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First Record of Native Egg Parasitoid, *Anastatus bifasciatus*, on *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae) Eggs in Türkiye

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ABSTRACT
Halyomorpha halys (Stål, 1855) (Hemiptera: Pentatomidae), the brown marmorated stink bug (BMSB), is an alien invasive pest that originated from South Asia (China, Japan, Taiwan, Korea) Many studies have shown that the number of invasions by pests has increased significantly in the last 5 centuries and the reason for this situation is directly related to human activities. *Halyomorpha halys* is considered to be a dangerous pest insect species due to economic losses in agricultural areas of the countries where it is distributed. It has been observed that the pest can have 1–2 generations per year and each egg mass contains of an average of 28 eggs and, furthermore, causes significant losses in many crops such as bean, hazelnut and pear. This study was conducted between July and August in 6 different areas and a total of 18 egg masses that were thought parasitized were collected from grape vine, tree of heaven, kiwi and hazelnut trees) BMSB infested fields and kept in tubes to determine if they had parasitoids. Emerged parasitoids were fed with the 10% honey water solution and bred in tubes. In this study, *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) was recorded as the first parasitoid of Halyomorpha halys in Türkiye.

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Halyomorpha halys (Stål, 1855) (Hemiptera: Pentatomidae) Yumurtalarında Yerli Yumurta Parazitoiti olarak *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae)'un Türkiye'de ilk Kaydı

ÖZET
Halyomorpha halys (Stål, 1855) (Hemiptera: Pentatomidae), Kahverengi Kokarca (BMSB), Güney Asya (Çin, Japonya, Tayvan, Kore) kökenli istilacı bir zararlıdır. Birçok çalışma ve araştırma, zararlıların istilalarının son 5 yüzyılda önemli ölçüde arttığını ve bu durumun nedeninin doğrudan insan faaliyetleri ile ilgili olduğunu göstermektedir. *H. halys*, yayıldığı ülkelerin tarım alanlarındaki ekonomik kayıplar nedeniyle tehlikeli tarımsal zararlı böcek türleri kategorisinde yer almaktadır. Zararlının yılda 1-2 döl verebildiği ve her yumurta paketinin ortalama 28 yumurtadan oluştuğu ve ayrıca fasulye, fındık gibi birçok üründe önemli kayıplara neden olduğu görülmüştür. Çalışma Temmuz-Ağustos ayları arasında 6 farklı alanda yürütülmüş ve parazitlendiği düşünülen toplam 18 yumurta paketi bulaşık olan üzüm bağı, kokar ağaç, kivi ve fındık bahçelerinden toplanarak parazitoit olup olmadığına bakmak için tüplere konulmuştur. Parazitli yumurtalardan çıkan parazitoitler koloni devamlılığını sağlamak için %10 su-bal solüsyonu ile beslenmiştir Bu çalışmada Türkiye'de *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae), *Halyomorpha halys* üzerinde kaydedilen ilk parazitoid olarak rapor edilmiştir.

Entomoloji

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INTRODUCTION

Halyomorpha halys, which is a polyphagous pest native to Asia, was first detected in Istanbul in Türkiye by Cerci & Kocak in 2017. In other studies conducted in the same year and it was detected in Kemalpaşa, Hopa and Arhavi districts of Artvin, in 2018 Borcka district in Artvin and Fındıklı and Ardeşen districts in Rize, then in 2019 in Artvin city centre, Trabzon city centre, Esiroglu district, Akcaabat district and Tirebolu district in Giresun, and the pest continues to spread rapidly (Gokturk & Tozlu, 2019).

Halyomorpha halys adults and nymphs damage host plants by piercing their surface and injecting digestive enzymes or sucking fluids (Rice et al., 2014). Scars, pits, deformities and colour changes on fruit surfaces occur as a result of the pest feeding. These crops are not desirable due to losses in their quality (Hedstrom et al., 2014). Studies have shown that *H. halys* is a polyphagous pest and their population density reaches high levels on some plants such as kiwi, hazelnut (Ak et al., 2018), apple (Morrison et al., 2016), pear (Maistrello et al., 2017), peach (Blaauw et al., 2015), bean, tomato, pepper (Nielsen et al., 2011), corn, grape (Smith et al., 2014), walnut, blackberry, catalpa (*Catalpa bignonioides*), maple and weeds. Considering the current distribution areas of this pest and climatic suitability, the Black Sea Region's agricultural production is under threat in Türkiye. Additionally, considering climate change data on a global scale, it is likely that the other regions are at risk as well (Kistner, 2017). Reports from North America indicate that *H. halys* could potentially be responsible for 70% of economic loss in fruits and vegetables. In addition, the overwintering aggregation buildings cause fear and anxiety in people. In a particularly heavy infestation (26205 adult) brown marmorated stink bugs were collected inside a house in 181 days (Inkley, 2012).

After a long period of mating and preoviposition, the females lay their eggs from July to August (Funayama & Zoology, 2002; Hoebeke & Carter, 2003). Females typically lay clusters of 28 eggs on the underside of leaves (Kawada et al., 1983; Nielsen et al., 2008). In temperate climates the number of eggs laid peaks in July and ends at the end of August. The eggs laid by the females on the underside of the leaves are initially light green and turn white before hatching (Figure 1).

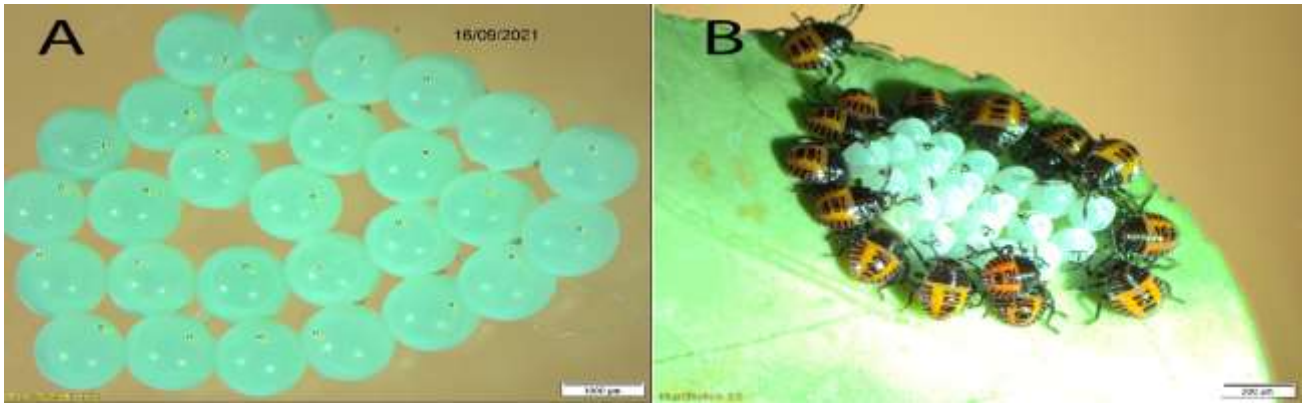

Figure 1. *Halyomorpha halys* eggs mass (A) Two days old *Halyomorpha halys* eggs (B) Newly emerged *H.halys* instars

Şekil 1. Halyomorpha halys yumurta paketi (A) 2 günlük *Halyomorpha halys* yumurtası (B) Nimf çıkışı olmuş yumurta paketi

Halyomorpha halys has 5 nymphal stages. The first nymphs have a black head, red eyes and reddish bodies and emerge 3–6 days after the eggs are laid and feed on their eggs to ingest gut symbionts (Taylor et al., 2014). The first instars moult into the second instars 3–5 days later and disperse to feed on plants. After 12–13 days, they reach the third instars that have a brown colour and the fourth and fifth instars emerge after 20 and 27 days later respectively (Hoebeke & Carter, 2003; Haye et al., 2014).

When the pest invades a new area, the lack of natural enemies eventually leads to population outbreaks. Taking into account that the pest is an invasive species that spreads rapidly, some control methods (mechanical and cultural control) can be difficult to suppress the pest. Additionally, many chemical compounds have been tested on H. halys, and some of them are applied in the field. However, despite intense chemical applications in many countries, no significant success has been achieved. (Leskey et al., 2012; Kuhar & Kamminga, 2017). Many pesticide applications are required because the pest is active 8–9 months of the year in natural areas, and 5–6 months in agricultural

areas (Haye et al., 2014), and pesticide half-lives are short. In addition to being a problem for the environment and human health, this method is not sustainable because of its high costs. Therefore, biological control can be the best option in terms of permanent, economic, eco-friendly and long term solutions.

Egg parasitoids are the most effective biological control method against the brown marmorated stink bug (Yang et al., 2009). BMSB eggs have been parasitised by Trissolcus japonicus (Ashmead) (Hymenoptera, Scelionidae), Trissolcus cultratus (Mayr) (Hymenoptera: Scelionidae), Anastatus bifasciatus, Trissolcus semistriatus (Nees) (Hymenoptera: Scelionidae), Trissolcus scutellaris (Thomson) (Hymenoptera: Scelionidae) and Telenomus chloropus (Thomson) (Hymenoptera: Telenominae) in the field and under laboratory conditions in the regions where the pest has spread (Yang et al., 2009; Lee et al., 2013; Haye et al, 2015), with parasitism rates for Trissolcus japonicus as high as 80% in China (Yang et al., 2009).

The most common egg parasitoid identified from BMSB eggs in Europe is Anastatus bifasciatus (Abram et al., 2017; Federico et al., 2017; Bout et al., 2021) and this species has potential to be mass reared for biological control (Haye et al., 2015; Federico et al., 2017; Stahl et al. 2019; Sabbatini-Peverieri et al., 2020). Moreover, while only a few native egg parasitoid species can reach adulthood on viable brown marmorated stink bug eggs, Anastatus bifasciatus is also one of them (Haye et al., 2015, Roversi et al., 2016). For this reason, A.bifasciatus can be considered a promising native candidate for augmentative biological control. On the other hand, its wide host range (around 50 hosts in the orders Hemiptera and Lepidoptera) can cause debate about the mass release of the parasitoid.The aim of this study is to detect native egg parasitoids of Halyomorpha halys in Türkiye.

MATERIAL and METHOD

Survey Studies

To detect egg parasitoids of the pest, BMSB eggs were collected in the hazelnut, tree of heaven, kiwi and corn areas in Artvin, Borçka, Hopa, Kemalpaşa, Arhavi, Rize, Fındıklı, Pazar, Ardeşen, Samsun, Terme, especially from areas where the chemicals are not applicated, survey studies were conducted every week from the beginning of July to the end of August.

Storing of *Halyomorpha halys* Eggs and Rearing Adult Parasitoids

H. halys egg masses found in the hazelnut, tree of heaven, kiwi and corn fields from the beginning of July to the end of August were taken together with the leaves, placed in 50 ml centrifuge tubes, covered with the fine mesh of an insect net, and then brought to the laboratory and setting in 50*50 cm metal insect cages made by us for this purpose (Figure 2). Egg masses were kept at 16:8 h L:D photoperiod, 25 °C ± 2, and 70% RH (Cira et al., 2018) (Figure 2) and checked twice a day to determine the emergence of parasitoids. The emerged parasitoids was recorded daily and wasps were placed in 70 ml glass test tubes that were covered by an insect net. The first emerged wasps were placed to 80% alcohol and stored at -20 °C prior to being sent for identification and the others were fed with 10% honey solution once every two days.

Figure 2. 50 ml centrifuge tubes (A,B), parasitized eggs (C) and 50x50 cm *Halyomorpha halys* cages (D)
Şekil 2. 50 ml santifirüj tüpü (A,B) parazitlenmiş yumurta paketi (C) ve 50x50 *Halyomorpha halys* kafesleri (D)

Identification of the Parasitoid

The morphological identification of the specimens was made with the aid of a stereomicroscope (Olympus SZ61) by the entomologists at the research institute by using the identification key of (Peng et al.,2020) in the "plazi.org" taxonomic treatments database (Figure 3).

The egg masses that were collected from the field were kept in the lab and parasitoids emergence was observed following the second day. Parasitoids were put in 50 ml test tubes in 80% alcohol to Elijah J. Talamas for molecular identification and the specimens were confirmed *Anastatus bifasciatus*, again using the characters in (Peng et al,2020).

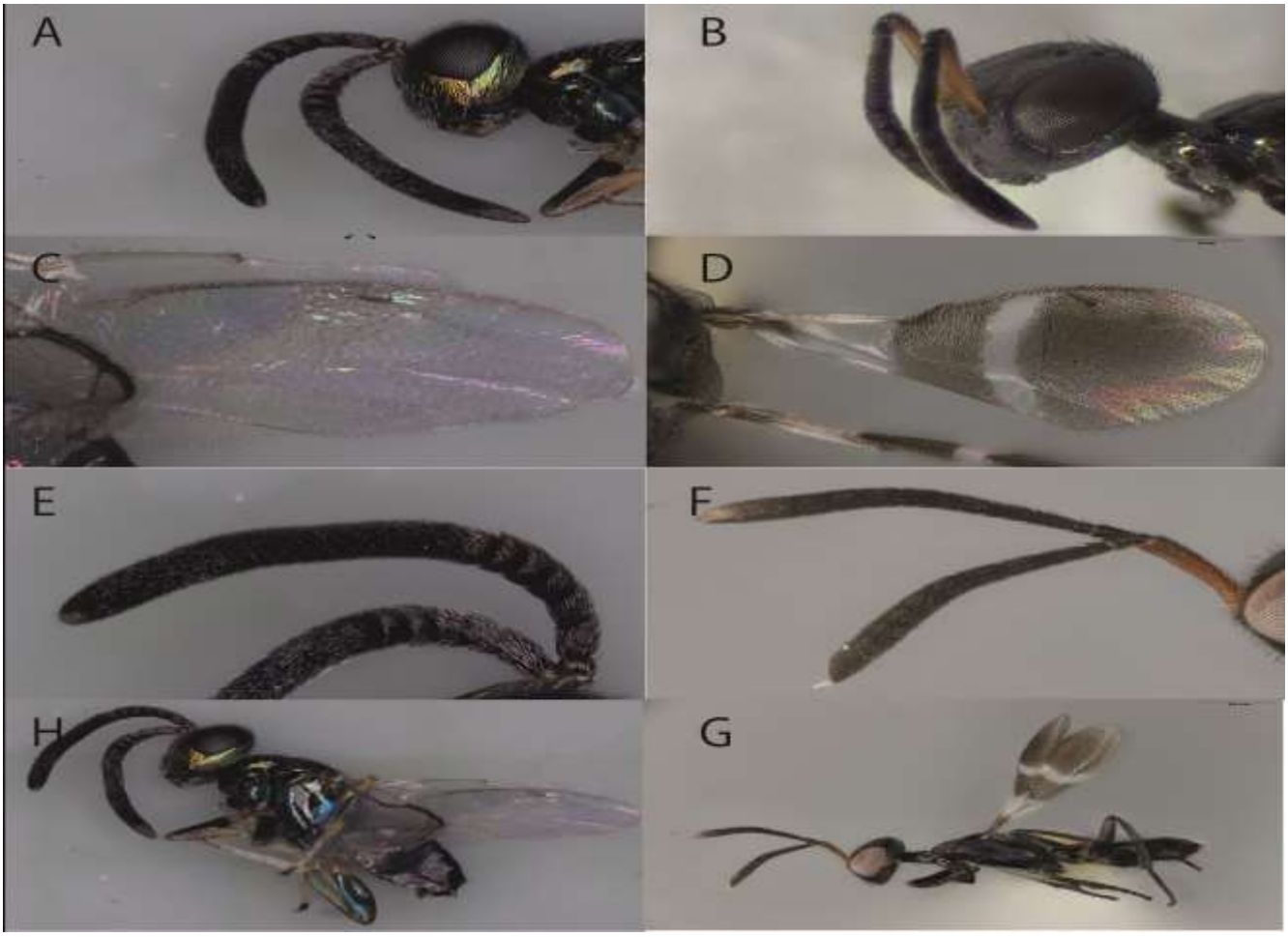

Figure 3. *Anastatus bifasciatus* Adult (A) Male Head, (B) Female Head , (C) Male Wing, (D) Female Wing, (E) Male Antennae, (F) Female Antennae, (H) Male Adult , (G) Female Adult

Şekil 3. Anastatus bifasciatus Ergini (A) Erkek Başı, (B) Dişi Başı, (C) Erkek Kanadı, (D) Dişi Kanadı, (E) Erkek Anteni, (F) Dişi Anteni, (H) Erkek Ergin Birey, (G) Dişi Ergin Birey

RESULTS

18 egg masses of the pest were collected during the survey studies that were conducted from the beginning of July to the end of August in Artvin, Borçka, Hopa, Kemalpaşa, Arhavi, Rize, Fındıklı, Pazar, Ardeşen, Samsun and Terme districts, however, only 4 of these egg packages were determined to be parasitized. One of these masses was found in Muratlı in Borçka, one in Arhavi, and two in Tekkeköy in Samsun (Table 1). Due to the height of the trees in the Black Sea Region, relatively short plants were preferred, and this was restricted to finding more egg masses.

During the survey, one parasitized egg mass was detected on cherry laurel (*Prunus laurocerasus*) in the first week of august in Gelemen, Tekkeköy/SAMSUN (41,232460, 36,506128) , and another egg mass was detected in the vineyard in the same area (41,229216, 36,502072). On the other hand, there was no emergence from the egg masses collected from tree of heaven in Arhavi (41,339088, 41,295133) and Borçka (41,479124, 41,711898). The egg masses were dissected to see if they are parasitized by a parasitoid, and it was observed that there was some embryo development in total 7 eggs in both masses however they could not reach the adults (3 embryos in Arhavi sample, 4

embryos in Borçka sample). Emerged parasitoids were identified as *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) and this was recorded as the first natural egg parasitoid detected against *Halyomorpha halys* in Türkiye.

Each egg masses consisted of 28 eggs and all of these were parasitized and hatched. Parasitoid emergence continued for almost 3 weeks, and during this period males and females' photos were taken and the emergence of the parasitoids were recorded. It was observed that the females emerged earlier than the males. 5 male and 23 female wasps emerged from the egg mass obtained from the coordinates 41,232460, 36,506128 (Tekkeköy), and 6 male and 22 female wasps emerged from the egg mass obtained from the coordinates 41,229216, 36,502072 (Tekkeköy).

Table 1. Detected parasitized Halyomorpha halys egg masses and locations where they were found
Çizelge 1. Tespit Edilen Parazitli Halyomorpha halys Yumurta Paketleri ve Bulundukları Yerler

City	District	The number of parasitized egg masses	Date	Coordinates (x,y)	Species
ARTVİN	Arhavi	1	12/07/2021	41,339088 41,295133	No emergence
ARTVİN	Borçka	1	13/07/2021	41,479124 41,711898	No emergence
SAMSUN	Tekkeköy	1	19/08/2021	41,232460 36,506128	*Anastatus bifasciatus*
SAMSUN	Tekkeköy	1	19/08/2021	41,229216 36,502072	*Anastatus bifasciatus*

DISCUSSION

Anastatus species are commonly encountered parasitoids of insects in the orders Hemiptera, Lepidoptera, Blattodea, Orthoptera and Mantodea (Narasimham & Sankaran 1982; Askew, 2005; Stahl et al., 2018). They are also tested and used on different pests for biological control in Europe (Stahl et al., 2018), and, *A. bifasciatus* is among the few native egg parasitoid species that can develop on live *H. halys* eggs (Haye et al., 2015), and is considered a potential candidate for augmentative biological control against *H. halys*. *Anastatus* has been used successfully in augmentative biological control against *H. halys* and *Tessaratoma papillosa* (Dru.) (Heteroptera: Tessaratomidae) in peach orchards and woodlands in China, and the goal is to reach more than 50% parasitism rate (Huang et al., 1974; Hou et al., 2009). Additionally, *Anastatus* sp. has been released against the first and second generations of *H. halys* in Pekin and the rates of the parasitism were 64.7% and 52.6%, respectively (Hou et al., 2009). In a 3-year study that was conducted to evaluate the effect of egg parasitoids on *H. halys* in Northern Italy, 1,826 *H. halys* egg masses were collected and the parasitism rate of *Anastatus bifasciatus* was found to be 12% in 2016 and 2017, and 21% in 2018. (Stahl et al., 2018). Therefore, suppressing the pest's spreading rate can be possible if some precautions can be taken to protect the wasp's population in nature. Besides, *Anastatus bifasciatus* can be reared and released to increase the population and have a higher parasitism rate. Nevertheless, when its wide host range is considered, host choice tests should be conducted to prevent non-target effects of the parasitoid. Lastly, similar survey studies should be conducted in the same and different areas to detect if there are more native natural enemies in Türkiye. Moreover, the study can shed light on future studies.

The study will help to mass-rear *A. bifasciatus* in the Agricultural Research Institute, Türkiye and its inundative release for biological control on *H. halys*.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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