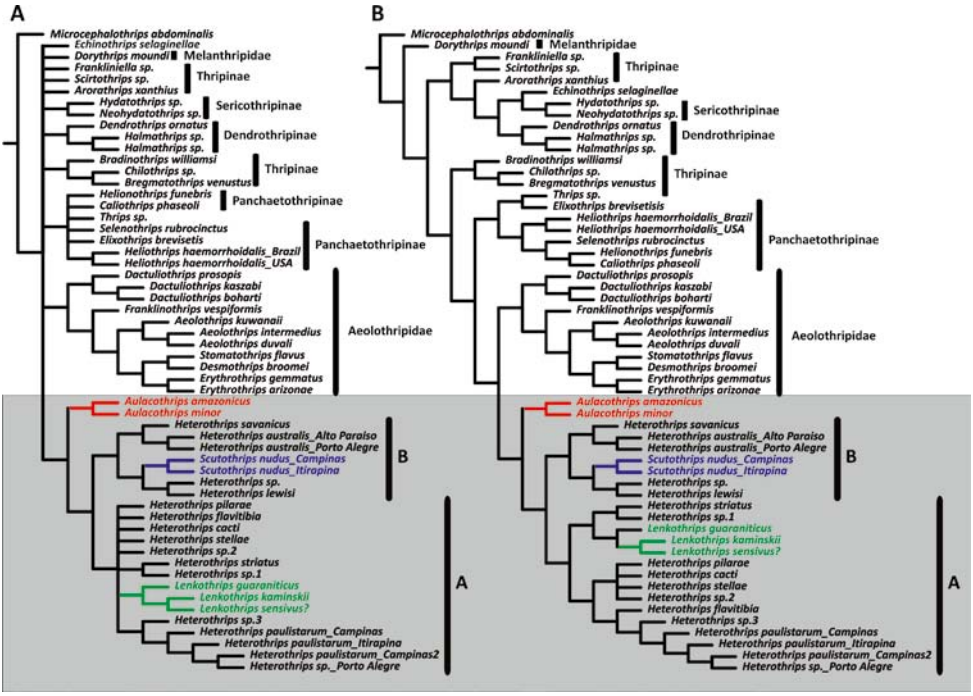


Fig. 28. **(a)** Consensus of the 30 optimal trees obtained of the analysis of the molecular data set under K8, K10, and K12 with L-INS-i alignment strategy. **(b)** Consensus of the 18 optimal trees obtained of the analysis of the molecular data set under K8, K10, and K12 with Q-INS-i alignment strategy.

When the morphological data set was analyzed alone under the three concavity values (K8, K10, K12), the strict consensus showed *Aulacothrips* as the sister group of *Selenothrips rubrocinctus* (Fig. 29a). *Aulacothrips* + *S. rubrocinctus* together represent the sister group of *Echinothrips selaginellae* + *Bradinothrips williamsi*. Neither one of the *Heterothrips* + *Scutothrips* + *Lenkothrips* groups A and B were recovered.

Results from the comparisons between the various combinations of molecular/morphological data (molecular, morphological, and combined data sets), continuous character coding (scaled from 0–1, logged, and logged then scaled from 0–1), concavity values (K8, K10, K12), and alignment strategies (Q-INS-i; L-INS-i) are displayed in supplementary material 5. When the total evidence trees were compared using SPR distance (similarity) a maximum of 23 SPR swaps (K8 MM_L vs. K8 MM_Q) and a minimum of 0–2 SPR swaps (K10 MM_Q vs. K8 MM_Q) were needed to change one tree into the other (Supplementary material 5). The most similar trees to the reference trees were obtained under K12. The other comparison measures (i.e. distortion coefficient, number of nodes of the consensus trees) presented high values across all different settings (Supplementary material 5).

When the reference trees were compared with the trees obtained using molecular data, the trees were similar and only required 4–15 SPR swaps to convert one tree into

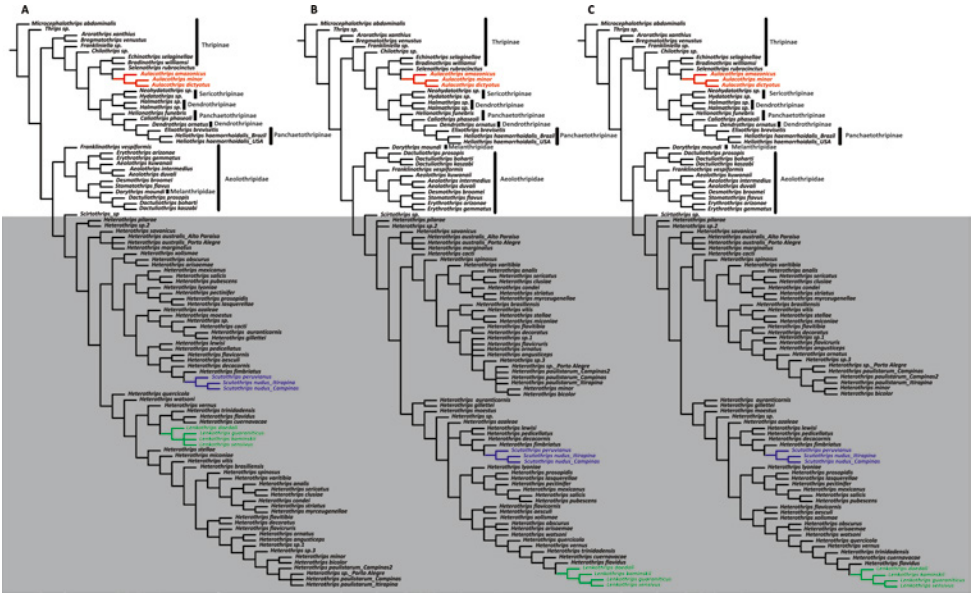


Fig. 29. Consensus of the optimal trees obtained of the analysis of the morphological data set under K8, K10, K12 and continuous characters: **(a)** scaled from 0 to 1, **(b)** logged; and **(c)** logged and scaled from 0–1.

another. The distortion coefficient similarly demonstrated high concordance with the reference trees although the number of nodes shared was low for all the comparisons (Supplementary material 5).

In general, the morphology-based trees were very different from the total evidence reference trees, 52 to 62 SPR swaps to convert one tree into the other, low values for the distortion coefficient, and relatively low shared nodes. When the morphology trees using different codifications for the continuous characters were compared, between 20 and 30 SPR swaps were needed to convert a tree obtained using continuous characters scaled from 0–1 into a tree using either logged or logged continuous characters scaled from 0–1. Re-scaling logged values from 0–1 did not result in much of a difference versus non-re-scaled logged values, 0–8 SPR swaps, and high values for the distortion coefficient and the number of nodes of the consensus trees (Supplementary material 5).

Discussion

Our results support the monophyly of Heterothripidae proposed by other authors (Mound et al. 1980; Morris & Mound, 2003; Mound & Morris, 2003, 2007; Buckman et al. 2013). According to our analysis, Heterothripidae is separated into two major groups: the ectoparasite genus *Aulacothrips* and a second group that includes the Heterothripidae that feed on flower tissue (*Heterothrips*, *Lenkothrips* and *Scutothrips*). The first group shows a series of adaptations to this life style, making their members

morphologically different from the rest of the family (Figs. 1, 6, 9, 13, 16, 20, 23). The second group comprises the paraphyletic genus *Heterothrips* that also includes species of *Lenkothrips* and *Scutothrips*. Members of the flower-feeding group are similar in general appearance but differ in the shape and arrangement of the sensoria on antennal segments III–IV (Figs. 1–5), the sculpture on the thorax and abdomen (Figs. 13–15 and 22–25), and the distribution of body microtrichia. A potentially shared trait between the two groups is that both show some degree of host-specificity (Mound & Marullo 1996; Cavalleri et al. 2010; Pereyra & Cavalleri 2012; Cavalleri & Mound 2014).

Despite the distinctive antennal sensoria, the general life style of *Lenkothrips* species is similar to *Heterothrips* and our analyses show that *Lenkothrips* is nested within *Heterothrips* and should probably be synonymized with that genus. *Scutothrips* had originally been suggested to be the sister group of *Aulacothrips* (Mound & Marullo 1996) since they share strong body reticulation and a prominent triangular metanotum. However, this proposed sister group relationship was not recovered in any of our analyses and we did not find characters that would distinguish *Scutothrips* from *Heterothrips*. We recovered a clade, 'group A', within the flower feeding Heterothripidae with taxa that are characterized by a comb of microtrichia on the posteromarginal margin of the abdominal tergites in all the analyses (Fig. 27).

Regarding the *Aulacothrips* clade, we can see an increase in surface and complexity of the sensoria on antennal segments III–IV (ch 23) from the base to the top of the clade (Fig. 27). *Aulacothrips amazonicus* has antennal sensoria that are less developed compared to other *Aulacothrips* species which is likely related with host specificity as finding hosts for *A. amazonicus* may not require as much effort (Cavalleri et al. 2012). Instead, the more developed sensoria observed in *A. minor* might be essential in searching for available hosts, particularly during seasons where hosts are less abundant (Cavalleri et al. 2012). Similarly, *A. dictyotus* possess extraordinarily large sensoria, this may be related to his need to find a specific host (Cavalleri et al. 2012). Interestingly, the sensoria in some species of *Aulacothrips* (*A. amazonicus*, *A. levinotus*) and *Lenkothrips* are similar despite these genera being distantly related and with different life styles. Almost all *Lenkothrips* species are associated with shrubs and vines and are restricted geographically to the Northern part of South America (Cavalleri & Mound 2014). They possess sensoria that are more complex in comparison to the sensoria of other flower-inhabiting Heterothripidae, a trait that could be associated with host plant specificity (Cavalleri & Mound 2014). On the other hand, *A. amazonicus* and *A. levinotus* have sensoria that are less developed compared to other *Aulacothrips* species, probably related also with host specificity as previously mentioned.

Host associations are not well documented for many thrips and this is particularly true for Heterothripidae (Mound & Teulon 1995; Mound 2013). The bulk of known host associations for Heterothripidae is limited to certain Argentinean, Brazilian and Central America species, but many of these records only mention on which plant species a specimen was collected, but not if this plant represents a breeding host for the species. For a better understanding of host patterns in a phylogenetic framework, further field work is needed to establish host plant associations.

This study is the first phylogenetic analysis for Heterothripidae, as well as the first study that uses both molecular and morphological evidence to test the monophyly and internal relationships for a family of Thysanoptera. Even if this is an advance in the knowledge of the phylogeny of Heterothripidae, we believe that additional morphological studies need to be conducted to include more characters in the data matrix. Additionally, a more comprehensive sample of sequence data would be desirable. As a first approximation to the phylogeny of the family, we here suggest new relationships that can be further investigated for confirmation: the paraphyly of *Heterothrips* with respect to *Lenkothrips* and *Scutothrips*; and the sister taxon relationship between the ectoparasitic and flower-inhabiting clades in Heterothripidae.

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