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Comparison Between Egg Batch and Egg Characteristics of the Two Pine Processionary Moth Species, *Thaumetopoea wilkinsoni* and *T. pityocampa* in Turkey

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Abstract

Pine processionary moths (*Thaumetopoea wilkinsoni* and *T. pityocampa* [Lepidoptera: Notodontidae]) are among the most important forest pests in the Mediterranean basin. Being close relatives yet having significant genetic differences, the two species has been shown to hybridize under laboratory conditions and in nature in Turkey. Ongoing research has focused on the comparison of the larval and adult morphological traits between the two species without any apparent interest in egg characteristics. In this study, we aimed to reveal differences in egg batch and egg characteristics between the two species. We also compared these characteristics among altitudes. For this purpose, we collected 61 and 11 egg batches from *T. wilkinsoni* and *T. pityocampa* ranges from different altitudes (30-260 m) in Turkey, respectively. Two months after sampling, when parasitoid emergence nearly completed, we removed scales on the egg batches and measured the following traits: egg arrangement, egg batch length, egg batch circumference, clutch size, egg diameter, and larval emergence, unhatched egg and parasitism ratios. We used *t*-test and Mann-Whitney U test for statistical comparisons between species and between altitudes. We found significant differences between the two species in terms of egg arrangement, egg diameter, larval emergence and unhatched egg ratios. We also found significant differences among altitudes in terms of egg batch circumference, clutch size and egg diameter. We conclude that egg characteristics, particularly those related to fecundity and survival, have much more to reveal about the differences between the two species and among different altitudes. Our results also show that pine processionary moth species complex could be a good biological system for studying insect reproductive ecology and evolution, such as trade-offs between egg and clutch sizes. A larger sampling will greatly favor testing hypothesis relevant to the dynamics of pine processionary moth reproduction.

Keywords: Pine processionary moth, *Thaumetopoea wilkinsoni*, *T. pityocampa*, egg batch, egg characteristics, altitude.

Türkiye'deki İki Çam Kese Böceği Türü, *Thaumetopoea wilkinsoni* ve *T. pityocampa*'nın Yumurta Koçanı ve Yumurta Özelliklerinin Karşılaştırılması

Öz

Çam kese böcekleri (*Thaumetopoea wilkinsoni* ve *T. pityocampa* [Lepidoptera: Notodontidae]) Akdeniz havzasındaki en önemli orman zararlılarındandır. Yakın akraba olsalar da genetik olarak oldukça farklılaşmış olan bu iki tür laboratuvar koşullarında ve Türkiye'de doğada melezleşmektedir. Şu anda devam etmekte olan çalışmalar iki türün larva ve ergin evredeki morfolojik özelliklerinin karşılaştırılmasına odaklanmıştır ancak yumurta koçanı ve yumurta özellikleri büyük ölçüde ihmal edilmiştir. Bu çalışmada, iki türün yumurta koçanı ve yumurta özelliklerinin karşılaştırılması amaçlanmıştır. Aynı zamanda farklı yükseklikler arasında da karşılaştırmalar yapılmıştır. Bu amaçla *T. wilkinsoni* ve *T. pityocampa*'nın Türkiye'deki yayılış alanlarından ve farklı yüksekliklerden (30-260 m) sırasıyla 61 ve 11 yumurta koçanı toplanmıştır. Örnekleme yapıldıktan iki ay sonra, parazitoit çıkışı büyük ölçüde tamamlandığında, yumurta koçanlarını kaplayan pullar uzaklaştırılmış ve şu özellikler ölçülmüştür: Yumurta düzenlenişi, koçan uzunluğu, koçan çevre uzunluğu, yumurta sayısı, yumurta çapı ve tırtıl çıkış, açılmamış yumurta ve parazitlenme oranları. Tür ve yükseklikler arası istatistiksel karşılaştırmalar için *t*-testi ve Mann-Whitney U testi kullanılmıştır. Sonuç olarak iki tür arasında yumurta düzenlenişi, yumurta

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çapı, tırtıl çıkış ve açılmamış yumurta oranları bakımından anlamlı farklılıklar tespit edilmiştir. Ayrıca yükseklikler arasında da koçan çevre uzunluğu, yumurta sayısı ve yumurta çapı bakımından anlamlı farklılıklar tespit edilmiştir. Sonuç olarak özellikle fekondite ve sağ kalım ile ilgili yumurta özelliklerinin iki tür ve de yükseklikler arasındaki farklılıklara ilişkin çok daha fazla bilgi barındırdığı söylenebilir. Sonuçlarımız ayrıca çam kese böceği tür kompleksinin böceklerdeki üreme ekolojisi ve evrimi çalışmaları için, örneğin yumurta sayısı ile yumurta büyüklüğü arasındaki ödünleşimin çalışılması için iyi bir biyolojik sistem olduğunu göstermektedir. Daha büyük bir örnekleme çam kese böceklerinin üreme dinamiklerine ilişkin hipotezlerin sınanmasını büyük ölçüde kolaylaştıracaktır.

Anahtar Kelimeler: Çam kese böceği, *Thaumetopoea wilkinsoni*, *T. pityocampa*, yumurta koçanı, yumurta özellikleri, yükseklik.

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1. Introduction

Fecundity is the number of offspring produced by an individual insect, whereas fertility is the number of viable offspring produced (Awmack and Leather, 2002). As fecundity and fertility are two important components of population growth, they are of particular interest in planning insect pest management programs (Krebs, 2008).

Pine processionary moths, *Thaumetopoea wilkinsoni* and *T. pityocampa* (Lepidoptera: Notodontidae) are among the most destructive defoliators of pine in the easternmost Mediterranean Basin (Kerdelhué et al., 2009). Their caterpillars also cause allergy in human and mammalian pets (Battisti et al., 2017). Accordingly, they are of high management concern in most Mediterranean countries and they also threaten northern latitudes due to the consequences of climate change on species boundaries (Battisti et al., 2005; Roques et al., 2015). The two species were recently found to co-occur in a contact zone and hybridize in Turkey (İpekdal et al., 2015). They also have been shown to hybridize to produce viable and fertile offspring under the laboratory conditions (Petrucco-Toffolo et al., 2017). The fact that they have well-separated genomes but highly similar morphological and behavioral features motivated researchers to pursue morphological and behavioral differences in previously unfocused characteristics. Ongoing studies deal with larval and adult morphological traits, whereas, to the best of our knowledge, there is not any study focusing on the differences in the egg batch and egg characteristics between the two species.

In this study, we aimed to compare eight characteristics or measures of egg batches and eggs of the pine processionary moths, namely, egg arrangement, egg batch length and circumference, clutch size, egg diameter, and larval emergence, unhatched egg and parasitism ratios by using samples collected from *T. wilkinsoni* and *T. pityocampa* ranges in Turkey. This is the first attempt dealing with interspecific differences in these characteristics in the pine processionary moth species complex.

2. Materials and Methods

2.1. Materials

We collected egg batch samples from *Pinus nigra* Arnold in 2017 between October 23rd and 25th in Bartın and Edirne. We visited 5 and 2 locations and collected 61 *T. wilkinsoni* and 11 *T. pityocampa* egg batches from different altitudes (30-260) in Bartın and Edirne, respectively (Figure 1, Table 1) (see İpekdal et al. (2015) for molecular taxonomic identification of the pine processionary moth species in Turkey).

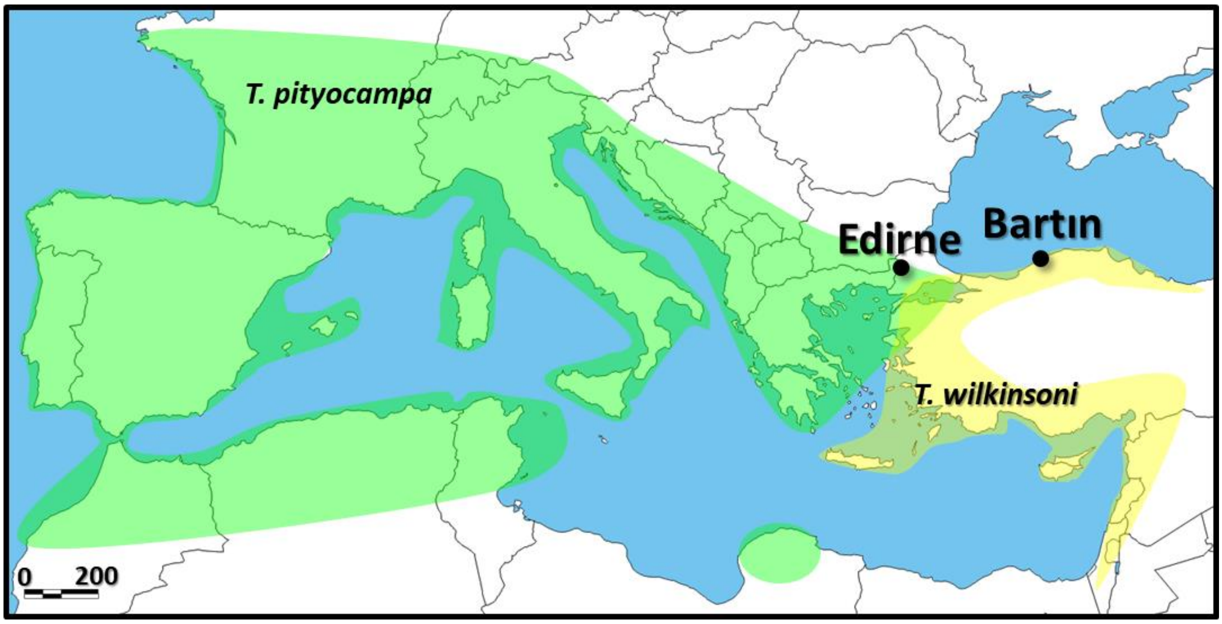

Figure 1. Distribution of pine processionary moth species and sampling locations.

We chose eight characteristics or measures of egg batches and eggs of the pine processionary moths: egg arrangement, egg batch length and circumference, clutch size, egg diameter, and larval emergence, unhatched egg and parasitism ratios. Twisted (relative to straight) egg arrangement was a trait that we observed frequently in

Turkish forests in *T. wilkinsoni* range, but we could not find any published record from *T. pityocampa* range. Therefore, we hypothesized that twisted egg arrangement could be a *T. wilkinsoni* trait and we tested this hypothesis. Other characteristics that we measured were related mainly to the fecundity and survival of the species which have great impact on the population sizes of these pests, and thus they are relevant to the level of damage caused by them. We measured and compared these characteristics between the two species. We also evaluated the effects of altitude on these characteristics.

Table 1. Details of egg batch samplings.

Location	Coordinate	Altitude (m)	Species	Number of egg batches
Bartın	41°39'6.44"N 32°22'0.70"E	107	*T. wilkinsoni*	10
Bartın	41°39'7.28"N 32°22'4.63"E	255	*T. wilkinsoni*	12
Bartın	41°39'0.12"N 32°22'7.05"E	30	*T. wilkinsoni*	11
Bartın	41°38.293"N 32°26,753"E	260	*T. wilkinsoni*	15
Bartın	41°38.768"N 32°26.270"E	95	*T. wilkinsoni*	13
Edirne	41°23.680"N 26°47.593"E	112	*T. pityocampa*	6
Edirne	41°23.302"N 26°48.145"E	118	*T. pityocampa*	5

2.2. Methods

The egg batches were placed in 25 ml glass tubes plugged with cotton and kept under room temperature. Two months after sampling, when parasitoid emergence nearly completed, we removed scales on the egg batches and measured eight characteristics of the egg batches and the eggs, namely, egg arrangement, egg batch length, egg batch circumference, clutch size, egg diameter, ratio of larval emergence, unhatched egg and parasitism ratios.

The length and circumference of the batches were calculated by using a string and a millimetric ruler. For the length of the batch, first we measured the length from three different aspects of a batch and then averaged the three measurements. Arrangement of eggs (spiral/straight) were recorded and counted healthy/parasitoid emergences and unhatched eggs under a magnifying glass.

The diameter of eggs was calculated through four steps as described below: First, we placed the egg batch horizontally on a cross-sectional paper and took a picture of the batch. Second, we transferred the picture to Paint, the graphics/painting utility of Microsoft Windows, and drew a horizontal 5 mm line as a scale. We noted the pixel values (x, y) of this line. Third, we drew a horizontal line on the egg batch as long as its diameter and noted the pixel values of this line. Fourth, we transformed these values to millimeters. In order to do this, we first calculated the pixel length by using the scale: We squared and summed the pixel values of the 5 mm line scale (Pythagorean Theorem). We did the same calculation for all egg measurements. Then for each egg, we multiplied the pixel length of the egg diameter with the ratio of scale length (5 mm) to the scale pixel length as shown at the formula below and Figure 2.

$$\text{Egg diameter in mm} \quad = \quad \text{Pixel length of the egg diameter} \times \text{Scale ratio}$$

$$= \quad (x^2 + y^2) \times \frac{\text{scale length in mm}}{\text{pixel length of the scale}}$$

2.3. Statistical Analyses

We tested statistical significance of interspecific and interaltitudinal comparisons of egg batch and egg characteristics by using statistical packages implemented in R (R Core Team, 2013). To test normality, we used Shapiro-Wilki normal distribution test. For normally distributed data, we used *t*-test and otherwise Mann-Whitney U test (p = 0.05).

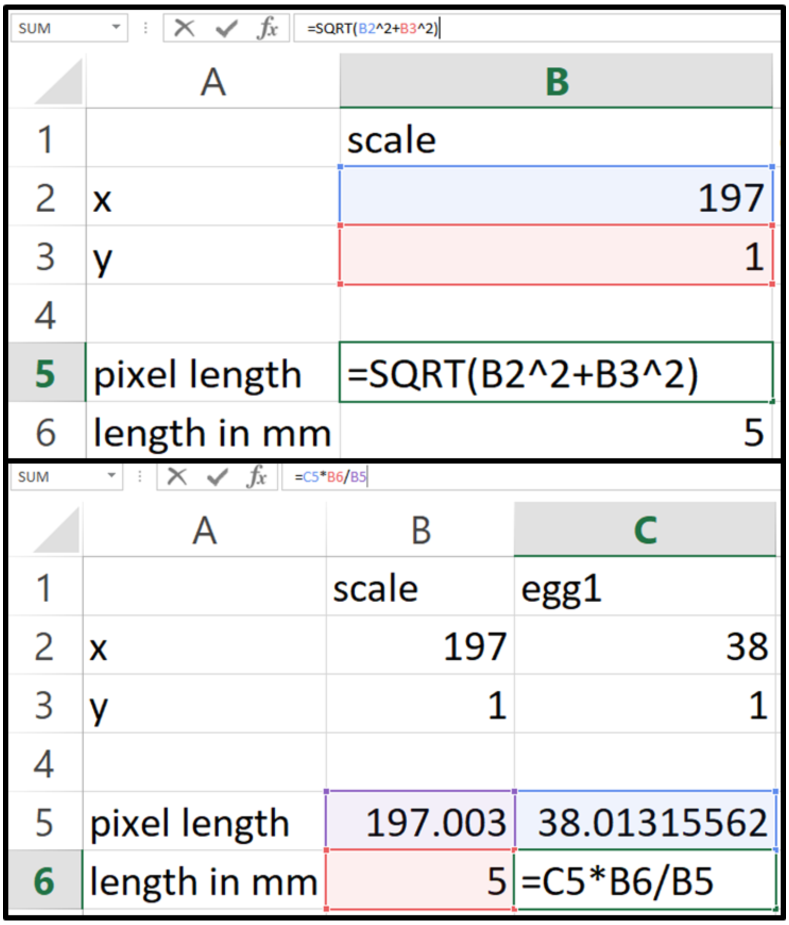

Figure 2. Calculation of the egg diameter from pixels.

3. Results and Discussion

We compared egg batch and egg characteristics (egg arrangement, egg batch length and circumference, clutch size, egg diameter, and larval emergence, unhatched egg and parasitism ratios) between the two pine processionary moth species, *Thaumetopoea wilkinsoni* and *T. pityocampa*, and among different altitudes (<100 m, 100 m - 200 m, and >200 m). Statistical significance of these comparisons is given Table 2 as a summary.

Table 2. Comparison of egg batch and egg characteristics between species and altitudes

Characteristic	**Interspecific**	**Interaltitudinal**			
		< 100 m	**> 100 m**	**< 200 m**	**> 200 m**
Egg arrangement	Twisted in *T. wilkinsoni*	No difference			
Egg batch length	No difference	No difference			
Egg batch circumference	No difference	-	Longer	-	Longer
Clutch size	No difference	-	Higher	-	-
Egg diameter	Longer in *T. pityocampa*	Longer	-	-	-
Larval emergence ratio	Higher in *T. wilkinsoni*	No difference			
Unhatched egg ratio	Higher in *T. pityocampa*	No difference			
Parasitism ratio	No difference	No difference			

Table 3. Comparison of egg batch and egg characteristic averages between species.

Characteristic	*T. wilkinsoni*	*T. pityocampa*
Egg batch length (mm)	32.310	32.000
Egg batch circumference (mm)	12.180	11.650
Clutch size (number of eggs)	288.707	294.250
Egg diameter (mm)*	0.833	0.868
Larval emergence ratio (%)*	88.5%	68.9%
Unhatched egg ratio (%)*	5.9	24.2
Parasitism ratio (%)	4.9	7.3

* statistically significant difference

Table 4. Comparison of egg batch and egg characteristic averages between species and altitudes.

Characteristic	*T. wilkinsoni*				*T. pityocampa*	
	< 100	> 100	< 200	> 200	< 100	> 100
Egg batch length	32.286	32.324	32.625	31.923	33.300	30.700
Egg batch circumference	10.905	*12.919	11.938	*12.500	11.600	11.700
Clutch size	274.619	*296.703	292.375	284.192	280.500	*308.000
Egg diameter	*0.859	0.817	0.844	0.820	0.875	0.862
Larval emergence ratio	91.7	86.7	85.8	91.8	84.4	53.5
Unhatched egg ratio	5.6	6.1	7.6	3.8	11.1	37.3
Parasitism ratio	4.1	5.3	5.1	4.6	5.3	9.4

* statistically significant difference

Egg arrangement: We found that twisted egg arrangement was more common in *T. wilkinsoni* egg batches ($p = 2.2 \times 10^{-16}$) (Table 2). Only a few studies cited this egg arrangement character of the processionary moths (Acatay, 1953; Avcı and Ölmez, 2016; Besçeli, 1969; Çanakçıoğlu and Mol, 1998). Although we are not sure why some egg batches are twisted, while others have a straight arrangement, a possible explanation could be related to needle morphology. As the female follows the needles during egg laying, egg batches on twisted needles eventually look twisted (pers. com. A. Battisti).

Egg batch length: We could not find any difference in the egg batch lengths (Table 2) between the two species ($p = 0.831$) (Table 3) or among different altitudes ($p = 0.582$) (Table 4). Egg batch length is a highly variable characteristic in the pine processionary moths (24-46 mm for *T. wilkinsoni*; 20-32 mm for *T. pityocampa*) (e.g., Acatay, 1953; Avcı & Ölmez, 2016; Kitt & Schmidt, 1993; Mirchev et al., 2007, 2017; Özkazanç, 1987; Schmidt et al., 1999) and it seems not only the fecundity but also several other factors (such as host tree needle thickness [Arnold and Torres, 2006]) have an effect on it. Unfortunately, our sampling size did not allow us understanding the impact of these factors as such a variability necessitates larger data set.

Egg batch circumference: We could not find any difference in the egg batch circumferences between the two species ($p = 0.278$) (Table 3). On the other hand, we found that egg-batch circumferences tend to be significantly larger above 100 m of altitude in comparison to the egg-batch circumference below 100 m ($p = 4.37 \times 10^{-5}$) and over 200 m ($p = 0.025$) (Table 4). Thus, we can conclude that egg batch circumference in the pine processionary moths increases with increasing altitude. Egg batch circumference (or egg batch diameter) is a highly variable characteristic in the pine processionary moths similar to the egg batch length. One of the main factors determining the circumference of an egg batch seems to be the needle thickness (Acatay, 1953). The thicker or the more the needles are used as egg laying substrate, the longer the egg batch circumference becomes. On the other hand, we are not aware of the factors that can contribute to egg batch circumference in different altitudes (such as possible variation in egg size). Once again, in order to resolve this variable character, we need a larger data set collected separately from different host tree species and altitudes.

Clutch size vs egg diameter: Although we could not find any difference in number of eggs between the two species ($p = 0.603$) (Table 3), we found that the number of eggs tend to be higher over 100 m ($p = 0.045$) (Table 4). Avcı (2000), Avcı and Ölmez (2016) and Özkazanç (1987) showed that there was a positive correlation between the clutch size and altitude. However, factors causing this apparent correlation is not clear.

We found a significant difference between two species in terms of egg diameter ($p = 0.003$). Our results show that *T. pityocampa* have bigger eggs (Table 3). We also found that the egg diameter tends to be bigger below 100 m ($p = 0.003$) (Table 4).

Clutch size and egg diameter are measures of fecundity together as there is a well-known trade-off between them (Wilson and Wessels, 1994). Because the total amount of energy that an insect can allocate to its eggs is limited, an increase in clutch causes a decrease in egg size and vice versa (Bernays and Chapman, 1994; Stearns, 1992). Perez-Contreras and Soler (2004) found a negative correlation between clutch size and egg size in *T. pityocampa*. Despite the fact that we found a difference in egg size between the two species, we could not find a difference in clutch sizes which was expected according to the above-mentioned studies. On the other hand, we found a negative correlation between the two characteristics when the altitude was taken as the parameter. Our results showed that eggs below 100 m are bigger but in smaller quantity, whereas those above 100 m are smaller but in bigger quantity. In accordance with this result, Özkazanç (1987) found a positive correlation between altitude and egg productivity in *T. wilkinsoni*.

Larval emergence vs unhatched egg ratio: Larval emergence ratio was higher in *T. wilkinsoni* ($p = 0.005$) and accordingly, unhatched egg ratio was higher in *T. pityocampa* ($p = 0.001$) (Table 3). We could not find any statistical correlation between these ratios and altitude ($p = 0.190$ and $p = 0.680$, respectively). On the other hand, larval emergence ratio seems to decrease and unhatched egg ratio to increase with increasing altitude in *T. pityocampa*.

Larval emergence studies in the relevant literature generally report annual comparisons and number of studies dealing with larval emergence comparisons among altitudes. In one such studies (Avcı and Ölmez, 2016) no difference has been found among different altitudes. Larval emergence ratio must be under the influence of several different factors from genetics to climate and to resolve the causes of the observed emergence ratios in this study is beyond the scope of this study.

Parasitism ratio: We found neither interspecific nor interaltitudinal difference in parasitoid emergence ratio ($p = 9.39 \times 10^{-7}$ and $p = 0.130$, respectively).

Records of parasitism on *T. pityocampa* eggs reveal a significant variability between 2.9 % and 72 % (Bellin et al., 1990; Schmidt et al., 1990). Similarly, *T. wilkinsoni* egg parasitism is quite variable with so far recorded extremes of 5 % and 65 % (Acatay, 1953; Avcı, 2000; Avcı and Oğurlu, 2002; Avcı and Ölmez, 2016; Can and Özçankaya, 2003; Mirchev et al., 2004, 2007; Nasr et al., 2013; Özkan, 1997; Sarıkaya, 2004; Şimşek et al., 2017). Causes of temporal and spatial variability of pine processionary moth egg parasitism is widely unknown. One of the factors is thought to be egg size as parasitism of smaller eggs is lower than larger eggs (Perez-Contreras and Soler, 2004). We found that egg size in *T. pityocampa* population sampled in this study is bigger than that of *T. wilkinsoni*; and that egg size *T. wilkinsoni* eggs below 100 m of altitude is bigger than that above 100 m. Although we expected correlated results in parasitism ratios (such as lower parasitism in *T. wilkinsoni* and in high elevation eggs due to smaller egg size), we could not find any significant relationship. On the other hand, parasitism rate in *T. pityocampa* (7.3 %) was slightly higher than that of *T. wilkinsoni* (4.9 %) (Table 3). Another factor that could possibly affect the parasitism rate is altitude, however, evidence is contradictory. For example, Avcı and Ölmez (2016) found a negative correlation between parasitism and altitude, whereas Tsankov et al. (1999) recorded parasitism ratios of 4.5 % and 16.4 % at altitudes of 300 m and 500 m, respectively. Sarıkaya (2004) also recorded higher parasitism at higher altitudes.

4. Conclusion and Future Perspectives

This study is the first attempt to compare the egg batch and egg characteristics between the two pine processionary moth species, *Thaumetopoea wilkinsoni* and *T. pityocampa*. We found significant differences between the two species in terms of egg arrangement, egg diameter, larval emergence and unhatched egg ratios. We also compared egg batch and egg characteristics intraspecifically among different altitudes. We found significant differences among altitudes in terms of egg batch circumference, clutch size and egg diameter.

Our sampling size was limited by the low population sizes of the processionary moths in the year of sampling and by the topography of the study regions where no pine locality presents above 300 m. Our results, on the other hand, show that the characteristics focused in this study could reveal more information if a larger data set could be acquired. Some of these characteristics (such as egg arrangement) would probably not be relevant to the understanding of the biology of the species that could further contribute to management practices, but some others (such as clutch/egg size) would. Therefore, these characteristics should be considered again in studies with larger sampling sizes.

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