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Chromosomal analysis of some Egyptian diving beetles (Coleoptera: Dytiscidae)

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ABSTRACT

For the first time, the karyological data on nine African dytiscid beetles is represented. The studied species include: *Hydrovatus cuspidatus* (Kunze), *Hydroporus humilis* klug, *Nebrioporus insignis* (Klug), *Nebrioporus walkeri* (vand der Barnden), *Nebrioporus lanceolatus* (Walker), *Agabus biguttatus* (Olivier), *Colymbetes piceus* Klug, *Eretes sticticus* (Linnaeus) and *Hydaticus decorus* Klug. The data providing useful evidence of chromosomal stability of *Hydrovatus cuspidatus* over a wide geographical area. *Hydroporus humilis* showed 17 pairs of autosomes and sex chromosome. All species of *Nebrioporus* have standard number of chromosome 24 pairs of autosomes plus XX/X0 sex chromosomes and very little differences have been shown between the three Egyptian species. For *Agabus biguttatus*, the karyotype of 21 pairs of autosomes plus XX/X0 appears normal for genus *Agabus*. For *Colymbetes piceus* and *Hydaticus decorus*, it has been found that diploid number of chromosomes of both species are 20 pairs of autosomes plus XX/X0 sex chromosomes. The world-wide species *Eretes sticticus* has been confirmed to be 2n=43 (21+X0).

KEYWORDS: Coleoptera, Dytiscidae, chromosomes, Egypt

INTRODUCATION

Karyotypes of Coleoptera were first reported in the literature late in the 19th century by Carnoy and early in the 20th century by Henking and Stevens cited in (Makino 1951). During recent years there has been an increase in the studies of cytogenetics of Coleoptera in general and Adephaga in particular. Smith & Virkki (1978) listed all published information on Coleoptera cytogenetics up to that date and Serrano & Yadav (1984) summerized published and unpublished chromosome data for the aquatic Coleoptera up to March 1983. They list data for a total of 426 species, the vast majority of which belong to the Carabidae. Most cytological studies on Adephaga were done mainly on the fauna of Europe (Angus 1983, 1986, 1988, 1989; Angus & Pazos 1991; Shaarawi & Angus 1991a,b; Bilton 1992) and Asia (Yadav & Karamjeet 1981a,b,c; Yadav *et al.* 1984). In spite of many taxonomic studies on African aquatic beetles there is not any published attempt to explore the karyology of the African fauna.

The sex determining mechanism occurs in different forms in Coleoptera (Smith and Virkki 1978). The majority of organisms rely on chromosomes for sex determination. The system usually comprises a pair of chromosomes called the X and Y chromosomes. Normally females have 2X chromosomes (XX) and males XY.

In many Coleoptera the Y-chromosome is very small and associates with the X during meiosis by means of a nucleolus. At first metaphase the small y-chromosome often has the appearance of a small item being parachuted down, using the X-chromosome as a parachute (John & Lewis 1960), and this arrangement is gradually written XYp or Xyp. In many Adephaga including most Dytiscidae so far studied, there is no y-chromosome. Females have XX, and males have only one X-chromosome, this arrangement being written X0. In some genera, such as *Deronectes* Sharp, the X-chromosome fuses with an autosome to give a neo-

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XY system (Nilsson & Angus 1992), with X+ autosome becoming neo-X and the autosome alone the neo-Y.

Recently, Bilton (1992) has recorded the multiple sex chromosome, X_1X_2Y (male) - $X_1X_1X_2X_2$ (female) to the three species of genus *Noterus* (family: Noteridae, tribe: Noterini) and similar mechanisms are known in Cicindelidae and 2 species of family: Carabidae. Similarly, the chromosomes of Egyptian *Synchortus imbricatus* (Coleoptera: Noteridae) comprised 12 pairs of autosomes plus sex chromosomes which are X_1X_2Y (male) and $X_1X_1X_2X_2$ (female) Unlike *Noterus*, in *S. imbricatus* the Y-chromosome is the largest in the nucleus and is largely heterochromatic (Ahmed *et al.* 1997).

Multiple sex chromosomes occur when extra chromosomes have been incorporated into the sex determining system (Charlesworth *et al.* 1987). Such situations can arise from the incorporation of autosomes or via the fragmentation or duplication of the original sex chromosomes themselves (White 1973). Such systems are widespread in the animal kingdom, but show no clear pattern in their phylogenetic distribution (Bilton 1992).

MATERIALS AND METHODS

Table 1 gives details of the material used. Adult beetles were kept in the laboratory in small aquaria (plastic sandwich boxes) until they were used.

Chromosomes preparation: Chromosomes were prepared by acetic acid spreading (Angus 1982), mainly from mid-gut and testis tissues. This method was modified for use on adult beetles following Shaarawi & Angus (1989).

For mid-gut chromosomes to be available the beetles should have been feeding for at least about 24 hours. This induces mitosis of mid-gut crypt cells, to replace cells lost in the process of enzyme secretion during digestion (Chapman 1982). Meiotic chromosomes can most readily be observed in the testes of sexually mature male beetles.

C-banding: C-banding is often very useful, as it demonstrates constitutive heterochromatin. Slides are kept for 24 hours, then treated for about 7 min. in a saturated solution of barium hydroxide at room temperature (20-25°C). They are then rinsed in three changes of distilled water buffered to pH 6.8 with Sørensen before being transferred to double strength Salt Sodium Citrate (2X SSC: 0.3 M sodium chloride with 0.003 M trisodium citrate) at 60°C, and left for 1 hour. They are then rinsed in three changes of distilled water at pH 6.8. They are then stained.

Staining: All slides, both unbanded and C-banded, were stained in about 1% Giemsa stain in distilled water at pH 6.8. Slides were stained for about 10 min., then rinsed in distilled water (un-buffered), shaken dry and dried vertically in racks.

Examination and photography: Preparations were examined using a Leitz Orthoplan Photomicroscope, using a Leitz Fluortar 100/1.32 oil immersion lens and a Zeiss precision interference filter No. 467808 to give a monochromatic green light. Partial closure of the condenser sometimes helps to give a bolder image. Photographs were taken using Agfa-Gevaert Copex Pan film. For most purposes the automatic exposure was set at 9 DIN, but this was increased to 12 DIN for C-banded preparations. The film was developed for 2 min. at 20 C using Agfa-Gevaert G 141 C developer, mixed 1 part developer to 4 parts water. Prints were made at a magnification of 3000 x, with No. 3 grade paper suitable for most purposes. C-banded preparations were on softer paper (No.1 or No. 2 grade).

Table 1. Species of dytiscid beetles used for chromosomal analysis

Species	Locality of Origin	Tissue used
Hydrovatus cuspidatus (Kunze)	- Egypt: 10th of Ramadan. Sinai, St. Katherine. - Corsica: Pinarelli near Porto veccio.	Mid-gut, Testis, Ovary
Hydroporus humilis klug	- Egypt: Sinai, St. Katherine.	Mid-gut, Testis, Ovary
Nebrioporus insignis (Klug)	- Egypt: Sinai, St. Katherine.	Mid-gut Testis
Nebrioporus walkeri (vand der Barnden)	- Egypt: Sinai, St. Katherine.	Mid-gut, Testis
Nebrioporus lanceolatus (Walker)	- Egypt: Sinai, St. Katherine.	Mid-gut, Testis
Agabus biguttatus (Olivier)	- Egypt: 10th of Ramadan. Sinai, St. Katherine.	Mid-gut, Testis
Colymbetes piceus Klug	- Egypt: Sinai, St. Katherine.	Mid-gut
Eretes stictcus (Linnaeus)	- Egypt: 10th of Ramadan.	Mid-gut, Testis
Hydaticus decorus Klug	- Egypt: Sinai, St. Katherine.	Mid-gut, Testis

RESULTS

This is the first karyological data on some species of African dytiscid beetles including: *Hydrovatus cuspidatus*, *Hydroporus humilis*, *Nebrioporus insignis, Nebrioporus walkeri*, *Nebrioporus lanceolatus* (Hydroporinae), *Agabus biguttatus, Colymbetes piceus* (Colymbetinae), *Eretes sticticus, Hydaticus decorus* (Dytiscinae). The data are presented in Table 2 and Figures 1-4.

Hydrovatus cuspidatus (Fig. 1): Mitotic preparations are shown in Figs. 1.1-1.4. Corsican material is shown in Figs. 1.1,2,3, while Fig. 1.4 represents Egyptian material. The karyotype is identical in both localities, The diploid number of autosomes is 16 pairs and the sex chromosome system is X0 in male and XX in female. The autosomes 1 to 6 are mostly metacentric while 7 to 16 are slightly submetacentric and show apparently gradual decrease in size. The X chromosome is clearly metacentric.

Hydroporus humilis (Fig. 2): Mitotic preparations are shown in Figs. 2.1, 2.2. The diploid number of chromosomes differentiates into 17 pairs of autosomes and sex chromosomes which are X0 in male and XX in female. This results in a karyotype of 2n = 34 + XO in males and 2n= 34 + XX in females. The autosomes are mainly metacentric or nearly so. The first two pairs of autosomes are almost equal in length and the largest element in the nucleus. The rest of the autosomes show fairly gradual decrease in size. X chromosome is submetacentric and clearly larger than the last autosomal pair.

Genus: *Nebrioporus* (Fig. 3): Four species have been confirmed to be in the Egyptian fauna. Three species were available for chromosomal analysis, *N. walkeri* Van den Branden, 1885; *N. lanceolatus* (Walker 1871) and *N. insignis* (Klug 1834).

Mitotic preparations are shown in (Figs. 3.1, 3.2 *N. walkeri*; 3.3, 3.4 *N. lanceolatus* & 3.5 *N. insignis*). In all species the preparations are from mid gut and testis of adult beetles as in Table 1. The three species have the same diploid number of chromosomes which is (2n = 24 + XO) in male and (2n = 24 + XX) in female.

The sequence of relative lengths and centromere portions of the autosomes appears to be virtually identical in all three species. The last three pairs of autosomes (pairs 22, 23 and 24) are tiny, more or less a dot- like appearance. Seven pairs of autosomes are clearly acrocentric (pairs 6, 7, 9, 10 and 14-16 in the sequence of decreasing relative lengths) in the three species, in addition to one pair more (pair 3) in *N. insignis*. On the other hand, the first two pairs of autosomes are apparently metacentric in the three species. In all species X-chromosome is acrocentric and about the same length as the middle sized autosomes.

Table 2: Chromosome number and sex mechanism of some Egyptian dytiscid beetles. Data in parentheses are inferred from information on the opposite sex.

Species	Diploid no. of chromosomes		Sex mechanism		Karyotype
	male	female	Male	female	
Hydrovatus cuspidatus	33	34	X0	XX	2n = 33 (16 + X0), male 2n = 34 (16 + XX), female
Hydroporus humilis	35	36	X0	XX	2n = 35 (17 + X0), male 2n = 36 (17 + XX), female
Nebrioporus insignis	49	(50)	X0	XX	2n = 49 (24 + X0), male {2n = 50 (24 + XX),female}
Nebrioporus walkeri	49	(50)	X0	XX	2n = 49 (24 + X0), male {2n = 50 (24 + XX) female}
Nebrioporus lanceolatus	49	(50)	X0	XX	2n = 49 (24 + X0), male {2n = 50 (24 + XX) female}
Agabus biguttatus	43	(44)	X0	XX	2n = 43 (21 + X0), male {2n = 44 (21 + XX) female}
Colymbetes piceus	41	(42)	X0	XX	2n = 41 (20 + X0), male {2n = 42 (20 + XX) female}
Eretes sticticus	43	(44)	X0	XX	2n = 43 (21 + X0), male {2n = 44 (21 + XX) female}
Hydaticus decorus	41	(42)	X0	XX	2n = 41 (20 + X0), male {2n = 42 (20 + XX) female}

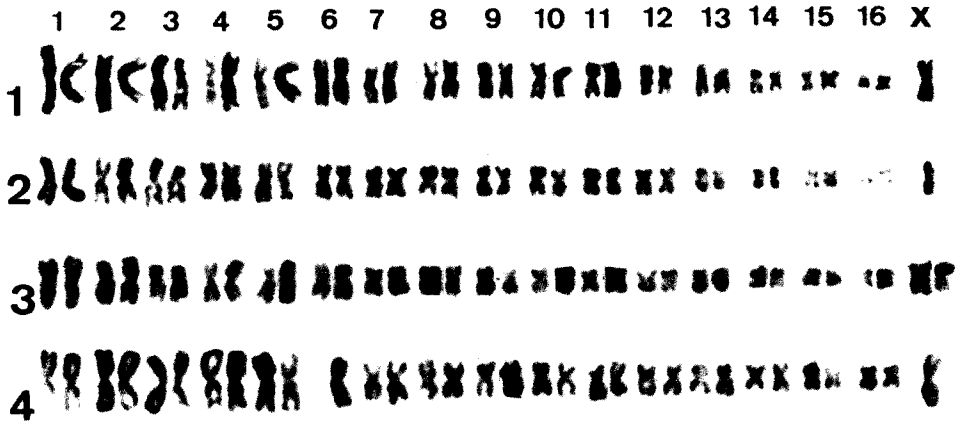

Figure 1: Mitotic chromosomes of *Hydrovatus cuspidatus*.1, 2, 3, 4 males, mid-gut nuclei, unbanded. The autosomes 1 to 6 are mostly metacentric while 7 to 16 are slightly submetacentric and show apparently gradual decrease in size. The X chromosome is clearly metacentric. 1, 2, 3 Corsican material, 4, Egyptian material. Karyotype with simple sex chromosome system 2n = 2n = 33 (16 + X0) in males and in 2n = 34 (16 + XX) in females. Scale line represents 5µm.

Figure 2: Mitotic chromosomes of *Hydroporus humilis*.1, male, mid-gut nuclei, unbanded. 2, female, ovary, unbanded. The autosomes are mainly metacentric or nearly so. The first two pairs of autosomes are almost equal in length and the largest element in the nucleus. The rest of the autosomes show fairly gradual decrease in size. X chromosome is submetacentric and clearly larger than the last autosomal pair. Karyotype with simple sex chromosome system 2n = 35 (17 + X0) in males and 2n = 36 (17 + XX) in females. Scale line represents 5µm.

Agabus biguttatus (Fig. 4): Mitotic preparations are shown in Figs. 4.5, 4.6. The karyotype comprises 21 pairs of autosomes and sex chromosomes which are X0 in male and XX in female. Two pairs of autosomes (pairs 3 and 4) are clearly submetacentric, while all the others are more or less metacentric. The autosomes 1 to 13 show a slight decrease in size, while the rest group of autosomes is about the same size. The X chromosome is submetacentric.

Colymbetes piceus (Fig. 4): Mitotic preparations are shown in Fig. 4.4. The karyotype possesses 20 pairs of autosomes and sex chromosomes which are X0 in male and almost certainly XX in female. The autosomes are small to medium sized, mainly submetacentric or nearly so except for first pair that is metacentric. The X chromosome is about the same length as in first autosome but clearly submetacentric.

Eretes stictcus (Fig. 4): Mitotic preparations are shown in Figs. 4.1, 4.2. The diploid number of chromosomes contains 21 pairs of autosomes and sex chromosomes that are X0 in male and XX in female. This results in a karyotype of 2n = 42 + X0 in males and almost certainly 2n = 42 + XX in females. The autosomes are differentiated into 11 pairs of metacentric (pairs 1-3, 5-8, 10, 11, 14, 15), 5 pairs of submetacentric (pairs 4, 9, 12,13, 16) and 5 pairs of acrocentric (pairs 17-21). The X chromosome is relatively of medium size and distinctly submetacentric.

Hydaticus decorus (Fig. 4): Mitotic preparations are shown in Fig. 4.3. The karyotype consists of 20 pairs of autosomes and sex chromosome that are X0 in male and almost certainly XX in female. The diploid number of chromosomes is 2n = 40 + XO in male and almost certainly 2n = 40 + XX in female. The autosomes are mostly metacentric except for pairs 1, 3-5 and 12, 13 which are submetacentric. They show clearly decrease in length with last pair (20) of very tiny size. The first pair is distinctly of second constriction and second one is acrocentric. X chromosome is submetacentric.

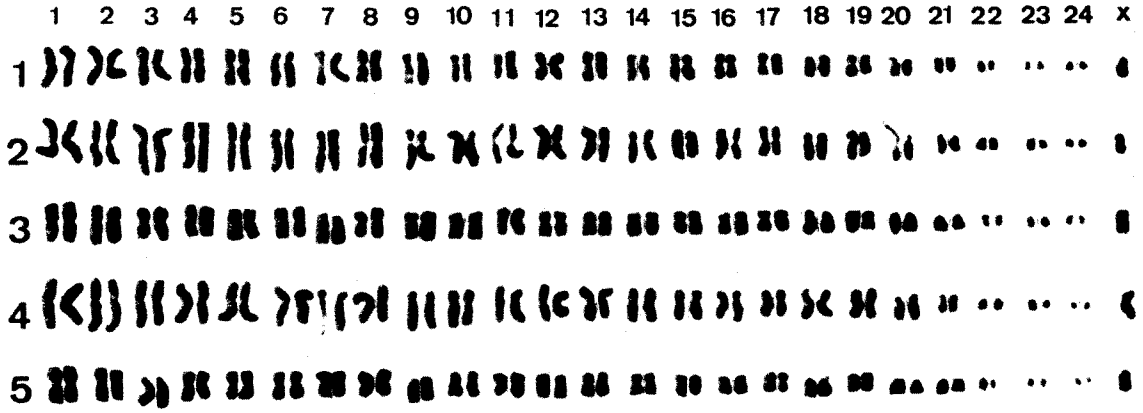

Figure 3: Mitotic chromosomes of genus *Nebrioporus*. 1,2 males, mid-gut nuclei, unbanded, *N. walkeri*. 3,4 males, unbanded, *N. lanceolatus*. 3, mid-gut. 4, testis. 5, males, mid-gut nuclei, unbanded, *N. insignis*. The sequence of relative lengths and centromere portions of the autosomes appears to be virtually identical in all three species. The last three pairs of autosomes (pairs 22, 23 and 24) are tiny, more or less a dot-like appearance. Seven pairs of autosomes are clearly acrocentric (pairs 6, 7, 9, 10 and 14-16 in the sequence of decreasing relative lengths) in the three species, in addition to one pair more (pair 3) in *N. insignis*. On the other hand, the first two pairs of autosomes are apparently metacentric in the three species. In all species X-chromosome is acrocentric and about the same length as the middle sized autosomes. Karyotype with simple sex chromosome system 2n = 49 (24 + X0) in males and {2n = 50 (24 + XX) } in females. Scale line represents 5µm.

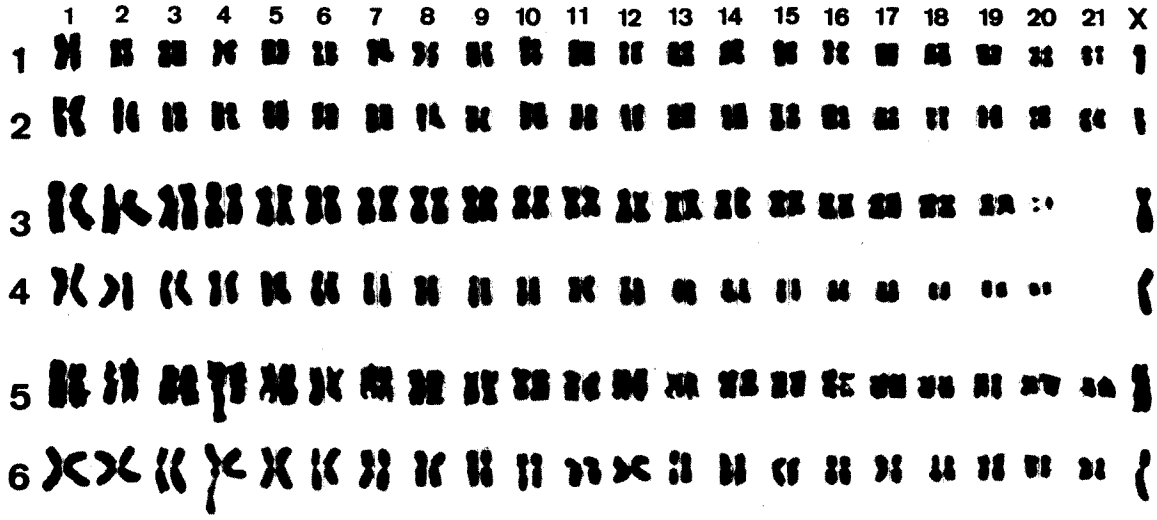

Figure 4: Mitotic chromosomes. 1,2 *Eretes sticticus*, males, mid-gut nuclei, unbanded. The autosomes are differentiated into 11 pairs of metacentric (pairs 1-3, 5-8, 10, 11, 14, 15), 5 pairs of submetacentric (pairs 4, 9, 12,13, 16) and 5 pairs of acrocentric (pairs 17-21). The X chromosome is relatively of medium size and distinctly submetacentric. Karyotype with simple sex chromosome system 2n = 43 (21 + X0) in males and {2n = 44 (21 + XX)} in females. 3, *Hydaticus decorus*, males, testis nuclei, unbanded. The autosomes are mostly metacentric except for pairs 1, 3-5 and 12, 13 which are submetacentric. They show clearly decrease in length with last pair (20) of very tiny size. The first pair is distinctly of second constriction and second one is acrocentric. The X chromosome is submetacentric. Karyotype with simple sex chromosome system 2n = 41 (20 + X0) in males and {2n = 42 (20 + XX) } in females.4, *Colymbetes piceus*, The autosomes are small to medium sized, mainly submetacentric or nearly so except for first pair which is metacentric. The X chromosome is about the same length as in first autosome but clearly submetacentric.Karyotype with simple sex chromosome system 2n = 41 (20 + X0) in males and {2n = 42 (20 + XX) } in females. 5,6 *Agabus biguttatus*, males, testis nuclei, unbanded. Two pairs of autosomes (pairs 3 and 4) are clearly submetacentric, while all the others are more or less metacentric. The autosomes 1 to 13 show a slight decrease in size, while the rest group of autosomes are about the same size. The X chromosome is submetacentric. Karyotype with simple sex chromosome system 2n= 43 (21 + X0) in males and {2n = 44 (21 + XX)} in females. Scale line represents 5μm.

DISCUSSION

Dytiscids possess simple sex-chromosome mechanisms (Yadav *et al.* 1984) with predominant formula X0 formula in male (Smith & Virkki 1978) which frequently gives rise to species with neo XY (White 1973). All investigated species have typical formula X0 of sex-chromosome mechanism.

The range of haploid chromosome number in the present investigations shows a wide distribution in subfamily Hydroporinae: n = 17 in *Hydrovatus cuspidatus* to n = 25 in the three species, *Nebrioporus insignis, Nebrioporus walkeri*, and *Nebrioporus lanceolatus*. Consequently, genus *Nebrioporus* has the biggest number of chromosomes. Actually, no clear picture with regard to a primitive dytiscid karyotype and the direction it might have taken during the course of evolution emerges from the available data. Smith (1950) has proposed that the primitive coleopteran karyotype contained nine pairs of autosomes. As more karyological information becomes available, it seems that for the Adephaga higher numbers are usual, even in the more primitive groups. More data are needed before any conclusion can be reached.

Hydrovatus cuspidatus: There is no published information on the chromosomes of *Hydrovatus*. However, Angus (unpublished data) has chromosomes of Corsican material of *H. cuspidatus*. The Egyptian material agrees with that from Corsica, providing useful evidence of chromosomal stability over a wide geographical area.

Hydroporus humilis: A simple dytiscid karyotype with 17 pairs of autosomes and the XX / X0 sex system normal in the Adephaga (Serrano & Yadav 1984).

Balke & Fery (1993) have shown that *H. humilis* is closely related to *H. tessellatus* Drapiez, and comparison of the karyotype would be of great interest. Unfortunately, attempts to obtain English *H. tessellatus* were unsuccessful because of the summer drought of 1995.

There are no published data on *Hydroporus* chromosomes, but Angus (unpublished) has 20 pairs of autosomes plus X0/XY for English *H. cantabicus* Sharp and Swedish *H. melanarius* Sturm.

Nebrioporus: The chromosome of this genus are of particular interest as it is the one case so far known where a large dytiscid genus appears to have a standard number of chromosomes 24 pairs of autosomes, ranging from medium sized metacentric to small (dot-like) acrocentric plus XX / X0 sex chromosomes (Nilsson & Angus 1992)

The karyotypes of the three Egyptian species *Nebrioporus insignis, Nebrioporus walkeri* and *Nebrioporus lanceolatus* confirm to this pattern, and show very little difference between the three species. The fourth Egyptian species, *N. cerisyi* was not available for study but Nilsson & Angus (1992) mentioned 50 chromosomes in an Israeli female- apparently 24 pairs of autosomes plus XX.

Agabus biguttatus: The karyotype of 21 pairs of autosomes plus XX / X0 appears normal for *Agabus* (Fery & Nilsson 1993 quoting unpublished data from Angus). At present no chromosome data on non-Egyptian *Agabus biguttatus* are available, but they would clearly be of great interest in view of the ecological peculiarities of some Egyptian *Agabus biguttatus.*

There are a number of published records of XY sex chromosome systems in *Agabus*. Thus, Suortti (1971) recorded *A. sturmii* Gyll. and *A. bipustulatus* (L.) as having 21 bivalents at meiosis, including Xyp sex-mechanisms; Yadav *et al.* (1984) recorded Indian *A. conspersus* Marsham as having 19 pairs of autosomes plus XY, and Smith (1953) recorded *A. confinis* Gyll. as having 20 pairs plus XY. However, Angus (unpublished) found that English material of the first three species has 21 pairs of autosomes plus XX / X0, and that a Swedish female of *A. confinis* had 44 chromosomes. There is thus at present no good reason to believe that any *Agabus* has an Y-chromosome.

Colymbetes piceus: The cytology of the genus Colymbetes Clairville was first studied by Günthert (1910) who described the oogenesis of *Colymbetes fuscus* Aubé, mentioned 2n = 35-37 (female) and drew comparison between his and Giardina's results (1901). Smith & Virkki (1978) reported *C. paykulli* Er. as having 17 pairs of autosomes plus Xyp, *C. striatus* L. as having 19-21 pairs plus XY and recorded polymorphism in *C. paykulli*. There are no published data on the chromosomes of *C. piceus* and in this study it has been found that diploid number of chromosomes 2n = 41 (20+ X0) (male).

Eretes sticticus: There are two previous records on the chromosome number of *Eretes sticticus*, one by Joneja (1960) as 2n = 41 (20 + X0) (male) and other one by Yadav *et al.* (1984) as 2n = 43 (21 + X0) (male). The chromosome number given by Yadav et al. (1984) agrees with the present study.

Hydaticus decorus: Joneja (1960) recorded the chromosome number of two species of genus *Hydaticus* Leach: *H. fabricii* Macl as 2n = 41 (20 +X0) (male) and *H. leander* Rossi as 2n = 45 (22 +X0) (male). However Yadav *et al.* (1984) reported 2n = 41 (20 +X0) (male) for *H. leander* and gave the chromosome number for two more species, *H. vittatus* F. as 2n = 45 (22 + X0) (male) and *H. luczonicus* Aubé as 2n = 41 (20 +X0) (male). There is no published data

on the chromosomes of *H. decorus* and according to this study it has been found a diploid number of chromosomes 2n = 41 (20+ X0) (male).

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الملخص العربى

التحليل الكروموسومى لبعض أنواع خنافس الغوص المصرية (رتبة غمدية الأجنحة - فصيلة دايتسكيدى)

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من خلال هذه الدراسة، ولأول مرة، يتم تناول التركيب الكروموسومى لتسعة أنواع من خنافس الغوص العالمية والتى تنتشر فى البيئة المصرية، وقد شملت الدراسة الأنواع التالية: *هيدروفاتس كاسبيدياتس*، *هيدروبورس هيوميليس*، *نبروبورس إنسيجنيس*، *نبروبورس واكارى*، *نبروبورس لانسيولاتس*، *أجابس بيجاوتاتس*، *كوليمبيتيس بيكيوس*، *وهاديتيكاس ديكوراس*، *إريتاس ستيتيكاس*. وكانت النتائج على النحو التالى:-

١- ثبات التركيب الكروموسومى للنوع *هيدروفاتس كاسبيدياتس* وذلك فى المناطق الجغرافية المتباعدة (لا يوجد تأثير للعزل الجغرافى على التركيب الكروموسومى لهذا النوع).

٢- أن النوع *هيدروبورس هيوميليس* يحتوى على ١٧ زوج من الكروموسومات الجسدية بالإضافة إلى زوج من الكروموسومات الجنسية

٣- أن الثلاثة أنواع المصرية من جنس *نبروبورس* تحتوى على ٢٤ زوج بالإضافة إلى الزوج الجنسى، وأن هناك إختلافات طفيفة بين الأنواع المصرية الثلاثة.

٤- بخصوص النوع *أجابس بيجاوتاتس* فيحتوى على ٢١ زوج بالإضافة إلى الزوج الجنسى، وهذا يتفق مع التركيب الكوموسومى لجنس *أجابس* بشكل عام.

٥- بخصوص كل من *كوليمبيتيس بيكيوس* و*هاديتيكاس ديكوراس* فيحتوى على ٢٠ زوج بالإضافة إلى الزوج الجنسى.

٦- أكدت دراسة التحليل الكروموسومى للنوع المصرى *إريتاس ستيتيكاس* تطابقه مع التركيب الكروموسومى لهذا الجنس الواسع الإنتشار فى العالم (٢١ زوج جسدى + زوج جنسى).