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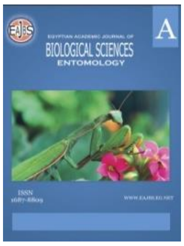

Morphometric Comparison Between Different Populations of Darkling Beetles "*Pimilia nilotica* (Coleoptera: Tenebrionidae)" In Egypt

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ABSTRACT

Insects include almost 60% of the described eukaryotic animals worldwide. However, the morphological measurements and assessment of the effects of isolation and habitat still lack many insect groups. The current study aimed to compare the morphometric measurements of dissimilar populations of darkling beetles "*Pimelia nilotica*" in Egypt. A variable comparison of morphometric variations was taken by a threaded micrometer for six different eco-geographical regions from Egypt fall under the Western Mediterranean Coastal Desert, Western Desert Oases, El-Faiyum Depression, the Eastern Desert, Nile Delta, and South Sinai. Twenty-one morphometric measurements were taken in the currents study. Traits best reminiscent of the excellence of populations were distinguished by cluster analysis, and also by the principal component analysis. The primary and second discriminants scores (score 1 and score 2) recorded 76.4% and 23.8%, respectively, of the full variation in samples. Multiple discriminant analysis disclosed clear morphometric differences between Western Desert, Eastern Desert, El-Faiyum Depression, and South Sinai populations. Traits clarifying the maximum of the changeability among populations were those associated with the morphological estimations.

INTRODUCTION

The location of Egypt between the faunal areas of the world is fairly unique since it consolidates the qualities of both Palaearctic and Afrotropical districts. It generally belongs to the Palaearctic; however, the Afrotropical elements seem to be more frequent than normally suspected (Steyskal and El Bialy, 1967). Egypt is a portion of the Great Desert Belt with a generally warm and rainless atmosphere. Just the Coastal Strip, Gebel Elba, and higher mountains of southern Sinai get relatively a great rainfall, which reflects their composition of flora and fauna. Some ecologists divide Egypt into eight ecological regions, in particular: The Coastal Strip, lower Nile Valley and Delta, upper Nile Valley, El-Faiyum Depression, Eastern Desert, Western Desert, Gebel Elba, and Sinai (Larsen, 1991; El Hawagry and Gilbert, 2014).

Although insects include almost 60% of the described eukaryotic animals worldwide, the morphological measurements and comparison of the effect of isolation and habitat still lack for most insect groups, including the beetles (Coleoptera) (Riad, 2019). Family Tenebrionidae is one of the largest families of the order Coleoptera, which includes more than 20000 known species of nearly 1700 genera of worldwide distribution (Booth *et al.*, 1990 and Bream *et al.*, 2019). Despite the fact that among all families of beetles, Tenebrionidae (darkling beetles) positions 7th as far as species diversity, the absence of information of the phylogeny and systematics of this group and its subfamilies and clans (for example, it's monophyly) has been questioned (Hassan *et al.*, 2017a). *Pimelia nilotica* (Sénac, 1884) (Coleoptera: Tenebrionidae) is a typical black darkling beetle found in the great Sahara (the Middle East and northern Africa) (Ghahari *et al*., 2010a; 2010b). *Pimilia* adaptation to arid climates and desert environments allows surviving and production in the dunes, morphological adaptations including lipid layer, epicuticle, fused sclerites, and subelyteral cavity. These morphological adaptations make it a good example of studying isolated areas and the effect of isolation (Hassan *et al.*, 2015; Riad, 2019).

The morphometrics has been considered as the first method used in the biological studies for discovering biodiversity (Wanek and Sturmbauer, 2015) and resolving phylogenies (Klingenberg and Marugan-Lobon, 2013). The morphometric measurements are ordinarily utilized in the integrated approach to systematics sideways through molecular information (Klingenberg and Marugan-Lobon, 2013), which may result in taxonomical revision (Ober and Connolly, 2015).

The most significant questions are which morphological characteristics would be taken for assessment. The redundancy of utilization of many characters can lead to a disturbance of obtaining results to resolve the phylogeny dependent on the morphological differences. Linear discriminant analysis (LDA) is one of the utmost communal devices for discovery appropriate measurements (van Rensburg *et al.*, 2003). Morphometric measurements might be helpful in establishing phylogeny, especially of species that are difficult to recognize because of few or even no diagnostic traits (De Bivort *et al.*, 2010; Navia *et al.*, 2015). The present study aimed at elucidating potential sub-specific differences in different Egyptian populations of *P. nilotica* in different ecoregions in Egypt by using the cluster and LDA.

MATERIALS AND METHODS

The Study Area:

The study was conducted from 2018 to 2019 at 14 different localities of Egypt (Table 1). Pitfall traps were used to collect the *P. nilotica* populations from the study sites. Three different localities were selected at each site. Traps were designed and distributed, as well as the collection of samples was performed according to the method reported in Hassan *et al* (2017). The identification of specimens was based on the keys provided by Löbl and Smetana (2008). The collected specimens were deposited at Al-Azhar University Zoological Collection, Faculty of Science, Al-Azhar University. The geographical position and altitude of each site were recorded using a Garmin eTrex 30 GPS; the study area map was designed with ArchGis 10.2 software programs. The study area was classified into 6 different eco-geographical regions of Egypt (Table 1 and Fig. 1).

Table 1: Ecoregions, coordinates, and museum numbers of specimens of *Pimilia nilotica* collected during the period from 2018 to 2019.

Locality		Coordinations		Specimen Numbers	Museum Number
		Latitude	Longitude		
Bagush, North Coast, Matruh	WMCD	31.10422 N	27.41474 E	36	IC01890 - IC01926
Mersa Matruh, Matruh		31.30902 N	27.29444 E	23	IC01927 - IC01949
Siwa Oasis	WDO	29.18073 N	25.47638 E	11	IC01950 - IC01961
Bahariya Oasis		28.27596 N	28.80067 E	13	IC01961 - IC01974
Farafra Oasis		27.07763 N	27.97546 E	15	IC01974 - IC01989
Dakhla Oasis		25.49454 N	28.97892 E	17	IC01989 - IC02006
Kharga Oasis		24.67444 N	30.60799 E	16	IC02006 - IC02022
El-Faiyum Depression		29.27999 N	30.57639 E	45	IC02022 - IC02067
Wadi El-Tarfa, Ras Gharib	ED	28.26184 N	32.16267 E	19	IC02067 – IC02086
Wadi Dabr, Marsa Allam		25.15159 N	34.39105 E	23	IC02086 - IC02109
10th of Ramadan City	ND	30.32248 N	31.78177 E	23	IC02109 - IC02132
El Salhiya City, Sharquiya		30.85409 N	32.06419 E	29	IC02132 - IC02161
Sharm El Sheikh, South Sinai	SS	27.84990 N	34.22448 E	22	IC02161 - IC02183
Taba, South Sinai		29.53391 N	34.70312 E	26	IC02183 – IC2209

IC: Insect Coleoptera, WMCD: Western Mediterranean Coastal Desert, WDO: Western Desert Oases, ED: Eastern Desert, ND: Nile Delta, SS: South Sinai.

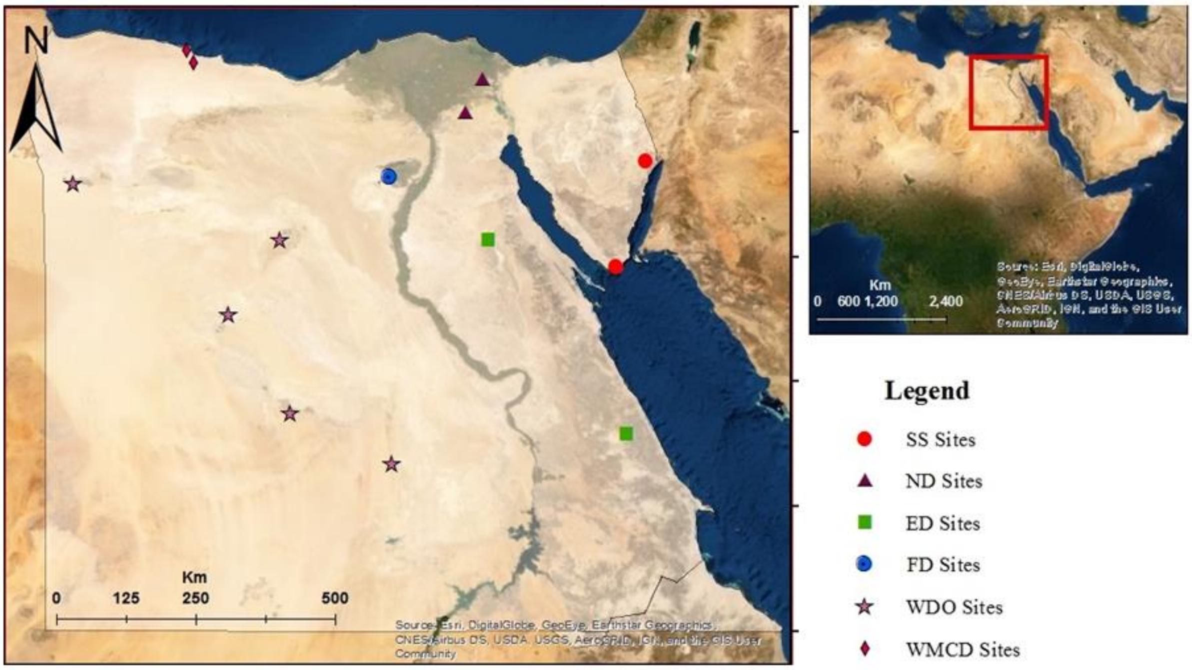

Fig. 1: Collection sites of *Pimilia nilotica* in different eco-geographical regions in Egypt. WMCD: Western Mediterranean Coastal Desert, WDO: Western Desert Oases, FD: El-Faiyum Depression, ED: Eastern Desert, ND: Nile Delta, SS: South Sinai,

Specimens' Measurements:

Morphometric analyses were performed on all 318 specimens of *P. nilotica*. For each specimen, an eyepiece micrometer was used to measure 17 absolute morphometric characters and 4 ratios (Table 2).

Table 2: Morphological measurements used in this study and their definition.

Symbol	Definition
AL	Antenna length, the total length of pedicel plus flagellum
HL	Head length, taken dorsally from the anterior border of the head to the margin of pronotum
HW	Head width, measured behind the eyes
PL	Pronotum length, taken dorsally from the anterior to the posterior border at scutellum
PW	Pronotum width (maximum), taken perpendicular to the pronotum length
EL	Elytron length, the distance between the anterior border of the scutellum and posterior border of the elytron
EW	Elytron width, the maximum width of elytron
BL	Body length = HL + PL + EL
FF	Fore femur length
FT	Fore tibia length
FTa	Fore tarsus length
MF	Mesofemur length
MT	Mesotibia length
MTa	Mesotarsus length
MeF	Metafemur length
MeT	Metatibia length
MeTa	Metatarsus length

Statistical Analysis:

The variances in each morphometric measurement between samples were examined with F- test (with Dunns post-hoc test) using the statistical package for social sciences (SPSS) computer software (version 20, SPSS Inc, Chicago, Illinois, USA) according to the method of Levesque (2007). The LDA was conducted in the NCSS11 statistical analysis software (NCSS, LLC, Kaysville, UT, USA). Probability at $P<0.05$ is considered significant.

RESULTS

A comparison of *P. nilotica* populations collected from six different eco-geographical areas based on 17 absolute morphometric characters and 4 ratios indicated significant differences between the six studied populations. Regarding the total width of elytra, the Eastern Desert, Nile Delta, and South Sinai populations were recorded the lowest for elytra width, also recorded high ratios of elytron width/elytron length. Regarding the El-Faiyum Depression population was recorded the highest for elytra width (Table 3).

Comparing all morphological characters, using cluster analysis showed that the six sampled populations are clustered in three discrete groups (Fig. 2). The first cluster consisted of the populations of the Eastern Desert, Nile Delta, and South Sinai. The second cluster contained Western Mediterranean Coastal Desert and Western Desert Oases populations. The third cluster contained the El-Faiyum Depression population only.

The LDA based on 21 variables showing significant intergroup variables indicated that the first and second discriminant scores (score 1 and score 2) recorded 76.4% and 23.8%, respectively, of the total variation in samples (Table 4). Factor loading values for each measurement are shown in Table (5).

According to LDA, a clear similarity between the four populations of Western Mediterranean Coastal Desert, Western Desert Oases, the Eastern Desert, and Nile Delta was detected (Fig. 3). These specimens distinctly separated from South Sinai specimens, also, El-Faiyum Depression population appeared as a distinct group different from all other populations. According to both the cluster and LDA statistical analysis, the populations of *P. nilotica* from South Sinai and El-Faiyum Depression showed a higher phenetic distance from the other studied populations.

Table 3: Morphometric measurements (mm) for *Pimilia nilotica* specimens from six different ecoregions areas of Egypt.

	WMCD (n=59)	WDO (n=72)	FD (n=45)	ED (n=42)	ND (n=52)	SS (n=48)	F value
TL	32.79±0.08	32.80±0.10	32.60±0.10	32.70±0.10	32.80±0.10	32.79±0.10	23.768
AL	10.39±0.08	10.40±0.10	10.42±0.10	10.30±0.10	10.40±0.10	10.39±0.10	13.818
HL	3.79±0.08	3.40±0.10	3.41±0.10	3.40±0.10	3.50±0.10	3.39±0.10	92.421
HW	7.49±0.08	7.50±0.10	7.40±0.10	7.40±0.10	7.50±0.10	7.49±0.10	21.555
PL	6.21±0.07	6.10±0.10	6.11±0.10	6.10±0.10	6.20±0.10	6.21±0.08	24.398
PW	12.19±0.08	12.20±0.10	12.21±0.10	12.10±0.10	12.20±0.10	12.19±0.10	32.353
EL	22.39±0.08	22.40±0.10	22.41±0.10	22.30±0.10	22.40±0.10	22.49±0.10	31.039
EW	17.79±0.08	17.80±0.10	17.82±0.10**	17.60±0.10 **	17.70±0.10 *	17.69±0.10*	2.482
FF	8.66±0.05	8.66±0.05	8.63±0.05	8.57±0.05	8.67±0.05	8.65±0.05	78.062
FT	6.69±0.08	6.70±0.10	6.50±0.10	6.60±0.10	6.70±0.10	6.69±0.10	38.461
Fta	4.51±0.09	4.52±0.10	4.50±0.10	4.50±0.10	4.52±0.10	4.49±0.10	31.901
MF	10.68±0.09	10.68±0.10	10.64±0.10	10.58±0.10	10.68±0.10	10.69±0.10	5.117
MT	7.76±0.11	7.77±0.12	7.67±0.12	7.67±0.12	7.77±0.12	7.79±0.10	8.788
Mta	6.49±0.08	6.50±0.10	6.60±0.10	6.40±0.10	6.40±0.10	6.49±0.10	31.229
MeF	11.29±0.08	11.30±0.10	11.20±0.10	11.40±0.10	11.30±0.10	11.29±0.10	22.927
MeT	10.49±0.08	10.50±0.12	10.60±0.12	10.40±0.12	10.42±0.12	10.49±0.10	9.083
MeTa	6.09±0.08	6.10±0.10	6.12±0.10	6.12±0.10	6.10±0.10	6.09±0.10	4.668
HW/HL	2.15±0.03	2.21±0.03	2.15±0.03	2.24±0.03	2.14±0.03	2.25±0.04	56.450
PW/PL	1.96±0.01	2.00±0.02	2.02±0.02	1.95±0.01	1.97±0.01	2.04±0.01	72.502
EW/EL	0.79±0.00	0.79±0.00	0.70±0.00	0.76±0.00	0.79±0.00	0.79±0.00	13.668
EL/PL	3.61±0.03	3.67±0.04	3.60±0.04	3.60±0.04	3.61±0.04	3.69±0.04	9.840

Data are presented as mean ± standard deviation. Values with different letters in the same row were significantly different. P<0.05* = significant. WMCD: Western Mediterranean Coastal Desert, WDO: Western Desert Oases, FD: El-Faiyum Depression, ED: Eastern Desert, ND: Nile Delta, SS: South Sinai, TL: total length, AL: antenna length, HL: head length, HW: head width, PL: pronotum length, PW: pronotum width, EL: elytron length, EW: elytron width, FF: fore femur length, FT: fore tibia length, FTa: fore tarsus length, MF: mesofemur length, MT: mesotibia length, MTa: mesotarsus length, MeF: metafemur length, MeT: metatibia length, MeTa: metatarsus length, HW/HL: head width/head length, PW/PL: pronotum width/pronotum length, EW/EL: elytron width/elytron length, El/PL: elytron length/pronotum length.

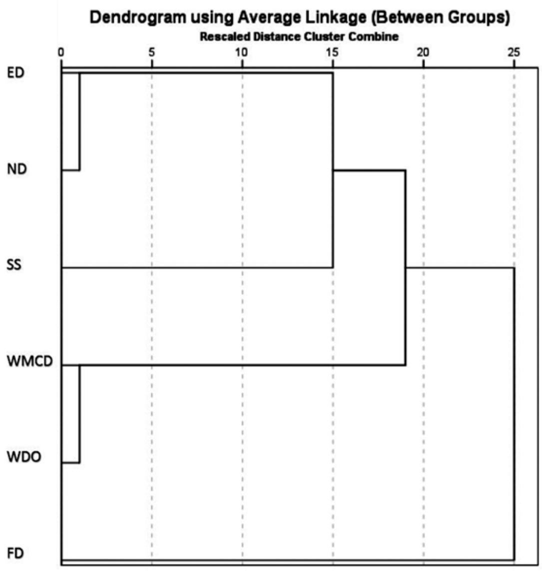

Fig. 2: A dendrogram showing the similarity between *Pimilia nilotica* populations of Western Mediterranean Coastal Desert (WMCD), Western Desert Oases (WDO), El-Faiyum Depression (FD), Nile Delta (ND), Eastern Desert (ED), and South Sinai (SS) based on 17 morphological measurements and 4 ratios.

Table 4: Results of linear discriminant analysis based on 21 morphologic variables.

Linear Discriminant Analysis		
	Score 1	Score 2
Eigen value	11.73	3.42
Explained (%)	76.4	23.8
Cumulative (%)	77.6	49.2

Table 5: Factor loading values for each morphological character

Character	Score 1	Score 2
TL	0.0003441	-0.002178
AL	0.0022505	-0.002995
HL	-0.0062981	0.0008794
HW	-0.0023295	-5.092334
PL	-0.0035364	2.5096337
PW	-0.0003441	0.0021747
EL	6.9486440	0.0018662
EW	-0.0019531	-0.0160443
FF	-0.0010464	0.0185923
FT	-0.0003441	0.0021783
Fta	-0.0014749	1.6948125
MF	-0.0006352	-0.0011271
MT	-0.0005876	0.0138356
Mta	-0.0019172	3.1919205
MeF	0.0002972	-3.8741540
MeT	-0.0001989	2.4429E-0
MeTa	0.0014212	0.0009456
HW/HL	0.0039018	-0.0006467
PW/PL	0.0018896	-0.0001006
EW/EL	-7.6252458	-0.0006771
EL/PL	0.0039537	-0.0002024

TL: total length, AL: antenna length, HL: head length, HW: head width, PL: pronotum length, PW: pronotum width, EL: elytron length, EW: elytron width, FF: fore femur length, FT: fore tibia length, FTa: fore tarsus length, MF: mesofemur length, MT: mesotibia length, MTa: mesotarsus length, MeF: metafemur length, MeT: metatibia length, MeTa: metatarsus length, HW/HL: head width/head length, PW/PL: pronotum width/pronotum length, EW/EL: elytron width/elytron length, El/PL: elytron length/pronotum length

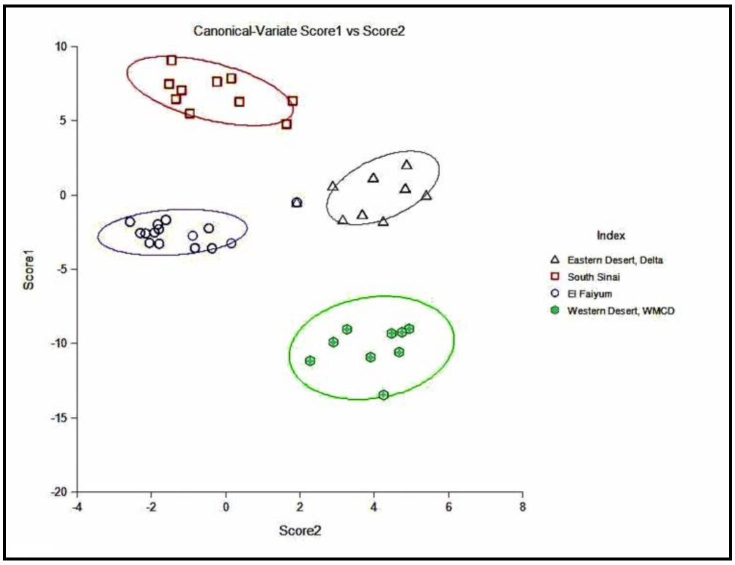

Fig. 3: Principle component analysis of the morphological measurements of the eight *Pimilia nilotica* populations

DISCUSSION

When comparing geographically separated populations by employing morphometric information set, it will exert some influence on the ascertained variations (Mamuris *et al.*, 1998). The current study attempted to minimize additional variances through size standardization, data transformation, and by acting separate LDA analysis. Extensive variation in morphometric variables occurred between the intentional populations. Besides, within the Western Desert studied populations, the Siwa, Bahariya, Farafra, Kharga Oases, and Western Mediterranean Coastal Desert populations were most similar. Within the Eastern Desert populations, Ras Gharib and Mersa Allam populations were also greatest similar, whereas the South Sinai and El-Faiyum Depression populations of dark battles were recorded dissimilar. This is often supported by not only the cluster analysis but also by LDA.

The variables of primary importance in separating the South Sinai and El-Faiyum Depression populations were associated with the large size of *P. nilotica* specimens in comparison with different populations (Hassan *et al.*, 2015). However, the comparatively high discriminant of the Western and Eastern Deserts, South Sinai, and El-Faiyum Depression populations variables suggested representation of subspeciation of *P. nilotica*. These four ecogeographical areas are separated from one another by huge geographical barriers that prevent the transformation of the studied species between them, additionally to the limited home range of this species (Badry *et al.*, 2017).

At present, the Nile River and its narrow floodplain act as a barrier for desert fauna dispersion separating the Western and Eastern Deserts in Egypt. Actuality the closest to the Nile Valley and subsequently relating to the River Nile via an enormous water system channel (Bahr Yusuf), El-Faiyum Depression is considered as a portion of the Nile Section. The lowermost part of the depression is involved by a shallow saline lake “Qarun Lake”, which is about 4.5 m under sea level and almost 200 km^2 in area. The depression has a whole area of around 1700 km^2. Its floor simply over the lake level is near 23 m above sea level (Ball, 1939). El-Faiyum Depression population of *P. nilotica* was isolated during this space from alternative desert populations studied in this study. In early geological time, lakes and wetlands of the Ismuth of Suez (which is now traversed by the Suez Canal) expanded greatly and extensive marshland conditions developed close this gate to Africa. The Gulf of Suez, by its shallow outline, appears to have stayed an uncovered bowl all through the majority of the Pleistocene, and until about 14-15 thousand years past, as soon as sea levels rose overhead near 50 m, linking the Sinai Peninsula to the Eastern Desert (Derricourt, 2005; Bailey *et al*., 2007 and Haggag *et al.*, 2018). While through drier times of the Pleistocene, the Gulf of Suez was compact in the zone also the Sinai Peninsula was readily accessible from the Eastern Desert with the two areas making one, to a great extent persistent dry zone (Riad, 2019). This can be possibly the explanation for the similarity between South Sinai and Eastern Desert *P. nilotica* populations.

During forceful atmosphere changes, a few species and populaces will endure exclusively in spaces with an extra steady climate, named refugia. There are a few impacts on a few species, for example, Carabidae and Tenebrionidae (Hassan *et al.*, 2017a; 2017b and Riad, 2019). It is very conceivable that the procedures of variation and speciation in genus *Pimelia* were intensely affected by contractions and succeeding expansions of series of populaces owing to the climate alteration. For instance, two populaces of one species may have been isolated in two dissimilar refugia, which can affect in allopatric speciation. Isolation in mountainous refugia will result in patterns in morphological variety, which is in numerous aspects constant with phylogeny (Ober and Connolly, 2015; Badry *et al.*, 2018). However, all distinguished *Pimelia* species have similar ecological niches, which can lead to similar adaptations to the environment. That's why the likelihood that a lot of parts of morphology don't reflect phylogeny, yet basically adapt to the basic condition, which is necessary to be considered (Hassan *et al.,* 2017).

Spatial and temporal expansion and contraction of desert conditions within the Sahara seem to act as a very important driver of faunal diversification and speciation events. Palaeoclimatic cycles continually adjusted the boundaries between the desert, alternative environments, and their associated biodiversity (Dumont, 1982; Le Houerou, 1992; 1997; Drake *et al.*, 2011). Vicariance events associated with Saharan aridity episodes become the most diversification force for post-Pleistocene allopatry (Douady *et al.*, 2003; Nyari *et al.*, 2010; Bream *et al.*, 2017). Such events are believed to result in allopatric isolation, which in turn induced the interruption of gene flow, and therefore the evolution of freelance lineages or new species.

The response of a given animal taxon to Saharan vicariant events varies in line with the habitat requirements. Throughout humid periods, desert-adapted animals become restricted to remaining desert habitat fragments or the remaining arid core of the Sahara. In their isolation, they are likely to undergo morphological and genetic allopatric diversification (Boratynski *et al.,* 2012). During a subsequent arid episode, isolated populations of desert-adapted species can expand their ranges, presumably merging the various meta-populations into larger populations. If the previous allopatric divergence was not enough to result in reproductive isolation, gene mixing will take place and a uniform population with a free gene flow will result (Riad, 2019). Desert oases depressions assume a key role in expansion designs over the Sahara by performing as environmental refugia for several species and facilitating gene flow throughout ideal climatic situations.

CONCLUSION

Traits connected with morphometric measurements mentioned in the current study may be a suitable way for differentiation between darkling beetles. El-Faiyum Depression and South Sinai populations of *P. nilotica* showed different morphological measurements from the other populations in Egypt. They are most distinct from different populations. Additional analysis of *P. nilotica* phylogeny is required to verify the data obtained in the current study.

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ARABIC SUMMARY

مقارنة مورفومترية بين مجموعات مختلفة من الخنافس الداكنة
"*PIMILIA NILOTICA* (COLEOPTERA: TENEBRIONIDAE)"
في مصر

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هدف الدراسة الحالية إلى مقارنة القياسات المورفومترية لمجموعات مختلفة من الخنافس الداكنة " *Pimelia nilotica*" في مصر. تم إجراء مقارنات متنوعة للاختلافات المورفومترية للخنافس الداكنة من ست مناطق جغرافية مختلفة من مصر تقع في الصحراء الغربية للبحر الأبيض المتوسط، وواحات الصحراء الغربية، ومنخفض الفيوم، والصحراء الشرقية، ودلتا النيل، وجنوب سيناء. وتم في الدراسة الحالية إجراء واحد وعشرون قياس مورفومتري, وسجلت درجات التمييز الأولية والثانية ٧٦,٤٪ و ٢٣,٨٪, على التوالي, من الاختلاف الكامل في العينات. وكشف التحليل التمييزي المتعدد عن اختلافات مورفومترية واضحة بين الصحراء الغربية, والصحراء الشرقية، ومنخفض الفيوم، وسكان جنوب سيناء. وكانت الصفات التي توضح معظم التباين بين السكان هي تلك المرتبطة بالقياسات المورفولوجية.
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