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New Data on the distribution and biology of *Machiloides tenuicornis* Stach, 1930 (Microcoryphia: Meinertellidae) in the Iberian Peninsula (western Palaeartic)

Mª José Notario-Muñoz[1], Rafael Molero-Baltanás[1], Carmen Bach de Roca[2] and Miquel Gaju-Ricart[1,*]

[1] Department of Zoology, University of Córdoba, C-1 Campus Rabanales, 14014 Córdoba. Spain
[2] Department of Animal and Vegetal Biology and Ecology, Autonomous University of Barcelona, 08193 Bellaterra (Barcelona), Spain
* Corresponding author, e-mail: ba1garim@uco.es

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Abstract

New data on the geographic distribution and biology of *Machiloides tenuicornis* Stach, 1930 is given. This species is the only representative of the genus in the Palaearctic and occurs in the central part of the Spanish Pyrenees. The study of samples collected has allowed us to study their life cycle and mode of reproduction. The number of males and females in the samples show that 33.3% are bisexual populations and 66.6% are only female populations. The study of the length of all individuals of each sample suggests that the specimens hatch at the end of spring or beginning of summer and can live at least two years. Finally, analysis of the geographical distribution of bisexual and female populations suggests a possible ecological pattern of geographical parthenogenesis.

Keywords *Machiloides tenuicornis* | geographic distribution | life cycle | geographic parthenogenesis

1. Introduction

The genus *Machiloides* Silvestri, 1905 comprises 33 species distributed worldwide, mainly in the southern hemisphere (South America, Africa, Madagascar and Australia), but also in the northern hemisphere where it is represented by only three species: two in the eastern Nearctic (occidental coast of North America) and one in the western Palaearctic (north of Iberian Peninsula).

From a phylogenetic point of view, the genus *Machiloides* can be considered to be the most primitive in the family Meinertellidae (Sturm 1984, Sturm & Bach 1993, Sturm & Machida 2001), since it shows several plesiomorphic characters. It is very likely that they are close to the species *Cretaceomachilis libanensis* Sturm & Poinar, 1998, which is the oldest fossil known within the family Meinertellidae (Sturm & Poinar 1998). At the basis of the genus could be *Machiloides tenuicornis* Stach, 1930 because it shows a striking accumulation of plesiomorphic characteristics (Sturm & Bach 1993) and its mating behaviour must be regarded as the most primitive indirect mode of sperm transfer (Sturm 1986). It is the only representative in the Palaearctic and its distribution is reduced to the Spanish central Pyrenees. The species was formerly described by Stach in 1930 with one male from the Lerida province. Bach (1984) described the female as a result of numerous samples (only females) from the Huesca province; Bach & Gaju (1983) widened its distribution to the east (Gerona province); Fanciulli et al. (1988) described the female morphology under the scanning electron microscope and, because of the absence of males in many samples, suggested that *M. tenuicornis* could reproduce parthenogenetically.

Now, the collection of new material and the revision of some previously published samples have provided information on the habitat, life cycle, sex-ratio and geographical distribution of *M. tenuicornis*.

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2. Material and methods

We studied the samples listed in Table 1, belonging to published data, and newly collected material (Tab. 2) where the province, locality, place, date, number of specimens and reference number of each collection are shown. All samples are deposited in the collection in the Zoology Department of the University of Córdoba (UCO).

Samplings were conducted during the summer (July, August and September) in different years (1982, 1988, 1989, 1992, 1993, 2008 and 2012); the specific dates are shown in Table 2. Most of the samplings occurred during the night because many Meinertellidae remain hidden under the stones in the day. At night they move to the surface of the stones, rocks or the soil to eat, making it very easy to collect them. In this case, only 15–20 were captured per site in order to maintain the populations.

Our experience in sampling indicate that an entomological aspirator is more suitable for collecting specimens, in order to preserve the material in good condition. The specimens were placed in small glasses filled with 70 % ethanol and labelled.

In the laboratory the specimens were separated by sexes under a stereoscopic microscope and their body length measured, from the frons until the end of the Xth abdominal tergite, over millimetre paper (Fig. 1). As the characters used in the taxonomy of the group can be observed only under an optical microscope, to confirm that the specimens belong to the studied species, some

Table 1. Bibliographic references on *M. tenuicornis* Stach, 1930, with data on number of specimens collected. With grey background: localities with males.

Province	Reference	Place	♂♂	♀♀	Juv
Lérida	Stach, 1930 Abhand. Senk. Naturf. Gesell	Monte Claverol	1	0	0
	Bach y Gaju, 1983	La Coma (Pujol del Racó)	0	5	0
	Act. l Cong. Iber. Entom. León	Congost de Collegats	4	2	0
		Camino de Sossis	5	5	0
		Monte Claverol	3	2	0
	Sturm, 1986 Braunschw. Naturk. Schr.	Monte Claverol	6	5	0
Huesca	Bach, 1984 Misc. Zool	De Bielsa a Parzán	0	19	0
		Ctra. Torla a Ordesa	0	1	0
Gerona	Bach y Gaju, 1983	Montaña de Urús	0	3	0
	Act. l Cong. Iber. Entom. León	Torre de Riu	0	6	0

Table 2. New data on *M. tenuicornis* Stach, 1930. With grey background: localities with males. Ref: Number of collection in UCO Zoology Department.

Province	Locality	Place	Date	♂♂	♀♀	Juv	Ref
Huesca	Arcusa		9-7-92	0	1	5	M0485
	Boltaña	Road from Güaso to Boltaña	18-9-93	0	7	0	M0721
	Sabiñánigo	Pto. Serrablo, 1km before Campodabre	18-9-93	0	1	2	M0712
	Torla	Camping	13-7-92	0	4	1	M0476
Lérida	La Coma	Cal Andal	29-8-86	0	11	5	M1827
		Cal Andal	20-8-88	0	1	6	M1828
		Pujol del Racó	31-7-82	0	6	3	M1095
	Sort	Close to the village	11-8-89	2	4	2	M1829
		Direction to Rialp, near Camping	15-8-08	10	7	0	M1831
		Direction to Rialp, near Camping	26-7-12	6	4	5	M1834
	Vall de Cardós		19-7-92	0	0	8	M0553

of them were dissected, mounted in Tendeiro liquid and dried in a drying oven for a week, and subsequently observed using an optical microscope.

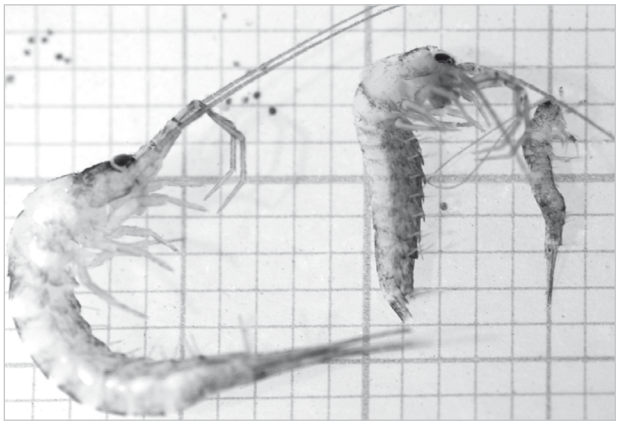

Figure 1. Specimens of *Machiloides tenuicornis* over millimetre paper prepared for their measurement**.**

3. Results

The literature review provides information on the worldwide distribution of the genus *Machiloides* (Fig. 2) and its plesiomorphic morphological characteristics: e.g. frons moderately protruded, lateral ocelli sole shaped (Fig. 3), article 3 of labial palp not distinctly broadened, sexual dimorphic characteristics of maxillary palps weakly developed, coxal stylets on legs II and III and one pair of coxal vesicles on abdominal segments I–VII.

New samples (Tab. 2) allowed us to update the distribution of *M. tenuicornis*. The species occupies an area of ca. 200 km E-W and 40 km N-S in the Spanish central Pyrenean area within the provinces Lerida, Gerona and Huesca (Fig. 4).

Their typical natural habitat (Fig. 5) is forest areas with piles of stones or rocks. The specimens are found in gaps between the stones or under them; they can be hidden among the lower vegetation always close to stones, rocks or soil. This species has colonized a new habitat, living in the stone walls near roads or fields, and at night are on the rocks, from the surface to the bottom (Fig. 6).

The study of the material collected revealed the proportion of sexes by sample. Only 33.33 % of the

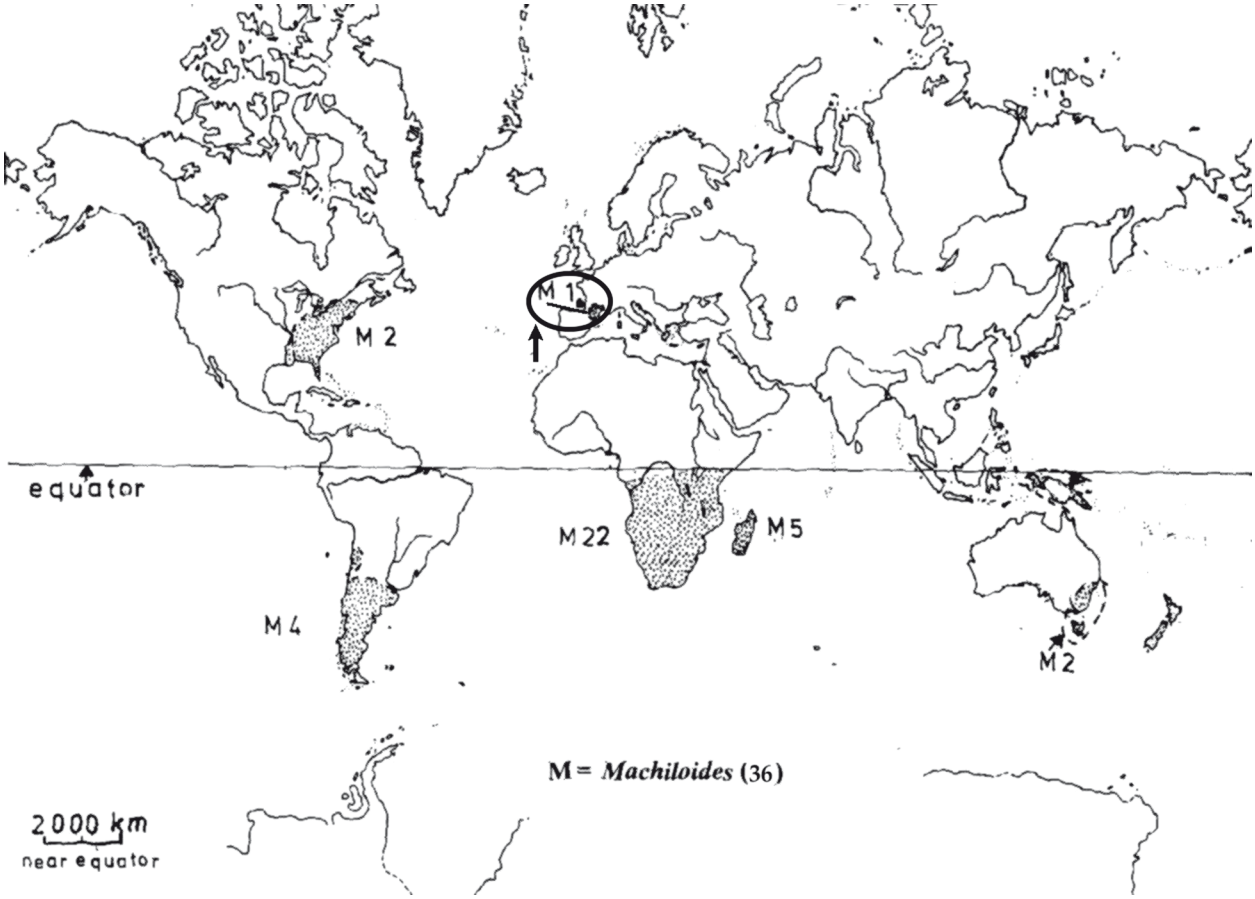

Figure 2. Distribution map of *Machiloides* throughout the world. The arrow and ellipse indicate the distributional area of *M. tenuicornis*. (Modified from Sturm & Machida 2001).

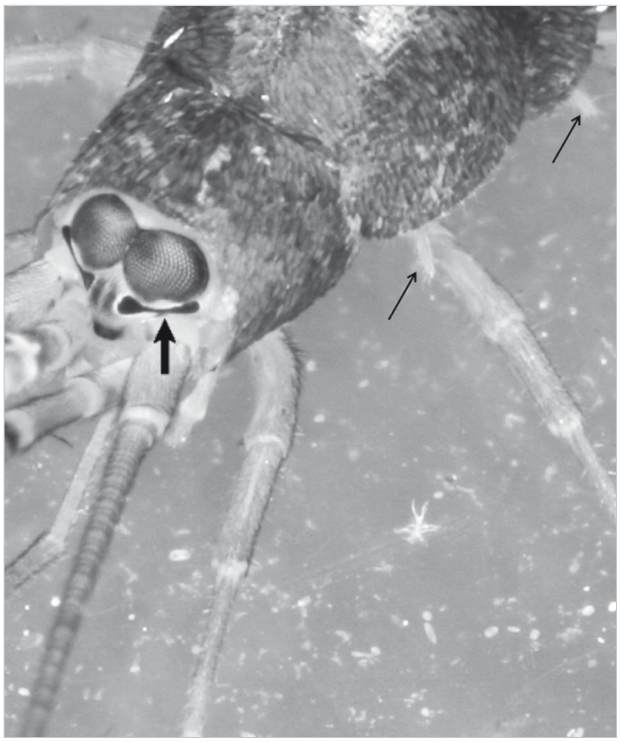

Figure 3. Frontal view of *M. tenuicornis* showing the shoe-shaped lateral ocelli (large arrow) and coxal stylets on legs II and III (thin arrow).

samples were bisexual populations, all of which were located in the Lerida province, occupying the centre of the distributional area of the species (Fig. 4). All the remaining samples (66.66%) were composed only of females and were distributed at both sides of the area with bisexual populations.

At the beginning of summer, we captured juveniles together with adults (clearly larger), we did not find any specimen of intermediate size (subadults); among the adults different sizes were found (Fig. 7). A similar life cycle was observed in both populations (bisexual and only females). The sample ref. M0553 from Vall de Cardós (Lérida) is included in the area of bisexual populations (Fig. 7), although only juveniles had been captured, because the locality is much closer to this area than the localities with only females.

4. Discussion

By the length of the juveniles we believe that the new generation hatches at the end of spring or the beginning of summer and, due to the different sizes of the adults in most of samples, we can assure that they can survive for

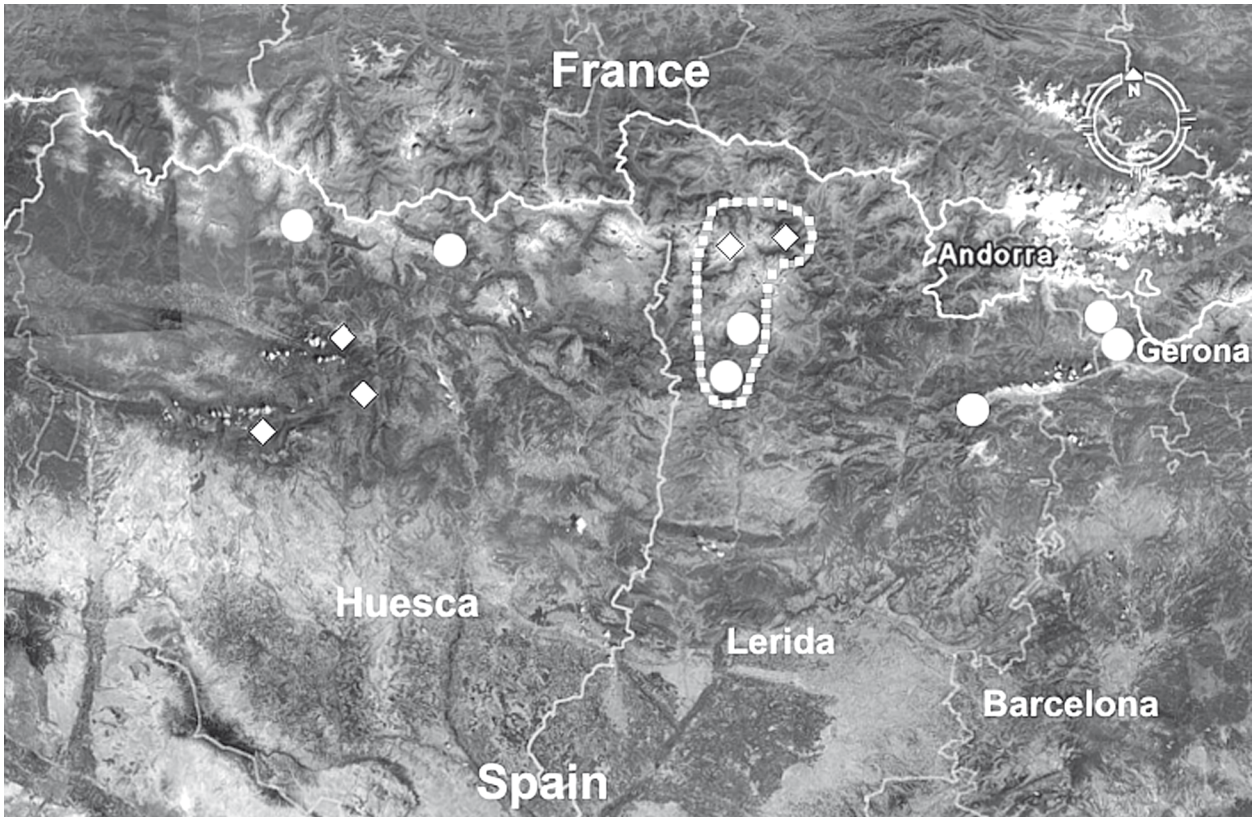

Figure 4. Localities where *M. tenuicornis* were found. The diamonds represent new localities. The circles indicate previously known localities. The discontinuous white line encloses the bisexual populations (Source: Google 2012).

Figure 5. *M. tenuicornis'* natural habitat.

Figure 6. *M. tenuicornis'* newly colonized habitat.

two years at least, the small individuals (ca. 8.5–9 mm) being those born the previous year and the largest being those hatched two years earlier.

Machiloides tenuicornis Stach, 1930 is a peculiar species endemic to the central Pyrenees. It has adapted to live in habitats modified by man, such as stone walls near roads or fields. Its life cycle can attain more than a year like other species of high mountains (Sturm & Machida 2001). Analyzing the size of the specimens we believe that, in some cases, three generations can coexist, so they can live two or more years.

Considering the presence of many samples consisting only of females occupying marginal areas of its general distributional area and geographically separated from bisexual populations in core habitats, we believe that in the marginal populations (only females) the species can reproduce by parthenogenesis. Parthenogenetic reproduction is widespread in nature; in some species males are unknown, but in others, depending on their life cycle, generations are parthenogenetic or bisexual. This type of reproduction would be most advantageous in marginal or newly colonized habitats where environmental factors are the dominating influence and only poor individual interactions exist (Haag & Ebert 2004). Only one specimen is needed to start or disperse a population.

This fact is reported also in Machilidae: *Trigoniophthalmus alternatus* (Silvestri, 1904) shows parthenogenetic populations in Europe and in the north-eastern United States; the extreme scarcity of males found among numerous specimens examined suggest this mode of reproduction. In this species, females from northern Switzerland were isolated in early juvenile stages in the laboratory; they were unlikely to have been fertilized and nonetheless developed eggs. Parthenogenesis was thus experimentally demonstrated based on this observation (Wygodzinsky 1941). *Dilta hibernica* (Carpenter, 1907) has populations consisting only of females in central and northern Europe (Meisch 1977), although mixed populations have been found in Luxembourg, so that the hypothesis of a general parthenogenesis across northern Europe is rejected. In another species, *Petrobius brevistylis* Carpenter, 1913, the sex ratio in some populations from North America and North Europe is highly skewed towards females; so that occasional reproduction through parthenogenesis is indicated (Wygodzinsky & Schmidt 1980). *Pedetontus saltator* Wygodzinsky & Schmidt, 1980, was described only by females, the authors also suggest their reproduction by parthenogenesis.

Among Meinertellidae, *Machiloides petauristes* Wygodzinsky & Schmidt, 1980, from New Jersey (eastern Nearctic), could be another case of parthenogenesis, represented at the moment only by females.

The case of *M. tenuicornis* could be related to an ecological pattern named "geographical parthenogenesis", which refers to a distribution pattern in species with mixed reproductive systems that are found in habitats at high altitudes or/and latitudes, where the mixed populations occupy the center of its general distributional area and the parthenogenetic populations in marginal areas (Morgan-Richards et al. 2010). Parthenogenetic individuals may be able to colonize marginal habitats, because it is not necessary to find a male for reproduction (Haag & Ebert 2004). This pattern reflects an association between parthenogenesis and environments that were strongly affected by the Pleistocene glacial cycles (Kearny 2005) (Fig. 8).

As we have pointed out, the bisexual populations of *M. tenuicornis* were found in the central part of its distributional area while the parthenogenetic populations were found in marginal areas, so the distribution pattern is similar to that described by Morgan-Richards et al. (2010). Moreover, the singular environmental pyrenaic region, where the species is endemic, was affected by the Pleistocene glacial cycles.

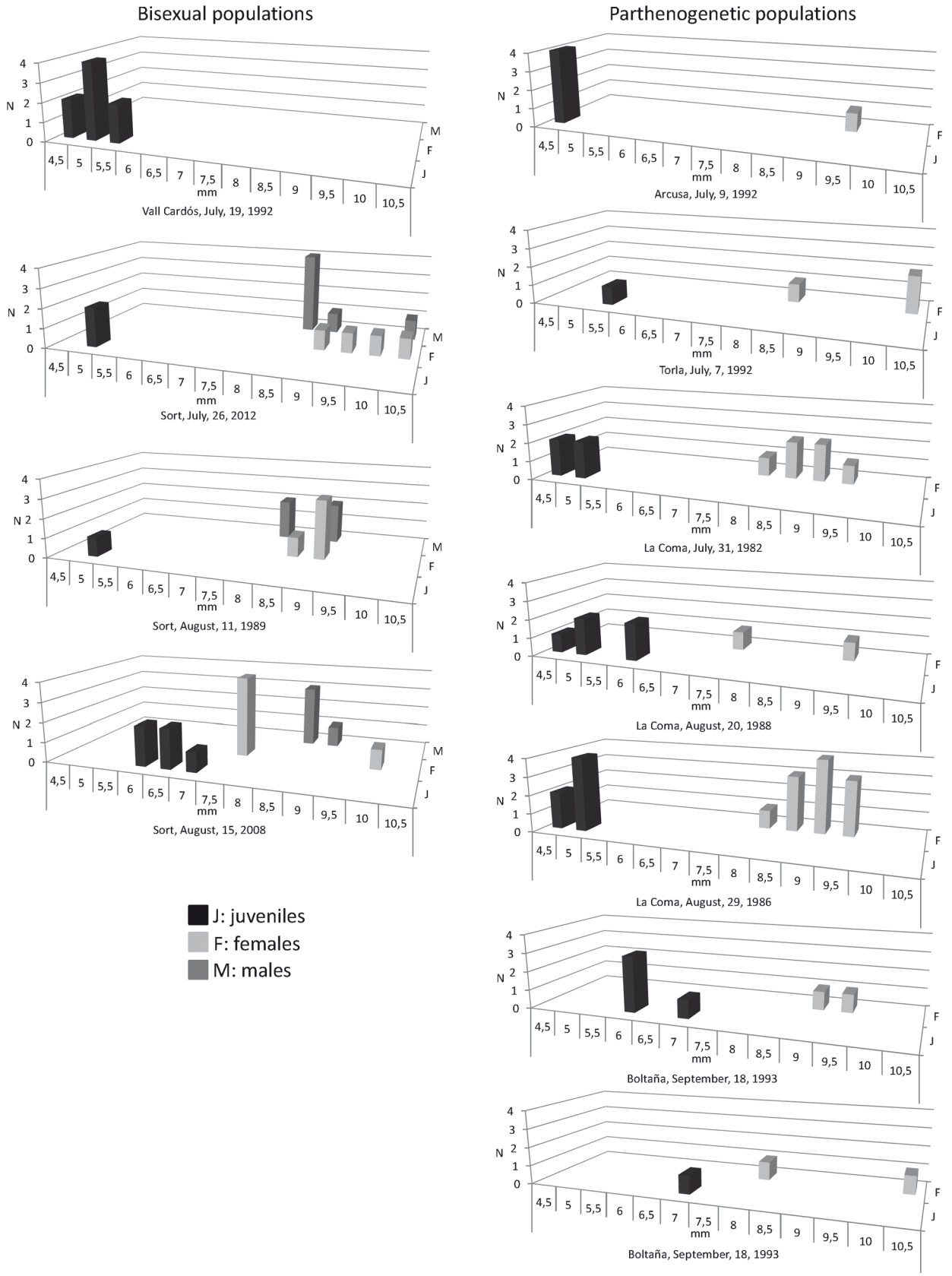

Figure 7. Comparison of life cycles from bisexual and parthenogenetic populations. To make the samples comparable, they have been ordered by month, day and year. N: number of specimens. mm: body length in mm.

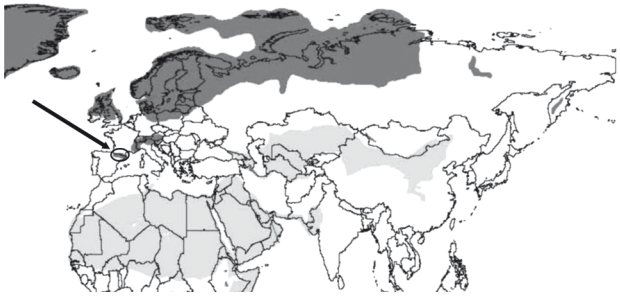

Figure 8. Regions severely affected by ice (dark grey) or desertification (light grey) during the late Pleistocene. The ellipse and arrow show the Pyrenean region. (Modified from Kearny 2005).

The ecological pattern of geographical parthenogenesis seems clear, but we believe that studies by molecular phylogeny are necessary to establish the spatial distribution of genetic variability and clarify this hypothesis. Some research on this aspect revealed that, in geographical parthenogenesis, sexual forms are located in central areas with high levels of genetic diversity, while parthenogenetic populations are located in marginal areas and showed low levels of variability (Rodriguero et al. 2009)

Summarizing, the general distribution area of *M. tenuicornis* is quite defined, with a clear separation between bisexual and parthenogenetic populations.

The life cycle seems to be well established, but more sampling in other seasons and laboratory studies will provide more information about when sexual maturity is acquired, the longevity of adults and the abiotic factors necessary for hatching.

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