Description of *Achromadora gracilis* n. sp. and observations on *A. micoletzkyi* (Stefanski, 1915) Van der Linde, 1938 and *Ethmolaimus multipapillatus* Paramonov, 1926 (*Nematoda: Chromadorida*) from Spanish lakes (*)

Descripción de *Achromadora gracilis* n. sp. y observaciones de *A. micoletzkyi* (Stefanski, 1915) Van der Linde, 1938 y *Ethmolaimus multipapillatus* Paramonov, 1926 (*Nematoda: Chromadorida*) en lagos españoles (*)

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SUMMARY

Three species of *Chromadorida* have been found for the first time in continental waters in Spain. Two of these belonging to the genus *Achromadora* Cobb, 1913, were found in high-mountain lakes in Sierra Nevada Mountains (Granada) at an altitude of over 2700 m.a.s.l.: *Achromadora micoletzkyi* (Stefanski, 1915) Van der Linde, 1938 and *A. gracilis* n. sp. The third species, *Ethmolaimus multipapillatus* Paramonov, 1926 was found in the hypersaline lake Fuente Piedra in Archidona (Málaga Province). A description of *A. gracilis* n. sp. is given. In addition, environmental date of two congeneric species (*A. gracilis* and *A. micoletzkyi*) are discussed. Supplementary morphological-anatomical and environmental data are provided for *E. multipapillatus*.

RESUMEN

Tres especies de chromadóridos, dos de *Achromadora* Cobb, 1913 y una de *Ethmolaimus* De Man, 1880, han sido encontradas en lagos de características fisico-químicas extremas. *Achromadora micoletzkyi* (Stefanski, 1915) Van der Linde, 1938 y *Achromadora gracilis* n. sp. se encuentran en lagunas de alta montaña en Sierra Nevada, a una altitud por encima de 2700 m.s.n.m. *Ethmolaimus multipapillatus* Paramonov, 1926 se ha encontrado en la laguna de Fuente Piedra en Archidona (Málaga), laguna hipersalina, con una conductividad de unos 180000 µS/cm. De *A. micoletzkyi* se destacan algunos datos morfológico-anatómicos y ecológicos de interés, se describe *A. gracilis* sp. n. y se aportan datos morfológico-anatómicos y ecológicos de *E. multipapillatus* que completan su conocimiento.

INTRODUCTION

During two nematode surveys conducted in southern Spain, the first in 20 high-mountain lakes in the Sierra Nevada Mountains (Granada Province) and the second in 8 hyper-salinized lakes in the Campillo and Archidona area (Málaga Province), six species of Chromadorida Chitwood, 1933, were found. *Achromadora ruricola* (de Man, 1880) Micoletzky, 1925 was mentioned in Gadea (1955); *A. terricola* (de Man, 1880) Micoletzky, 1925 was studied in Ocaña, *et al.* (1990), and *Ethmolaimus pratensis* de Man, 1880 was studied in Ocaña & Morales (1992). These three species are well known worldwide and, as mentioned here, were found previously in Spanish continental waters.

Taxonomical and ecological data are provided for the three other found species of *Chromadorida*. *Achromadora gracilis* n. sp. is described by the first time. *A. micoletzkyi* (Stefanski, 1915) Van der Linde, 1938 is a cosmopolitan species repeatedly described in detail, recently by Eyualem & Coomans (1995). Therefore, in the present paper, the only anatomical data provided are those of table I, for comparison with findings by other authors. Both of the *Achromadora* species were found together and in large numbers in 12 of 20 lakes studied. The physico-chemical characteristics of the lakes and other data, which might explain the concurrence of these two congeneric species, are provided in tables II and III. *Ethmolaimus multipapillatus* was recently considered by Jensen (1994) and Platt (1982, 1985). Our study helps complete and broaden what is already known about this species.

MATERIAL AND METHODS

Bimonthly samples were collected from the 20 high-mountain lakes in the Sierra Nevada from July through September 1990 and 1991. In addition, 150 cc of sediment were collected at a depth of between 3-5 cm in the littoral

zone. Samples were likewise collected every three months from the lakes in Archidona (e.g. Lake "Fuente Piedra") during 1992 and 1993. Nematodes were extracted from the substrate samples following the Baermann method (1917) modified by Hooper (1986). Extracted material was fixed with 4% acetic formalin and mounted as described by Seinhorst (1962) (modified version) in anhydrous glycerine. Nematode measurements are given as ranges, followed by means and standard deviations in parentheses.

In situ temperature, conductivity and water pH measurements were taken, as were separate water samples for laboratory determination of the dissolved oxygen following the Winkler method. Data on Cl^-, CO_3^{2-}, SO_4^{2-} from Lake Fuente Piedra are reported by Monterrubio & Ocaña (1995). Data on chlorophyll **a** are reported by Sánchez-Castillo (1986).

***Achromadora micoletzkyi* (Stefanski, 1915) Van der Linde, 1938** (Fig. 1 A, B, Table I)

Differential morphological data: Morphological characteristics are in general agreement with those reported by other authors: Schneider (1925) (described as *Achromadora tenera*, a synonym of *A. micoletzkyi*); Altherr (1952) (described as *A. inermis*, a synonym of *A. micoletzkyi*) and especially with the most recent study published by Bongers (1988), Zeidan & Geraert (1989), particularly the latter in the Wadigalol and Kas populations, and Eyualem & Coomans (1995), big form (see table I). Nevetheless, we wish to stress the greater body thickness found in populations from our lakes (smaller **a** index) and overall longer tails, even in comparison with populations found in springs also situated in the province of Granada (Ocaña, 1987).

Locality and habitat: Samples collected from sandy sediments of 15 high-mountain lakes in Sierra Nevada with pH, temperature, conductivity and oxygen concentration values ranging between 5.7 to 8.2, 6 to 18.7°C, 7 to 60 µS/cm and 5.1 to 9.3 mg/l, respectively.

Voucher specimens: Specimens of *Achromadora micoletzkyi* are on slides 93-100, deposited in the nematology collection of the Dpto. Biología Animal y Ecología, Universidad de Granada.

***Acromadora gracilis* n. sp.** (Fig. 1 C, D, E)

Females (n = 9): L = 640-850 (752 ± 78) µm; a = 29.2-39 (35.2 ± 3.1); b = 5.7-6.8 (6.4 ± 0.5); c = 3.8-6 (4.6 ± 0.7); c' = 9.4-14.8 (12.7 ± 1.4); V = 42-48 (44 ± 2.6) %.

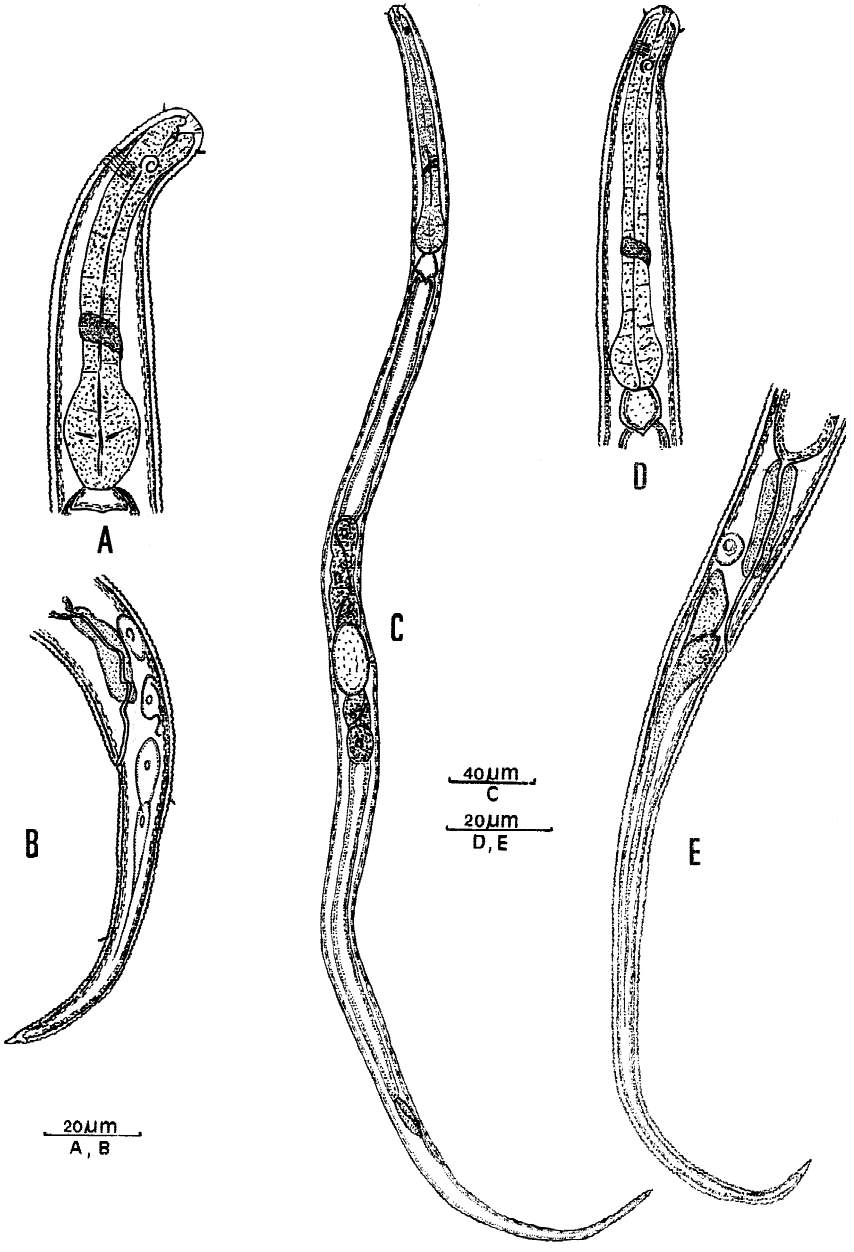

Fig. 1.—*Achromadora micoletzkyi (*Stefanski, 1915) Van der Linde, 1938: A) Anterior region. B) Tail. Achromadora gracilis n. sp.: C) Female. D) Anterior region. E) Tail.
Fig. 1.—*Achromadora micoletzkyi* (Stefanski, 1915) Van der Linde, 1938: A) Región anterior. B) Cola. *Achromadora gracilis* n. sp.: C) Hembra. D) Región anterior. E) Cola.

Table I.—Measurements of *Achromadora. micoletzky* females as given by various authors. Abbreviation.- L, a, b, c, c', V: de Man index; bw: corresponding body width; hw: head width; lcs: length of longer cephalic setae; lst: length of stoma; amph: width of amphids; b: pharyngeal bulb; phl: pharyngeal length; rl: rectum plus pre-rectum length; abw: anal body width; sl: spinneret length.

Tabla I.—Medidas aportadas por distintos autores para las hembras de *Achromadora micoletzky*. Abreviaturas.- L, a, b, c, c', V: índices de de Man; bw: correspondiente anchura del cuerpo; hw= anchura de la cabeza; lcs: longitud de las sedas cefálicas más largas; lst: longitud del estoma: amph: diámetro de los anfidios; b: bulbo faríngeo; phl: longitud de la faringe; rl: longitud del recto más el prerecto; abw: anchura del cuerpo a nivel del ano; sl: longitud del espinerete.

	Sierrra Nevada lakes (n=18)	*Eyualem & Coomans (1995) (n=10; big form)*	*Zeidan & Geraert (1989) (n=13)*	*Bonger (1988) (n=?)*	*Ocaña (1987) (n=10; Granada's spring)*	*Altherr (1952) (n=3)*	*Schneider (1925) (n=43)*
L (mm)	0.5-0.7	0.5-0.6	0.5-0.6	0.4-0.7	0.4-0.8	0.4	0.5-0.6
a	16-23	20-23	21-29	20-30	18-32	26-30	19-30
b	5.5-6.7	5-5.9	5.2-6.4	5-7	5.7-7.4	5-5.7	5.3-6.6
c	5.1-6.7	5.4-7.8	5.9-7.9	6-8.5	6.1-8	8-8.5	6.4-7.5
c'	4.9-6.9	4.3-7.4	5-6.4	5	4.2-6.3	6	6.5
V (%)	41-45	39-43	40-46	42-52	41-51	48-51	43-45
hw (µm)	8.6-10.9	11-13	9-12	10	10.2-12.2	—	—
lcs/hw	1/4-1/5	1/3	1/4	1/4	1/3-1/4	1/3	1/4
lst/hw	0.8	0.5-0.8	—	0.9	0.7	1	1.2
amph/bw	1/3-1/4	1/3	1/5	1/3	1/4-1/5	1/3	1/3
amph/hw	0.9-1.3	0.6-0.9	1	1.3	1-1.2	1-1.4	1
b/phl	1/4-1/5	1/6	1/5	—	1/6	—	1/6
Eggs (µm)	42-54x23-28	53x20	38-79x15-21	—	—	—	—
rl/abd	2.2-3.2	2.8-3.5	2.7-5	—	2.8-3.2	3	2.5
sl (µm)	3.5-4.3	—	—	—	3.8-4.2	—	—

Holotype: L = 732 µm; a = 37; b = 6; c = 4.9; c' = 12.3; V = 46 %.

Description: Body curved ventrally or frequently straight and tail ventrally arcuate. Annulated cuticle, with transverse rows of punctations. Ten annuli measure 5-7 µm at the base of the pharynx, 7-9 µm at mid-body and 8-9 µm at anus level. Somatic setae are distributed over all parts of the body, with the highest number occurring in the posterior half of the body. Vacuolated lateral glandular bodies absent. Rounded head crowned by 6 lips with small papillae. Head width 6.5-9.5 µm. Very short cephalic setae (not seen in all specimens), 1/7-1/8 the head width. Stoma conical, 5.5-8 µm deep, with one small dorsal and another small subventral tooth at posterior end.

Amphids spiral with two coils, 2-3 µm in diameter (25-33 % head width at the same level). Amphids situated 1.2-1.5 times head width from anterior end invariably behind the base of the stoma, 1/2-1 time head width measured from stoma base. Chromadoride-like pharynx, 1/6-1/7 body length with bulb 1/6 pharynx length. Nerve ring at mid-pharynx level. Pharyngo-intestinal junction simple.

Female reproductive system didelphic, amphidelphic with reflexed ovaries, anterior ovary branch usually longer than the posterior, simple transverse vulva. Vulva-anus distance always more than twice the tail length.

Eggs (n = 4) range from more elongated (50-62 x 16-18 µm) to somewhat rounded (35 x 18 µm). Rectum muscular, nearly 3.2-3.8 times anal body widths long, composed of two parts, the anterior one of which is sometimes called prerectum. Tail very elongate conoid, 3.5-4 times rectal lengths and with two or three distinct caudal glands terminating in a tubular spinneret with 3-3.5 µm long.

Remarks: Of the *Achromadora* species described to date, *Achromadora longicauda* Schneider (1937) is the most similar to *A. gracilis* n. sp. in that both are the only species of the genus with a long tail (**c** less than 6). Nevertheless, the differences between the two species are clear. Differences were found in body length (L = 0.350 mm (n=1) vs. 0.66-0.85 mm (n=9)), and in the de Man **a** index (23 vs. 29-39) and in the different prerectum shape (triangular in *A. longicauda*, cylindrical in *A. gracilis*).

Locality and habitat type: Samples collected from sandy sediment of 16 high-mountain lakes in Sierra Nevada. pH, temperature, conductivity and oxygen concentration values for these lakes ranged between 5.3 to 8.5, 4 to 23°C, 7 to 60 µS/cm and 5.1 to 9.3 mg/l, respectively. The holotype was found in Rio Seco Alberge lake.

Etymology: The name of *Achromadora gracilis* refers to the extremely delicate and svelte aspect of this species.

Environmental data of congeneric Achromadora *species*: Physico-chemical data for those lakes in which both of the *Achromadora* species simultaneously

appeared are given in table II. These data are representative of those obtained for all of the lakes in the Sierra Nevada Mountains. According to our findings, in accordance with those reported by Morales Baquero (1988), the lake water in the Sierra Nevada can be characterized as low mineralization, relatively variable pH (tending towards basic), moderately cold temperatures during the thawing season and high oxygen concentration.

The appearance of paired congeneric species of *Achromadora* (together in 12 of the 20 high-mountain oligotrophic lakes) is not at all unusual. In the case of the two congeneric chromadoride species, the mechanism used to avoid competition may a consequence of differences in trophic strategies. Both species could be considered unicellular eucaryote feeding (Yeates *et al.*, 1993). This is evident that when the chlorophyll **a** concentration is higher (Munawar & Burns, in Sánchez Castillo, 1986, discovered a direct relationship between chlorophyll **a** and the populations of Chlorophycea and Cyanophycea). In high-mountain lakes "Rio Seco Inferior", "Rio Seco Alberge", lake 5 of "7 Lagunas" and Lake "Yeguas", the concentration of chlorophyll **a**, and therefore of unicellular algae, is adequate for the trophic maintenance of both species. In addition, when the algal concentration is greater (higher value of chlorophyll **a**), as occurred in Río Seco Inferior, the population of *Achromadora micoletzkyi* increases considerably.

Voucher specimens: specimens of *Achromadora gracilis* n. sp. are deposited on slides 101-105 in the nematology collection of the Dpto. Biología Animal y Ecología, Universidad de Granada (holotype in slide 101). One slide with two females is deposited in the collection of the Instituut voor Dierkunde, Rijksuniversiteit Gent, Belgium.

Ethmolaimus multipapillatus **Paramonov, 1926** (Fig. 2)

Males (n = 18): L = 1300-1900 (1633 ± 191) µm; a = 25-27 (26 ± 0.9); b = 7.7-10.2 (8.9 ± 0.7); c = 10.2-16 (14 ± 1.4); c' = 2.9-4 (3.2 ±0.3); Spicules= 39-53 (45.8 ± 5.7) µm; Preanal supplements= 16-25.
Females (n = 7): L = 900-1700 (1228 ± 330) µm; a = 25-30.3 (28 ± 2.2); b = 7.5-9.8 (8.1 ± 0.8); c = 9-14 (9.8 ± 3.2); c' = 3.5-4.1 (3.7 ± 0.2); V = 51-54 (52 ± 1.1) %.

Additional description: Body ventrally curved, posterior end extremely curved and even coiled in males. Males somewhat larger than females. Annulated cuticle, punctated with dots in transverse rows, width of the cuticle at the base of the pharynx is 0.8-1.2 µm, 1.8-2.2 µm at mid-body and 1.2-1.4 µm at anus level. Ten annuli measure is 12-17 µm at base of the pharynx, 16-27 µm at mid-body and 12-19 at anus level. Small-sized vacuolated lateral glandular

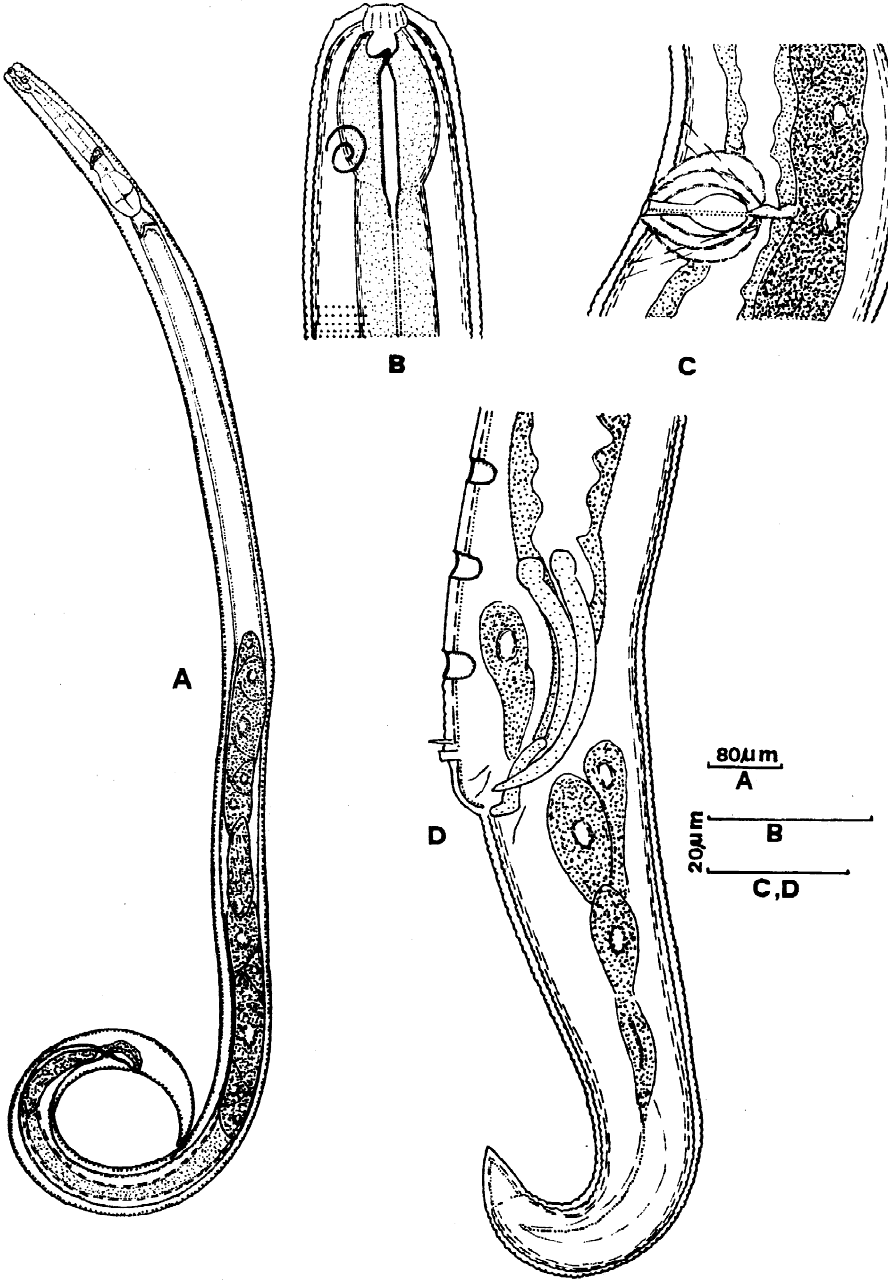

Fig. 2.—*Ethmolaimus multipapillatus* Paramonov, 1926: A) Male. B) Anterior region. C) Vulval region. D) Male posterior region.
Fig. 2.—*Ethmolaimus multipapillatus* Paramonov, 1926: A) Macho. B) Región anterior. C) Región vulvar. D) Región posterior del macho.

bodies occasionally present at anterior body half. Rounded head region not offset, 16-22 µm in diameter. Six inner labial and six outer labial papillae in two circlets in front of the four cephalic setae. Stoma with vestibulum sclerotized and striated, anterior portion of buccal cavity cup-shaped, posterior portion cylindrical, separated by three teeth (1 more developed dorsal, 1 less developed ventral, 1 subventral). Stoma 18-22 µm deep. Spiral amphid (1.5 whirls), 4-5.2 µm in diameter, approximately 25 % body width at same level, situated at mid- or mid-posterior stoma level. Chromadoride-like pharynx, 1/8-1/10 body length. Pharyngeal musculature expanded round the buccal cavity, then cylindrical with posterior portion enlarged to a bulb. Terminal bulb approximately 1/4 pharynx length. Nerve ring situated at mid-anterior level of the pharynx. Cardia tear-shaped.

Female reproduction system didelphic, amphidelphic with straight ovaries equally developed. Both ovaries 260-350 µm long, situated left of gut. Transversal vulva, somewhat oblique, well muscularized, bottle-shaped musculature surrounding vagina.

Developed male reproduction system with seminal vesicle and distal spermatheca well noticeable. Anterior testis usually more developed than the posterior, with a length of 259-418 µm v.s. 152-326 µm posterior testis. Anterior testis left, posterior right of gut. Copulatory apparatus comprised of two spicules and a somewhat sclerotized ventral ala (piece also present in other species of same genus —Jensen, 1979—). Curved, uniformly thick spicules, tapering towards distal end, round capitulum; 39-53 µm long. Preanal supplements of varying number (16-25), most frequently 22-23. Ethmolaimide-like gubernaculum. Several males with a cylindrical pre-cloacal piece which differed from the rest of the supplements (see fig. 2D), and an occasional seta (this pre-anal piece is not mentioned in other descriptions but can be seen in drawings by Paramonov (1929); Platt (1985) and Jensen (1994). Conical tail, somewhat longer than the female tail. Spinneret 4.7-6.2 µm long.

Differential diagnosis: The only descriptions of this species available to date are those reported by Paramonov (1929) and Gerlach (1953). Paramonov's (1926) original description is vague and extremely general. Platt (1982) provides de Man ratios obtained from bibliographical data, rather than from specimens examined by him. Platt (1985) and Jensen (1994) give only a brief taxonomic discussion. The most striking difference between the data of Paramonov (1929) and Gerlach (1953) and ours, is the length of Paramanov's specimens in comparison to ours (1000 µm vs. 1228 µm mean for females; 907 µm vs. 1633 µm mean for males), whereas total specimen length reported by Gerlach is more similar to our findings (830-990 µm vs. 900-1700 µm females; 1230 µm vs. 1633 µm males). Spicule length is notably longer in the Spanish specimens (32-35 µm for Paramonov; 32 µm in Gerlach vs. 39-53 µm). The

Table II.—Limnological data from the lakes studied. Oxygen concentration, pH, conductivity and temperature values are the minimum and maximum recorded in each lake.
Tabla II.—Datos limnológicos de los lagos estudiados. Rangos de variación de la concentración de oxígeno, pH, conductividad y temperatura obtenidos en cada lago.

Lake	*O_2(mg/l)*	*pH*	*Conductivity (µS/cm)*	*Temperature (ºC)*
Lake 1 at Siete Lagunas	4-7.9	6.2-8-6	17-42	9.6-15
Lake 3 at Siete Lagunas	6.7-7.1	6.1-7.7	8-34	8-13.7
Lake 5 at Siete Lagunas	6.3-7.6	6.2-8.2	26-41	13-18.2
Aguas Verdes	7.9-8	6-8	22-27	5-15
Yeguas	6.6-9.3	6-7.9	28-60	6-14.8
Caldera	7-7.5	7-9.7	20-25	13-16
Río Seco Inferior	6.3-6.7	5.6-9.1	9-30	11-20.6
Río Seco Albergue	6.7-7.6	6.5-8.7	9-15	11-18
Virgen Inferior	5.1-7.6	6.1-7.8	28-43	6.2-20
Virgen Intermedia	7.3-8.2	6.3-7.7	33-60	6-16
Virgen Superior	5.2-10.2	5.7-7.7	45-68	7-11.5
Lanjarón 2	5.6-7.9	5.9-7.3	7	12-16
Fuente Piedra	7.6	7.6	180000	21.4

Table III.—Number of *Achromadora micoletzky* and *A. gracilis* specimens reported in the lakes studied and data on chlorophyll a. Chlorophyll a data extracted from Sánchez Castillo (1986).
Tabla III.—Número de individuos encontrados en cada laguna de *Achromadora micoletzky* y *A. gracilis*, acompañados de los correspondientes datos de clorofila a. Los datos de clorofila a están extraídos de Sánchez Castillo (1986).

Lakes	*A. micoletzkt*	*A. gracilis*	*Chlorophylla (µg/l)*
Lake 1 at Siete Lagunas	3	1	0.7-5.1
Lake 3 at Siete Lagunas	5	12	0.8-1.7
Lake 5 at Siete Lagunas	14	2	0.8-6.4
Aguas Verdes	11	6	0.2-2.4
Yeguas	15	1	0.9-5.2
Caldera	3	1	0.3-1
Río Seco Inferior	55	5	1.5-6.3
Río Seco Alberge	15	2	0.7-4.4
Virgen Inferior	6	1	—
Virgen Intermedia	1	7	0.5-1.4
Virgen Superior	2	11	0.2-1.1
Lanjarón 2	8	6	—

range of variation in the number of pre-anal pieces is further complemented with the Spanish data: 20-24 for Paramonov (1929); 24 for Gerlach (1951); 17-24 for Platt (1985), and 16-25 for our material.

Locality and habitat: Collected from muddy sediment in Lake "Fuente Piedra" (Archidona, Málaga) in 1992 and beginning of 1993 before a period of extreme drought. The mean anionic concentration during sampling period were: Cl^- (90,900 ppm); Co_3H^- (216 ppm); CO_3^{2-} (1.5 ppm); SO_4^{2-} (8187 ppm). These data complement those already given for the same lake in table II.

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