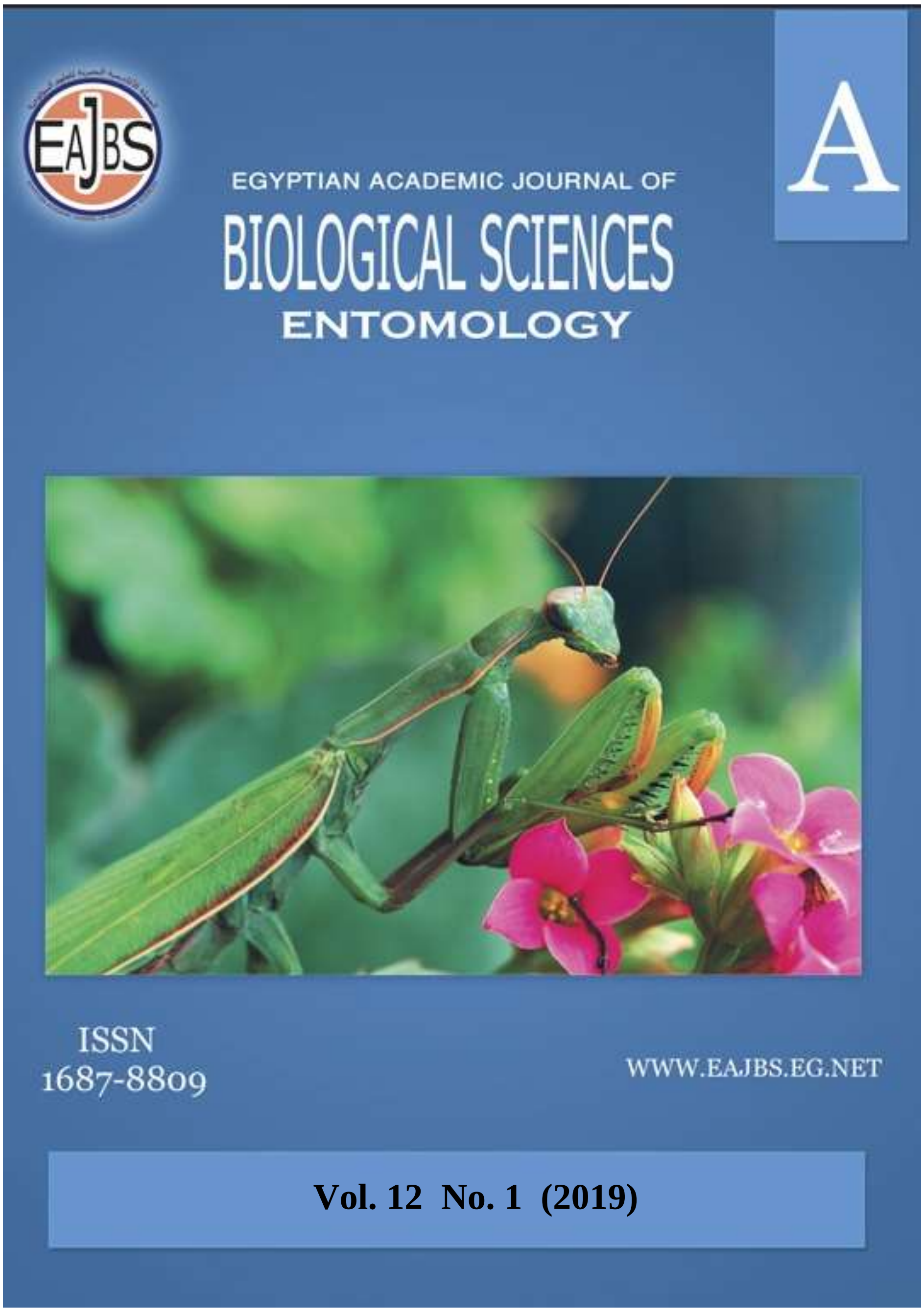

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Morphometric Comparison Between Different Isolated Populations of *Ocnera sparsispina* (Coleoptera: Tenebrionidae) in Egypt

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ABSTRACT

The present study aimed to compare morphometric measurements of different populations of *Ocnera sparsispina* in Egypt. A multivariate comparison of morphometric differences was undertaken by eyepiece micrometer on five different ecogeographical regions from Egypt fall under; Western Mediterranean Coastal Desert, Western Desert Oases, El Faiyum Depression, Nile Delta, and Sinai. Seventeen absolute morphometric characters and 4 ratios for 412 specimens undertaken. Traits best corresponding to the distinction of populations were distinguished by cluster analysis and the linear discriminant analysis. The first and second discriminant scores (score1 and score2) recorded 77.3% and 22.7%, respectively, of the total variation in samples. Multiple discriminant analysis revealed clear morphometric differences between the *Ocnera sparsispina* populations collected from 5 different ecogeographical regions of Egypt, and they are clustered in two discrete groups.

INTRODUCTION

Egypt occupies the northeastern part of the African continent. It is roughly quadrangular, extending about 1073 km from north to south and about 1229 km from east to west. Thus, the total area of Egypt is a little more than one million square kilometers (1,019,600 km^2) occupying nearly 3% of the total area of Africa (Abu Al-Izz, 1971). The position of Egypt amongst the faunal regions of the world is a rather anomalous one since it combines the characteristics of both Palaearctic and Afrotropical regions. It has generally been considered to belong to Palaearctic, but there is evidence that the Afrotropical element is much greater than usually thought (Steyskal and El Bialy, 1967). Ecologists divide Egypt into 8 ecological zones namely; 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).

Family Tenebrionidae (darkling beetles) is one of the largest families of order Coleoptera, including about 20000 known species in nearly 1700 genera of worldwide distribution (Booth *et al.*, 1990). *Ocnera sparsispina* (Coleoptera: Tenebrionidae) is a typical black darkling beetle found in the deserts of the Middle East and northern Africa (Krasnov and Ayal, 1995). During the last two decades, it has been collected from a few localities within several countries including Egypt, Sinai, Palestine and Iran (Zalat *et al.*, 2008). Despite the general abundance and widespread distribution of this species, little works have been published on its ecology and behavior**,** and *O. sparsispina* are feeding on decaying plants.

Morphometry has been one of the first method used in biological studies for discovering biodiversity (Wanek and Sturmbauer, 2015) and resolving phylogenies (Klingenberg and Marugan-Lobon, 2013). Morphometric measurements are widely used in the integrative approach to systematics along with molecular data (Ober and Connolly, 2015) which may result in taxonomical revision (Grobler *et al.*, 2006).

One of the most important questions is which morphological traits should be chosen for analysis. Redundancy of used traits can lead to disturbance of obtained results in an attempt to resolve phylogeny based on morphological variation. Recently in Egypt, some researchers began using morphometric measurements to measure the extent to which the desert beetles were morphologically affected by isolated areas in different ecoregions between western, eastern deserts and Sinai Peninsula (Hassan *et al.*, 2017 a, b). The present study aims to elucidate potential sub-specific differences of different Egyptian populations of *O. sparsispina* by using the cluster and linear discriminant analysis (LDA).

MATERIALS AND METHODS

The Study Area:

The present study was conducted during the period from spring 2015 to summer 2018 at 14 different localities of Egypt (Table 1 and Fig. 1). Pitfall traps were used to collect the *O. sparsispina* populations of the study localities. Each trap consisted of a rounded plastic jar with a depth of 13 cm and an upper opening of 5.7 cm in diameter (Forsythe, 1987). Three different sites were selected in each locality, they have chosen to represent as much as possible the local habitat heterogeneity. Sixty pitfall traps were divided into three sites, twenty traps per each site were placed late afternoon at 16:00 PM until the following sunrise, it was checked whenever possible. Trapping was conducted for two to three consecutive nights at each trapping locality, or until live specimens were collected. Many specimens and that found under stones were collected by hand.

Collected specimens were kept in alcohol 70% and taken to the laboratory for examination and processing. Taxonomic identification of the collected specimens was based on keys provided by Lobl and Smetana (2008). The collected specimens were deposited at Al-Azhar University Zoological Collection (AUZC), Faculty of Science, Al Azhar University, Cairo, Egypt. The geographical position and altitude of each site were recorded using a Garmin eTrex 30 GPS.

The study area was classified into 5 different ecogeographical regions of Egypt; Western Mediterranean Coastal Desert (North Coast and Mersa Matruh), Western Desert Oases (Siwa, Bahariya, Farafra, Dakhla and Kharga Oases), El Faiyum Depression, Nile Delta (10^{th} of Ramadan and El Salhiya deserts) and South Sinai (Saint Catherine and Taba Protectorate and Sharm El Sheikh wadis) (Table 1).

Table 1: Ecogeographical regions, coordinates and number of specimens of *O. sparsispina* collected during the period from spring 2015 to summer 2017.

Locality	Coordination		No. of specimens	Museum number
	Latitude	Longitude		
Saint Katherine, South Sinai	27.84990 N	34.22448 E	39	IC00055 - IC00094
Sharm El Sheikh, South Sinai	27.82994 N	34.20170 E	31	IC00095 - IC00126
Taba Protectorate, South Sinai	29.53391 N	34.70312 E	45	IC00127 - IC00172
Taba Protectorate, South Sinai	28.89613 N	34.92268 E	25	IC00173 - IC00198
10th City, Sharquiya	30.32248 N	31.78177 E	26	IC00199 - IC00225
El Salhiya, Sharquiya	30.85409 N	32.06419 E	43	IC00226 - IC00269
Bagush, North Coast, Matruh	31.10422 N	27.41474 E	9	IC00270 - IC00279
Mersa Matruh, Matruh	31.30902 N	27.29444 E	8	IC00280 - IC00288
Bahariya Oasis	28.27596 N	28.80067 E	34	IC00289 - IC00323
Farafra Oasis	27.07763 N	27.97546 E	43	IC00324 - IC00367
Dakhla Oasis	25.49454 N	28.97892 E	29	IC00368 - IC00397
Kharga Oasis	24.67444 N	30.60799 E	25	IC00398 - IC00423
Siwa Oasis	29.18073 N	25.47638 E	46	IC00424 - IC00470
El Faiyum Depression	29.27999 N	30.57639 E	9	IC00471 - IC00480

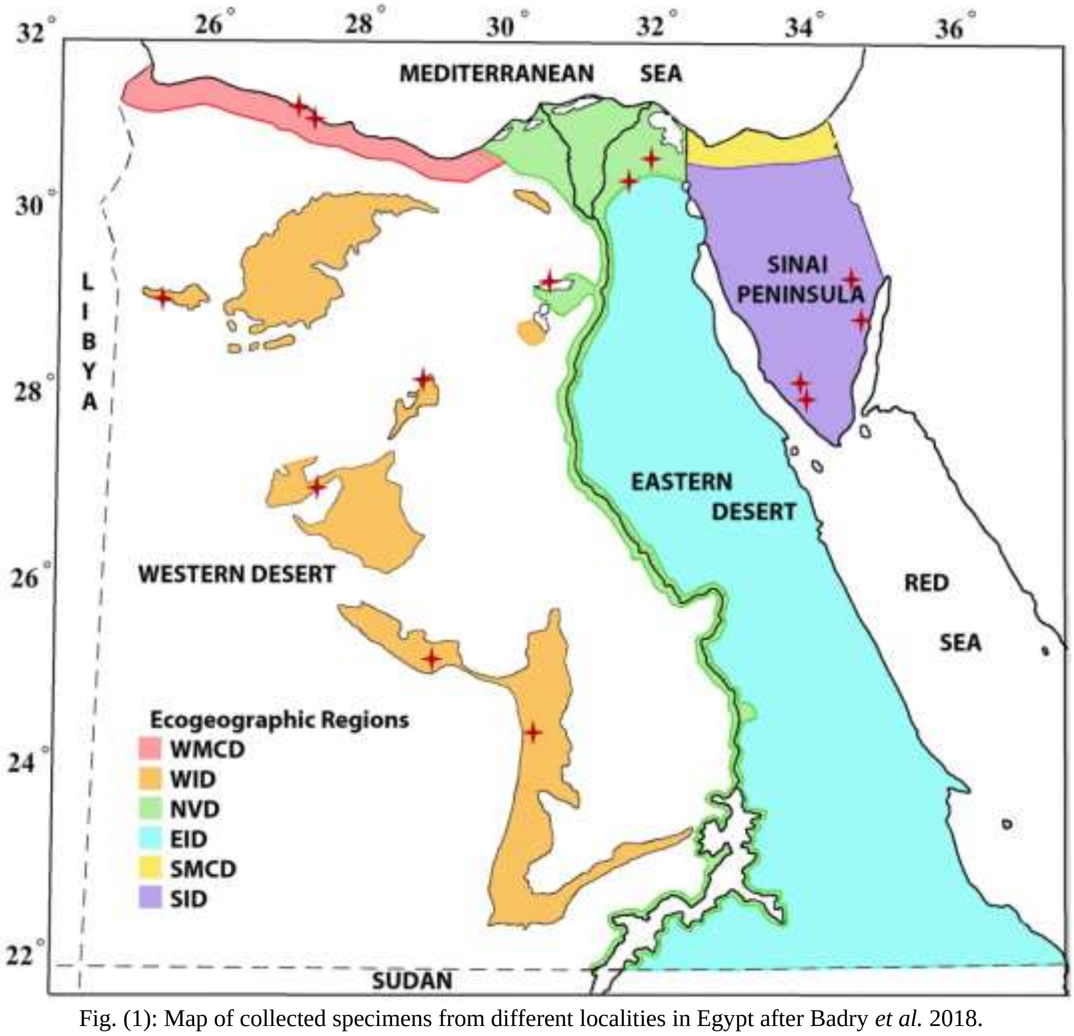

Fig. (1): Map of collected specimens from different localities in Egypt after Badry *et al.* 2018.

Specimens Measurements:

Morphometric analyses were performed on all 412 specimens of *O. sparsispina*. For each specimen, using eyepiece micrometer we measured 17 absolute morphometric characters and 4 ratios (Table 2)

Table 2: Morphological measurements used in this study and its 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**, the maximum pronotum width, taken perpendicular to the pronotum length.
EL	**Elytron Length**, the distance between the anterior border of the scutellum and the posterior border of the elytron.
EW	**Elytron Width**, the maximum width of elytron.
BL	**Body Length**, Head length + Pronotum length + Elytron length.
FF	**Fore Femur length.**
FT	**Fore Tibia length.**
FTa	**Fore Tarsus length.**
MF	**Meso Femur length.**
MT	**Meso Tibia length.**
MTa	**Meso Tarsus length.**
MeF	**Meta Femur length.**
MeT	**Meta Tibia length.**
MeTa	**Meta Tarsus length.**

Statistical Analysis:

Linear discriminant analysis (LDA) was conducted in NCSS11 statistical software. The differences in each morphological measurement between samples were examined with F- test, One-way ANOVA (analysis of variance) using SPSS (Statistical package for social sciences) computer software package, version 20 according to Levesque (2007).

Probability at $P \leq 0.05$ and at $P \leq 0.01$.

*=significant at $P \leq 0.05$ or $P \leq 0.01$ and non-significant at $P > 0.05$.

RESULTS

Data in Table (3) show the comparison of morphological measurements of *Ocnera sparsispina* populations collected from 5 different ecogeographical regions; Western Mediterranean Coastal Desert (WMCD), Western Desert Oases (WDO), El Faiyum Depression (FD), Nile Delta (ND) and South Sinai (SS). Morphometric measurements show a significant difference between the five studied populations. Regarding the total lengths, the (SS) population mean values were 4.58 mm ± 0.07, and that of (WDO) population mean values was 7.79 mm ± 0.08, that difference was statistically significant. The highest mean lengths of pronotum, elytron, forefemur, foretarsus, mesofemur, mesotibia and mesotarsus were recorded for the (WDO) population. The highest mean values of antenna length, foretarsus length, metatarsus length, and ratios of head width/head length and pronotum width/pronotum length were recorded for SS population (Table 3).

Table 3: Morphological measurements of *O. sparsispina* specimens from the five ecogeographical regions in Egypt, all measurements were in mm.

	WMCD	Western Desert Oases	El Faiyum Depression	Nile Delta	South Sinai	F Value
TL	19.48±0.11 (19.30-19.60)17	19.48±0.11 (19.30-19.60)177	19.46±0.12 (19.30-19.60)9	19.47±0.11 (19.30-19.60)69	19.45±0.12 (19.30-19.60)140	1.025
AL	10.31*±0.07 (10.20-10.40)17	10.35*±0.13 (10.20-10.70)177	10.61*±0.08 (10.50-10.70)9	10.30*±0.07 (10.20-10.40)69	10.29*±0.07 (10.20-10.40)140	22.596
HL	2.59±0.10 (2.40-2.70)17	2.59±0.09 (2.40-2.70)177	2.58±0.07 (2.50-2.70)9	2.58±0.10 (2.40-2.70)69	2.57±0.10 (2.40-2.70)140	1.248
HW	7.80*±0.08 (7.70-7.90)17	7.79*±0.08 (7.70-7.90)177	7.77*±0.07 (7.70-7.90)9	7.67*±0.17 (7.40-7.90)69	7.49*±0.08 (7.40-7.80)140	166.55 7
PL	2.59*±0.09 (2.40-2.70)17	2.59*±0.09 (2.40-2.70)177	2.57*±0.09 (2.40-2.70)9	2.65*±0.14 (2.30-2.90)69	2.76*±0.13 (2.30-2.90)140	39.985
PW	7.39*±0.09 (7.20-7.50)17	7.39*±0.09 (7.20-7.50)177	7.37*±0.09 (7.20-7.50)9	7.31*±0.13 (7.00-7.50)69	7.18*±0.09 (7.00-7.30)140	81.069
EL	13.90±0.08 (13.80-14.00)17	13.90±0.08 (13.80-14.00)177	13.88±0.07 (13.80-14.00)9	13.89±0.07 (13.80-14.00)69	13.88±0.07 (13.80-14.00)140	.637
EW	11.30±0.08 (11.20-11.40)17	11.30±0.08 (11.20-11.40)177	11.28±0.07 (11.20-11.40)9	11.29±0.07 (11.20-11.40)69	11.28±0.07 (11.20-11.40)140	.637
FF	6.90±0.08 (6.80-7.00)17	6.90±0.08 (6.80-7.00)177	6.88±0.07 (6.80-7.00)9	6.89±0.07 (6.80-7.00)69	6.88±0.07 (6.80-7.00)140	.637
FT	4.92*±0.09 (4.80-5.10)17	4.87*±0.14 (4.50-5.10)177	4.58*±0.07 (4.50-4.70)9	4.59*±0.07 (4.50-4.70)69	4.58*±0.07 (4.50-4.70)140	155.46 7
Fta	3.10±0.08 (3.00-3.20)17	3.10±0.08 (3.00-3.20)177	3.08±0.07 (3.00-3.20)9	3.09±0.07 (3.00-3.20)69	3.08±0.07 (3.00-3.20)140	.637
MF	7.90±0.08 (7.80-8.00)17	7.90±0.08 (7.80-8.00)177	7.88±0.07 (7.80-8.00)9	7.89±0.07 (7.80-8.00)69	7.88±0.07 (7.80-8.00)140	.637
MT	7.00*±0.08 (6.90-7.10)17	6.98*±0.09 (6.80-7.10)177	6.88*±0.07 (6.80-7.00)9	6.69*±0.07 (6.60-6.80)69	6.68*±0.07 (6.60-6.800)140	304.32 3
Mta	4.60±0.08 (4.50-4.70)17	4.60±0.08 (4.50-4.70)177	4.58±0.07 (4.50-4.70)9	4.59±0.07 (4.50-4.70)69	4.58±0.07 (4.50-4.70)140	.637
MeF	8.70±0.08 (8.60-8.80)17	8.70±0.08 (8.60-8.80)177	8.68±0.07 (8.60-8.80)9	8.69±0.07 (8.60-8.80)69	8.68±0.07 (8.60-8.80)140	.637
MeT	9.49*±0.10 (9.30-9.60)17	9.49*±0.09 (9.30-9.60)177	9.48*±0.07 (9.40-9.60)9	9.19*±0.07 (9.10-9.30)69	9.18*±0.07 (9.10-9.30)140	299.39 3
MeTa	4.80±0.08 (4.70-4.90)17	4.80±0.08 (4.70-4.90)177	4.78±0.07 (4.70-4.90)9	4.79±0.07 (4.70-4.90)69	4.78±0.07 (4.70-4.90)140	.637
HW/ HL	3.01*±0.08 (2.93-3.21)17	3.01*±0.08 (2.93-3.21)177	3.01*±0.06 (2.93-3.08)9	2.97*±0.12 (2.74-3.21)69	2.92*±0.11 (2.74-3.17)140	15.852
PW/PL	2.85*±0.07 (2.78-3.00)17	2.86*±0.06 (2.78-3.00)177	2.87*±0.06 (2.78-3.00)9	2.77*±0.15 (2.52-3.09)69	2.61*±0.12 (2.52-3.09)140	105.70 9
EW/ EL	0.81±0.00 (0.81-0.81)17	0.81±0.00 (0.81-0.81)177	0.81±0.00 (0.81-0.81)9	0.81±0.00 (0.81-0.81)69	0.81±0.00 (0.81-0.81)140	.000
EL/P L	5.35*±0.16 (5.19-5.75)17	5.37*±0.16 (5.19-5.75)177	5.41*±0.16 (5.19-5.75)9	5.26*±0.27 (4.76-6.00)69	5.05*±0.25 (4.75-6.04)140	42.067

Data shown as mean ± SD (range) number of specimens (P<0.05 * = significant), Where, **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**: forefemur length, **FT**: foretibia length, **FTa**: foretarsus 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 and **El/PL**: elytron length/pronotum length.

Using cluster analysis, the morphological measurements used in Figure (2) showed the dissimilarity between the *Ocnera sparsispina* populations collected from 5 different ecogeographical regions of Egypt. Figure 2 shows the comparison between the five sampled populations of *Ocnera sparsispina*. They are clustered in two discrete groups. The first cluster consisted of the populations of the WMCD, WDO, and FD. The second cluster contained ND and SS populations.

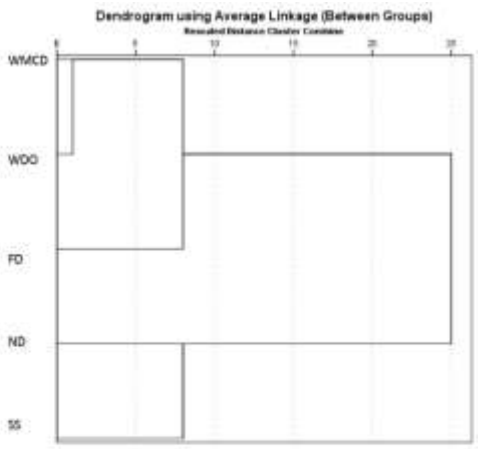

Fig. 2: A dendrogram showing the similarity between *O. sparsispina* populations of Western Mediterranean Coastal Desert (WMCD), Western Desert Oases (WDO), El Faiyum Depression (FD), Nile Delta (ND) and South Sinai (SS) based on 17 morphological measurements and 4 ratios.

The linear discriminant analysis (LDA) of *Ocnera sparsispina* populations based on 21 morphometric variables was carried out. The analysis generated two linear discriminant analyses for all samples. The first and second discriminant scores (score1 and score2) recorded 77.3% and 22.7%, respectively, of the total variation in samples (Table 4). Factor loading values for each measurement is shown in Table (5).

Table 4: Linear discriminant analysis based on 21 morphologic variables

	Eigenvalue	**% explained**	**% cumulative**
Score1	12.73	77.3	77.5
score2	3.72	22.7	49.0

Table 5: Factor loading values for each morphological character in LDA

Character	Score1	Score2
TL	0.00034415	-0.0021784
AL	0.00225053	-0.0029955
HL	-0.00629881	0.00087894
HW	-0.00232195	-5.0923345
PL	-0.00353764	2.50963347
PW	-0.00034415	0.00217847
EL	6.94864406	0.00186662
EW	-0.00195391	-0.01604432
FF	-0.00104664	0.01859238
FT	-0.00034415	0.00217843
Fta	-0.00147499	1.69481205
MF	-0.00063452	-0.00112714
MT	-0.00058769	0.01383256
Mta	-0.00191742	3.19192305
MeF	0.00029732	-3.87415405
MeT	-0.00019896	2.4429E-05
MeTa	0.00142123	0.00094556
HW/HL	0.00390185	-0.00064067
PW/PL	0.00188926	-0.00010064
EW/EL	-7.62524558	-0.00067721
EL/PL	0.00395237	-0.00020234

Based on the cluster analysis mentioned above, the two WMCD and WDO populations were grouped together as WMCD/WDO. Using the linear discriminant analysis, Figure 3 shows a clear similarity between WMCD/WDO and FD populations. While ND is close to SS population. According to both the cluster and linear discriminant analyses, the populations of *Ocnera sparsispina* from South Sinai and Western Desert Oases has a higher phenetic distance from the other studied populations.

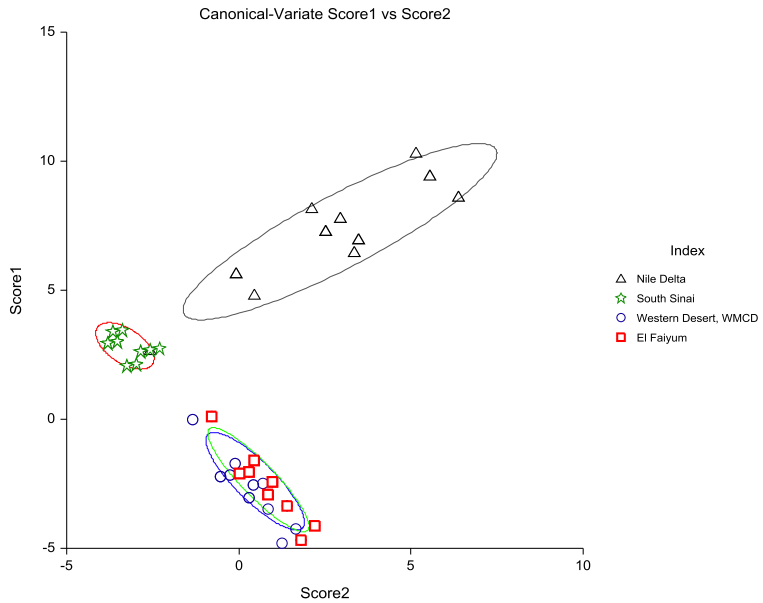

Fig. 3: Linear discriminant analysis of the morphological measurements of *O. sparsispina* populations.

DISCUSSION

When comparing geographically separated populations by means of a morphometric dataset can exert some influence on the observed differences (Mamuris *et al.*, 1998). The present study attempted to minimize additional variances through size standardization, data transformation and by performing separate LDA analysis.

Extensive variation in morphometric variables existed between the studied populations. In addition, Within the Western Desert oases and WMCD studied populations of *Ocnera sparsispina* were most similar because these areas contain asimilar sand vegetation, and there are no major barriers to groups moving between them. Whilst the Nile Delta and El Faiyum Depression populations were most dissimilar, this difference and similarity are due to the existence of large natural barriers that isolated populations in each ecoregion, with some minor climatic factors that led to these differences. The variables of primary importance in separating the South Sinai population was related to the larger size of *O. sparsispina* populations when compared with other populations except for Nile Delta. However, the relatively high discriminant of the (Western Mediterranean Coastal Desert, Western Desert oases and El Faiyum Depression), Nile Delta and South Sinai populations variables, may represent speciation of *O. sparsispina*.

These three ecogeographical areas are separated from each other by huge geographical barriers which prevent transformation of the studied species between them in addition to the limited home range of this species, these results were agreed with Angilletta and Dunham (2003); Shingleton *et al.*, (2007); Arthur *et al.*, (2008) and Hassan *et al.,* (2017 a, b), where they have studied the different ecogeographical populations of tow beetle’s species in Egypt.

Now, the Nile River and its narrow floodplain act as a barrier for desert faunal dispersion separating the Western and Eastern Deserts in Egypt. Being the nearest to the Nile Valley and after relating to the River Nile by a large irrigation canal (Bahr Yusuf), El Faiyum Depression is considered as a part of the Nile Region. The lowest part of the depression is occupied by a shallow saline lake – Qarun Lake – which is about 4.5 m below sea level and about 200 km^2 in area. The depression has a total area of about 1700 km^2. Its floor just above the lake level is about 23 m asl (Ball, 1939). El Faiyum Depression population of *O. sparsispina* are isolated in this area from other desert population studied in this study.

In early geological time, lakes and wetlands of the Ismuth of Suez (which is now traversed by Suez Canal) expanded greatly and extensive marshland conditions developed closing this gate to Africa. The Gulf of Suez, with its shallow profile, appears to have remained an exposed basin throughout most of the Pleistocene, and until about 14,000–15,000 years ago, when sea levels rose above about 50 m, linking the Sinai Peninsula to the Eastern Desert (Derricourt, 2005; Bailey *et al*., 2007). Derricourt (2005) suggests that during drier periods of the Pleistocene, the Gulf of Suez was reduced in area and the Sinai Peninsula was readily accessible from the Eastern Desert with the two regions forming one, largely continuous arid zone. This is maybe the reason for similarity between South Sinai and Eastern Desert *O. sparsispina* populations.

During drastic climate changes, many species and populations can survive only in areas with a more stable climate, called refugia. Throughout Quaternary, there were many such places in Egypt and surrounding areas. It is quite possible that processes of differentiation and speciation in genus *Ocnera* were heavily influenced by contractions and subsequent expansions of ranges of populations due to climate change. For example, two populations of one species may have been isolated in two different refugia which may lead to allopatric speciation. Isolation in mountainous refugia can lead to patterns in morphological diversity which are in many aspects consistent with phylogeny (Ober and Connolly, 2015). However, all distinguished *Ocnera* species have similar ecological niches, which may result in similar adaptations to the environment. That is why the possibility that many aspects of morphology do not reflect phylogeny but simply adaptation to the common environment, must be considered.

Spatial and temporal expansion and contraction of desert conditions in the Sahara appear to have acted as an important driver of faunal diversification and speciation events. Palaeoclimatic cycles continually adjusted the boundaries between the desert, other environments and their associated biodiversity (Dumont, 1982; Le Houerou, 1992; 1997; Drake *et al.,* 2011). Vicariance events associated with Saharan aridity episodes become the main diversification force for post-Pleistocene allopatry (Douady *et al.*, 2003; Nyari *et al.,* 2010). Such events are believed to have resulted in allopatric isolation, which in turn induced the interruption of gene flow and the evolution of independent lineages or new species.

The response of a given animal taxon to Saharan vicariant events varies according to the taxon’s habitat requirements. During 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 morphologic and genetic allopatric diversification (Boratynski *et al.,* 2012). During a subsequent arid episode, isolated populations of desert-adapted species will expand their ranges, possibly merging the different meta-populations into larger populations. If the previous allopatric divergence was not sufficient to result in reproductive isolation, genetic mixing will take place and a uniform population with a free gene flow will result. Desert oases depressions play a key role in diversification patterns across the Sahara by acting as ecological refugia for many species and facilitating gene flow during favorable climatic conditions.

CONCLUSION

Traits connected with morphometric measurements mentioned above are good characters for the differentiation between darkling beetles. Western Desert Oases and South Sinai populations of *O. sparsispina* have been shown to differ morphologically from the other populations in Egypt. They are most distinct than other populations. Further analysis of *O. sparsispina* phylogeny is needed to confirm the present study.

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ARABIC SUMMERY

مقارنة مورفومترية بين مختلف العشائر المنعزلة من خنفساء أوكنيرا سبارسيسبينا (غمدية الأجنحة: تينيبريونيدي) في مصر

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تهدف الدراسة الى مقارنة القياسات المورفومترية لمختلف العشائر من خنفساء *أوكنيرا سبارسيسبينا* في مصر. وأجريت مقارنة المتغيرات المتعددة من الإختلافات المظهرية بإستخدام عدسة عينية قياسية لعشائر خمس مناطق معزولة جغرافياً في مصر تندرج تحت صحراء الساحل الشمالي الغربي، منخفضات الصحراء الغربية، منخفض الفيوم، دلتا النيل وسيناء. أخذت سبعة عشر قياساً مظهرياً مطلقاً وأربع قياسات نسبية لأربعمائة وإثنتي عشر عينة. وقد أختبرت هذه القياسات لتمييز العشائر عن طريق التحليل العنقودي وتحليل المكون الرئيسي. حيث سجلت محاور المكون الرئيسي الأول والمكون الرئيسي الثاني ٧٧،٣ % و ٢٢،٧% على التوالي من إجمالي الإختلاف في العينات. بيّن تحليل التمايز المتعدد أن هناك اختلافات مورفولوجية واضحة بين عشائر *أوكنيرا سبارسيسبينا* المختلفة المجمعة من خمس مناطق جغرافية منعزلة في مصر، والتي انعزلت الى مجموعتين منفصلتين في تحليل التمايز.