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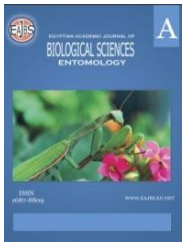

Morphological and Molecular Studies on Certain Species of Family Anthicidae (Order: Coleoptera) In Egypt

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ABSTRACT

The Family Anthicidae are of the heteromerous group of beetles recognized by their superficial shape, which resembles ants and have an appearance of small carabids. Six species are compared within genera, *Anthicus*, *Cyclodinus*, *Striticomus,* and *Omondus* (Family: Anthicdae), the male genital structure used to represent morphological character and (RFLP) molecular data. The Subgenera, *Cyclodinus*, *Striticomus,* and *Omondus* were promoted to genera. The results revealed that the molecular data are emphasized the elevations of *Cyclodinus*, *Striticomus*, and *Omondus*, these subgenera to status genera. Whereas species not changed in its scientific name.

INTRODUCTION

The Anthicidae (ant mimic) small size, usually being in or around flowers and foliage of plants and trees. Many species are predaceous and several species were found associated with crops but none serious pests (Imms, 1937), from the taxonomic point of species all over the world is approximately 85 species known to occur in Egypt (Alfieri, 1976). The Palearctic anthicids are treated taxonomically, and the list of species together with their distribution and taxonomical notes (Uhmann, 1995). Certain subgenera of Anthicidae are promoted to genus rank by (Uhmann, 1998). A key was designed for diagnosis genera by Turkey *et al.,* (2005). The molecular genetic technique can overcome certain of the problems associated with the morphological character. Insect samples can be collected and stored in alcohol (Sperling *et al.*, 1994) and DNA characters are independent of like-stage or DNA extracted from a small amount of tissue that can be used for PCR-RFLP reaction, polymerase chain reaction and restriction fragment length polymorphisms (Crozier, 1993). However, tissues such as genitalia can be saved as morphological vouchers, PCR-RFLP, and have been successfully used for molecular diagnostics of gypsy moth (Pfifer, 1995) blow-flies (Sperling *et al*., 1994) and screw-worm (Taylor *et. al*., 1996). The purpose of this study was to access available mtDNA, molecular marker as a source of diagnostic polymorphism for six species of Anthicidae. This research also provides data for molecular systematics for Anthicidae in view of the difficulties associated with identification by morphological characters. According to the present investigation, we used the molecular marker of mtDNA obtained by PCR-RFLP as a helpful and quick identification method together with the male genital structure to confirm of elevation of subgenera to genera rank.

MATERIALS AND METHODS

Collection of Specimens:

Samples of anthicid beetles were collected from various locations; Gabel Asfar, Desert road, and Baharieh Oases, by using the light trap and then killed by Sodium Cyanide. The collected specimens were transferred to the lab for examination and identification of species. Samples were put in absolute alcohol and transported to the laboratory dead and stored at -4 ºC until analysis.

Identification and Morphological Studies:

The study of the morphological characters was made in the Plant Protection Department, Faculty of Agriculture, Al-Azhar University.

Preparation of Genitalia And Microscopic Slides:

The entire beetles were soaked in 10% KOH solution for 24 hours to dissolve excess tissue and transferred to a glass containing distilled water for rinsing, then, transferred to 50% ethyl alcohol for dehydration of water gradually till the absolute alcohol grade. Cleared in xylene and transferred to clove oil for easily dissecting. Fine dissecting needles are used for separation of male and female genitalia. A stiff camel's hair-brush was used to remove excess tissues from the abdomen and genitalia were examined and photographed in glycerol by a camera attached to Olympus SZ61 Stereomicroscope.

Molecular Data:

Some specimens were preserved in Eppendorf contain 95% Ethyl alcohol and deposited at -4 ºC until using in the molecular investigation.

I-**DNA Isolation**:

1- Smash the head and thorax of specimens for obtaining enough quantities of DNA.
2- Addition 500µl buffer B (25 mM EDTA, 75 mM $NaCl_2$, 10 mM Tris HCl, pH 7.5) for keep the pH at 7.5.
3- Addition 25µl 20% sodium dodecyl sulfate (SDS) to block its action through make bonds with enzymes.
4- Addition of 50µl proteinase k (0.02mg/µl, 1mg proteinase k) for extraction the DNA through penetration the cell walls and lay down extracted protein.
5- Shaking strongly by vibrator.
6- Incubate the sample at 50 ºC for one night.
7- Add 400µl saturated $NaCl_2$ for osmosis pressure.
8- Shaking by vibrator.
9- Centrifugation at 8000 rpm and RT for 30 min.
10- Prepare the same number of tubes (epic), have the same data.
11-Addition of isopropanol cold to regulate space shape for DNA and elimination of protein.
12-Centrifugation at 8000 rpm and RT for 30 min.
13-Addition of 1 ml ethanol 70% cold for washing DNA.
14-Incubation at 50ºC for 15 min to remove the ethanol traces.
15-Addition 200µl TE-Buffer (sterile, 10mM Tris-HCl, 1mM EDTA, pH 7.5) forob tain the DNA solution.

II- Buffers:

Bromophenol blue	50 mg (0.25% final)
Sucrose	8 g (40% final)
Distilled water	Complete to 20 ml

The solution was stored at 4ºC.

III- Tris-Acetate EDTA (TAE; 50X stock):

Tris-base	242 g
Glacial acetic acid	57.1 ml

0.5 M EDTA (pH 8.0)	100 ml
Distilled water	Complete to 1 L
Working solution	1X

IV-Agarose Gel Electrophoresis: (Sambrook *et al.* 1989)

Agarose was melted in 1X TAE buffer in a microwave oven and poured to form a horizontal slab gel. The DNA samples, as well as a DNA molecular size marker, 1 kb ladder (Gibco-BRL), were loaded in 1X agarose-gel loading buffer and run submerged in an electrophoresis tank in 1X TAE buffer at 5 volts/cm until the leading dye (bromophenol blue) reached near the end. The gel was then stained with Ethidium bromide solution (0.5 µg/ml) for 20-30 minutes, destained with water for 10-15 minutes and photographed using polaroid type 667 film.

V-Restriction Enzyme Digestion; (Sambrook *et al.* 1989).

After plasmid DNA preparation, and in order to identify the size of the insert cloned (EcoR1) in the pBluescript plasmid, the following digestion reaction was done by using one enzyme. A typical restriction enzyme.

DNA template	Y µl (~2µg)
10X Restriction enzyme reaction buffer	1µl
Restriction enzyme 1	X µl (~ 2 units)
Distilled water	Complete to 10µl

RESULTS AND DISCUSSION

A – Morphological characters of family Anthicidae

Members of the anthicidae, the ant-flower beetles, are moderate-sized, the family comprises about 3000 species under 40 genera (Booth, 1990). The herteomerous group of beetles recognized by their superficial shape, which resembles ants. They usually being in or around flowers and foliage of plants and trees, other species are associated with compost or straw. They are characteristically narrow-bodied beetles with a pronotum wide toward the anterior margin, constricted at base. Antennae thread-like to weakly clubbed, elytra long to elongate, sometimes with patches or spots are brown-orangish or yellow. Tarsal formula 5-5-4. The number of species about 85 species known to occur in Egypt (Alfieri, 1976).

key to the Genera

1.Pronotal base with two small tubercles (Fig. 1) ……..……...……*Cyclodinus*
. Pronotal base not as above ……..……..……..……2
2.Pronotum attenuates in an oblique line towards the base (fig.2) …...…….. *Anthicus*
. Pronotum attenuate sinuately towards the base (Figs. 3&4) ……..……..3
3.Sinuation of pronotum moderate, basal edge not extended to the middle (Fig. 3) ……..…….....*Omonadus*
. Sinuation of pronotum strong, basal edge extended to the middle of the pronotum (Fig.4) ……..……..……...……..…… *Striticomus*

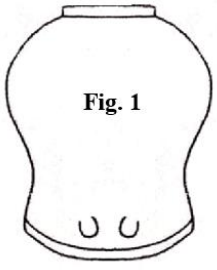


Fig. 1

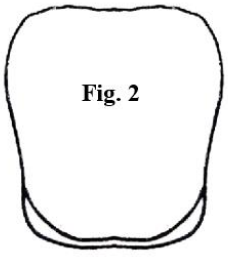


Fig. 2

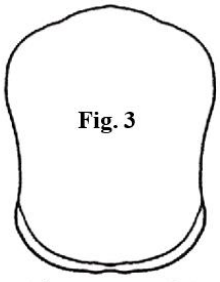


Fig. 3

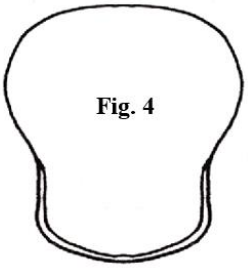


Fig. 4

The previous results revealed that just one taxonomic character which separate among four genera. These genera in previous years were subgenera but in the present time becomes

genera, the promotion for three subgenera to genera rank by just one taxonomic character (in pronotum part).

Morphology of Male Genital Structure of Species
(Figs. 5, 6, 7, 8, 9, and 10)

1-Anthicus crinitus

Male genitalia: phallobase (a) with median lobe elongated, slightly pointed at tip, apodeme (b) of male sternum nine rod-like bifurcated.

2-Anthicus dimidiatipennis

Male genitalia: phallobase (a) with median lobe, elongate, apex bent towards orifice, spoon-shaped, lateral lobes finger-like, shorter than median lobe provided by short hairs, apodeme (b) of male sternum nine rod-like bifurcated at apex, slightly chitininzed.

3-Cyclodinos deblis

Male genitalia: phallobase (a) with medium lobe ovate shaped at ventral side, lateral lobes rectangular, truncated at tip and longer than median lobe, apodeme (b) of male sternum nine rod-like strongly hooked at tip and more stout.

4-Cyclodinos larvipennis

Male genitalia: phallobase (a) with median lobe reflexed v-shaped at tip and longer than paramers apodeme (b) of male sternum nine very harrow, rod-like and hooked at tip.

5-Omandus floralis

Male genitalia: phallobase (a) with median lobe with an obvious rectangular orifice, terminal edge V-shaped, apodeme (b) of male sternum nine rod-like.

6-Striticomus modestus

Male genitalia: phallobase (a) with median lobe spoon-shaped from dorsal side and extended from ventral side to form truncated tip and longer than whether the ventral side or dorsal side. Lateral lobes fingers like, apodeme (b) of male sternum rod-like stout at tip.

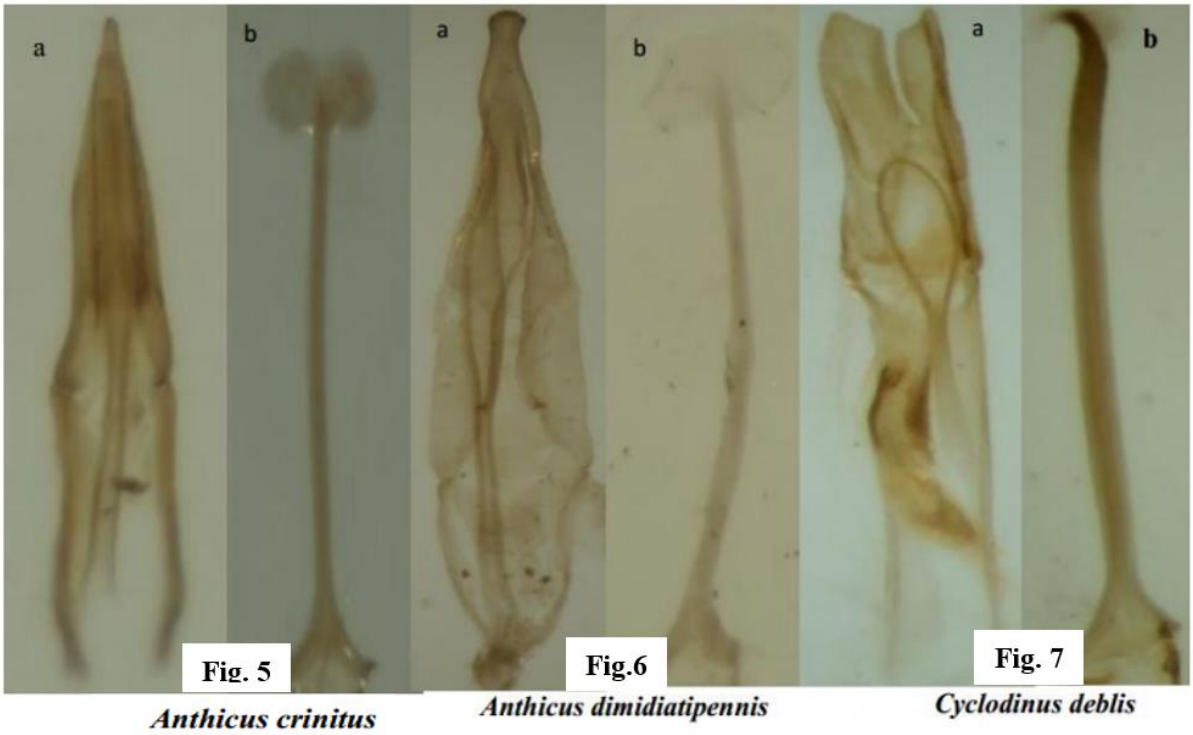
Molecular Data:

Among them, an appropriate restriction site for EcoR1, gave six distinct profiles of fragments for *Cyclodinus debilis* 2000 bp, *Anthicus crinitus* 12000 bp, *Cyclodinus larvipennis* 11000, *Omondus floralis* 10500, *Anthicus dimidiatipennis* 12000 bp, *Striticomus modestus*11000 (Figs. 11, and 12), but the differentiation among them bands we showed in the places and don't show in other places.

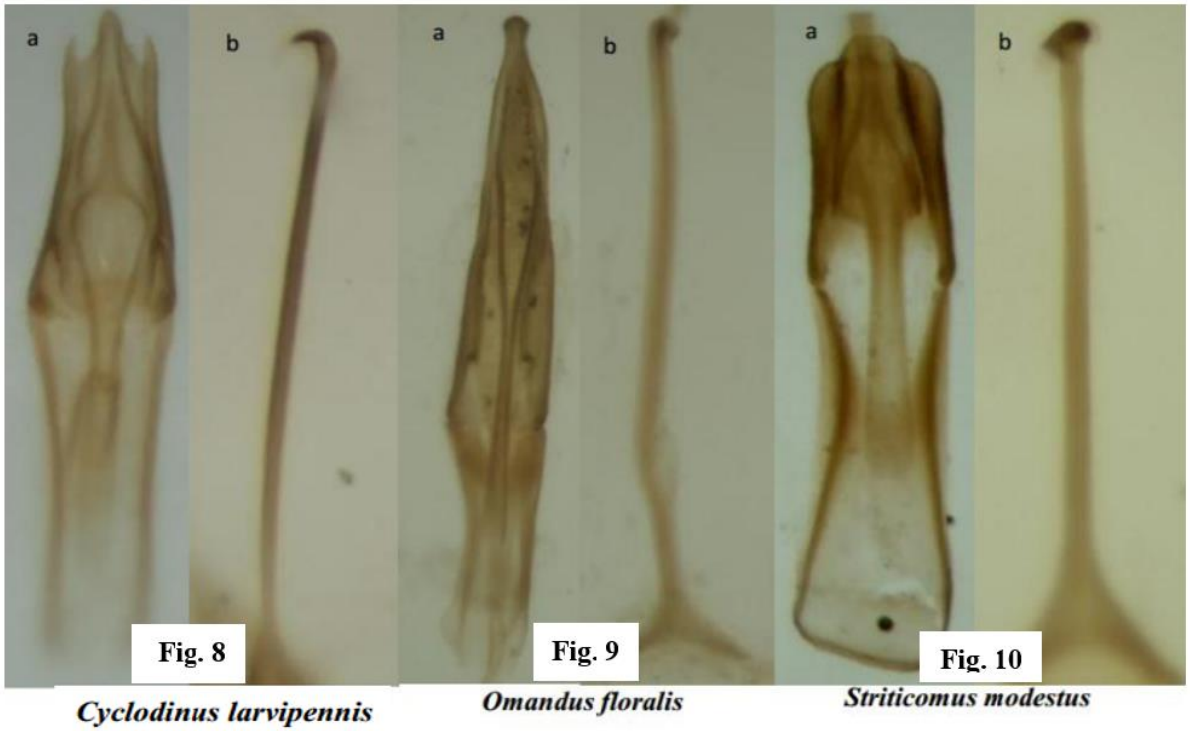
The restriction profiles for all Anthicidae samples were screened using agarose gels. Though the agarose gels provide better resolution for small fragments. This doesn't impair its use as a means of differentiation because the larger fragments are easily distinguishable in any system and there is only a small loss of resolution for fragments smaller than 1000 bp. One restriction enzyme EcoR1, was useful for distinguishing among six species (figures 11, and 12). The obtained data revealed that the species of *Cyclodinus deblis* different from other species. There are no difference between the two species of *Anthnicus crinitus* and *A. dimidiatepennis*. On the other hand, species of *Striticomus modestus* different from species *Omandus floralis*. The results of the molecular marker PCR-RFLP confirmed that all subgenera species not properly promoted to genera rank, because the *larvipennis* species morphologically belong to genus *Cyclodinus*, although, the molecular data cleared that this species belong to genus *Anthicus*.

However, PCR-RFLP method is reasonably fast, and user friendly in many laboratories involving species identification including insects (Clark *et al.*, 2001; Mukabana *et al.* 2002; Armstrong and Ball 2005; Oshaghi *et al*. 2006 a and b; Green Stone 2006; Oshaghi *et al.* 2008, 2009, and 2010; Kato *et al.* 2010; and Oshaghi *et al.* 2011).

Finally, this family needs more molecular studies to fill the lacking gaps in our knowledge. Also, more efforts are needed to update and correct status of genera and species.



Figs. 5, 6, and 7, genital structures phallobase (a), and apodeme (b) of three species of *Anthicus crinitus, A. dimidiatipennis*, and *Cyclodinus deblis*.



Figs. 8, 9, and 10, genital structures: phallobase (a), and apodeme (b) of three species of *Cyclodinus larvipennis, Omandus floralis, and Striticomus modestus*.

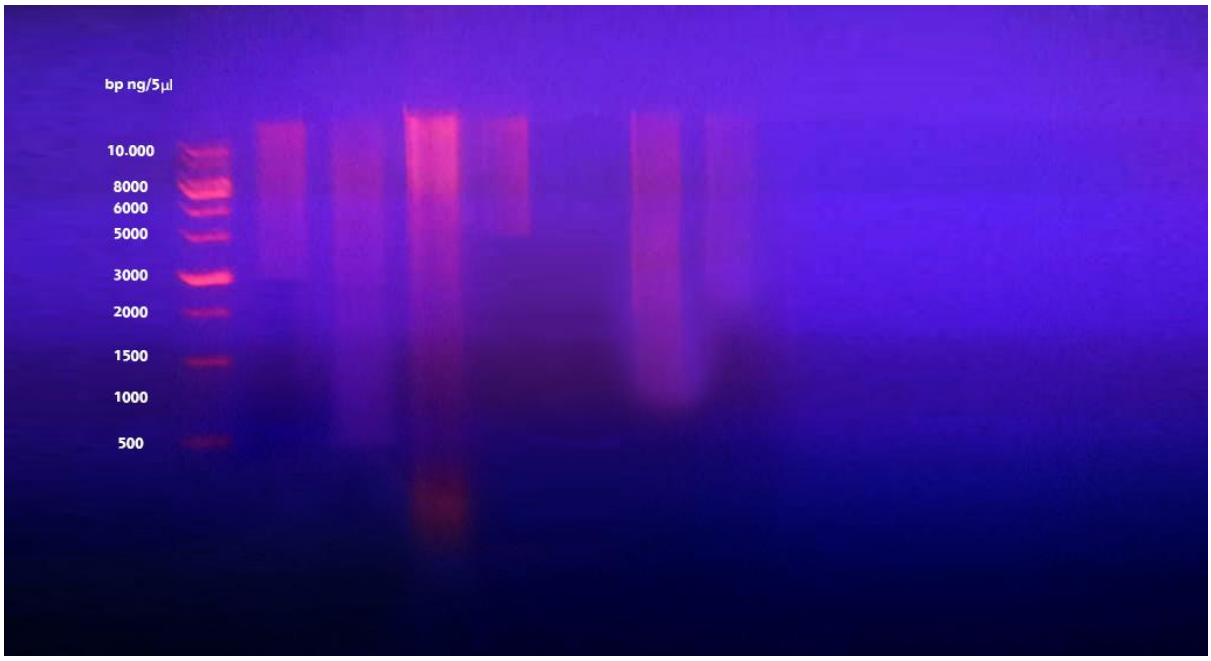


Fig. 11: gel profile for six species of *Anthicus crinitus, A. dimidiatipennis*; *Cyclodinos deblis, C. larvipennis*; *Omandus floralis*, and *Striticomus modestus* as arranged respectively.

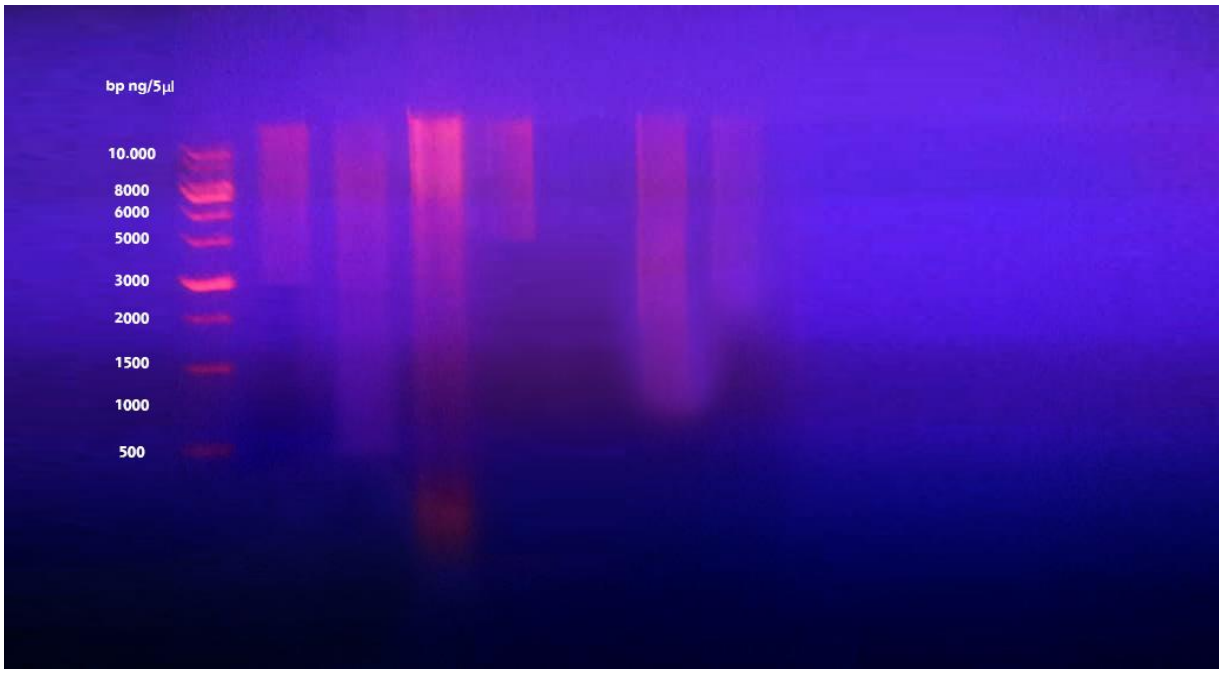


Fig. 12: gel profile for six species of *Anthicus crinitus, A. dimidiatipennis*; *Cyclodinos deblis, C. larvipennis*; *Omandus floralis*, and *Striticomus modestus* as arranged respectively.

REFERENCES

Alfieri, A. (1976): The Coleoptera of Egypt. Bull. Soc. Entomol. Egypt, 5: 145-152.

Armstong, KF, and Ball, S. L. (2005): DNA barcodes for biosecurity: invasive species identification. Philos Trans R Soc. Lon. B Biol. Sci. 360 (1462): 1813-1823. Review Erratum in: Philos Trans R Soc. Lon. B Biol. Sci. 360 (1464): 2373.

Clark, T. L., Meinke, L. J., and Foster, J. E. (2001): PCR-RFLP of the mitochondrial cytochrome oxidase (subunit 1) gene provides diagnostic markers for selected *Diabrotica* species (Coleoptera: Chrysomelidae). Bull. Entom. Res. 91 (6): 419-427.

Crozier, R. H. (1993): Molecular methods for insect phylogenetics. *In*. J Oakeshott and M. Whitten (ed.), Molecular approaches to fundamental and Applied Entomology, pp. 164-221. Springer-Verlag, New York.

EL-Torkey, A. M, Fadl, A. A., EL-Gharbawy, A. A. And ABDEL-DAYEM, M. S.(2005): A review of the Egyptian ant flower beetles (Anthicidae, Coleoptera). Bull. Ent. Soc.(115-141).

Imms, A. D. (1937): A general textbook of entomology. 727pp.

Kato, H., Gomez EA, Cáceres AG, Uezato H., Mimori T., and Hashiguchi Y (2010): Molecular epidemiology for vector research on leishmaniasis. Int J. Environ Res. Public Health. 7(3): 814-826.

Mukabana WR, Takken W, and Knols BG (2002): Analysis of arthropod blood-meal using molecular genetic markers. Trends Parasitol. 18(11): 505-509.

Oshaghi MA, Chavshin AR, Vatandoost H (2006): Analysis of mosquito blood-meal using RFLP markers. Exp. Parasitol. 114(4): 259-264.

Oshaghi MA, Rafinejad J, Choubdar N, Piazak N, Vatandoost H, Telmadarraiy Z, Mohtarami F, Ravasan NM (2001): Discrimination of relapsing fever *Borrelia persica* and *Borrelia microtti* by diagnostic species- specific primers and polymerase chain reaction-restriction fragment length polymorphism. Vector Zoonotic Dis. 11(3): 201-207.

Oshaghi MA, Rasolian M, Shirzadi MR, Mohtarami F, Doosti S (2010): First report on isolation of Leishmania tropica from sandflies of a classical urban Cutaneous leishmaniasis focus in southern Iran Exp. Parasitol. 126(4): 445-450.

Oshaghi MA, Ravasan NM, Hide M, Javadian EA, Rassi Y, Sedaghat MM, Mohebali M, Hajjaran H (2009): Development of species-specific PCR and PCR-restriction fragment length polymorphism assays for *L. infantum* / *L. donovani* discrimination. Exp. Parasitol. 122(1):61-65.

Oshaghi MA, Yaaghoobi F, Abaie MR (2006): Pattern of Mitochondrial DNA variation between and within *Anopheles stephensi* (Diptera: Culicidae) biological forms suggests extensive gene flow. Acta Trop. 99(2-3): 226-233.

Oshaghi MA, Yaghobi-Ershadi MR, Shemshad K, Pedram M, Amani H (2008): The *Anopheles superpictus* complex: introduction of a new malaria vector complex in Iran. Bull. Soc. Pathol Exot. 101(5):429-434.

Pfeifer, T. A., L. M. Humble, M. Ring, and T. A. Grigliatti (1995): Characterization of gypsy moth populations and related species using a nuclear DNA marker. Can. Entomol. 127:49-58.

Sperling, F. A. H, G. S. Anderson, and D. A. Hickey (1994): A DNA-based approach to the identification of insect species used for post-mortem interval estimation. J. Forensic Sci. 39:418-427.

Taylor, D. B., Szalanski, and R. D. Peterson (1996): identification of screwworm species by polymerase chain reaction-restriction fragment length polymorphism. Med. Vet. Entomol. 10: 63-70.

Uhman, G. (1995): Palaearctic Anthiciden (Coleoptera) des Ungarischen Naturwissen

Schaftlichen Museum, Budapest. 15. Beitrag Zur Kenntunis der Anthicidae Folia Entomological Hungarica, 36 (1): 177- 203.

Uhman, G. (1998): Anthicidae (Insecta: Coleoptera) from Saudi Arabia with the description of a new species. Fauna of Saudi Arabia, 17: 93-105.

ARABIC SUMMARY

دراسات مورفولوجية وجزيئية على بعض الأنواع من فصيلة أنثيسيدي (رتبة: غمدية الاجنحة) في مصر

محمد كامل عبيد

قسم وقاية النبات- كلية الزراعة- جامعة الازهر

تعتبر فصيلة أنثيسيدي مجموعة من الخنافس متباينة المعيشة وتعرف بأنها تشبه مظهرياً حشرات النمل والخنافس الارضية صغيرة الحجم، وقد تمت مقارنة ستة أنواع تتبع أربعة أجناس وهي أنثيكس وسيكلودينس وستريتيكومس واوماندوس وذلك بالاعتماد علي بعض الصفات المورفولوجية خاصة الاعضاء التناسلية الخارجية للذكور إلي جانب الدراسات الجزيئية حيث تم استخدام تقنية انزيمات القطع المتخصصة، واظهرت النتائج تأكيد تصعيد ثلاثة جنيسات إلي مرتبة الجنس وهي سيكلودينس وستريتيكومس واوماندوس واثبتت الدراسات الجزيئية وجود نوع لارفيبينس لا ينتمي إلي جنس سيكلودينس.