Aphids and their parasitoids on the Canary grass, *Phalaris canariensis* in Malta (Hymenoptera, Braconidae, Aphidiinae)

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ABSTRACT. *Adialytus ambiguus* and *Diaeretiella rapae* were reared from *Rhopalosiphum padi* on the Canary grass, *Phalaris canariensis* in Malta. The identity to species level of the *Adialytus* required confirmation via DNA analysis of the respective species group. Some ecosystem interrelationships derived from the determined food webs are discussed.

KEY WORDS. Molecular identification, *Adialytus ambiguus*, *Diaeretiella rapae*.

INTRODUCTION

In recent years, research on plant-aphid-parasitoid associations in Malta has revealed several faunal and ecological results new for this territory (Mifsud & Starý, 2012). The present account brings original information on the Canary grass, *Phalaris canariensis* L. and its aphid- parasitoid associations in Malta.

MATERIAL AND METHODS

The Canary grass and associated aphids were sampled within a framework of research work which is being carried out on this group of herbivorous insects in Malta. Aphid colonies were gently sampled together with a cut piece of plant and preserved at room temperatures in a plastic box to obtain the parasitoids by rearing them from the aphids. The emerged parasitoids were either sampled directly in ethanol containing vials or sampled subsequently from the dry litter on the box bottom.

Owing to a need to supplement the morphological evidence, characters in morphology, adult specimens of *Adialytus* were also examined by DNA detection methods. The DNA sequences of the reared *Adialytus* specimens were compared with the sequences of the two *Adialytus* species: *A. ambiguus* and *A. salicaphis*. Sequences of *Aphidius rhopalosiphi*, *Lysiphlebus orientalis* and *Praon yomenae* were used as outgroup taxa, sequences of which were retrieved from the gene bank. The sequences of *A. ambiguus* reared from *Sipha* and *A. salicaphis* were used from Stanković *et al.* (unpublished data).

Five specimens of *A. salicaphis* and four specimens of *A. ambiguus*, along with the *Adialytus* specimen on *Rh. padi* from Malta (SQ10) were used for the DNA analysis: SQ1: *A. ambiguus* from *S. maydis* on *Lolium perenne*, Greece, Kyparissia, 1.v.2010., leg. V. Žikić; SQ2: *A. salicaphis* from *Chaitophorus salicti* on *Salix caprea*, Serbia, Vlasina Lake, 6.viii.2010., leg. S. Stanković;

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SQ3: *A. salicaphis* from *Ch. niger* on *S. fragilis*, Serbia, Niš, Popovac, 4.vii.2010., leg. V. Žikić; SQ4: *A. salicaphis* from *Ch. populeti* on *Populus alba*, Serbia, Niš, Popovac, 25.v.2010., leg. V. Žikić; SQ5: *A. salicaphis* from *Ch. populeti* on *Populus alba*, Serbia, Niš, Popovac, 25.v.2010., leg. V. Žikić; SQ6: *A. salicaphis* from *Chaitophorus* sp. on *S. caprea*, Serbia, Dukat mt., 6.viii.2011., leg. S. Stanković; SQ7: *A. ambiguus* from *Sipha* sp. on *Dactylis glomerata*, Serbia, Vlasina Lake, 28.vi.2012., leg. V. Žikić; SQ8: *A. ambiguus* from *Sipha* sp. on *Arrenantherum elatior*, Serbia, Tara mt., 3.vii.2012., leg. S. Stanković; SQ9: *A. ambiguus* from *Sipha* sp. on *Zea mays*, Serbia, Niš, Popovac, 13.vii.2012., leg. S. Stanković.

All genomic DNA material was extracted from the wasps using KAPA Express Extract Kit. The sequence of the mitochondrial COI gene was amplified using two primers: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGCTGACCAAAAAATCA-3') (Folmer *et al*., 1994). PCR amplification of the DNA was done in a 25 µl volume which contained 1 µl of extracted DNA 12.5 µl 1 × KAPA2G Robust HotStart ReadyMix 1.25 µl (0.5 µm) of each primer and 9 µl of water. Thus prepared tubes were placed in the Eppendorf Mastercycler® using the following protocol: initial denaturation at 95°C for 5 min, 35 cycles consisting of 1 min at 94°C, 1 min at 54°C, 1.5 min at 72°C and the final extension at 72°C for 7 min. All PCR products were purified using QIAGEN QIAquick® PCR Purification Kit according to the manufacturer's instructions. The sequencing of the amplified DNA was done by Macrogen Inc. (Seoul, South Korea).

Editing of the sequences was done using software FinchTV (Geospiza, Inc., Seattle, WA) and thus prepared for the alignment which was performed in Clustal W program, part of the MEGA5 software (Tamura *et al*., 2011) which was also used for acquiring phylogenetic trees. The average genetic distance between obtained sequences was completed by Kimura's two-parameter method of base substitution by bootstrap method with 1000 replicates.

RESULTS

Adialytus ambiguus (Haliday, 1834)

Material examined. MALTA: Marsaskala, 20.iv.2013, 6 ♀♀ & 4 ♂♂ emerged from *Rhopalosiphum padi* on *Phalaris canariensis* (near human habitation on disturbed ground), leg. D. Mifsud (dry mounted); 6 ♀♀ & 1 ♂, same data (in alcohol).

Diaeretiella rapae (M´Intosh, 1855)

Material examined. MALTA: Marsaskala, 20.iv. 2013, 2 ♀♀ & 3 ♂♂ emerged from *Rhopalosiphum padi* on *Phalaris canariensis* (near human habitation on disturbed ground), leg. D. Mifsud (in alcohol).

Molecular identification of parasitoids

The sequences were aligned and trimmed to the length of 619 bp and used in genetic analysis. From the topology on the phylogenetic tree (Fig. 1), which was constructed using Maximum Likelihood (ML) method, is clear that specimen *A. ambiguus* reared from *Rh. padi* (SQ10) is clustered among other specimens of *A. ambiguus*, all reared from the genus *Sipha*.

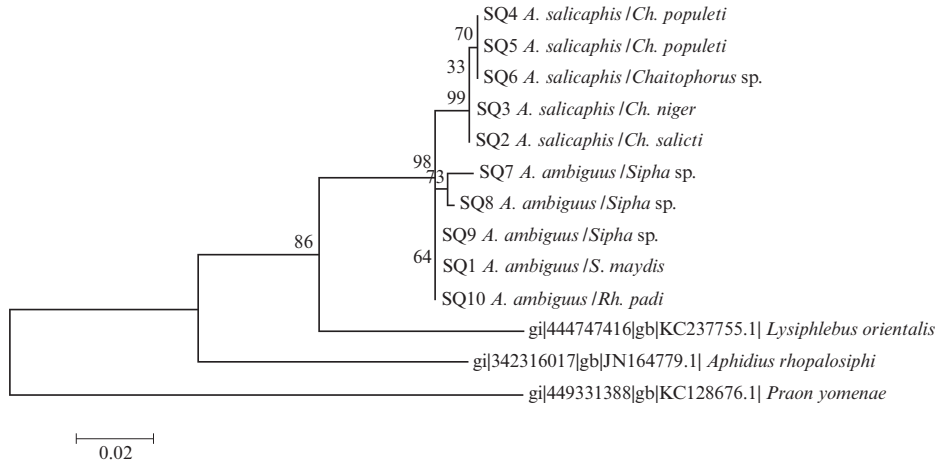

Figure 1: Phylogenetic tree with branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

According to Kimura's two-parameter model for computing the Pairwise distances, the specimens of *Adialytus* reared from *Rhopalosiphum padi* on the Canary grass are genetically closest to the specimens of *A. ambiguus* with the average distance of 0.37%. The average distance between this specimen and *A. salicaphis* is 1.1%. The molecular analysis of the mitochondrial gene COI, which is widely used as a barcoding gene (Derocles *et al*., 2012), showed that specimen of *A. ambiguus* reared from *Rh. padi* on the Canary grass does not differ genetically from the other specimens of *A. ambiguus* all reared from the aphid genus *Sipha*, i.e. the genetic difference is within the species level.

DISCUSSION

The Canary grass, *Phalaris canariensis* L. (Poaceae) is native of the Western Mediterranean basin but is now also grown commercially in several parts of the world. In Malta, this plant is best described as a casual species, often found growing close to man-made habitats.

The mentioned aphid parasitoids found in Malta during the present study differ in their host range and respective ecosystem relationships significantly. *Adialytus ambiguus* is a specific parasitoid of *Sipha* (Kavallieratos *et al*., 2004; Starý, 1976), and less frequent also of *Rhopalosiphum padi* on grasses (Michelena *et al*., 2004, on *Triticum aestivum*, Spain). *Diaeretiella rapae* is commonly associated with cabbage aphids, and from Malta it was to-date collected from *Lipaphis erysimi* on *Diplotaxis* and *Melanaphis donacis* on *Arundo donax* (Mifsud & Starý, 2011), but is known to occur on other aphids in other countries (e.g. Kavallieratos *et al*., 2004; Starý, 1976, 2006).

The identification of the parasitoid followed an up-dated key of aphid parasitoids from Iran, where *A. ambiguus* is also recorded (Rakhshani *et al*., 2012). However, it ought to be notified that the generic revision of *Adialytus* at World level has clarified some miss-understandings in species identification.

Adialytus ambiguus (Haliday), re-classified by Rakhshani *et al.* (2012) has been recorded as a parasitoid of *Sipha elegans* del Guercio in Iran. The same association was also recorded in Europe (Kavallieratos *et al*., 2004; Starý, 1976, 2006) but the parasitoid identification may be possibly somewhat misleading owing to an incorrect synonymy of *A. ambiguus* and *A. arvicola*. However, both species are also parasitoids of *Sipha* aphids respectively. Further taxonomic treatment is under current investigation (Stanković *et al*., 2013).

The plant-aphid-parasitoid (hyperparasitoid) associations have been classified as relatively simple and easy to determine some food webs in the ecosystems as well as the relationships between them. In an island environment such as Malta the fauna and flora prevailingly of Mediterranean origin is not numerous due to the insular phenomena. A respective increase of information on the tri-trophic (parasitoid-aphid-plant) associations has been believed to contribute to the knowledge of respective island ecosystems. It ought to be notified that the relations through the food webs in and between the ecosystems may be considerably different if the aphids and aphid parasitoids are considered. If exemplified by Canary grass situation, it is apparent that an analysis can be presented, as follows: *Phalaris canariensis* - *Rhopalosiphum padi* - *Adialytus ambiguus*. The aphid's relationship is specific to *Phalaris* and aphid's may relate to some other Poaceae. *Adialytus* parasitoid is specific in a similar manner, but its preferred hosts are *Sipha* aphids on Poaceae, its parasitization on *Rh. padi* is less frequent. *Diaeretiella rapae* may interact with the cabbage aphid, *Lipaphis erysimi* on Brassicas, and also with *Melanaphis donacis* on *Arundo donax*, and presumable also with other aphid species occurring in Malta.

ACKNOWLEDGEMENTS

The research by P. Starý was partially conducted with institutional support RWO: 60077344 and that by S. Stanković with Grant no. III43001 (Ministry of Education, Science and Technological Development, Republic of Serbia. We thank Prof. Vladimir Žikić for the loan of *Adialytus* spp. and Dr Nicolás Pérez Hidalgo (University of Leon, Spain) for the identification of the aphid samples. Finally we express our sincere thanks to Ž. Tomanović (Institute of Zoology, University of Belgrade) for supervising the manuscript.

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Received: May 30, 2013
Accepted: November 10, 2013