Bulletin of Insectology **77** *(2): 299-305, 2024*
ISSN 1721-8861 eISSN 2283-0332

First record of *Anagyrus tristis* (Hymenoptera Encyrtidae) from Mexico

Adriana ACEVEDO-ALCALÁ[1], J. Refugio LOMELI-FLORES[1], Esteban RODRÍGUEZ-LEYVA[1], Ariel W. GUZMÁN-FRANCO[1], Julio C. VELÁZQUEZ-GONZÁLEZ[2]
[1]*Colegio de Postgraduados, Posgrado en Fitosanidad, Entomología y Acarología, Montecillo, Texcoco, Estado de México, México*
[2]*Koppert México, Parque industrial El Marqués, Querétaro, México*

Abstract

Biological control has been frequently used against mealybugs in many places worldwide, and a correct identification of natural enemies is essential for its implementation. This study aimed at identifying the *Anagyrus* species (Hymenoptera Encyrtidae) associated with *Phenacoccus madeirensis* Green 1923 (Hemiptera Pseudococcidae) collected in bell peppers under greenhouse conditions in Texcoco, State of Mexico. A laboratory rearing of the parasitoid was established and some specimens were used for morphological and molecular characterization. DNA amplification was conducted using the 28S rRNA D2 region, recommended for *Anagyrus* species, and sequencing and editing were performed to compare it with the GenBank base. The parasitoid is identified as *Anagyrus tristis* Noyes et Hayat 1994 representing a new record for the American continent. Here the description of the male is included, and this parasitoid is associated with *P. madeirensis* for the first time. The study of biology and behaviour of *A. tristis* on *P. madeirensis* pave the way to determine its potential as a biological control agent of an important agricultural pest.

Key words: *Phenacoccus madeirensis*, biological control, male description.

Introduction

Mealybugs are pests that affect a wide variety of vegetables, fruits, and ornamental crops; they feed on sap, and high populations can weaken plants (Williams and Granara de Willink, 1992; Ben-Dov, 1994; Franco *et al*., 2009). In addition, they can also cause indirect harm, by transmitting viruses and by producing abundant honeydew on that stratify the sooty mould, reducing transpiration. Besides, their presence may result in the rejection of products in national and international markets (Mani and Shivaraju, 2016; Selvarajan *et al*., 2016). *Phenacoccus madeirensis* Green 1923 (Hemiptera Pseudococcidae) is an internationally relevant mealybug in open-field and greenhouse vegetables; this species is reported from more than 50 countries in Southeast Asia, North Africa, in the Mediterranean area, in Europe and Mexico (CABI, 2000; Lomeli-Flores *et al.*, 2021). *P. madeirensis* is a polyphagous species attacking over 170 host species in 45 botanical families (Ben-Dov *et al.*, 2014), primarily affecting ornamentals and vegetables such as *Capsicum annuum* L. and *Solanum melongena* L. (Solanales Solanaceae) (Tok *et al*., 2016). In some areas of Colombia, Brazil, and West Africa, *P. madeirensis* can cause economic losses ranging from 68 to 88% if some control measures are not adopted in time (Herren and Neuenschwander, 1991; Bellotti *et al*., 1999).

Biological control is considered one of the most desirable approach against many species of mealybugs, and it has been employed through introduction or augmentation of natural enemies (Franco *et al*., 2009; Beltra *et al*., 2013). Specifically, for *P. madeirensis* there are recorded 11 species of predators of the families Chrysopidae, Coccinellidae, and Phytoseiidae, along with 27 species of parasitoids of Eulophidae, Platygastridae, and Encyrtidae; eight species of *Anagyrus* Howard 1896 (Hymenoptera Encyrtidae) are standing out in the latter family (Shylesha and Jhosi, 2012; García Morales *et al*., 2016, Ashraf *et al*., 2024, Noyes, 2024). The genus *Anagyrus* comprises around 290 described species (Noyes, 2024), some of which have been successfully utilized alone or in combination, for the biological control of mealybugs on different crops (Noyes and Hayat, 1994; Guerrieri *et al*., 2009). A successful example is represented by *Anagyrus callidus* Triapitsyn, Anderson et Perring 2019 (referred as *Anagyrus kamali* Moursi) for the control of *Maconellicoccus hirsutus* Green 1908 (Hemiptera Pseudococcidae) in California, USA, in Mexicali Valley, Baja California, and Bahía de Banderas, Nayarit, Mexico, where it resulted in 97% reduction of pest populations (García-Valente *et al*., 2009; Perring *et al*., 2022; Lomeli-Flores *et al*., 2023). Other important species within the *Anagyrus* genus include *Anagyrus aegyptiacus* Moursi 1948, *Anagyrus dactylopii* Howard 1898, and *Anagyrus pseudococci* Girault 1915, all playing a significant role in mealybug control (Shylesha and Mani, 2016).

Currently, there are eight species of *Anagyrus* associated with *P. madeirensis* globally: *Anagyrus bermudensis* Kerrich 1982 and *Anagyrus loecki* Noyes et Menezes 2000, found in North and Central America (Chong, 2005); *Anagyrus elgeri* Kerrich 1982 in South America; *A. pseudococci* in America, Africa, Asia, and Europe; *Anagyrus amnestos* Noyes et Poorani 2013 in Asia, Europe, and North America (Rameshkumar *et al.*, 2013), and *Anagyrus qadrii* Hayat, Alam et Agarwal 1975 in some Asian countries (Shylesha and Jhosi, 2012; Kondo and Watson, 2022; Japoshvili *et al*., 2023). Of these, only *A. loecki* is reported in Mexico on *P. madeirensis* (Noyes, 2024).

Although, keys are available for the identification of Nearctic species of *Anagyrus* from North America and some areas of northern Mexico (Noyes, 1989; Noyes *et*

al., 1997), exploration of parasitoids within this genus and their hosts has been limited. Currently, only 26 species of *Anagyrus* are known from Mexico (Trjapitzin *et al*., 2008). This genus comprises a complex of morphologically similar species; for instance, the *A. pseudococci* complex exhibits morphotypes, complicating its identification, as it was recently confirmed by the definition of six different species within it (Trjapitzin *et al*., 2008; Sugawara *et al*., 2020). One of these species is *A. callidus*, frequently cited from Mexico as *A. kamali*, was used in national biological control program and integrated management of *M. hirsutus* (García-Valente *et al*., 2009; Lomeli-Flores *et al*., 2023). Despite minimal differences between species and the difficulty linked of a correct identification, it is fundamental a correct characterization to avoid the failure of biological control programs (Andreason *et al*., 2019; Sugawara *et al*., 2020). Therefore, this study aimed at the integrated characterization of a species of *Anagyrus* recently collected in Mexico associated with *P. madeirensis* in greenhouse vegetables.

Materials and methods

A colony of *P. madeirensis* was established on bell pepper plants under greenhouse conditions (25 ± 9 °C, 70 ± 10% RH) at Colegio de Postgraduados, Texcoco, State of Mexico. The plants were grown using peat moss and a hydroponic system with automated irrigation. Adult females and egg sacs of *P. madeirensis* were placed on 45-day-old bell pepper plants to allow development and reproduction on them. The plants were protected with fabric mesh to prevent the introduction of natural enemies into the mealybug-rearing.

Anagyrus was reared on parasitized *P. madeirensis* pupae collected from experimental greenhouses at the Colegio de Postgraduados (19°27'39.6"N 98°54'28.8"W 2,220 masl) on August 29, 2022. These pupae were placed in plastic containers (26 × 13 × 13 cm) with a ventilation hole (5 × 5 cm) sealed with mesh on the lid to allow ventilation. A cotton wick saturated with water was placed inside the container, along with honey drops on the walls to provide *ad libitum* feeding for emerging parasitoids. The cotton wicks and honey drops were replaced every 48 hours. In each container, 15 third-instar nymphs and 20 adult females of *P. madeirensis* were introduced every three days to maintain the parasitoid colony.

Morphological identification

A group of parasitoid adults underwent gradual dehydration in 80%, 90%, and 96% ethanol solutions, with each solution immersion lasting 30 minutes. Following the final ethanol solution, they were placed in a glass container with amyl acetate for 24 hours. After this period, the specimens were removed from the amyl acetate and air-dried at room temperature for 24 hours, diagnostic structures were subsequently removed for identification, placed on a microscope slide with a drop of glycerine, and covered with a coverslip. Another group of adult specimens was processed following the technique of Kosztarab (1963), with a few modifications, for mounting them on microscope slides in Canada balsam. The slide mounted samples were observed under an Optika B-510PH compound stereoscope. Photographs of diagnostic structures were taken using a Canon EOS T7 digital camera. Noyes and Hayat (1994) dichotomous key was used for the identification. The voucher specimens were deposited in the Insect Collection of the Colegio de Postgraduados under the accession number CAEM-Hy-022.

Molecular identification

The DNA extraction from individual adult specimens was performed using the Tissue and Insect DNA MicroPrep Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. The DNA quality was confirmed through agarose gel electrophoresis in 1.5% TAE buffer. The gel was stained with ethidium bromide (10 mg mL^{-1}) and photographed.

Partial sequences of the D2 domain of the 28S nuclear rRNA were obtained using the primers D2F and D2R, previously reported for some *Anagyrus* species (Andreason *et al*., 2019). Reactions were made in a final volume of 30 µL containing 1X PCR buffer (Tris-Cl, KCl, (NH4)2SO4, 15 mmol L^{-1} MgCl2; pH 8.7), 0.2 µM of each primer, 0.2 mM of dNTPs, 0.5 U of TaqDNA polymerase (Qiagen®, GmbH, Hilden, Germany), 1 mM of MgCl2 and 3 µL (approx. 30 ng) of DNA. The amplification conditions included an initial denaturation at 95 °C for 3 minutes, followed by 40 cycles of 94 °C for 45 seconds, 54 °C for 1 minute, and 72 °C for 90 seconds, with a final extension step at 72 °C for 10 minutes. The reactions were done using a MyCycler T100™ thermocycler (Bio-rad Laboratories, Inc., Hercules, CA, USA). For each set of reactions, a negative control (containing sterile distilled water) and a positive control (a sample with a history of successful amplification) were included.

The PCR products were sent to Macrogen Inc. (Geumchen-gu, Seoul, South Korea) for direct sequencing. The resulting sequences were edited using the BioEdit program (Hall, 1999). Multiple alignments were performed using Clustal W (Thompson *et al*., 1994) with the BioEdit program V. 7.2.5 (Hall, 1999). For analysis, sequences of the genus *Anagyrus* were retrieved from GenBank as reported by Triapitsyn *et al*. (2007; 2018), and Gillespie *et al*. (2005), with the following accession numbers: DQ667688 *A. kamali*, AY599315 *A. pseudococci*, MG731495 *Anagyrus quilmes* Triapitsyn, Logarzo et Aguirre 2014, MG731490 *Anagyrus cachamai* Triapitsyn, Logarzo et Aguirre 2014, KY211221 *A. tristis*, DQ667687 *Anagyrus agraensis* Saraswat 1975, and AY599308 *Cheiloneurus fulvescens* Hoffer 1957.

A pairwise genetic distances analyses between and within nucleotide sequences of the species studied were done in MEGA 11 (Tamura *et al*., 2011) using the Kimura two-parameter (K2P) model (Kimura, 1980). Standard error estimates were obtained by a bootstrap procedure with 1000 replicates.

The substitution model selection and cladogram inference were performed using IQ TREE with the maximum likelihood method (Trifinopoulos *et al*., 2016) (available at: http://iqtree.cibiv.univie.ac.at/). Branch robustness

was estimated through a bootstrap analysis with 5000 replicates using UFBoot2 (Hoang *et al.*, 2018). The phylogenetic tree was visualized and edited in iTOL (Letunic and Bork, 2021) (available at: https://itol.embl.de/), and the sequences were deposited in GenBank.

Results

All specimens of the parasitoid collected on *P. madeirensis* attacking greenhouse peppers at Colegio de Postgraduados were identified as *Anagyrus tristis* Noyes et Hayat 1994 (Hymenoptera Encyrtidae). The morphological identification was corroborated with molecular identification. The characteristics of females match the description by Noyes and Hayat (1994) and it was confirmed by the photographs of the diagnostic female features (figure 1). The male of this species was previously unknown. Material collected at Colegio de Postgraduados and laboratory rearing specimens show a 1:1 sexual ratio. A description of the male is included, following the descriptions for the *A. pseudococci* species complex (Andreason *et al*., 2019), and diagnostic features are illustrated (figure 2).

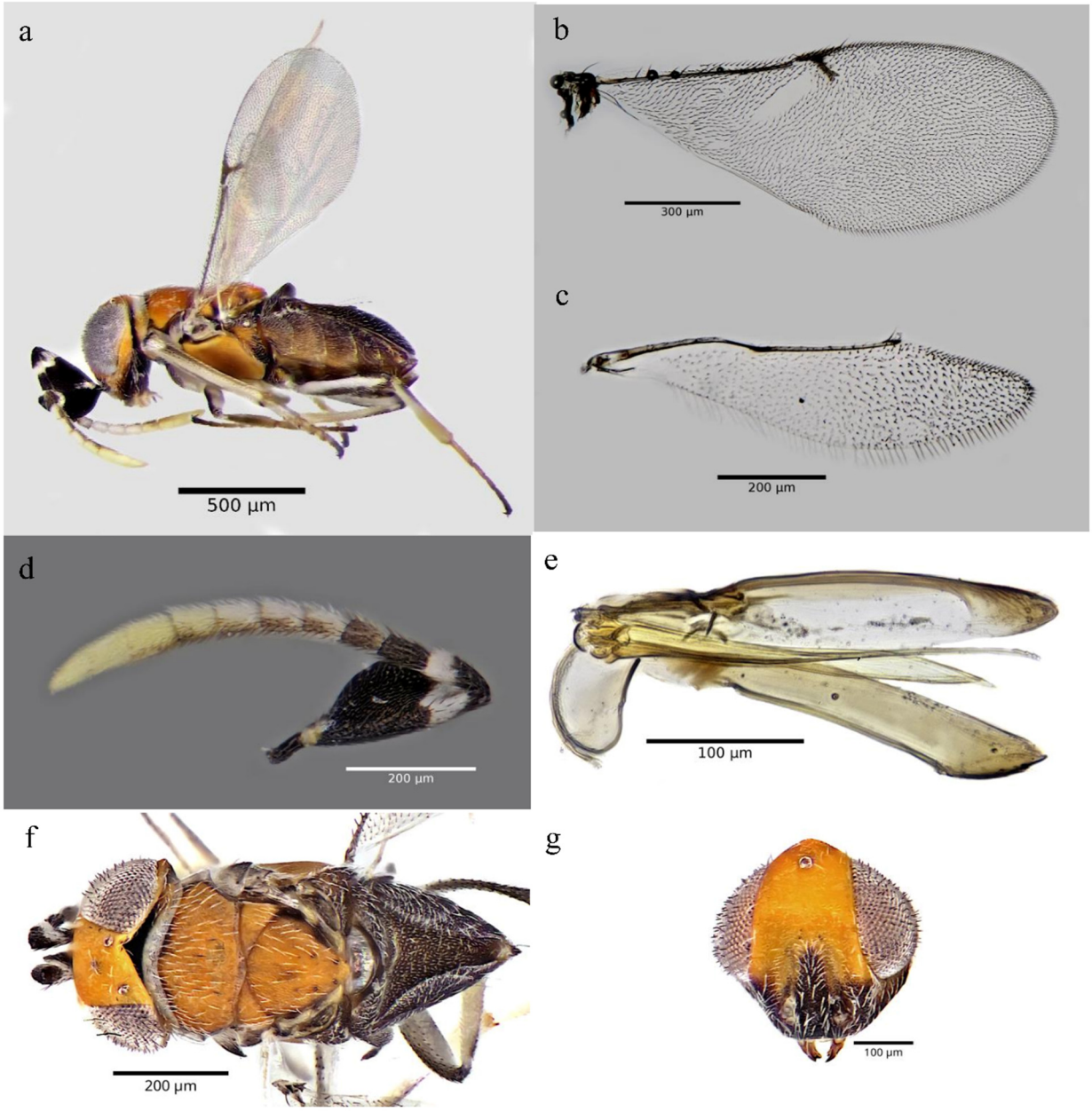

Figure 1. Female *A. tristis*: **a)** adult, **b)** anterior wing, **c)** posterior wing, **d)** antenna, **e)** genitalia, **f)** thorax and **g)** head.

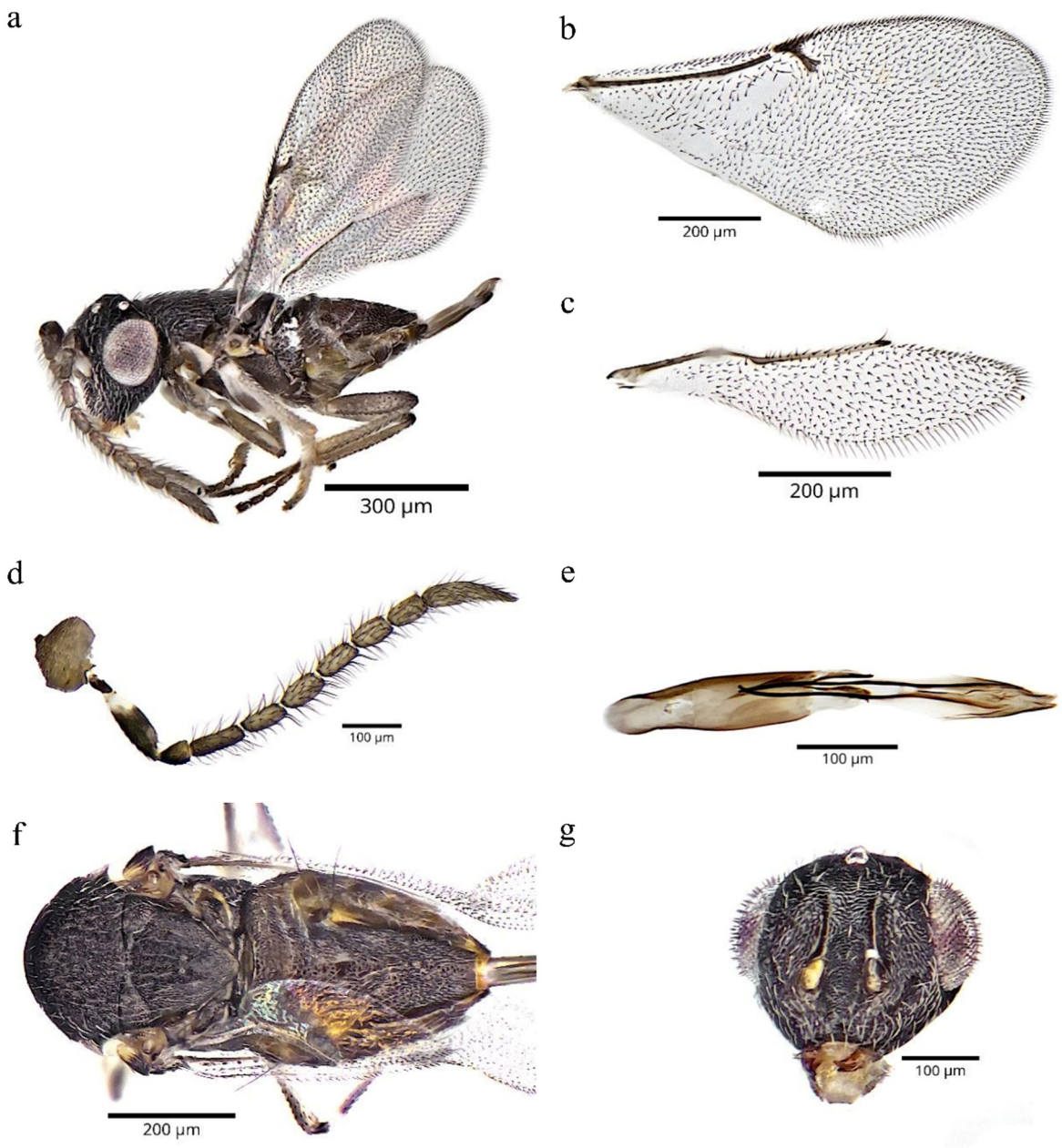

Figure 2. Male *A. tristis*: **a)** adult, **b)** anterior wing, **c)** posterior wing, **d)** antenna, **e)** genitalia, **f)** thorax and **g)** head.

Anagyrus tristis Noyes et Hayat 1994

F e m a l e d i a g n o s i s

A. tristis females can be differentiated from other species within the genus by the flagellum having the first two segments brown and the rest white, except for the apical segments of the club, which are yellowish. The prothorax is lighter than the rest of the thorax, which is orange-coloured, while the metasoma is dark brown (figure 1).

M a l e d i a g n o s i s

Body length of 0.77 mm (0.68-0.90 mm) (dry-mounted specimens); colour: body mostly black with dark brown parts; scape and pedicel darker than the rest of antenna; basal third of scape white, the remaining dark brown; tegula slightly lighter in colour than thorax and slightly yellowish at base; legs a little paler than the rest of the body; forewing hyaline; eyes abundantly hairy, with bristles longer than the width of ommatidia; scape 3.4× longer than broad (0.13 by 0.039 mm); pedicel 1.4× longer than broad (0.08 by 0.03 mm); all funicular segments longer than broad, F1 longest (0.08 by 0.031 mm); clava 2.6× longer than broad (0.15 by 0.11 mm) and longer than twice the 2nd funicular segment. Mesosoma is 1.6× longer than metasoma; forewing 2.7× longer than broad (0.838 by 0.311 mm); hindwing 4.2× longer than broad (0.559 by 0.132 mm). Genitalia length 0.22 mm.

Table 1. Percentage of divergence (± SE) for D2 region of the 28S rDNA sequences pairs within and between *A. tristis* and other members *Anagyrus* retrieved from GenBank and accession numbers are shown.

Species	*Anagyrus cachamai* MG731490	*Anagyrus agraensis* DQ667687	*Anagyrus kamali* DQ667688	*Anagyrus pseudococci* AY599315	*Anagyrus quilmes* MG731495	*Anagyrus tristis* KY211221	*Cheiloneurus fulvescens* AY599308
Anagyrus cachamai MG731490	N/C						
Anagyrus agraensis DQ667687	6.08 (1.44)						
Anagyrus kamali DQ667688	7.67 (1.61)	8.99 (1.76)					
Anagyrus pseudococci AY599315	7.33 (1.61)	8.64 (1.73)	4.82 (1.25)				
Anagyrus quilmes MG731495	4.83 (1.27)	6.71 (1.52)	4.82 (1.27)	3.03 (0.96)			
Anagyrus tristis KY211221	4.36 (1.20)	6.88 (1.55)	7.52 (1.65)	6.85 (1.57)	4.97 1.27)		
Cheiloneurus fulvescens AY599308	25.13 (4.00)	27.50 (4.33)	24.55 (3.77)	26.55 (4.12)	24.22 (3.73)	25.27 (3.87)	N/C

Molecular identification

The final lengths of the D2 region of the 28S rRNA sequences were 398, 418, 416, 576, 424, and 417 bp, and they are available in GenBank under the accession numbers PP501784, PP501785, PP501786, PP501787, PP501788, and PP501789, respectively.

Sequence diversity

The average mean divergence among species of the genus *Anagyrus* was 6% (SE = 1.44%). The genetic divergence of the sequences in this study ranged from 3.03% to 8.99% (table 1). The average divergence between the *A. tristis* sequences obtained in this study and those obtained from GenBank was 0.22% (SE = 0.13%), confirming the identity of the species.

Phylogenetic analysis

The phylogenetic tree inferred from the maximum likelihood analysis of the D2 region of the studied sequences consisted of two well-supported clades. One clade contained *A. tristis*; all *A. tristis* sequences grouped together with 100% probability, distinct from *A. cachamai* and *A. agraensis* with 99% probability. The other clade encompassed all other sequences. This analysis successfully supported the identification of *A. tristis* in our samples (figure 3).

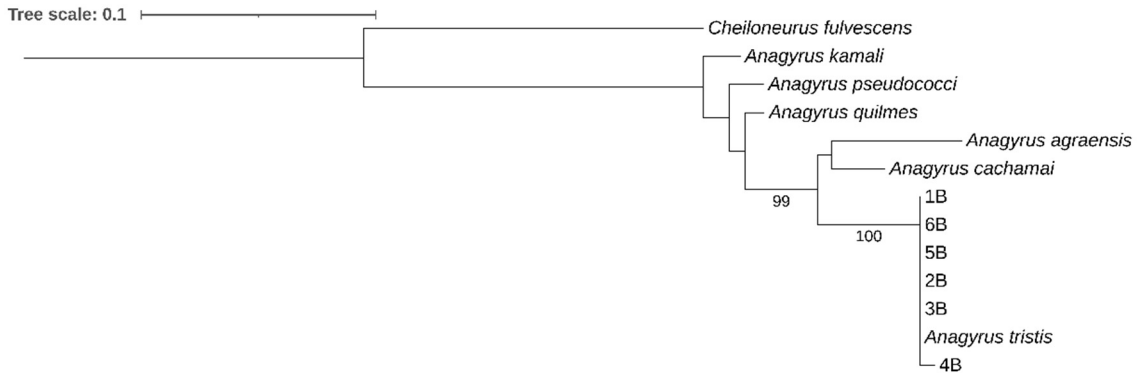

Figure 3. Dendrogram inferred from maximum likelihood (ML) analysis of the D2 region within the 28S rRNA data of *A. tristis*. The sequence *C. fulvescens* was used as an outgroup. Only bootstrap values above 85% are shown.

Discussion

A. tristis had previously only been recorded as parasitoid of the genus *Rastrococcus* Ferris in Indonesia (Noyes and Hayat, 1994), and more recently from *Phenacoccus solenopsis* Tinsley 1898 in Hainan, China (Chen *et al*., 2021). In this study, *A. tristis* is reported for the first time from Mexico and the American continent. Also, this is the first record of this species from *P. madeirensis*. Previously, only *A. loecki* had been reported as a parasitoid of *P. madeirensis* in Mexico (Noyes, 2024); but it is easily separated from *A. tristis* by its larger funicle segments and darker clava. The initial recovery of *A. tristis* specimens occurred in greenhouses where experiments were run with another parasitoid recently recorded from Mexico, *Dicarnosis ripariensis* Kerrich 1978 (Lomeli-Flores *et al*., 2021; Acevedo-Alcalá *et al*., 2024). Currently, laboratory trials are ongoing to characterize the biology, behaviour, and demographic parameters of this species, and to determine its potential as a biological control agent against *P. madeirensis.*

Further research is needed to determine the potential of natural enemies of mealybugs reported from North America (Chong, 2005; Lomeli-Flores *et al*., 2021; Acevedo-Alcalá *et al*., 2024), supported by a solid integrative characterization of involved species. It is known that *A. tristis* reproduces in at least three species of mealybugs (Noyes and Hayat, 1994; Chen *et al*., 2021), two of them are significant pests in various crops in North America. Therefore, ongoing efforts are essential in searching for and identifying natural enemies that could potentially contribute, in the medium term, to the control these economically important pests.

Acknowledgements

We express our gratitude to the National Council of Humanities, Sciences, Technologies, and Innovation (CONAHCYT), Mexico, for awarding a doctoral scholarship to the first author (CVU 926178). We also thank Jorge M. Valdez Carrazco for his contributions to photography and image processing. Furthermore, we extend our appreciation to Maribel Rivero Borja for her patience and guidance in DNA extraction procedures.

Adriana Acevedo-Alcalá (conceptualization, data curation, investigation, methodology, visualization and writing-original draft), J. Refugio Lomeli-Flores (conceptualization, data curation, investigation, supervision, review and editing), Esteban Rodríguez-Leyva (conceptualization, data curation, funding acquisition, investigation, project administration and writing original draft, review and editing), Ariel W. Guzmán-Franco (conceptualization, formal analysis, methodology, resources, software, review and editing), and Julio C. Velázquez-González (conceptualization, data curation, investigation, resources, and review and editing).

References

ACEVEDO-ALCALÁ A., RODRÍGUEZ-LEYVA E., LOMELI-FLORES J. R., GUZMÁN-FRANCO A. W., VELÁZQUEZ-GONZÁLEZ J. C., 2024.- Combining a predator and a parasitoid for biological control of *Phenacoccus madeirensis* (Hemiptera: Pseudococcidae).- *BioControl*, doi: 10.1007/s10526-024-10288-9.

ANDREASON S. A., TRIAPITSYN S. V., PERRING T. M., 2019.- Untangling the *Anagyrus pseudococci* species complex (Hymenoptera: Encyrtidae), parasitoids of worldwide importance for biological control of mealybugs (Hemiptera: Pseudococcidae): Genetic data corroborates separation of two new, previously misidentified species.- *Biological Control*, 129: 65-82.

ASHRAF R., ZEYA S. B., YADAV G. A. K., 2024.- Description of a new species of Anagyrini (Hymenoptera: Encyrtidae), with some records from India.- *Indian Journal of Entomology*, doi: 10.55446/IJE.2024.2142.

BELLOTTI A. C., SMITH L., LAPOINTE S. L., 1999.- Recent advances in cassava pest management.- *Annual Review of Entomology*, 44: 343-370.

BELTRA A., TENA A., SOTO A., 2013.- Fortuitous biological control of the invasive mealybug *Phenacoccus peruvianus* in Southern Europe.- *BioControl*, 58: 309-317.

BEN-DOV Y., 1994.- *A systematic catalogue of the mealybugs of the world (Insecta: Homptera: Coccoidea: Pseudoccocidae and Putoidae) with data on their geographical distribution, host plants, biology and economic importance*.- Intercept Ltd, Andover, UK.

BEN-DOV Y., MILLER D. R., GIBSON G. A. P., 2014.- *ScaleNet, a database of scale insects of the world*.- [online] URL: http://www.sel.barc.usda.gov/scalenet/scalenet.htm

CABI, 2000.- *Distribution maps of plant pests*.- [online] URL: http://www.cabi.org/dmpp

CHEN H. Y., LI H. L., PANG H., ZHU C. D., ZHANG Y. Z., 2021.- Investigating the parasitoid community associated with the invasive mealybug *Phenacoccus solenopsis* in Southern China.- *Insects*, 12 (4): 290.

CHONG J. H., 2005.- Biology of the mealybug parasitoid, *Anagyrus loecki*, and its potential as a biological control agent of the Madeira mealybug, *Phenacoccus madeirensis*. 186 p. *Doctoral thesis*, Athens, University of Georgia [online] URL: https://getd.libs.uga.edu/pdfs/chong_juang_h_200505_phd.pdf

FELSENSTEIN J., 1985.- Confidence limits on phylogenies: an approach using the bootstrap.- *Evolution*, 39: 783-791.

FRANCO J. C., ZADA A., MENDEL Z., 2009.- Novel approaches for the management of mealybug pests, pp. 233-278. In: *Biorational control of arthropod pests* (ISHAAYA I., HOROWITZ A., Eds).- Springer, Dordrecht.

GARCÍA MORALES M., DENNO B. D., MILLER D. R., MILLER G. L., BEN-DOV Y., HARDY N. B., 2016.- ScaleNet: a literature-based model of scale insect biology and systematics.- *Database*, 2016: bav118.

GARCÍA-VALENTE F., ORTEGA-ARENAS L. D., GONZÁLEZ-HERNÁNDEZ H., VILLANUEVA-JIMÉNEZ J. A., LÓPEZ-COLLADO J., GONZÁLEZ-HERNÁNDEZ A., ARREDONDO-BERNAL H. C., 2009.- Parasitismo natural e inducido de *Anagyrus kamali* sobre la cochinilla rosada en brotes de teca, en Bahía de Banderas, Nayarit.- *Agrociencia*, 43 (7): 729-738.

GILLESPIE J. J., MUNRO J. B., HERATY J. M., YODER M. J., OWEN A. K., CARMICHAEL A. E., 2005.- A secondary structural model of the 28S rRNA expansion segments D2 and D3 for chalcidoid wasps (Hymenoptera: Chalcidoidea).- *Molecular Biology and Evolution*, 22 (7): 1593-1608.

GUERRIERI E., CABALLERO-LÓPEZ B., SANS F. X., PUJADE-VILLAR J., 2009.- Encyrtidae (Hymenoptera, Chalcidoidea) colectados en Montblanquet (Lleida, Cataluña).- *Boletín de la Asociación Española de Entomología*, 33 (3): 389-397.

HALL T. A., 1999.- BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.- *Nucleic Acids Symposium*, 41: 95-98.

HERREN H. R., NEUENSCHWANDER P., 1991.- Biological control of cassava pests in Africa.- *Annual Review of Entomology*, 36: 257-283.

HOANG D. T., CHERNOMOR O., VON HAESELER A., MINH B. Q., VINH L. S., 2018.- UFBoot2: improving the ultrafast bootstrap approximation.- *Molecular Biology and Evolution*, 35: 518-522.

JAPOSHVILI G., YERLIKAYA H., KAYDAN M. B., 2023.- New records of mealybug (Hemiptera: Pseudococcidae) parasitoids belonging to the family Encyrtidae (Hymenoptera: Chalcidoidea) from Turkey.- *Zootaxa*, 5254 (4): 576-584.

KIMURA M., 1980.- A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences.- *Journal of Molecular Evolution*, 16: 111-120.

KONDO T., WATSON G. W., 2022.- *Encyclopedia of scale insect pests*.- CABI, Wallingford, UK.

LETUNIC I., BORK P., 2021.- Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation.- *Nucleic Acids Research*, 49: W293-W296.

LOMELI-FLORES J. R., LIMA-ESPINDOLA J., GONZÁLEZ-HERNÁNDEZ H., RODRÍGUEZ-LEYVA E., VELÁZQUEZ-GONZÁLEZ J. C., 2021.- New host record and distribution of *Dicarnosis ripariensis* Kerrich in Mexico.- *Southwestern Entomologist*, 46 (1): 191-196.

LOMELI-FLORES J. R., RODRÍGUEZ-LEYVA E., ARREDONDO-BERNAL H., BARRERA-GAYTÁN. J. F., GONZÁLEZ-HERNÁNDEZ H., BERNAL J. S., 2023.- Classical biological control experiences and opportunities from Mexico, a megadiverse country and center of crop domestication.- *Entomologia Generalis*, 44 (1): 63-80.

MANI M., SHIVARAJU C., 2016.- Methods of control, pp. 209-222. In: *Mealybugs and their management in agricultural and horticultural crops*.- Springer, New Delhi, India.

NOYES J. S., 1989.- The diversity of Hymenoptera in the tropics with special reference to Parasitica in Sulawesi.- *Ecological Entomology*, 14: 197-207.

NOYES J. S., 2000.- Encyrtidae of Costa Rica (Hymenoptera: Chalcidoidea), 1. The subfamily Tetracneminae, parasitoids of mealybugs (Homoptera: Pseudococcidae).- *Memoirs of the American Entomological Institute*, 62: 1-355.

NOYES J. S., 2024.- *Universal Chalcidoidea Database. World Wide Web electronic publication*.- [online] URL: http://www.nhm.ac.uk/chalcidoids

NOYES J. S., HAYAT M., 1994.- *Oriental mealybug parasitoids of the Anagyrini (Hymenoptera: Encyrtidae)*.- CAB International, Wallingford, UK.

NOYES J. S., WOOLLEY J. B., ZOLNEROWICH G., 1997.- Family Encyrtidae, pp. 70-320. In: *Annotated keys to the genera of Nearctic Chalcidoidea (Hymenoptera)* (GIBSON G. A. P., HUBER J. H., WOOLLEY J. B., Eds).- National Research Council, Ottawa, Canada.

PERRING T. M., ANDREASON S. A., ROLTSCH W., TRIAPITSYN S., GANJISAFFAR F., 2022.- Successful management of the pink hibiscus mealybug, *Maconellicoccus hirsutus*, in the Coachella Valley of California, pp. 151-160. In*: Contributions of classical biological control to the U.S. food security, forestry, and biodiversity* (VAN DRIESCHE R. G., WINSTON R. L., PERRING T. M., LOPEZ V. M., Eds).- FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA.

RAMESHKUMAR A., NOYES J. S., POORANI J., CHONG J. H., 2013.- Description of a new species of *Anagyrus* Howard (Hymenoptera: Chalcidoidea: Encyrtidae), a promising biological control agent of the invasive Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Sternorrhyncha: Pseudococcidae).- *Zootaxa*, 3717 (1): 76-84.

SELVARAJAN R., BALASUBRAMANIAN V., PADMANABAN B., 2016.- Mealybugs as vectors, pp. 123-130. In: *Mealybugs and their management in agricultural and horticultural crops* (MANI M., SHIVARAJU C., Eds)- Springer, New Delhi, India.

SHYLESHA A. N., JOSHI S., 2012.- Occurrence of Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae) on cotton in India and record of associated parasitoids.- *Journal of Biological Control*, 26 (3): 272-273.

SHYLESHA A. N., MANI M., 2016.- Natural enemies of mealybugs, pp. 149-171. In: *Mealybugs and their management in agricultural and horticultural crops* (MANI M., SHIVARAJU C., Eds)- Springer, New Delhi, India.

SUGAWARA Y., MITA T., TABATA J., UENO T., 2020.- Genetic and morphological approach to reappraising species validity in two different *Anagyrus* wasps (Hymenoptera: Encyrtidae) attracted by cyclolavandulyl butyrate.- *Entomological Science*, 23 (2): 152-164.

TAMURA K., STECHER G., KUMAR S., 2021.- MEGA 11: Molecular Evolutionary Genetics Analysis, Version 11.- *Molecular Biology and Evolution*, 38 (7): 3022-3027.

THOMPSON J. D., HIGGINS D. G., GIBSON T. J., 1994.- CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions specific gap penalties and weight matrix choice.- *Nucleic Acids Research*, 22: 4673-4680.

TOK B., KAYDAN M. B., MUSTU M., ULUSOY M. R., 2016.- Development and life table parameters of *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae) on four ornamental plants.- *Neotropical Entomology*, 45: 389-396.

TRIAPITSYN S. V., GONZÁLEZ D., VICKERMAN D. B., NOYES J. S., WHITE E. B., 2007.- Morphological, biological, and molecular comparisons among the different geographical populations of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), parasitoids of *Planococcus* spp., (Hemiptera: Pseudococcidae), with notes on *Anagyrus dactylopii*.- *Biological Control*, 41 (1): 14-24.

TRIAPITSYN S. V., AGUIRRE M. B., LOGARZO G. A., HIGHT S. D., CIOMPERLIK M. A., RUGMAN-JONES P. F., RODRIGUES J. C. V., 2018.- Complex of primary and secondary parasitoids (Hymenoptera: Encyrtidae and Signiphoridae) of *Hypogeococcus* spp. mealybugs (Hemiptera: Pseudococcidae) in the New World.- *Florida Entomologist*, 101 (3): 411-434.

TRIFINOPOULOS J., NGUYEN L. T., VON HAESELER A., MINH B. Q., 2016.- W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis.- *Nucleic Acids Research*, 44 (W1): W232-W235.

TRJAPITZIN V. A., MYARTSEVA S. N., RUÍZ-CANCINO E., CORONADO-BLANCO J. M., 2008.- *Clave de géneros de Encyrtidae (Hymenoptera: Chalcidoidea) de México y un catálogo de las especies. Serie avispas parasíticas de plagas y otros insectos*.- Universidad Autónoma de Tamaulipas, Ciudad Victoria, Tamaulipas, México.

WILLIAMS D. J., GRANARA DE WILLINK., 1992.- *Mealybugs of central and South América*.- CAB Int., Wallingford, UK.

Authors' addresses: J. Refugio LOMELI-FLORES (corresponding author: jrlomelif@hotmail.com), Adriana ACEVEDO-ALCALÁ (acevedo.adri.alcala@gmail.com), Esteban RODRÍGUEZ-LEYVA (esteban@colpos.mx), Ariel W. GUZMÁN-FRANCO (arielguzmanfranco@gmail.com), Colegio de Postgraduados, Posgrado en Fitosanidad, Entomología y Acarología, Montecillo, CP 56264 Texcoco, Estado de México, México; Julio C. VELÁZQUEZ-GONZÁLEZ (jvelazquez@koppert.com.mx), Koppert México, Circuito norte 82, Parque industrial El Marqués, CP 76246 Querétaro, México.

Received August 1, 2024. Accepted November 25, 2024.