

Variation of host plant-associated populations of Dubas bug, *Ommatissus lybicus* de Bergevin (Hem.: Tropicuchidae) in southern Iran

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Abstract

Dubas bug, *Ommatissus lybicus* de Bergevin (Hem.: Tropicuchidae), is a key pest of date palms in Iran. This pest was first reported on date palms in 1966 and has been established throughout the date producing regions in southern Iran since. Date palm is the only host plant of Dubas bug. It attacks almost all date palm varieties, esp. Mazafati variety in city of Bam, Kerman Province and other varieties such as Halilei and Karoot. Using geometric morphometric method on forewings, we studied the morphological characters of host-associated populations of this pest. Adult Dubas bugs were collected on three varieties of date palms, *Phoenix dactylifera* L., including: Mazafati, Halilei and Karoot from Bam in 2015. For the analysis of wing shape and wing size, MANOVA and ANOVA were done, respectively. An allometric analysis was performed on the shape and size variables. The results of MANOVA indicated a significant difference ($P < 0.05$) among wing shapes but no difference observed among wing sizes ($P > 0.05$). The regression of shape on the size variables showed no significant allometric growth ($P > 0.05$). The UPGMA tree, based on the Mahalanobis distances matrix, detected two distinct groups: the first group was a population feeding on the Mazafati variety with narrow wing shapes, and the second were populations feeding on Halilei and Karoot varieties with wide and shorter wing shapes. The observed forewing shape of individuals in population feeding on the Mazafati variety may change their flight abilities, and other biological aspects, such as survival.

Key words: date palm, *Phoenix dactylifera*, Tropicuchidae, biodiversity, Geometric morphometrics.

چکیده

تنوع جمعیت‌های میزبانی زنجره خرما، *Ommatissus lybicus* de Bergevin (Hem.: Tropicuchidae) در جنوب ایران

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زنجره خرما، *Ommatissus lybicus* de Bergevin (Hem.: Tropicuchidae)، آفت کلیدی خرما در ایران است که برای اولین بار در سال ۱۹۶۶ از روی خرما گزارش شد. این آفت اکنون در تمام نواحی تولیدکننده خرما در جنوب ایران مستقر شده است. خرما تنها میزبان زنجره خرما است. این آفت تقریباً به تمام رقم‌های خرما حمله می‌کند، به ویژه رقم مضافتی در استان کرمان (شهرستان بم)، و رقم‌های دیگر شامل هلیله‌ای و کروت. بنابراین مطالعه حاضر به منظور بررسی تفاوت‌های ریخت‌شناختی در جمعیت‌های میزبانی زنجره خرما با استفاده از روش ریخت‌سنجی هندسی روی بال جلو آفت انجام شد. به این منظور، حشرات بالغ زنجره خرما از روی سه رقم خرما، *Phoenix dactylifera* L.، شامل: مضافتی، هلیله‌ای و کروت از شهرستان بم در سال ۱۳۹۴ جمع‌آوری شدند. برای آنالیز شکل و اندازه بال بین گروه‌ها، آنالیزهای MANOVA و ANOVA انجام شد. سپس به منظور بررسی وجود رشد آلومتری، آنالیز رگرسیون روی متغیرهای شکل و اندازه طرح‌ریزی شد. نتایج MANOVA نشان داد که اختلاف معنی‌داری بین شکل بال وجود دارد ($P < 0.05$)، ولی در اندازه بال تفاوت معنی‌داری دیده نشد ($P > 0.05$). رگرسیون متغیرهای شکل روی اندازه، رشد آلومتری را نشان نداد ($P > 0.05$). آنالیز کلاستر با روش UPGMA و بر اساس ماتریس فواصل ماهالانوبیس دو گروه را مشخص کرد: گروه اول مربوط به جمعیت‌های تغذیه‌کننده روی رقم مضافتی با بال باریک‌تر؛ و گروه دوم مربوط به جمعیت‌های تغذیه‌کننده روی رقم‌های هلیله‌ای و کروت با بال عریض‌تر و کوتاه‌تر بود. شکل بال مشاهده شده در افراد متعلق به جمعیت‌های تغذیه‌کننده روی رقم مضافتی ممکن است توانایی‌های پرواز و ویژگی‌های دیگر بیولوژیکی مانند بقا را تغییر دهد. واژه‌های کلیدی: خرما، *Phoenix dactylifera*، Tropicuchidae، تنوع زیستی، مرفومتیک هندسی.

Introduction

Date palm is one of the most important crops with a long history in Iran and other parts of the world. Egypt, Iran, Saudi Arabia, Iraq and Pakistan are among the top date producing countries in the world (Faostat, 2016).

Dubas bug, *Ommatissus lybicus* de Bergevin (Hem.: Tropiciduchidae), is a key pest of date palm, *Phoenix dactylifera* L., and reported as a pest of date palm in 1919 from Iraq (Afshar, 1937) and now it is a common pest in the Middle East (Asche & Wilson 1989). The species was formerly known as *Ommatissus binotatus lybicus*, but later raised to species status (Asche & Wilson, 1989). This species is one of the major pests of date palms in the Middle East (Dlabola, 1979; Asche & Wilson, 1989; Wilson, 2010). Recently, Dubas bug has been considered as a serious pest on palm in Iran, causing economic losses in palm groves (Gharib, 1998; Arbabtafi *et al.*, 2015). It is reported from Bafgh, Kahkom, Bam, Bandar Abbas, Geno, Isin, Jahrom, Jandagh, Khorramshahr, Khur va Biabanak, Mehran, Shahdad and Tabas (Mozaffarian & Wilson, 2016) and generally in all palm growing areas of the country (Gharib, 1998). The nymphs and adults of this pest cause direct damage through feeding and producing honeydew leading to coverage of leaves, by sooty mold and reduction of photosynthesis rate (Hussain, 1963).

Presence of the pest on different hosts during its life cycle can affect the other biological aspects such as survival, physiology and morphology (Kim & McPherson, 1993). Many studies have been made for the discrimination of host-associated populations of major insect pests, such as *Ectomyelois ceratoniae* (Zeller) (Mozaffarian *et al.*, 2007), *Diaphorina citri* Kuwayama (Lashkari, 2013; García-Pérez *et al.*, 2013; Paris *et al.*, 2016), *Aphis craccivora* Koch. (Mehrparvar *et al.*, 2012) and *Brachycaudus elichrysi* (Kaltenbach) (Madjdzadeh *et al.*, 2009), which suggest morphological differences among pest populations.

Little is known about the intraspecific variation of Dubas bug on different date palm varieties such as Mazafati, Halilei and Karoot varieties in Bam. Date palm serves as the only host plant for Dubas bug. This study was carried out, with geometric morphometric approach, to investigate wing morphological characters of Dubas

bug populations feeding on different date palm varieties to improve the efficiency of the pest management programs against Dubas bug.

Materials and methods

The adults of Dubas bug were collected on common varieties of date palms (Halilei, Karoot and Mazafati) on the outskirts of the city of Bam (Kerman Province), in November 2015 coinciding the second generation of this pest. The sampling sites were selected far enough to reduce the effects of their ability to flight towards different varieties of host-plants. The distance between each site was about two kilometers (Table 1, Fig. 1). The climate in the collection sites is arid, with cool winter and very warm summer (A-C-VW) (Fig. 1).

To standardize the sample size for statistical analysis, only females from each host species were collected. Females were more frequent, with complete wings, which were suitable for geometric morphometric analysis.

A total of 40 female specimens with undamaged wings, from each population were randomly selected. Wing slides from the right forewing of each specimen were prepared using Canada balsam and their images captured by a stereomicroscope. In geometric morphometric analyses, 19 homologous landmarks (type I) were selected on the forewing (Fig. 2) and digitized by TpsDig program (Rohlf, 2004). The wings were aligned and the Partial warp scores (shape variables) were extracted by TpsRelw program. The wings were aligned and the Partial warp scores (shape variables) were extracted by TpsRelw program (Rohlf, 2010) which were used as the variables of shape in other analyses (Bookstein, 1991). Centroid sizes, as a size measure of a wing, (Slice *et al.*, 1996) were also calculated using the TpsRelw software and used as the measure of size among populations (Adams & Funk, 1997).

To detect any significant wing shape differences, multivariate analyses of variance (MANOVAs) and canonical variate analysis (CVA) procedures were designed and performed using SAS program (Ver. 9.1) (SAS Institute, 2003) and NTSYS-pc program (Ver. 2.10e) (Rohlf, 2000), respectively. The Mantel test was performed using the NTSYS-pc program. To detect the allometric relationship, a regression of shape variables on

size was performed using the TpsRegr program (Rohlf, 2009). The ANOVA procedure and subsequently Tukey pair-wise comparisons of wing size among populations were done in SAS program (Ver. 9.1) (SAS Institute, 2003).

Results

The superimposition of 120 specimens of *O. lybicus* from different host-associated populations showed the range of variation at each landmark (Fig. 3). The positive and negative extremes along the first relative warps, RW1, showed the directions of wing shape variation between the populations (Fig. 4).

Wings in the females associated with Mazafati variety are narrower than those in Halilei and Karoot varieties. In the populations feeding on Mazafati variety, the orientation of the scatters at the marginal landmarks numbers 1-7, specially 2-6 showed their inclination to the tip of the wing, therefore led to narrower-concave wings with relatively longer tips. In; on the other hand, in the

populations feeding on Halilei and Karoot, the orientation of the scatters at the marginal landmarks numbers 2-6 showed their inclination to the inner parts of the wings as defined a shorter, wider and convex wing shape.

The MANOVA for shape variables was statistically significant ($P<0.05$) (Table 2). CVA separated the populations and confirmed the results of MANOVA (Fig. 5), as the population feeding on Mazafati were placed in the right side of CVA plot and discriminated from the populations on the left, while the populations on Karoot and Halilei overlapped (Fig. 5).

The allometric analysis did not show allometric growth among populations ($P>0.05$) (Table 2).

The centroid size of the wings, in contrast to their shapes, was not significant ($F= 1.18$, $P = 0.3125$). Pairwise comparisons (using HSD post-hoc test) showed similarity in size of the wings of populations feeding on different date palm varieties (Fig. 6).

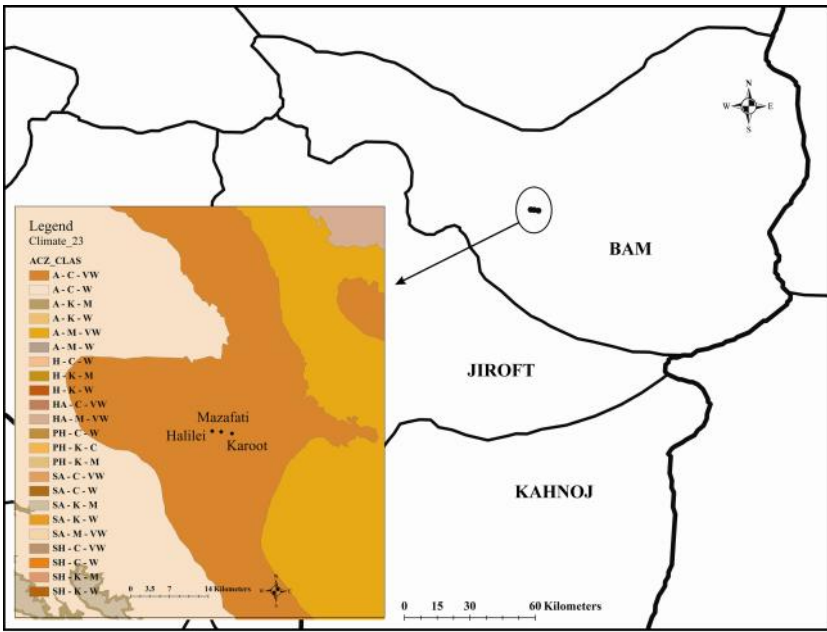


Fig. 1. Collection sites of Dubas bug, *Ommatissus lybicus* de Bergevin in Bam (from Mazafati, Karoot and Halilei varieties) based on 23 climate layers map of Iran.

Table 1. Collection sites, their geographic coordination and the number of examined specimens for Dubas bug, *Ommatissus lybicus* de Bergevin.

Host-Plant	Collection site	N.	E.	Altitude (m)	n.
Halilei	Bam	29°06'02"	58°18'50"	1114	40
Mazafati	Bam	29°05'58"	58°19'42"	1099	40
Karoot	Bam	29°05'48"	58°20'45"	1078	40

Table 2. MANOVA and Allometry tests in host plant populations of Dubas bug, *Ommatissus lybicus* de Bergevin.

	Wilks Lambda	Fs	P
MANOVA	0.7359	1.69	0.0372
Allometry	0.65144853	1.243	0.2131

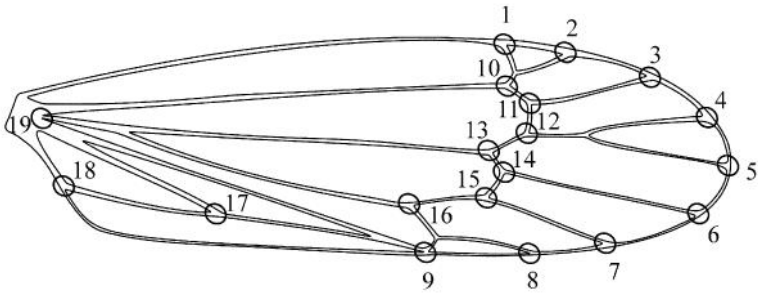


Fig. 2. Position of selected landmarks (circles) in the right forewing of Dubas bug, *Ommatissus lybicus* de Bergevin.

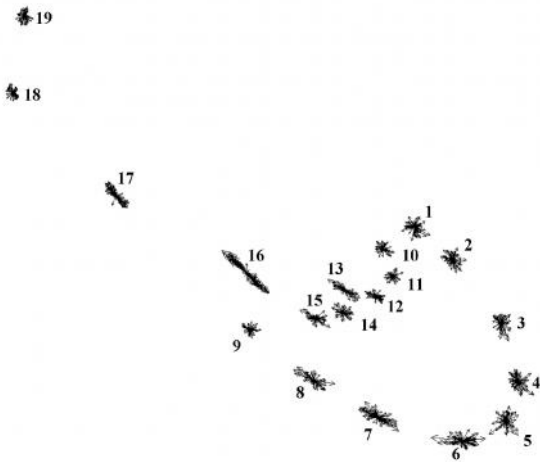


Fig. 3. Superimposed landmarks on the forewing of Dubas bug, *Ommatissus lybicus* de Bergevin.

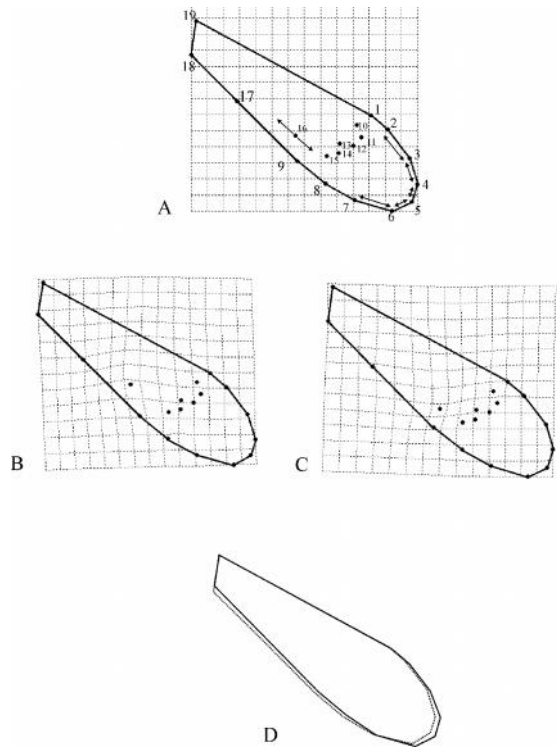


Fig. 4. Shape variation along the (A) consensus configuration, (B) negative and (C) positive extremes of RW1, and (D) superimposed wing shapes of negative (dotted outline) and positive (solid outline) of RW1.

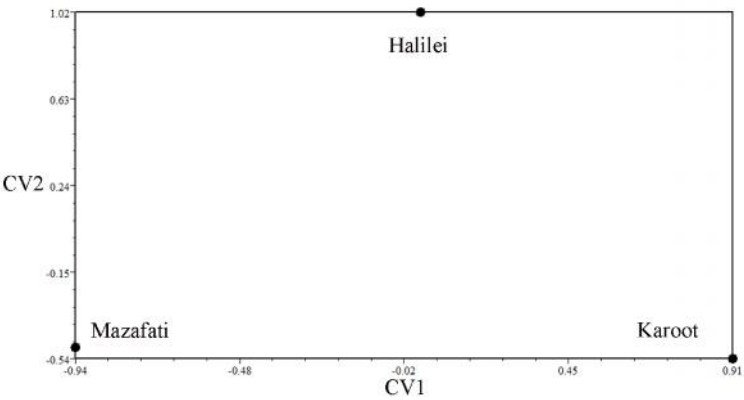


Fig. 5. Canonical variate analysis (2D plot of mean scores) of different host-associated populations of Dubas bug, *Ommatissus lybicus* de Bergevin, along the first two canonical variate axes (CVA1, CVA2). H: Halilei; K: Karoot; M: Mazafati variety.

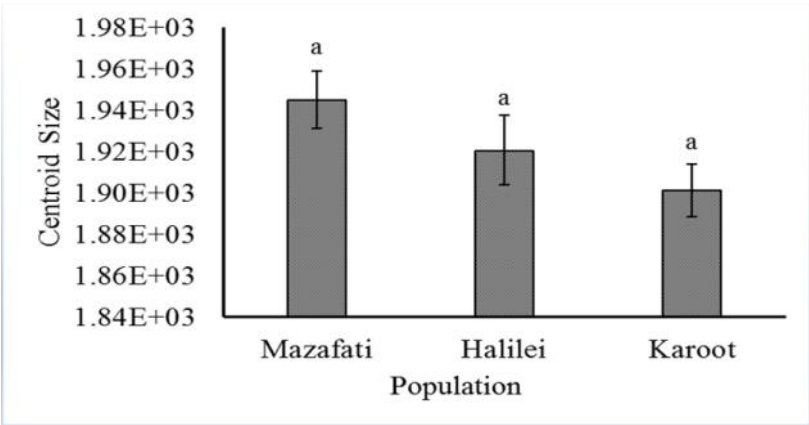


Fig. 6. Centroid size comparison of the host associated populations of Dubas bug, *Ommatissus lybicus* de Bergevin.

Discussion

The variation of wing shape in *O. lybicus* was clearly shown by the geometric morphometric method. Although we found differences in wing shape of the Host-associated populations, but there were not significant differences in the wing sizes.

Geometric morphometric analysis revealed that the specimens in the population feeding on Mazafati variety tend to have relatively narrow and elongated wings comparing to those feeding on other varieties. This pattern also slightly varied in the landmarks near the body but was strongly different in the landmarks associated with the tip of the wing. Similar pattern has been shown in the study of Rohlf and slice (1990) who used generalized least-squares method as a superimposition method on the wings of 127 species of north American mosquitoes. The variation in wing shape in host associated populations has also been shown in many other insects, such as *D. citri* (Paris *et al.*, 2016), which the wing shape of *D. citri* on *Murraya paniculata* (L.) was narrower on average. The patterns of wing shape in population feeding on Karoot are more similar to those feeding on Halilei variety. The wing shape similarity in populations feeding on Halilei and Karoot varieties likely results from similarity in quality of nutritional elements, which has been detected in some species, such as *D. citri* (Lashkari, 2013), *Daktulosphaira vitifoliae* Fitch. (Downie *et al.* 2001) and *Bemisia tabaci* (Gennadius) (Abdullahi *et al.* 2003).

The narrower wings and longer tips (that is observed in population feeding on Mazafati variety) can enhance the flight abilities of the individuals (Betts & Wootton, 1988). Theoretically, wings with longer shape will be more aerodynamic and more efficient than the shorter ones, because of lower pressure (Vogel, 1994) as exists in grasshoppers (Bai *et al.*, 2016). Narrower wings with high value of aspect ratio are suitable for long gliding which in all butterflies affects the amount of energy used for flight and their migration (DeVies *et al.*, 2010). Breuker *et al.* (2007) showed that changes in the shape of female’s forewing are associated with dispersal. Considering the female’s narrower wing shape, there appears changes in the forewings of individuals feeding on Mazafati variety is associated with dispersal and the result of natural selection for long gliding abilities facilitating their access to new areas and host plants. In the current study, 1) it seems that the population on Mazafati variety developed a better flight ability, due to its wing shape, rather than the other populations; 2) wing shape within *O. lybicus* populations is associated with the host plant on which they can establish themselves faster. Comparing with other varieties, the Mazafati variety has relatively more extended crown, consist of longer leaves and branches, which play an important role in preparing the moisture by providing more extended canopy (Fig. 7). It is demonstrated that relative humidity are positive association with Dubas bug density (Mahmoudi *et al.*, 2013). Mahmoudi *et al.* (2013) also found that Dubas bug

density is positive correlation with variables such as application of fertilizer and the number of date palm leaves. In Bam city, Mazafati is the most important date palm variety commercially cultivated, because it is suitable variety for this climate, therefore the farmers are tending more to this variety and provide better agronomic conditions (such as applications of fertilizer) for this variety. So, this variety is more fresh relative to the other varieties. Mahmoudi et al. (2013) find that better nutrished trees have significantly higher density of Dubas bugs.

Based on the presence of high density of the pest on Mazafati variety, it seems that this variety provides better conditions for the pest. Mahmoudi et al. (2015) studied demographic parameters of *O. lybicus* on five date palm varieties (Zahedi, Mazafati, Piarom, Khasi and Shahani) and mentioned that the mean fecundity per female was significantly higher on Zahedi (78.62 eggs) and Mazafati (68 eggs) than on Shahani (43.53 eggs) and Khasi (46.32 eggs). They found that Shahani and Khasi were highly resistant varieties comparing to Zahedi variety in response to Dubas bug attack (Mahmoudi et al., 2015). It is believed that Dubas bug adults prefer to feed on Mazafati. Although there were no significant differences between wing sizes of the populations, populations feeding on Mazafati had bigger wings that

suggests better nutritional capacities of this variety (Fig. 6). The individuals with narrower and longer tips can find the suitable host because of their better flight abilities. The results support the hypothesis that natural selection acts on the morphology of wing. More studies are required to evaluate flying, behavioral and demographic capacities to confidently confirm this hypothesis.

Allometric analyses showed isometric growth among studied populations. Shape variation was not accompanied with the size variation on different host plants likely due to its genetical basis. The result of the allometric analyses also indicates that natural selection is in process to create a population with high dependency on the host plant.

In this study, some important issue are suggested in the management of this pest: 1) the observed morphological variations may potentially influence the biological characteristics, therefore, the comparative demographic studies of Dubas bug on the studied varieties of date palm is recommended. 2) Molecular markers are needed. 3) Although the level of infestations on the Halilei and Karoot varieties was lower than those on Mazafati variety, control programs should not focus only on Mazafati variety as the density of pest populations may increase on other varieties.



Fig. 7. Varieties of date palm, A) Mazafati, B) Halilei, C) Karoot.

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