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**PORTRAIT OF *MARCHALINA HELLENICA* GENNADIUS (HEMIPTERA: MARGARODIDAE), THE MAIN PRODUCING INSECT OF PINE HONEYDEW-BIOLOGY, GENETIC VARIABILITY AND HONEY PRODUCTION**

**Çam Salgı Balını Üreten Esas Böcek Olan Çam Koşnili *Marchalina hellenica* Gennadius (Hemiptera: Margarodidae)'un Tanımlanması, Genetik Çeşitliliği ve Bal Üretimi**

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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**Key words:** *Marchalina hellenica*, Greece, Turkey, honeydew, pine tree.

**Anahtar kelimeler:** *Marchalina hellenica*, Yunanistan, Türkiye, Basra şebnemi, çam ağacı.

**ABSTRACT**

*Marchalina hellenica* is the main honeydew producing insect of pine trees. It is endemic to Greece and Turkey and introduced to the Italian island of Ischia. It has one generation per year and the adult females appear on the trees only after mid March. Studies on the genetic structure of the insect show that the low genetic variability may be due to the fact that it can not be dispersed long distances in correlation with the parthenogenetic reproduction. The amount of honeydew produced by the insect varies over the year and mainly depends on the size and age of the nymphs.

**Geographic distribution**

*Marchalina hellenica* is the main honeydew producing insect of pine trees. It resides mainly on *Pinus halepensis* (allepo pine) and *P. brutia* (calabrian pine) (Bodenheimer, 1953; Nikolopoulos 1959, 1964; Kailidis, 1965; Selmi, 1983; Gürkan and Boşgelmez, 1989; Pollini, 1998) and rarely on *P. pinea*, *P. nigra*, *P. maritima* and *P. silvestris* (Nikolopoulos 1964, 1965; Avtzi, 1985; Pollini, 1998). It is endemic to Greece and Turkey and introduced species in the island of Ischia in Italy (Kailidis, 1965; Nikolopoulos, 1965; Santas, 1979, 1983; Tranfaglia and Tremblay, 1984; Fimiani and Solino, 1994; Priore *et al.*, 1996; Pollini, 1998). Only recently it has been announced the establishment of *M. hellenica* on *Abies cephalonica* trees in Helmos Mountain in Greece (Bacandritsos *et al.*, 2004). A different species (*M. caucasica*) but with great similarities to *M. hellenica* has also been described in Caucasus Mountain by Hadzibeyli (1969).

**Biology and habitat**

*Marchalina hellenica* (initially described as *Monophlebus hellenicus*) belongs to the family Margarodidae (Marchalinidae by Koteja, 1996), of the Hemiptera-Coccoidea. It has one generation per year and the adult females appear on the trees only after mid March. Given that the male is rare, it has been suggested that the insect is produced mainly parthenogenetically and rarely bisexually (Nikolopoulos, 1964, 1965; Pollini, 1998; Erlinghagen, 2001). *M. hellenica* has three female nymphal instars (Gounari *et al.*, 2002a, 2002b, Gounari, 2006). The 1<sup>st</sup> and 2<sup>nd</sup> instar nymphs have antennae with 6 segments and are present from June to October, while the 3<sup>rd</sup> instar nymphs have antennae with 9 segments and the adults 11 segments (Gounari, 2006). The insects live in crevices, under the folds of the pine bark and are covered with a white cotton-like material that excretes (Fig. 1). The overall body colour is light yellow, and the adult females are apterous and

have no mouth parts (Fig. 2) while the mouthparts of the nymphal stages are very long, almost three times their body size (Fig. 3) and coiled inside their body when the insects are not feeding (Gounari, 2006). Males are apterous, elongated, light yellow in colour with very long antennae (Fig. 4) (Hatjina *et al.*, 2002, Hodgson and Gounari, 2006) and have 4 immature stages instead of 3 (Hodgson and Gounari, 2006).



**Fig. 1. Nymphs of *M. hellenica* in a row**



**Fig. 2. Adult female. Note the absence of mouthparts between the first pair of legs**



**Fig. 3. Nymph with its long mouthparts**

According to Gounari *et al.*, (2002a, 2002b) the 3<sup>rd</sup> instar nymphs undergo their last ecdysis from the end of March to almost the end of April, and then emerge as adults. The ecdysis can last for up to two days and during this phase the insect discards its integument together with its mouthparts. When the adult females appear on trees, they are usually looking for a new site to lay their eggs. *M. hellenica*

adults can move quite a distance in order to oviposit.



**Fig. 4. The male**

They have been observed moving to different branches or even to different trees which help to disperse the insects and assists in the colonization of new habitats. Each female can lay an average of 222 eggs, protected in a cotton wool ovisac which encloses the whole body of the insect (Fig. 5). Adult females can live for about one month and eggs need almost one month in order to hatch. During this time period one can simultaneously observe behavior of the adults, eggs and some of the new nymphs. The nymphs are called 'crawlers' in recognition of their activity as they search for a place on the bark to settle. Their body size is very small (about 1 mm) and they are very vulnerable to climatic conditions. At the time they undergo their second ecdysis they are almost double in size.



**Fig. 5. Adult female during oviposition**

Populations of *M. hellenica* that live on fir trees show some differences in their life cycle, especially on the number of eggs lay, which is very low compared to populations on pine trees (Bacandritsos *et al.*, 2004). Of course it has to be mentioned that fir trees grow in higher altitude than pine trees. The altitude difference probably influences the insect's habitat preferences. It should also be noted that the establishment of *M. hellenica* in fir trees is after anthropogenic intervention and

has not happened naturally during the last two centuries of coexistence.

### Genetic variability

The question that arises is whether geographically distant populations of the species are genetically divergent. The results of a study by Margaritopoulos *et al.* (2003) failed to reveal a specific marker enabling discrimination between the populations examined. However, data analysis, in the same study, for genetic polymorphisms showed a degree of both intra- and interpopulation genetic variation. The intrapopulation variation observed was associated with host type and region of origin. Preliminary research on the genetic structure of *M. hellenica* in Greece, using sequencing analysis, also showed low genetic variability between the populations studied (Bouga *et al.*, 2005). On the other hand, data from sequencing analysis of samples from *M. hellenica* from Greece and Turkey show that some populations can be discriminated (Bouga *et al.*, paper in preparation).

### Honey production

*M. hellenica* feeds on sap that it sucks from the tree and it produces a transparent, and at times, pinkish and reddish sweet droplets of honeydew (Fig. 6). Honeydew is the excess pine sap that the insects provide and it is the raw material collected vigorously by honey bees to be converted to pine honey. Pine honey represents almost 65% of the annual honey production in Greece (Santas, 1983; Thrasyvoulou and Manikis, 1996) and about 50% of the annual honey production in Turkey. Given the high percentage of pine honey in the annual honey production in both countries it is easy to understand the importance of the insect for the beekeeping industry. Large numbers of bee colonies are moved to pine forests during periods of heavy honeydew secretions, and the density of colonies can exceed the 225 hives/ Km<sup>2</sup> (Xidias, 1975).



**Fig. 6. Different colour of honeydew drops**

The amount of honeydew produced by the insect is different across various times of the year and

mainly depends on the size and age of the nymphs. Given that the adults have no mouthparts, they do not feed and eventually stop producing any honeydew. Additionally, when the 1<sup>st</sup> instar nymphs are very small, no honeydew is produced, at least in quantities large enough to be collected by honey bees. However, during summer, the body size of the nymphs increase and as well as their ability to suck and excrete honeydew improves. The amounts of honeydew are high enough for honey production after mid August, and this is known to beekeepers as the first 'honeydew flow'. During September, and while the insects are gradually developing into 2<sup>nd</sup> instar larvae, they stop feeding for a period of a few days. However, as not all of the insects undergo the ecdysis at the same time, honeydew production never completely stops but rather decreases slowly only to increase again after about 15-20 days (Gounari *et al.*, 2002; Gounari, 2006). This is known as the 'second honeydew flow'. From that time on honeydew production is continuous till early spring, but honey bees are collecting it only when weather conditions permit it (usually till late November). Nevertheless, it is not a rare phenomenon to see honey bees collecting honeydew in a sunny winter day, even when the environment contains snow! Early spring, when the temperatures are rising again, is time for the 'third honeydew flow' which ceases when the insects become adults. From the above description it is evident that the main honeydew flow is from September to November, although times and dates can vary considerably due to geographic areas and climatic conditions (Kailidis, 1965; Xidias, 1975; Santas, 1979, 1983; Selmi, 1983; Avtzis, 1985; Gürkan and Boşgelmez, 1989; Gounari, 2006).

Honeydew secretions are believed to be more stable over the years compared to various types of nectar flow and although during the recent years, extreme high temperatures have caused some problems in this stability, as overall, honeydew is the biggest honey source at least in Greece. Above that, pine forests can accommodate large numbers of honey bee colonies with no fear of extra-exploitation or competition and this is another great advantage for the beekeeping industry.

### Honeydew differences from pine honey

Pine honey has specific volatile, chemical and taste characteristics (Thrasyvoulou and Manikis, 1996; Sabatini *et al.*, 2001; Tananaki, 2004) which can be differentiated easily from other types of honey. Its

high viscosity, low sweetness, high mineral contents, together with its very low tendency to crystallize, makes it a valuable and good prize product for consumers showing preference to forest honeys. The main difference in honey produced from *M. hellenica* from pine trees and fir trees is the diastase and HMF content (both are higher in honey from fir trees, but within the EC requirements) (Bacandritsos *et al.*, 2004).

### Is *M. hellenica* a pest of pine trees?

As *M. hellenica* feeds on sap that it sucks from the trees, it is also considered as a pest (Çanakçıoğlu and Mol, 1998). However, the question which rises is: is this 'pest' harmful to pine trees and if yes to what extend? Not many researchers have tried to answer this question. In Italy, *M. hellenica* is considered as a dangerous pest (Fimiani and Solino, 1994) and Yeşil *et al.* (2005) showed that there is an effect of the parasitism of *M. hellenica* on the growth of pine trees but not that it 'kills' the trees. On the other hand, a study by Zafiri *et al.*, (2007), showed that the insect's stylet moves vertically through the tissues of the pine tree to the layer of phloem, where it moves in parallel without reaching the xylem. No widening of the initial bark cracks are observed where the insects are established. Further research is necessary to explore the possibility that parasitism of *M. hellenica* can considerably influence the development of the trees. So as further research is necessary on the possibility and the magnitude that the parasitism of *M. hellenica* can influence considerably the development of the trees, it is of great importance to protect the pine forests and to search for scientific evidence. The relationship between pine forests and *M. hellenica* is long and complicated especially so since other insects affect the development of the trees as do socio-economic parameters.

### Other sap sucking insects on pine

Apart from *M. hellenica*, a number of other sap sucking insects have been found on the barks of pine trees (Hatjina *et al.*, 2002). At least one aphid and two other insects have been observed to produce honeydew but in very small quantities. One of the insects is the *Phenacoccus yerushalmi* Ben Dov (Fig. 7) (Ben-Dov *et al.*, 2006) and the second is the *Palaeococcus* sp. (probably *P. fuscipennis* Burm (Fig. 8) (Hatjina *et al.*, in preparation). Both insects have been found in Greece and Turkey as well as in other countries of Mediterranean basin.



Fig. 7. *Phenacoccus yerushalmi* Ben Dov



Fig. 8. *Palaecoccus* sp. nymphs while secreting honeydew

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### GENİŞLETİLMİŞ ÖZET

**Amaç:** Bu derleme makalenin amacı salgı balı üretiminde esas rol oynayan ve Türkiye ve Yunanistan'da doğal olarak bulunan böcek olan *M hellenica*'nın tanımlanması, yayılışı ve biyolojisini kısaca açıklamaktır.

**Tartışma ve Sonuç:** *M hellenica* çam ağaçlarında daha çok *Pinus helepensis* ve *P brutia* üzerinde yaşarlar. *M hellenica* az da olsa Gökmar ve Ladin ağaçlarında bulunur Bodenheimer, 1953; Nikolopoulos 1959, 1964; Kailidis, 1965; Selmi, 1983; Gürkan ve Boşgelmez, 1989; Pollini, 1998). Bunun yanında başka bir tür olan ve *M hellenica*'ya oldukça benzeyen *M caucasica* Kafkas dağlarında belirlenmiştir (Hadzibeyli 1969). *M hellenica* çam balı üretiminde ana etken olan bir böcektir. Bu böcek Mart ortalarından sonra çam ağaçlarında

görölmeye başlar ve her yıl bir nesil üretirler. Ağaç kabuklarının altında ve küçük oyuklarda salgıladığı pamuk gibi bir örtü ile kendini saklar. Genel olarak bu böcekler ergin durumda iken açık sarı renkte olup erginlerinde ağız parçaları yoktur. Ergin olmayan böceklerde ağız parçaları çok uzun, vücudun 3 katı civarında ve beslenmediği zamanlarda vücudun iç kısmında kıvrılmış olarak bulunur (Gounari 2006).

*M hellenica*'da genetik varyasyon oldukça düşük olmasına rağmen son yıllarda Türkiye ve Yunanistan'dan alınan numunelerde popülasyonların ayırt edilebileceği görülmektedir (Bouga ve diğ. Yayınlanmamış). *M hellenica* çam ağaçlarında emerek beslenir, pembemsi ve kırmızımsı tatlı salgı damlacıkları çıkarır. Bu damlacıklar bal arıları tarafından çam balına dönüştürülür. İlk çam ana salgı akımı Ağustos ortasından sonra başlar ve ikincisi Eylül ayında başlar ve Kasım ayı sonlarına kadar devam eder. Erken ilkbaharda ise üçüncü ana salgı akımı başlar. Bu tarihler coğrafik bölge ve iklim koşullarına göre değişiklik gösterebilir (Kailidis, 1965; Xidias, 1975; Santas, 1979, 1983; Selmi, 1983; Avtzis, 1985; Gürkan ve Boşgelmez, 1989; Gounari, 2006). Salgı akımının ve böceğin yoğun olduğu bölgelerde arılar çok sayıda koloniyi fazla rekabet endişesi olmadan kullanarak üretim yapabilirler.

Çam balı kendine özgü kokusu, kimyası ve tat özellikleri ile ayrılır. Koyu kıvamlı, yüksek mineral içeriği, tatlılık oranı düşük, donmaya veya kristalize olmaya az meyilli olması nedeni ile orman balı tercih eden tüketiciler için değerli ve iyi bir ücretle talep edilmektedir.

*M hellenica* bazı araştırmacılar tarafından zararlı olarak (Çanakçıoğlu ve Mol 1998) tanımlanmış, fakat böceğin çam ağaçlarını öldürmediği rapor edilmiştir (Fimiani ve Solino 1994, Yeşil ve diğ. 2005). Son yıllarda Zafiri ve diğ. (2007) tarafından yapılan araştırmalarda böceğin çam ağaçlarında floem dokusuna doğru ksilem'e ulaşmadan dikine ve paralel olarak giderek ağacın kabuklarını fazla derinleştirmedeği tespit edilmiştir. Sonuçta *M hellenica* ve çam ağaçları arasındaki ilişki uzun bir geçmişe dayanan, sosyo-ekonomik parametreleri de içeren karmaşık bir ilişkidir.

Çam ağaçlarında *M hellenica* dışında az da olsa salgı balı üretiminde etken başka böcekler de bulunmaktadır.