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Replacement of *Megastigmus pistaciae* Walker (Hymenoptera: Megastigmidae) by *Eurytoma plotnikovi* Nikol'skaya (Hymenoptera: Eurytomidae) in pistachio orchards in southern Italy

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Abstract Investigating the effect of non-native herbivore species in the new areas is vital for understanding their effects on native crops and the interactions that the newly arrived species have with any natural native herbivores and predatory species. The pistachio-seed wasp *Eurytoma plotnikovi* Nikol'skaya (Hymenoptera: Eurytomidae) is an invasive species on pistachio groves in Sicily, southern Italy; thus, the current study investigated its effects on pistachio crops and the native pistachio-seed wasp *Megastigmus pistaciae* Walker (Hymenoptera: Megastigmidae). The results showed that fruits in *Pistacia vera* pistachio groves were infested only with *E. plotnikovi*, which had been able to replace *M. pistaciae* after only a decade. Female *E. plotnikovi* emerge between May and June, but the males are rarely seen. No natural enemies were detected for *E. plotnikovi* in the study sites, highlighting an important phytosanitary issue for pistachio nut production in Sicily.

Keywords Chalcid wasps · Ecological function · *Pistacia vera* · Seed-feeder · Seed wasp

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Introduction

The introduction of non-native species into the new areas far from their natural distribution requires research to determine any direct and indirect impacts they might have in their new environments (McNeely, 2001). Such introductions involve numerous different taxa that perhaps affect most significantly the agricultural industry (Sileshi et al., 2019). Insects include common invasive species, aided in particular by human interventions, such as through international trade or accidental introduction (Seebens, 2019; Venette & Hutchison, 2021). Invasive non-native species often have significant effects on native biodiversity species and ecosystems, posing serious problems for the conservation of populations (Walker & Steffen, 1997). Given the potential for non-native species to negatively influence native populations, communities, and ecosystems, it is vital to determine their occurrence and possible adverse impacts as soon as possible, particularly regarding native species with which they occupy the same ecological niche. The control or eradication of a non-native species is a priority if it negatively influences its host plant, particularly if crop plants are involved (Bonsignore et al., 2021).

Various species and subspecies of the pistachio tree genus *Pistacia* are grown in southwest Asia and in the Mediterranean basin. The seeds, gums, and galls produced by plants of this genus have been used in southwest Asia since the Epipaleolithic and Neolithic, as evidenced by archeological finds of *Pistacia* fruits,

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seeds, and charcoal remains (Rousou et al., 2021). Among the domesticated species, *Pistacia vera* L., is cultivated on a large scale in different areas in southwest Asia, the Mediterranean basin, and the USA (FAOStat, 2019). Over the past few decades, there has been a change in the framework of pests that can affect the seed production of *P. vera*, and several species of seed-feeders have been reported, including *Amyelois transitella* (Walker, 1863) (Lepidoptera: Pyralidae) and two pistachio-seed wasps, namely, *Megastigmus pistaciae* Walker (Hymenoptera: Megastigmidae) and the invasive species *Eurytoma plotnikovi* Nikol'skaya (Hymenoptera: Eurytomidae) (Jarraya & Helali, 1978).

Megastigmus pistaciae has been known for years throughout pistachio crops in Italy (De Stefani, 1917, Monastero, 1958–59; Nieves-Aldrey et al., 2008) and in all pistachio-producing countries (Jarraya & Bernard, 1971), including new production areas, such as California (Rice & Michailides, 1988). It has two generations per year with overwintering larvae produced from eggs of both generations (Jarraya & Bernard, 1971; Rice & Michailides, 1988). The second generation appears after shell hardening of pistachio seeds and thus, it is thought that the second generation is unable to predate on pistachio seeds (Taghizadeh, 1953).

Eurytoma plotnikovi expands the overall framework of seed-feeder species harmful to pistachio production; however, its occurrence in the various Mediterranean countries where pistachio production occurs is not always known. It is thought that, in the western Mediterranean (e.g., Jordan), *E. plotnikovi* was present since the early 1960s (Doğanlar et al., 2009) and was subsequently reported in Tunisia (Jarraya & Helali, 1978), Turkey (Uygun, 1994; Doğanlar et al., 2009), Iran (Basirat & Seyedoleslami, 2000), and Israel (Izhaki, 1998). The species is also reported in the Middle East and in China on *Pistacia chinensis* (Tian et al., 1994; Li et al., 2008; Tang et al., 2012). The occurrence of this seed wasp in Europe was first recorded from Greece and Italy only in 2009 (Mourikis et al., 1998; Longo & Suma, 2011), and was also recently reported from France (Rousse & Reynaud, 2022). From its initial occurrence in southwest Sicily, *E. plotnikovi* has spread to all areas of pistachio cultivation on the island, with significant detrimental effects on pistachio harvests. As a seed-feeder, *E. plotnikovi* is able to completely empty the seed cases, resulting in production losses as high as >80% of the entire production (Mourikis et al., 1998). Initially, it was unclear which wasp species was causing the damage to the pistachio fruits, *M. pistaciae* or *E. plotnikovi*. On hatching, eurytomid larvae feed on the developing endosperm of *P. vera* seeds. The wasp has one generation per year, and the final instar larvae over winter inside the seed case (Mohammadzadeh et al., 2017). The present study evaluated the occurrence of *M. pistaciae* in Sicily, after just over a decade from the first finding of *E. plotnikovi* by comparing infestation levels of the two species in different pistachio orchards in the area of the first original report of *E. plotnikovi* in Sicily. Natural enemies adapted to *E. plotnikovi* and their effectiveness to control this pest were also investigated.

Materials and methods

Observational orchards

Pistachio seeds were collected from trees in conventionally farmed orchards distributed in different areas (Table 1) within the geographical region where *E. plotnikovi* was first found by Longo and Suma (2011). The pistachio orchards occur within a Protected Designation of Origin (PDO) of 'Pistachio of Raffadali' that lies within a Mediterranean xeric-type climatic area. Pistachio groves (cv Napoletana) are obtained from the grafting of *P. vera* on *P. terebinthus* on regosol soils based on gypsum, chalky clays and calcareous substrates. The orchards are not irrigated and ranged in altitude from 250 to 550 m above sea level. The average temperature in the study area was ~18 °C with an average rainfall of 500 mm per year, mainly during the autumn, with a dry summer; the area experiences torrential precipitation events over a period of ~5 months (based on data from 1971 to 2000) (Arnone et al., 2013).

Table 1 Locations of pistachio grove sample sites

Area	Municipality	Latitude	Longitude
1	Raffadali (AG)	37°23'30" N	13°32'43" E
2	Alessandria della R. (AG)	37°33'15" N	13°31'17" E
3	S. Biagio Platani (AG)	37°31'50" N	13°30'49" E
4	Sant'Angelo Muxaro (AG)	37°27'41" N	13°33'25" E
5	S. Biagio Platani (AG)	37°29'49"N	13°30'31" E

Data collection

Fruit sampling and infestation by seed-feeders

Pistachio seeds that showed evidence of insect attack or other diseases affecting the seeds were collected. In September, mummified and empty pistachio seeds containing fully grown larvae of seed-feeding insects were collected from ~20 sites in each study location (Table 1). To separate the fruits with the aforementioned symptoms, in agreement with the farmers, all harvested seeds were added to drinking water and those that floated were removed; alternatively, the seeds were exposed to the action of a mechanical fan for <60 s and any that were blown by the fan were also removed. Then, in each study area, 400 seeds were randomly selected from these collected seeds ($N=2000$ in total). Seeds were placed individually in polystyrene containers with a fabric lid to allow gas exchanges. The containers were kept in the dark at room temperature and the position of the containers was changed weekly. Observations on the seeds and separation of any insects that emerged were performed weekly starting from May until August. All emerged insects were observed under a stereomicroscope, separated by site and date, and then preserved in absolute alcohol for storage.

Identification of emerging insects and data analysis

Each insect that emerged from a seed was observed under a stereomicroscope Olympus SZX9 at magnifications from 20 × to 60. Species were identified using a key for the Eurytomidae (Nikol'skaya, 1935; Zerova, 2017). The total number of pistaciae seeds collected and the percentage parasitization of seeds (i.e., the number of seeds from which a phytophagous or its parasitoid emerged) at different sites were used for analysis. SPSS v.23 (IBM, 2015) was used for data analyses and SigmaPlot 13.0 (SigmaPlot, 2018) was used to produce graphs. The images were taken with a Dino-Lite mod AM73915MZTL acquisition and processing system.

Results and discussion

The results highlighted the almost total presence of *E. plotnikovi* in all study sites, with clear differences between sites, accounting for 5.25% of damaged seeds in area 5 and 13.25% in area 1. The appearance of adult *E. plotnikovi* occurred during May and June (Fig. 1). *Amyelois transitella* (Walker, 1863) was present in low numbers only in one location (with only two specimens obtained). The study confirmed the scarcity of male *E. plotnikovi* (Fig. 2), which were mainly black except for their brownish yellow legs. The males had antenna with seven-segmented funicles and long pubescence (Fig. 3). In addition, their forewings had marginal veins that were longer than the stigmal vein.

Invasion by *E. plotnikovi* has resulted in the net reduction of *M. pistaciae* in the study area, as well as in other Sicilian pistachio cultivation areas where the presence of *M. pistaciae* is sporadic (personal observation). Despite the presence of many seed-feeding species belonging to the genus *Megastigmus* Dalman, 1820, which are able to feed on conifer seeds and as well as Anacardiaceae, Malvaceae, and Rhamnaceae (Roques et al., 1999, 2016; Roques & Skrzypczyńska, 2003), these species have not yet replaced native seed-feeding species. Therefore, *E. plotnikovi* is thought to compete more successfully with *M. pistaciae* in their invaded environment, probably because the native species is also exposed to natural enemies. Similar replacement has also been reported in Tunisia (Jarraya & Bernard, 1971; Braham, 2005; Braham et al., 2010) but needs to be verified in other areas where *E. plotnikovi* is now established. Diapause in *Eurytoma plotnikovi* depends on both temperature and photoperiod (Tzanakakis et al., 1992) and this species survives the winter either inside the fruits that remain on the trees after harvest or in those that have fallen on the ground. The final-instar larvae of *E. plotnikovi* are able to tolerate the low temperatures, experienced mainly during December–February (Mohammadzadeh et al., 2017). In mid-spring, the larvae, which cause significant damage to the growing seeds, pupate inside the nut and adults emerge through a round hole that they bore in the shell (Fig. 4). The seasonal flight period reported in Tunisia starts at the end of April and continues into June (Braham, 2016). Doğanlar and Doğanlar (2010) reported that the parasitoid *Gugolzia karadagae* (Hymenoptera: Pteromalidae) is able to parasitize up to 17.5% of *E. plotnikovi* (Doğanlar & Doğanlar, 2010). However, this parasitoid was not found in other areas investigated by the same authors. Therefore, the absence of information on

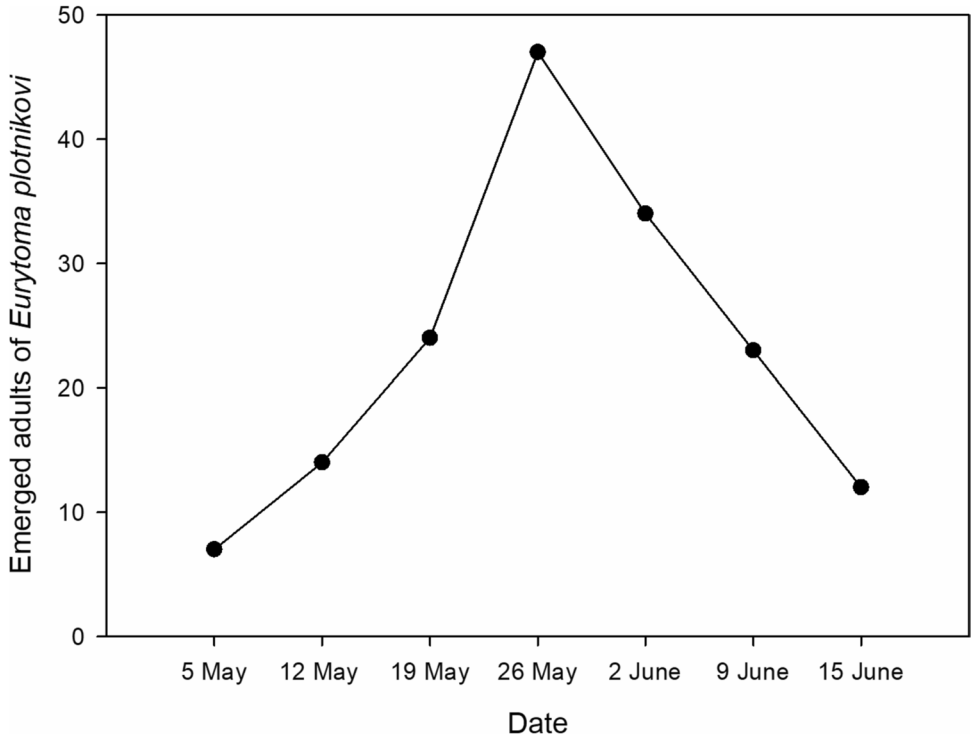

Fig. 1 Seasonal emergence (sum of five sampled areas) of *Eurytoma plotnikovi*

Fig. 2 Adult female (left) and adult male (right) *Eurytoma plotnikovi*

the interactions between *E. plotnikovi* and its natural enemies is a significant gap in our ability to develop biological control approaches for this pest.

The control of *E. plotnikovi* and others *Pistacia* pests in Sicily is generally based on the application of insecticide sprayings during late spring and July. Given the limited number of insecticides that can be used against this species in Europe (deltamethrin, lambda-cyhalothrin, etofenprox and those derived from natural products, such as spinosoids), and with authorizations that will be modified or revoked, further work is required to understand the life cycle and

Fig. 3 Forewing (left), and antennae (right) of male *Eurytoma plotnikovi*

Fig. 4 Bunch of pistachio fruit exhibiting effects of *Eurytoma plotnikovi* predation (above). Empty pistachio nut containing *E. plotnikovi* larva and seed remnants (bottom left). Mummified seed of *Pistacia vera* with round exit hole formed by emergence of adult *E. plotnikovi* (bottom right)

natural enemies of *E. plotnikovi* in more depth. One approach to reducing the presence of phytophagous insects is to bury any dropped fruit. However, this is not always possible in the study area because of the scattered presence of uncultivated pistachio trees, which supports the constant presence of *E. plotnikovi* in this region. In Sicily, growers use phytoiatric strategies in the most important areas of production, accentuating the alternation of production of pistachio groves through the removal of flowers during the

unloading years of production (Bonsignore, personal communication). Further investigations on other seeds of *Pistacia* species, such as *Pistacia lentiscus* and other Anacardiaceae are necessary to verify the presence of *M. pistaciae* and its possible interaction with *E. plotnikovi*.

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Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The author has no conflicts of interest to declare.

References

Arnone, E., Pumo, D., Viola, F., Noto, L. V., & La Loggia, G. (2013). Rainfall statistics changes in Sicily. *Hydrology and Earth System Sciences, 17*, 2449–2458.

Basirat, M., & Seyedoleslami, H. (2000). Biology of Pistachio seed wasp *Eurytoma plotnikovi* Nikoloskaya (Hym.: Eurytomidae) in Isfahan Province, Iran. *Journal of Science and Technology of Agriculture and Natural Resources, 4*(1), 137–147.

Bonsignore, C. P., Laface, V. L. A., Vono, G., Marullo, R., Musarella, C. M., & Spampinato, G. (2021). Threats posed to rediscovered and rare sage (*Salvia ceratophylloides*) by borer and seed feeders insect species. *Diversity, 13*(1), 33. https://doi.org/10.3390/d13010033

Braham, M. (2016). Contribution to a better understanding of flight pattern and egg-laying duration of the Pistachio seed wasp. Conference: XVI GREMPA Meeting on Almonds and Pistachios. *Options méditerranéennes*, *119*, 205–209.

Braham, M., Smiri, H., & Cherif, R. (2010). Geographic distribution and impact of *Eurytoma plotnikovi* Nik. (Hymenoptera: Eurytomidae) and *Megastigmus pistaciae* walk. (Hymenoptera: Torymidae), insects damaging pistachio nuts in Tunisia. *Options Méditerranéennes: Série A Séminaires Méditerranéens, 94*, 187–201.

Braham, M. (2005). Management of the pistachio seed wasp *Eurytoma plotnikovi* Nikolskaya (Hymenoptera, Eurytomidae) in Tunisia: Integration of pesticides sprays and other means of control. *International Pest Control, 47*(6), 319–324.

De Stefani, T. (1917). *Megastigmus ballestrerii*, a hymenopteron living in pistacio trees and turpentine tree in Sicily. *Bollettino del R Orto Botanico di Palermo, 4*, 101–131.

Doğanlar, M., & Doğanlar, O. (2010). Review of the species of *Gugolzia* Delucchi and Steffan (Hymenoptera: Pteromalidae) in Europe and Turkey, with description of new species. *Turkish Journal of Zoology, 34*(1), 23–34. https://doi.org/10.3906/zoo-0806-5

Doğanlar, M., Karadağ, S., & Mendel, Z. (2009). Notes on pistachio seed wasps from two locations in the east Mediterranean. *Phytoparasitica, 37*, 147–151.

FAOStat Statistical Database (2019). *Food and Agriculture Organization of the United Nations*. https://www.fao.org/faostat/en/#data/QC. Accessed 15 Dec 2019.

Izhaki, I. (1998). The relationships between fruit ripeness, wasp seed predation, and avian fruit removal in *Pistacia palaestina*. *Israel Journal of Plant Sciences, 46*(4), 273–278.

IBM Corp. (2015). Released: IBM SPSS Statistics for Windows, Version 23.0. IBM Corp. 2015.

Jarraya, A., & Bernard, J. (1971). Premières observations bioécologiques sur *Megastigmus pistaciae* en Tunisie. *Annales de l'Institut National de la Recherche Agronomique de Tunisie, 44*(3), 1–26.

Jarraya, A., & Helali, T. (1978). Contribution to the study of the insect fauna of pistachio. On the spatial distribution of *Megastigmus pistaciae* walk. (hym. Torymidae) and of *Eurytoma plotnikovi* Nik. (hym. Eurytomidae) in Tunisia. *Bulletin des Recherches Agronomiques de Gembloux, 13*(3), 215–252.

Li, J. X., Liu, Q. Z., Wang, X. Y., & Wang, J. Y. (2008). Major pests and their control of *P. chinensis*. *The Journal of Hebei Forestry Science and Technology, 4*, 102–103.

Longo, S., P. & Suma, P. (2011). First report of Eurytoma plotnikovi Nik. (Hymenoptera, Eurytomidae), a seed parasite

of pistachio, in Sicily (Italy). *Journal of Entomological and Acarological Research, Ser. II*(43), 333–336.

McNeely, J. (2001). Invasive species: A costly catastrophe for native biodiversity. *Land Use and Water Resources Research, 2*, 1–10.

Mohammadzadeh, M., Borzoui, E., & Izadi, H. (2017). Physiological and biochemical differences in diapausing and nondiapausing larvae of *Eurytoma plotnikovi* (Hymenoptera: Eurytomidae). *Environmental Entomology, 46*(6), 1424–1431. https://doi.org/10.1093/ee/nvx128

Monastero, S. (1958-59). Il più dannoso insetto del pistacchio (*Megastigmus pistaciae* Walker 1874) (Sin. Trogocarpus Ballesterri 1877). *Bollettino dell'Istituto di Entomologia Agraria e dell'Osservatorio di Fitopatologia di Palermo 3*, 107–138. (in Italiano).

Mourikis, P. A., Tsourgianni, A., & Chitzanidis, A. (1998). Pistachio nut insect pests and means of control in Greece. *Acta Horticulturae, 470*, 604–611.

Nieves-Aldrey, J. L., Nieves, M. H., & Gómez, J. F. (2008). Larval morphology and biology of three european species of Megastigmus (Hymenoptera, Torymidae, Megastigminae) parasitoids of gall wasps, including a comparison with the larvae of two seed-infesting species. *Zootaxa, 1746*, 46–60.

Nikol'skaya, M. N. (1935). Fista'skove semeedy i ich parazity (Hymenoptera, Chalcididae). *Zashchita Rasteniy, Leningrad, 1935*(1), 83.

Rice, R. E., & Michailides, T. J. (1988). Pistachio seed Chalcid, *Megastigmus pistaciae* Walker (Hymenoptera: Torymidae), in California. *Journal of Economic Entomology, 81*, 1446–1449. https://doi.org/10.1093/jee/81.5.1446

Roques, A., Copeland, R. S., Soldati, L., Denux, O., & Auger-Rozenberg, M. A. (2016). Megastigmus seed chalcids (Hymenoptera, Torymidae) radiated much more on Angiosperms than previously considered. I- description of 8 new species from Kenya, with a key to the females of Eastern and Southern Africa. *ZooKeys, 585*, 51–124. https://doi.org/10.3897/zookeys.585.7503

Roques, A., Markalas, S., Roux, G., Pan, Y., Sun, J., & Raimbault, J. P. (1999). Impact of insects damaging seed cones of cypress, *Cupressus sempervirens*, in natural stands and plantations of southeastern Europe. *Annals of Forest Science, 56*(2), 167–177. https://doi.org/10.1051/forest:19990208

Roques, A., & Skrzypczyńska, M. (2003). Seed-infesting chalcids of the genus Megastigmus Dalman, 1820 (Hymenoptera: Torymidae) native and introduced to the West Palearctic region: Taxonomy, host specificity and distribution. *Journal of Natural History, 37*(2), 127–238. https://doi.org/10.1080/713834669

Rousou, M., Parés, A., Douché, C., Tengberg, M., et al. (2021). Identification of archaeobotanical Pistacia L. fruit remains: Implications for our knowledge on past distribution and use in prehistoric Cyprus. *Vegetation History and Archaeobotany*. https://doi.org/10.1007/s00334-020-00812-z

Rousse, P., & Reynaud, P. (2022). First report of the pest pistachio-seed wasp *Eurytoma plotnikovi* (Hymenoptera: Eurytomidae) in France. *EPPO Bulletin, 52*, 456–459.

Seebens, H. (2019). Invasion ecology: Expanding trade and the dispersal of alien species. *Current Biology, 29*(4), 120–122. https://doi.org/10.1016/j.cub.2018.12.047

Sigmaplot 13.0. (2018). Systat Copyright © Systat Software.

Sileshi, G. W., Gebeyehu, S., & Mafongoya, P. L. (2019). The threat of alien invasive insect and mite species to food security in Africa and the need for a continent-wide response. *Food Security, 11*, 763–775. https://doi.org/10.1007/s12571-019-00930-1

Taghizadeh, F. (1953). *Important pests of Pistachio trees and method of control*. U.S. Overseas Mission-Iran Pest control Branch Ministry of agriculture, Theran.

Tang, M., Zhang, P., Zhang, L., Li, M., & Wu, L. (2012). A potential bioenergy tree: *Pistacia chinensis* Bunge. *Energy Procedia Part B, 16*, 737–746.

Tian, S. B., Qin, X. R., & Zhao, X. (1994). Infestation characteristics of the larvae of *Eurytoma plotnikovi* and their control. *Plant Protection China, 20*(2), 15–16.

Tzanakakis, M. E., Veenendaal, R. L., & Veerman, A. (1992). Effects of photoperiod and temperature on the termination of diapause in the univoltine seed wasp *Eurytoma plotnikovi*. *Physiological Entomology, 17*, 176–182.

Uygun, N. (1994). *Bahçe Bitkileri Zararlıları [Insect pests of horticulture]. No. 26. Adana*. Ç.Ü. Agriculture Faculty Press. (Turkish, with English abstract).

Venette, R. C., & Hutchison, W. D. (2021). Invasive insect species: Global challenges, strategies & opportunities. *Front Insect Science, 1*, 650520. https://doi.org/10.3389/finsc.2021.650520

Walker, B. H., & Steffen, W. (1997). An overview of the implications of global change for natural and managed terrestrial ecosystems. *Conservation Ecology*, *1*(2). http://www.consecol.org/vol1/iss2/art2. Accessed June 2023

Zerova, M. D. (2017). A key to the Palaearctic genera of the family Eurytomidae (Hymenoptera, Chalcidoidea), with review of trofic associations. *Ukrainska Entomofaunistyka, 8*(2), 41–47.