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CHIRONOMUS (CHIRONOMUS) APRILINUS MEIGEN, 1818
(DIPTERA CHIRONOMIDAE), FIRST RECORD FROM ITALY:
CYTOTAXONOMY AND ECOLOGY

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Boggero A., Ruocco M., Shokri M., Gjoni V., Ansaloni I., Zaupa S., Montagna M., Rossaro B. – *Chironomus (Chironomus) aprilinus* Meigen, 1818 (Diptera Chironomidae), first record from Italy: cytotaxonomy and ecology

Larvae, pupae and adult males of two populations of *Chironomus (Chironomus) aprilinus* Meigen, 1818 from two saline habitats in North and South Italy (Poiano spring and Giammatteo channel respectively) are described. The species can be easily identified at the larval stage because of the unequal length of ventral tubuli. The examination of pupal exuviae, adult male and salivary gland chromosomes confirmed the species identification. The two habitats differ in location and in salinity, the former (Poiano) is an inland spring in Northern Apennines (Emilia) with high salinity (12000 mg L⁻¹), the latter (Giammatteo) is a channel near Lecce (Puglia) with a relatively low salinity content (1720 mg L⁻¹) feeding a coastal lagoon (Acquatina). On the basis of the present information no substantial differences except in some morphometric measures were apparent between the two populations, despite the different salinity of the two habitats.

KEY WORDS: taxonomy, Chironominae, ecology.

INTRODUCTION

Inland saline waters are unique habitats with typical assemblages and organisms tolerant to adverse and fluctuating salinity and main ions conditions. Among the benthic communities, species diversity in these types of waters is very low and often dominated by few tolerant species, which can however occur in high numbers.

Chironomus (Chironomus) aprilinus Meigen, 1818 is a senior synonym of *C. halophilus* Kieffer, 1911 and of *Chironomus obscurus* Goetghebuer, 1921. It is widespread in West Palaearctic (www.fauna-eur.org; DE JONG *et al.*, 2014) and extends to its most Oriental areas as the Aral Sea (ALADIN *et al.*, 2005). In the European Mediterranean area it is reported only from Spain, France, Croatia and Turkey, but it is also reported from North Africa (RAMDANI *et al.*, 2001). The adult male (STRENZKE, 1959), pupal exuviae (LANGTON & VISSER, 2003) and larvae (THIENEMANN, 1954, WEBB & SCHOLL, 1985) were well described. The karyotype was described from different European populations (KEYL & KEYL, 1959; MICHAILOVA, 1989).

The species is characterized by the unequal length of ventral tubuli. Morphological modifications of ventral tubuli in relation to oxygen concentration were also reported (HAAS, 1957).

The aim of the present paper is to give additional information about this species, considering all the three metamorphic stages and polytene chromosomes. The species was collected in saline ponds of Northern and Southern Italy, representing the first finding of the species in Italy.

MATERIALS AND METHODS

STUDY AREA

The Poiano spring (44° 23' 24.63" N - 10° 26' 23.70" E, altitude 406 m a.s.l.) (Fig. I, 1) is the main karst spring of Northern Apennines and Emilia-Romagna (CHIESI *et al.*, 2010). It is located in the upper Secchia Valley within the National Park of Appennino Tosco-Emiliano and it is one of the Sites of Community Importance (SCI) "Gessi Triassici" (IT4030009) of the Natura 2000 network. Its catchment is characterized by an extended karst area, 22 km² of gypsum (BERTOLANI, 1949). The spring has an average discharge of over 400 L s⁻¹, with almost constant water temperatures (10 °C) and mean annual salinity of about 12000 mg L⁻¹. A remarkable characteristic of the spring is that it is a salty gypsum/anhydrite karst spring with a high NaCl content (about 6000 mg L⁻¹) and a mean dissolved gypsum estimated in 3200 mg L⁻¹ (FORTI *et al.*, 1986). The NaCl can have variations which are related to active halokinetic movements (CHIESI *et al.*, 2010). An artificial pond (surface area: 1500 m², mean depth: 1 m), constantly fed by spring waters and presenting an inlet and an outlet, is present downstream the spring (Fig. I). The bottom area showed carpets of *Chara canescens* Loiseleur 1810, *Chara vulgaris* L. 1753, *Tolypella glomerata* (Desvaux) Leonhardi 1863, and scarce *Zanichellia palustris* L. (RUOCCO & ANSALONI, 2015).

The Acquatina lagoon (Fig. I, 2) is a coastal marsh lake located on the Adriatic Sea shore of the Salento peninsula about 13 km North-East of Lecce (40° 26' 32.82" N - 18° 14' 14.48" E, altitude 0 m a.s.l.) with mean depth of 1.2 m.

Figure I – Location of the sampling stations in the two Italian areas; 1. Poiano, 2. Acquatina

Temperature and salinity range between 8.8-25.3°C, and 20500-38000 mg L⁻¹ respectively (SCHIROSI *et al.*, 2010; VERSCHUT *et al.*, 2015). In the Acquatina lagoon dissolved inorganic nitrogen (DIN = NH₄⁺, NO₂⁻, NO₃⁻) showed concentrations ranging from 8.2 to 37.5 µM in February 1995, orthophosphate showed mean concentration values of about 0.20-0.05 µM in 1991. In March 2007, DO concentrations varied from 7.40 to 8.14 mg L⁻¹, whereas in July 2007 DO ranged from 5.9 to 7.3 mg L⁻¹.

The sediments of the lake are colonized by *Cymodocea nodosa* (Ucria) Ascherson 1870 and *Ruppia* sp., one of the principal energy sources for detritus-based communities, while on the banks there are dense swamps of *Spartina juncea* Fabre and *Phragmites australis* (CAPPELLO *et al.*, 2005).

The principal freshwater inputs are a lateral ramification of the Giammatteo channel (on the northern boundary of the lake) and rainfalls. The watershed of the Giammatteo channel is fed by precipitations and groundwaters. The input from the channel is highly variable, depending on seasonal precipitation amount and intensive agricultural use. This freshwater channel (salinity 1720 ± 20 mg L⁻¹; pH 7.52 ± 0.02 measured with YSI 556 MPS multiprobe during April-June 2012) with a depth of 30-70 cm is characterized by boulders and dense accumulations of leaf detritus. The leaf detritus mainly consists of *Phragmites australis* (Cav.) Trin. ex Steudel (Poaceae) and *Posidonia oceanica* (L.) Delile (Posidoniaceae) (VERSCHUT *et al.*, 2015).

SAMPLING AND ANALYSIS OF SPECIES

Samples for Chironomids larvae and pupae were taken through a hand-net (250 µm mesh size) according to STOREY *et al.* (1991) for about 1 to 3 min (BIGGS *et al.*, 1998) in three different stations in the Poiano pond (Fig. I) in winter in December 2014 and in January 2016 and in the Acquatina lagoon and in the Giammatteo channel representing different bottom textures; next to the same sampling stations, adults chironomids were collected through the use of an entomological net. Specimens were preserved in the field with 5% formalin or in 70% ethanol

and sorted under a stereo-microscope. Some adults were directly extracted from the pharate pupae.

Morphological taxonomy was performed through the use of a microscope. The identified species are available as alcohol-preserved sample and permanent slides in the collection of the Department of Life Sciences, University of Modena and Reggio Emilia and in the Department of Food, Nutrition and Environmental Sciences (DeFENS), University of Milano.

To confirm the identification of *C. aprilius*, the sampled larvae were reared to adults, larvae in the 4th stage and prepupae were collected in the field and preserved in acetic alcohol (1: 3) to extract the polythene chromosomes in the salivary glands to cytotaxonomically confirm the identification.

The adult males were cleared in KOH, all stages were rinsed in acetic acid and in a mixture of xylene:phenol 3:1 and mounted in Canada Balsam. Morphological terminology and abbreviations follow STRENZKE (1959), SÆTHER (1980), WEBB & SCHOLL (1985), SHOBANOV (2000), KOWNACKI *et al.* (2016).

For cytogenetic analysis, about 20 larvae fixed in a 3:1 mixture of 96% ethanol and glacial acetic acid were kept in a refrigerator. Polytene chromosome squashes from salivary glands were prepared using the routine aceto-orcein method (KEYL & KEYL, 1959; MICHAILOVA, 1989). Chromosome arms A, E and F were mapped according to KEYL (1962), and arms C and D according to DÉVAI *et al.* (1989), using *Chironomus piger* Strenzke 1956 banding sequence as a standard.

RESULTS

MORPHOLOGY

Adult male – A detailed description is in STRENZKE (1959). The present description is based on two adults collected in Poiano (16.XII.2014 and 26.I.2016).

Antennal ratio = AR 3.61 (1507/417 µm). Back ground colour of head and thorax light yellow, vittae dark brown,

Figure II – 1. Male genitalia of *C. aprilinus*, Poiano 21.I.2016 (photo); 2. Male genitalia of *C. aprilinus*, Poiano 12.XII.2014 (drawing)

lucent, thorax between vittae darker. Scutellum light yellow, with a narrow anterior margin only a bit darker. Postnotum dark, only moderately lighter anteriorly. Mesosternum dark, but dorsal part (about 1/3) lighter. 25 dorsocentral, 15 acrostichals, 6 prealars, 38 scutellars.

Wing length about 6 mm. All legs yellow, except tarsomeres 2-5 of P_{II} and P_{III} . Setae $Ta_{I,P_{III}}$ 236 μm long, BR about 7. Hypopygium (Fig. II 1, 2): Tergite IX with 5 setae in a large white spot and 4 setae in a small white spot. Superior volsella as in *C. riparius* Meigen 1804, but without the medial krenulate margin characterizing *C. riparius*. Gonostylus slender at apex, with 7 robust median setae and many small thin setae along its medial margin. Anal point with a narrow base and a rounded apex of the *halophylus* type.

Pupa (Fig. III 1, 2, 3, 4) (described on the basis of pupal exuviae collected in Poiano in 16.XII.2014). All characters well fit with the description of the genus *Chironomus* (LANGTON & VISSER, 2003), with a thoracic horn divided into many long filaments, cephalic tubercles conical, thorax strongly granulate anteriorly; armament of tergites not very extensive, pleura of segment IV extensively armed; characteristic of the species are shagreen of sternite II with a definite gaps, intersegmental spinules IV/V and V/VI small, tergite VIII with a spur.

Larva (Fig. IV 1, 2, 3, 4) (described on the basis of about 20 larvae collected in Poiano in 16.XII.2014 and in Giammatteo channel in 9-25.V.2016). Antenna composed of 5 segments, antennal segments from Poiano population: 135.87, 29.41, 11.72, 12.34, 7.57 μm long, AR 1.6 (135.87/84.8 μm), antennal blade 60-67 μm , accessory blade 13.97 μm , ring organ 59.39 μm distant from base of antenna, small sensillum on the first antennal segment 136.87 from base. In Giammatteo population antennal segments 141.42, 33.93, 15.90, 14.71, 7.48 μm long, AR 1.8 (141.42/78.17 μm), ring organ 41.91 μm from base.

Pecten epipharyngis composed of 15 teeth, mentum 200 μm large, with one median tooth 23 μm large, 6 lateral teeth, with small first lateral teeth, clearly shorter than the second lateral teeth. The larvae belong to the *thummi*

Figure III – 1. Thorax with thoracic horn; 2. Intersegmental points between tergites V and VI; 3. Pleura of tergite IV; 4. VIII tergite and anal lobes of pupal exuviae of *C. aprilinus*, Poiano 12.XII.2014

Figure IV – 1. Antenna, 2. Mandible, 3. Ventromental plates, 4. Caudal end of larva, Giammatteo, Acquatina lagoon, 15.V.2016

group, because of the absence of lateral tubuli at the caudal end of 10th segment (7th abdominal) and belong to the *halophilus* type because of the presence of short anterior and long posterior ventral tubuli on 11th segment (8th abdominal) (THIENEMANN, 1954). Other diagnostic characters of the larva are the ventral tubuli rounded at apex, 4th mandibular tooth pale, ventromental plate 200 µm large, striae not extending close to outer hook series, ventromental plates with about 30 outer hooks (WEBB & SCHOLL, 1985).

Karyotype (Fig. V 1, 2): the species is easily separated from other species of the genus *Chironomus* thanks to the presence of a well defined karyotype. The chromosome set is

$2n = 8$. The species belongs to the *pseudothummi* cytocomplex, with chromosome arm combinations: AE, BF, CD, G (KEYL, 1962; MICHAILOVA, 1989). The chromosomes AE (Fig. VI, 1), BF (Fig. VI, 2), CD (Fig. VI, 3) and G (Fig. V, 3) of the two populations are mapped and compared with the standard maps (KEYL, 1962; DEVAI *et al.*, 1989). The G chromosome is characterized by the presence of a nucleolus on one margin and by two Balbiani rings in the centre and on the other margin (KEYL & KEYL, 1959). In the examined populations the C chromosome is sometime characterized by an heterozygous inversion which is observed in both populations, but is more frequent in the Poiano population (Fig. VI, 3). Other minor differences between the two populations can be observed examining Fig. VI.

Figure V – 1. Karyotype of *C. aprilius*, Giammatteo channel, 15.V.2016; 2. Karyotype of *C. aprilius*, Poiano, 16.I.2016; 3. Chromosome G, upper Giammatteo channel, 15.V.2016, lower Poiano, 16.I.2016, N, Nucleolus, B, Balbiani ring, the map according to Keyl & Keyl (1959) is given in centre of the figure

Figure VI – 1. Chromosome AE, upper Giammatteo channel, 15.V.2016, lower Poiano, 16.I.2016; 2. Chromosome BF, upper Giammatteo channel, 15.V.2016, lower Poiano, 16.I.2016; 3. Chromosome CD, upper Giammatteo channel, 15.V.2016, lower Poiano, 16.I.2016. The white arrows indicate centromeres. The maps of the chromosomes are according to Keyl (1962) for chromosomes A, E, F and to Devai (1989) for chromosomes B, C, D.

DISTRIBUTION AND ECOLOGY

The distribution of this species (see Introduction) can now be enriched adding two new stations, both in Italy, the former in Northern Apennines (Emilia), in an inland saline spring, the latter in Salento (Puglia) in a mesohaline channel, located close to marine ecosystems. These two additional stations do not modify the known distribution, which can be confirmed to be West Palaearctic. It is of interest to emphasize the presence of the species both far from coastal regions, and nearby the coastal marine areas.

C. aprilius does not inhabit the highest saline waters, but both mesohaline and polyhaline habitats and it can be considered relatively euryhaline. A recent ecological study of the species in relation to its adaptations to variation in salinity and high sulphates concentrations (MATENA *et al.*, 2016) confirmed that *C. aprilius* prefers waters with moderate salinity comprised between 3000 and 13000 mg L⁻¹ and can be also collected in continental areas very far from marine coasts as in Czech Republic (MATENA *et al.*, 2016).

The salinity values measured in Poiano (12000 mg L⁻¹) are in the known range of tolerance experimented by *C. aprilius*. In the present research the range of salinity is extended to relatively low values, below 2000 mg L⁻¹ in Giammatteo; it is here emphasized that in the nearby Aquatina lagoon, with a much higher salinity (20000-40000 mg L⁻¹), *C. salinarius* Kieffer, 1915 is the dominant species. These results confirm that the species tolerates high salinity, but its range of tolerance is inferior to other halobiont species as *C. salinarius*. The mean/optimum values reported for the two species *C. aprilius* and *C. salinarius* are 16900 and 41100 mg L⁻¹ respectively (ZINCHENKO & GOLOVATYUK, 2013).

Our samples confirm that the species is found in a relative wide range of salinity, tolerating low to moderate saline conditions and that it prefers soft muddy bottoms where the species frequents vegetated areas (NICOL, 1935).

CONCLUSIONS

The first junior synonym (*C. halophilus*) emphasize that the species live in brackish waters. The species was classified as an halobiont species (THIENEMANN, 1954) due to its resistance to increase in blood osmotic concentrations (CHENG, 1976), but more recent results (CHENG, 1976; MATENA *et al.*, 2016) suggest *C. aprilius* being an halophilous species regularly inhabiting coastal brackish ecosystems, preferring brackish to saline waters. The species can be found far from marine coasts.

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