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MARIANA FLORES LINDNER

INVESTIGAÇÃO MORFOLÓGICA E MOLECULAR DOS TRIPES DO FOLHIÇO DA TRIBO GLYPTOTHRIPINI (THYSANOPTERA: PHLAEOTHRIPIDAE)

PORTO ALEGRE

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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutor em Biologia Animal.

Área de concentração: Biologia Animal

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Orientador: Prof. Dr. Adriano Cavalleri
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SUMÁRIO

RESUMO

A tribo Glyptothripini é um dos grupos mais diversos de tripes encontrados em folhiço. Possui mais de 150 espécies descritas em 20 gêneros, sendo a maioria registrada nas Américas. Apesar desta aparente riqueza, o conhecimento deste grupo é extremamente limitado: pouco se sabe sobre o hábito de vida e ecologia destes tripes, bem como suas relações filogenéticas. A morfologia é bastante variável, mas pouco compreendida, com muitas dificuldades para a identificação e delimitação de espécies. Este trabalho objetiva explorar algumas destas limitações e maneiras de resolvê-las, especialmente quanto à questão de identificação de espécimes.

Avaliamos três grandes aspectos: a representatividade e utilidade de sequências de DNA Barcode como uma biblioteca de referência para identificação de tripes; as informações que DNA Barcode podem prover para Glyptothripini; e um resumo detalhado da diversidade morfológica registrada no gênero *Glyptothrips*, um dos maiores da tribo.

Estudamos o banco de dados de DNA Barcode disponibilizado na plataforma online BOLD quanto à representatividade em Thysanoptera, presença de Barcode Gaps em diferentes gêneros, bem como se estas sequências permitiriam identificações corretas de novas sequências. Verificamos que apenas 5% das espécies válidas de tripes estão representadas neste banco de dados, e que muitas sequências apresentam incongruências com as identificações morfológicas, dificultando o uso delas como referência para identificação. Porém, Barcode Gaps foram identificados na maioria dos gêneros avaliados, indicando que a diferenciação entre espécies do mesmo gênero parece ser clara na maioria dos casos.

Avaliamos as distâncias genéticas dentro de Glyptothripini, com base no marcador molecular "Citocromo c Oxidase subunidade I" (COI). *Glyptothrips* e *Terthrothrips* apresentaram as distâncias interespecíficas médias mais altas dentre todos os gêneros de Phlaeothripidae analisados. Inferência Bayesiana deste gene recuperou os dois gêneros mencionados mais *Eurythrips* como grupos parafiléticos. Estas constatações sugerem que a informação evolutiva presente no gene COI não concorda com a atual definição destes gêneros, baseada em informações limitadas da morfologia.

Também resumimos todo o conhecimento sobre a variação morfológica dentro do gênero *Glyptothrips*. Esta variação foi detalhada em uma discussão das principais estruturas e comparações entre espécies, bem como ilustrada com fotos de espécimes-tipo. Por fim, criamos uma chave de identificação para as espécies registradas na América do Sul, e uma tabela listando diversos caracteres diagnósticos para todas as espécies, para facilitar futuros esforços

de identificação de espécimes de *Glyptothrips*.

Concluímos que, atualmente, nem morfologia sozinha nem dados moleculares disponíveis são suficientes para identificar espécimes de Glyptothripini com segurança; porém, ambas as fontes de dados possuem potencial se usadas em conjunto. Morfologia é essencial para associar o conhecimento e nomes atuais aos dados moleculares que estão sendo gerados; e estes dados moleculares poderão testar as definições criadas com base em morfologia, e modificá-las quando necessário.

Palavras-chave: Identificação taxonômica; DNA Barcode; morfologia; *Eurythrips*; *Glyptothrips*; *Terthrothrips*.

ABSTRACT

The tribe Glyptothripini is one of the most diverse groups of leaf litter thrips. It has over 150 species described in 20 genera, with most of them recorded from the Americas. Despite this apparent richness, the knowledge regarding this group is extremely limited: almost nothing is known about the biology and ecology of these thrips, nor about their phylogenetic relationships. Morphology is highly variable, but barely understood, with many difficulties for specimen identification and species delimitation. This work aims to explore some of these limitations and ways to address them, focusing mainly on specimen identification.

We evaluated three main aspects: representativity and utility of DNA Barcode sequences as a reference library for thrips identification; the information DNA Barcode can provide to tribe Glyptothripini; and a detailed description of the morphological diversity within the genus *Glyptothrips*, one of the largest in the tribe.

We studied the DNA Barcode database available online at BOLD, to assess its representativity, the presence of Barcode Gaps in different genera, and if these sequences would allow correct identifications for new sequences. We verified that only 5% of valid thrips species are represented in this database, and many of the morphological identifications are incongruent with the molecular data, limiting their usage as a reference library. However, Barcode Gaps were identified for most of the evaluated genera, suggesting that differentiation between congeneric species may be clear in most cases.

We evaluated the genetic distances within this group, utilizing a fragment of the Cytochrome *c* Oxidase subunit I (COI) gene. *Glyptothrips* and *Terthrothrips* had the highest mean interspecific distances among all analysed Phlaeothripidae genera. Bayesian Inference analysis of this gene recovered the two genera above plus *Eurythrips* as paraphyletic groups. These observations suggest that the evolutionary information available in COI does not agree with the current definition of these genera, based on limited morphological data.

We also summarized all morphological variation knowledge within genus *Glyptothrips*. This variation was described in a discussion of the main body structures and comparisons between species, and illustrated with photographs of type specimens. Finally, we created an identification key for the South American species, and a table listing many diagnostic characters for all species, to facilitate future identification efforts of *Glyptothrips* specimens.

We conclude that, currently, neither morphology alone nor available molecular data are sufficient to identify specimens of Glyptothripini safely; however, both sources of data have a lot of potential if utilized together. Morphology is essential to match the current names and

knowledge to the newly generated molecular data; and this molecular information could test the morphology-based definitions, modifying them whenever needed.

Keywords: Specimen identification; DNA Barcode; morphology; *Eurythrips*; *Glyptothrips*; *Terthrothrips*.

INTRODUÇÃO GERAL

A Ordem de insetos Thysanoptera é composta por cerca de 6.400 espécies, organizadas em nove famílias com representantes atuais e seis famílias com representantes fósseis (THRIPS WIKI, 2023). São insetos com tamanho variando de menos de um milímetro a 1,5 milímetros, aparelho bucal picador-sugador assimétrico e asas franjadas (MOUND & MARULLO, 1996). Diversos hábitos alimentares são registrados na ordem: fungivoria e fitofagia são os mais comuns, mas algumas espécies predadoras e ectoparasitas também são conhecidas (MOUND & MARULLO, 1996; CAVALLERI et al., 2010). Apesar dos tripes que vivem e se alimentam em plantas serem mais conhecidos, as espécies que se alimentam de fungos são a maioria dentro da ordem (MOUND, 2018; THRIPS WIKI, 2023). Tripes fungívoros ocorrem em pelo menos quatro das nove famílias atuais, com Phlaeothripidae, a maior família de Thysanoptera, reunindo a grande maioria destes insetos (THRIPS WIKI, 2023).

Phlaeothripidae é tradicionalmente dividida em duas subfamílias: Idolothripinae, com mais de 80 gêneros e pouco mais de 760 espécies, e Phlaeothripinae, com quase 380 gêneros e mais de 3.000 espécies atuais (MOUND, 2013; THRIPS WIKI, 2023). O primeiro destes táxons reúne espécies comumente coletadas em galhos e folhas mortas, com muitas espécies que se alimentam pela ingestão de esporos fúngicos inteiros (MOUND & PALMER, 1983; MOUND & MARULLO, 1996). Já a segunda subfamília é composta por uma grande variedade de hábitos, com tripes se alimentando de folhas, fungos, ou mesmo predando outros animais (incluindo diferentes espécies de tripes) (MOUND & MARULLO, 1996).

A taxonomia de Phlaeothripinae é confusa, com um grande número de gêneros constituídos por uma única espécie, alguns gêneros com muitas espécies morfologicamente similares, e poucas informações acerca das relações taxonômicas (MOUND & MARULLO, 1996; THRIPS WIKI, 2023). Muitas propostas de grupos de gêneros, como tribos e subtribos, foram feitas ao longo de décadas de estudos desta ordem, e tanto a composição como a aceitação destes grupos é variável. Mais recentemente tem sido sugerida de uma classificação mais subjetiva, que dividiria os membros de Phlaeothripinae em três grandes grupos ou "linhagens": "linhagem *Haplothrips*", reunindo espécies que vivem em flores e/ou predadoras; "linhagem *Liothrips*", composta por diversos tripes que se alimentam em folhas; e "linhagem *Phlaeothrips*", que representaria as espécies fungívoras, mas acaba sendo o grupo que reúne todas as espécies que não se encaixam nas outras duas linhagens (MOUND & MARULLO, 1996). Destes três agrupamentos, apenas a "linhagem *Haplothrips*" foi formalmente definida como a tribo Haplothripini (MOUND & MINAEI, 2007; CAVALLERI et al. 2016); as outras

duas linhagens seguem sem uma definição clara de suas características e membros, e são raramente utilizadas em trabalhos recentes.

Além destas linhagens, muitos outros agrupamentos de gêneros e espécies de Phlaeothripinae são mencionados na literatura, baseando-se principalmente em características morfológicas e/ou hábitos alimentares para suas definições (PRIESNER, 1960; STANNARD, 1970; OKAJIMA, 1981; entre outros). Dentre estes, podemos destacar a tribo Glyptothripini, um dos grupos de tripes que vivem em folhiço com maior riqueza (MOUND, 1977). Boa parte das mais de 150 espécies desta tribo são encontradas nas Américas (THRIPS WIKI, 2023), mas existem espécies exclusivamente asiáticas ou africanas, e pelo menos uma espécie (*Tylothrips osborni*) está sendo reportada de diferentes partes do mundo, potencialmente introduzida pela ação humana (YIĞIT et al., 2021).

Os membros da tribo Glyptothripini possuem, em geral, corpo coberto por forte esculturação e olhos globosos, de tamanho reduzido. Outras características comuns neste grupo são a ausência de cílios duplicados na margem posterior das asas anteriores, cerda anteromarginal do pronoto reduzida, e ocorrência de múltiplas formas alares (STANNARD, 1955; MOUND, 1977). Porém, diversos gêneros e espécies da tribo apresentam exceções a uma ou mais destas características, portanto não há uma diagnose morfológica clara e definitiva para a tribo. Mesmo as definições dos gêneros não são claras, com diversas espécies apresentando combinações de caracteres morfológicos intermediárias entre dois ou mais gêneros (MOUND, 1977). Um exemplo ocorre com os gêneros *Eurythrips* e *Terthrothrips*, os maiores da tribo: a maioria das espécies de *Eurythrips* possuem antenas mais curtas, asas menores ou mesmo ausentes, olhos reduzidos e a cabeça mais quadrada, com margens laterais quase retas; já a maioria das espécies de *Terthrothrips* possuem antenas muito alongadas, asas bem desenvolvidas, olhos grandes e cabeça alongada, com margens curvadas (GERDES,1984). A comparação entre espécies nestes dois extremos sugere que se trata de táxons diferentes, mas diversas espécies dentro destes dois gêneros possuem caracteres mais intermediários, e a separação delas em um ou outro gênero é por vezes arbitrária (MOUND, 1976; MOUND, 1977).

Casos como este são comuns em Glyptothripini, e contribuem para dificultar a identificação de gêneros e espécies dentro deste grupo. Outro agravante é a falta de chaves dicotômicas ou outras ferramentas de identificação para a maioria das espécies: existe uma chave para os gêneros da tribo (MOUND, 1977) e algumas para espécies (STANNARD, 1955; MOUND, 1976; entre outros), mas estas são em sua maioria limitadas a áreas geográficas específicas e/ou desatualizadas com relação à composição atual dos gêneros. Além disso,

existem poucas coleções de referência com material deste grupo: a grande maioria dos espécimes-tipo (pelo menos 100 holótipos) se concentram em um único museu, o National Museum of Natural History (NMNH) dos Estados Unidos, com poucos espécimes depositados em outros museus (THRIPS WIKI, 2023). No Brasil, a coleção de Thysanoptera da Universidade Federal do Rio Grande do Sul (UFRGS) possui alguns espécimes identificados como pertencentes à tribo Glyptothripini, mas a falta de acesso aos espécimes-tipo e de material de referência não permitiu a identificação até nível de espécie para a maioria dos indivíduos.

Outro fator limitante para a taxonomia e sistemática de Glyptothripini (e de Thysanoptera como um todo) é a dependência de dados morfológicos (MOUND & MORRIS, 2007): toda a taxonomia da ordem foi construída com base na morfologia, principalmente externa, de espécimes montados em lâminas de microscopia (MOUND & MARULLO, 1996). Tal preparação permite a preservação dos espécimes por um longo tempo e a observação de caracteres morfológicos em microscopia óptica, porém limita ou mesmo elimina a obtenção de dados de outras fontes. A preparação para montar e preservar um tripes envolve a dissolução de seu conteúdo interno, removendo a possibilidade de estudar a morfologia interna ou obter dados moleculares de espécimes de museu (MOUND & MARULLO, 1996; KUMAR et al., 2014). Lâminas de microscopia também não podem ser utilizadas em métodos com maior capacidade de aumento de imagem, como Microscopia Eletrônica de Varredura (MEV) e Microscopia Eletrônica de Transmissão (TEM).

Apesar da problemática apresentada acima, o surgimento de novas tecnologias e metodologias nos últimos anos têm aumentado as possibilidades de estudo dos tripes, e diversas ferramentas que podem auxiliar na identificação dos espécimes estão sendo estabelecidas (GHOSH et al., 2021). Uma destas ferramentas é o DNA Barcode, uma sequência genética específica que pode servir como um código de barras para identificar espécimes (HEBERT et al., 2003). Esta ferramenta propõe a comparação de um trecho específico de DNA presente nos seres vivos (em animais, o fragmento 5’ do gene mitocondrial Citocromo *c* Oxidase I - COI) entre o espécime a ser identificado e um banco de dados de referência, onde maior similaridade indica maior proximidade evolutiva (HEBERT et al., 2003). Com o desenvolvimento de metodologias de extração e sequenciamento de DNA cada vez mais rápidas e eficientes, a utilização de dados moleculares como base para uma ferramenta de identificação vem se tornando uma opção cada vez mais atraente (por exemplo, KARIMI et al., 2010; MARULLO et al., 2020). O fato de que qualquer cientista capaz de obter sequências de DNA poderia comparar estas a uma biblioteca de referência e obter uma identificação, sem a necessidade de especializar-se no táxon a ser estudado, também contribui para o interesse em DNA Barcodes.

Atualmente o principal banco de dados de DNA Barcode encontra-se na plataforma “Barcode of Life Data System” (BOLD, 2023): são mais de 14 milhões de sequências de DNA Barcode, representando cerca de 250 mil espécies animais, 72 mil espécies vegetais e 25 mil espécies de fungos e outros seres (em 25 de setembro de 2023). Este banco de dados apresenta ferramentas para auxiliar na identificação de espécimes com base neste fragmento de DNA, e também para propor delimitação de espécies com base nos dados disponíveis na plataforma. O BOLD é constantemente atualizado com contribuições de diversas partes do mundo, e os dados inseridos passam por um processo de curadoria e filtragem, visando garantir um controle de qualidade das sequências disponibilizadas (RATNASINGHAM & HEBERT, 2007).

Porém, um sistema de identificação com base em DNA Barcode só poderá funcionar se for suportado por uma ampla biblioteca de referência. Sequências de qualidade, associadas a nomes corretos, amostrando uma boa variedade de regiões, são essenciais para a construção de uma biblioteca confiável de DNA Barcode. Tendo isso em mente, o primeiro capítulo desta tese avalia a qualidade dos dados atualmente disponibilizados para Thysanoptera no banco de dados do BOLD. São verificados o número de espécies e regiões geográficas representadas, se há distinções claras entre espécies, e se o banco de dados permite a identificação correta de sequências de COI por comparação com o banco de dados.

Ao avaliar as sequências de DNA disponibilizadas em plataformas online, verificou-se a quase total ausência de qualquer informação genética para a tribo Glyptothripini no maior bancos de dados moleculares, o GenBank (NLM, 2023). No BOLD, apenas nos últimos meses algumas sequências de membros da tribo foram publicadas (BOLD, 2023; acesso em setembro de 2023). Esta falta de dados moleculares para este grupo de tripes só contribui para as limitações no estudo desta tribo, como discutido acima. No capítulo 2, sequências parciais de COI foram obtidas pela primeira vez para diversos espécimes de Glyptothripini, e avaliadas quanto à similaridade genética para verificar se haveria concordância com a classificação atual, baseada em morfologia.

Por fim, é necessário começar a associar os dados mais recentes, provenientes de diferentes fontes (molecular, ecológica, geográfica, etc.) com o conhecimento já disponibilizado na literatura, em sua maioria baseado em morfologia. No capítulo 3, é apresentada uma revisão morfológica detalhada de *Glyptothrips*, o gênero-tipo de Glyptothripini. A variação observada é discutida e ilustrada com fotografias de todas as espécies do gênero. Além disso, uma chave de identificação para as espécies registradas no Brasil é disponibilizada, para auxiliar nos esforços de identificar espécies de *Glyptothrips* com maior segurança.

OBJETIVOS

Três grandes objetivos nortearam o desenvolvimento desta tese, gerando cada um dos três capítulos aqui apresentados: (1) avaliar os dados de DNA Barcode disponíveis para Thysanoptera, quanto à qualidade e potencial de uso como biblioteca de referência; (2) explorar o uso de dados moleculares para estudar a taxonomia de Glyptothripini, verificando se a informação disponível em DNA Barcode concordaria com os dados morfológicos; e (3) estudar a variação morfológica dentro de *Glyptothrips*, gerando ferramentas de identificação e permitindo a posterior combinação destes dados com outras fontes de informação biológica.

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Capítulo 1

TINY INSECTS, BIG TROUBLES - A REVIEW OF BOLD'S COI DATABASE FOR THYSANOPTERA (INSECTA)

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Tiny insects, big troubles - A review of BOLD's COI database for Thysanoptera (Insecta)

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Abstract

DNA Barcoding is an important tool for disciplines such as taxonomy, phylogenetics, and phylogeography, with BOLD being the largest database of partial cytochrome *c* oxidase subunit I (COI) sequences. We provide the first extensive revision of the information available in this database for the insect order Thysanoptera, to assess: how many COI sequences are available; how representative these sequences are for the order; and the current potential of BOLD as a reference library for specimen identification and species delimitation. The COI database at BOLD currently represents only about 5% of the over 6,400 valid thrips species, with a heavy bias towards a few species of economic importance. Clear Barcode gaps were observed for 24 out of 33 genera evaluated, but many outliers were also observed. We suggest that the COI sequences available in BOLD as a reference would not allow for accurate identifications in about 30% of Thysanoptera species in this database, which rises to 40% of taxa within Thripidae, the most sampled family within the order. Thus, we call for caution and a critical evaluation in using BOLD as a reference library for thrips Barcodes, and future efforts should focus on improving the data quality of this database.

Key words: Barcode gap; DNA Barcoding; Aeolothripidae; Phlaeothripidae; Thripidae; *Frankliniella*

Short title: A review of BOLD's COI database for Thysanoptera

Introduction

Since its first suggestion in the early 2000s (Hebert et al., 2003), DNA Barcoding has received much attention due to its versatility as a global bioidentification system. The proposal of using a specific DNA sequence as a type of barcode for all life forms, allowing for quick comparisons and easier identification of specimens, is attractive in the context of fewer taxonomists and less time available for the careful study of specimens. In 2007, the Barcode of Life Data System (BOLD) was created as a freely available online workbench for collecting, analysing, and sharing DNA Barcodes (Ratnasingham & Hebert, 2007).

In 15 years of existence, BOLD has gathered almost 14 million DNA sequences of over 345 thousand animals, plants, and fungi species (as of May 16th, 2023; BOLD, 2023). It is a valuable repository allowing the association of voucher pictures with sequence data, which increases the repeatability and verification of information, two fundamental principles of scientific work (Vink et al., 2012; Bianchi & Gonçalves, 2021b). BOLD also provides its own tool for species delimitation, the Barcode Index Number (BIN), which is based on cluster analysis of the sequences in the database, and compatible with the constant inclusion of new data (Ratnasingham & Hebert, 2013). Each BIN can represent a potential species, allowing the evaluation of such units and their use in the lack of a well-developed taxonomic frame.

However, some authors have pointed out some limitations currently found in BOLD for specific taxa (Sonet et al., 2013; Lis et al., 2016; Gonçalves et al., 2021; Bianchi & Gonçalves, 2021a), and even questioning the quality of the data added to this database. For example, it has been shown that there are problems in the acquisition of reference data and its curation in BOLD and GenBank, as well as in the production of sequences to assess the reference data (Meiklejohn et al., 2019; Pentinsaari et al., 2020). Thus, the efforts to improve the quality of data of these online databases must be continuous, and should include revision and curation of available data.

While DNA Barcoding has been extensively utilised in many taxa, for the insect order Thysanoptera (popularly known as thrips; Fig. 1) it is still a rather incipient tool. With over 6,400 species in the order and a cosmopolitan distribution, Barcode data is available only for a few species, most of them with some importance for agriculture (e.g., Karimi et al., 2010; Chakraborty et al., 2019). In fact, only a few works deal with a large variety of thrips taxa, and most studies focus on a limited geographical area (Iftikhar et al., 2016; Tyagi et al., 2017) or a specific family (Marullo et al., 2020). Still, partial cytochrome *c* oxidase subunit I (COI) sequences, especially at the 5’ portion (COI-5P), have shown potential to be a useful

identification tool for these insects, as shown in the recent revision of Ghosh et al. (2021) of molecular and electronic identification tools.

Thysanoptera specimens offer difficulties and limitations for their DNA extraction and sequencing. Most preserved specimens no longer contain any source of DNA, thus molecular studies of thrips require freshly collected specimens. Their small size requires the usage of whole specimens for DNA extraction, and some procedures can easily damage the thrips, hampering specimen usage for molecular and morphological data concurrently. Finally, thrips often yield low quantities of DNA, further complicating molecular analyses (Dickey et al., 2015).

This work aims to evaluate the available COI sequences for Thysanoptera in BOLD. Despite the existence of other databases for genetic sequences, such as GenBank, our focus on BOLD data is due to its emphasis on DNA Barcodes and implementation of several quality control steps. The objectives of this study were: (1) investigate the representativity of BOLD sequences compared to the valid taxa within Thysanoptera; (2) identify Barcode gaps at the generic level; and (3) assess the correct identifications of thrips specimens using DNA Barcodes. After these analyses, we highlight some taxa within Thysanoptera that need a careful taxonomic revision, sequences whose identity may need to be re-evaluated, and suggest ways to improve the overall quality of the database available in BOLD.

Material and Methods

The workflow described below follows and adapts the methodology utilised in Gonçalves et al. (2021) and Bianchi & Gonçalves (2021a).

Data acquisition and filtering

All sequences available on BOLD labelled as "Thysanoptera" were manually downloaded in November 2021 (database 0). We curated this original database to remove sequences which did not fit the criteria needed for our analyses, and Table 1 lists how many sequences were removed at each step. The filtering steps are as follows: (1) removal of sequences of genes other than COI-5P; (2) all sequences without species-level identification removed, and names corrected whenever needed (synonymy, misspellings); (3) removal of all genera with a single species, as the PCI (Probability of Correct Identification) analysis requires all genera to have at least two species; (4) remaining sequences divided into families and aligned using MAFFT v7.0 (Katoh et al., 2019); (5) alignments were trimmed to the canonical barcode region (Hebert et al., 2003) using as reference the BOLD entry MAIMB460-09 (*Thrips palmi*), and all sequences with less than 400 bp were removed; (6) sequences were separated by genus; (7) genera with less than two species, or lacking any species with two or more sequences, were removed, to ensure intra- and interspecific comparisons for Barcode gap analysis. With these steps, Databases 1, 2, and 3 were generated (Table 2). All sequences were treated by their species name only, with subgenera or subspecies not being considered. Table 1 lists how many sequences, families, genera and species labels were available after each filtering step. All databases utilised in this work is given in Supplementary file 1. A dataset on BOLD has been generated with most sequences downloaded in November 2021, and is available at the following DOI: dx.doi.org/10.5883/DS-THRIPS21.

Representativity of Thysanoptera data on BOLD

We assessed the representativeness of Database 1 for Thysanoptera taxa by determining the number of families, genera, and species included. We also examined the distribution of sequences within these taxa to identify any potential biases. Geographical distribution data from databases 0 and 3 were obtained to generate global heat maps, to evaluate shifts in distribution patterns before and after filtering steps. The maps were created with MapChart, available at https://www.mapchart.net.

Barcode Gap Analysis

To evaluate the presence of Barcode gaps in Thysanoptera, we used the function dist.dna() of the R package ape (Paradis & Schliep, 2019) on database 3 to estimate pairwise uncorrected p-distances for all sequences within each genus (Supplementary File 2). We used uncorrected p-distances because they yield better or similar results when compared to other nucleotide substitution models, such as Kimura 2-parameter (Collins et al., 2012; Srivathsan & Meier, 2012). Intra- and interspecific distances were then represented in a boxplot for each evaluated genus, using the base R function 'boxplot()'. This allows the automatic identification of outliers, which represent comparisons between two sequences whose distances fall outside the extent of the whiskers (Fig. 2).

The boxplots allow visualisation of the Barcode gap, which were classified into one of the following three categories: Good, when there was no overlap between intraspecific and interspecific boxplots; Intermediate, when there was an overlap between boxplot whiskers only; and Poor, when the boxplot boxes overlapped (Badotti et al., 2017; Bianchi & Gonçalves, 2021a).

Many of the boxplot graphs showed at least one outlier. To analyse these, we listed the intraspecific outliers above the upper whisker limit, and the interspecific outliers below the lower whisker limit (Supplementary Files 3-4). These were chosen due to their potential overlap with interspecific distances and intraspecific distances, respectively. Supplementary file 5 lists all the genera with outliers and how representative they are concerning the number of potential comparisons and sequences available.

Finally, to demonstrate the potential of outlier comparisons in detecting taxonomic inconsistencies, we conducted a detailed examination of select outliers for *Aeolothrips* Haliday, 1836 and *Frankliniella* Karny, 1910. These genera were chosen due to the abundance of available sequences, their economic importance and their history of challenging taxonomy.

Probability of Correct Identification (PCI) Analysis

To evaluate if the available sequences in BOLD allow for the correct identification of COI sequences within Thysanoptera, we calculated the Probability of Correct Identification (PCI) (Supplementary file 6) utilising database 2. The PCI is a "discrete species assignment" and considers the maximum intraspecific distance and the minimum interspecific distance (or nearest-neighbour distance) for each recognized species (Erickson et al., 2008). Then, these values are visualised in a scatterplot, where each dot represents a species name (Collins &

Cruickshank, 2012). By drawing in the graph a line where x=y, it is possible to divide the species dots between two groups. Those above the x=y line have the nearest neighbour distance higher than the maximum intraspecific distance, and thus are considered to provide a "correct" identification (since there is a clear gap between the species and the closest one, thus a clear delimitation of that species). Those dots below the x=y line have the nearest neighbour distance lower than the maximum intraspecific distance, and thus are considered to provide an "incorrect" identification (since there is an overlap between the species and the closest neighbour, therefore a query sequence could fall in this overlap and have an uncertain identity). By calculating the number of points above the line in relation to the total number of points in the graph, it is possible to calculate the PCI for a given taxon. Thus, PCI calculations were performed for Thysanoptera as a whole and for three families (Aeolothripidae, 12 species names; Phlaeothripidae, 52 species names; and Thripidae, 96 species names).

Results

Representativity of Thysanoptera data on BOLD

A total of 30,581 sequences were obtained from BOLD, of which about one third had any image record, and only 5% were barcode compliant. After removing non-COI sequences and those lacking species identification (steps 1 and 2 of the filtering procedure), 11,096 sequences remained, representing seven families, 115 genus labels, and 297 species labels (Table 1). The overall representativity of these sequences was low, with less than 15% of genera and 5% of valid thrips species (*sensu* ThripsWiki, 2023) (Table 3). Representativity of species varied in each family and subfamily, but most families had only a third or less of their genera represented in BOLD (Table 4).

Out of the 11,096 sequences analysed, almost 90% belonged to Thripidae species (Fig. 3A). Three genera comprise nearly 70% of the sequences: *Taeniothrips* Amyot & Serville, 1843 (32.81%), *Thrips* Linnaeus, 1758 (18.67%), and *Frankliniella* (17.59%) (Fig. 3B). The species with most sequences in BOLD, *Taeniothrips inconsequens* (Uzel, 1895), represents almost 30% of all records in this database (Fig. 3C). On the other hand, almost 70% of the species names have less than ten sequences each, and 27.6% of the species labels have a single COI sequence under their name (Supplementary file 7).

We also identified errors in at least 35 records, such as species labels with outdated names, typos, and even a sequence belonging to a beetle species mistakenly listed as a member of Thysanoptera. A complete list of the errors detected on the sequences obtained in November 2021 can be found in Supplementary File 8.

Geographical distribution data was available for 28,922 out of the 30,581 Thysanoptera sequences downloaded from BOLD (database 0), representing 69 countries which contributed with at least one sequence (Fig. 4). Canada alone comprised about 40% of the total, with 11,756 sequences. The five countries with the most sequences (Canada, Costa Rica, South Africa, Australia, and the United States) gather over 80% of the sequences (Fig. 4A).

Barcode Gap

A total of 33 genera belonging to families Aeolothripidae, Phlaeothripidae and Thripidae could be evaluated for the presence and quality of Barcode gaps. Of these, 24 genera were classified as having a Good Barcode gap, four Intermediate, and five a Poor gap (Fig. 5).

The median intraspecific distance varied widely among genera, with some presenting a median of 0% (i.e., *Franklinothrips* Back, 1912, *Hoplothrips* Amyot & Serville, 1843 and

Orothrips Moulton, 1907), seven genera above 4%, and *Pseudodendrothrips* Schmutz, 1913 above 23%. The average median intraspecific distance was 2.49%.

The median interspecific distance also varied greatly among genera, with the lowest value being 3.44% for *Odontothrips* Amyot & Serville, 1843, and the highest value being 22.89% for *Pseudodendrothrips*. The average median interspecific distance was 13.27% (Table 5).

Boxplot Outliers

Among the 33 analysed genera, 22 exhibited outliers in the boxplots, indicating pairwise comparisons that fell at the extreme ends of the observed data range (Fig. 5); and 19 genera had at least one outlier in the range listed by our R script (Table 6, Supplementary Files 4-5).

Aeolothrips *outliers*

We found 2,256 intraspecific outliers for *Aeolothrips*, of which 1,727 outliers (those above the dotted line on Figure 2) were visually inspected. These outliers exclusively involved comparisons between sequences of *Aeolothrips intermedius* Bagnall, 1934, which could be assigned to three distinct sequence clusters (Supplementary File 9).

We also observed low interspecific distances involving some *Aeolothrips* sequences. The only sequence identified as *Aeolothrips melaleucus* Haliday, 1852 (BOLD ID: GBMIN39680-13) exhibited distances ranging from 0.17% to 1.73% when compared to sequences of *Aeolothrips fasciatus* (Linnaeus, 1758), whose highest observed intraspecific distance was 2.92%. Similarly, two entries of *Aeolothrips mongolicus* Pelikan, 1985 (BOLD ID: GBMIN91243-17 and GBMIN91244-17) displayed distances varying from 0.15% to 4.85% when compared to sequences of *A. intermedius*, and they even clustered with some *A. intermedius* sequences within the same BIN (BOLD:AAU0572).

Frankliniella *interspecific outliers*

In the case of *Frankliniella*, a total of 16,717 interspecific outliers were identified, out of which 5,403 (the ones which directly overlapped with the intraspecific boxplot) were considered for analysis. We observed outlier comparisons between five species pairs (Table 7). Moreover, the single sequence identified as *F. minuta* (Moulton, 1907) (BOLD ID: GBA8033-12) was identical to several sequences of *F. schultzei* (Trybom, 1910). Similarly, sequences labelled as *F. citripes* Hood, 1916 (BOLD ID: GBA8030-12) and *F. borinquen* Hood, 1942 (BOLD ID: GBMHT2007-19) exhibited very low distances when compared to *F. insularis*

(Franklin, 1908) and *F. occidentalis* (Pergande, 1895), respectively. A complete list of the outlier comparisons between *Frankliniella* sequences is available in Supplementary File 9.

PCI

The highest PCI value was observed for Aeolothripidae, with 83.33% of species labels allowing for "correct" identifications (= maximum intraspecific distance < nearest neighbour distance). Meanwhile, the lowest value was observed for Thripidae, with 58.33% of species labels allowing for "correct" identifications (Fig. 6). The complete list of species names evaluated, and their maximum intraspecific and nearest neighbour distance values, can be found in Supplementary File 10.

Discussion

Thysanoptera data on BOLD

While there were over 30,000 sequences available on BOLD for Thysanoptera in November of 2021, only about a third of them matched the criteria to be included in the Barcode gap and PCI analyses performed in this work; moreover, the sequences we utilised have a very limited representativity for Thysanoptera genera and species (about 15% and 5% of valid taxa, respectively). This is similar to what was observed for Pentatomomorpha (about 6% of valid species; Bianchi & Gonçalves, 2021a) and Orthoptera (about 3% of valid species; Timm et al., 2022), but much less than what is available for insect groups with a higher focus on molecular studies, such as Apidae (around 17% of valid species; Gonçalves et al., 2022) and Lepidoptera (almost two-thirds of valid species; Mutanen et al., 2016). The usage of COI in Thysanoptera is usually focused on identification or population studies of a few pest species (e. g. Leão et al., 2017; Chakraborty et al., 2019; further references in Ghosh et al., 2021).

Despite fungivorous thrips species representing about 50% of the current diversity in the order, most of them lack sequences in BOLD. For example, there is no molecular information for the single extant species of Uzelothripidae, whose relationships within the order are still unknown. Subfamily Idolothripinae, which is the only group of thrips able to ingest and process whole fungal spores, is also underrepresented in BOLD.

Many sequenced specimens also lack any image records, and the available digital photographs were taken on a stereomicroscope or without enough magnification to examine thrips morphological traits. While it is possible to identify potential species units by utilising only the molecular data, for many taxa, including thrips, most species are still defined only by morphological traits. A good molecular library can work independently from morphology data, but we are still very far from using the BOLD database as a reliable identification tool for Thysanoptera. The lack of good quality pictures associated with the sequences or even voucher specimens hinders the possibility of reviewing and correcting potential misidentification. The lack of sequence metadata supporting taxonomic identification plays against the basic scientific principle of reproducibility (Bianchi & Gonçalves, 2021b) and compromises the utility of reference sequences.

While many countries contributed sequences to BOLD's Thysanoptera database, most of these sequences are concentrated in a small number of countries (Fig. 4). Most of Africa and several countries in Asia, Europe, and Latin America do not have any data added to BOLD.

After filtering our data, all sequences from 15 countries were removed, and the remaining data is even more concentrated on Canada, the largest source of sequenced thrips specimens.

Barcode Gap

A Good Barcode gap was observed for most of the genera, allowing species identification for these taxa based on this gap. However, many of them also had multiple outliers, which could potentially cloud identification efforts by increasing the observed intraspecific and interspecific ranges, creating overlaps. While the median intraspecific distance was below 1% in most genera classified as Good, both *Kladothrips* Froggatt, 1906 and *Stenchaetothrips* Bagnall, 1926 had a median intraspecific distance above 4%. If one were to use an arbitrary cut-off value to separate sequences into species for these groups (e.g., 2-3%; Hebert et al., 2003), they would split a single species into different names. We recommend caution in using arbitrary distance values for thrips species delimitations without a proper sampling and previous evaluation of the intraspecific diversity of the target group.

The median intraspecific distance in genera with Intermediate or Poor gaps was high in comparison to those genera with Good gaps, and in those cases the Barcode gap may not be a reliable tool for species delimitation. Within the genera with Intermediate Barcode gap, *Frankliniella* and *Scirtothrips* Shull, 1909 have a high number of species distributed worldwide (236 and 108, respectively; ThripsWiki 2023) as well as complex taxonomy (Mound & Palmer, 1981; Cavalleri & Mound, 2012), and some potential cryptic species (Rugman-Jones et al., 2010; Dickey et al., 2015).

Boxplot outliers

The Barcode gap analysis resulted in frequent outlier comparisons, which can demonstrate the necessity of re-examining the sequences involved or even a taxonomic revision of some groups, especially when there are overlaps between intraspecific and interspecific distances.

For *Aeolothrips intermedius*, one sequence (BOLD ID: GBMNC48112-20) had very high distances (above 20%) when compared to most the other sequences identified as *A. intermedius* (Supplementary File 9), suggesting this sequence is not conspecific with the other *A. intermedius* specimens. The other two observed sequence clusters also separate into different BINs (Group 1 = BOLD:ACD4587; Group 2 = BOLD:AAZ8618 and BOLD:AAU0572; see Supplementary File 9 for full composition of these groups), which indicates that what is currently identified morphologically as *A. intermedius* may represent 3 or 4 distinct species

when utilising this COI fragment as reference. Tyagi et al. (2017) found support for two species within *A. intermedius* collected from India, when conducting single-locus delimitation. We also observed that the single sequence of *A. melaleucus* (BOLD ID: GBMIN39680-13) and the two sequences of *A. mongolicus* (BOLD ID: GBMIN91243-17 and GBMIN91244-17) need revision, as they may represent specimens of *A. fasciatus* and *A. intermedius*, respectively. None of these specimens have photos on BOLD, so we are unable to compare their morphologies to see if they match the identity suggested by molecular data.

Regarding *Frankliniella*, we suggest that at least the sequences GBMHT2007-19, GBA8030-12, and GBA8033-12 (labelled *F. borinquen*, *F. citripes* and *F. minuta*, respectively) are misidentified. Unfortunately, there are no available images of these records to verify their identity.

Taxonomic incongruencies may be the most probable explanation for many of the observed high intraspecific distances and outliers. Misidentification of thrips species are frequent, especially in groups with high reliance on minute and similar looking morphological characters, such as *Frankliniella* and *Scirtothrips.* Alternatively, cryptic species (i.e. when distinct species are lumped under the same name due to a lack of morphological, ecological or biological distinction) could also explain a high intraspecific variation, due to molecular divergence that has not been translated into phenotypic differences yet (Struck et al., 2018; Struck & Cerca De Oliveira, 2019). However, we cannot discard the possibility of the taxonomy being correct, and the high intraspecific variation in COI being explained by other underlying causes. Geographic distribution and events can have an influence, by allowing or limiting contact and genetic exchange between different populations. The presence of parasites able to affect the host's reproduction, such as *Wolbachia* bacteria, could also influence the genetic composition of a species (Xiao et al., 2012). Further studies can explore in more detail these or other potential explanations to the observed variation, but it is important to consider all available hypotheses and test them when reviewing the highly diverging sequences.

PCI

The PCI analysis indicates that in over 30% of the cases, identifying Thysanoptera species using the sequences as a reference library could lead to incorrect names, if using the "nearest neighbour" distance value as a cut-off. This is worrisome especially for Thripidae, the second largest family within the order and the one with the most sequences in BOLD: more than 40% of the species names analysed returned as "incorrect" identifications. Furthermore, many of the Thripidae species labels with intraspecific and interspecific distances overlapping

belong to large genera, with complex taxonomy (e. g. *Frankliniella*, *Scirtothrips*, *Thrips*). This could support the hypothesis of incorrect identifications of some reference specimens included in BOLD, but the possibility that multiple cryptic species may be under the same name cannot be discarded (Rebijith et al., 2014; Dickey et al., 2015; Tyagi et al., 2017; see discussion above for other potential causes).

Curiously, *Haplothrips* Amyot & Serville, 1843 (PCI=66.67%) and *Thrips* (PCI=45.45%), despite their low PCI values, were both considered Good in the Barcode gap analysis, although with many outlier comparisons each. This suggests that most of the sequences within these genera have low enough distances for observing a clear Barcode gap between species; however, the PCI analysis can detect when there are a single or few sequences with a high intraspecific distance or low interspecific distance to another sequence.

The PCI analysis does not identify the causes for "incorrect identifications" but can be used to detect taxa with a low percentage of correct identifications, which can then be further explored to identify such causes. A few potential causes for the "incorrect identifications" include identification errors in the reference sequences, taxonomic incongruencies, human error during DNA extraction, sequencing or upload to databases, among others (Mutanen et al., 2016).

Conclusion

Undoubtedly BOLD serves as a valuable tool for various molecular studies, offering a freely accessible COI sequence library for many taxa and enabling specimen identification and species delimitation. However, caution is advised when using BOLD data, particularly for Thysanoptera, as the representativity of thrips species in the database is low, with the majority lacking COI data. Additionally, the sampling effort has been limited to specific regions, restricting the usefulness of BOLD as a reference database for many geographical areas. Our analysis revealed a clear Barcode gap for most genera, yet numerous potential misidentifications and cryptic diversity were identified. We propose prioritising non-destructive DNA extraction methods and improving the photographic record to enhance taxonomic analysis. The hardest part—creating a global and freely accessible database of Barcode data—is done. It is up to us, researchers who use this database and populate it with new data, to work on identifying and correcting the inconsistencies and limitations currently present in BOLD, so that it can reach its full potential as a DNA-based species identification tool.

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Conflicts of Interest

Conflicts of interest: The author(s) declare none.

Supplementary Files

All supplementary files for this work can be found in the following Google Drive folder: https://drive.google.com/drive/folders/1-HXpVW68naWePwutjA7hrkiAugVQJqWe?usp=sharing

Supplementary File 1: All databases utilised for this work (ZIP folder containing 39 TXT/FASTA files, 5,528 KB).

Supplementary File 2: Script for R utilised to generate the Boxplot graphs for Barcode gap analysis. Script by L.T.G., annotations translated to English by M.F.L (R file, 3 KB).

Supplementary File 3: Script for R utilised to list the outlier dots observed in the boxplot graphs, for further analysis. Script by L.T.G., annotations translated to English by M.F.L (R file, 4 KB).

Supplementary File 4: Lists of all outliers detected per genera (ZIP folder containing 29 TXT files, 2,352 KB).

Supplementary File 5: Summary of outlier pairwise comparisons detected in the boxplot analyses (PDF file, 38 KB).

Supplementary File 6: Script for R utilised to perform the PCI analysis. Script by L.T.G., annotations translated to English by M.F.L (R file, 3 KB).

Supplementary File 7: Number of COI sequences per species label in database 1, and relative representativity of each (PDF file, 162 KB).

Supplementary File 8: List of errors observed in the Thysanoptera records downloaded from BOLD in November 2021 (PDF file, 76 KB).

Supplementary File 9: Excel sheet listing *Aeolothrips intermedius* sequences with high intraspecific distance, and *Frankliniella* spp. sequences with low interspecific distance (Excel XLSX file, 100 kb).

Supplementary File 10: Excel sheet with all PCI comparisons performed (Excel XLSX file, 67 kb).

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Figures

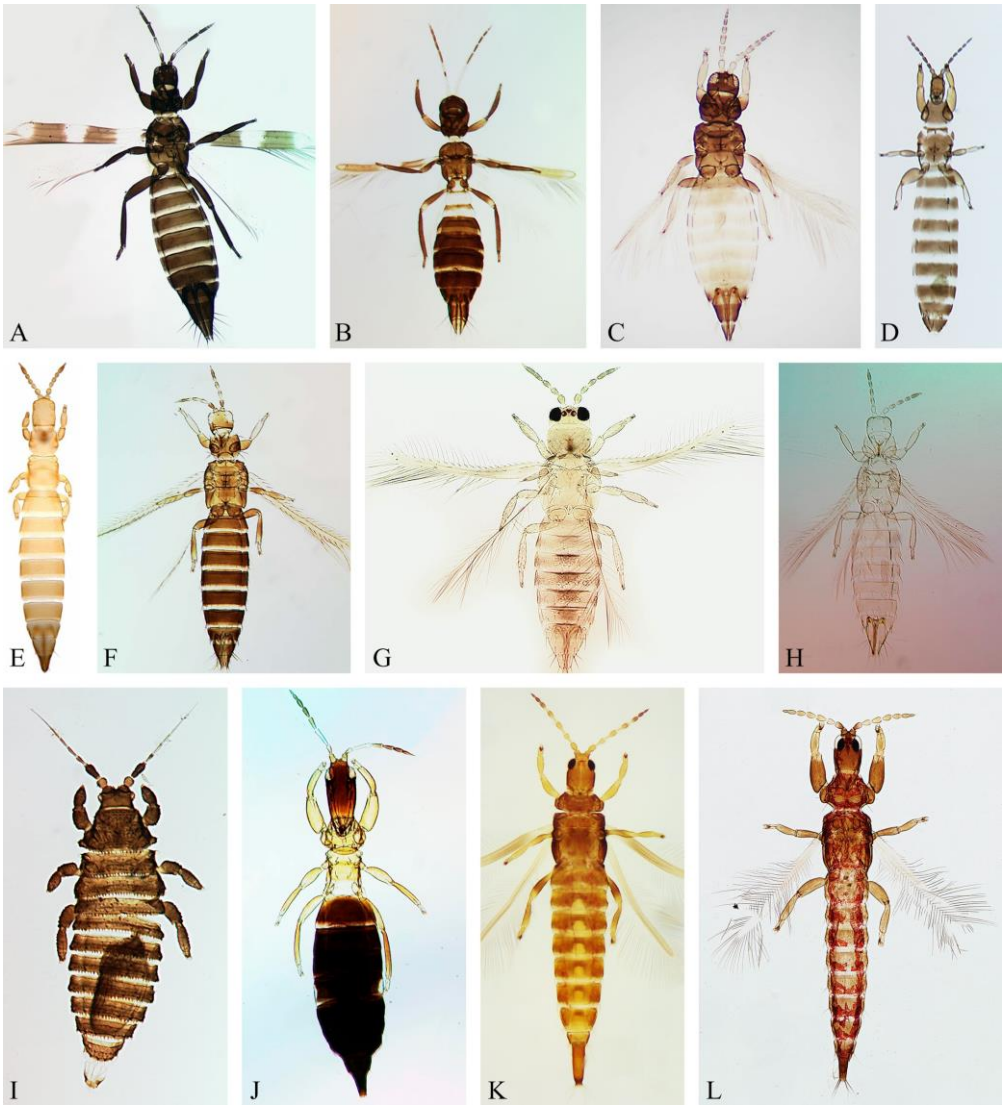

Figure 1: Mounted specimens of a variety of Thysanoptera species and families. A-B: Aeolothripidae; A: *Aeolothrips fasciatus* (Linnaeus, 1758), B: *Franklinothrips vespiformis* (Crawford DL, 1909). C: Heterothripidae, *Heterothrips bicolor* Hood, 1954. D: Merothripidae, *Merothrips brunneus* Ward, 1969. E-H: Thripidae; E: *Aptinothrips stylifer* Trybom, 1894, F: *Frankliniella occidentalis* (Pergande, 1895), G: *Scirtothrips dorsalis* Hood, 1919, H: *Thrips palmi* Karny, 1925. I: Uzelothripidae, *Uzelothrips scabrosus* Hood, 1952. J-L: Phlaeothripidae; J: *Compsothrips graminis* (Hood, 1936), K: *Eschatothrips decoratus* Hood, 1957, L: *Haplothrips dissociatus* Cavalleri, Lindner & Mendonça, 2016. Photos A and E are from the site Thrips of California 2012 (Available at https://keys.lucidcentral.org/keys/v3/thrips_of_california/Thrips_of_California.html; accessed on September 16th, 2022), remaining photos from The Thrips of Brazil (Available at http://thysanoptera.com.br/home; accessed on September 16th, 2022).

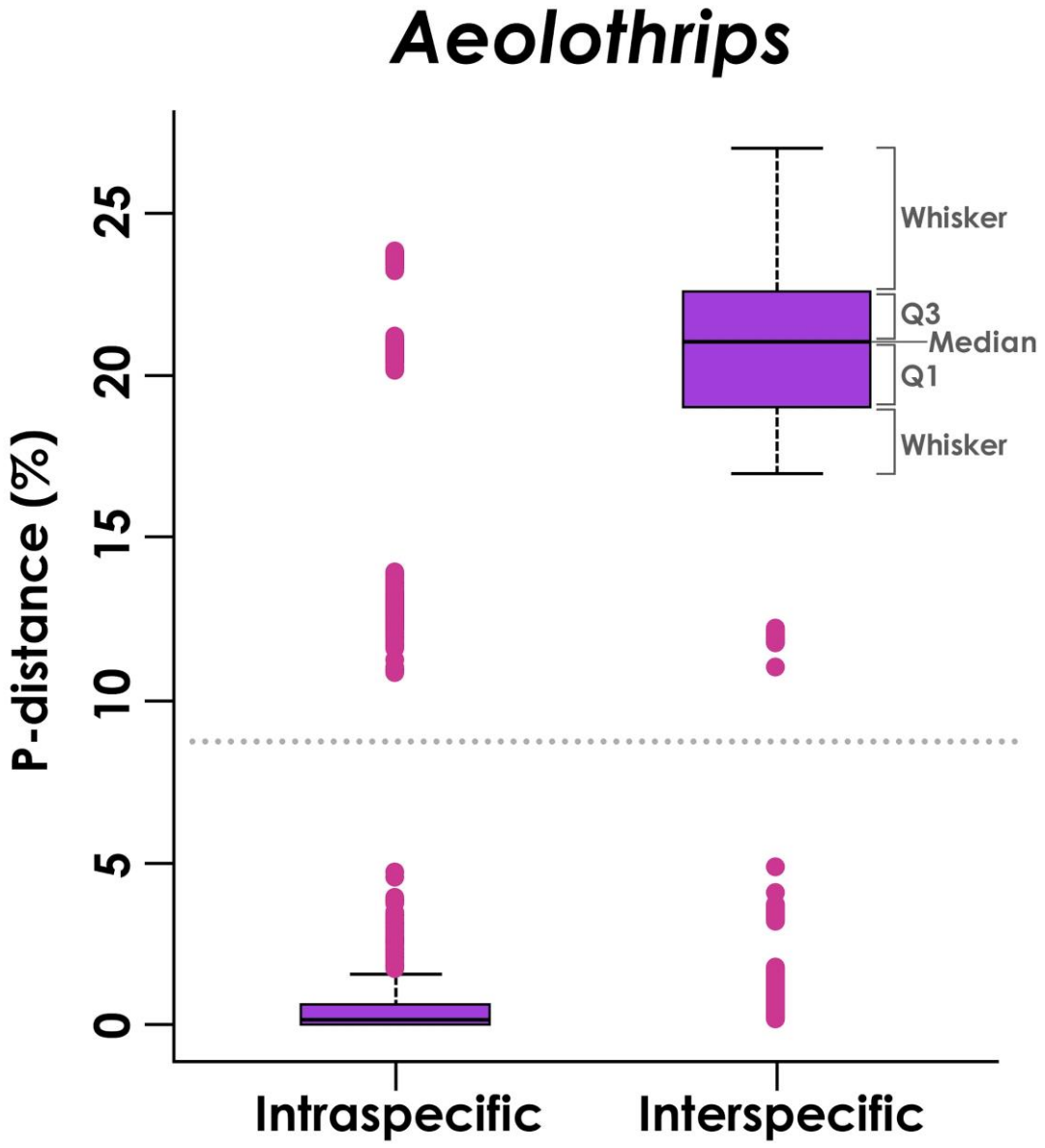

Figure 2: Example of a Boxplot graph, indicating its parts. The outliers are represented as pink dots. The horizontal dotted line represents the cut-off area we delimited for selecting outliers for visual inspection in this genus. The boxplots generated illustrate the lower (Q1) and upper (Q3) quartiles as the two parts of the box, divided in the middle by the median value of the observed data. The whiskers represent ± 1.5 Interquartile Range (IQR), the range from the lower limit of the boxplot (25%) to the upper limit of the boxplot (75%).

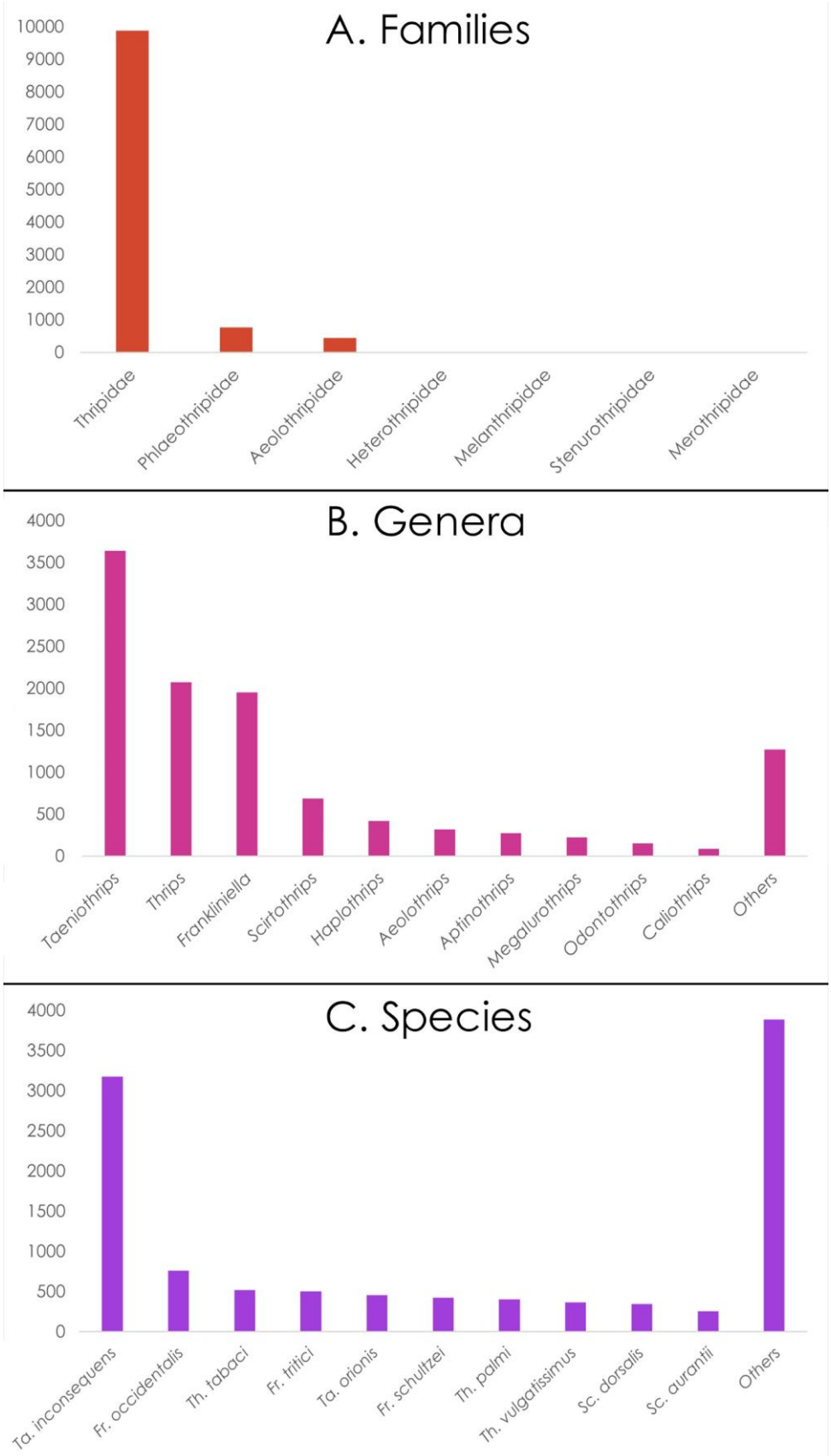

Figure 3: Distribution of Thysanoptera COI sequences (post filtering step 2) from BOLD, in different taxonomic levels. A: Family; B: Genus; C: Species.

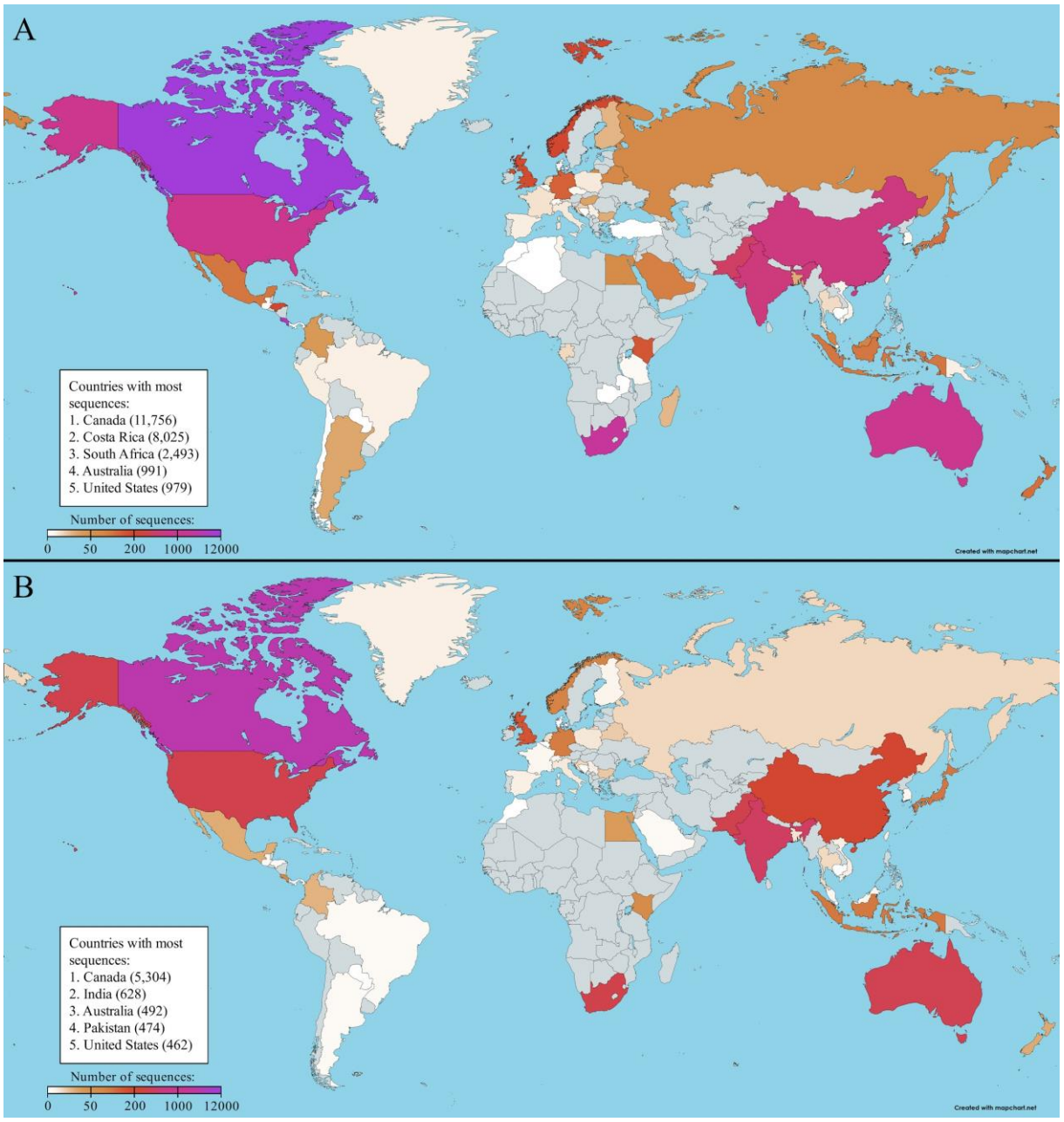

Figure 4: Heat maps showing the countries with most (purple) to least (white) Thysanoptera sequences added to BOLD. A: Geographical distribution of all Thysanoptera sequences downloaded from BOLD, before filtering (database 0). B: Geographical distribution of remaining Thysanoptera sequences, after all filtering steps (database 3). Countries in grey have zero sequences in the evaluated database. Image created with MapChart, available at https://www.mapchart.net.

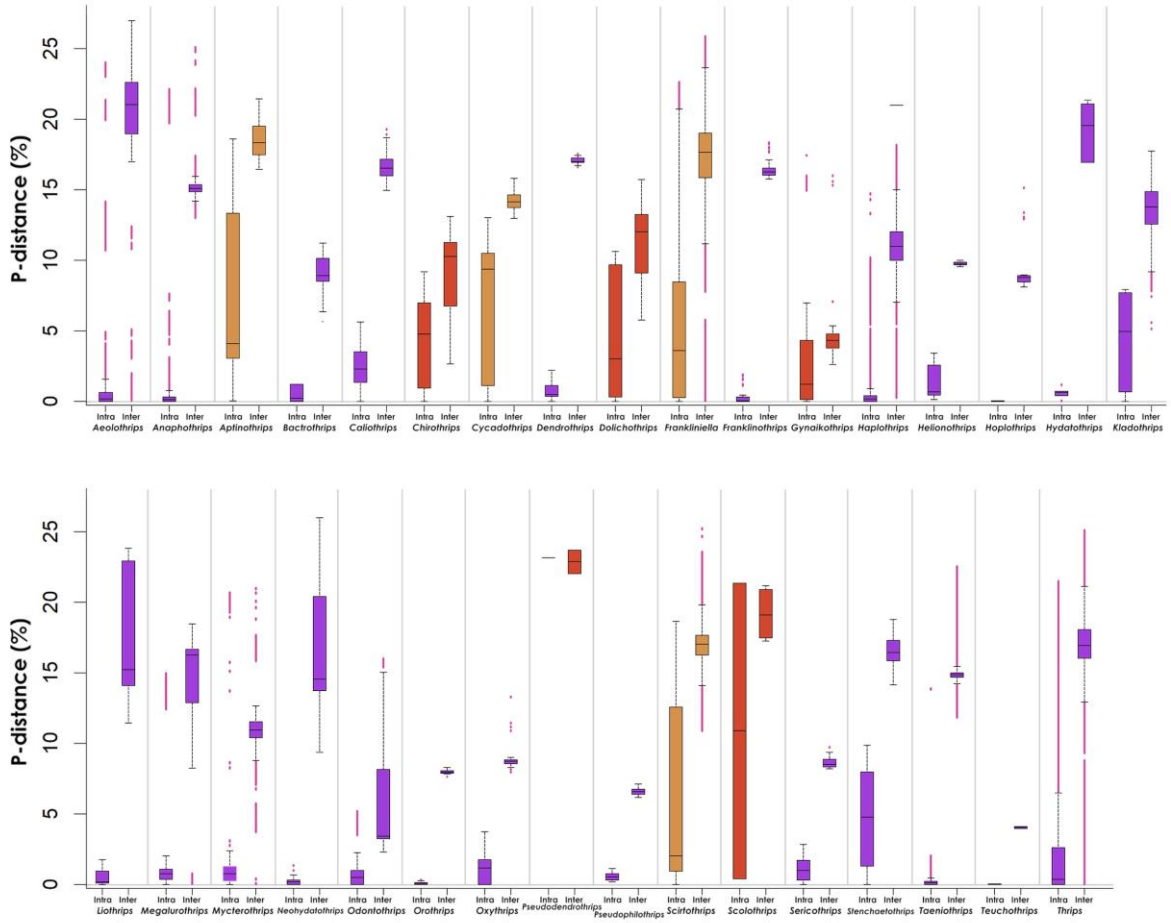

Figure 5: Boxplot graphs for 33 Thysanoptera genera, showing distribution of intraspecific and interspecific distances. Genera considered Good are in purple, Intermediate in orange, and Poor in red. Pairwise comparisons which escape the area of significance indicated by the whiskers (±1.5 IQR), considered outliers, are represented in pink.

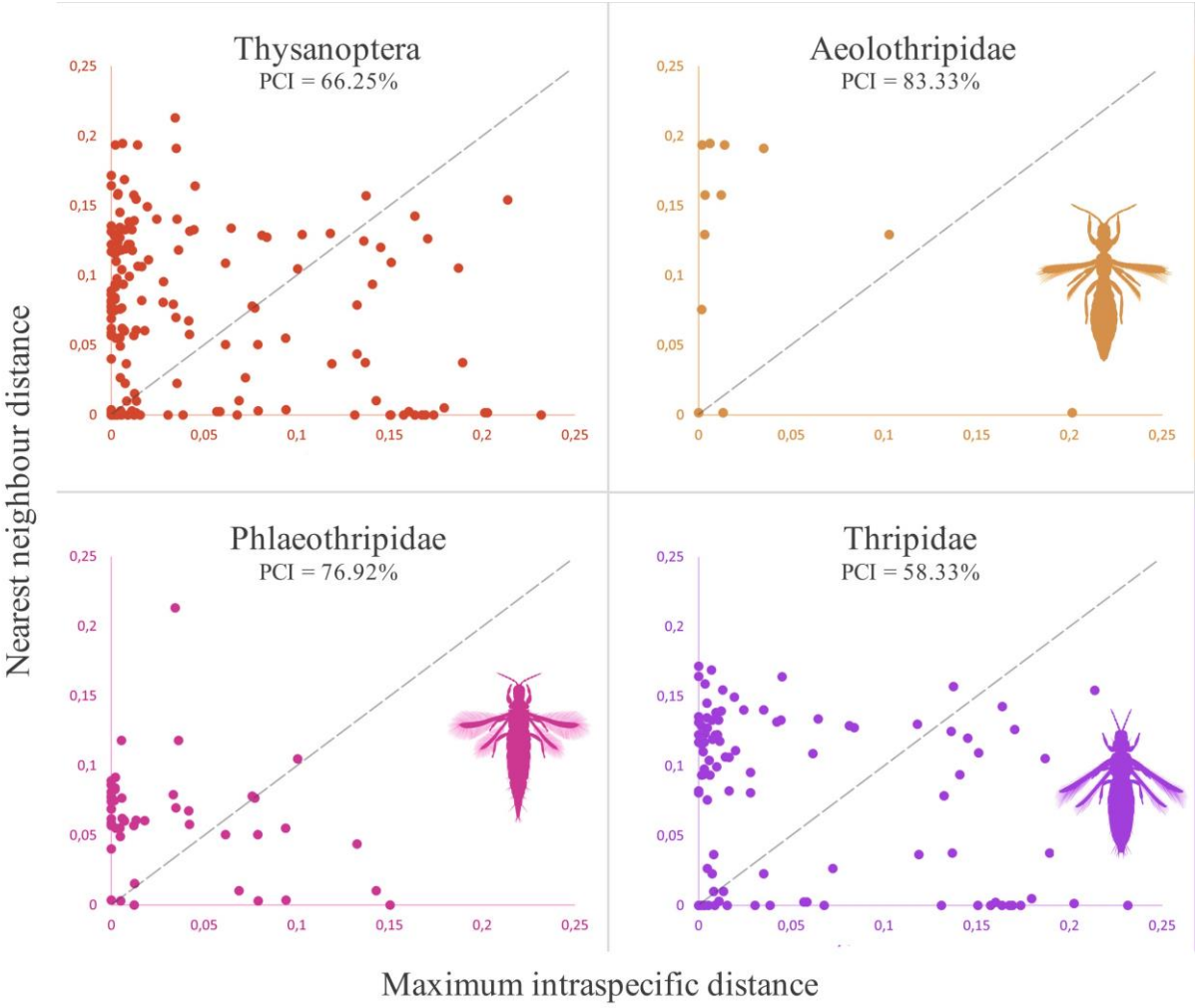

Figure 6: Probability of Correct Identification Analysis for order Thysanoptera, and for Aeolothripidae, Phlaeothripidae and Thripidae. Each dot represents a species, and dots above the dashed line represent species where a correct identification, using the evaluated COI sequences as reference, would be possible (maximum intraspecific distance < nearest neighbour distance).

Tables

Table 1: Number of sequences and taxon representativity on BOLD, after each filtering step.

Filtering step	N Sequences (% from total)	N Families	N Genus labels	N Species labels
0. Data labelled "Thysanoptera" downloaded from BOLD (Nov. 2021)	30,581 (100)	7	139	323
1. Sequences other than COI-5P removed	29,920 (97.84)	7	125	300
2. Sequences lacking species identification removed	11,096 (36.29)	7	115	297
3. Genera with a single species removed	10,434 (34.12)	4	37	219
4. Sequences separated by family and aligned	10,434 (34.12)	4	37	219
5. Sequences with less than 400 bp removed	9,816 (32.10)	4	37	198
6. Sequences separated into genera	9,816 (32.10)	4	37	198
7. Genera with less than two species, or only with singleton species, removed	9,810 (32.08)	3	33	193

Table 2: Databases generated in this work, filtering steps completed on each, and analyses performed with them. All databases are available in Supplementary file 1.

	Filtering steps applied	**Analyses performed**
Database 0 (raw data from BOLD)	None	Distribution map A
Database 1 (COI sequences with ID)	1-2	Representativity
Database 2 (COI sequences by family)	1-5	Probability of Correct Identification (PCI)
Database 3 (COI sequences by genera)	1-7	Barcode gap analysis Boxplot outliers Distribution map B

Table 3: Number of Thysanoptera taxa with at least one sequence after filtering step 2 (database 1), compared to the number of taxa currently accepted in the order (following ThripsWiki 2023).

	Taxa with sequences (valid taxa in the order)	**Barcode coverage (%)**
Suborders	2 (2)	100
Families	7 (9)	77.78
Genera	115 (787)	14.61
Species	297 (6,414)	4.63

Table 4: Number of Thysanoptera genera and species with at least one sequence after filtering step 2, compared to the number of genera and species currently accepted (following ThripsWiki 2023).

	Genera		Species	
	With sequences (valid)	**% from valid**	**With sequences (valid)**	**% from valid**
Suborder Terebrantia	75 (330)	22.72	191 (2,615)	7.30
Family Aeolothripidae	8 (23)	34.78	19 (220)	8.64
Family Heterothripidae	1 (4)	25	2 (89)	2.25
Family Melanthripidae	2 (4)	50	2 (70)	2.86
Family Merothripidae	1 (3)	33.33	1 (18)	5.56
Family Stenurothripidae	1 (3)	33.33	1 (6)	16.67
Family Thripidae	62 (288)	21.53	166 (2,206)	7.52
Subfamily Dendrothripinae	2 (13)	15.38	4 (111)	3.60
Subfamily Panchaetothripinae	13 (42)	30.95	17 (146)	11.64
Subfamily Sericothripinae	3 (3)	100	13 (174)	7.47
Subfamily Thripinae	44 (230)	19.13	132 (1,775)	7.44
Suborder Tubulifera	40 (458)	8.73	106 (3,812)	2.78
Family Phlaeothripidae	40 (458)	8.73	106 (3,812)	2.78
Subfamily Idolothripinae	5 (82)	6.10	10 (744)	1.34
Subfamily Phlaeothripinae	35 (376)	9.31	96 (3,068)	3.13

Table 5: Barcode gap category and median intra- and interspecific distances of the evaluated genera.

Genus	Category	Median intraspecific distance (%)	Median interspecific distance (%)
Aeolothrips	Good	0.17	21.03
Anaphothrips	Good	0.17	15.09
Aptinothrips	Intermediate	4.12	18.36
Bactrothrips	Good	0.26	8.98
Caliothrips	Good	2.29	16.51
Chirothrips	Poor	4.80	10.28
Cycadothrips	Intermediate	9.29	14.10
Dendrothrips	Good	0.46	16.90
Dolichothrips	Poor	3.06	12.04
Frankliniella	Intermediate	3.58	17.65
Franklinothrips	Good	0	16.26
Gynaikothrips	Poor	1.24	4.34
Haplothrips	Good	0.15	10.97
Helionothrips	Good	0.70	9.70
Hoplothrips	Good	0	8.79
Hydatothrips	Good	0.70	19.55
Kladothrips	Good	4.89	13.77
Liothrips	Good	0.20	15.25
Megalurothrips	Good	0.77	16.25

Mycterothrips	Good	0.78	10.97
Neohydatothrips	Good	0.15	14.51
Odontothrips	Good	0.48	3.44
Orothrips	Good	0	7.90
Oxythrips	Good	1.20	8.86
Pseudodendrothrips	Poor	23.15	22.87
Pseudophilothrips	Good	0.54	6.59
Scirtothrips	Intermediate	1.99	16.98
Scolothrips	Poor	10.90	19.12
Sericothrips	Good	0.94	8.52
Stenchaetothrips	Good	4.79	16.51
Taeniothrips	Good	0.16	14.87
Teuchothrips	Good	0	4.09
Thrips	Good	0.33	16.95

Table 6: List of genera with observed outliers in the boxplot graphs.

Genus	Type of Outlier			
	Below Intraspecific boxplot [1]	**Above Intraspecific boxplot [2]**	**Below Interspecific boxplot [2]**	**Above Interspecific boxplot [1]**
Aeolothrips		x	x	
Anaphothrips		x	x	x
Bactrothrips			x	
Caliothrips				x
Dendrothrips			x	x
Frankliniella		x	x	x
Franklinothrips		x		x
Gynaikothrips		x	x	x
Haplothrips		x	x	x
Hoplothrips				x
Hydatothrips	x	x		
Kladothrips			x	
Megalurothrips		x	x	
Mycterothrips		x	x	x
Neohydatothrips		x		
Odontothrips		x		x
Orothrips		x	x	
Oxythrips			x	x

Scirtothrips		x	x
Sericothrips			x
Taeniothrips	x	x	x
Thrips	x	x	x

[1] Outliers not listed by the R script. [2] Outliers listed by the R script.

Table 7. *Frankliniella* species with analysed interspecific outlier comparisons.

Species	N sequences involved in outliers	N sequences	Compared to	N comparisons	Average distance (%)
F. bispinosa	16	18	*F. tritici*	4,530	4.544
F. borinquen	1	17	*F. occidentalis*	642	2.272
F. citripes	1	1	*F. insularis*	39	0.316
F. insularis	39	41	*F. citripes*	39	0.316
F. minuta	1	1	*F. schultzei*	164	0
F. occidentalis	642	762	*F. borinquen, F. panamensis*	670	2.519
F. panamensis	3	26	*F. occidentalis*	28	8.188
F. schultzei	164	424	*F. minuta*	164	0
F. tritici	284	505	*F. bispinosa*	4,530	4.544

Capítulo 2

DNA BARCODING IN THE LEAF-LITTER THRIPS OF THE TRIBE GLYPTOTHRIPINI (THYSANOPTERA: PHLAEOTHRIPIDAE)

Artigo a ser submetido no periódico Insect Systematics and Evolution.

Normas para os autores: https://brill.com/view/journals/ise/ise-overview.xml?contents=artsub

Capítulo 3

MORPHOLOGICAL IDENTIFICATION OF *GLYPTOTHRIPS* SPECIES (THYSANOPTERA: PHLAEOTHRIPIDAE)

Artigo publicado no periódico Zootaxa. DOI: https://doi.org/10.11646/zootaxa.5375.1.2

Normas para os autores: https://www.mapress.com/zt/about/submissions

Morphological identification of *Glyptothrips* species (Thysanoptera: Phlaeothripidae)

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Abstract

Glyptothrips is a New World genus with 14 species of fungivorous Phlaeothripidae found in leaf litter. Their identification is a complex task, due to outdated descriptions, lack of identification keys and images, and limited collection of specimens. Here we provide an identification key for the nine *Glyptothrips* species recorded in South America, based on literature data and type specimens observations. A table with many diagnostic characters for all species in the genus is given, and we discuss the morphological variation observed in this group. Finally, many species are illustrated for the first time, to facilitate comparisons and identification efforts.

Key words: Identification key, Glyptothripini, leaf litter, fungivorous

Running Title: Morphological identification of *Glyptothrips*.

Introduction

Since the proposal of tribe Glyptothripini in 1927 by Priesner, this group has been utilised to place a variety of leaf-litter Phlaeothripinae members. Initially only those species with strongly reticulated heads and small bulbous eyes were included in this tribe, but subsequent revisions (Stannard 1955; Mound 1976; Mound 1977) added and removed many genera and species that did not fit this simple description. As a result, the current definition of Glyptothripini lacks a proper diagnosis. The latest formal revision of this group (Mound 1977) left the tribe with a total of 21 genera, of which 20 are still valid and composed of 152 species (ThripsWiki 2023).

Three species of *Glyptothrips* were originally described within this genus; the other 11 species were described in five different genera of Glyptothripini, before being transferred to their current genus (ThripsWiki 2023). In describing *Glyptothrips* and its type species in 1912, *G. flavescens* Hood, the author indicated the 7-segmented antennae as one of the main diagnostic traits, along with the 'long tube', bulbous eyes, and strongly defined dorsal sculpture (Hood 1912, p.116–117). However, some of these traits are not present in current members of the genus. Many characters have variable states within the genus, such as colouration, the degree of separation between antennomeres VII–VIII, length and shape of tip of the pterothoracic ventrolateral setae, and even the appearance of body sculpture (Mound 1977; Mound & Marullo 1996).

Glyptothrips is more commonly compared to *Eschatothrips* Stannard and *Orthothrips* Priesner, due to the strong body reticulation, and to *Tylothrips* Hood due to the presence of well-developed pterothoracic ventrolateral setae (Mound 1977; Mound & Marullo 1996). It is separated from the first two genera more readily by the tube appearance: *Eschatothrips* species usually have a thick tube with convex margins, covered in strong ridges or reticulation, whereas *Orthothrips* species have slender tubes, longer than the head, and sculptured near the base. In contrast, *Glyptothrips* members have mostly smooth tubes with straight margins, and length similar to head length or shorter. The body sculpture in *Glyptothrips* species is usually formed by strong hexagonal reticulation; meanwhile *Tylothrips* species usually have weaker sculpture or even smooth dorsal surfaces, but forms whose character states are intermediate between both genera are known (Mound 1977).

The species currently included in *Glyptothrips* are restricted to the New World (Figs 1–3): five species (*G. arkansanus* Hood, *G. claviger* (Hood), *G. floridensis* (Stannard), *G. interior* (Stannard), *G. reticulatus* Watson) were collected only in the United States (Fig. 1), and eight species (*G. bucca* (Hood), *G. divergens* (Hood), *G. fuscipes* (Hood), *G. hylaeus* (Hood), *G. longiceps* (Hood), *G. saltuarius* (Hood), *G. silvaticus* (Hood), *G. subcalvus* (Hood)) have been

recorded only from Brazil (Mound 1977; ThripsWiki 2023) (Fig. 2). However, *G. flavescens*, the type species of *Glyptothrips* and originally described from the United States, is here newly recorded in southern Brazil, being the only species so far collected in both regions. This disjunction is most likely the result of sampling efforts biases, due to researchers who described and studied *Glyptothrips* species (mainly J. D. Hood and L. J. Stannard) focusing on North American fauna, with Hood also having access to leaf litter collections performed by F. Plaumann in southern Brazil (Hood 1954; Hood 1957b). *Glyptothrips* species are among the most frequent thrips in the leaf litter in northeastern and southern Brazil (Pinent *et al.* 2006; Santos *et al.* 2020), but they usually cannot be identified at species level.

Identifying *Glyptothrips* species is a very imprecise task, considering the available taxonomic information. Most of the original descriptions are outdated, lacking informative illustrations; the latest revision of the tribe was made over 45 years ago (Mound 1977), and there are no identification keys for the genus with its current composition. The only keys including *Glyptothrips* species are either limited to a few North American species (Stannard 1955; 1957; 1968) or have outdated taxonomy (Mound 1976).

We aim to provide the following new tools to aid in identifying *Glyptothrips* species: (1) an identification key to the South American species of *Glyptothrips;* (2) a brief comparative diagnosis for all species, including a table compiling some characters states of all species; (3) a discussion of the morphological variation observed in the species included in the genus; and (4) photographs of type specimens whenever possible, to better illustrate the morphological traits of *Glyptothrips* species.

Material and Methods

Material studied. We followed the taxonomic treatment of ThripsWiki (2023) for the current composition of *Glyptothrips* and its synonyms. Literature data (original descriptions, revision works, illustrations) consulted are listed on Table 1.

We studied type specimens for most species of *Glyptothrips*, which are deposited at the Smithsonian National Museum of Natural History (NMNH), held by the United States Department of Agriculture (USDA), and at Illinois National History Survey (INHS) (Table 1). The holotypes of two synonyms of *G. reticulatus* Watson, *Eurythrips sculpturus* Hood and *Eurythrips silvarum* Hood, have also been studied.

Finally, we observed specimens collected from Brazil in the past twenty years, all deposited at Universidade Federal do Rio Grande do Sul (UFRGS).

Identification Tools. An identification key has been created through observations of type specimens and literature data, and complemented with observations of some non-type specimens for a few taxa. We have chosen to only include species recorded in South America for this key, due to the limited access to some North American species and difficulties to include them in the key. A variety of diagnostic morphological characters have been organised into Table 2, as a complementary identification tool. This table has been created from mainly literature data, but tested with specimens observations whenever possible.

Finally, brief comments are given to each species, listing some of their main diagnostic traits and comparisons to other similar *Glyptothrips*. However, we were unable to observe a large number of individuals for the majority of the species, thus our proposed identification tools do not consider potential intraspecific variations, unless stated otherwise.

Photographs. Specimens were photographed with the usage of a Nikon DS-Fi1 camera attached to a Nikon Eclipse 80i microscope with phase contrast and DIC.

The images with different focal points were manually edited in the software Krita (available at https://krita.org/), which was also used to create the plates in this work.

Nomenclature and abbreviations. We followed Mound & Marullo (1996) and Bhatti (1998) for most morphological nomenclature, and the term “pore plates” is used instead of “glandular areas”.

Figure 4 provides an illustrated guide to many of the setae mentioned in this work.

The following abbreviations are used: PO - postocular setae; AM - pronotal anteromarginal setae; AA - pronotal anteroangular setae. Setae organised in a row or group will be named from the innermost pair towards the most external or apical pair as S1, S2, S3... Sn.

Measurements. We utilise the following delimitations for the measurements mentioned in this work:

Head length: measured dorsally across the middle of the structure; from tip of interantennal projection to basal limit of head capsule.

Head width: measured dorsally, across the widest area of the head (not considering the eyes region).

Tube length: measured dorsally across the middle of the structure; from base of tube to the tip, including membranous area, but not including setae.

Tube width: measured dorsally, at the widest sub basal area.

Seta length: measured from base of seta (not considering the pore at the base of seta) to tip, including dilated or capitate tips if present. For curved setae, an approximation of its length can be done by doing two or more partial measures, accompanying the curve.

Results

Identification key to *Glyptothrips* species from South America

1. Tube at least 1x as long as head or longer (Fig. 19) 2
- Tube shorter, at most 0.9x as long as head (Fig. 47) 3

2. Pterothoracic ventrolateral setae thick and capitate (Fig. 21); antennae with 8 elongate segments (Fig. 23), antennomere IV with 4 sense cones; head more than 1.4x as long as greatest width, genae mostly straight (Fig. 20) ..... *G. divergens*
- Pterothoracic ventrolateral setae acute, indistinct from discal setae (Fig. 26); antennae with 7 globose segments (Fig. 26), antennomere IV with 2 or 3 sense cones; head less than 1.35x as long as greatest width, genae curved ..... *G. flavescens*

3. Head with PO at least 0.8x as long as the dorsal length of the compound eye (Fig. 32)..... 4
- PO very small or scarcely visible, less than 0.5x as long as the dorsal length of the compound eye (Fig. 58) 6

4. Pterothoracic ventrolateral setae thin and acute, indistinct from meso-metasternal setae; fore femora without any thick and capitate setae (Figs 9–10); antennomeres II–IV without capitate setae (Fig. 12), antennomere IV with 3 sense cones ..... *G. bucca*
- Pterothoracic ventrolateral setae thick and capitate, distinct from meso-metasternal setae (Fig. 33); fore femora with at least one thick and capitate setae (Figs 31, 63); antennomeres II–IV with at least one pair of capitate setae each (Fig. 35), antennomere IV with 4 sense cones 5

5. Legs mostly brown (Figs 31, 33); antennomeres III and IV bicolored, yellow on thinner basal area and brown elsewhere (Fig. 35); PO setae straight (Fig. 32); males with a large pore plate covering most of sternite VIII (Fig. 36) ..... *G. fuscipes*
- Legs mostly yellow (Figs 62–63); antennomere III mostly yellow, much paler than the mostly brown antennomere IV (Fig. 64); PO setae curved; males with a slender transverse pore plate on sternite VIII .....*G. silvaticus*

6. Antennomere IV with 2 sense cones; head less than 1.2 times as long as greatest width, lateral margins greatly curved into a constrict basal neck (Fig. 67); males with a transverse pore plate

on abdominal sternite VIII, anterior margin concave, not covering the whole sternite (Figs 70–72) ..... *G. subcalvus*

- Antennomere IV with 4 sense cones; head at least 1.25 times as long as greatest width, lateral margins straight without a constrict basal neck (Figs 38, 48, 58); males with a large pore plate covering most of abdominal sternite VIII (Fig. 40) 7

7. Head dorsal sculpture transversely elongate (Fig. 48); tube less than 0.7 times as long as head (Fig. 47)..... *G. longiceps*

-. Head dorsal sculpture with equiangular reticles (Figs 38, 58); tube about 0.8–0.9 times as long as head (Figs 37, 57) 8

8. Head about 1.25–1.35x as long as greatest width (Fig. 38) ..... *G. hylaeus*

- Head about 1.45–1.55x as long as greatest width (Fig. 58)..... *G. saltuarius*

***Glyptothrips* Hood, 1912**

There is not a unique morphological trait used to diagnose *Glyptothrips*. However, the following combination of character states seems to be useful to distinguish a member of the genus, as it is currently defined, from other Glyptothripini: (1) body strongly reticulate (Figs 6, 15, 58), except the tube which has at most some weak sculpture basally (Figs 16, 45, 59); (2) pronotal AM reduced (Figs 20, 32, 69); (3) pterothoracic ventrolateral setae usually thick and capitate (Figs 21, 33, 62), except in *G. arkansanus*, *G. bucca*, *G. flavescens* and *G. reticulatus*.

Besides these three traits, all observed *Glyptothrips* species seem to share the following character states: head incut behind globose/moruloid eyes (Figs 26, 48); PO setae and all pronotal setae except AM well-developed with dilated tips (Figs 15, 32); antennomeres II–IV with at least one pair of capitate setae (except in *G. bucca*) (Fig. 35); prosternal basantra present; tube with straight sides, tapering (Figs 7, 11); fore tarsi armed with a tooth (except in *G. arkansanus*) (Figs 42, 48); fore wings without any duplicated cilia.

Below we further discuss the variation in character states observed within *Glyptothrips* species, for many traits useful to identification:

Colouration: the colour of body structures can be variable and is heavily influenced by how the specimen was prepared before slide mounting: many *Glyptothrips* species were described from individuals not macerated in NaOH prior to mounting. Several type specimens were also initially mounted in Hoyers, a mounting media not as durable as Canada Balsam, so

much of the colour information could have been modified or lost (e.g. Figs 28–30). However, some patterns can still be useful to eliminate possible identifications. The body colour in original descriptions varies from mostly yellowish species, such as *G. interior* (Fig. 41) and *G. silvaticus* (Fig. 61); to darker brown species, like *G. claviger* (Fig. 14) and *G. subcalvus* (Fig. 66). However, some macerated specimens which have been structurally identified as *G. subcalvus* have a lighter, yellowish colour (e.g. Figs 69–72).

The antennae seem to be more frequently a similar colour to the head, getting progressively darker past antennomeres IV or V. It is also common for the darker antennomeres (especially IV–VI) to have the basal areas and/or pedicels lighter (Figs 25, 35, 43). A few species escape this general pattern, though: *G. arkansanus* has the antennae mostly yellow, with only the last antennomere slightly darker (Fig. 5); and *G. subcalvus* has the first two antennomeres a very pale yellow, much lighter than the head (Figs 66, 68).

Head and thorax are more commonly the same colour (Figs 9, 41), or the thorax is partially or fully darker than the head (Figs 47, 57). The abdomen is frequently paler or gets progressively paler towards the terminal segments (Fig. 31). Some interesting colouring patterns can be seen in the abdomen: there are species with a darker antecostal ridge and/or spot on tergites II–VII, such as *G. divergens* (Fig. 19), *G. interior* (Fig. 41), *G. saltuarius* (Fig. 57) and *G. silvaticus* (Fig. 61); species with two longitudinal pale lines separating the tergites in three thirds (very apparent in *G. arkansanus* (Figs 5–7), which also has the posteromedian area of the tergite much paler; these lines are present but not as apparent in *G. flavescens* (Fig. 26)); and in *G. subcalvus* the median third of tergites seems to be darker than the rest of the abdomen (Fig. 66). The tube is also variable between species, but more commonly a similar (Figs 7, 27) or darker colour to the previous abdominal segments (Figs 22, 65). The basal and/or apical areas of the tube are frequently lighter or darker than the middle of the tube (Figs 45, 73).

Head: all *Glyptothrips* species have the head fully covered by strong reticulation, both on dorsal and ventral surfaces (Figs 6, 20, 67). The genae varies from almost straight, like in *G. divergens* (Fig. 20) or *G. hylaeus* (Fig. 38), to strongly curved, like in *G. bucca* (Fig. 10) or *G. subcalvus* (Fig. 67). Observed length x greatest width ratio from original descriptions vary from 1 (*G. floridensis*) to 1.5 (*G. longiceps*). However, this character should be observed with caution, as the ratio can be impacted by the slide mounting, and ranges can overlap between species (Table 2).

Most species in the genus have small and acute postocellar, occipital and head lateral setae, but *G. floridensis* escapes this pattern: it is illustrated (Stannard 1955) with the occipital and many lateral setae as long and capitate as the PO. The occipital setae have dilated tips as

well in *G. claviger* (Fig. 15) and *G. interior* (Fig. 42), but neither of these species has the head lateral setae thickened or capitate. Length of PO varies from very short and inconspicuous like in *G. divergens* (Fig. 20), to as long or longer than the compound eye like in *G. silvaticus* (Fig. 63) (Table 2).

Antennae: the type species, *G. flavescens*, has only 7 antennal segments due to the fusion of antennomeres VII and VIII (Fig. 26); however, only *G. arkansanus* approximates this characteristic in the genus, having antennomeres VII–VIII broadly joined, but still separated by a suture. All other *Glyptothrips* species have VII and VIII clearly separated (Figs 12, 23, 35). The most common sense cone formula in *Glyptothrips* is 3 sense cones on antennomere III and 4 on IV. However, reductions in the number of sense cones are known in *G. bucca*, *G. flavescens*, *G. interior*, *G. reticulatus* and *G. subcalvus* (Table 2).

The shape of antennomeres is also variable within the genus, from very short and globose antennomeres in *G. flavescens* (Fig. 26) and *G. arkansanus* (Fig. 5), to elongate antennomeres like in *G. divergens* (Fig. 23) and *G. fuscipes* (Fig. 35). Some species are also intermediate between these extremes, such as *G. bucca*, *G. subcalvus* (short but not globose antennomeres), *G. hylaeus* or *G. silvaticus*.

Prothorax: most species have the pronotum reticulate (Figs 6, 20), but the sculpture can be less apparent in some species (Fig. 69). The epimeral sutures are usually incomplete. The position of AA setae seems to be variable: while positioned at or very close to the anterior angles of pronotum in the majority of *Glyptothrips* species, at least in *G. flavescens* (Fig. 26) and *G. floridensis* this pair of setae arises close to AM.

Pterothorax: meso- and metanotum are both clearly sculptured, usually with equiangular reticles without any internal markings (Figs 6, 21). However, at least *G. flavescens* and some specimens of *G. claviger* have “tubercles” in the metanotum, in place of some of the sculpture lines (Fig. 26). The ventrolateral setae are thick and capitate in most *Glyptothrips* (Figs 21, 62), with the only exceptions listed in the proposed diagnosis above.

Legs: all legs usually have similar sizes and sculpturing, and are covered in many acute setae (Figs 5–6, 14–15). The fore tibia does not have tubercles. In *G. fuscipes*, all femora and the fore tibiae have at least one or two elongate, thick and capitate setae (Figs 31, 33); most other *Glyptothrips* species lack such setae, although a short but capitate seta has been seen in the fore femora of some specimens of *G. claviger*, *G. longiceps*, *G. reticulatus*, *G. silvaticus* (Fig. 63) and *G. subcalvus*.

Wings: there are multiple wing forms within the genus, with macropterous (Fig. 5), micropterous (Fig. 15) and apterous (Fig. 55) specimens of *Glyptothrips* being known. Five

species (*G. arkansanus*, *G. flavescens*, *G. reticulatus*, *G. saltuarius* and *G. subcalvus*) are known to have two or more wing morphs.

The degree of wing development is frequently associated to some changes in character states, especially when comparing macropterous (eyes sometimes larger, ocelli present, pelta not as wide) with apterous (eyes sometimes reduced, ocelli reduced or absent, pelta wide and rectangular or oval in shape) specimens (Mound 1977, 2005; Mound & Marullo 1996; personal observations). Thus, caution is required when comparing those characters in different wing morphs, as they can lead to incorrect assumptions of species delimitations.

Abdomen: the pelta is highly variable, and at least partially associated with the degree of wing development. The most common shape seems to be a broad oval or rectangular plate (Figs 10, 26, 44, 69), however some species may have a triangular, trapezoidal (Figs 15, 36) or hat-shaped (Fig. 6) pelta.

Tergites II–IX are sculptured, with the median posterior area weaker or sometimes smooth (Figs 6, 44, 58). The three main setal pairs on tergite IX are well developed, with S2 reduced in males (Fig. 45). Setae S1 are most frequently dilated or capitate (Figs 45, 73), while S2 are more frequently acute or blunt (Table 2).

The tube is mostly smooth, with weak lines of sculpture basally in many *Glyptothrips* species (Figs 7, 16, 39), but never with strong reticulation as in *Chamaeothrips* Hood or *Eschatothrips* species. The tube is usually shorter than the head or almost as long as the head; only two species (*G. divergens* and *G. flavescens*) have recorded specimens where the tube is slightly longer than the head (Figs 19, 25).

***Glyptothrips arkansanus* Hood, 1957a**

(Figs 5–8)

This species is most similar to *G. flavescens*, the type species of *Glyptothrips*. However, *G. arkansanus* can be differentiated by having a suture between antennomeres VII–VIII (whereas in *G. flavescens* both antennomeres are fully fused), the abdominal pale lines much more apparent (Figs 6–7) than in *G. flavescens*, and the antennae mostly yellow (Fig. 5), while it is mostly brown from antennomere III onwards in G. *flavescens*. *Glyptothrips arkansanus* also lacks the "tubercles" on pronotal and metanotal sculpture, which is characteristic of *G. flavescens*.

Known wing forms: macropterous and micropterous.

Specimens studied. Lectotype f#; United States of America, Arkansas, Fayetteville, on *Andropogon virginicus* L., 16.iii.1957 (W.H. Whitcomb), at NMNH.

Paralectotypes 1 m# and 1 f#, same collection data and depositary as lectotype.

***Glyptothrips bucca* (Hood, 1957b)**

(Figs 9–13)

This species is unique in lacking capitate setae on antennomeres II–IV (Fig. 12), which is present in all other *Glyptothrips* species; and in the shape of male pore plate, which is a small circle medially on sternite VIII (Fig. 13). Another unusual trait in *G. bucca* is the reduction in the number of sense cones on antennomere IV, from four to three. Only some individuals of *G. flavescens* share this character, and both species can be easily differentiated by number of antennomeres (seven in *G. flavescens*, eight in *G. bucca*) and thorax sculpture (*G. bucca* has a reticulate dorsal surface (Fig. 10), while *G. flavescens* has the pronotal and metanotal sculpture modified into "tubercles").

Known wing forms: micropterous.

Specimens studied. Holotype f#; Brazil, Santa Catarina, Nova Teutônia, under fallen leaves, iv.1954 (F. Plaumann), at NMNH.

Allotype m#, same collection data and depositary as holotype.

***Glyptothrips claviger* (Hood, 1941)**

(Figs 14–18)

This North American species has a mostly dark brown colouration and occipital setae capitate (Fig. 15), a combination of character states only present elsewhere in *G. floridensis* - however, the latter has the head lateral setae also thickened and capitate, which is not present in *G. claviger*. These two species can be further differentiated by the colouration of antennomere III, which is a similar shade of brown to other antennomeres in *G. claviger* (Fig. 18), but bright yellow in *G. floridensis* (Fig. 28). Further traits which can differentiate these species can be seen in Table 2.

Known wing forms: micropterous.

Specimens studied. Holotype f#; United States of America, New York, Oswegatchie, on dead grass and debris, 26.viii.1940 (J.D. Hood), at NMNH.

Paratypes 1 m# and 1 f#, same collection data and depositary as lectotype.

***Glyptothrips divergens* (Hood, 1957b)**

(Figs 19–24)

This species has a rather elongate head, almost 1.5 times as long as the greatest width (Fig. 20). Only *G. longiceps* and *G. saltuarius* have similarly elongated heads within *Glyptothrips*. This species can be differentiated by the large tube, which is actually longer than the head (Fig. 19), while the tube is shorter than the head in the other two species. A unique trait within *G. divergens* is the shape of male pore plate: a transverse ellipse in the middle of abdominal sternite VIII (Fig. 24). This contrasts with the more common pore plate shapes in *Glyptothrips* (Table 2).

Known wing forms: macropterous.

Specimens studied. Paratypes 1 m# and 1 f#; Brazil, Santa Catarina, Nova Teutônia, under fallen leaves, ii.1953–x.1955 (F. Plaumann), at NMNH.

***Glyptothrips flavescens* Hood, 1912**

(Figs 25–27)

Comments. The type species of *Glyptothrips*, but with several unique traits. Besides the 7-segmented antennae (only seen in *G. arkansanus*), it lacks thick and capitate ventrolateral setae, has the AA setae closer to the reduced AM (only seen elsewhere in *G. floridensis* and *G. interior*), and the sculpture on thorax and abdomen bears multiple tubercles (Fig. 26).

Known wing forms: macropterous, micropterous and apterous.

Specimens studied. Holotype f#; United States of America, Illinois, Grand Tower, in sweepings from grass, 10.vii.1909 (C.A. Hart), at NMNH.

Paratype 1 f#, United States of America, Illinois, Putaski, in sweeping from grass, 28.vi.1909 (Cleared in KOH, remounted 1952), at NMNH.

***Glyptothrips floridensis* (Stannard, 1955)**

(Figs 28–30)

This seems to be the only species within *Glyptothrips* where the body is mostly dark brown and the lateral head setae are thick and capitate, similarly to the PO setae - however, in one observed specimen from the type series, the head lateral setae are not as developed (Fig. 29) as indicated in the holotype's illustration (Stannard 1955). The pronotal AA setae also arise closer to the reduced AM, a trait only seen elsewhere in the lighter-coloured *G. flavescens* and *G. interior*. *Glyptothrips floridensis* also seems to share some similarities with *G. claviger*, and both species are differentiated in the latter's comments.

Known wing forms: micropterous.

Specimens studied. Holo- or Paratype 1 m#; United States of America, Florida, Everglades National Park, Long Pine Key Hammock, from forest leaf mould, 24.xii.1952 (Richards & Stannard), at INHS.

1 f# (listed as "paratype" in the slide label, but not mentioned in the original description); United States of America, Florida, Gainesville, from damp leaves along stream in Catarata Glen. 1.xii.1926? or 12.i.1926? (D.M. Bates), at INHS.

***Glyptothrips fuscipes* (Hood, 1954)**

(Figs 31–36)

This species can be differentiated from most other *Glyptothrips* species by its brown colouration (including legs, which are usually yellow or at least lighter than the body in other species; Figs 31, 33), and presence of rather thick and elongate capitate setae in all femora and fore tibiae (Fig. 31). Other species in the genus may present this type of thickened setae at least on fore femora (e.g. *G. claviger*, *G. silvaticus*), but they are not as elongate and numerous as in *G. fuscipes*.

Known wing forms: micropterous.

Specimens studied. Holotype f#; Brazil, Santa Catarina, Nova Teutônia, viii.1952 (F. Plaumann), at NMNH.

Paratypes 1 m# 1 f# , same collection data and depositary as holotype.

***Glyptothrips hylaeus* (Hood, 1950)**

(Figs 37–40)

This species seems to lack a highly distinctive trait, and most of its morphological character states are shared with a variety of other *Glyptothrips* species. The head shape (Fig. 38) seems to be intermediate between the shorter and strongly rounded head in one extreme (e.g. *G. claviger*, *G. subcalvus*), and the elongate head with straight genae in the other extreme (e.g. *G. divergens*, *G. longiceps*). *Glyptothrips hylaeus* seems to be quite similar to *G. saltuarius*, with the only clear difference between type specimens being the head length in relation to head width (Table 2).

Known wing forms: macropterous.

Specimens studied. Holotype m#; Brazil, São Paulo, Franco da Rocha, Serra da Cantareira, dead leaves on ground in shade of low trees, 11.vi.1948 (J.D. Hood, F. Lane and L.T. Filho), at NMNH.

***Glyptothrips interior* (Stannard, 1955)**

(Figs 41–46)

This mostly yellow species is described as one of the lightest *Glyptothrips* species, with only tips of antennae and tube, wing pads and median spots on abdominal tergites being brown (Fig. 41). It also has the postocellar and occipital setae thickened and capitate (Fig. 42), a trait only seen elsewhere in *G. claviger* and *G. floridensis*, two mostly dark brown species. *Glyptothrips interior* is also one of few species in the genus with a reduced number of sense cones on antennomere IV (Table 2), being differentiated from the others by its light colouration and capitate occipital and pterothoracic ventrolateral setae.

Known wing forms: micropterous.

Specimens studied. Paratype m#; USA, Illinois, Monticello, Allerton Estate, woodland debris, 14.iv.1942 (M. O. Farrar), at INHS.

***Glyptothrips longiceps* (Hood, 1954)**

(Figs 47–50)

This is one of the species with the longest head in relation to its greatest width, with a ratio of 1.5x or longer in all observed specimens (Fig. 48). Only *G. divergens* and *G. saltuarius* have similarly elongated heads within *Glyptothrips*. This species can be differentiated from the other two by the appearance of head sculpture, which is transversely elongate (Fig. 48), and the rather short tube in relation to head (less than 0.7x as long as head, Fig. 47). Both *G. divergens* and *G. saltuarius* have equiangular reticulation on head, and the tube proportionally longer (Table 2). *Glyptothrips longiceps* also seems to be the only one of the three long headed *Glyptothrips* species where individuals may bear thickened and capitate setae on the fore legs (Fig. 48).

Known wing forms: macropterous.

Specimens studied. Holotype f#; Brazil, Santa Catarina, Nova Teutônia, viii.1952 (F. Plaumann), at NMNH.

1 m# and 1 f# (non-types); same location as holotype, under fallen leaves, ix.1952, at NMNH.

***Glyptothrips reticulatus* Watson, 1934**

(Figs 51–56)

Glyptothrips reticulatus has been described over a large series of specimens, and Stannard (1955) indicates that this series represents multiple species. Besides that, two other species have been synonymized with *G. reticulatus* (*Eurythrips sculpturus* Hood and *Eurythrips silvarum* Hood; Mound 1976), casting even more confusion over the identity of this name. The original

description says it is a “brownish yellow” species, while one of the synonyms (*E. silvarum*) is described as “chestnut brown”. Here we mostly considered the original description for the proposed key and diagnosis, but also considered type specimens of the two synonyms for some observations. *Glyptothrips reticulatus* has only two sense cones on antennomere IV and lacks a capitate ventrolateral seta, while having antennomeres VII and VIII fully distinct.

Known wing forms: macropterous, micropterous and apterous.

Specimens studied. Paratypes 2 m# 4 f#; United States of America, Florida, from a variety of localities in ferns, pine needles and/or dry leaves, between 1929–1934, at NMNH.

1 f# (holotype of the synonymized *Eurythrips sculpturus*); United States of America, Louisiana, Tallulah, in humus, 11.v.1934 (J.W. Folson), at NMNH.

1 f# (holotype of the synonymized *Eurythrips silvarum*); United States of America, New York, Tompkins County, Enfield Glenn State Park, on fallen pine seedless, 15.ix.1940 (F.R. Nevin), at NMNH.

***Glyptothrips saltuarius* (Hood, 1957b)**

(Figs 57–60)

This is one of the species described to have the largest head length to greatest width ratio within the genus (Fig. 58), together with *G. divergens* and *G. longiceps*. It can be differentiated from the other two species by having setae S2 of abdominal tergite IX in females with dilated tip, instead of acute or at most blunt. *Glyptothrips saltuarius* is also quite similar to *G. hylaeus*, with only head length separating both species.

Known wing forms: macropterous and micropterous.

Specimens studied. Holotype f#; Brazil, Santa Catarina, Nova Teutônia, under fallen leaves, ii.1954 (F. Plaumann), at NMNH.

Allotype m#, same collection data and depositary as holotype (but dated x.1955).

***Glyptothrips silvaticus* (Hood, 1957b)**

(Figs 61–65)

This is a paler species when compared to some other *Glyptothrips*, with a greyish yellow or light brown colour (Fig. 61), contrasting to the dark brown or golden yellow shades more commonly seen in the genus. The sternal pore plate on males is a narrow transverse band, a trait more commonly seen in North American *Glyptothrips* species; the only other Brazilian species with this type of pore plate is *G. subcalvus*, a much darker species with several morphological differences to *G. silvaticus* (Table 2). It also bears thickened and capitate setae on all femora

(Fig. 63) like *G. fuscipes*, but they are not as straight and elongate as in that mostly dark brown species.

Known wing forms: micropterous.

Specimens studied. Lectotype f#; Brazil, Santa Catarina, Nova Teutônia, under fallen leaves, vi.1954 (F. Plaumann), at NMNH.

***Glyptothrips subcalvus* (Hood, 1954)**

(Figs 66–73)

This species has some interesting colour patterns, which are unique within the genus. The first two antennomeres are a very pale yellow, in contrast to the remaining antennomeres and head, which are dark brown in non-macerated specimens (Figs 66–68). The legs are also highly contrasting, with all tarsi, extreme apex and basal area of tibiae, and apical third or fourth of femora as pale as the basal antennomeres, and the rest of the legs dark brown (Fig. 66); most other *Glyptothrips* have the legs in some shade of yellow, with at most the junctions between segments slightly paler. Another unusual character state in this species is the presence of only two sense cones on antennomere IV, which is only seen elsewhere in *G. flavescens*, *G. reticulatus* and *G. interior* (all of which with much paler abdomens). The first two also lack a thickened and capitate pterothoracic ventrolateral setae (present in *G. subcalvus*, Fig. 69), and the latter has dilated occipital setae (not dilated in *G. subcalvus*).

Known wing forms: micropterous and apterous.

Specimens studied. Holotype f#; Brazil, Santa Catarina, Nova Teutônia, viii.1952 (F. Plaumann), at NMNH.

Paratype 1 f#, same collection data and depositary as holotype.

Five m# (non-types), same collection data and depositary as holotype (but dated vii.1953, v.1954, v.1955, viii 1955, x.1955).

Acknowledgements

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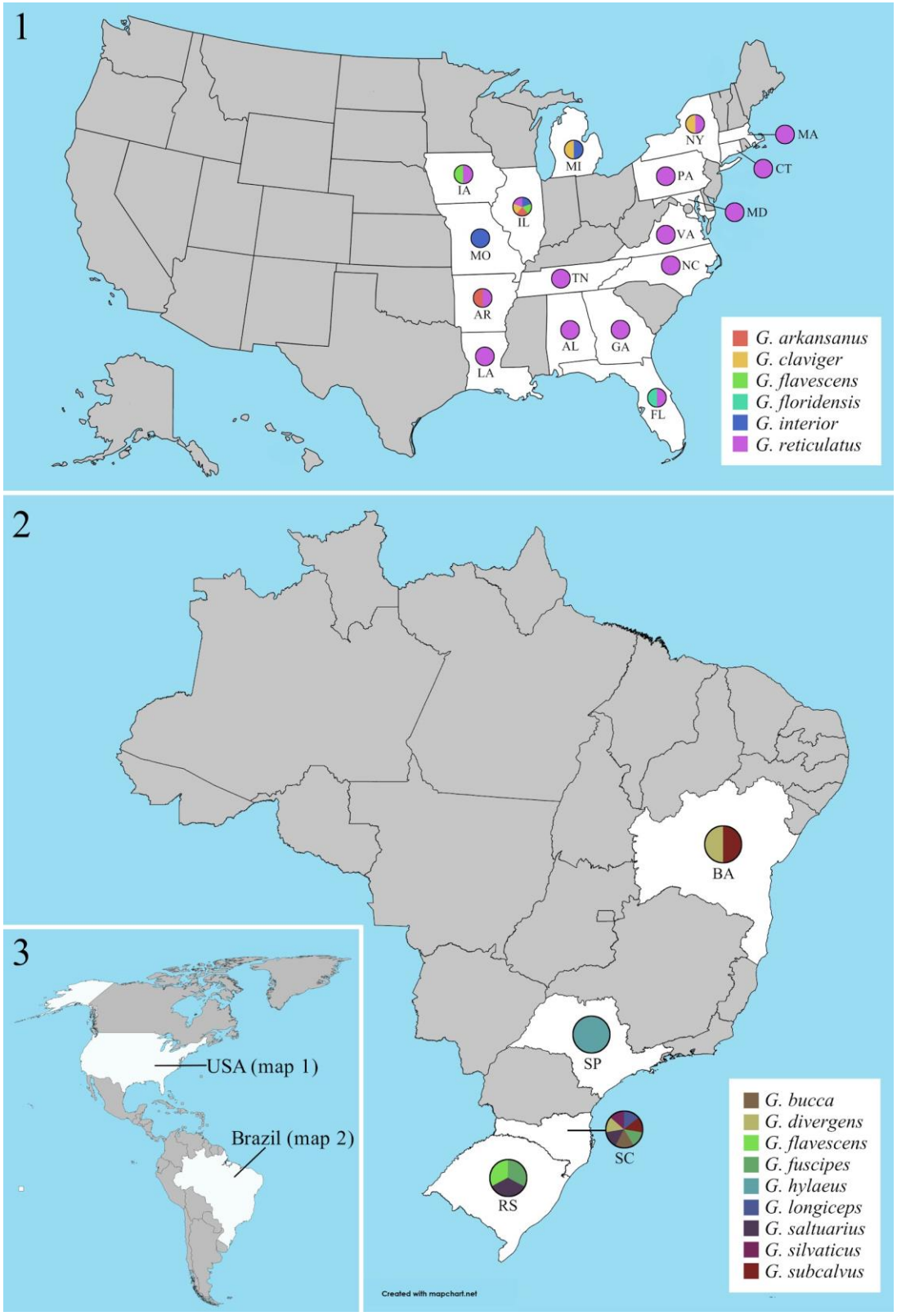
Figures



Figures 1–3. *Glyptothrips* species distribution, from literature data. 1: United States of America, species recorded per state. 2: Brazil, species recorded per state. 3: Americas, with the two countries with *Glyptothrips* records highlighted.

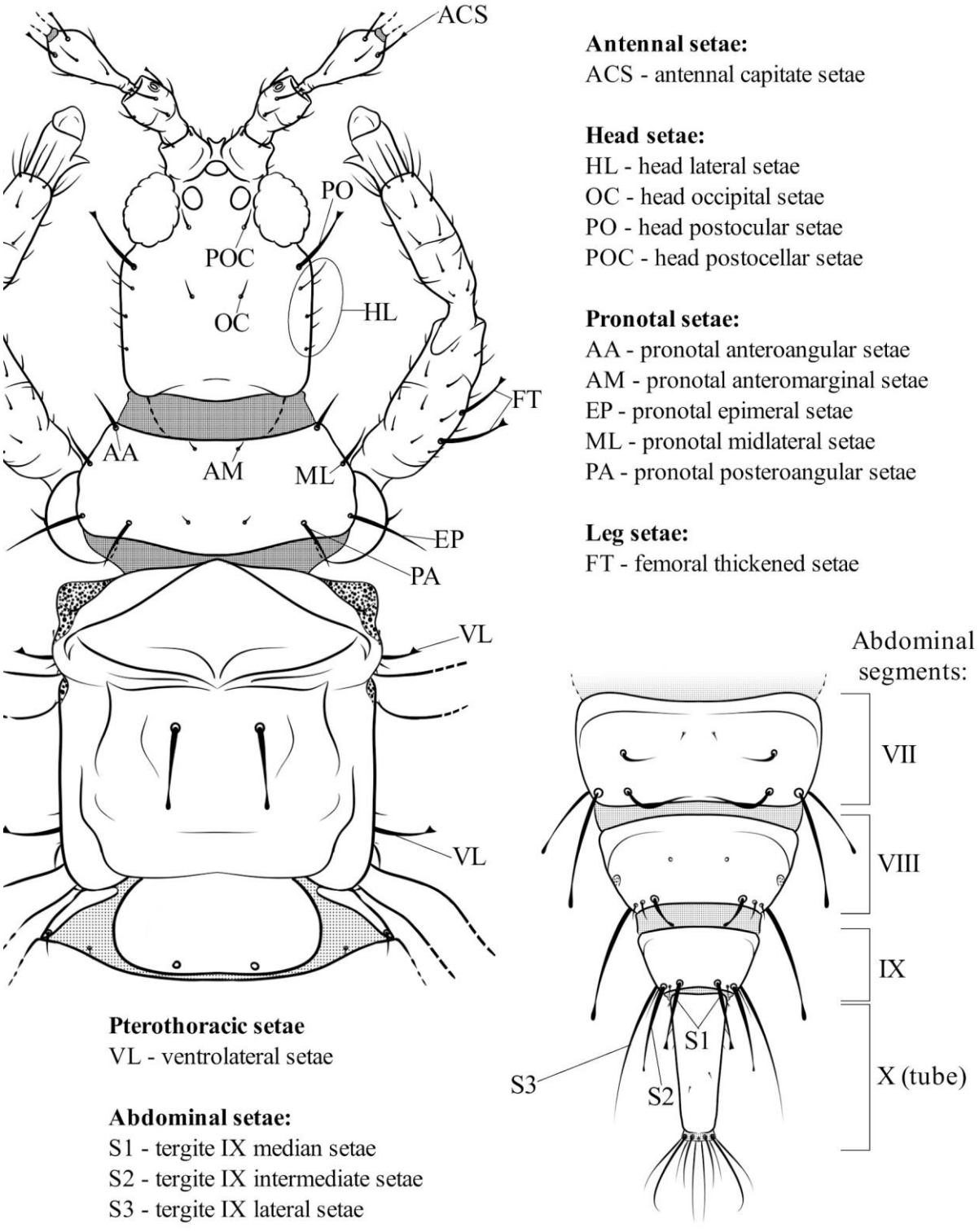
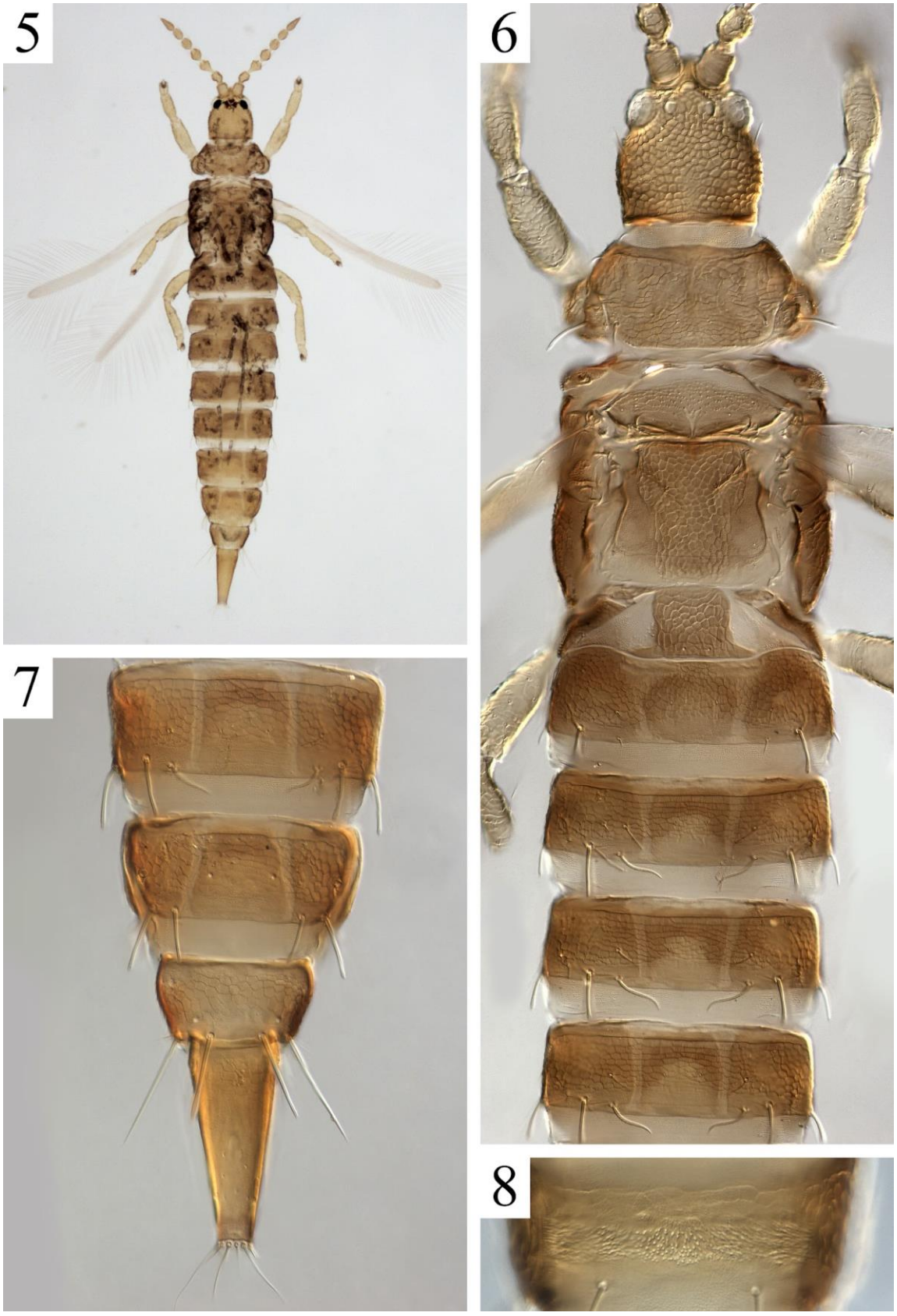
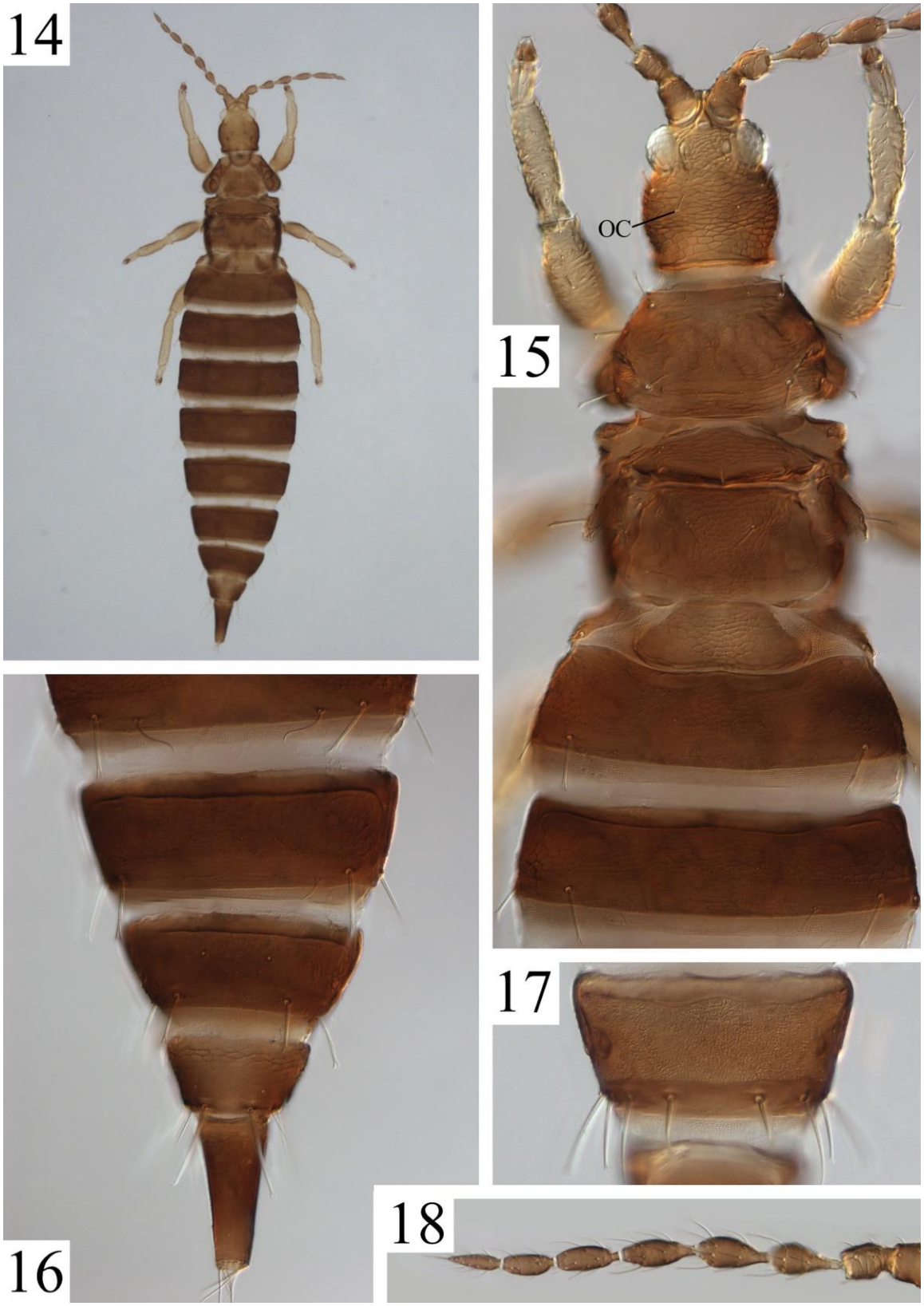


Figure 4. Thysanoptera chaetotaxy. Only setae mentioned in this work are indicated.

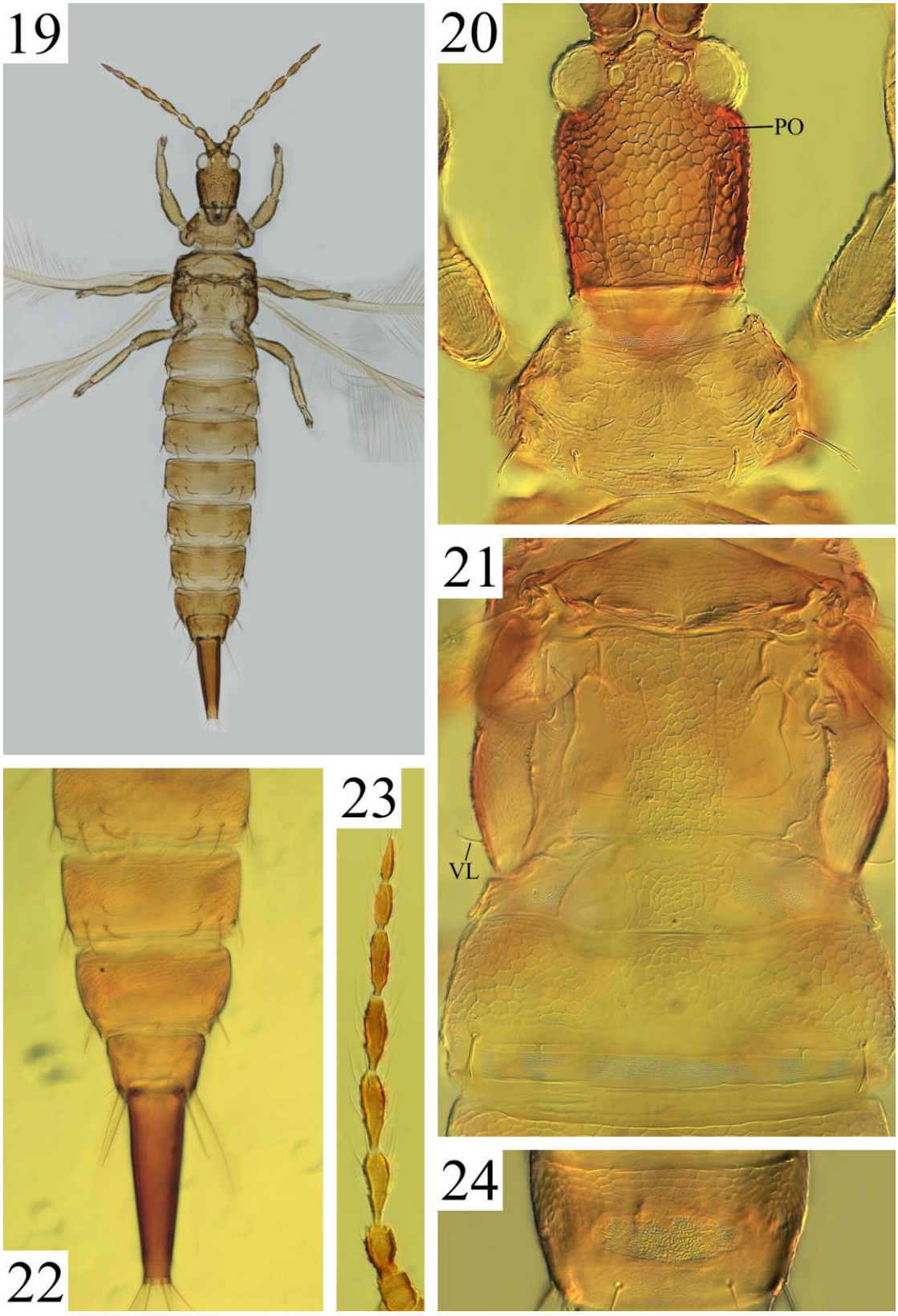


Figures 5–8: *Glyptothrips arkansanus*, female lectotype and paralectotypes. 5: Female. 6. Head, thorax and abdominal segments I–V. 7: Abdominal segments VII–X, dorsal view. 8: Male pore plate on abdominal sternite VIII, a narrow transverse band medially.

Figures 9–13: *Glyptothrips bucca*, female holotype and male allotype. 9: Male, dorsal view. 10: Female head, thorax, and abdominal segments I–III. 11: Female abdominal segments VI–X. 12: Female antenna. 13: Male pore plate on abdominal sternite VIII, a circular plate medially.

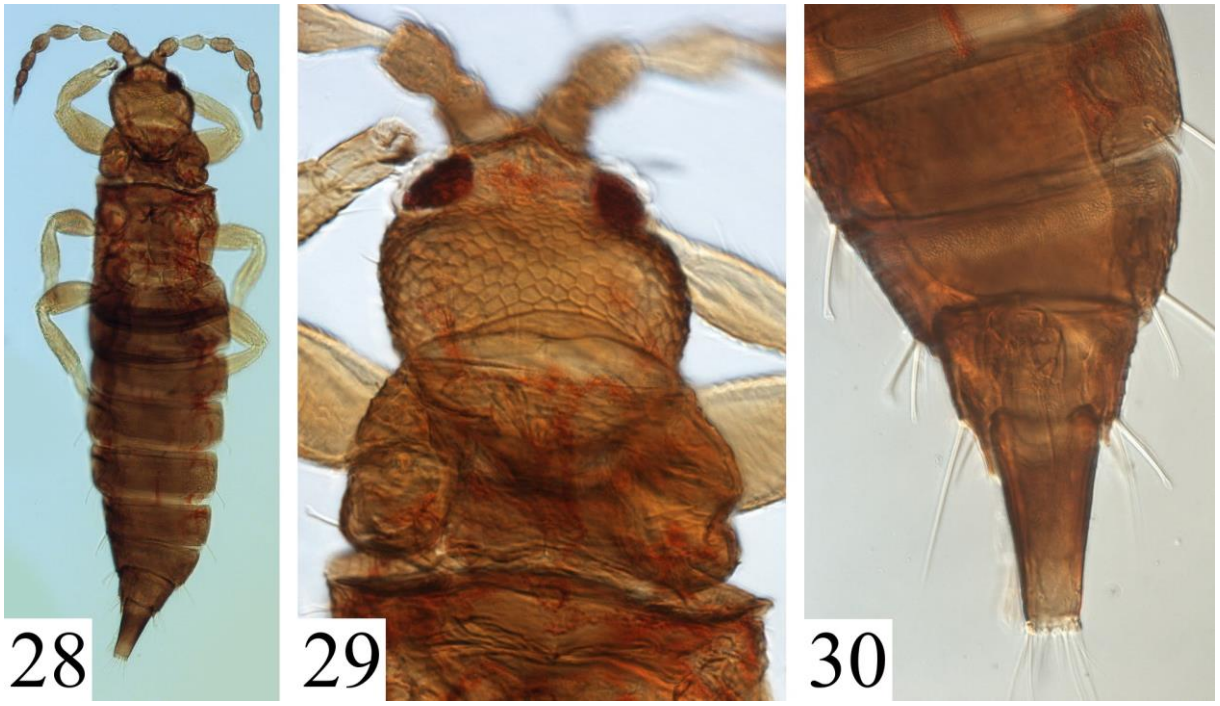


Figures 14–18: *Glyptothrips claviger*, female holotype and paratypes. 14: Female. 15: Head, thorax and abdominal segments I–III; OC - occipital setae. 16: Abdominal segments VI–X. 17: Male pore plate on abdominal sternite VIII, a large plate covering most of the sternite. 18: Antenna.

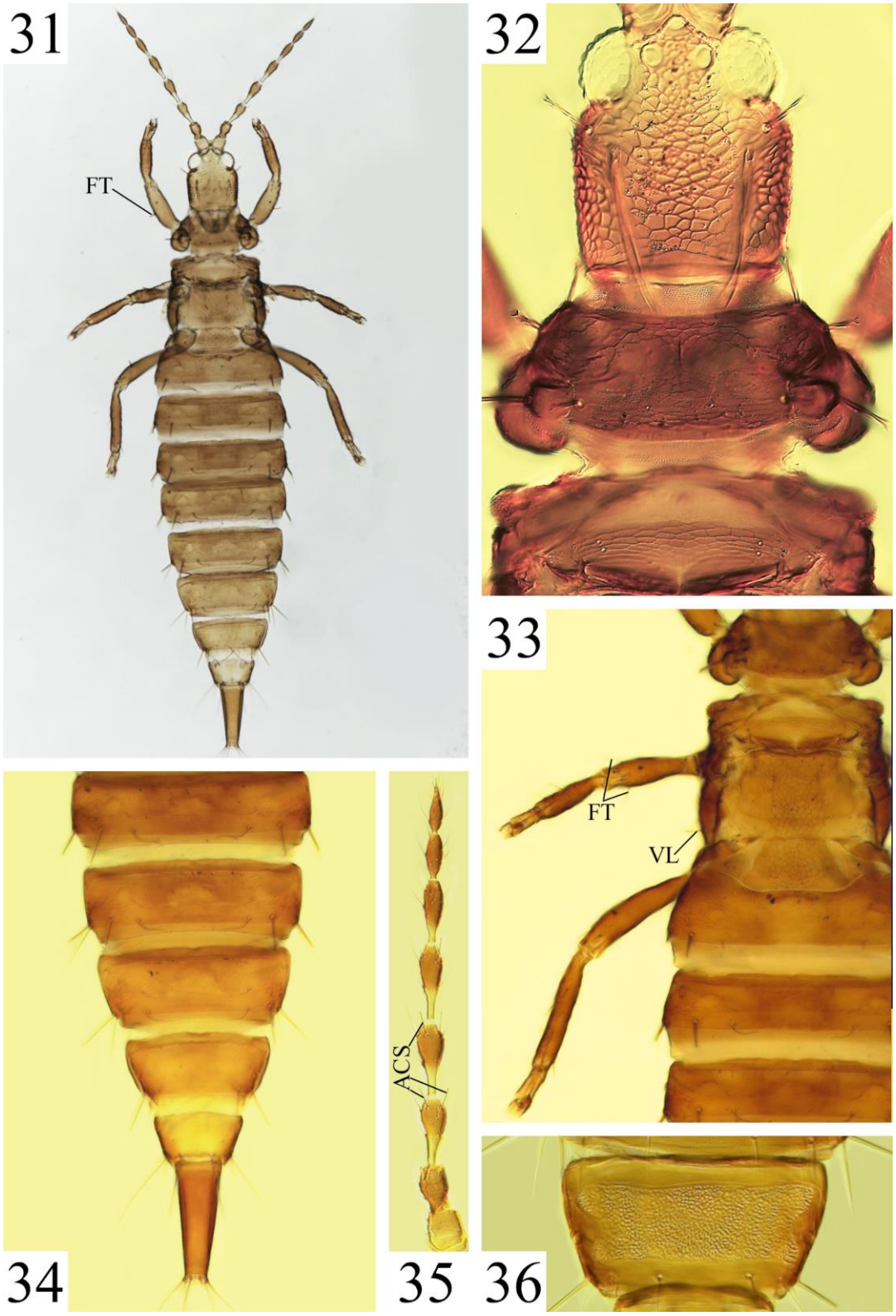


Figures 19–24: *Glyptothrips divergens*, female and male paratypes. 19: Female. 20: Head and pronotum; PO - postocular setae. 21: Thorax and abdominal segments I–III; VL - ventrolateral setae. 22: Abdominal segments VI–X. 23: Antenna. 24: Male pore plate on abdominal sternite VIII, a transverse ellipse medially.

Figures 25–27: *Glyptothrips flavescens*, female holotype and female paratype. 25: Female. 26: Head, thorax and abdominal segments I–IV. 27: Abdominal segments VI–X.

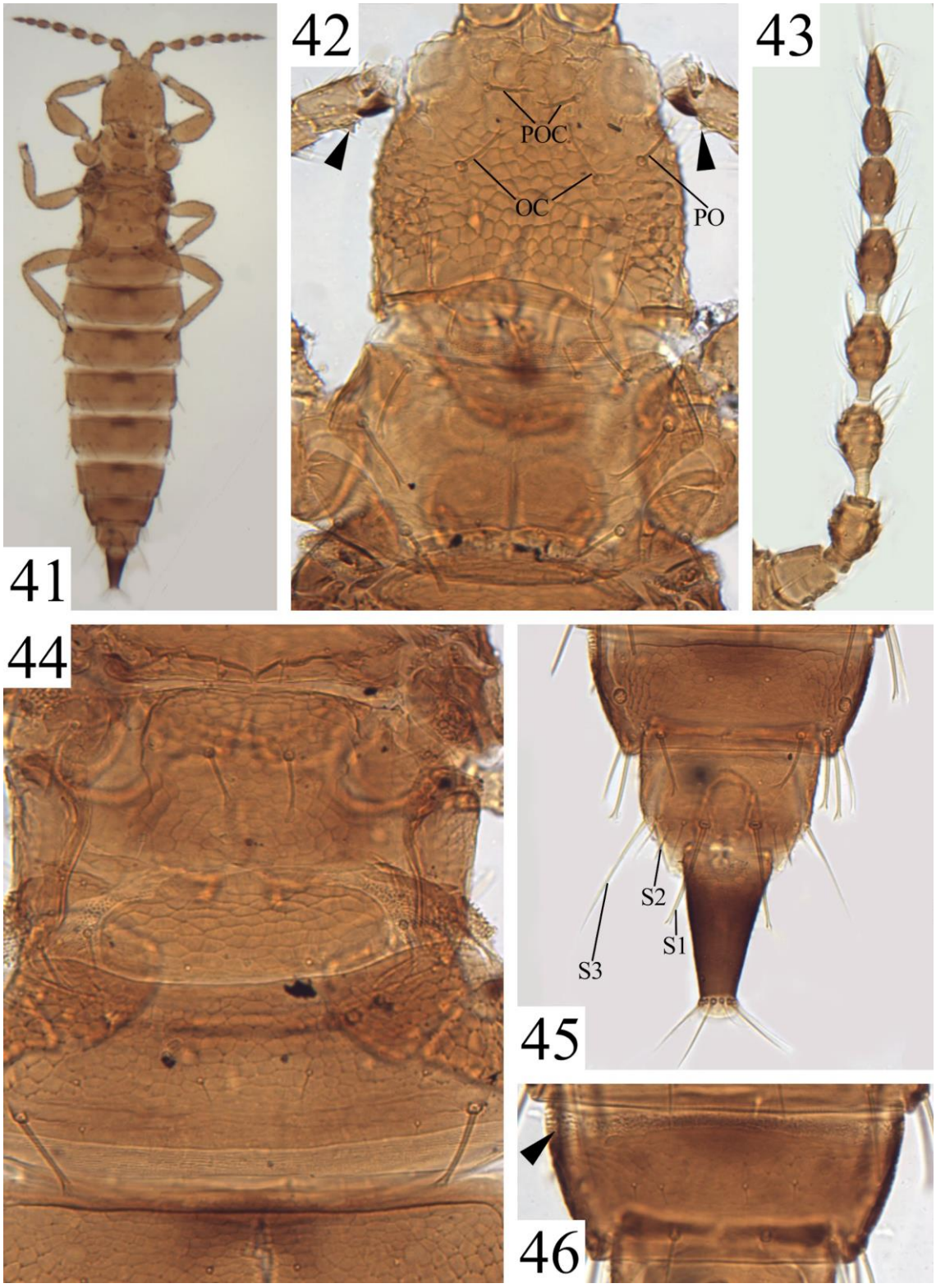


Figures 28–30: *Glyptothrips floridensis*, male from type series. 28: Dorsal view. 29: Head and prothorax. 30: Abdominal segments VI–X, ventral view; sternite VIII with a sub basal pore plate.

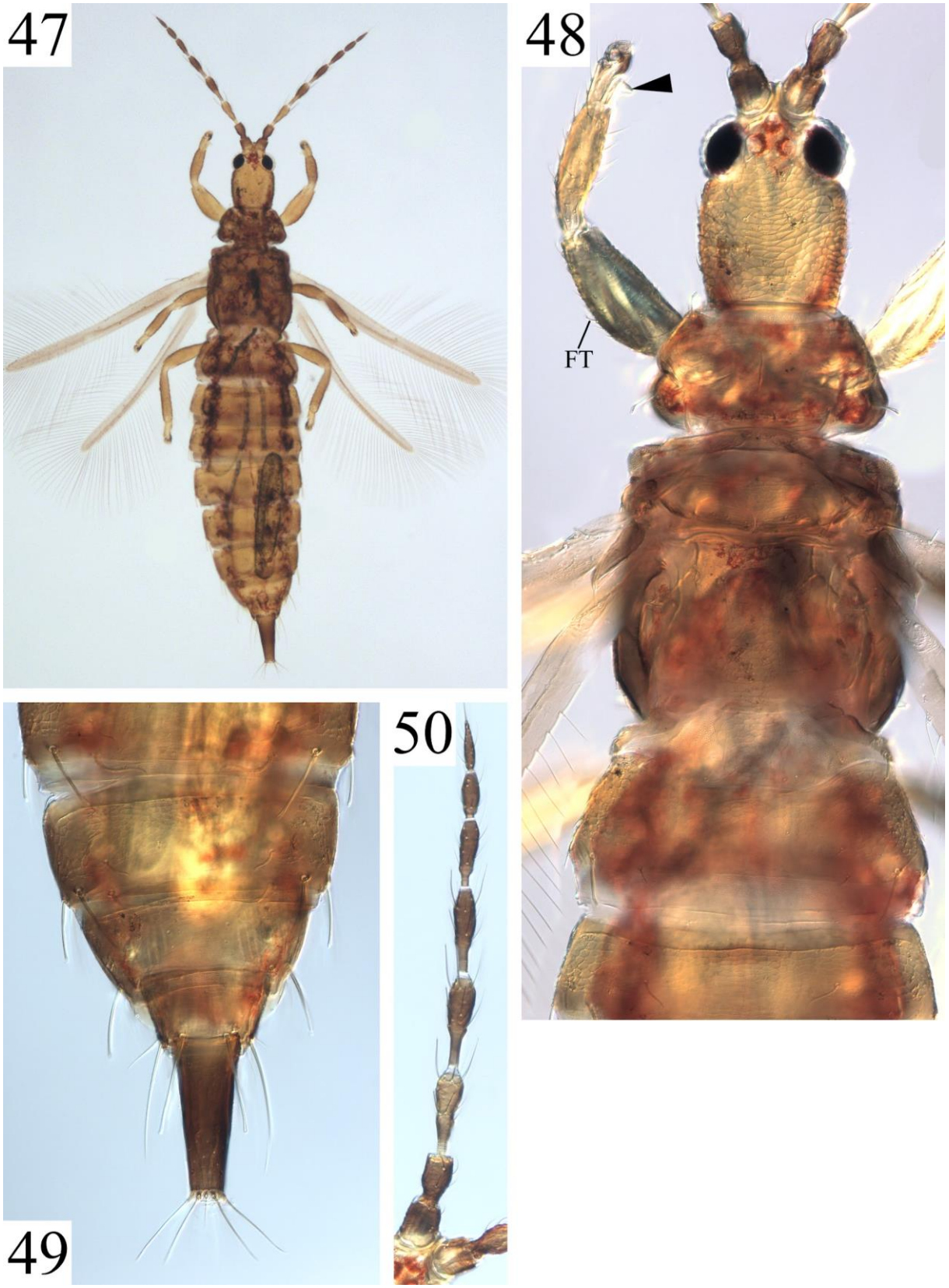


Figures 31–36: *Glyptothrips fuscipes*, female holotype and male paratype. 31: Female. 32: Head, pro- and mesonotum. 33: Thorax, mid and hind legs, and abdominal segments I–IV. 34: Abdominal segments V–X. 35: Antenna. 36: Male pore plate on abdominal sternite VIII, a large plate covering most of the sternite. ACS - antennal capitate setae; FT - femoral thickened setae; VL - ventrolateral setae.

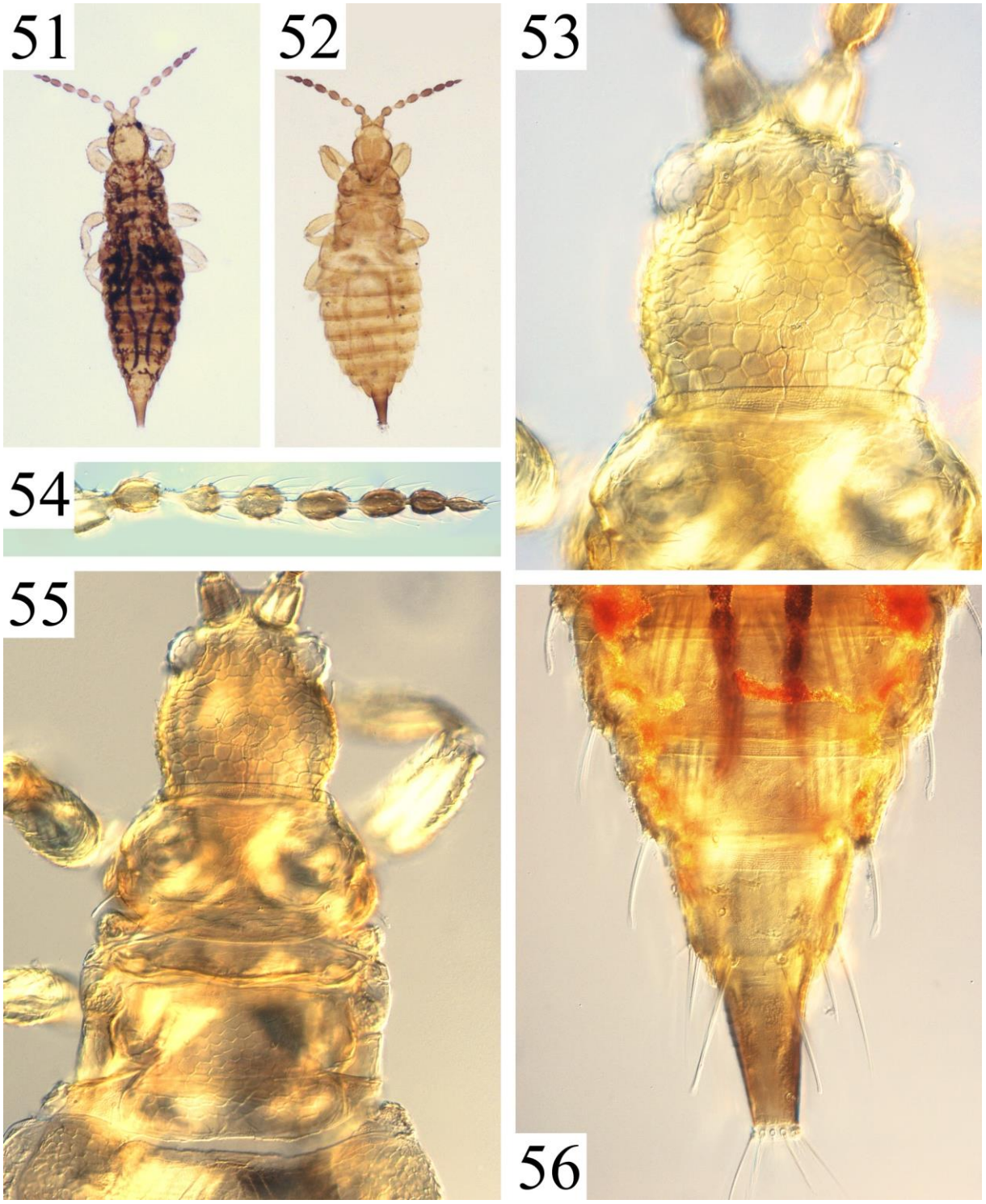
Figures 37–40: *Glyptothrips hylaeus*, male holotype. 37: Male. 38: Head, thorax and abdominal segments I–III. 39: Abdominal segments VII–X. 40: Male pore plate on abdominal sternite VIII, a large plate covering most of the sternite.



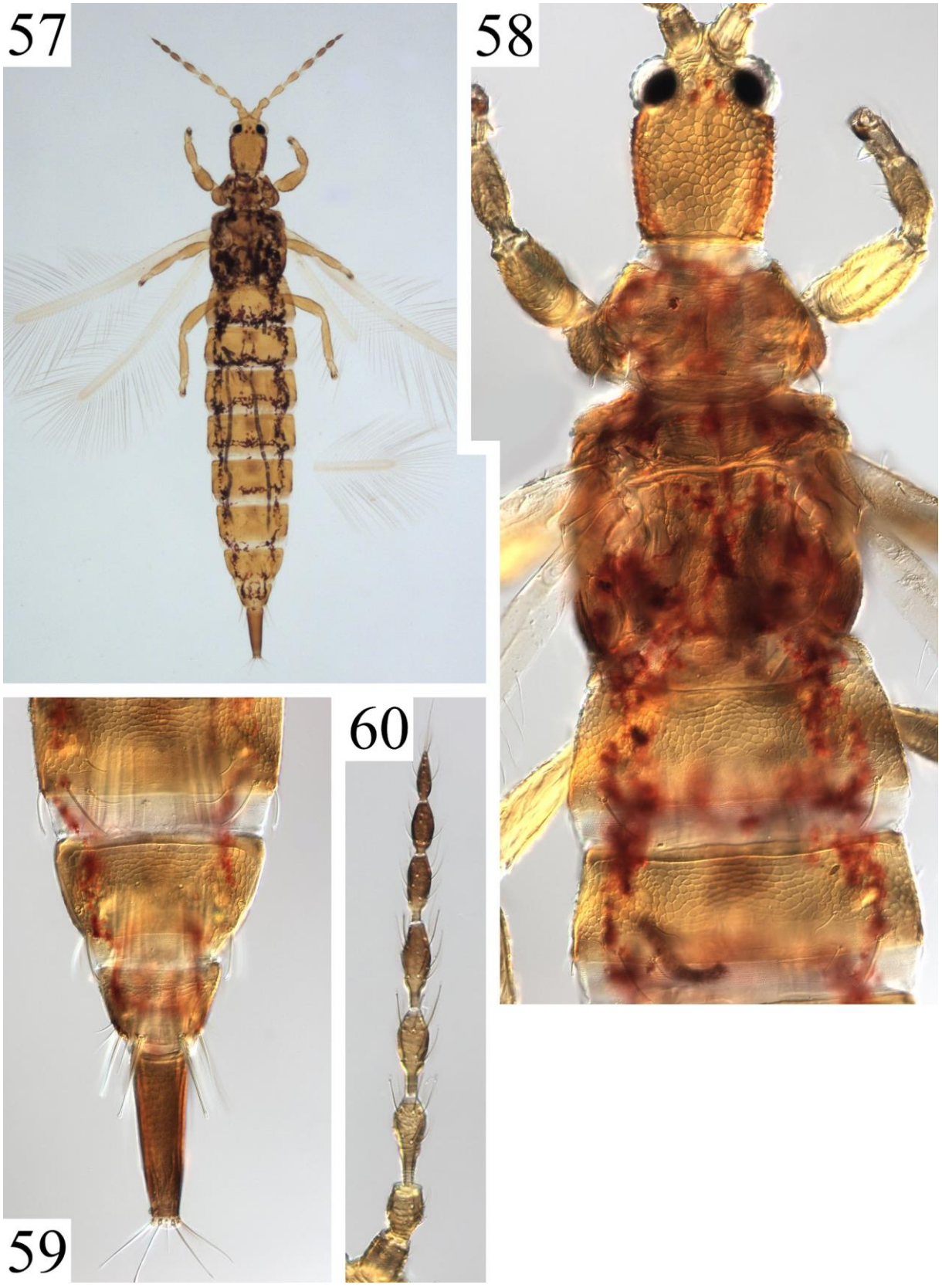
Figures 41–46: *Glyptothrips interior*, male paratype. 41: Male. 42: Head and prothorax; fore tarsal tooth indicated by arrow heads. OC - occipital setae, PO - postocular setae, POC - postocellar setae. 43: Antenna. 44: Pterothorax and abdominal segments I–III. 45: Abdominal segments VIII–X, with tergite IX setae indicated. 46: Male pore plate on abdominal sternite VIII, a narrow transverse band sub basally (arrow head).



Figures 47–50: *Glyptothrips longiceps*, female holotype. 47: Female. 48: Head, thorax and abdominal segments I–III; fore tarsal tooth indicated by arrow head. FT - femoral thickened setae. 49: Abdominal segments VI–X. 50: Antenna.

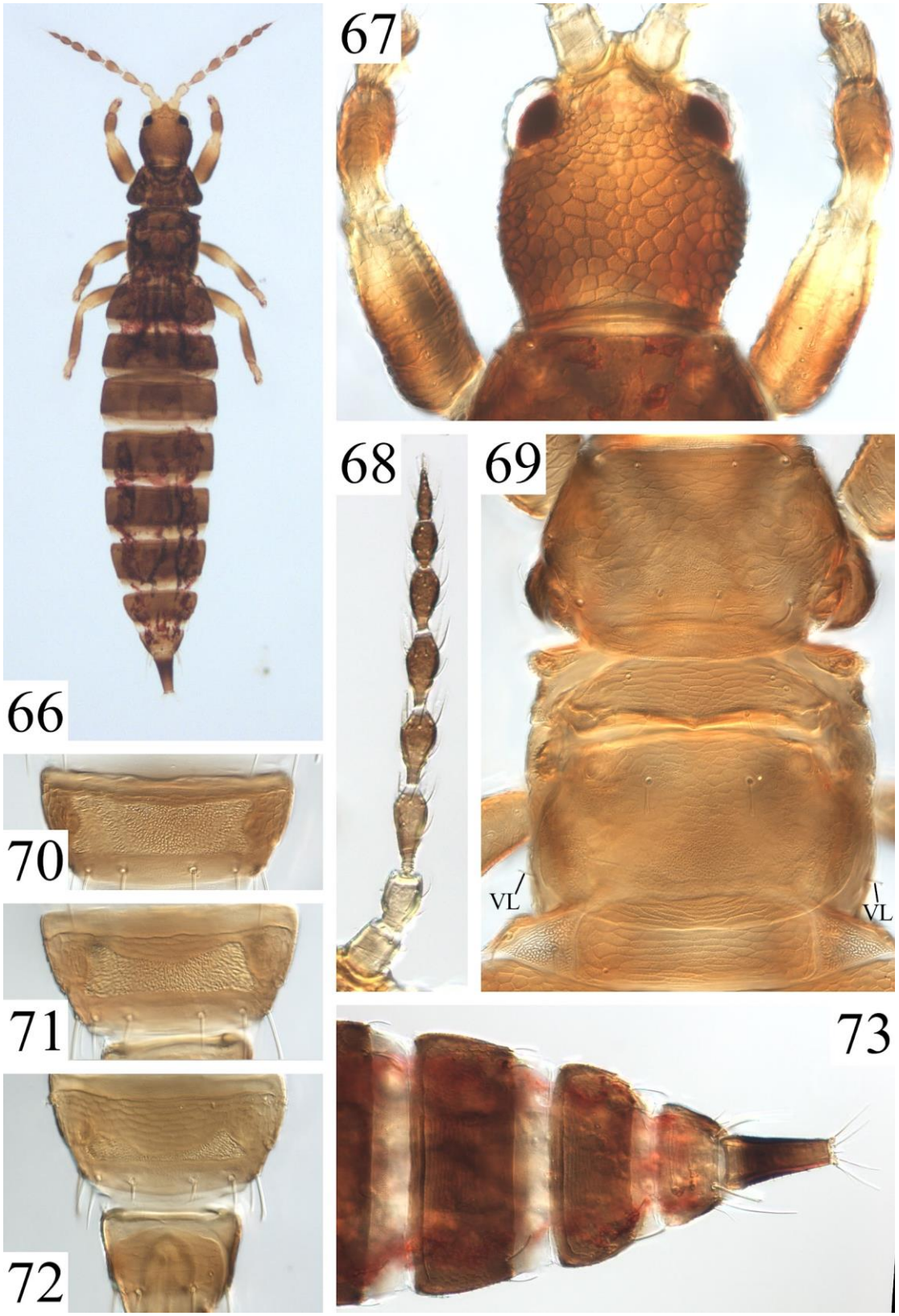


Figures 51–56: *Glyptothrips reticulatus*, female and male paratypes. 51: Male. 52: Female. 53: Head and pronotum. 54: Antenna. 55: Head, thorax and abdominal segments I–II. 56: Abdominal segments VI–X.



Figures 57–60: *Glyptothrips saltuarius*, female holotype. 57: Female. 58: Head, thorax and abdominal segments I–III. 59: Abdominal segments VII–X. 60: Antenna.

Figures 61–65: *Glyptothrips silvaticus*, female holotype. 61: Female. 62: Head, thorax and pelta; VL - ventrolateral setae. 63: Fore leg; FT - femoral thickened setae. 64: Antenna. 65: Abdominal segments VII–X.



Figures 66–73: *Glyptothrips subcalvus*, female holotype, female paratype, and male non-types. 66: Female. 67: Head and fore legs. 68: Antenna. 69: Thorax and pelta; VL - ventrolateral setae. 70–72: Male pore plate on abdominal sternite VIII, showing variation in size and form between different males. 73: Abdominal segments VI–X.

Tables

Table 1: List of *Glyptothrips* species and material studied for each.

Species	Literature data	Type specimens
G. arkansanus	Original description; Stannard 1968; Mound 1977.	f# lectotype, 1f# and 1m# paralectotypes, deposited at NMNH
G. bucca	Original description; Mound 1976, 1977.	f# holotype, 1m# allotype, deposited at NMNH
G. claviger	Original description; Stannard 1955, 1957, 1968; Mound 1976, 1977.	f# holotype, 1f# and 1m# paratypes, deposited at NMNH
G. divergens	Original description; Mound 1977.	1f# and 1m# paratypes, deposited at NMNH
G. flavescens	Original description; Watson 1934, 1935; Stannard 1955, 1957, 1968; Mound 1977.	f# holotype, 1f# paratype, deposited at NMNH
G. floridensis	Original description; Stannard 1957; Mound 1976, 1977.	m# type, deposited at INHS
G. fuscipes	Original description; Mound 1977.	f# holotype, 1f# and 1m# paratypes, deposited at NMNH
G. hylaeus	Mound 1977.	m# holotype, deposited at NMNH
G. interior	Original description; Stannard 1957, 1968; Mound 1976, 1977.	m# paratype, deposited at INHS
G. longiceps	Original description; Mound 1977.	f# holotype, deposited at NMNH
G. reticulatus	Original description; Hood 1941; Stannard 1955, 1957, 1968; Mound 1976, 1977.	*Eurythrips sculpturus* Hood (synonym) f# holotype, *Eurythrips silvarum* Hood (synonym) f# holotype, deposited at NMNH
G. saltuarius	Original description; Mound 1977.	f# holotype, m# allotype, deposited at NMNH
G. silvaticus	Original description; Mound 1976, 1977.	f# holotype, deposited at NMNH
G. subcalvus	Original description; Mound 1976, 1977.	f# paratype, deposited at NMNH

Table 2: Diagnostic characters for *Glyptothrips* species.

	Head, dorsal length x width ratio[1]	PO length x eye dorsal length ratio[1]	Antennomeres, sense cone formula	Antennomeres VII–VIII, junction	Pterothoracic ventrolateral setae	Legs, presence of thick and elongated capitate setae	Abdominal tergite IX, tip of dorsal posterior setae	Abdominal sternite VIII in males, pore plate appearance	Tube length x head dorsal length ratio [1]
G. arkansanus	1.14–1.24	0.77–0.83	III: 3 IV: 4	Partially fused, with visible suture	Acute, indistinct from other meso-metanotal ventral setae	Absent	S1: Dilated S2: Acute to blunt	Narrow transverse plate medially	0.87–1.07
G. bucca	1.23–1.27	0.91–1.19	III: 3 IV: 3	Fully distinct	Acute, indistinct from other meso-metanotal ventral setae	Absent	S1: Dilated: S2: Acute to blunt	Circular pore plate medially	0.67
G. claviger	1.08–1.33	0.42–0.71	III: 3 IV: 4	Fully distinct	Thickened and with dilated tip	Present in all femora	S1: Dilated S2: Dilated	Large, covering most of sternite	0.76–0.80
G. divergens	1.44–1.46	~0.22	III: 3 IV: 4	Fully distinct	Thickened and with dilated tip	Absent	S1: Dilated S2: Acute to blunt	Elliptical pore plate medially	1.23
G. flavescens	1.14–1.32	0.64–0.80	III: 2 or 3 IV: 2 or 3	Fully fused, usually without a suture	Acute, indistinct from other meso-metanotal ventral setae	Absent	S1: Blunt to dilated S2: Acute to blunt	Narrow transverse plate medially	1.04–1.23
G. floridensis	~1.05 (estimated from the figure in Stannard 1955)	~0.5	III: ? IV: ?	Fully distinct	Thickened and with dilated tip	Present at least on fore femora	S1: Dilated S2: Dilated	Narrow transverse plate subbasally	~0.92

	Head, dorsal length x width ratio[1]	PO length x eye dorsal length ratio[1]	Antennomeres, sense cone formula	Antennomeres VII–VIII, junction	Pterothoracic ventrolateral setae	Legs, presence of thick and elongated capitate setae	Abdominal tergite IX, tip of dorsal posterior setae	Abdominal sternite VIII in males, pore plate appearance	Tube length x head dorsal length ratio [1]
G. fuscipes	1.27–1.38	0.70–0.88	III: 3 IV: 4	Fully distinct	Thickened and with dilated tip	Present on all femora and fore tibiae	S1: Dilated S2: Acute to blunt	Large, covering most of sternite	0.89
G. hylaeus	1.27–1.3	~0.34	III: 3 IV: 4	Fully distinct	Thickened and with dilated tip	Absent	S1: Dilated S2: Variable, from acute to dilated	Large, covering most of sternite	0.81
G. interior	~1.04	~0.89	III: 2 IV: 2	Fully distinct	Thickened and with dilated tip	?	S1: Dilated S2: ?	Narrow transverse plate subbasally	~0.64
G. longiceps	1.5–1.56	0.18–0.27	III: 3 IV: 4	Fully distinct	Thickened and with dilated tip	Present on fore femora of some specimens	S1: Dilated S2: Acute to blunt	Large, covering most of sternite	0.63–0.68
G. reticulatus	1.06–1.27	~0.35 (estimated from original description)	III: 1 IV: 2	Fully distinct	Acute, indistinct from other meso-metanotal ventral setae	Present on fore femora of some specimens	S1: Blunt S2: Acute to blunt	Narrow transverse plate subbasally	0.65–0.76
G. saltuarius	1.51–1.52	0.32–0.39	III: 3 IV: 4	Fully distinct	Thickened and with dilated tip	Absent	S1: Dilated S2: Dilated	Large, covering most of sternite	0.81–0.84
G. silvaticus	1.28–1.31	~0.83	III: 3 IV: 4	Fully distinct	Thickened and with dilated tip	All femora	S1: Dilated S2: Acute to blunt	Narrow transverse plate medially	0.86–0.88

	Head, dorsal length x width ratio[1]	PO length x eye dorsal length ratio[1]	Antennomeres, sense cone formula	Antennomeres VII–VIII, junction	Pterothoracic ventrolateral setae	Legs, presence of thick and elongated capitate setae	Abdominal tergite IX, tip of dorsal posterior setae	Abdominal sternite VIII in males, pore plate appearance	Tube length x head dorsal length ratio [1]
G. subcalvus	1.06–1.15	0.37–0.42	III: 2 IV: 2	Fully distinct	Thickened and with dilated tip	Present on fore femora of some specimens	S1: Dilated S2: Dilated	Narrow transverse plate medially; anterior margin is concave, and in some specimens the pore plate is almost U-shaped	0.57–0.68

[1]All given ratios and measures were obtained from original descriptions and/or the individuals listed in the "observed specimens" section under each species in the manuscript. Intraspecific variation has not been studied for most species due to the limited number of individuals available.

CONSIDERAÇÕES FINAIS

Um sistema de classificação baseado em relações filogenéticas necessita de diversas fontes de dados, para gerar uma visão mais completa dos processos evolutivos envolvidos. Por muito tempo, a principal (e muitas vezes única) fonte de informação para estabelecer relações entre espécies de tripes foi a morfologia, que ainda é a base de todo o sistema de classificação da ordem. Estudos realizados nas últimas décadas têm desenvolvido novas técnicas para estudar os seres vivos, gerando novas fontes de dados capazes de contribuir para o entendimento da evolução de um grupo. Dados moleculares, especialmente certas sequências genéticas como COI ou microssatélites, têm se mostrado altamente informativos para uma grande variedade de táxons, inclusive Thysanoptera.

DNA Barcode é uma ferramenta com grande interesse e potencial, mas que ainda tem um longo caminho a seguir: o principal banco de dados de DNA Barcode (BOLD, 2003) ainda apresenta diversos problemas que limitam seu uso dentro de Thysanoptera. Apenas 5% das espécies de tripes estão representadas neste banco de dados, portanto a imensa maioria destes insetos não poderia ser identificada por comparação com estas sequências. Além disso, diversas sequências estão potencialmente identificadas erroneamente, e há indícios de que muitas espécies definidas com base em morfologia não condizem com delimitações propostas com base em sequências de COI. Para tornar o DNA Barcode uma ferramenta realmente eficiente de identificação em Thysanoptera, primeiramente necessitamos de mais espécies sequenciadas. Um maior foco deve ser dado em obter informações de táxons pouco estudados e coletados, e também na curadoria dos dados já disponíveis. Assim como morfologia conta apenas uma parte da história, dados moleculares também são um capítulo da história evolutiva de um grupo. Ambos devem ser utilizados em conjunto com outras fontes de dados para a criação de um sistema de classificação mais próximo da história evolutiva, e ferramentas de identificação mais eficazes e confiáveis.

Até o presente trabalho, apenas dados morfológicos disponíveis em descrições e revisões estavam disponíveis para a tribo Glyptothripini. Ampliamos o escopo destes dados, ao sequenciar COI para vários espécimes de diferentes gêneros de Glyptothripini, e avaliar a potencial contribuição deste gene para o entendimento da taxonomia e evolução do grupo. Observamos a necessidade de revisão das relações internas, especialmente para os gêneros que conseguimos amostrar mais de uma espécie: *Eurythrips*, *Glyptothrips* e *Terthrothrips*.

Por fim, também facilitamos o acesso aos dados morfológicos e melhoramos a

qualidade da informação disponível para o gênero *Glyptothrips*. Esperamos que a chave de identificação e ilustração da diversidade morfológica observada sejam ferramentas úteis e eficazes para futuros estudos do gênero, bem como uma boa base para identificações iniciais de espécimes. Porém, não achamos que nossa revisão morfológica deva ser a última palavra na classificação do gênero - o capítulo 2 apresenta indícios de que as espécies de *Glyptothrips* podem não formar um grupo monofilético, e acreditamos que futuros estudos, unindo morfologia e DNA, trarão novidades na classificação do grupo.

É necessário aumentar a qualidade das descrições morfológicas com imagens, ferramentas de identificação e informações sobre a variação intra e interespecífica. Além disso, devemos incluir outras fontes de dados sempre que possível - seja o sequenciamento de genes de interesse, a descrição da distribuição geográfica, observações da biologia e ecologia da espécie, entre outros. O foco não deve mais ser apenas dar nomes para espécimes que parecem diferentes, mas entender as entidades que estão sendo nomeadas como unidades evolutivas com um papel no meio ambiente.