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First record of *Colpodidium caudatum* (Ciliophora, Colpodidiidae) in Europe

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ABSTRACT

The present study documents the discovery of the first European population of *Colpodidium caudatum* (Ciliophora, Colpodidiidae) in a water drain in a school playground in Manzanares (Ciudad Real, Spain). This species has been documented on every continent except Antarctica and Europe, until now. The ciliate was isolated from wet run-off soil collected from the water drain and was grown in semi-permanent cultures in the laboratory. The infraciliature of the ciliate was revealed using silver carbonate impregnation and cell measurements were taken from living and silver-impregnated specimens. A comparative analysis of published data from various populations of *C. caudatum* across the globe showed high intraspecific morphological variability in this species. To differentiate between species within the *Colpodidium* genus, a dichotomous key is presented. This investigation shows that *C. caudatum* is a ciliate that is found all over the world and is particularly associated with terrestrial habitats that are periodically flooded.

1. Introduction

Free-living ciliates are phagotrophic protists with remarkable adaptability to diverse environmental conditions. This is particularly true in terrestrial environments, and it is primarily attributed to the ability of soil ciliates to produce resting cysts (Esteban and Fenchel, 2020). As cysts, soil ciliates can endure extended periods of drought and heat stress (Oshima et al., 2020) but develop population growth when moisture is reestablished (Finlay et al., 2000). As vital contributors to the soil's nutrient network (Acosta-Mercado and Lynn, 2004), they play a crucial role in the mineralization of organic matter, preying upon smaller organisms and serving as prey for larger predators (Finlay,et al., 2000; Hu et al., 2023).

Resting cysts are easily dispersed within and across environments and their development into population growth will depend on whether the places to which they are dispersed meet their growth requirements or not (Esteban and Fenchel, 2020; Fenchel et al., 2019; Finlay, 2002). Despite the ongoing challenge of undersampling issues in ciliates' biogeography studies, the expansion of geographical sampling ranges has revealed new records of 'endemics' in previously unexplored locations around the world (e.g. Olmo and Esteban, 2000; Hines et al., 2016,2020). In this study, the occurrence of the ciliate *Colpodidium caudatum* is reported for the first time in Europe.

The genus *Colpodidium* was established by Wilbert (1982) with the description of *C. caudatum*, discovered in soils from Afghanistan. Wilbert (1982) classified the genus within the family Colpodidae (Class Colpodea) based on what he described as somatic dikinetids and two ciliary fields located within an inconspicuous vestibulum. In 1983, de Puyrotac et al. erected the family Colpididiidae for *Colpodidium* Wilbert, 1982, and in 1990, upon further examination of the infraciliature, Foissner (XXIV Congress of the International Association of the Theoretical and Applied Limnology, 1989, Munich, Germany – see Foissner, 1990) found that the somatic kineties of *C. caudatum* were actually monokinetidal, the ciliate had three adoral membranelles and a tightly irregular silverline system. Thus, Foissner (1990) suggested that the ciliate was more closely related to the family Furgasonidae (Order Nassulida) than to the Order Colpodida. Later, the species was found in Kenya, Namibia, Australia, Japan, Tibet, China, Costa Rica, Galápagos and Venezuela (Foissner, 1995,1998,2016; Foissner et al., 2002), but never in Europe. In 1995, a population of *C. caudatum* from African samples (Mombasa, Kenya) was the subject of further morphological investigations (Foissner, 1995) using a diversity of microscopic techniques that resulted in the transfer of the family Colpodidiidae from the class Colpodea to the Nassophorea. The species' phylogeny was finally confirmed by Breiner et al. (2008) through the analysis of the 18S ribosomal DNA (SSrDNA) which established its classification within the Nassophorea. The molecular phylogeny of the genus was further confirmed by Yildiz (2021).

The aim of this study is to describe a population of *C. caudatum*

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Table 1
Morphometric characteristics of the Spanish population of *Colpodidium caudatum* Wilbert, 1982; x̃, arithmetic mean; M, median; SD, Standard Deviation; SE, Standard error of the arithmetic mean; CV, coefficient of variation; Min, minimum; Max, maximum; n, number of cells examined.

	x̃	M	SD	SE	CV	Min	Max	n
Body length	65.8	67	10.4	1.9	15.8	48	89	30
Body width	34.5	34	6.16	1.1	18.0	23	45	30
Body length/width ratio	1.9	1.9	0,15	0,2	7.5	1.8	2.4	30
Distance from anterior end of the cell to macronucleus	41.9	42.5	7.0	1.3	16.7	28	57	30
Distance from anterior end of the cell to excretory pore of contractile vacuole	42.5	42.0	6.7	1.2	14.5	32	57	30
Distance from posterior end of the cell anterior to the summit of the paroral membrane	26,8	26	4.3	0.8	16.2	18	37	30
Macronucleus length	16.2	14.5	3.9	0.7	24.6	12	20	30
Macronucleus width	14,6	15	2.7	0.5	18.9	10	22	30
Number of macronuclei	1.0	1.0	0.0	0.0	0.0	1.0	1.0	30
Number of micronuclei	1.0	1.0	0.0	0.0	0.0	1.0	1.0	7
Number of somatic kineties	21.1	21	1.4	0.3	6.5	21	23	30
Number of somatic kineties between the anterior part of the cell and the paroral membrane	8.5	9	0.8	0.2	9.6	7	10	30
Number of kinetids in a dorsal kinety	30.6	31	2.1	0.4	7.0	27	35	30
Number of ciliary rows in nassulid organelle 3	20	20	0.8	0.4	4.0	19	21	4
Number of dikinetids in the paroral membrane	16.7	16	1.8	0.3	11.8	14	19	30

discovered in Spain using silver impregnation and other techniques. By comparing the results with published records of the species from other parts of the world, this investigation aims at gaining insights into the morphological diversity and geographic distribution of *C. caudatum*. This research provides new morphological and ecological data that contribute to a better understanding of this ciliate species. Additionally, this study includes a comprehensive review of the genus *Colpodidium* and provides a dichotomous key for species identification, which can be a valuable resource for future research.

2. Results

2.1. Description of the Spanish population of Colpodidium caudatum

Table 1 shows the morphometric characteristics of the European population of *Colpodidium caudatum* found in Spain. Living cells of *C. caudatum* measure 48–89 μm in length and 23–45 μm in width (Table 1). The shape of the living organism is variable, ranging from crescent-shaped to ovoid. The macronucleus is globular with an irregular surface, located in the posterior half of the cell and below the oral cavity (Figs. 2-4). The micronucleus is difficult to observe but is spherical and positioned close to the macronucleus. A conspicuous contractile vacuole is present just below the oral opening, in a subequatorial position, with a single excretory pore (Fig. 4). The cytopyge is located below the excretory pore, and along the middle of the cell (Fig. 4). The organism's cortex is thick and covered with cilia. Mitochondria of varied shapes are visible in some silver-carbonate impregnated specimens (Olmo and Fernández-Galiano, 1997), mostly elongated in shape and located beneath the cortex, forming a complex and developed chondriome (Fig. 4B, C). Extrusomes have not been observed, either in vivo or through silver impregnation. The cytoplasm is colourless, with numerous small particles, especially in the posterior part of the cell (Supplementary Material). Food vacuoles containing bacteria and digested material are also observed in the cytoplasm (Fig. 4).

The ciliate swims rapidly, rotating along its longitudinal axis (Supplementary Material). The somatic cilia are approximately 5 to 10 μm long. Silver impregnated specimens have 21–23 somatic kineties with 27 to 35 kinetids in each (Table 1, Figs. 3-4). Each kinetid consists of a large granule formed by the kinetosome, from which the cilium originates, along with a short kinetodesmic fibre to its left and another transverse fibre to its right, forming an angle of approximately 120 degrees between the two fibres (Figs. 3-4). Additionally, each kinetid contains a smaller granule, likely corresponding to a parasomal sac that is visible in some silver-impregnated individuals (Fig. 4). This arrangement can mistakenly give the impression that kineties consist of two kinetids instead of one. The kinetids within each somatic kinety are closer to each other at the anterior part of the kinety (Fig. 4A). This arrangement is particularly conspicuous in the kinetids of kinety 1 (K1, immediately to the right of the paroral membrane), which are more closely positioned to each other at the anterior stretch of the kinety (Figs. 2-4).

The somatic kineties are fully bipolar on the dorsal side of the cell (Figs. 2, 3), while on the ventral side, the kineties located to the left of the oral area present a slight rotation in its anterior part, resulting in a narrow and almost straight preoral suture, although occasionally it may be more curved (Figs. 2, 4). This rotation is also recognisable in the living organism (Supplementary Material).

There are four postoral kineties (PO1-4) (Figs. 3-4). The first postoral kinety (PO1) begins below the top end of the paroral membrane (PM); the second postoral kinety (PO2) starts at the oral cavity near the pharyngeal opening, and the third postoral kinety (PO3) emerges at the end of adoral organelle 3 (also known as the nassulid organelle 3, NO3). Finally, postoral kinety 4 (PO4) is very short and located to the left of the excretory pore (Figs. 2, 4). The ciliate lacks a caudal cilium.

In living organisms, the oral area is positioned at the cell equator (Figs. 2-4; Supplementary Material) but in some cells it appears slightly below the centre of the cell (Fig. 4 A, B) or above (Fig. 3). The oral infraciliature consists of the paroral membrane (PM) and three nassulid organelles (NO1-3) (Figs. 2, 4). NO3 is the longest, consisting of approximately 20 rows of three kinetosomes each (Table 1, Figs. 2, 4 C, D). NO2 is formed by approximately eight kinetosomes arranged in zigzag. NO1 is reduced to one pair of kinetosomes (Table 1, Figs. 2, 3). This pair is not always detectable in the silver-impregnated specimens and its location in the oral area is variable (Figs. 2, 4).

The oral cavity is tube-shaped and contains a long, slightly curved NO3 that enters the infundibulum-like mouth opening (Figs. 2, 4). The typical nassulid pharyngeal basket is inconspicuous and hardly recognisable in living and in silver-impregnated specimens. The paroral membrane is located on the right side of the oral cavity, and it curves over the oral area (Figs. 3-4). It is composed of about 16 dikinetids. The distance between the dikinetids gradually increases from the beginning to the posterior or final part of the paroral membrane (Figs. 3-4).

The staining method of Klein (1958) did not produce reliable results, making it impossible to confirm the presence of a silverline system (argyrome) in the European population of *C. caudatum*.

2.2. Ecology of the Spanish population of Colpodidium caudatum

The Spanish population of *C. caudatum* was discovered in a water drain located in a playground at IES Azuer School (Manzanares, Ciudad Real, Spain), where water and soil accumulate after rainfall, but otherwise the drain is typically dry throughout the year. The initial collection was made in May 2021, and subsequent collections have been conducted

Table 2
Morphometric data of the different world populations of *Colpodidium caudatum* Wilbert, 1982 described thus far.

	Afghanistan (Wilbert, 1982)	Kenya (Foissner, 1995)	Namibia (Foissner, 1995)	China (Foissner, 1995)	Spain (present research)
Body shape	Fusiform-ovoid thin	Slightly reniform	Reniform	Very fusiform	Variable; from reniform to slightly fusiform
Live cell length	40–50	55–70	40–55	70–90	45–80
Live cell width	15–20	25–35	15–25	25–40	20–40
Mean cell size after silver impregnation	45 × 15	55 × 22	47 × 18	76 × 28	67 × 34
Macronucleus, length	10	10	–	11	14.5
Distance from anterior cell end to excretory pore	14	25	–	45	42
Number of somatic kineties	19–20	16–19	20–25	19–23	19–23
Number of somatic kineties between anterior cell end and paroral membrane	8	5–7	5–6	8–11	7–10
Number of kinetids in a dorsal kinety	25–31	23–32	19–28	27–44	27–35
Number of ciliary rows in nassulid organelle 3	?	15	12–14	19–22	19–21
Number of dikinetids in the paroral membrane	18	12–14	10–13	14–19	14–19
Cortex	Rigid	Rigid	Rigid	Very flexible	Rigid
Dorsal kineties' shape	More or less straight	More or less straight	More or less straight	Spiral	More or less straight
Shape of the preoral suture	Slightly curved, starts on the right side	More or less straight; starts ventrally	More or less straight; Starts ventrally	Spiral, begins dorsally	Slightly curved, begins ventrally
Number of cells examined	25	13	21	15	30

Table 3
Comparison of the morphometric data of *Colpodidium* species. Data based on silver-impregnated, mounted specimens. Lengths and distances expressed in µm. *C. viride* is not included due to lack of available data of silver impregnated specimens. [1]Values for *C. caudatum* have been extracted from different populations (our data; Wilbert, 1982; Foissner, 1995; Foissner et al., 2002) and are shown as maximum and minimum values; [2]Yildiz (2021); [3]Foissner et al. (2002).

	C. caudatum[1]	*C. zelihayildizae*[2]	*C. horribile*[3]	*C. trichocystiferum*[3]	*C. microstoma*[3]	*C. bradburyarum*[3]
Body, length	45–76	40–60	50–63	35–50	53–78	43–68
Body, width	15–34	15–21	48–68	18–26	21–35	24–39
Body length/width ratio	1.8–2.4	2.3–3.4	1.7–2.1	1.7–2.2	1.9–2.8	1.5–1.9
Anterior end of body to macronucleus, distance	28–57	40–24	8–26	6–29	36–53	16–31
Anterior end of body to excretory pore of contractile vacuole, distance	32–57	28–40	34–40	23–29	23–22	21–34
End of the body anterior to the summit of the paroral membrane, distance	18–37	19–26	23–28	14–20	15–21	11–20
Macronucleus, length	12–20	6–11	10–15	9–13	11–16	7.5–11.5
Macronucleus, width	10–22	5–9	9–13	8–13	10–14	6–10
Macronucleus, number	1	1	1	1	1	1–3
Micronucleus, number	1	1	1	1	1	–
Somatic ciliary rows, number	16–25	19–22	23–26	17–18	23–26	21–25
Somatic ciliary rows between the anterior part of the body and the paroral, number	5–11	8–9	10–13	6–7	–	–
Number of kinetids in a dorsal kinetic	19–35	27–35	19–28	12–14	25–37	16–23
Number of ciliary rows in nassulid organelle 3	12–22	16–17	13–19	9–11	10–12	18
Number of dikinetids in the paroral membrane	12–19	20	19–33	15–19	8–10	19

regularly on or after rainy days. Dry material consisting mainly of leaves and soil that had accumulated in the drain was also collected. However, there were occasions after abundant rainfall and residual water in the drain in which the ciliate was not found. This may suggest that the species is associated with soil habitats rather than water environments. These findings further support our view that *C. caudatum* is a ciliate species associated with terrestrial habitats and requires flooded soils to flourish. Considering the organic matter content in its environment, *C. caudatum* can be classified as a species ranging between polysaprobic and alpha-mesosaprobic, as also indicated by the presence of *Metopus* sp. and numerous heterotrophic flagellates.

Other ciliate species found alongside *C. caudatum* include *Vorticella* sp., *Cyrtolophosis mucicola*, *Drepanomonas revoluta*, *Colpoda steinii*, *Colpoda inflata*, *Dileptus* sp., *Metopus* sp., and various species of hypotrichs and scuticociliates. Other identified microscopic organisms found in the samples include naked amoebae, rotifers, nematodes, and abundant heterotrophic flagellates, particularly a lanceolate-shaped heterotrophic euglenid.

2.3. Revision of the genus Colpodidium

The discovery of the first European population of *Colpodidium caudatum* and the addition of new morphological and ecological data prompted us to undertake a revision of the genus *Colpodidium* (Table 3).

Since the initial description of *C. caudatum* by Wilbert (1982), its taxonomic classification has been a subject of debate. Wilbert initially assigned *C. caudatum* to the family Colpodidae within the order Colpodida and class Colpodea, based on the description of dikinetids in each kinety and the presence of an inconspicuous vestibule, both of which are typical characteristics of Colpodids (Wilbert, 1982). In 1989, de Puytorac et al. assigned the species to a new family, Colpodidiidae. Foissner (1990) discovered that the dikinetids were actually monokinetids, and although silver impregnation revealed two impregnated granules, one actually corresponded to base of the cilium (the kinetosome) and the other to a parasomal sac. Moreover, the presence of argyrome and the arrangement of the contractile vacuole pore and cytopyge as well as the characteristics of the ciliate's oral infraciliature, led Foissner (1995) to conclude that *C. caudatum* should be included within the Class Nassophorea. Further ontogenetic data obtained from a population of *C. caudatum* from Namibia allowed for the establishment of a new order

Fig. 1. (**A**) Sampling location in the school playground of IES Azuer (Manzanares, Ciudad Real, Spain). (**B**) Drain from where the samples containing *Colpodidium caudatum* were collected.

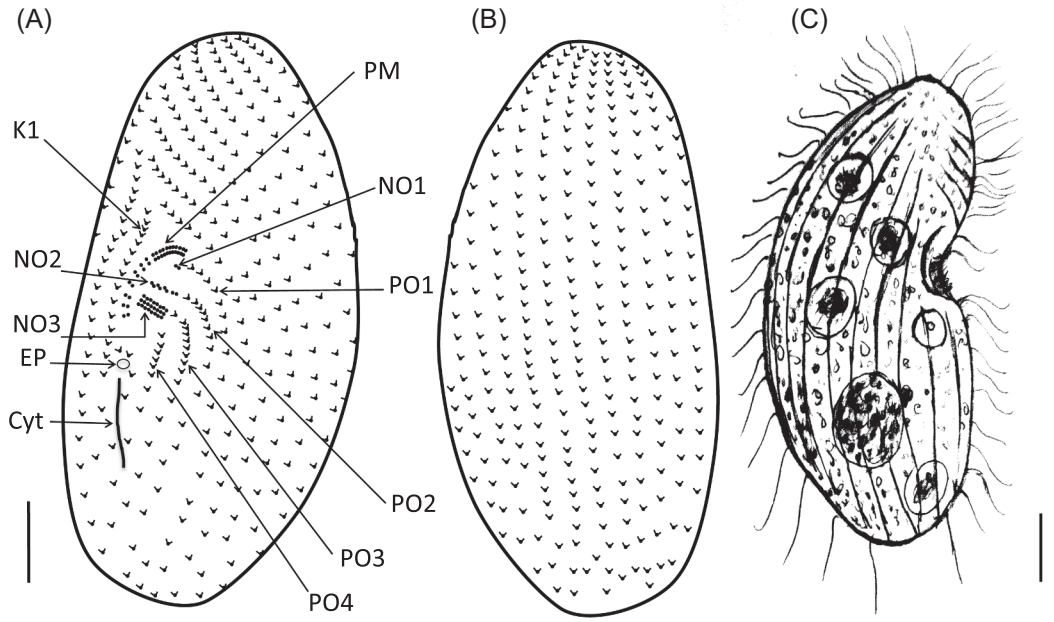

Fig. 2. Line drawings of the European strain of *Colpodidium caudatum*. (**A**) and (**B**) show the ventral side (**A**) and dorsal side (**B**) of a silver carbonate impregnated individual. (**C**) Overall cell shape of the living organism. Cyt, Cytopyge; EP, Excretory Pore; K1, Somatic Kinety 1; NO1, NO2, NO3, Nassulid Organelles 1, 2 and 3; PM, Paroral Membrane; PO1, PO2, PO3, PO4, Postoral Somatic Kineties 1, 2, 3, and 4. Scale bar: 10 μm.

Colpodidiida, within the Class Nassophorea (Foissner et al., 2002). In an effort to confirm these phylogenetic relationships, Breiner et al. (2008) analyzed the 18S ribosomal DNA (SSrDNA) of *C. caudatum* and confirmed its classification as a nassulid (Order Nassulida). The molecular phylogeny of the genus was further confirmed by Yildiz (2021).

Therefore, the current taxonomic classification of *Colpodidium caudatum* is as follows: Phylum: Ciliophora; Class: Nassophorea; Order:

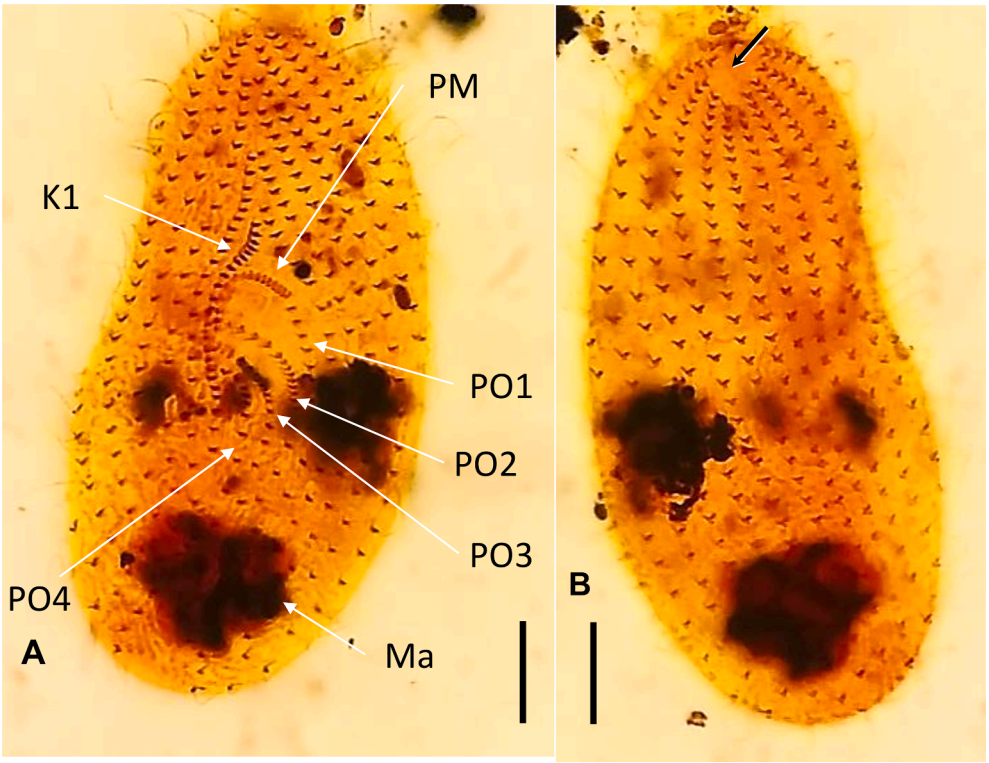

Fig. 3. Silver carbonate impregnated individuals of a European population of *Colpodidium caudatum*. (**A**) Ventral side of the cell. (**B**) Dorsal side of the same cell. Arrow to the polar cap at the anterior end of the cell. K1, somatic kinety 1; Ma, Macronucleus; PM, Paroral Membrane; PO1-PO4, Postoral somatic kineties 1 to 4. Scale bars: 10 μm.

Colpodidiida; Family: Colpodidiidae; Genus: *Colpodidium*; Species: *Colpodidium caudatum.*

The genus *Colpodidium* currently includes six species: *Colpodidium caudatum*, *C. horribile*, *C. trichocystiferum*, *C. microstoma*, *C. bradburyarum*, *C. viride* and *C. zelihayildizae*. Table 3 and Fig. 5 provide information for distinguishing between different species of *Colpodidium*.

3. Discussion

Comparison of the European population of *Colpodidium caudatum* with isolates from other world geographical locations.

Table 2 summarizes the morphological characteristics of the Spanish isolate along with isolates of *C. caudatum* from around the world. As shown in Table 2, the cell size range of the Spanish isolate includes the cell sizes described for all the other isolates. In our isolate, we observed two distinct populations: individuals of smaller size (cell size range 50–55 μm) and individuals of larger cell size (55–90 μm). The Afghan and Kenyan isolates fit within the former, whereas the Chinese and Namibian isolates fit within the latter, although the Chinese isolate can reach a slightly larger size (Table 2).

Regarding body shape, most world populations of *C. caudatum* have been described as reniform or fusiform. However, the Spanish isolate showed high shape variation, ranging from crescent-shaped (fusiform) with rounded ends to nearly ovoid. These variations in cell shape were dependant on the specific environment and feeding conditions of the specimens studied. The location of the oral cavity also varied considerably amongst the different world isolates, although in most of them it is in the middle or upper half of the cell, both of which have been observed in the Spanish populations (Figs. 3-4).

When analysing the data related to somatic infraciliature, we also observed overlap in the number of somatic kineties with the world isolates (16 to 25 in the Spanish populations), as well as in the number of kinetids in the dorsal kinety and the number of dikinetids of the paroral membrane (Table 2). One difference between the Chinese isolate and other isolates is the highly spiralled pattern of the dorsal kineties, unlike the typically less curved or almost straight form observed in other populations (see Figs. 3 and 4). However, interpreting this feature accurately can be challenging as it is influenced by the specimen's orientation and view angle on the microscope slide – see examples in the different specimens of Figs. 3 and 4. These findings suggest a substantial transition in most of the analyzed characteristics among the studied populations, indicating strong intraspecific and interpopulation variability in *C. caudatum*, as previously demonstrated by Foissner et al. (2002). Furthermore, these observations suggest that *C. caudatum* may represent a sister species complex (Foissner et al., 2002), like in *Paramecium caudatum, P. aurelia*, or *Tetrahymena pyriformis*.

In our study, we also revised the published data of *C. caudatum* from Benin (Africa) previously described by Dragesco and Dragesco-Kernéis (1986). Based on the similarities in several characteristics, particularly the elongated paroral membrane, it appears highly likely that this population is conspecific with *C. horribile*, as noted by Foissner et al. (2002).

The morphological characteristics described for *C. zelihayildizae* from Turkey (Yildiz, 2021) fall within the range of variation observed in *C. caudatum* from Spain except for the length of the macronucleus and one dikinetid in the paroral membrane (Tables 2, 3). At morphological level, both species overlap. However, the lack of molecular data from the Spanish strain makes it premature to consider *C. zelihayildizae* a junior synonym of *C. caudatum*.

3.1. Biogeography of Colpodidium caudatum

C. caudatum was initially discovered in a grassland soil in Afghanistan in 1978 by Wilbert (1982). Subsequently, Foissner (1995, 2016) and Foissner et al. (2002) found it in soil samples from Kenya, Namibia, Australia, Japan, Tibet, Costa Rica, Venezuela, Galápagos and China, but never in Europe. We have also found it (Hines and Esteban

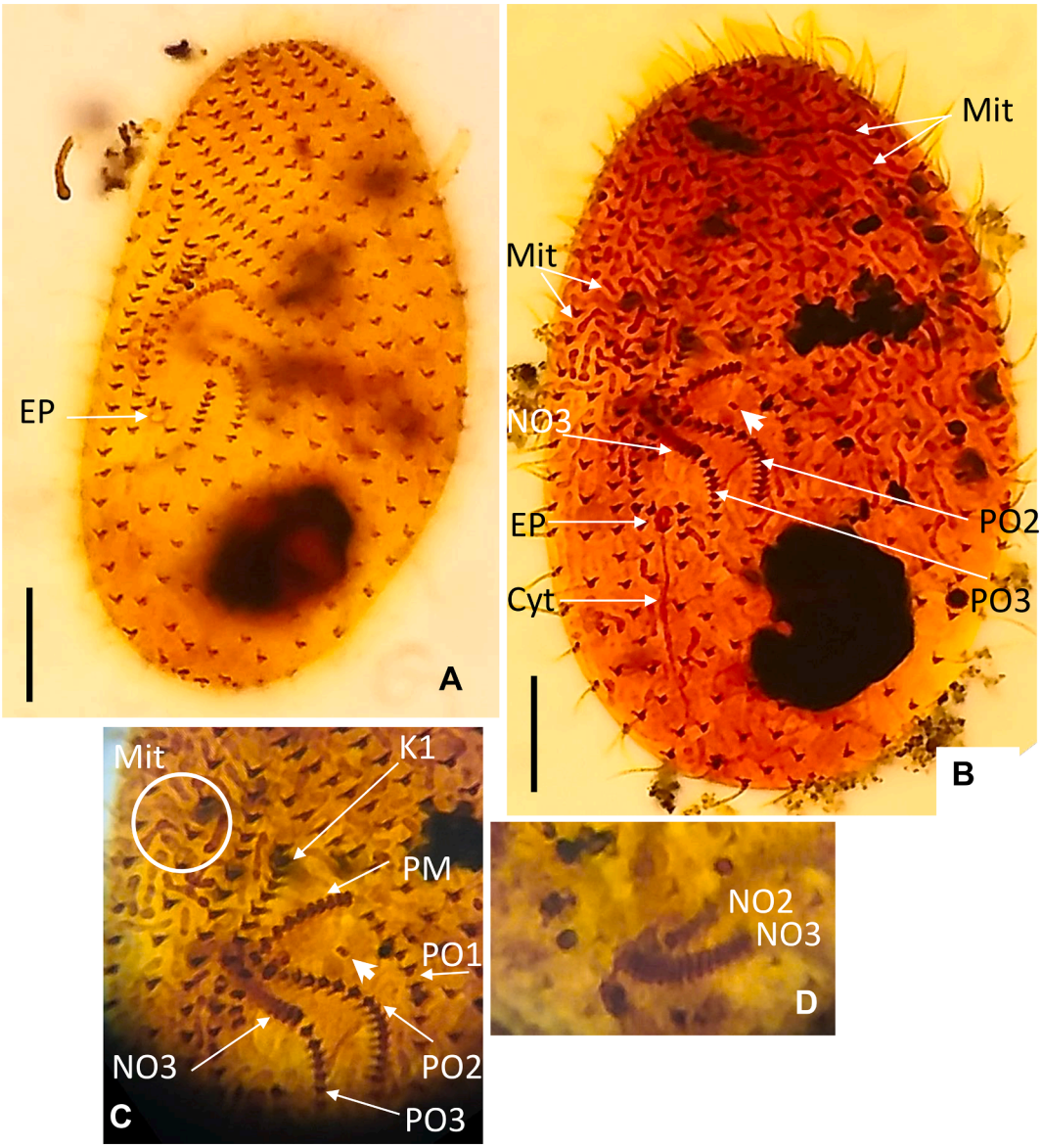

Fig. 4. Silver carbonate impregnated individuals of a European population of *Colpodidium caudatum*. (**A**), (**B**) ventral side of two different individuals. (**B**) Arrowhead to what probably is the nassulid organelle 1. (**C**), (**D**) Close-up of the oral area showing the arrangement of the oral infraciliature. (**C**) Mitochondria clearly visible beneath the cell membrane (white circle shown to pinpoint some of them); Arrowhead to the nassulid organelle 1 formed by one pair of kinetosomes, (D) Close-up of nassulid organelles 2 and 3. Cyt, Cytopyge; EP, Excretory Pore; K1, Somatic Kinety 1; Ma, Macronucleus; Mit, Mitochondria; NO2, NO3, Nassulid Organelles 2 and 3; PM, Paroral Membrane; PO1, PO2, PO3, Postoral Somatic kineties 1, 2 and 3. Scale bars: 10 μm.

unpub. observations) in soils from Florida (USA). Our study of the Spanish isolate documents, for the first time, the presence of *C. caudatum* in Europe. According to Foissner (1995), this species is presumed to be common in his samples. However, our evidence suggests that this may not be the case, at least in Europe.

C. caudatum, although rare, supports the cosmopolitan nature of ciliates (Finlay, 2002; Fenchel et al., 1997; Fenchel et al., 2019). The record from Spain expands the known geographical distribution of *C. caudatum* to all continents except Antarctica. It is a mystery why this species, described as common in other parts of the world (Foissner, 1995), has not been recorded in Europe prior to our discovery. It is also paradoxical that Wilhelm Foissner, despite studying soils in Europe for over 50 years, never encountered it. There are two possible explanations for this puzzle: (1) Temperature probably is a key environmental factor affecting the population growth of this ciliate species. For example, a population of *C. caudatum* in dry soil samples from Florida has been found (USA, Hines and Esteban unpublished results) after rewetting, enriching, and incubating the soil for two days at 30 °C. The isolate from Spain was observed in its active form in freshly collected samples during warm months of the year, indicating that the species might be a mesophile (i.e., organisms that grow best at 20 to 45 °C); (2) *C. caudatum* may not be a soil ciliate per se. The ciliate can indeed form resting cysts that aid its dispersal and survival during drought periods. However, the active form of the ciliate requires flooded soils to flourish, suggesting that *C. caudatum* is primarily a soil-dwelling ciliate that thrives in temporarily flooded areas with abundant water availability. Further research is needed to investigate soils in general, temperature, and the 'seedbank' of ciliates.

4. Conclusions

This study reports the first record of *Colpodidium caudatum* in Europe, a ciliate that is typically found in flooded soil environments. The Spanish isolate shows significant intraspecific morphological variability, with

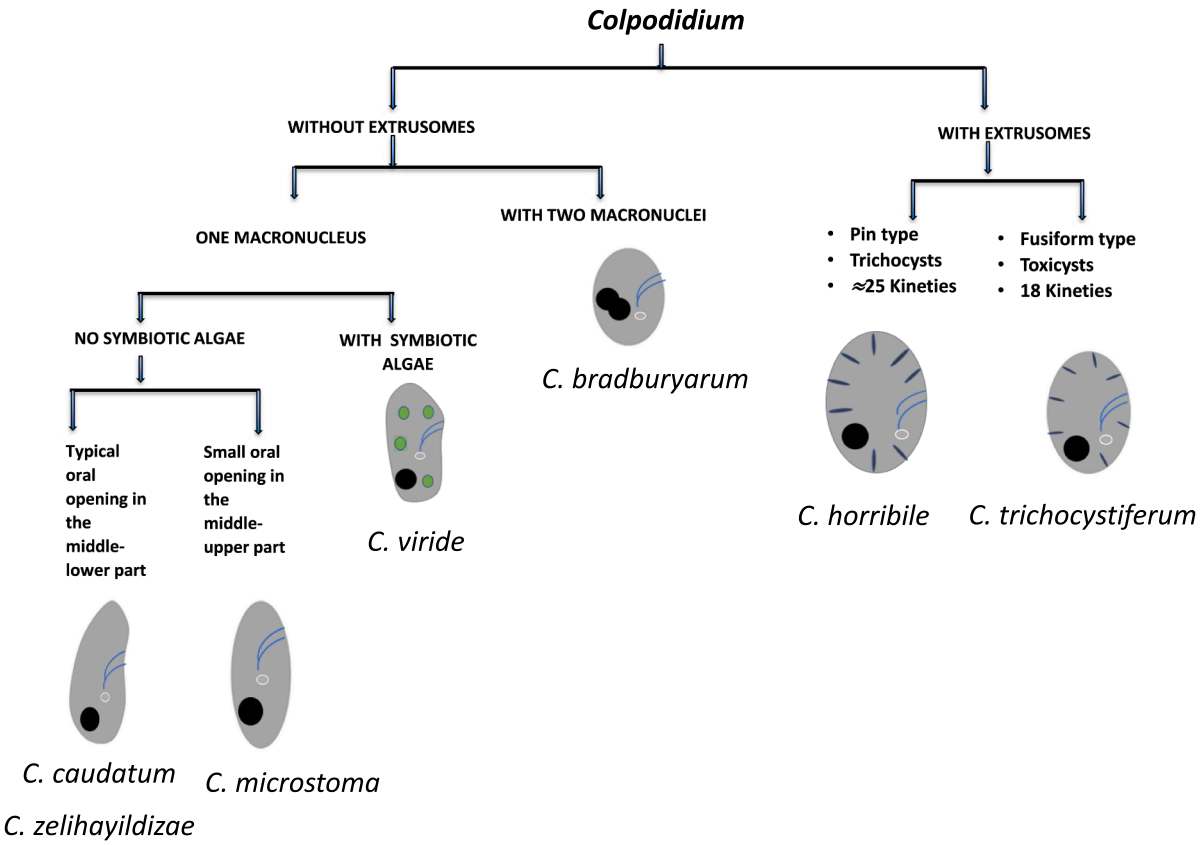

Fig. 5. A dichotomous key for identifying species belonging to the genus *Colpodidium* Wilbert, 1982.

overlaps observed with populations from other regions of the world. However, at the current stage of knowledge, these variations are not substantial enough to warrant the separation of distinct species. The Spanish isolate displays the greatest morphological variation, likely due to the higher number of cells that have been analysed.

Further genetic studies are required to determine the precise taxonomic position of *C. caudatum*. These studies will contribute to a more comprehensive understanding of the species and its relationship to other closely related organisms.

C. caudatum has a cosmopolitan distribution and is uncommon in Europe. It is a remarkable example of the biogeography and distribution of microbial eukaryotes and how their biogeography is shaped by environmental factors. Protistologists have examined hundreds, if not thousands, of European soil samples for almost a century, but specific growth and environmental conditions such as temperature or time of year when sampling took place may have prevented its discovery in Europe. Consequently, this ciliate can be regarded as an emblematic or flagship species.

Our findings confirm that *C. caudatum* is primarily a soil-dwelling ciliate that thrives in temporarily flooded areas with ample water availability. These environmental conditions provide optimal conditions for growth and reproduction.

The study of the Spanish population of *C. caudatum* reveals major insights into its intraspecific variability, highlights the need for further genetic investigations, demonstrates its cosmopolitan nature, and reinforces its association with soil habitats and water availability.

5. Methods

Samples were collected from a water drain located in the playground of the IES Azuer school in Manzanares, Ciudad Real (Spain, 38.9953° N, 3.3601° W) (Fig. 1A). The water drain is 50 cm × 50 cm, and 17 cm deep, and easily accessible by lifting the grid cover (Fig. 1B). After rainfall, the water drain contains residual water and wet soil from the run-off; during dry weather, dry soil and leaves may accumulate over time (Fig. 1B). The first sample collection took place in May 2021, and subsequent collections up until May 2023 were conducted regularly on rainy days, totalling six times. *Colpodidium caudatum* was found thriving in the samples of water and soil collected with a 250 ml glass container; sometimes the ciliate would be detected in these wet samples after two or three days of leaving them at room temperature. In other occasions, dry soil with dried leaves was collected from the dry water drain using a spoon and placing the dry material in sterile Petri dishes to which Lanjarón(R) mineral water was added in enough volume to produce a slurry (Finlay et al. 2006). The Petri dishes were left at room temperature and checked every day for *C. caudatum* growth. The ciliate would grow in these Petri dishes after two or three days, but not always, which suggests that the ciliate must have been present as cysts. In occasions when there was abundant rainfall and substantial residual water in the drain, we did not find the species, suggesting that it is a ciliate associated with soil habitats rather than water environments.

Cultures were prepared by enriching the water samples or re-wetted soil with one or two boiled wheat grains. The cultures were not always successful and those that were, lasted a few weeks only. Attempts were made to obtain a pure culture of this protozoon, but the cultures would not last more than two or three weeks.

The silver carbonate impregnation method of Fernández-Galiano (1994) was used to reveal the pattern of the somatic and oral infraciliature. The method described by Klein (1958) was followed to reveal the silverline system (argyrome).

CRediT authorship contribution statement

Juan Rodero Madrid: Methodology, Investigation, Visualization, Writing – original draft. **José Luis Olmo:** Investigation, Data curation, Supervision, Writing – original draft, Writing – review & editing.

Genoveva F. Esteban: Investigation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

The supplementary material should take the reader to a video of the ciliate https://www.youtube.com/watch?v=Hib_C3n3fHs.

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