MOLECULAR PHYLOGENY OF GALL-FORMING APHIDS TRIBE FORDINI (HOMOPTERA, APHIDIDAE, ERIOSOMATINAE) ON *PISTACIA* TREES FROM KURDISTAN REGION NORTH OF IRAQ

H. B. Ali R. S. Kamal H. Y. Fadhil
Assist. Prof. Assist. Lecturer. Assist. Prof.
Dept. Biol. Coll. Sci. University of Baghdad.
Corresponds author e-mail: hayder.ali1130@yahoo.com

ABSTRACT

Four aphid species belonging to the Tribe Fordini (Homoptera, Aphididae, Eriosomatinae) induce galls on *Pistacia* trees in North Iraq (Kurdistan) were recorded in this study. Molecular phylogeny of Iraq Fordini species was constructed for These species (*Forda riccobonii, Paracletus cimiciformis, Baizongia pistaciae and Forda marginata*), based on sequences of mitochondrial Cytochrome Oxidase I (*COI*) gene of these aphid species to detect the relationship and taxonomy of Iraqi species for tribe Fordini. Our results confirmed that each of *Forda* and *Paracletus* are a sister genus, or monophyletic group by designing in same clade, while *B. pistaciae* is separate from other clades, supporting the taxonomic division of the tribe Fordini into two subtribes (Fordina and Baizongina).

Key words: fordini, molecular phylogeny, (COI) gene, gall aphids, *Pistacia*

مجلة العلوم الزراعية العراقية -2020 :51(عدد خاص):184-190 **علي وآخرون**

علاقات النشوء الجزيئية لحشرات المن المكونة للعقد قبيلة Fordini (Homoptera, Aphididae, Eriosomatinae) على أشجار الفستق من منطقة كردستان شمال العراق

حيدر بدري علي رؤيا صفوان كمال حُلا يونس فاضل
استاذ مساعد مدرس مساعد استاذ مساعد
قسم علوم الحياة/ كلية العلوم – جامعة بغداد

المستخلص

اربعة انواع من حشرات المن المكون للعقد على اشجار الفستق تعود لقبيلة Fordini (Homoptera, Aphididae, Eriosomatinae) قد سجلت في هذه الدراسة. علاقات النشوء الجزيئية لانواع هذه القبيلة في العراق تمت دراستها في الانواع (*Forda riccobonii, Paracletus cimiciformis, Baizongia pistaciae and Forda marginata*) اعتمادا على تتابعات الجين mitochondrial Cytochrome Oxidase I (COI) للتحري عن علاقات الانواع العراقية لقبيلة Fordini وتصنيفها. اكدت نتائج هذه الدراسة على ان كل من الجنس *Forda* و *Paracletus* هي اجناس شقيقة عن طريق انضمامها في مجموعة وحيدة الاصل (clade) في حين انفراد النوع *B. pistacia*e في مجموعة اخرى مما يدعم تصنيف هذه القبيلة الى تحت قبيلتين هما (Fordina and Baizongina).

الكلمات المفتاحية: قبيلة Fordini ، العلاقات الجزيئية الجينية، جين COI ، المن المكون للعقد، الفستق.

***Received:16/5/2019, Accepted:24/9/2019**

INTRODUCTION

The genus *Pistacia* belongs to a cosmopolitan family "Anacardiaceae, this family include about 70 genera and over 600 species (24). The species of the genus *Pistacia* are dioecious trees or shrubs and characterized as xerophytic trees which and growing up to 8–10m height. In Iraq AL-Saghir and Porter (4) listed three *Pistacia* species, *Pistacia atlantica* Desf., *Pistacia eurycarpa* Yalt. and *Pistacia khinjuk* Stocks. The subfamily Eriosomatinae has recently been placed with family the Aphididae (16, 17). It was previously placed in the family Pemphigidae (= Eriosomatidae). There are about 19 aphid genera with 107 valid species belong to the tribe Fordini (Subfamily Eriosomatinae) (5, 6, 28), according to Inbar, (19) about 30 of these species are gall-inducing aphids belong to this tribe. The division of subtribes in Fordini is confusing. Some believe that Fordini includes two subtribes, Fordina and Baizongina (14, 37), others suggest it is divided into Fordina and Melaphidina (8, 18, 34). Almost all the species in Fordini are gall-forming and stimulate species-specific galls on their primary hosts, whereas the primary hosts for all species are full within two genera, *Pistacia* and *Rhus*, which belong to Anacardiaceae. Galls are the characteristic of aphids (29). Their position, morphology and structure of these galls are important aphid appearing. Among the whole Aphididae, only Eriosomatinae, Hormaphidinae and some species of Aphidinae produce galls (7), Galls of Fordini are significantly variable in morphology and position, nevertheless fascinatingly, are species-specific (32), since the gall founder, fundatrix forms a unique-shaped gall in a species-specific manner, the galling aphid species can usually be identified exclusively based on the gall morphology (21). Martinez, (23) and Wool, (33) reported about 15 species of aphids on *Pistacia* trees induce species-specific gall. The more common galls are those of Slavum wertheimae [H.R.L.], Smynthurodes betae West, Forda riccobonii [Stefani] and Geoica sp,.The geographic distribution of these aphid species everywhere in the Mediterranean Basin, from Morocco and Spain to east Afghanistan and Iran, agrees with the distribution of host *Pistacia* sp. trees (9, 14, 36). In India Chakrabarti, (10, 11) listed four species of aphids on *Pistacia* trees included *Forda riccobonii*; *F. marginata*; *Baizongia pistaciae* and *Geoica urticularia*. In Turkey 7 aphid species were Identified forming galls on *Pistacia* species. *Forda formicaria, Smynthurodes betae*, *Aploneura lentisci*, *Geoica utricularia*, *Baizongia pistaciae*, *F. Magenta* and *Rectinasus buxtoni*. (12) Ortiz-Rivas *et al.* (26) conducted a survey to explain the phylogenetic relationships among 10 Fordina species present in the Iberian Peninsula and the Canary Islands when they studied the implications for the taxonomy of genera *Forda* and *Paracletus* Mróz and Depa, (25) proved the usefulness of molecular analysis for solving taxonomic problems in aphids when they studied the DNA sequences of Mediterranean populations of *Paracletus cimiciformis* in aphid specimens morphologically identical with *Forda rotunda* Theobald, 1914. Li *et al*. (22) analyzed the molecular phylogeny of the Subfamily Eriosomatinae (also called Pemphiginae) which traditionally consists of 3 tribes, Eriosomatini, Pemphigini, and Fordini based on morphological and biological evidence. In Iraq, Aphid fauna induce species-specific gall almost poorly known, they are known mainly by insufficient surveillance lists, three aphids forming galls on *Pistacia khinjuk* where listed by Daoud and El-Haidari, (13); Al-Ali, (1) in North of Iraq the species are *Baizongia pistaciae, Geoica articular* and *Forda formicary.* The present study aimed to determine forming galls aphid in the North of Iraq, and clarifying the evolutionary relationships among the Tribe Fordini, and further investigating the relationships within and between *Forda, Paracletus* and *Baizongia*. This study represented the first phylogenetic study to this group in Iraq, preceded by few studies on other groups such as: Ali *et al.*, (2) on the widow spider in Iraq; Al-Saad and Aletby, (3) on the red palm weevil; Faraj *et al.,* (15) on the protozoan parsites causing Equine babesiosis in Baghdad city and few others.

MATERIALS AND METHODS

Sampling

Specimens for DNA extraction and amplification of mitochondrial Cytochrome

Oxidase I (*COI)* gene were collected from recent years, mostly from different types of galls on the infected primary hosts *Pistacia* trees (figure 1). COI gene was applied widely in the molecular phylogenetic studies of aphids and are easy to use in PCR. (22). All specimens were collected from different locations in Duhok, Erbil and Sulaimaniya provinces/ Kurdistan region North of Iraq during Summer seasons 2012-2013. Collection information of these specimens, including locations, sample numbers, and collection dates, (shown in table 1). Specimens were stored at (95–100)% ethanol at 4°C. (25). All specimens were deposited in the Iraq National History Research center and Museum, Department of Entomology, University of Baghdad. The specimens were identified according to their main morphological diagnostic features (7, 31). The extraction of total genomic DNA using the Geneaid DNA Mini extraction kit for (Tissue), according to the standard protocol recommended by the manufacturer, with some modification. Extracted DNA was being stored at -20°C until use. the whole aphid individual was selected for molecular experiments in 10 replicates for each per specimen.

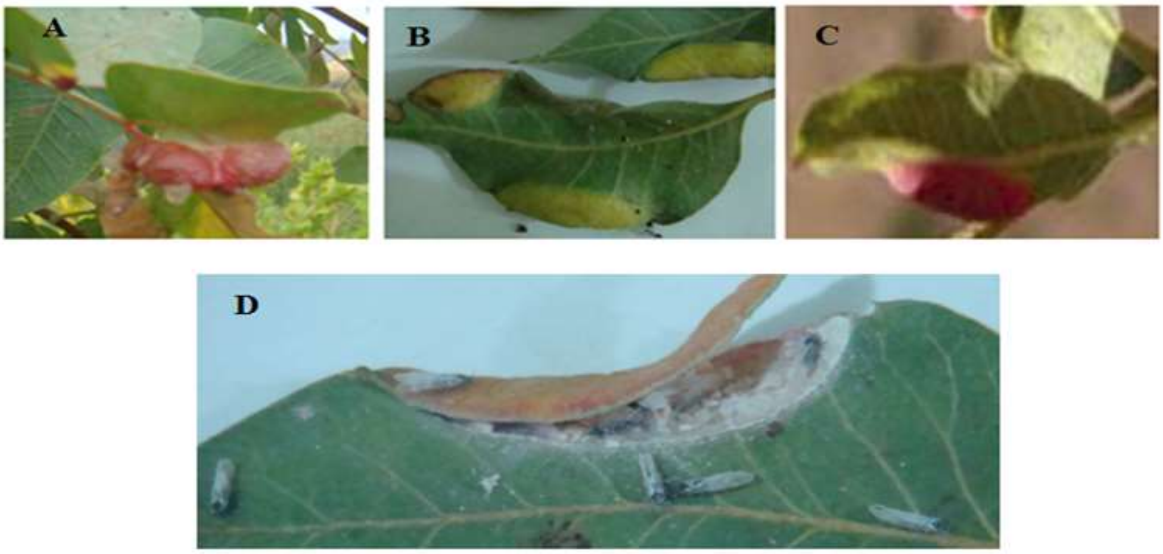

Figure 1. Shape of *Pistacia* trees-feeding aphids galls. (A,B and C), Pea/Margin galls on the blade of leaves by: A, *Forda riccobonii* from Erbil and Duhok provinces; B, *Paracletus cimiciformis* from Erbil, Sulaimaniya and Duhok provinces; and C, *Forda marginata* from Duhok province); D) Alate form of *Paracletus cimiciformis* from opening matured gall.

Table 1. Summary of collecting data relative to species (*Ford riccobonii, Paracletus cimiciformis, Baizongia pistaciae and F. marginata*) included in the present study.

Species	Host	Locality, Province	Number of collecting galls	date
Forda riccobonii	*Pistacia khinjuk*	Erbil and Duhok	6 Erbil (1) and Duhok (5)	14/05/2012 10/6/2013
Forda marginata	*Pistacia* Sp.	Duhok	3	12/6/2013
Paracletus cimiciformis	*Pistacia khinjuk*	Erbil and Duhok	12 Erbil (4) and Duhok (8)	15/5/2012 13/6/2013 14/6/2013
Paracletus cimiciformis	*Pistacia* sp.	Sulaimaniya	2	10/5/2012 11/5/2012
Baizongia pistaciae	*Pistacia khinjuk*	Duhok	3	16/5/2012

Sequencing

The classical method of CTAB (hexadecyltrimethylammonium bromide) extraction was reliable to obtain whole-genome DNA from each sample. The gene *COI* was amplified, and sequenced directly with an automated sequencer. The gene was amplified with LepF (5′ATTCAACCAATCATAAAGATATTGG-3′) (forward primer) and LepR (5′-TAAACTTCTGGATGTCCAAAAAATCA-5′) (reverse primer) (22). Complete sequences were deposited in GenBank under accession numbers given in table 2, together with the accession numbers of the sequences used for data comparison from the NCBI databases from different countries.

RESULTS AND DISCUSSION

The problems of morphological taxonomy of the gall-forming species identity, is confusing since they very closely related species, which are usually morphologically very similar and thus, making it difficult for identifying these species. (25, 27, 30). So the present study was done to avoid these problems.

Table 2. Summary of data relative to specimens analyzed and accession numbers for the mitochondrial DNA Cytochrome Oxidase I (*COI*) gene sequences

Organsims	**Accession no. local samples**	**Accession no. of reference samples of GenBank**	**Nucleitid no. of GenBank**	**Score/Identities**
Forda riccobonii	**MK111622.1**	**EU701669.1**	**41-637**	**588/(99%)**
Forda marginata	**MK111626.1**	**EU701668.1**	**50-637**	**576/(99%)**
****Paracletus cimiciformis***	**MK111623.1**	**JQ241782.1**	**30-623**	**585/(99%)**
*****Paracletus cimiciformis***	**MK111625.1**	**JQ241782.1**	**43-620**	**440/(92%)**
Baizongia pistaciae	**MK111624.1**	**KY841873.1**	**46-449**	**377/(98%)**

*** specimens from Erbil and Duhok provinces.**
**** specimens from Sulaimaniya province.**

The comparison of the studied sequence specimens with deposits (COI) gene copies of the same species in the NCBI database showed high identity (98-99%) with the reference species with accession number (table 2) (*Forda riccobonii* EU701669.1- Israel, *Forda marginata* EU701668.1 Canada, **Paracletus cimiciformis* JQ241782.1- Poland and *Baizongia pistaciae* KY841873.1- Canada), while the specimen ** *Paracletus cimiciformis* which collected from Sulaimaniya Province showed identity (92%) which differ from specimen * *Paracletus cimiciformis* this probably because of the polymorphism of their nucleotide as proofed by the alignment matrix, thus GenBank providing this specimen in different accession number. The results of phylogenetic analysis for a segment of 513 bp of the mitochondrial barcoding gene cytochrome oxidase I (COI) obtained with both applied markers indicated that sequences of studied specimens with those deposited in the data bank belonging to species (*Ford riccobonii, Paracletus cimiciformis, Baizongia pistaciae and F. marginata*). The present phylogenetic analyses support the taxonomic division of the tribe Fordini into two subtribes (Fordina and Baizongina) by creating two clades, the first one represents two sister groups, the first one includes *Ford riccobonii* and *F. marginata* and the second includes *P. cimiciform* which represents a sister group to the genus *Forda* sp. or monophyletic group, while the species *B. pistaciae* individually with the second clade. Comparing our molecular phylogenetic analysis with previous studies our results are in agreement with the phylogeny of Ortiz-Rivas *et al*. (26) which presented that each of *Forda* and *Paracletus* are a sister genera when they studied Molecular phylogeny of Iberian Fordini, they phylogeny analysis suggests that the group can be divided into two main clades. One clade is composed of aphids inducing small, low-capacity galls including genera *Forda, Paracletus* and others, while the second clade is composed of species inducing larger galls such as *B. pistaciae* and other species, although they are detecting others genes. Similar results are showen by Mróz and Depa, (25). Although Zhang and Qiao, (35) suggest Fordini consisting of two subtribes, Fordina and Melaphidina. They concluded that in Fordina, two groups: one is represented by *Forda*, and the other is represented by *Baizongia*. The second clade included the species (*B. pistaciae* and *Slavum wertheimae*), the molecular relationships among them would agree with previous literatures (21, 26, 35). The present study concludes that each of *Forda* and *Paracletus* are a sister genus, or monophyletic group by designing in the same clade while, *B. pistaciae* separately with other clades, supporting the taxonomic division of the tribe Fordini into two subtribes (Fordina and Baizongina).

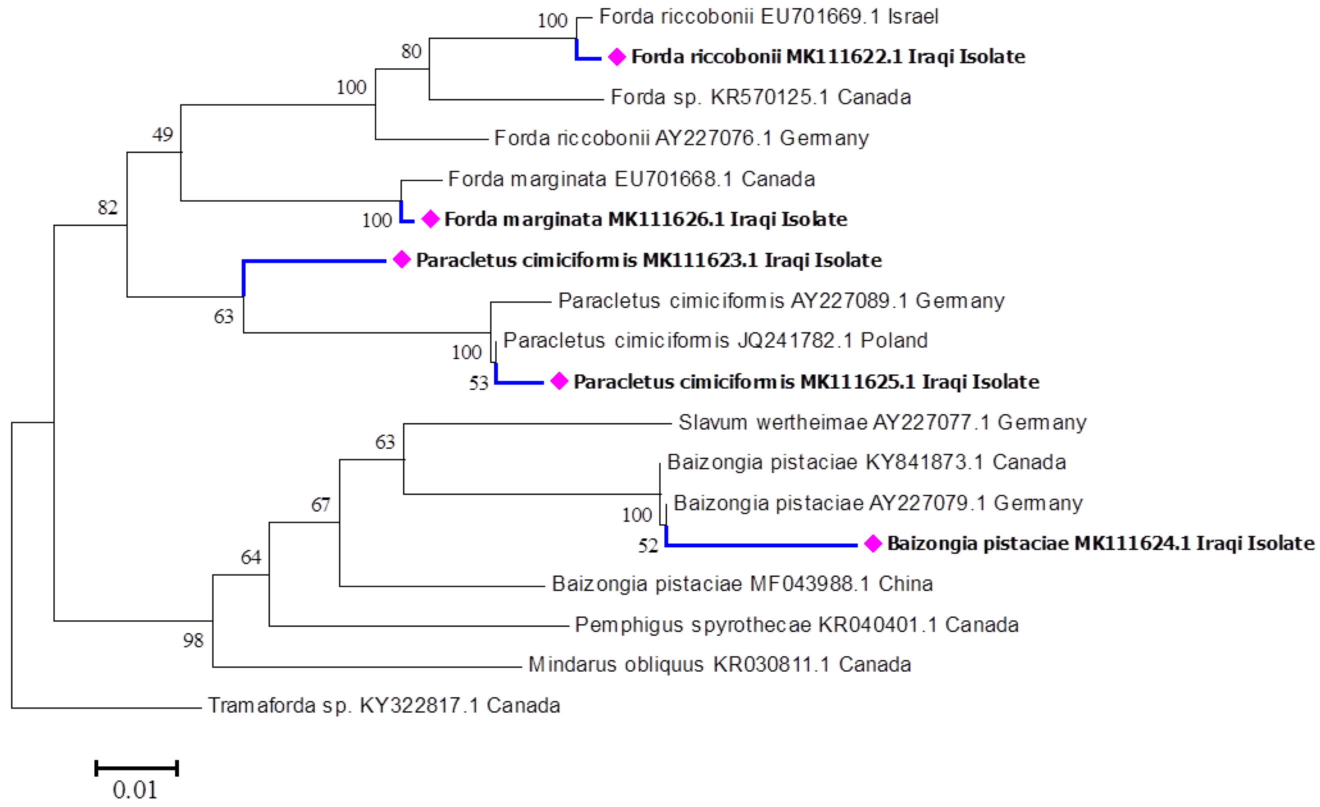

Figure 2. Neighbour joining phylogenetic tree constructed of Cytochrome Oxidase I gene sequences from galls forming aphids collected from different locations in Duhok, Erbil and Sulaimaniya, with their accession numbers; local samples in Bold, reference samples of GenBank from different countries. A measure of distance between taxa is indicated by the scale bar below the tree

REFERENCES

1. Al-Ali, A.S., 1977. Phytophagous and entomophagous insects and mites of Iraq Natural History Research Center 33:1-142

2. Ali, H. B., H. Y. Fadhil and I. M. Baker 2018. Taxonomic and molecular study of the widow spider genus *Latrodectus* Walckenaer, 1805 (Araneae: Theridiidae) in Iraq. Pakistan Entomologist. 40(1):25-29

3. Al-Saad, L. A. and M. A. Aletby 2018. Molecular diagnosis and comparison study to the red palm weevil *Rhyncophorous ferrugineus* (olivier, 1790) in Basrah province – Iraq. Iraqi Journal of Agricultural Sciences 49(2):228- 234

4. AL-Saghir, M. G. and D. M. Porter 2012. Taxonomic revision of the genus pistacia L. (Anacardiaceae), American Journal of Plant Sciences, 3: 12-32

5. Barjadze, Sh., S. Halbert, D. Matile and R. Ben-Shlomo 2018. A new genus of gall-forming aphids of tribe Fordini Baker, 1920 (Hemiptera: Aphididae: Eriosomatinae) from Near East, Annales de la Société entomologique de France, https://doi.org/10.1080/00379271.2018.1532814

6. Blackman, R.L. and V.F. Eastop 2018. Aphids on the world's plants: an online identification and information guide. Accessed 2018 May 30. http://www.aphidsonworldsplants.info

7. Blackman, R. L. and V. F. Eastop 1994. Aphids on the World's Trees. An Identification and Information Guide 1–1016 (CABI,).

8. Blackman, R.L. and V.F. Eastop 1984. Aphids on the World's Crops: an Identification and Information Guide. 2nd ed. Chichester, John Wiley and Sons. pp: 466

9. Bodenheimer F and E. Swirski 1957. Aphidoidea of the Middle East. Weizmann Science Press, Jerusalem, pp: 378

10. Chakrabarti, S. 1987. Biosystematics of gall aphids (Aphididae, Homoptera) of western Himalaya, India. Proceeding of the Indian Academy of Sciences (Animal Science), 96: 561–572

11. Chakrabarti, S. 2007. Diversity and biosystematics of gall-inducing aphids

(Hemiptera: Aphididae) and their galls in the Himalaya. Oriental Insects, 41: 35–54

12. Çulcu, M. and C. Mart 2015. Distribution and natural enemies of gall-forming Aphids on Pistachio leaves in Gaziantep and Şanlıurfa provinces. Bitki Koruma Bülteni, 55(3): 171-185.

13. Daoud, A. K. and H.S. El-Haidari 1968. Recorded aphids of Iraq. Iraq Natural History Museum 24: 1-37

14. Davatchi, A.G. 1958. Etude biologique de la faune entomologique des Pistacia sauvages et cultives. Revue de Pathologie Vegetale et d' Enomologie Agricole de France 37, 1–166

15. Faraj A. A., , B. F. Hade and A. M. Al-Amery 2019. Conventional and molecular study of *babesia* spp. of natural infection in dragging horses at some areas of Baghdad City, Iraq. Iraqi Journal of Agricultural Sciences, 50(3):909- 915

16. Favret, C. 2019. SF Aphid: Aphid Species File (version 5.0, Jun 2018). In: Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist (Roskov Y., Ower G., Orrell T., Nicolson D., Bailly N., Kirk P.M., Bourgoin T., DeWalt R.E., Decock W., Nieukerken E. van, Zarucchi J., Penev L., eds.). Digital resource at www.catalogueoflife.org/annual-checklist/2019. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-884X

17. Favret C. and D. C. Eades 2011. "Taxa display: family Aphididae Latreille". aphid.speciesfile.org. Retrieved October 19

18. Ghosh, A.K. 1984. Fauna of India, Homoptera, Aphidoidea, Subfamily Pemphiginae.. Calcutta, Zoological Survey of India. pp: 429

19. Inbar, Moshe 2008. Systematics of Pistacia: Insights from Specialist Parasitic Aphids. Taxon, 57(1) 238-242

20. Inbar, M., M. Wink, and D. Wool 2004. The evolution of host plant manipulation by insects: molecular and ecological evidence from gall-forming aphids on Pistacia. Molecular Phylogenetics and Evolution. 32:504-511

21. Kutsukake, M., K. Uematsu and Fukatsu, T. 2019. Plant Manipulation by Gall-Forming Social Aphids for Waste Management. Frontiers in Plant Science. 10:933

22. Li, Xing-Yi, Jiang, Li-Yun, Qiao, Ge-Xia. 2014. Is the subfamily Eriosomatinae (Hemiptera: Aphididae) monophyletic? Turkish Journal of Zoology, 38: 285-297.

23. Martinez, Jean-Jacques Itzhak, 2009. Population ecology of *forda riccobonii* (Hemiptera: Pemphigidae) on *Pistacia Atlantica.* Oriental Insects, 43:1, 241-251

24. Mitchell, J.D. and S.A., Mori, 1987. The cashew and its relatives (Anacardium: Anacardiaceae). Memoirs of the New York Botanical Garden. 42, 1–76

25. Mróz E. and L. Depa 2012. Molecular identification of atypical morph of *Paracletus cimiciformis* von Heyden, 1837 described as *Forda rotunda* Theobald, 1914 (Hemiptera: Aphidoidea: Eriosomatidae). Genus 23(1): 17-24

26. Ortiz-Rivas B., D. Martinez-Torres and N. P. Hidalgo 2009. Molecular phylogeny of Iberian Fordini (Aphididae: Eriosomatinae): implications for the taxonomy of genera *Forda* and *Paracletus*. Systematic Entomology 34: 293–306

27. Rakauskas, R., J. Turčinavičiené and J. Bašilova 2011. How many species are there in the subgenus Bursaphis (Hemiptera: Sternorrhyncha: Aphididae)? Co-I evidence. European Journal of Entomology. 108: 469-479

28. Remaudière, G. and M. Remaudière 1997. Catalogue des Aphididae du Monde. INRA, Paris, pp: 473

29. Stone, G.N. and K. Schönrogge 2003. The adaptive significance of insect gall morphology. Trends in Ecology and Evolution 18: 512–522

30. Wang, J. and G. Qiao 2009. DNA barcoding of genus *Toxoptera* Koch (Hemiptera: Aphididae): identification and molecular phylogeny inferred from mitochondrial CO I sequences. Insect Science, 2009: 475–484

31. Wojciechowski ,W., Ł. Depa, J. Halgoš, I. Matečný, J. Lukáš and M. Kanturski 2016. Aphids of Slovakia. Distributional Catalogue, Checklist, keys and List of Host Plants. Comenius University in Bratislava Faculty of Natural Sciences, Bratislava, pp: 344

32. Wool, D. 2004. Galling aphids: specialization, biological complexity, and variation. Annual Review of Entomology, 49: 175–192

33. Wool, D. 2013. Aphid-induced galls on Pistacia in the natural Mediterranean forest of Israel: Which, where, and how many? Israel Journal of Zoology 41(4): 591-600

34. Zhang, G.X., G.X. Qiao, T.S. Zhong, and W.Y. Zhang 1999. Fauna Sinica Insecta Vol. 14. Homoptera: Mindaridae and Pemphigidae. Beijing, Science Press. pp: 356

35. Zhang, H.C. and G.X. Qiao 2007. Molecular phylogeny of Fordini (Hemiptera: Aphididae: Pemphiginae) inferred from nuclear gene EF-1 *a* and mitochondrial gene COI. Bulletin of Entomological Research, 97: 379–386

36. Zohary, M. 1952. A Monographical Study of the Genus *Pistacia*. Palestine Journal of Botany, Jerusalem Series, 5: 187-228

37. Zwölfer, H. 1957. Zur Systematik Biologie und Okologie unterirdisch lebender Aphiden (Homoptera: Aphidoidea) (Anoeciinae, Tetraneurini, Pemphigini und Fordinae). Teil. II. Tetraneurini und Pemphigine. Zeitschrift fu¨r Angewandte Entomologie 40: 528–575.