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Preliminary results of the shape analysis of pine processionary moth scales in Turkey

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Abstract: Larvae of the two pine processionary moth (PPM) sister species, *Thaumetopoea wilkinsoni* and *T. pityocampa*, causes severe allergies in mammals and are dangerous defoliators of Mediterranean pines. While *T. pityocampa* occurs mainly in Europe, *T. wilkinsoni* occurs in Turkey and the Middle East. Recent studies showed that ranges of the two species are in contact in Turkey. Female moths of the two species cover their eggs with scales on their abdomens. These scales are believed to protect eggs from parasitism. Although *T. wilkinsoni* and *T. pityocampa* exhibit highly similar morphologies, few studies have suggested that scale morphology could be used for species identification. However, our field observations in Turkey do not confirm this suggestion. Nevertheless, statistical approaches have never been used on this issue. In this study, we aimed at solving this contradiction by digitizing PPM scale shape photographs taken standardly from 60 individuals collected from different locations in Turkey and Cyprus and by subjecting them to geometric morphometry approaches. We conducted shape analysis by using TPS software and Elliptic Fourier Outline Analysis which is a sensitive method for morphometric computations. Results did not reveal any correlation between scale morphology and species taxonomy. This result could be interpreted as an insufficiency of scale morphology being able to be used as a taxonomic character to separate *T. wilkinsoni* and *T. pityocampa* in Turkey. However, it should be kept in mind that these two species have a contact zone in Turkey, which might cause hybridization. This phenomenon could completely change morphological patterns. In order to reach more accurate results, this study should be repeated with samples from the entire ranges of the two species.
Keywords. *Thaumetopoea wilkinsoni*, *T. pityocampa*, Scale, Shape analysis, Geometric morphometry, Turkey

Türkiye'deki çam kese böceği pullarının şekil analizinin ilk sonuçları

Özet: İki çam kese böceği (ÇKB) türü olan *Thaumetopoea wilkinsoni* ve *T. pityocampa*'nın larvaları memelilerde ciddi alerjik reaksiyonlara neden olmakla kalmaz, aynı zamanda Akdeniz çamlarının da önemli zararlılarıdır. *T. pityocampa* daha ziyade Avrupa'da, *T. wilkinsoni* ise Türkiye ve Orta Doğu'da yayılış gösterir. Yakın zamanlarda yapılan çalışmalar iki türün yayılış alanlarının Türkiye'de kesiştiğini göstermiştir. Dişi güveler yumurtalarını abdomenlerinde bulunan pullarla örter. Bu pulların yumurtaları parazitoidlerden koruduğu düşünülmektedir. Her ne kadar *T. wilkinsoni* ve *T. pityocampa* morfolojik olarak birbirine çok benzese de birkaç çalışmada pul morfolojisinin tür ayrımında kullanabileceği öne sürülmüştür. Ancak arazi gözlemlerimiz bu iddiayı desteklememektedir. Bununla birlikte bu konu ile ilgili olarak şimdiye kadar herhangi bir istatistiksel çalışma yapılmamıştır. Bu çalışmada konuya ilişkin sorulara cevap aramak amacıyla Türkiye ve Kıbrıs'ta farklı alanlardan toplanan 60 bireye ait fotoğraflanmış pullar dijitalleştirilerek geometrik morfometri yaklaşımlarıyla incelenmiştir. TPS yazılımı kullanılarak şekil analizi ve morfometrik hesaplamalarda kullanılan hassas bir yöntem olan Eliptik Fourier Anahat Analizi yapılmıştır. Sonuçlar pul morfolojisi ile tür taksonomisi arasında herhangi bir uyum göstermemiştir. Bu sonuç pul morfolojisinin Türkiye'de yayılış gösteren *T. wilkinsoni* ve *T. pityocampa*'yı birbirinden ayırt etmeye yetecek bir taksonomik karakter olmadığı şeklinde yorumlanabilir. Ancak bu iki türün Türkiye'de melezleşme ile sonuçlanabilecek bir temas kuşağının bulunduğu unutulmamalıdır. Bu durum morfolojik örüntüleri önemli ölçüde değişkliğe uğratmış olabilir. Daha doğru sonuçlara ulaşmak için bu çalışma her iki türün de tüm yayılış alanlarını kapsayacak şekilde genişletilmelidir.
Anahtar kelimeler: *Thaumetopoea wilkinsoni*, *T. pityocampa*, Pul, Şekil analizi, Geometrik morfometri, Türkiye

1. Introduction

The pine processionary moth (hereafter PPM) species, *Thaumetopoea wilkinsoni* (in Turkey and the Middle East) and *T. pityocampa* (Europe and north Africa) (Lepidoptera, Notodontidae) are the most important defoliators of pine in the Mediterranean Basin (Carus, 2004; Masutti and Battisti, 1990) and their larval setae cause severe allergic reactions in mammals (Denis and Schiffermüller, 1776; Rodríguez-Mahillo et al., 2012; Vega et al., 1997). These two species' ranges contact in northwestern Turkey and studies show

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evidences for hybridization (İpekdal, 2012; İpekdal et al., 2015).

PPM is a univoltine organism. Its gregarious larvae feed on the pine host during the winter, descend to the soil in spring for pupation, and adults fly in late summer or early autumn depending mostly to latitude and altitude; and females lay their eggs on pine needles immediately after mating (Avcı and Oğurlu, 2002; Avcı, 2000; Démolin, 1969; Halperin, 1990). Female moth covers its oviposited eggs with the scales on its abdomen. These scales probably protect the eggs from parasitoids and desiccation (Avcı, 2000; Floater, 1998, Mirchev et al., 2004, Özkazanç, 2002, Schmidt, 1990).

T. wilkinsoni and *T. pityocampa* exhibit highly similar morphologies, but few studies have suggested that scale morphology could be used for species identification (Doğanlar et al., 2005; Tsnakov et al., 1991). Although our field observations do not confirm this suggestion (İpekdal, 2012) statistical approaches have never been used so far to solve this ambiguity.

In this study we aimed to quantify shapes of the scales collected from different localities in Turkey and Cyprus. This study is the first attempt to investigate scale morphology statistically and to reveal its suitability as a taxonomic character. Our results did not reveal any correlation between scale morphology and species taxonomy.

2. Material and methods

We collected 60 PPM egg batches from 13 localities in Turkey and one in Cyprus. According to İpekdal (2012) 45 of them were identified as *T. wilkinsoni*, 12 as hybrids, and 3 as *T. pityocampa*. We picked off 20 scales per egg batch and fixed them on microscope glasses by using Entellan (Merck). We took photographs of the fixed scale preparations under a Leica DC300-MZ75 digital dissection microscope at Hacettepe Uni. Dept. of Biology and Ecological Sciences Research Lab. by using a magnification of 0.32 and scaling of 1mm. Then the two-dimensional outline of the scale was digitized. The starting point of the outline was defined at the peak point of the scale (Figure 1).

For each scale, 64 points at equally spaced intervals along the outline were sampled using TpsDig2 (Rohlf, 2010). This set of *x, y*-coordinates was then analysed using an Elliptical Fourier Analysis (EFA) using the software EFAwin (Ferson et al., 1985). This method is based on separate Fourier decompositions of the incremental changes along *x* and *y* as a function of the cumulative length along the outline (Kuhl and Giardina, 1982). The outline is approximated by a sum of trigonometric functions of decreasing wavelength: the harmonics. Any harmonic corresponds to four coefficients: A_n and B_n for *x*, and C_n and D_n for *y*, defining an ellipse in the *xy*-plane.

The first ellipse corresponds to the best-fitting ellipse to the outline. Its major axis was taken as new *x*-axis to adjust the orientation of the outline (Rohlf, 1990). Its area was used to standardize the Fourier coefficients (FCs) for size differences in order to eliminate isometric size effects and to concentrate on shape information only. Since the coefficients A_1, B_1 and C_1 correspond to residuals after standardization (Crampton, 1995; Renaud et al., 1996) they were not included in the subsequent statistical analysis. The coefficient D_1 still retains information about the elongation of the outline (Michaux et al., 2007). It was thus included in the statistical analyses.

In order to evaluate the adequate threshold harmonic for the analysis of PPM scale, the shape of one UM1 was measured ten times, providing an estimate of the measurement error for each harmonic (Figure 2). Measurement error was estimated as the percentage of error for the amplitude (square root of the sum of the squared FCs). In our case, measurement error was low (<5%) until the fifth harmonics and abruptly increased thereafter (Figure 2). The content of information of each harmonic provided information about the amount of shape information provided by that harmonic (Crampton, 1995). The amplitude of each harmonic wass cumulated over the total range of harmonics, and the information brought by each harmonic wass then estimated as the percentage of this sum represented by its harmonic. Each of the five first harmonics increased significantly the amount of shape information up to 97.07% of the total information (Figure 2). The plateau reached afterwards showed that the subsequent harmonics brought almost no further relevant shape information. Hence, considering the set of the first five harmonics appeared as a good compromise between measurement error, information content, and the number of variables to be considered. A data set of 17 variables (20 FCs minus A_1, B_1 and C_1) was thus retained for subsequent analyses.

Statistical analyses: Principal component analysis (PCA) was performed on the set of 17 FCs in order to test differences between the two species *T. wilkinsoni* and *T. pityocampa*. All statistical analyses were performed by using SYSTAT v.11.

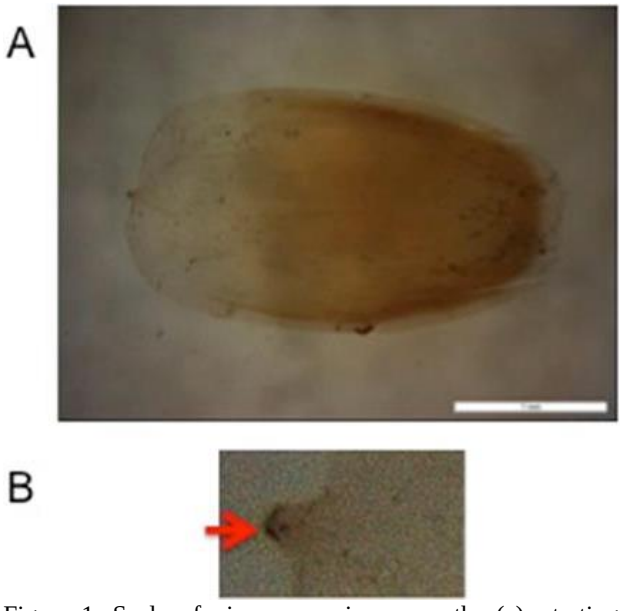

Figure 1. Scale of pine processionary moths (a), starting point of digitization of outline analysis on a scale (b)

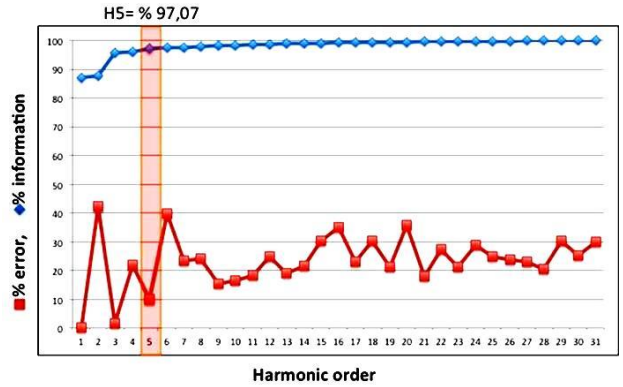

Figure 2. Measurement error (red squares below) and cumulative power (blue diamonds above) as a function of the harmonic order for the elliptic Fourier transform of PPM scale

3. Results and discussion

The first axes of principal component (PC1), based on 17 variables came from elliptic fourier outline analysis of scale measurements, accounted for 94.6% of the total morphometric variation and seemed to express the overall shape of the scale with the populations of *T. wilkinsoni* and *T. pityocampa* and their hybrids.

Our results did not reveal any significant correlation between scale morphology and taxonomy. However, we found a slight differentiation between *T. wilkinsoni* and *T. pityocampa* on the first axis of PCA (Figure 3); yet it is not enough to seperate the two PPM species and their hybrids. Thus we can conclude that scale morphology is an insufficient character to be used for seperating *T. wilkinsoni* and *T. pityocampa* in Turkey. However, it should be kept in mind that these two species have a contact zone in Turkey. Hybridization could completely change morphological patterns. Furthermore, our sampling size could cause biased results. Therefore, in oreder to reach more accurate results, this study should be repeated with a larger sampling size from the entire ranges of the two species.

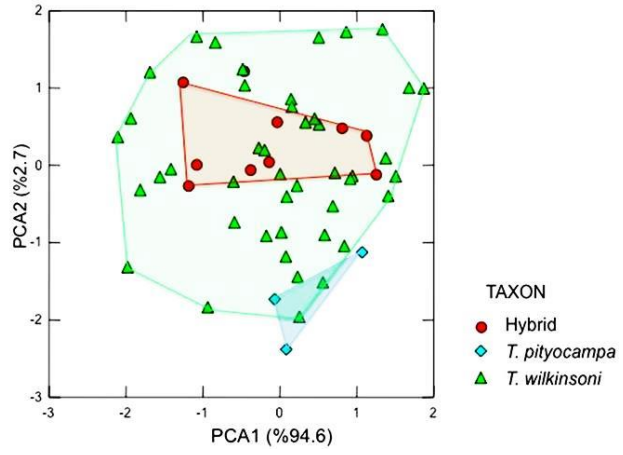

Figure 3. Shape variation of the three different groups of PPM scale in Turkey and Cyprus, displayed on the first two axes of a PCA on the Fourier coefficients

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