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First record of *Thrips origani* (Thysanoptera: Thripidae), from the Americas and illustrated key for the known species of *Thrips* from Argentina

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Primer registro de *Thrips origani* (Thysanoptera: Thripidae) para las Américas y clave ilustrada para las especies de *Thrips* conocidas de Argentina

RESUMEN. En este trabajo, basado en la caracterización morfológica y molecular, se registra por primera vez a *Thrips origani* Priesner (Thysanoptera: Thripidae) en América, causando daños a cultivos de *Origanum vulgare* L. en La Consulta, Mendoza, Argentina. Se proporciona una clave ilustrada para ayudar a la identificación morfológica de las cinco especies del género *Thrips* L. que se sabe que están presentes en Argentina y se han agregado secuencias parciales del gen de la subunidad I de citocromo oxidasa de especímenes de tres especies de *Thrips* de Argentina, incluido *T. origani*, a un banco de datos internacional.

PALABRAS CLAVE. Clave Morfológica. COI. Orégano. Trips Plaga.

ABSTRACT. In this work, based on morphological and molecular characterization *Thrips origani* Priesner (Thysanoptera: Thripidae) is recorded for the first time from the Americas, causing damage to cultivated *Origanum vulgare* L. in La Consulta, Mendoza, Argentina. An illustrated key is provided to aid morphological identification of the five species of the genus *Thrips* L. now known to be present in Argentina and partial sequences of the cytochrome oxidase subunit I gene from specimens of three species of *Thrips* from Argentina, including *T. origani*, have been added to an international data bank.

KEYWORDS. COI. Morphological Key. Oregano. Thrips Pest.

The genus *Thrips* L. (Thysanoptera: Thripidae) has 303 valid species (Thrips Wiki, 2023), of which 62 species (43 endemic, 18 introduced, and one Holarctic) were recorded from the Americas by Nakahara (1994). It can be distinguished from other genera of Thripidae by the following features: antennae 7- or 8-segmented, segment I without median dorso-apical setae, III and IV each with forked sense cones (Fig. 2i-k); ocellar setae pair I absent, ocellar setae III subequal to or longer than ocellar setae II; median pair of setae on posterior margin of pronotum subequal to or longer than submedian pair (Fig. 1d); ferna entire, undivided; mesosternum with spinula, metasternum without spinula; presence of paired ctenidia laterally on abdominal tergites V-VIII, terminating laterally at tergal setae S3 on tergites VI-VII; ctenidia on tergite VIII posteromesad of the spiracles; tergite X split longitudinally in both sexes (Bhatti, 1980; Mound, 2002; Masumoto & Okajima, 2013; Tyagi & Kumar, 2015).

Only four species from the genus *Thrips* have been recorded from Argentina: *Thrips tabaci* Lindeman, *Thrips simplex* (Morison), *Thrips australis* (Bagnall) and *Thrips trehernei* Priesner (de Borbón, 2009). The onion thrips (*T. tabaci*), a widely distributed, polyphagous pest species, has been characterized as containing at least three biotypes (leek-associated arrhenotokous L1-biotype, leek-associated thelytokous L2-biotype and tobacco-associated arrhenotokous T-biotype); however, the adults are morphologically indistinguishable (Iftikhar et al., 2016;

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Farkas et al., 2020). In 2016, Panonto & Bauzá reported for the first time a species of the genus *Thrips* that damage oregano (*Origanum vulgare* L.) crop in the province of Mendoza, without confirming the identity of the species.

The objectives of this work were to identify the species of thrips present on the oregano and then provide an illustrated morphological key to the *Thrips* species present in Argentina, and also to obtain and analyze a partial sequence of cytochrome oxidase I for these species. As a result of this work, we record the presence of *Thrips origani* for the first time anywhere in the Americas.

The identity of the thrips on oregano in Argentina

Specimens were collected from oregano being grown in Mendoza, Argentina, and these were then cleared with 10 % KOH, dehydrated with increasing concentrations of ethanol, transferred to clove oil and mounted onto slides in Canada Balsam (Mound & Marullo, 1996). The specimens were observed under a microscope with phase contrast at 400x and identified as *T. origani* using keys to European species of *Thrips* (zur Strassen, 2003; Mound et al., 2018). To confirm the identification, images were taken (Fig. 1 c-d; Fig. 2 a, e, f, i) and matched with slide-mounted specimens of *T. origani* that had been collected from wild-growing *Origanum vulgare* in England. As a result, *T. origani* is here recorded for the first time from both Argentina and the Americas.

Thrips origani is found across western, central, and eastern continental Europe, plus the United Kingdom, Norway, the Azores and Madeira (zur Strassen, 2003; Mound et al., 2018). It is host specific to *O. vulgare*, a woody perennial with a native range across the Palaearctic from Macaronesia to China (POWO, 2024). It is widely cultivated as a culinary herb, but not only has *T. origani* not previously been reported as a pest of cultivated oregano (in Europe or elsewhere), the thrips is very rarely mentioned in the literature at all. A problem with thrips causing damage to cultivated oregano in Mendoza dates back to at least 2011, with 40 % of growers in one survey reporting a problem (Panonto & Bauzá, 2016). The damage is manifested in spring (from September onwards); new shoots are attacked leaving the top of the stems brownish with only a few deformed flowers appearing.

La Consulta, Mendoza, is a locality situated in the centre-west of Argentina. The climate semi-arid and cold corresponds to the BSK type according to Köppen-Geiger classification (Peel et al., 2007). It has a great thermal amplitude, with cold nights and warm days. It is separated from Chile and the Pacific Ocean by a high part of the Los Andes range. By contrast, for example, in the maritime climate of England, *T. origani* is a mid-summer species, its visibility coinciding with the flowering season of wild *O. vulgare* on dry grasslands, particularly those on chalk geology.

Morphological identification of *Thrips origani* Priesner

This species is morphologically similar to two other species found in its home range in Europe, *Thrips tabaci* and a rarely collected host-specialist of the woodland plant *Euphorbia amygdaloides*, *Thrips euphorbiicola* Bagnall. These three species all have closely spaced rows of microtrichia on the abdominal pleurotergites (Fig. 2e); the posteromarginal comb on tergite VIII complete with long, fine, microtrichia; the anterior pairs of pores on tergite IX not developed; and second-instar larvae with the lateral spiracles on tergite II absent (this is unusual within the genus *Thrips*). However, *T. origani* is morphologically distinct from these other two species, with 1-5 discal setae present on each of sternites III-VI (Fig. 2a), strong dark antecostal ridges on the sternites (Fig. 2a) and tergites, and variable brown and yellow mottling on the tergites. Males are unknown, the species reproducing by thelytokous parthenogenesis.

Examined Material

NEW RECORDS: Argentina, Mendoza, La Consulta (-33.7625, -69.1153): 9 females on *Origanum vulgare*, 21.x.2014, and 7 females on *Origanum vulgare*, 13.iii.2023 (coll. S. Panonto). The specimens are deposited in the Thysanoptera collection of the Estación Experimental Agropecuaria, Mendoza, INTA.

Key to the species of *Thrips* present in Argentina:

1. Abdominal pleurotergites with closely spaced rows of microtrichias (Fig. 2e); abdominal sternite VII without discal setae (Fig. 2a-b).....2

-. Abdominal pleurotergites without closely spaced rows of microtrichia (Fig. 2d); abdominal sternite VII with many discal setae (Fig. 2c).....3

2. Abdominal sternites with a few discal setae (Fig. 2a); antennal segments III-V uniformly brown, III slightly paler than IV-V (Fig. 2i); on *Origanum vulgare* .....*T. origani*

-. Abdominal sternites without discal setae (Fig. 2b); antennal segments III-V, usually slightly bicoloured, darker at apex than base; polyphagous.....*T. tabaci*

3. Forewing first vein with continuous row of setae (Fig. 2g); female and male yellow to brown, typically yellow with brown post-occipital ridge on head and brown spots on tergites (Fig. 1a); metanotum with campaniform sensilla (Fig. 1e); on *Eucalyptus* spp..... *T. australis*

-. Forewing first vein with discontinuous row of setae (Fig. 2h); female and male dark brown (Fig. 1b); metanotum without campaniform sensilla 4

4. Antenna 8-segmented; antenna brown, except segment III yellow (Fig. 2k); reticulate metanotum, many of the reticles with internal markings (Fig. 1f) ; on *Gladiolus* spp .....*T. simplex*

-. Antenna 7-segmented, antennal segments III to base of VI paler than the rest of the antennal segments (Fig. 2j); metanotum, with reticles lacking internal markings; on Asteraceae ..... *T. trehernei*

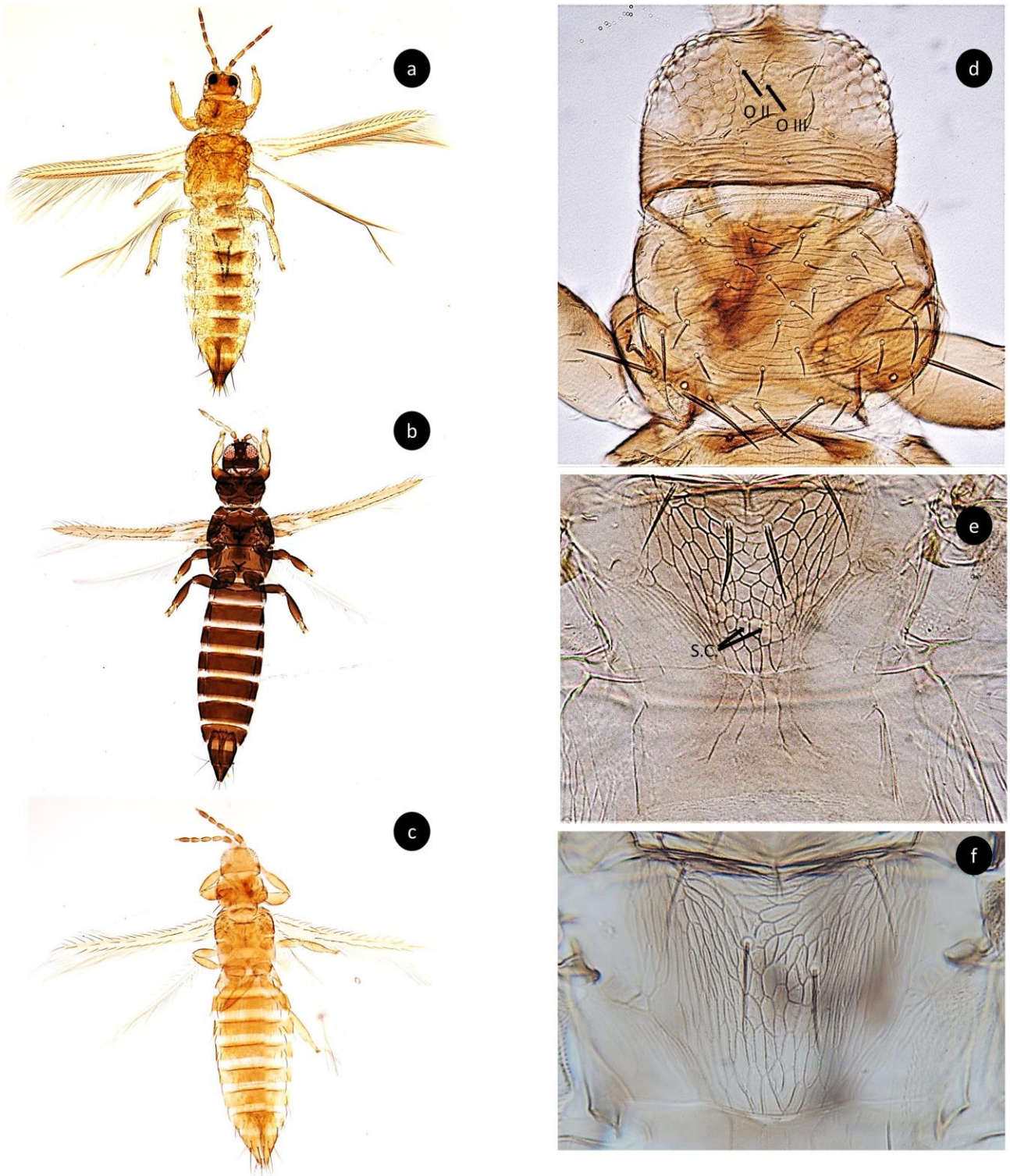

Fig. 1. Species of the genus *Thrips*. a-c female adults' full body (a) *T. australis* (b) *T. trehernei* (c) *T. origani*; (d) head with ocellar setae pair I absent, ocellar setae III subequal to or longer than ocellar setae II and pronotum with median pair of setae on posterior margin longer than submedian pair of *T. origani;* e-f metanotum (e) *T. australis*, campaniform sensilla present (f) *T. simplex*, reticles with internal markings.

Molecular studies

Four specimens were individually examined under a stereoscopic microscope with 160x magnification and placed in identifiable vials (labeled with a "sample number"). Specimens originally collected from La Consulta, Mendoza, were reared on oregano plants and collected for morphological and molecular characterization (INTA CdB 147 and INTA CdB 148). Additionally, DNA was also extracted from one specimen collected on *Senecio subulatus* D. Don ex Hook. & Arn. from El Carrizal, Mendoza, and previously identified under a stereoscopic microscope as *T. tabaci* (INTA CdB 35) and one specimen collected from an unidentified Asteraceae from Mar del Plata, Buenos Aires, and classified as *T. trehernei* (INTA CdB 131).

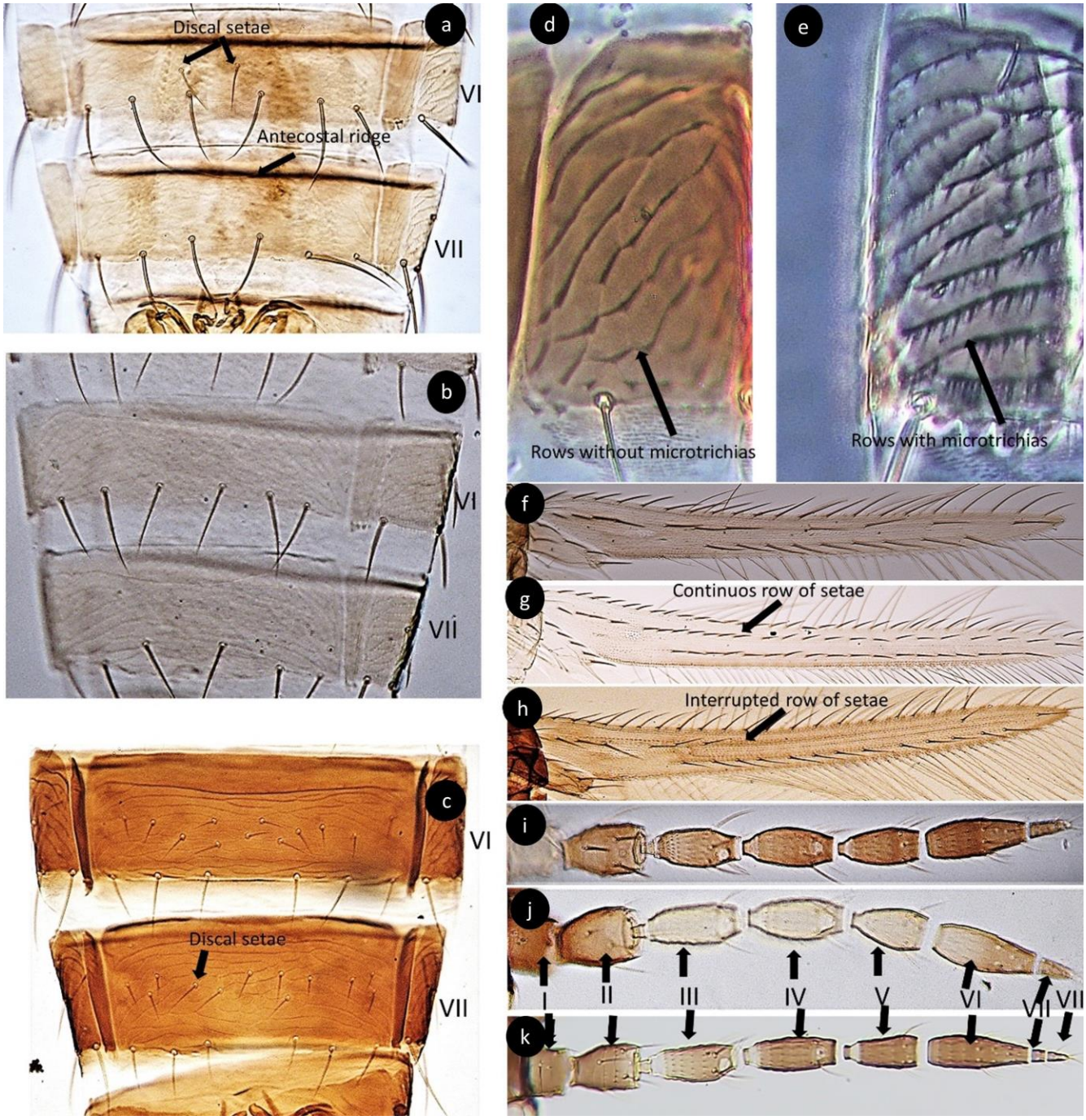

Fig. 2. Species of the genus *Thrips*. a-c sternites VI-VIII (a) *T. origani*, sternites VI with few discal setae and VI without discal setae (b) *T. tabaci*, sternites VI-VII without discal setae (c) *T. trehernei*, sternites VI -VII with many discal setae; d-e. pleurotergites (d) *T. trehernei*, rows without microtrichias (e) *T. origani*, rows with microtrichias, f-h forewings setae distribution (f) *T. origani*, (g) *T. australis* (h) *T. trehernei*; i-k antenna, number of segments and colour (i) *T. origani* (j) *T. trehernei* (k) *T, simplex*, antenna 8-segmented.

Partial sequence of COI

Specimens of thrips were collected in tubes containing 100 % ethanol. The species were recognized using morphological characters observable under a stereoscopic microscope at 160x magnification. Specimens were preserved in a freezer at -20 ºC until the moment of their use. DNA extraction was performed following the protocol proposed by Rugman et al. (2006) and modified by Kadirvel et al. (2013). A whole specimen was placed in a 1.5 ml Eppendorf tube with 100 µl of TNES (50 mM Tris [pH: 7.5], 400 mM NaCl, 20 mM EDTA, and 0.5 % SDS) and ground with the help of a plastic tip. The ground-down tissue was transferred to a 0.5 ml Eppendorf tube and 0.85 µl of proteinase K (20 mg/ml) was added. The sample was then incubated in a bath at 37 ºC for 18 h. The protein was precipitated with the addition of 28 µl of 5 M NaCl and centrifuged for 5 min at 13,000 rpm. The supernatant was transferred to a new 0.5 ml Eppendorf tube, one volume of

100 % ethanol previously chilled in a freezer at -20 ºC was added, and the tube incubated for one hour at room temperature. A precipitation step was done by centrifugation for 5 min at 13,000 rpm. The supernatant was discarded, 200 µl of 70 % ethanol was added to wash, and another centrifugation was carried out for 5 min at 13,000 rpm. The supernatant was carefully discarded, and the pellet was allowed to air dry. Once dry, the DNA was resuspended in 20 µl of sterile water and stored in a freezer at -20 ºC until PCR was carried out. The following primers were used: forward C1-J-1718 GGAGGATTTGGAAATTGATTAGTTCC and reverse C1-N-2329: ACTGTAAATATATGATGAGCTCA, cited by Simon et al. (1994), to obtain the partial sequence of the mitochondrial COI gene. A total volume of 40 µl PCR reagents was used, containing a 4 µl sample with 10 ng/µl DNA, 4 µl 1X PCR buffer, 1µl MgCl2 [50 mM], 0.6 µl [10 µM], dNTPS, 0.4 µl [100 µM] of forward and reverse primers and 0.3 µl Platinum Taq polymerase, and 29 µl sterile distilled water. The PCR reaction was performed in an Eppendorf Mastercycler nexus model thermocycler. The program consisted of 94 ºC for 10 minutes, followed by 4 cycles of 94 ºC for 40 seconds, 45 ºC for 40 seconds, 72 ºC for 1 minute, then 35 cycles of 94 ºC for 40 seconds, 51 ºC for 40 seconds, 72 ºC for 1 minute, and finally 15 minutes at 72 ºC. A 1 % agarose gel (TBE) was run at 90 volts for 30 minutes to confirm the amplification of the fragment sought and bands of approximately 650 bp were visualized, using a 100 bp ladder (Invitrogen™) as marker. The positive samples were purified according to the manufacturer's protocol with a Qiagen purification kit (QIAquick PCR Purification Kit), and they were sent to the laboratory of the Genomics Unit, Institute of Biotechnology, CICVyA - CNIA - INTA, where they were sequenced by capillary electrophoresis.

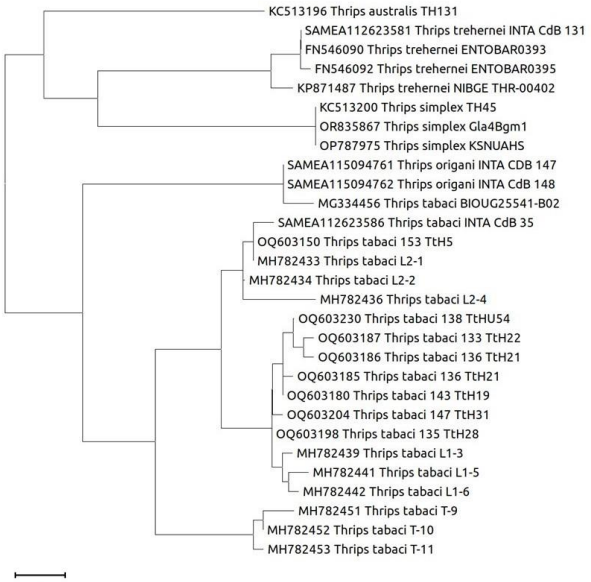

Fig. 3. Maximum Likelihood phylogenetic tree (1000 bootstrap) obtained from COI gene partial sequences.

Table I. List of partial COI sequence codes used for construction of the phylogenetic tree, ordered by thrips species.

Code *	Classified as	Host plant	Country
KC513196.1	*T. australis*	-	-
SAMEA115094761	*T. origani*	*Origanum vulgare*	Argentina
SAMEA115094762	*T. origani*	*Origanum vulgare*	Argentina
OP787975.1	*T. simplex*	*Gladiolus*	India
KC513200.1	*T. simplex*	*Gladiolus*	-
OR835867.1	*T. simplex*	*Gladiolus*	India
OQ603230.1	*T. tabaci*	*Allium sp*	Argentina
OQ603204.1	*T. tabaci*	*Allium sp*	Argentina
OQ603198.1	*T. tabaci*	*Allium sp*	Argentina
OQ603187.1	*T. tabaci*	*Allium sp*	Argentina
OQ603186.1	*T. tabaci*	*Allium sp*	Argentina
OQ603185.1	*T. tabaci*	*Allium sp*	Argentina
OQ603180.1	*T. tabaci*	*Allium sp*	Argentina
OQ603150.1	*T. tabaci*	*Allium sp*	Argentina
SAMEA112623586	*T. tabaci*	*Senecio subulatus*	Argentina
MG334456.1	*T. tabaci* ?	-	Canada
MH782439.1	*T. tabaci* L1	*Allium puerro*	Hungary
MH782441.1	*T. tabaci* L1	*Allium puerro*	Hungary
MH782442.1	*T. tabaci* L1	*Allium puerro*	Hungary
MH782433.1	*T. tabaci* L2	*Brassica oleracea*	Hungary
MH782434.1	*T. tabaci* L2	*Brassica oleracea*	Hungary
MH782436.1	*T. tabaci* L2	*Brassica oleracea*	Hungary
MH782451.1	*T. tabaci* T	*Nicotiana tabacum*	Hungary
MH782452.1	*T. tabaci* T	*Nicotiana tabacum*	Hungary
MH782453.1	*T. tabaci* T	*Nicotiana tabacum*	Hungary
FN546090.1	*T. trehernei*	-	UK
KP871487.1	*T. trehernei*	-	Pakistan
FN546092.1	*T. trehernei*	-	Croatia
SAMEA112623581	*T. trehernei*	Asteraceae	Argentina

* Code from GenBank or European Nucleotide Archives (ENA)

Four partial sequences contributed by us and deposited in the European Nucleotide Archives (ENA) and 25 obtained from GenBank (including three sequences for each biotype of *T. tabaci* , all available sequences of the genus *Thrips tabaci* filtered as cytochrome oxidase subunit I and Argentina, three sequences for *T. simplex,* three for *T. trehernei* and the only one available for *T. australis*) (Table I) were edited using BioEdit Sequence Alignment Editor (Hall, 1999) to conserve only the regions with good sequencing quality and that showed consensus in both directions. A BLASTN analysis (Zhang et al., 2000) was carried out using the consensus sequences of COI amplicon as query. Target sequences with the greatest similarity were retrieved from the GenBank database, and all taxa were aligned with ClustalW. Phylogenetic trees were constructed with MEGA11 software (Tamura et al., 2011). The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei

model (Tamura & Nei, 1993). The sequences were deposited in the EMBL-EBI database (PRJEB59814).

Molecular characterization

The phylogenetic tree for a partial sequence of cytochrome oxidase I, obtained for species of *Thrips* including those identified in Argentina up to May 23rd 2024 (Fig. 3) shows that both isolates of *T. origani* locate on the same clade distinct from *T. tabaci* (biotypes L1, L2 and T), *T. australis, T. simplex* and *T. trehernei* sequences, but show similarity (0.99) with one specimen classified as *T. tabaci* (sequence code in GenBank, MG334456). This sequence differs by more than 10 % from other *T. tabaci* COI sequences (598 sequences available in GenBank), which suggests a possible misidentification of a *T. origani* specimen labelled as having been collected in Canada.

The presence of *T. origani* in Argentina was reported to Sistema Nacional de Vigilancia y Monitoreo (SINAVIMO) of Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA), Identificaction number 1306.

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