

Article

Conservation of Protists: The Krauthügel Pond in Austria

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Abstract: Although constituting more than 100,000 described species, protists are virtually ignored within the arena of biodiversity conservation. One reason is the widespread belief that the majority of protists have cosmopolitan distributions, in contrast to the highly heterogeneous biogeography of the “mega-Metazoa”. However, modern research reveals that about one third of the known protists have restricted distributions, which endorses their conservation, at least in special cases. Here, we report what probably ranks as the first successful conservation intervention focused directly on known protist diversity. It is justified by unique species, type localities, and landscape maintenance as evidence for legislation. The protected habitat comprises an ephemeral pond, which is now a “Natural Monument” for ciliated protozoa. This wetland occupies a natural depression on the Krauthügel (“cabbage hill”) south of the fortress of Salzburg City. When filled, the claviform pond has a size of $\sim 30 \times 15$ m and a depth rarely surpassing 30 cm. Water is present only for some days or weeks, depending on heavy and/or prolonged rain. The pond occupied an agricultural field where root and leafy vegetables were cultivated for possibly more than 200 years. In the 1960s, this area became a grassland utilized as an autumn pasture, but was abandoned in the 1990s. Repeated sampling between 1982 and 2012 recovered a total of at least 150 ciliate taxa, of which 121 were identified to species level. Eight species were new to science, and an additional 10 poorly known species were

reinvestigated and neotypified with populations from the Krauthügel pond. Both endemism and type localities justify the argument that the “integrative approach” in biodiversity and conservation issues should include protists and micro-metazoans. We argue that Krauthügel holds a unique reference node for biodiversity inventories to obtain the baseline knowledge—which is the prerequisite to monitor ecosystem integrity—and detect and evaluate impacts of natural and anthropogenic disturbances.

Keywords: ciliates; protist endemism; integrative biodiversity and conservation approach; Salzburg; type locality

1. Introduction

Rarely, if ever, do we consider microscopic organisms, such as protists and small pluricellular eukaryotes (e.g., rotifers, mosses), in conservation issues. Microscopic inconspicuousness is one obvious reason for their neglect. An academic argument justifies least concern for these “micro-biota”. This assumes the known species have cosmopolitan distributions [1–3]. In contrast, biogeographical knowledge of plants, vertebrates and the better known Insecta (e.g., Lepidoptera, Odonata) is not only more comprehensive and precise; but a high degree of endemism is well documented in such “Mega-Metazoa”, and it is not surprising that protected area design has been informed primarily by these surrogate taxa [4,5]. However, two recent books [6,7] on the distribution and biogeography of microscopic organisms demonstrate convincingly that their biogeographical patterns approximate those of Metazoa. About one third of mapped species exhibit endemism, confirming the moderate endemism model [8]. Accordingly, biodiversity conservation should include these minute but genetically highly diverse organisms, especially in the framework of “integrative approaches” (for reviews, see [3,5]).

The major issues in protist conservation have been discussed previously [4,9]. At least one protist species becomes extinct with each metazoan lost to extinction; change or loss of habitats; loss of type localities; obviously, extinction of a species, and/or clade, equates to the irreversible loss of unique genetic resources. Extreme losses of this evolutionary potential can be gauged against the ancient history of protists, given that the start of the Eukaryotic radiation is estimated at ~1,400 Ma, in the mid-Proterozoic, in response to unique biogeochemical opportunities inaugurated on Earth’s first supercontinent [10].

Ultimately, in conservation biology, practical success subsumes theory. Here we report on a successful case of conservation of the ciliates of an ephemeral pond in the town of Salzburg. This pond is type locality for 18 ciliate species, but was suddenly filled with earth in the course of an outdoor art project. When we recognized this crisis in mid-2010, we applied for conservation, and succeeded in convincing the landowner to remove the earth. We hope that our effort will stimulate colleagues to try the same for other small habitats with great diversity of certain groups of protists and/or microscopic pluricellular organisms.

Figure 1. The Krauthügel pond when filled.



Figure 2. The Krauthügel pond when dry.



2. Study Area and Methods

2.1. The Krauthügel Pond

The Krauthügel pond is at the southern foot of the fortress of Hohensalzburg, *i.e.*, near the center of the town of Salzburg at the northeast margin of estate 2324 KG, E13°02'40.3" N47°47'30.7", 429.7 m above the Adriatic Sea. Here is a small agricultural area on a hill called “Krauthügel” (cabbage hill). The temporary pond occupies a natural depression overgrown by herbaceous vegetation, except in the barren center (Figures 1 and 2). Water is present only for some days or weeks, depending on short, but heavy, rains (mainly thunderstorms) or long periods of rain. When filled, the pond is clavate and has a size of about 30 × 15 m and a depth rarely surpassing 30 cm (Figure 1).

The Krauthügel has experienced a shifting history of landuse. It occupied an agricultural field where root and leafy vegetables (e.g., cabbage) were cultivated between 1789 and 1960. Then, it became a pasture for cows, whose excrements strongly eutrophied the pond water. This caused population blooms of a diversity of autotrophic and heterotrophic protists. Grazing of the pasture ceased after 1990. In medieval times, the pond was possibly influenced by an open canal (Alm canal) that supplied the town with drinking water. Most recently in the 20th century, expansion of the road network and urbanization culminated in landscape fragmentation to isolate the Krauthügel from the local drainage network. Taken together, the historical evidence indicates that the natural landscape depression produced an ephemeral pond of variable size and structure over several centuries. Although the geomorphology of this portion of the landscape has not been studied directly, the Krauthügel appears to be an abandoned stream infilled by surface runoff, and anthropogenic activities appear to have contributed to its formation. The original drainage channel and depression was likely modified in the Middle Ages with expansion of agriculture in the environs of the medieval town.

2.2. Methods

We investigated the ciliates of the Krauthügel pond sporadically between the years 1982 and 2012, using orthodox collection methods. The most intense investigation periods were the years 1982–1983; 2005–2007 when *Meseres corlissi*, one of the new species discovered in the pond, was subject of a focal research program of the Austrian Science Fund [11]; and 2010–2012 when the conservation procedure started.

Limnetic habitats: Plankton (mesh size 12 µm) and bottom mud; mud and water from cow steps and the agricultural machinery; infusions set up with the material collected and often enriched by some squashed wheat grains.

Soil-water habitats: During the Science Fund project, about 70 soil samples (0–10 cm) from the pond margin were investigated for ciliates, using the non-flooded Petri dish method described by Foissner [12]. Briefly, this simple method involves placing 20–500 g of air-dried soil in a Petri dish (13–18 cm wide, 2–3 cm high) and saturating, but not flooding it, with distilled water. These cultures were analyzed for ciliates by inspecting about 2 ml of the run-off on days 2, 7, 14, 21, and 28.

The ciliates of all samples were first inspected *in vivo*. When there was indication that an unknown or poorly known species was present with sufficient abundance, it was investigated using the methods described by Foissner [13], *i.e.*, detailed live observation, various silver impregnation techniques, and

scanning electron microscopy. Preparations of these species were deposited in a museum (for details, see Chapter 3.1.).

3. Faunistics of the Ciliate Community of the Krauthügel Pond

3.1. Remarkable Species

All holotypes, paratypes, neotypes and vouchers have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (Biology Centre of the Museum of Upper Austria, Linz (LI). Present curator: Dr. Erna Aesch; e-mail: e.aesch@landesmuseum-linz.ac.at). The taxa are ordered alphabetically.

Acropisthium mutabile Perty, 1852 [14]. A highly variable, possibly globally distributed haptorid ciliate whose identity is threatened. Thus, it is here neotypified with protargol-impregnated specimens deposited by Foissner [15]: inv. nos. 1984/ 44, 45.

Actinorhabdos trichocystiferus Foissner, 1984 [15] (Figure 3b). An inconspicuous haptorid ciliate with highly distinct, awl-shaped extrusomes first described from the Krauthügel pond: inv. nos. 1984/ 29, 30 (protargol impregnation). Recently re-discovered in a litter and soil sample from the restinga on the outskirts of the town of Rio de Janeiro, Brazil.

Apertopathula implicata (Kahl, 1930) [16] Foissner & Oertel 2009 [17]. One of the “difficult” spathidiid haptorids redescribed and neotypified by Foissner & Oertel [17]: inv. nos. 2013/ 28–32 (protargol impregnation). A very rare, microaerobic species as yet reported only from Germany (pond in the botanical garden of the town of Hamburg) and the Krauthügel pond. Thus, possibly restricted to the holarctic.

Apodileptus visscheri visscheri Dragesco, 1963 [18]. A “difficult” dileptid haptorid redescribed and neotypified by Vďačný & Foissner [19], using a population from the Krauthügel pond: inv. nos. 2011/ 218–225 (protargol impregnation). A voucher population from Lower Austria has the inv. nos. 2011/ 210–217. This is the nominal subspecies which differs by the shape of the extrusomes from *A. visscheri rhabdoplites* Vďačný & Foissner, 2012.

Cyrtolophosis mucicola (Stokes, 1885) [20]. Redescribed by Foissner [21].

Didinium nasutum (Mueller, 1773) [22] Stein, 1859 [23]. Redescribed by Foissner [15]: inv. nos. 1984/ 28.

Dileptus anatinus (Golińska, 1971) [24]. Redescribed by Vďačný & Foissner [19]: inv. nos. 2013/ 25–27 (protargol impregnation).

Enchelyodon anulatus (Foissner, 1984) [15] (Figure 3d). A highly characteristic haptorid ciliate with several globular macronuclear nodules each having a ring-shaped nucleolus. First described from the Krauthügel pond: inv. nos: 1984/ 42, 1984/ 43 (protargol impregnation). No other record known.

Enchelydium piliforme (Kahl, 1930) [16,25] Foissner, 1984 [15]: inv. nos. 1984/24, 1984/25 (protargol impregnation).

Frontonia angusta angusta (Kahl, 1931) [26]. The nominal subspecies has been redescribed and neotypified by Foissner *et al.* [27], using the population from the Krauthügel pond: inv. nos. 2002/ 628–630 (Chatton- Lwoff silver nitrate impregnation).

Furgasonia theresae (Fabre- Domergue, 1889) [28] Foissner *et al.*, 2002 [27]. This nassulid ciliate has been neotypified by Foissner *et al.* [27], based on the population from the Krauthügel pond described by Foissner [29]: inv. nos. 1988/ 29, 1988/ 30 (Chatton- Lwoff silver nitrate impregnation).

Fuscheria nodosa salisburgensis Gabilondo & Foissner, 2009 [30] (Figure 3g). A new haptorid subspecies first described by Gabilondo & Foissner [30] from the Krauthügel pond: inv. nos. 2010/52–61. No other record known.

Halteria grandinella (Mueller, 1773) [22] Dujardin, 1841 [31]. Ontogenesis described by Petz & Foissner [32].

Maryna ovata (Gelei, 1950) [33] Foissner, 1993 [34]. Redescribed by Foissner [34].

Maryna umbrellata (Gelei, 1950) [33] Foissner, 1993 [34]. This colpodid ciliate will be neotypified with the population from the Krauthügel pond.

Meseres corlissi Petz & Foissner, 1992 [32] (Figure 3a). This oligotrich ciliate was discovered in the Krauthügel pond: inv. nos. 1993/ 52, 1993/ 53 (protargol impregnation). It served as an example for ciliate distribution in a research program of the Austrian Science Fund [11]. Likely a cosmopolitan.

Papillorhabdos multinucleata Foissner, 1984 [15] (Figure 3f). This haptorid ciliate was discovered in the Krauthügel pond: inv. nos. 1984/ 26 (protargol impregnation). No other record known.

Perispira ovum Stein, 1859 [35]. A rare haptorid ciliate that will be redescribed and neotypified with the population from the Krauthügel pond.

Protospathidium serpens (Kahl, 1930) [25] Foissner, 1981 [36]. This haptorid ciliate was re- studied and neotypified by Xu & Foissner [37]: inv. nos. 2007/ 133–135 (protargol impregnation). It is a common mud and soil ciliate composed of cryptic species differing in the morphology of the resting cyst.

Pseudochilodonopsis algivora (Kahl, 1931) [26] Foissner, 1979 [38]. Redescribed by Foissner [39].

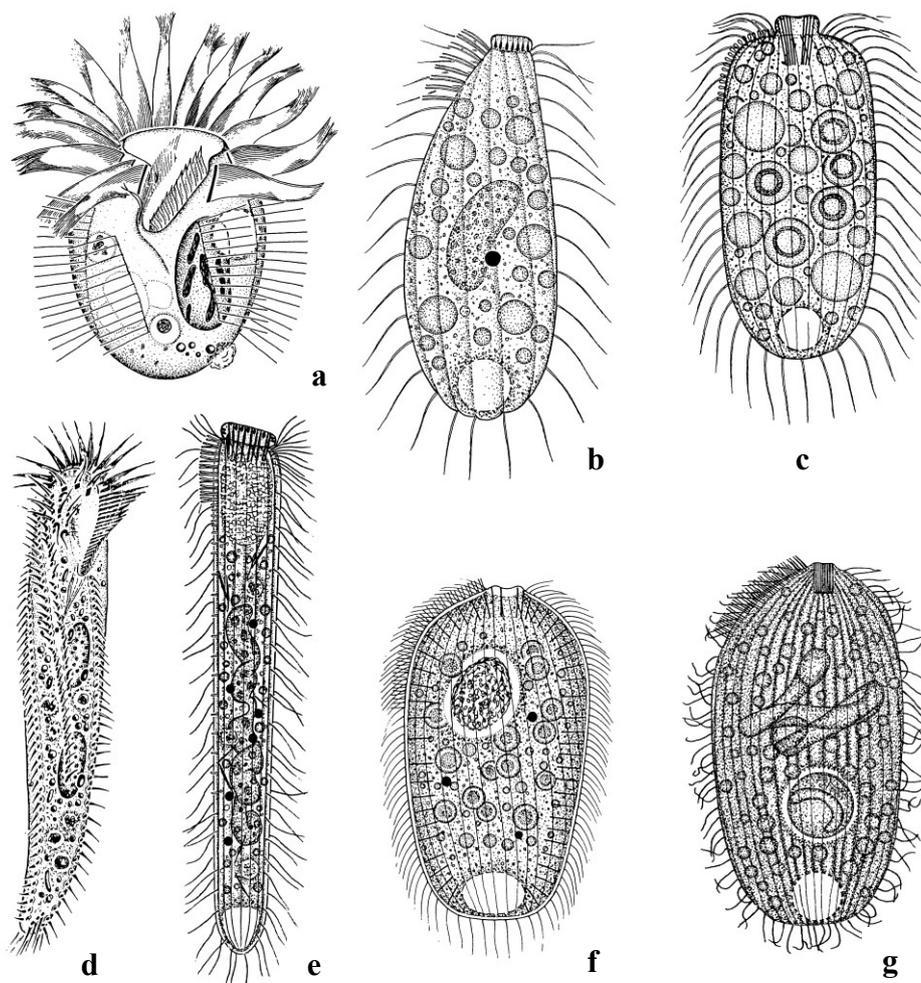
Pseudouroleptus procerus Berger & Foissner, 1987 [40] (Figure 3d). This hypotrich ciliate was discovered in a soil sample from the margin of the Krauthügel pond: inv. nos. 1986/ 61, 1986/ 62. Later recorded from the Palaeotropis [41].

Psilotricha succisa (O. F. Müller, 1786) [42] Foissner, 1983 [43]. Redescription and ontogenesis by Foissner [43]. Identity threatened by similar populations from Japan and Africa. Thus, here neotypified with the population from the Krauthügel pond: inv. nos. 2013/ 20–24 (protargol impregnation).

Semispathidium pulchrum Foissner, Hess & Al- Rasheid, 2010 [44] (Figure 3e). This conspicuous haptorid ciliate has as yet not been found at any other locality: inv. nos. 2011/392–397.

Tillina magna Gruber, 1880 [45]. This species belongs to the class Colpodea. Recent morphological and molecular studies showed the existence of several similar species threaten the identity of the Gruber species. Thus, we neotypify the species with specimens from the Krauthügel pond (slide 1984/ 28 Chatton- Lwoff silver nitrate method and with Figures 148r–y in Foissner [34], based on material from the Krauthügel pond.

Figures 3. Ciliate species discovered in the Krauthügel pond. For details, see chapter 3.1. (a). *Meseres corlissi* Petz & Foissner, 1992; length about 80 μm . (b). *Actinorhabdos trichocystiferus* Foissner, 1984; length about 50 μm . (c). *Enchelyodon anulatus* Foissner, 1984; length about 50 μm , (d). *Pseudouroleptus procerus* Berger & Foissner, 1987; length about 170 μm . (e). *Semispithidium pulchrum* Foissner, Hess & Al- Rasheid, 2010; length about 150 μm . (f). *Papillorhabdos multinucleatus* Foissner, 1984; length about 80 μm . (g). *Fuscheria nodosa salisburgensis* Gabilondo & Foissner, 2009; length about 115 μm .



3.2. Species Numbers and Community Structure

Over 30 years, we recorded about 150 ciliate taxa, of which 121 were identified to species level (Table 1). Ten of the 121 species were originally undescribed but two of them have been later described from other sites (*Bilamellophrya hawaiiensis*, *Coriplites grandis*), leaving the seven species shown in Figure 3 and at least one further new species among the unidentified ones (Table 1). Thus, so far, 10 new ciliate taxa have been discovered in this small, ephemeral pond.

Five species have been previously neotypified from populations of the Krauthügel pond, and five further species are neotypified in the present study or will be neotypified in forthcoming studies (*Acropisthium mutabile*, *Maryna umbrellata*, *Perispira ovum*, *Psilotricha succisa*, *Tillina magna*). Thus, the Krauthügel pond is type locality for 18 ciliate species. Additionally, seven species were redescribed from the Krauthügel pond (Table 1).

The ciliate community of the Krauthügel pond is a mixture of species from stagnant and permanent running waters (e.g., *Amphileptus pleurosigma*, *Cinetochilum margaritaceum*, *Euplotes affinis*, *Paramecium caudatum*, *Steinia sphagnicola*, *Uroleptus gallina*), soil and moss (e.g., *Colpoda maupasi*, *Drepanomonas pauciciliata*, *Euplotes muscicola*, *Grossglockneria* spp., *Mykophagophrys terricola*, *Spathidium claviforme*), and ephemeral waters (*Bresslaua* spp., *Bursaria truncatella*, *Furgasonia* spp., *Maryna* spp., *Opisthonecta* spp., *Tillina magna*), the latter belonging to the classes Colpodea (*Bresslaua*, *Bursaria*, *Maryna*, *Tillina*), Nassophorea (*Furgasonia*) and Oligohymenophorea (*Opisthonecta*).

Table 1. Ciliate species recorded from the Krauthügel-pond.

	Taxa
*	<i>Acropisthium mutabile</i> Perty, 1852
	<i>Actinorhabdos trichocystiferus</i> Foissner, 1984
	<i>Amphileptus pleurosigma</i> (Stokes, 1884) Foissner, 1984
**	<i>Apertospathula implicata</i> (Kahl, 1930) Foissner & Oertel, 2009
**	<i>Apodileptus visscheri visscheri</i> (Dragesco, 1963) Vďačný & Foissner, 2012
	<i>Arcuospathidium cooperi</i> Foissner, 1996
	<i>Askenasia volvox</i> (Eichwald, 1852) Kahl, 1930
	<i>Aspidisca cicada</i> (Mueller, 1786) Claparède & Lachmann, 1858
	<i>Balantidion pellucidum</i> Eberhard, 1862
	<i>Bilamellophrya hawaiiensis</i> Foissner, Agatha & Berger, 2002
	<i>Blepharisma steini</i> Kahl, 1932
	<i>Blepharisma undulans</i> Stein, 1867
	<i>Bresslaua insidiatrix</i> Claff, Dewey & Kidder, 1941
	<i>Bryometopus atypicus</i> Foissner, 1980
	<i>Bryometopus pseudochilodon</i> Kahl, 1932
	<i>Bursaria truncatella</i> Mueller, 1773
	<i>Bursellopsis truncata</i> (Kahl, 1927) Corliss, 1960
	<i>Chilodonella uncinata</i> (Ehrenberg, 1838) Strand, 1928
	<i>Chilodontopsis depressa</i> (Perty, 1852) Blochmann, 1895
	<i>Cinetochilum margaritaceum</i> (Ehrenberg, 1831) Perty, 1849
	<i>Colpoda aspera</i> Kahl, 1926
	<i>Colpoda cucullus</i> (Mueller, 1773) Gmelin, 1790
	<i>Colpoda inflata</i> (Stokes, 1884) Kahl, 1931
	<i>Colpoda lucida</i> Greeff, 1888
	<i>Colpoda maupasi</i> Enriques, 1908
	<i>Colpodidium bradburyarum</i> Foissner, Agatha & Berger, 2002
	<i>Colpodidium caudatum</i> Wilbert, 1982
	<i>Colpodidium microstoma</i> Foissner, Agatha & Berger, 2002
	<i>Coriplites grandis</i> Oertel <i>et al.</i> , 2008
	<i>Cyclidium glaucoma</i> Mueller, 1773
	<i>Cyrtolophosis minor</i> Vuxanovici, 1963
***	<i>Cyrtolophosis mucicola</i> Stokes, 1885
	<i>Deviata bacilliformis</i> (Gelei, 1954) Eigner, 1995

Table 1. Cont.

Taxa	
***	<i>Didinium nasutum</i> (Mueller, 1773) Stein, 1859
***	<i>Dileptus anatinus</i> Golińska, 1971
	<i>Dileptus margaritifer</i> (Ehrenberg, 1833) Dujardin, 1841
	<i>Drepanomonas pauciciliata</i> Foissner, 1987
***	<i>Enchelydium piliforme</i> (Kahl, 1930) Foissner, 1984
	<i>Enchelyodon anulatus</i> Foissner, 1984
	<i>Engelmanniella mobilis</i> (Engelmann, 1862) Foissner, 1982
	<i>Epispathidium ascendens</i> (Wenzel, 1955) Foissner, 1987
	<i>Epistylis alpestris</i> Foissner, 1978
	<i>Euplotes affinis</i> (Dujardin, 1841) Kahl, 1932
	<i>Euplotes muscicola</i> Kahl, 1932
**	<i>Frontonia angusta angusta</i> Kahl, 1931
	<i>Furgasonia rubens</i> (Perty, 1852) Jankowski, 1964
**	<i>Furgasonia theresae</i> (Fabre-Domergue, 1889) Foissner, Agatha & Berger, 2002
	<i>Fuscheria nodosa salisburgensis</i> Gabilondo & Foissner, 2009
	<i>Gastrostyla mystacea mystacea</i> (Stein, 1859) Sterki, 1878
	<i>Grossglockneria acuta</i> Foissner, 1980
	<i>Grossglockneria hyalina</i> Foissner, 1985
***	<i>Halteria grandinella</i> (Mueller, 1773) Dujardin, 1841
	<i>Holophrya teres</i> (Ehrenberg, 1833) Foissner, Berger & Kohmann, 1994
	<i>Holosticha stueberi</i> Foissner, 1987
	<i>Homalogastra setosa</i> Kahl, 1926
	<i>Kahliella simplex</i> (Horváth, 1934) Corliss, 1960
	<i>Kahlilembus attenuatus</i> (Smith, 1897) Foissner, Berger & Kohmann, 1994
	<i>Leptopharynx costatus</i> Mermod, 1914
	<i>Maryna lichenicola</i> (Gelei, 1950) Foissner, 1993
***	<i>Maryna ovata</i> (Gelei, 1950) Foissner, 1993
*	<i>Maryna umbrellata</i> (Gelei, 1950) Foissner, 1993
	<i>Meseres corlissi</i> Petz & Foissner, 1992
	<i>Metopus hasei</i> Sondheim, 1929
	<i>Metopus inversus</i> (Jankowski, 1964) Foissner & Agatha, 1999
	<i>Metopus minor</i> Kahl, 1927
	<i>Metopus palaeformis</i> Kahl, 1927
	<i>Monodinium balbiani</i> Fabre-Domergue, 1888
	<i>Monomacrocaryon terrenum</i> (Foissner, 1981) Vďačný <i>et al.</i> , 2012
	<i>Mykophagophrys terricola</i> (Foissner, 1985) Foissner, 1995
	<i>Odontochlamys alpestris</i> Foissner, 1981
	<i>Opisthonecta minima</i> Foissner, 1975
	<i>Opisthonecta bivacuolata</i> Foissner, 1978
	<i>Oxytricha opisthomuscorum</i> Foissner <i>et al.</i> , 1991
	<i>Papillorhabdos multinucleatus</i> Foissner, 1984
	<i>Paracolpoda steinii</i> (Maupas, 1883) Lynn, 1978

Table 1. Cont.

	Taxa
	<i>Paramecium aurelia</i> -complex
	<i>Paramecium caudatum</i> Ehrenberg, 1833
	<i>Paramphisiella caudata</i> (Hemberger, 1985) Foissner, 1988
*	<i>Perispira ovum</i> Stein, 1859
	<i>Plagiocampa difficilis</i> Foissner, 1981
	<i>Platyophrya vorax</i> Kahl, 1926
	<i>Podophrya fixa</i> (Mueller, 1786) Ehrenberg, 1833
	<i>Protocyclidium terrenum</i> Alekperov, 1993
**	<i>Protospathidium serpens</i> (Kahl, 1930) Foissner, 1981
**	<i>Pseudochilodonopsis algivora</i> (Kahl, 1931) Foissner, 1979
	<i>Pseudochilodonopsis piscatoris</i> (Blochmann, 1895) Foissner, 1979
	<i>Pseudomicrothorax dubius</i> (Maupas, 1883) Penard, 1922
	<i>Pseudoplatyophrya nana</i> (Kahl, 1926) Foissner, 1980
	<i>Pseudouroleptus procerus</i> Berger & Foissner, 1987
	<i>Pseudourostyla franzi</i> Foissner, 1987
***	<i>Psilotricha succisa</i> (Mueller, 1786) Foissner, 1983
	<i>Rostrophryides africana</i> Foissner, 1987
	<i>Sagittaria hyalina</i> Foissner, Czapik & Wiakowski, 1981
	<i>Sathrophilus muscorum</i> (Kahl, 1931) Corliss, 1960
	<i>Semispathidium pulchrum</i> Foissner, Hess & Al-Rasheid, 2010
	<i>Spathidium claviforme</i> Kahl, 1930
	<i>Spathidium procerum</i> Kahl, 1930
	<i>Spathidium puteolagri</i> Baumeister in Kahl (1930)
	<i>Spathidium rusticanum</i> Foissner, 1981
	<i>Spathidium spathula</i> (Mueller, 1773) Moody, 1912
	<i>Steinia platystoma</i> (Ehrenberg, 1831) Diesing, 1866
	<i>Steinia sphagnicola</i> Foissner, 1989
	<i>Sterkiella histriomuscorum</i> (Foissner, Blatterer, Berger & Kohmann, 1991) Foissner, Blatterer, Berger & Kohmann, 1991
	<i>Strobilidium caudatum</i> (Fromental, 1876) Foissner, 1987
	<i>Stylonychia mytilus</i> -complex
	<i>Stylonychia notophora</i> Stokes, 1885
	<i>Tetrahymena pyriformis</i> -complex
	<i>Tetrahymena rostrata</i> (Kahl, 1926) Corliss, 1952
*	<i>Tillina magna</i> Gruber, 1880
	<i>Tokophrya infusionum</i> (Stein, 1859) Bütschli, 1889
	<i>Uroleptus gallina</i> (Mueller, 1786) Foissner, Blatterer, Berger & Kohmann, 1991
	<i>Urosoma acuminata</i> (Stokes, 1887) Kahl, 1932
	<i>Urosoma emarginata</i> (Stokes, 1885) Berger, 1999
	<i>Urostyla grandis</i> Ehrenberg, 1830
	<i>Urotricha farcta</i> Claparède & Lachmann, 1859
	<i>Urotricha globosa</i> Schewiakoff, 1892
	<i>Vorticella convallaria</i> -complex
	<i>Vorticella (Echinovorticella) echini</i> (King, 1931) Foissner, Agatha & Berger, 2002

Table 1. Cont.

Taxa
<i>Vorticella infusionum</i> -complex
<i>Vasicola ciliata</i> Tatem, 1869
<i>Woodruffides metabolicus</i> (Johnson & Larson, 1938) Foissner, 1987
<i>Epistylis</i> sp. n.?
<i>Notohymena</i> sp. n.?
<i>Odontochlamys</i> n. sp.?
<i>Spathidium</i> -like gen. n., sp. n. (description in preparation)
<i>Spathidium</i> spp.
<i>Urotricha</i> sp. n.?
<i>Epispathidium</i> cf. <i>amphoriforme</i>
<i>Nassula</i> spp.

Bold = new species, * species neotypified in this paper, ** species neotypified previously, *** redescribed species. References to the authors and combining authors of the species are provided in the literature section but only when it is a new, a neotypified, or a redescribed species. For most others, this information can be found in the reviews of Foissner [41] and Foissner *et al.* [27,46,47].

3.3. Distribution

Four of the ten new ciliate taxa from the Krauthügel pond have also been recorded from other biogeographic regions: *Actinorhabdos trichocystiferus* was re-discovered in a soil sample on the outskirts of Rio de Janeiro (Foissner, unpubl.); *Bilamellophrya hawaiiensis* has been discovered in arable soil from Hawaii [27]; *Coriplites grandis* was discovered in soil south of the town of Cairns, Australia [48]; and *Meseres corlissi* has been recorded from China, Australia, Africa, and South America [11].

Figure 4. Officially protected area. Green outline: buffer zone. Blue outline: Krauthügel pond. The arrow denotes the house (frequently wrongly called “house of the hangman”) where the watchman of the Krauthügel lived.



3.4. Official Recognition

After two positive reviews, our application was accepted by the mayor of the town of Salzburg. On 19.01.2012 and 15.02.2012, he declared the “Ephemeral pond on the Krauthügel” as a “Natural Monument for Single-celled Organisms”, protecting the pond and a buffer zone (Figure 4), altogether an area of 5,290 m² on plot 2324 KG [49].

4. Discussion

4.1. Number of Ciliate Species in Other Well-Investigated Ephemeral Waters

Studies of the kind presented are very rare because ephemeral waters are not very attractive for limnologists and well-trained ciliate taxonomists were always an exception. Further, the data are hardly comparable because of great differences in the number of waters investigated, the number of samples taken, the time range samples were studied, and the methods used for ciliate identification.

Nonetheless, a considerable amount of data has been accumulated (for a review, see [50]), and thus we put together the most similar investigations in Table 2. This shows that the total number of species in the Krauthügel pond is in the upper range, possible because of the high age of the pond and the detailed

Table 2. Number of ciliate species in well-investigated ephemeral waters.

	Brief site description	Number of species	% new species	Authors
1.	Many ephemeral waters sampled across Central Europe ^a	69	10	[51]
2.	Five ephemeral, eutrophic pasture ponds, each investigated several times during two years, on a mountain meadow in Hungary	75	45	[52–54]
3.	785 ephemeral waters, mainly road and meadow puddles in Bavaria, Germany	132	12	[55]
4.	An arctic tundra pond sampled several times ^b	~30	0?	[56]
5.	23 alpine, eutrophic pasture ponds in Austria, most investigated 1 to 2 times (but see next entry)	107	33	[57]
6.	Many samples from pasture pond 1 in the series mentioned above	54	17	[57]
7.	Four soil samples from a seasonally inundated grassland in New Zealand	57	0?	[58]
8.	Many samples from an experimental ricefield in Italy studied for four years	60	0?	[59]
9.	One sample from a few road puddles within the Bambatsi guest house area (Namibia, Southwest Africa)	130	14	[27]
10.	Two meltwater ponds in southern Ontario (Canada) investigated weekly for 98 and 34 days, respectively	145	0?	[60]
11.	Nine Lajas (granitic lithotelmes in Venezuela), each investigated one time (Foissner, unpubl.)	103	9	
12.	Present study	~121	8	

^a Including seven new species discovered by Bresslau [61] in lawn infusions and mentioned by Spandl [51] in the addendum to the ciliate list; ^b Four species added for four “ssp.”

investigations, while the percentage of new species is in the middle range, possibly because Bresslau [61], Gelei [33,52], Gruber [45], Dingfelder [55], and Foissner [57] have already described many species characteristic for ephemeral waters in central and eastern Europe. Our unpublished data from ephemeral waters of Venezuela and Australia indicate that many species wait to be discovered [30,62].

4.2. Conservation Arguments

4.2.1. Endemism

Usually, conservation appeals are based on the narrow distribution of one or several species and their habitat, or of species and habitats endangered by human activities [63]. In the Krauthügel case, five of the 10 new species have not been reported from any other locality. Although we cannot exclude future discoveries in other biogeographic regions, presently they have to be considered as endemics. Accordingly, the likely endemism of some of these new species recorded only in the Krauthügel habitat was accepted by the reviewers of our application.

4.2.2. Type Locality and Landscape Diversity

The epistemological credentials of the Type Locality [64] underpins the second main argument in our application to conserve the Krauthügel. Small, ephemeral waterlands are especially prone to being “flattened” by anthropogenic agencies, because they occupy landforms that decrease the economic value of farmland. In case of the Krauthügel, this would mean the loss of the type localities of eight new species and 10 neotype localities.

The type locality is the site where a previously unknown organism was first discovered and redescribed for the first time [64]; it is critical to taxonomy as the geographical place location of capture or collection of the name-bearing type of a nominal species or subspecies. For protists and several small pluricellular eukaryotes, the type locality is much more important than in larger organisms because their presence and distribution cannot be seen with the naked eye but needs complex instruments and methods. Unfortunately, habitat destruction, which is responsible for one third of biodiversity loss globally [63], has erased many protist type localities. In ciliates, the biggest loss occurred by draining of the wetland on the outskirts of the town of Hamburg (Germany), where Alfred Kahl discovered more than hundred new species [65]. In their epistemological impacts on scientific knowledge, loss of type localities and their populations characterize immediate depredations of the Second Alexandrian Tragedy—the extirpation of biological knowledge [66,67]. Anthropogenic threats to biotic habitats that qualify as type localities constitute poorly appreciated conservation targets. Obviously, the future integrity of the natural science collections will vouch for the biotic uniqueness of the Krauthügel.

When the type specimen has been lost or not deposited, as for most protists, the identity of the species may be threatened forever. Therefore, a neotype should be established [68]. The site of the neotype becomes the new type locality [64]. Thus, the Krauthügel pond qualifies as the type locality for eight new species and for 10 neotypified species. This criteria and value of the type locality extends to future research, when we acknowledge that phylotyping [69] of these known ciliates will provide an essential benchmark for future explorations of cryptic and ‘unculturable’ biodiversity in the Krauthügel.

We note this landform occupies a very restricted natural setting, but argue that the merits of preserving it outweigh impacts of human interference on the natural environment. The position and context of the Krauthügel does raise questions as to what extent humans should interfere technologically, or otherwise, with natural environmental processes to ensure persistence of a natural ecosystem that otherwise would likely be altered or obliterated by not only anthropogenic but equally natural processes. Here, a buffer zone has been established, largely to prevent incursions by human activity, but it has nonetheless altered natural geomorphological processes that can be expected to have altered the site. There was a heavy loss of small permanent and ephemeral waters during the past fifty years when agriculture became industrialized. This had many negative consequences, of which the loss of local biodiversity and monotonization of landscapes were the most serious ones [58]. Without the pond, the Krauthügel, which is heavily used by walkers, would be exposed to both of these undesirable impacts.

Conservation is frequently associated with limited agriculture and thus financial loss for the owner. In the Krauthügel case, this played no role because the protected area and the buffer zone should be farmed as usual. Accordingly, the problems discussed by Hansen and De Fries [70] do not apply.

4.3. A Benchmark for Future Biodiversity Characterization

Legislated preservation of the Krauthügel prioritizes the landform's microbiota as a keystone target for metagenomic surveys [71,72]. Phylotyping [69] will characterize the known species of protozoa in the context of the sympatric cryptic and 'unculturable' biodiversity. Such an exercise will constitute an inaugural landmark in a research strategy that integrates new discoveries of microbial diversity with published knowledge. We argue for an expansion of this exercise beyond its inaugural application to the Krauthügel.

Focal metagenomic characterizations of the type localities of protists (Table 2) across landscapes will open the way for a scientifically robust strategy to quantify the known *versus* unknown microbial diversity. This approach can profitably integrate rarefaction methods [73] to quantify new discoveries of cryptic taxa. Metagenomic characterization of formally described microbial taxa (pertinently ciliates) will provide the benchmark to evaluate regional, continental and global patterns of microbial biodiversity. Moreover, repeated metagenomic surveys of these focal and associated habitats will provide the foundation of data detailing the intricacies of ecosystem dynamics, which are barely explored, let alone, understood. Such baseline knowledge of biodiversity is the prerequisite for future monitoring, to detect and evaluate impacts of natural and anthropogenic disturbances within and across the landscape mosaics in which keystone habitats are embedded. As argued by Cotterill *et al.* [4], the importance of this strategy is underscored by the key roles of ciliates (and protists) in nearly all ecosystems, notably as predators in food webs, and especially in agroecolandscapes and hydrological systems.

4.4. Taxonomy Informs Conservation

There is an increasing number of studies that argue for preservation of microbial diversity [4,74,75]. Further, there are many studies that find a "high molecular diversity" in a variety of habitats. Unfortunately, most of these studies did not include morphological investigations leaving the species unnamed. However, in practice, conservation decisions need named species because these reflect refined

knowledge of biodiversity (indeed, the species list was important for the Krauthügel project, and its knowledge continues to improve). Thus, we fully support the appeal of Mace [76] that solving conservation issues need a more intimate collaboration among conservation biologists, taxonomists and legislators, but, above all, increased investment in taxonomists with relevant and high-quality skills. High quality scientific taxonomy should inform conservation decisions and not the other way around [77].

5. Conclusions

We have invoked evolutionary novelty (local endemism), preservation of taxonomic knowledge (type locality), and landscape maintenance as arguments for the conservation of a small, ephemeral pond. Although its spatial extent is relatively miniscule when considered against established protected area networks, this does not undermine its relevance to ecosystem management and biodiversity research. Considerable ciliate endemism and a focal type locality are the criteria of biotic and epistemic uniqueness, respectively, which underpins the conservation arguments. Legislation that conserves the Krauthügel serves to maintain the ecological integrity of a benchmark habitat. The case of the Krauthügel exemplifies a previously unappreciated role of such benchmark landforms as repositories of focal data. They hold a key role in integrated research to advance the frontiers of knowledge of microbial biodiversity. Arguments for monitoring programmes that seek to quantify ecosystem integrity further underscore the values of maintaining high fidelity knowledge of microbial habitats.

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