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Synonymy between *Pholidoptera ebneri* Ramme, 1931 and *Pholidoptera stankoi* Karaman, 1960 (Orthoptera Tettigoniidae)

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Abstract

Pholidoptera ebneri was described from Albania in 1931. About a quarter of a century later, *P. stankoi* was described from the Republic of North Macedonia, assuming that a different mountain range should host different species of this genus. In the absence of reliable differentiation criteria, for decades entomologists have assigned *P. stankoi* to North Macedonia and Greece and *P. ebneri* to Albania. Considering proximity of the type localities and the basic morphological similarity of the species, we decided to carry out a morphological and acoustic study of these two species. In addition, we compared the songs with other species of the aptera/macedonica group. According to our results, we can state that *P. ebneri* and *P. stankoi* are the same species. The oldest name, *P. ebneri*, has priority and will be used as the species name. This species is clearly distinct from the other species of the aptera/macedonica group, but this group still needs to be taxonomically reviewed for some other species or subspecies.

Keywords: Albania, bioacoustics, morphology, North Macedonia

Zusammenfassung

Pholidoptera ebneri wurde 1931 in Albanien beschrieben und etwa ein Vierteljahrhundert später wurde *Ph. stankoi* in der Republik Nordmazedonien beschrieben, wobei angenommen wurde, dass verschiedene Gebirge auch verschiedene Arten dieser Gattung beherbergen sollten. Mangels verlässlicher Unterscheidungskriterien ordneten Entomologen jahrzehntelang *Ph. stankoi* Nordmazedonien und Griechenland und *Ph. ebneri* Albanien zu. Da die Typuslokalitäten nahe beieinander liegen, beschlossen wir, eine morphologische und akustische Untersuchung der beiden Arten durchzuführen. Darüber hinaus haben wir die Gesänge mit denen anderer Arten der aptera/macedonica-Gruppe verglichen. Nach unseren Ergebnissen können wir sagen, dass es sich bei *Ph. ebneri* und *Ph. stankoi* um die gleiche Art handelt. Der zuerst vergebene Name *Ph. ebneri* bleibt erhalten. Diese Art ist eindeutig von anderen Arten der aptera/macedonica-Gruppe zu unterscheiden, aber der Status weiterer Arten oder Unterarten dieser Gruppe muss noch genauer untersucht werden.

Schlüsselwörter: Albanien, Bioakustik, Morphologie, Nordmazedonien

Introduction

Pholidoptera ebneri was described by Ramme in 1931 based on three specimens collected in August 1918 in the mountains of Mali i Polisit (Librazhd district) in eastern Albania between 1200 m and 1600 m above sea level, close to the border with the Republic of North Macedonia (Ramme 1931).

In August 1955, Zora Karaman collected three specimens of the group *P. macedonica* Ramme, 1928, on Mt. Karaorman in the Republic of North Macedonia, and Mladen Karaman attributed them to a new species naming it *P. stankoi* (Karaman 1960). He did not give further details on the location and altitude of his discovery. This massif is located only about sixty kilometres from the area where *P. ebneri* was found. In his diagnosis, the author compared the titillators of his specimens with those of *P. aptera* (Fabricius, 1793) and of *P. macedonica,* but not with *P. ebneri.* He assumed that, with the exception of *P. macedonica*, all species of the *macedonica* group are associated with separate massifs: *P. ebneri* to Mali i Polisit Mt in Albania; in Bulgaria *P. rhodopensis* Maran, 1953 to the Rhodopes Mts, *P. buresi* Maran, 1957 to the Pirin Mts and *P. hoberlandti* Maran, 1957 to the Rila Mts. Subsequently, the last two taxa were synonymised with *P. rhodopensis* (Chobanov 2011).

Since its description, *P. stankoi* has also been reported from several mountain ranges in central and northwestern Greece (Willemse 1976, 1977,1984; Heller 1988; map in Willemse et al. 2018) and also from a valley not far from Karaorman Mt in the Republic of North Macedonia (Warchałowska-Śliwa et al. 2017). Findings of *P. stankoi* on Mt. Athos (Tilmans, Willemse & Willemse 1989) were later listed under *P. macedonica* by Willemse & Willemse (2008). Willemse et al. (2018) do not use the name *P. ebneri* and mention the data from Albania under the name *P. stankoi,* stating that the status of the subspecies of *P. macedonica* and the relationship with *P. stankoi* need to be re-evaluated. Lemonnier et al. (2014) attribute their unpublished data from Republic of North Macedonia to *Pholidoptera ebneri*/*stankoi*. All of these new data invalidate the initial assumption by M Karaman

(1960), that *P. stankoi* was endemic to Karaorman Mt and thus open the way to a synonymy with *P. ebneri.*

Kaltenbach (1965) published comparative figures of *Ph. ebneri* and *Ph. stankoi* (from the literature) and a discrimination table based on the descriptions by the two authors. Harz (1969) suggested a dichotomous key. The shape of the male subgenital plate and the size of the teeth on titillators - mentioned by Kaltenbach as diagnostic - do not differ between the specimens we analysed, and the different morphological criteria proposed by Harz (1969) to distinguish *P. stankoi* from *P. ebneri*, , do not seem to be reliable. Therefore, in this study, we considered all records of the two species as a lump "ebneri/stankoi", including the two types, and by statistical analysis of morphometric and song measurements we tested whether or not the resulting data formed two clusters, each one including each type, thus revealing two different species or only one.

Material and Methods

Morphological study

The general principle is to undertake a statistical analysis on a substantial number of specimens distributed across the range of *P. ebneri/P. stankoi*, based on the morphological characteristics used by the two authors of the species. The resulting cloud of data will then be examined to ascertain whether it exhibits two clusters or a single cluster. Table 1 lists the characters used by the authors of both species to characterise the types and by other authors to distinguish the two species. Kaltenbach (1965) was the first to propose comparative measurements on males. Harz (1969) proposed a dichotomous key based on the shape of the titillators in males, which is used to distinguish the two taxa. On page 337, the author provides drawings of the titillators of *Ph. ebneri* and *Ph. stankoi* (after Karaman 1960). In the case of females, the length of the pronotum and the ovipositor were employed as diagnostic characters. During the course of this study certain criteria were excluded because they are not informative for distinguishing the samples under examination from other taxa, such as the shape of the male cerci, the 10^{th} tergite of the male and the subgenital plate of the female.

For the males and females that we have in collection (dry and alcohol), we measured the lengths of: the body, the pronotum, the tegmina (uncovered part), the posterior femur for both sexes, and the ovipositor for the female. The body length could differ between dry specimens and some of the alcohol-fixed dissected animals (to extract male gonads) especially if it remained in alcohol for a long time. Although we tried to reshape the body of wet specimens before measurement, we accept that the variance of this variable could be higher than in reality. The samples were collected in three countries and their locations are shown in figure 1.

Table 1: Morphological characteristics used by the authors of the species. M = Measurement, D = Drawing.

Characteristics	Authors / year						
	P. ebneri				*P. stankoi*		
	Ramme 1931	Ramme 1951	Kaltenbach 1965	Harz 1969	Karaman 1960	Kaltenbach 1965	Harz 1969
♂							
Body length	M		M		M	M	
Pronotum length	M		M		M	M	
Hind femur length	M		M		M	M	
Hind tibia length	M						
Tegmina length	M		M		M	M	
Titillators		D		D	D		D
Cercus	D			D	D		
Subgenital plate	D						
10th tergite	D				D		
♀							
Body length	M				M		
Pronotum length	M			M	M		M
Hind femur length	M				M		
Hind tibia length	M						
Ovipositor length	M			M	M		M
Subgenital plate	D			D			

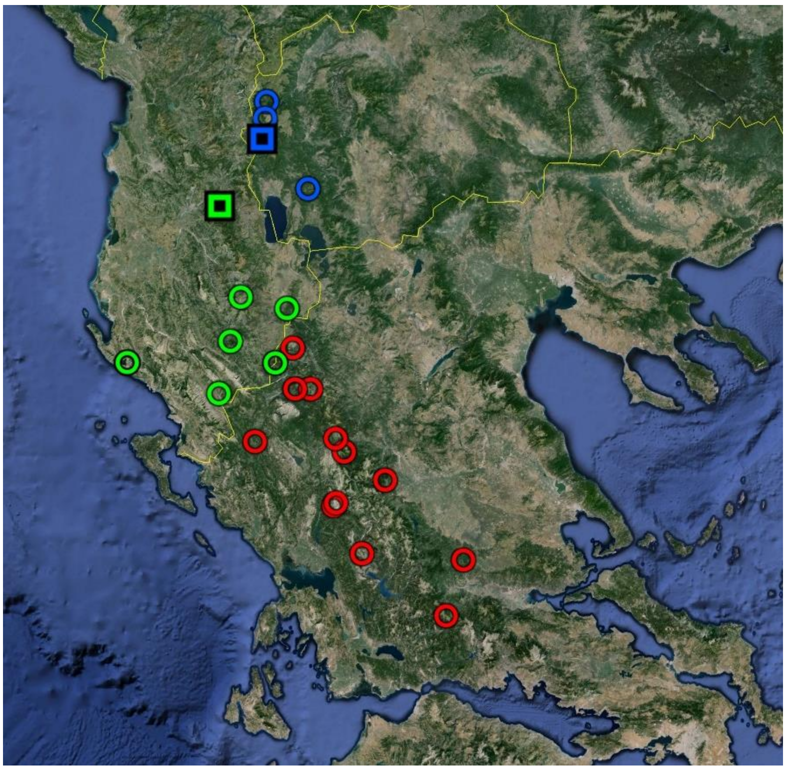

Fig. 1: Map of the study area. Square = type locality of *Ph. ebneri* (green) and *Ph. stankoi* (blue). Circle = localities of *Ph. ebneri/stankoi* specimen studied, green for Albania, blue for Republic of North Macedonia, red for Greece.

Analyses of the measurements were carried out using Excel and XLStat. Based on the morphological parameters, we used the Expectation-Maximisation (EM) algorithm (Dempster et al. 1977) on all samples, including the measurements provided by the authors of *P. ebneri* and *P. stankoi*, in order to extract clusters, if any. It is a method that aims to classify the samples by giving to them a probability of belonging to a subgroup. The Normalised Entropy Criterion (NEC) resulting of this processing was analysed as well as the Maximum *a posteriori* (MAP) graphs.

For the males we worked with 56 samples with 3 parameters (body length, pronotum length, uncovered part of tegmina length) and with a subset of 45 samples with 4 parameters (including the posterior femur length). The 56 samples are divided into 27 from Albania, 7 from the Republic of North Macedonia, and 22 from Greece. The 45 samples are divided into 17 from Albania, 7 from the Republic of North Macedonia, and 21 from Greece. For the females we worked with 12 samples, each sample characterised by 4 parameters (body length, pronotum length, hind femur length, ovipositor length). The 12 samples are divided into 7 from Albania, 2 from the Republic of North Macedonia, and 3 from Greece.

The graphical presentation of the results is carried out through a Principal Component Analysis (PCA) which presents the samples in a simple way, especially when the number of variables is greater than 3, by transforming the set of its variables into a smaller one that still contains most of the information of the large initial one, through a graph, usually in 2 dimensions, with main factors as axes. This is done by finding the best plane representation (R^2) of the data of R^k (k=4 in this analysis) that maximises the variance of the projected data on this plane (i.e. total projected distances to R^2 as close as possible to the total distances from the centroid in R^4).

Titillators (epiphallic sclerites in male genitalia) were extracted from randomly selected specimens, macerated in 10% potassium hydroxide and photographed for comparison.

Bioacoustics

For this study we evaluated own unpublished sound data and all published recordings of the calling songs of *Pholidoptera stankoi* and of *P. ebneri* with a total of 17 singing males (5 from Albania, 1 from Republic of North Macedonia, and 11 from Greece). For comparison, published data and own unpublished recordings of related species of the *Pholidoptera aptera/macedonica* group were also checked (Table 2) including similarly singing forms (see Heller 1988, p. 123) (4 males of *P. lucasi* and 3 males of *P. macedonica cavallae* from Greece). For making the recordings, a variety of microphones and analogue tape recorders as well as digital devices were used. Subsequently, all analogue recordings were digitised. Given that the songs under consideration invariably exhibited robust audio characteristics, it was deemed appropriate to utilise an audio frequency range of microphones. It was therefore anticipated that the microphones used would not exert any distorting effects on the temporal pattern of the sampled songs.

Table 2: Localities of the other species where songs were recorded and evaluated.

Species	Country	Locality	Source
Pholidoptera group "m-a-r"			
P. macedonica	Albania	Galichica Mt., Pikina voda	own recording
P. macedonica	Albania	Galichica Mt., Pikina voda	own recording
P. macedonica	North Macedonia	Nidzhe Mountains, above Skochivir village	own recording
P. macedonica	Greece	Drama, Kato Vrondou	OSF Heller
P. macedonica	North Macedonia	Korab Mt., Strezhimir	own recording
P. macedonica	Greece	Drama, Makedonia, near Elatia	own recording
P. macedonica	Greece	Drama, Makedonia, near Elatia	own recording
P. macedonica	Greece	Makedonia	Willemse et al. 2018
P. aptera aptera	Austria	Carintia, Turracher Höhe, Kormulde	OSF Ingrisch
P. aptera bulgarica	Bulgaria	East Rhodope Mts: Boubino village	own recording
P. aptera bulgarica	Bulgaria	Strandzha Mts., Malko Tarnovo - Gradishteto hill	own recording
P. aptera bulgarica	Greece	Makedonia	Willemse et al. 2018
P. aptera goidanichi	Italy	Apennine Mountains, N of Gaviserri, near Stia	OSF Horvat & Ivcovic
P. aptera karnyi	Serbia	Bojanine vode	OSF Ingrisch
P. aptera karnyi	Bosnia-Herzegovina	Durmitor, Celine	OSF Ingrisch
P. aptera karnyi	Bulgaria	Ruy Mt.	own recording
P. aptera karnyi	Bulgaria	Uzana place	own recording
P. aptera spp.	Bulgaria	Sushtinska Sredna Gora Mts	own recording
P. aptera spp.	Bulgaria	Lyulin Mt., Vladaya gorge	own recording
P. cf. aptera	Bulgaria	Pirin area. near Javorov	own recording
P. cf. aptera	North Macedonia	Maleshevska Planina mt., Ploshchica Place	own recording
P. rhodopensis	Bulgaria	Pirin Mts., above Bansko, Shili-garnika & above Bunderishka Polyana	
Pholidoptera lucasi			
P. lucasi	Greece	Evia, Dirphys area. near Katafigion	own recording
P. lucasi	Greece	Evia, Dirphys area. near Katafigion	own recording
P. lucasi	Greece	Evia, Dirphys area., pass above Steni Dirfios	own recording
P. lucasi	Greece	Pilio Mt., West of Zagora	own recording
Pholidoptera macedonica cavallae			
P. macedonica cavallae	Greece	Kavalla, Pangaion area	own recording
P. macedonica cavallae	Greece	Kavalla, Pangaion area	own recording
P. macedonica cavallae	Greece	Kavalla, Pangaion area	own recording

Song measurements were obtained using the audio editing software Audacity (http://audacity.sourceforge.net/), AmadeusII and AmadeusPro (http://www.hair-ersoft.com). Oscillograms of the songs were prepared using Turbolab (Bressner Technology, Germany). From each recording session, the mean of the echeme repetition rate is given with standard deviation, n=10 per series. Since the temporal pattern of the song changes with temperature, syllable and echeme repetition rates are presented in relation to this parameter, mostly measured as temperature of the ambient air, only in a few cases corrected by adding three degrees to compensate the effect of special recording lamp (Heller 1988). The temperature dependency of these rates is typically linear (Walker, 1975), allowing the calculation of regression lines.

With reduced number of data, any error on the temperature associated to some of the data could slightly impact the slope of the regression line. To eliminate the dependency of temperature, we introduced the ratio of SRR and ERR as new variable and analysed the structure of grouping of this variable, to confirm and/or complement the conclusions.

Bioacoustic terminology

Syllable is the sound produced during one cycle of movements, generated by opening and closing of the tegmina; **syllable period** is the time period measured from the beginning of a syllable to the beginning of the next (reciprocal value: syllable repetition rate [SRR]). A first-order assemblage of syllables is called **echeme** (=chirp; short series of syllables) with the echeme repetition rate (ERR) calculated as above. **Recording session** refers to all sound files of one animal obtained at the same date and the same temperature.

Results

Morphological analysis

The results of the expectation-maximization (EM) inference algorithm are as follows: With the 56 samples of males composed of 3 parameters, whatever the Gaussian mixture model used (equal or varying volume, round, equal or varying shape, and axis parallel, equal or varying orientation), we did not find any grouping within the set of the samples (NEC higher than 1). With the 45 samples of males composed of 4 variables (posterior femur length added), the algorithm did not converge before 100 iterations. It can be concluded that no cluster is present within the set of samples. However, it is notable that some samples collected in Greece at low altitude exhibit a longer posterior femur in average than the rest of the samples. With the 12 samples of females composed of 4 variables, the algorithm converged after 24 iterations to a structure that was not significant: the two distinct species types in question are found to fall within the same cluster. The remaining samples are distributed across the two clusters, exhibiting no discernible geographical base, and the MAP classification is not statistically significant with samples exhibiting substantial overlap. The low number of samples does not allow deeper analysis. Furthermore, females show quite a large variability, which makes it challenging to differentiate them from females of *P. macedonica*. Only the examination of a male from the same station can confirm the identification by analysing the shape of the titillators (Ramme 1951, Harz 1969).

Figure 2 shows the cloud of dots of the 45 samples of males fitted with 4 variables, along the main factors with two orthogonal presentations of the 3-dimensional structure: F1/F2 and F1/F3. F1+F2+F3 cover 91% of the total variance of the set of data. The letter E indicates the sample described by Ramme as *P. ebneri*, and the two letters S indicate the two samples described by Karaman as *P. stankoi*. Up to now all authors of respective data systematically labelled the specimens collected in North Macedonia as *P. stankoi*, and the specimens from Albania as *P. ebneri*. The specimens collected in Greece were always labelled *P. stankoi* by convention but those could had been labelled *P. ebneri*, as discrimination criteria are not reliable. Therefore, we showed the ellipses per country, instead of Albania in one side and the two other countries together in the other side in figure 2 with a confidence interval of 95%. These ellipses overlap but with the Greek ellipse slightly elongated due to particularly long hind femur of samples of the low altitude collected near Ioannina (at 500 m asl) and Karditsa (at 700 m asl). Hence, we analysed the relationship of all the samples with the altitude through a polynomial regression (5 degrees)(Figure 3). The conclusion is that the altitude alone cannot explain this phenomenon, and we should find other environmental covariates to better understand these variations (climate, temperatures, hygrometry, etc.). However, these samples are located far from the locations of the two respective types, and do not change our conclusion concerning the similarity between the two taxa.

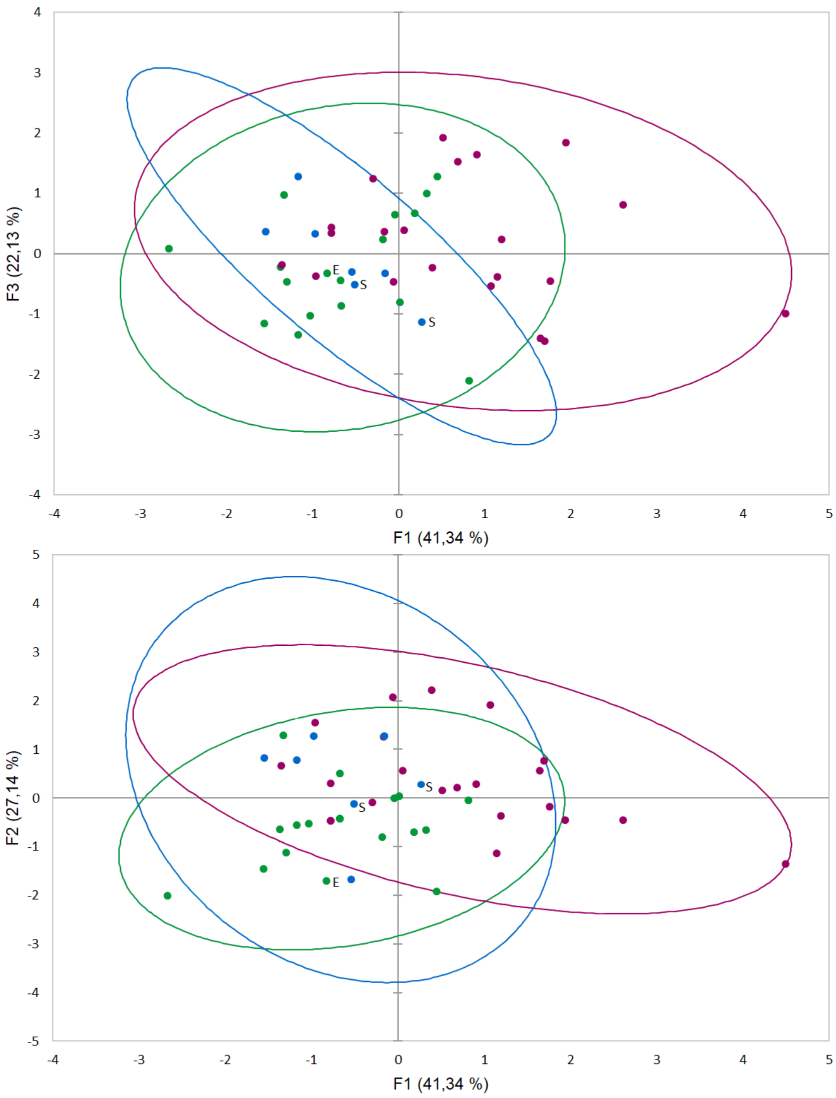

Fig. 2: PCA of samples in F1/F2 and F1/F3. In blue, samples from Republic of North Macedonia; in green, from Albania; in red, from Greece.

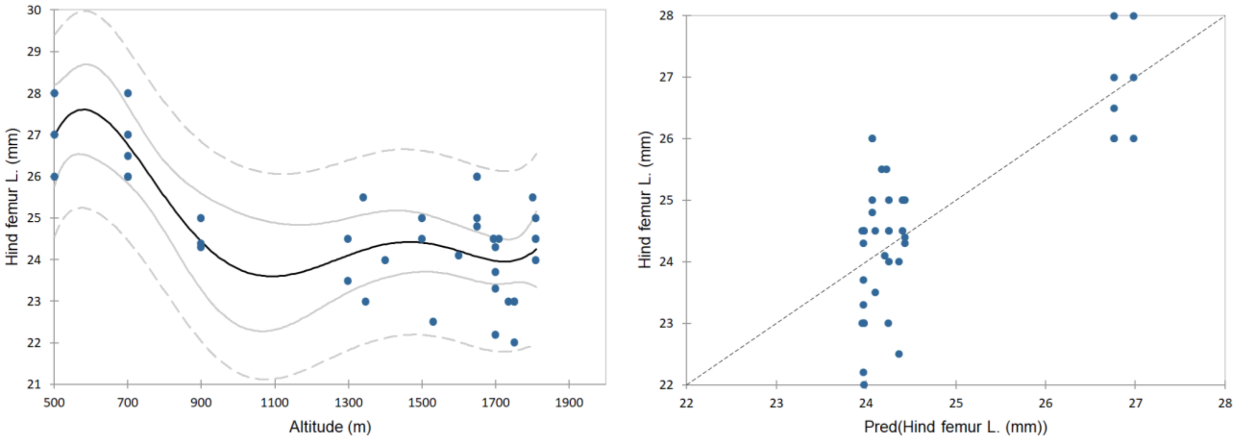

Fig. 3: Polynomial regression of the length of posterior femur by the altitude.

Figures 4 and 5 show examples of titillators. We note a great variability within the same population, sometimes even between the two titillators of the same individual. The average shape characterises *P. ebneri/stankoi*. Thanks to this average and variability, it should be noted that the titillators of *P. ebneri* presented in Harz (1969) on page 357 are not clearly distinguishable from those of *P. stankoi* on page 337. Both belongs to this average shape according to potential variability.

Fig. 4: Titillators arms, general shape: A = Mali-i-Polisit, AL; B = Ribnichka Reka valley, NMK; C = Cika Mt, AL; D = Valtou Mt, GR; E = Ostrovica Mt, AL; F = Ohrid, NMK; G = Karditsa, GR; H = Evritania, GR.

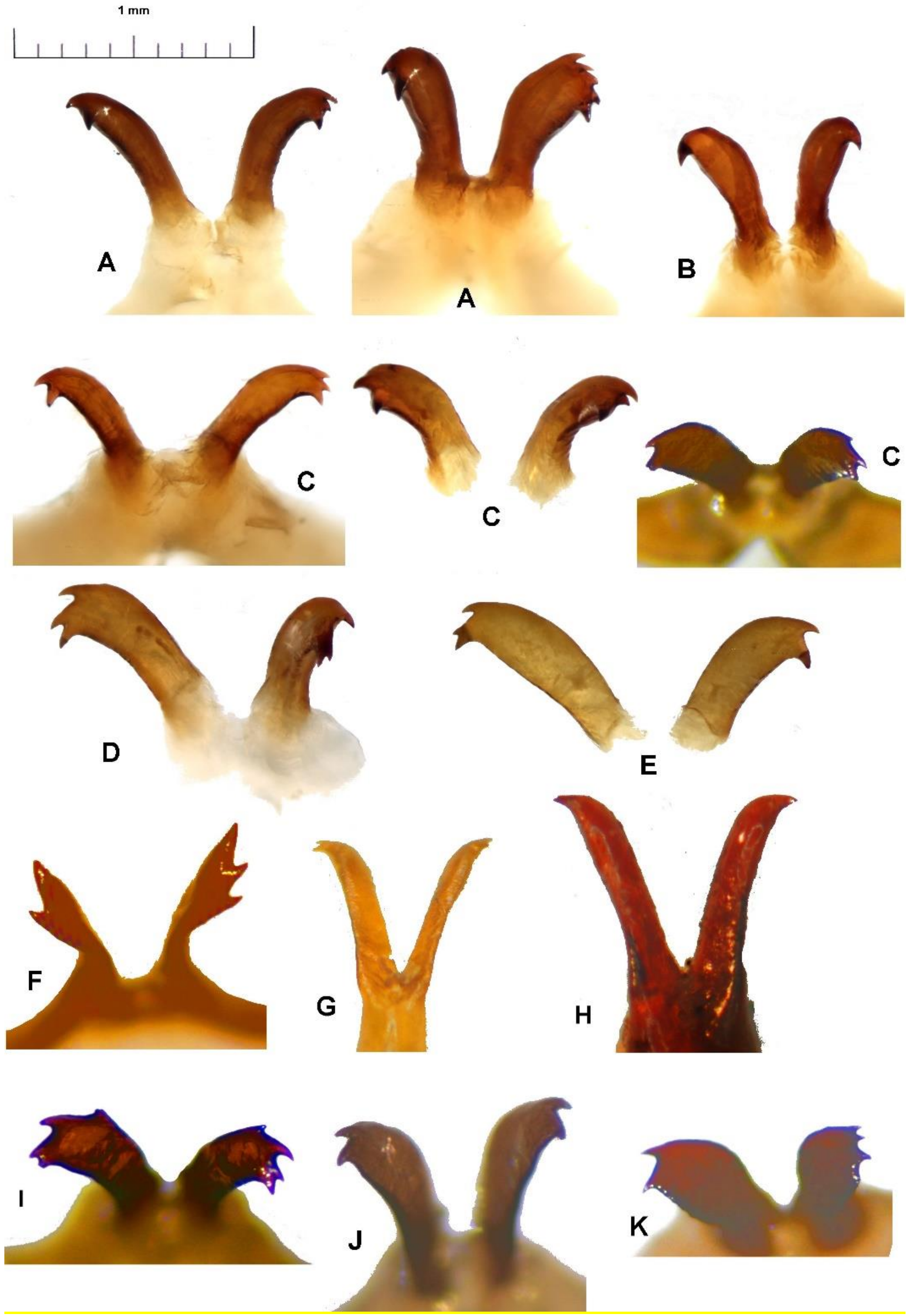

Fig. 5: Titillators arms, tips: A = Mali-i-Polisit, AL; B = Ribnichka Reka valley, NMK; C = Cika Mt, AL; D = Valtou Mt, GR; E = Ostrovica Mt, AL; F = Ohrid, NMK; G = Karditsa, GR; H = Evritania, GR; I = Permet, AL; J = Buretos Mt, AL; K = Kamenik Mt, GR/AL.

Bioacoustical analysis

In all species of the *Pholidoptera aptera* group sensu lato the calling song consists of sequences of three- to four-syllabic echemes, repeated at intervals of several seconds (Heller 1988). The length of these sequences is relatively variable, typically consisting of 20-30 echemes. Often within a sequence a slight ritardando can be recognized. Based on song pattern, within the group several subgroups can be recognized, differing in syllable (SRR) and echeme (ERR) repetition rate. The song of the specimen collected at the type locality of *P. ebneri* (Fig. 6) clearly belongs to one subgroup which can be separated from the others by a distinctly higher ERR (Fig. 7). All specimens of the formerly called *P. stankoi* belong to the same subgroup. Since in insects all song pattern related parameters depend on body temperature, it is necessary to have at least an estimate of this external factor, e.g. by measuring the ambient air temperature. Besides specimens of the formerly called *P. stankoi* the subgroup contains only species occurring on the West coast of the Aegean sea, i.e. *P. lucasi* and animals from Mt. Pangaion in Greece identified as *P. macedonica cavallae*. The songs of the members of this subgroup can at present not be discriminated, except perhaps those of *P. macedonica cavallae*, which may show slightly lower ERRs. It should be noted that up to now in Greece Western (and Southern) of Florina no specimens singing with a low ERR have been recorded (see Table 2 and Heller 1988) although animals identified as *P. macedonica* seem to be quite widespread. In addition, syntopic occurrences of slow and fast singing forms were not found anywhere as yet. In SRR the species of the *P. ebneri* subgroup sing also faster than the representatives of the other two subgroups (Fig. 7), as also described earlier (Heller 1988, fig. 12), but the difference is not large enough to allow always a safe identification.

The analysis of the variable “SRR div ERR” lead to the same conclusion (Table 3) with, on one side the *P. ebneri* group (“e”), and on the other side *P. aptera* group (“a”) clearly separated, and intermediate positions for *P. lucasi* and *P. macedonica cavallae*. The lower reliability for these two later taxa is linked to the low number of samples. Within the *P. ebneri* group, it was not possible to separate two sub-groups (i.e. *P. ebneri* and *P. stankoi*).

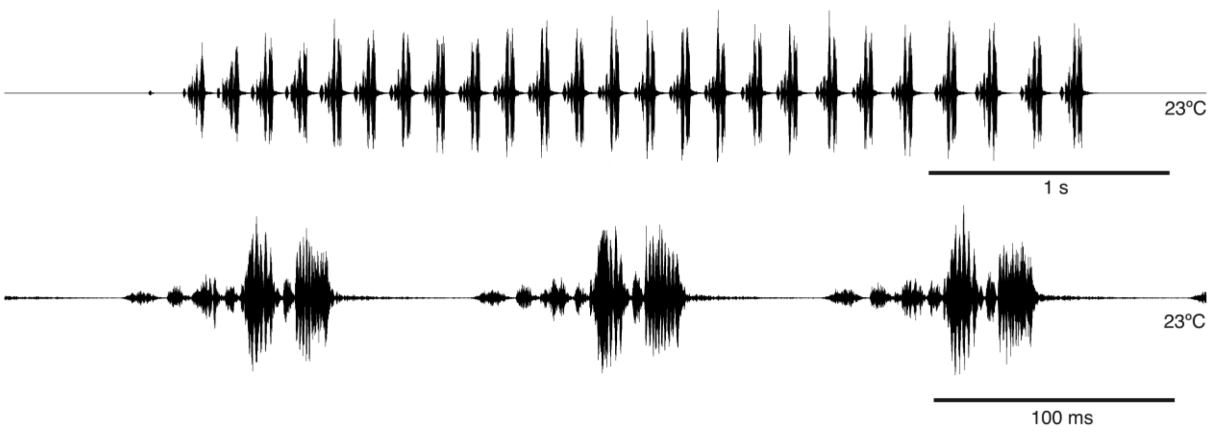

Fig. 6: Oscillograms of the calling song of *Pholidoptera ebneri* from the type locality. 5 s and 500 ms sections, respectively.

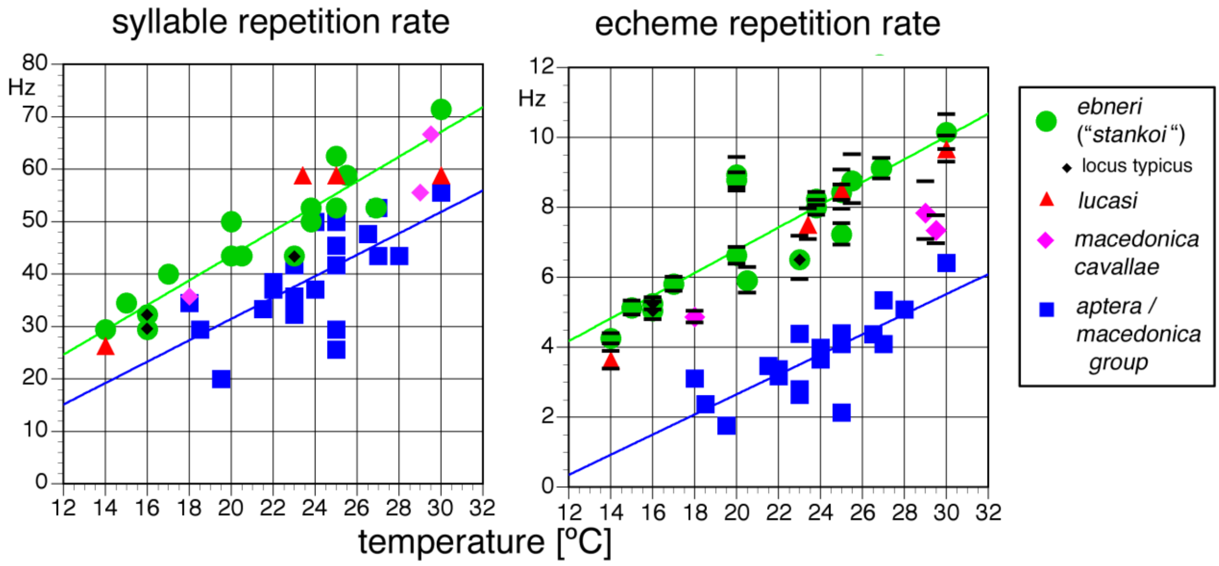

Fig. 7: Syllable repetition rate (SRR) on the left and echeme repetition rate (ERR) on the right, of the calling song of members of the *Pholidoptera aptera* group s.l., depending on temperature. Each point represents a different recording session. On the ERR graph, the standard deviation of each result is represented by horizontal black stripes.

Table 3: ANOVA analysis of SRR/ERR variable. SE = Standard error, Lower = lower threshold at 95%, Upper = upper threshold at 95%. Significant results in bold.

Species	Mean SRR/ERR	SE	Lower	Upper	P	Comment
Group *Pholidoptera aptera/ macedonica/rhodopensis*	10.773	0.266	10.235	11.310	**<0.001**	Group "a"
Pholidoptera macedonica cavallae	7.671	0.721	6.216	9.127	0.576	Hypothesis that data cannot be separated from group "a" or group "e" cannot be excluded
Pholidoptera lucasi	7.029	0.625	5.769	8.289	0.066	Hypothesis that data cannot be separated from group "e" cannot be excluded
Pholidoptera ebneri	6.503	0.303	5.892	7.115	**<0.001**	Group "e"

Discussion

In the light of the presented results, we can state that *P. ebneri* and *P. stankoi* are the same species. As *P. ebneri* is described earlier, this name has a priority. The literature list for *P. ebneri*, should now include *P. stankoi* syn.nov. as follows: Karaman 1960 ["Karaorman-Gebirge 21.VIII.55. leg. Dr. Z. Karaman."]; Kaltenbach 1965; Us & Matvejev 1967; Harz 1969; Willemse 1976; Willemse 1977; Willemse 1984; Heller 1988; Chobanov & Mihajlova 2010; Lemonnier et al. 2014; Warchałowska-Śliwa et al. 2017; Willemse et al. 2018.

Despite the low altitude of occurrence and longer hind femora, specimens from Ioannina and Karditsa area do not differ acoustically from the other studied specimens of *P. ebneri*. Specimens from the latter localities should be investigated further to better understand the relationship of some environmental covariates with their size parameters.

P. lucasi has a song which is very similar to that of *P. ebneri* but the genitalia are distinct (Willemse 1976). The situation with *P. macedonica cavallae* is more difficult because the song is also quite similar, but data is based on a very low number of records. The differences in male genitalia are much less pronounced than between *P. lucasi* and *P. ebneri*. We prefer to retain its current taxonomic status until more data are available.

Our study has focused on the particular taxonomic case concerning the status of *P. ebneri* and *P. stankoi*, syn.n. but comparisons with other closely related taxa opened new questions. As a result, a revision of the *P. aptera* group including *P. macedonica, P. rhodopensis, P. lucasi*, and all the subspecies of *P. aptera*, including DNA study, needs to be carried out in order to better clarify their taxonomic status.

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