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A cytogenetics study of *Hydrodroma despiciens* (Müller, 1776) (Acari: Hydrachnellae: Hydrodromidae)

Serap Tutgun Onrat[1], Ferruh Aşçı[2] and Muhlis Özkan[3]

[1]Department of Molecular Biology, Faculty of Science, Afyon Kocatepe University, Afyon, Turkey
[2]Department of Zoology, Faculty of Science, Afyon Kocatepe University, Afyon, Turkey
[3]Education Faculty, Uludağ University, Bursa, Turkey
Corresponding author: S.T. Onrat
E-mail: tutgunonrat@yahoo.com

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ABSTRACT. The karyotypes of water mites (Acari: Hydrachnellae: Hydrodromidae) are largely unknown. The present investigation is the first report of a study designed to characterize the chromosomes of water mites. The study was carried out with specimens of *Hydrodroma despiciens* collected from Eber Lake in Afyon, Turkey. Several different methods were tried to obtain chromosomes of this species. However, somatic cell culture proved to be the most effective for the preparation of chromosomes. In the present study, we determined the diploid chromosome number of *Hydrodroma despiciens* to be 2n = 16. However, a large metacentric chromosome was found in each metaphase, which we believed to be the X chromosome. We could not determine the sex chromosomes of this species. This study is the first approach to the cytogenetic characterization of this water mite group. Furthermore, these cytogenetic data will contribute to the understanding of the phylo-

genetic relationship among water mites. To our knowledge, this is the first report on the cytogenetics of water mites.

Key words: Chromosomes, *Hydrodroma despiciens*, Hydrachnellae, Acari

INTRODUCTION

Water mites are known as Hydracarina, Hydracnidia or Hydrachnellae. This group belongs to the phylum Arthropoda, class Arachnida, order Acarina, and contains 40 families. Thus, approximately 5000 water mite species are known (Smith and Cook, 1991). However, there have not been enough studies on water mites in Africa, Asia and South America. Therefore, the total number of species may be more than the number mentioned above. Water mites live in lakes, ponds, streams, and lentic waters. They also live in hot water resources, waterfalls, underground water, and seas (Cook, 1974).

Water mites have a complex life cycle. Their eggs are founded in water bodies and on aquatic plants. They live in many animals as exoparasites in the larval term. They are characterized by having three pairs of legs in the larval form. Sexual dimorphism has often been observed in water mites (Smith, 1998).

Hydrodroma despiciens (Müller, 1776) is a common species of water mites (Özkan, 1981). There have been many studies on the anatomy and morphology of this species (Lang, 1905; Schmidt, 1935; Besseling, 1940; Bader, 1954; Meyer, 1983). Recently, there have been many investigations regarding their developmental biology (Meyer, 1985). Some research has concentrated on egg and larval development, nymph morphological structure, and habitats of adults (Sokolow, 1924, 1925, 1954, 1977; Prasad and Cook, 1972). Morphological information of this species and its family has been nearly completed.

The cytogenetics of water mites has expanded considerably over the past few decades. Nevertheless, the karyotype of most groups is still poorly understood or is completely unavailable. Cytogenetic studies have concentrated on hard and soft mites. Recently, similar research has been done on Acarida (Bernini, 1986). The present study was designed to resolve systematics problems of all acari species.

Given the huge variation in chromosome complements among insects, karyotypical techniques have proved to be extremely useful in systematics studies at both the micro- and macroevolutionary levels in several orders such as Hymenoptera (Gokhman and Quicke, 1995), Orthoptera (White, 1973; John, 1983; Westerman and Hewitt, 1985) and in various genera of Diptera including *Drosophila*, *Chironomus* and *Aedes* (King, 1993; MacGregor, 1993).

In this study, a chromosome study was conducted using the somatic cell culture method in the species *Hydrodroma despiciens* belonging to the Hydrodromidae family. Many cytogenetic methods were tried: first, individuals were fixed in 3:1 (absolute ethanol:glacial acetic

acid); mites were dissected out and slides were performed by the squash method in iron propionic hematoxylin (Nùñez, 1968; Rodrý´guez Gil et al., 2002). Secondly, the material was previously stained with hematoxylin and then squashed under a coverslip (Guerra, 1983). Third, the material was first squashed in 45% acetic acid and then stained with hematoxylin (Fujii and Guerra, 1998).

Although the Hydrodromidae is a large family whose members provide ecological benefits, there are several species that remain cytogenetically unknown, requiring further cytogenetic studies of this family. The aim of the present study was to determine the karyotype of *Hydrodroma despiciens* using the somatic cell culture method.

MATERIAL AND METHODS

Collection of specimens

Samples were collected from Eber Lake in Afyon, Turkey and preserved and prepared according to the methods described by Özkan (1981).

Morphological examination

Sample figures were drawn and the dimensions were measured in microns.

Chromosome preparations

We modified and updated methods for Hydrachnellae chromosomes and used the somatic cell culture method. The culture medium was Drosophila Schneider Medium or TC-100 Insect Medium containing 10% heat-inactivated fetal bovine serum, 2% 1/100 penicillin/streptomycin (10,000 units/mL stock) (Gibco/Lifescience), 2% 1/100 glutamine (200 mM stock) (Gibco/Lifescience), 0.35 g $NaHCO_3$, 8.1 mL 2 N NaOH and 2.25 g NaCl, to which 1000 mL distilled water was added, and the final medium was sterilized by filtration. PHA-M (Cat. No. 12-006-1H) was added to the medium at 2-4 mL per 100 mL. Mites were then squashed in water on the slides and then put in a glass or plastic tube along with 5 mL of medium. Cell culture was carried out at 28°C for 48 h. A volume of 0.2 mL colcemid solution (Cat. No. 12-004-1) was added to each culture tube, and the culture was incubated for another 15-30 min. The culture was then transferred to a centrifuge tube and spun at 1200 rpm for 5 min. The supernatant was removed and the cells were resuspended in 5-10 mL hypotonic 0.075 M KCl (Cat. No. 12-005-1). The cell suspension was incubated at 28°C for 10-12 min and then spun at 1200 rpm for 5 min, after which the supernatant was removed. The cell pellet was agitated and 5-10 mL of fresh, ice-cold fixative (1:3, acetic acid:methanol) was added drop-by-drop. After allowing to stand 10 min at 4°C, the fixative solution was changed three times using centrifugation. The cell pellet was resuspended in a small volume (0.5-1 mL) of fresh fixative, and cells were dropped onto a clean slide which was allowed to air dry. Preparations were dried overnight and stained with 5% Giemsa solution in Sörensen's phosphate buffer, pH 6.8, for 30-40 min. Preparations were inspected using an Olympus BX 50 microscope and an oil immersion objective, and the best metaphases were photographed.

RESULTS

Morphological results

The following measurements were determined according to the results drawn: round body, 1330/1180 µm: lengths of the coxae, 250-330-350-400 µm; acetabular plates, 220-300 µm; excretory pore, 80 µm; lengths of the leg segments; LegI: 60-130-100-180-270-240 = 980 µm, LegII: 80-140-150-300-360-280 = 1310 µm, LegIII: 90-150-150-300-370-290 = 1350 µm, LegIV: 130-210-220-390-410-330 = 1690 µm; capitulum, 230-340 µm; dorsal lengths of palp segments: 50-70-40-150-80 = 390 µm; ventral lengths of palp segments: 50-40-42-100-70 = 302 µm; heights of palp segments: 70-60-63-50-20 µm; chelicerae, 320 µm; strong chelicerae claws, 60 µm (Figures 1 and 2).

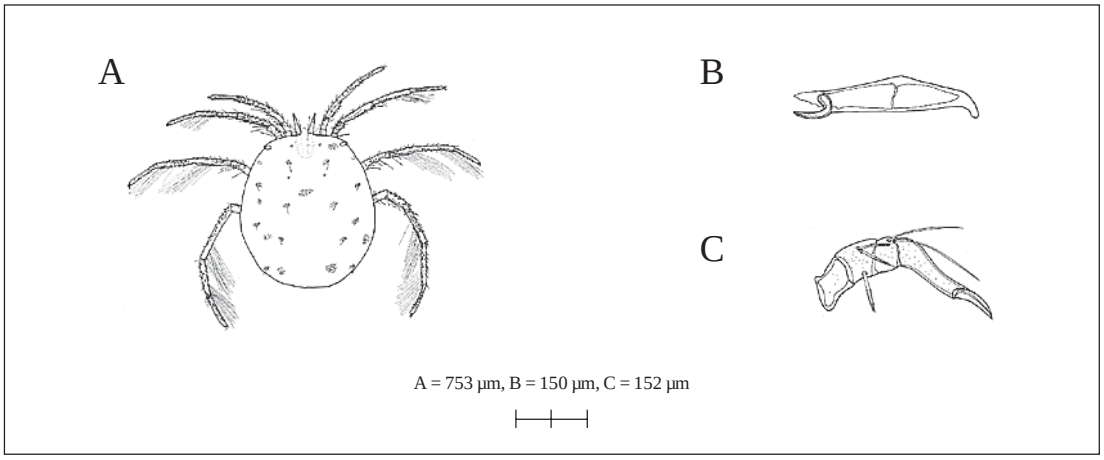

Figure 1. *Hydrodroma despiciens*, **A.** ♀ Body, dorsal view, **B.** Cheliserae, **C.** Palp.

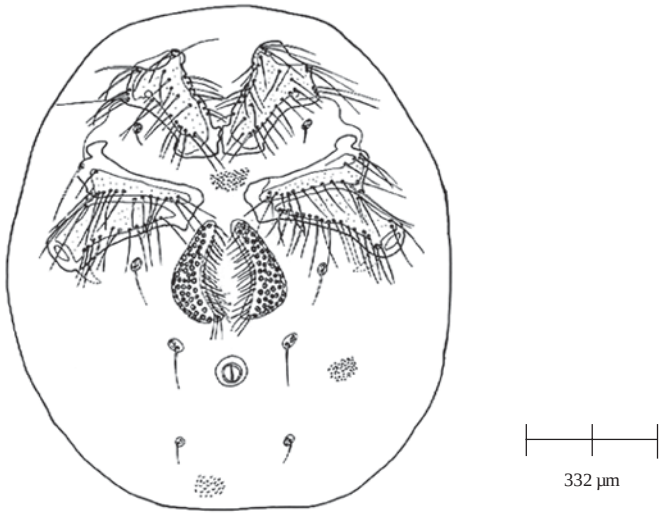

Figure 2. *Hydrodroma despiciens*. ♀ Body, ventral view.

Cytogenetic results

Hydrodroma despiciens has a diploid number of 2n = 16 in the mitotic phase of somatic cells. The morphological constitution of the chromosomes is metacentric, submetacentric, acrocentric, and telocentric (Figure 3a,b,c,d,e,f).

We experimented several methods for preparing water mite chromosomes. We tried Sharma's method, but found it too complicated, and therefore it was not used. The squash method used by Maddison (1982) may be practicable for water mites. The results were informative for water mite chromosomes; however, there were many metaphase plates and it was difficult to count the chromosomes. The embryonic cell suspension method (splash method) used by Matsumoto (1977) and Rowell (1985) can only determine the 2*n* number, but not the sex-determining mechanism. This method was very difficult to determine chromosomes in water mites, since we could not obtain embryonic cells of water mites. Also, the smear method is not applicable for water mite chromosomes. The cytogenetic methods we tried were as follows: first, individuals were fixed in absolute ethanol:glacial acetic acid (3:1). Mites were then dissected out and slides were performed by the squash method in iron propionic hematoxylin (Nùñez, 1968). The dissection of water mites was very difficult, because of the very small size of water mites. Secondly, the material was first stained with hematoxylin and then squashed under a coverslip (Guerra, 1983), but staining was not effective. Thirdly, the material was first squashed in 45% acetic acid and then stained with hematoxylin (Fujii and Guerra, 1998), and this method proved to be practical.

Among the classical cytogenetic methods we tried, somatic cell culture was the most informative; thus, this method may be developed by researchers to study water mite chromosomes.

DISCUSSION

This study presented, for the first time, the karyotype of water mites of the family Hydrodromidae. A cytogenetic study has not been carried out in water mites until now, despite that systematics studies have been ongoing for the past 250 years. The aim of this study was to resolve systematics problems by using cytogenetic data which would be beneficial to taxanomists studying this area. We used several different methods in our experiments for this purpose, but the best results were obtained using the somatic cell culture method. First, we found a 2n = 16 diploid chromosome number in *Hydrodroma despiciens*, but we could not determine the sex chromosomes. On the other hand, we determined one large chromosome in each metaphase. We believe that this large chromosome is probably an X chromosome. As a result of this study, we can use somatic cell culture methods to obtain data on *Hydrodroma despiciens* chromosomes.

There are many methods for preparing chromosomes of spiders and various insect groups, but we have no knowledge of any methods for water mites other than the thin paraffin section method used by Sharma (1950) and the squash method used by Maddison (1982). The embryonic cell suspension method (splash method) used by Matsumoto (1977) and Rowell (1985) can only determine the 2*n* number but not the sex-determining mechanism. All these methods are unsuitable for field use. Cokendolpher and Brown (1985) developed an air-dry method, which has been used by many authors (Tugmon et al., 1990; Gorlova et al., 1997). It is

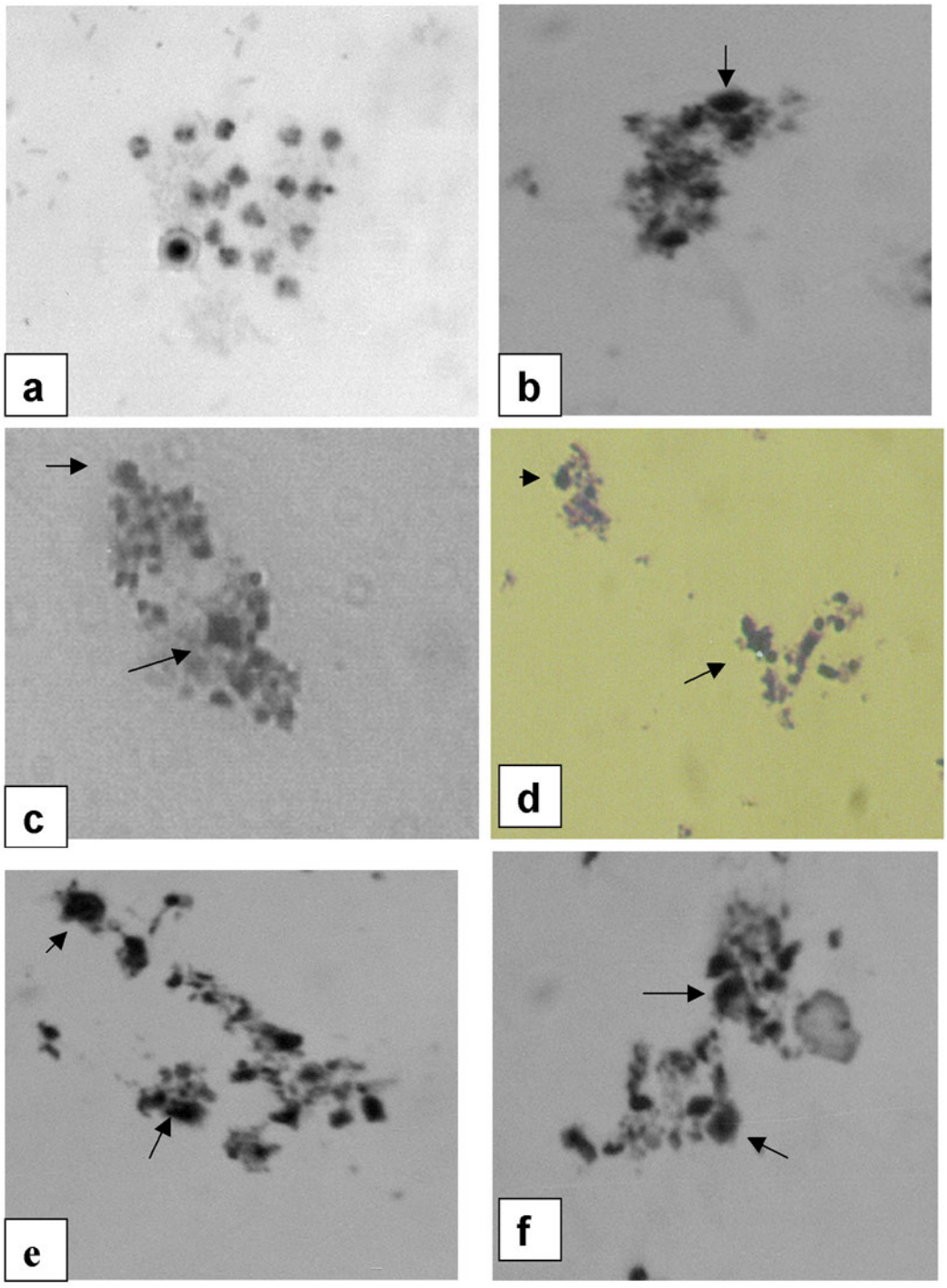

Figure 3. I- We obtained the best of metaphase chromosomes by using the somatic cell culture method **(a).** Metaphase chromosomes have been obtained by using some other methods **(b, d, e, f), II-** Anaphase phases **(c), III-** Large chromosomes (arrows, probably X chromosomes) (100X, oil immersion).

a modified cell suspension method, but avoids dropping the cells. Instead, a glass rod is used to macerate the tissue and spread the dissociated cells by tilting the slide back and forth. No true smear method is used in the study of chromosomes. The improved air-dry method using fine needles to macerate (smear) the tissues, developed by Imai et al. (1977) with Australian ants and elaborated by Luykx (1983) with the wood roach, is considered a needle smear method in contrast to that of Cokendolpher and Brown (1985). Chen (1999) followed the procedures of Luykx (1983) but removed the gonads and transferred them to a slide before hypotonic treatment in a humid chamber.

As a result of this study, the somatic cell culture method was found to be the most informative method among the cytogenetic methods examined.

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