Chapter 2

Sampling, collection of material for preparations, identification, description, species concept, types[1, 2]

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2.1 Sampling and sample processing

The material collected usually includes mineral topsoil (0–5 cm, rarely up to 10 cm depth) with fine plant roots, the humic layer, and the deciduous and/or grass litter from the soil surface. In soil with few organic materials and very sandy habitats, litter was sieved off the sand with an ordinary kitchen sieve (1 mm mesh-size), so that the final sample consisted of about 80% litter and 20% sand and gravel. Usually, 10 small subsamples were collected with a small shovel from an area of about 100 m^2 and mixed to a composite sample. Bark samples were usually taken from one to three trees. The bark was collected with a knife, selecting for regions grown with mosses or lichens and/or containing some soil.

Generally, a "good" sample consists of 50% litter, humus and roots and 50% mineral soil. The litter and humus are very important because they release many nutrients when the sample is rewetted, stimulating growth of bacteria, fungi, flagellates, and amoeba, that is, the main food of ciliates. The nutrient increase obviously decouples microbiostasis, as explained in Foissner (1987).

All samples were air-dried for at least one month and then sealed in plastic bags. Such samples can be stored for years without significant loss of species, provided they are from arid or temperate environments (Foissner 1997). This is emphasized by the Australian investigations: there is no correlation between storage time and species number; indeed, the richest samples are those stored for over four years (see also Foissner et al. 2002).

All collections were analyzed with the "non-flooded Petri dish method", as described by Foissner (1987, 1992). The technique is not perfect but likely the best available for biodiversity assessment of soil ciliates at large. The protocol is simple (see Fig. 262 in Foissner et al. 2002):

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[1] Note by H. Berger: This chapter is basically from Foissner et al. (2002) with several modifications. Wilhelm Foissner made some corrections which I included. However, the manuscript was not finished, especially as concerns hints to specific samples from Australia. I had to delete these hints because I could not reconstruct what he exactly meant. I made the reference section for this chapter.

[2] This chapter should be referenced as follows: Foissner W. (2021): Sampling, collection of material for preparations, identification, description, species concept, types. – In: Foissner W. & Berger H. (Eds): Terrestrial ciliates (Protista, Ciliophora) from Australia and some other parts of the world. — Series Monographiae Ciliophorae, Number 5: 9–20.

W. Foissner & H. Berger (Eds), *Terrestrial ciliates (Protista, Ciliophora) from Australia and some other parts of the world*, Series Monographiae Ciliophorae, Number 5

1 Put the material in a Petri dish and spread it over the bottom of the dish in at least a 1 cm, better 2–3 cm thick layer. As concerns the Australian samples, sufficient material was available to fill a 2 cm high Petri dish 13 cm across or, rarely, a 3 cm high dish 18 cm in diameter. Basically, a large Petri dish (18 cm) is preferable because it provides more material for preparations.

2 Slightly over-saturate but do **not** flood the sample with distilled water. Water should be added to the sample until 5–20 ml will drain off when the Petri dish is tilted (45°) and the soil gently pressed with a finger. Complete saturation takes up to 12 h, so check cultures after this time. Never flood the sample, that is, do not make an Aufguss ("infusion") because then only a few common species will develop. Further, the material should have been dry for at least one month.

3 Cover Petri dish and pinch a clip between bottom and lid to enable gas exchange. Generally, care must be taken that the samples do not putrefy. This happens rather easily with saline material, soil containing animal excrements or, in "ordinary" samples, if the litter is very easily decomposable. In this case, change the water in the sample and do not cover it for some days so that plenty of air is available; further, slightly under-saturate sample with water. Heavily saline soil (≥20‰) should be "washed", if no ciliates develop. Saturate the sample with water, as described above. After 2–3 d, remove the percolate and saturate again with water. Repeat two to four times, until ciliates begin to develop.

4 A distinct succession occurs in the rewetted samples. Thus, they must be inspected on days 2, 6/7, 13/14, 21/22, and 30. Later inspections usually add only few species, likely because microbiostasis (ciliatostasis; see Foissner 1987) increases and metazoan (rotifers, nematodes) and protozoan (mainly heliozoans!) predators often became abundant. For inspection, the Petri dish is tilted some seconds and a rather large drop (~0.3 ml) of the drained water ("soil percolate") taken with a Pasteur pipette and inspected for species; several such drops must be investigated from different sites of the Petri dish, until the last drop adds but few species.

5 Rainforest samples: When rainforest litter and soil samples are air-dried for the non-flooded Petri dish method, then one must consider that the resting cysts of rainforest protists are much weaker than those from moderate or hot and dry regions. There is a rapid loss of species in samples older than nine month (Foissner 1997, 2011), and new data from Venezuela (Foissner 2016) and Australia show great loss within a month.

In Mérida (Venezuela) and Borneo (Foissner 2011, 2016), I had the opportunity to look at fresh samples. They were full of ciliates, similar as in a mesotrophic river! The negative influence of prolonged drying becomes obvious also in the samples from the Australian and Tasmanian rainforests. Those which were investigated within a year provided much more species than those investigated 4–6 years after collection.

These observations provide two recommendations: soil and litter ciliates from everwet rainforests should be investigated on site without any culture method or within about two months after air drying when the non-flooded Petri dish method is used. Up to one year drying is possible for rainforests whose litter and upper soil layer become dry during the dry season.

6 Other culture methods: The non-flooded Petri dish cultures, as described above, provided about 90% of the material contained in the monograph. The rest is from a variety of ordinary "limnetic" cultures. First, clone cultures were made in the usual way by transferring individual specimens into various media, preferable Eau de Volvic (French table water), either pure or mixed with soil extract in a ratio of 10:1 and enriched with a crushed and two uncrushed wheat grains to stimulate growth of indigenous food organisms, viz., bacteria and small flagellates; occasionally, selected food items were added, for instance, filamentous cyanobacteria for several nassulids. Second, 2 ml of the percolate (together with all organisms) were mixed with 8 ml Eau de Volvic and enriched with wheat grains, as described above. Of course, such cultures contain a variety of

ciliates, and sometimes interesting species develop for a while. Third, a Petri dish was filled with 10–20 ml culture medium plus some wheat grains. Then some grams of soil were added as an inoculate to a small site of the Petri dish, taking care not to distribute it throughout the medium. Such cultures were sometimes helpful for strongly saline material (>20‰), which is set up with artificial sea water. Fourth, if the sample is very saline (>20‰), it may occur that no ciliates develop. Such samples can be "washed" every third day with fresh table water, which decreases the salt concentration. Frequently, ciliates appear after the third or fourth wash! See also item 3 above.

7 Problems: Data of the kind presented are highly dependent on the (non-flooded Petri dish) method used to reactivate ciliates from the air-dried samples, that is, to stimulate them to leave the resting cysts and to reproduce to detectable numbers. I highlight this problem, although it was discussed in detail by Foissner (1987, 1997, 1999), because it heavily influences data analysis and interpretation. Specifically, it causes undersampling of species which are rare and/or have special demands (Foissner 1997), and explains the phenomenon that 30–40% undescribed species are found in large sample collections, such as the Australian and Namibian material while the individual samples contain only one or two new species (Foissner 1999).

Usually, soil ciliates are not active but encysted, producing a "hidden biodiversity". The resting cysts can survive for years (Foissner 1987), provided they are from specimens living in dry or temperate climates, where soils desiccate from time to time; in humid environments, such as rain forests, the resting cysts are not adapted to survive longer dryness, and thus the non-flooded Petri dish method does not work (Foissner 1997). But even with "optimal" samples, the non-flooded Petri dish method is selective, that is, cannot provide an environment suitable for all kind of ciliates. This becomes evident when the same site is investigated several times over a year (Foissner 1999), or a single sample is manipulated to encourage encysted and more rare forms of ciliates to emerge (Esteban et al. 2000). Figure 1 shows that a single sample from a certain site collects only about one

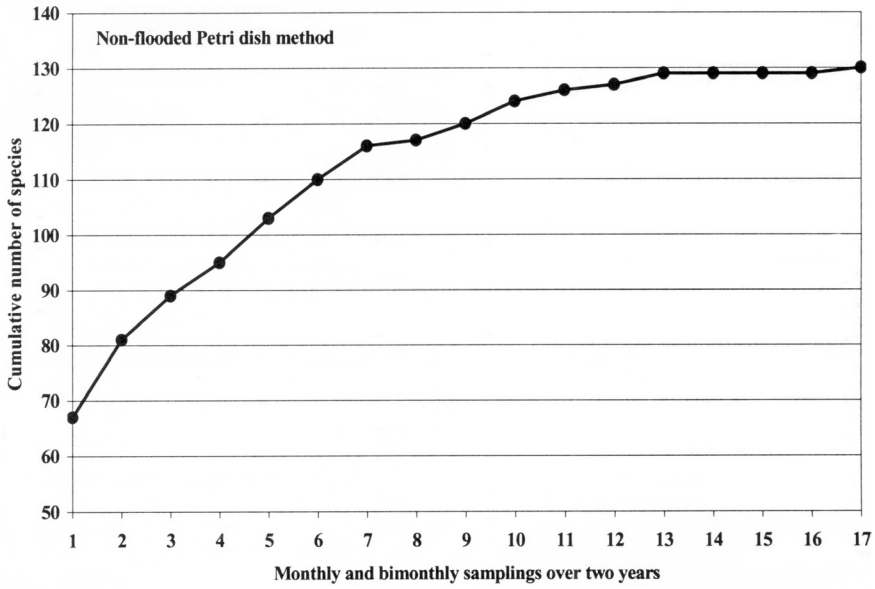

Fig. 1. Cumulative number of species obtained with the non-flooded Petri dish method (Foissner 1987) in 17 monthly and bimonthly samplings from a 100 m^2 area of beech forest soil in Austria (from Foissner et al. 2002). The curve flattens distinctly at sample number 13, indicating that further effort hardly will increase species number significantly. However, direct investigation of fresh samples after rainfalls provided 30 further species. Accordingly, the total number approaches 160 species, which is far from the total number (about 1500) of soil ciliates known. Thus, I do not agree with the hypothesis of Finlay & Esteban (1998) that "all species of freshwater protozoa could eventually be discovered in one small pond".

third of the species present, that is, the number found in 17 replicates distributed over two years (further examples, see Foissner 1987). Likely, this applies also to the Australian samples.

Fortunately, there is evidence that the situation changes if many samples are analyzed from a not too large area, that is, if the Australian samples are considered as some sort of replication.

Even if a certain ciliate excysts, we can hardly recognize it among the mass of soil particles. To be seen, it must reproduce to a detectable number. And even if this occurs, there remains the problem of recognizing it as a distinct taxon among hundreds of individuals from other species, many of which look alike. Only when experience, live observation, and silver impregnation are combined, reliable species lists will emerge.

In sum, there is convincing evidence that the non-flooded Petri dish method is selective, that is, reactivates only a rather small, undefined fraction of the resting cysts present in a sample, and undescribed species or species with specialized demands are undersampled. Thus, the real number of species, described and undescribed, is considerably higher in the samples investigated. Unfortunately, a better method for broad analysis of soil ciliates is not known. On the other hand, about 800 new ciliate species were discovered by Foissner (1998, 2016) and colleagues with this simple method, suggesting, inter alia, that a considerable amount of the hidden biodiversity can be revealed by investigating large numbers of samples.

2.2 Collection of material for preparations

If a "difficult" species is noted, which happens in more than 70% of the samples, material for preparations must be collected. To obtain many specimens, the Petri dish is tilted (45°) several times for a minute or so and the percolating soil water collected with a Pasteur pipette from several sites of the dish. If only little water (<10 ml) drains from the sample and/or the species of interest is very rare, it should be sprinkled with 10–15 ml distilled water. This will cause an osmotic shock, detaching or rinsing many specimens from the soil particles and capillaries within about 10 min. Then, the procedure described above is repeated, that is, the Petri dish is tilted several times and the percolating soil water added to the first collection. Finally, the soil sample is again saturated with clean table water (e.g. Eau de Volvic) and stored for the next investigation. Certainly, these procedures strongly change the milieu, and thus a rather different ciliate community may develop, possibly containing further "difficult" species. If so, the whole procedure is repeated, and so on.

Much care must be taken to keep the percolate clean of large (>2 µm) soil particles, which would disturb the investigation of the preparation, while particles smaller than 2 µm hardly disturb, if not too numerous. To achieve clean material, note the following advices:

1 Usually, the percolating soil water which contains the organisms will be clean because the soil particles soon become stabilized by microbial activities, mainly by fungal hyphae and bacterial mucilage. Thus, extreme care must be taken not to destroy the soil structure developed in the non-flooded Petri dish culture. Accordingly, the Petri dish must be handled gently and, if necessary, distilled water sprinkled softly on the surface. To increase percolation, mild finger pressure on the soil may be applied. Depending on the material sampled, the percolate has a light brown to orange colour (from lignins, humus colloids, etc.), which does not disturb the preparations (but see below).
2 The percolate is now gently shaken and large soil particles allowed to settle for about one minute. Then, the supernatant, which is now ready for preparations, is collected with a Pasteur pipette. Be careful not to lose bottom-dwellers. Occasionally, it may be helpful to sieve the percolate through a plankton net with 50–100 µm mesh-size or to concentrate it by mild centrifugation (max. 2000 min^{-1} for a few seconds), especially for preparations with expensive chemicals (osmium tetroxide in Chatton-Lwoff silver nitrate impregnation).

2.3 Identification of species

Provided sufficient experience, many ciliate species can be identified from life at low magnification (×100–200). Of course, details must be checked at high magnification (×1000, oil immersion), preferably with interference contrast optics. All "difficult", new, or supposedly new species must be treated with the silver impregnation techniques described in Chapter 3. Accordingly, my species lists are a mixture of taxa identified in vivo and/or silver preparations. However, I emphasize that all species were seen in silver slides, at least from one site. Thus, seen on a whole, all species were observed in vivo and in silver preparations. Identification literature is highly scattered and cited in the reference section (see Chapter 4) and Foissner (1998). Still indispensable are Kahl's monographs (Kahl 1930, 1931, 1932, 1935) and, for colpodids and hypotrichs, the recent reviews by Foissner (1993) and Berger (1999, 2006, 2008, 2011); for euplotids, peritrichs and suctorians, the reviews by Curds (1986) and Warren (1986) are very useful. Keys for limnetic (Berger & Foissner 2003) or marine (Carey 1992) ciliates are almost useless because only about 25% of the species occur both in terrestrial, limnetic, or marine habitats.

2.4 Description of species, morphometry, and illustrations

The monograph contains mainly species observed both in vivo and in silver preparations, as minimal requirements for a solid description, morphometry, and illustrations; light and scanning electron micrographs were prepared whenever possible.

Species descriptions were performed in telegramese style, as is good practice among experienced taxonomists, a fact often overlooked by protozoologists, who prefer prose style, which makes the description unnecessarily long and circumstantial. Furthermore, each of the new or improved taxa is headed by a brief "diagnosis", containing only those features which, in my opinion, separate the species from its nearest relatives, as already emphasized by Linne. A "diagnosis" is not an abbreviated description, as is often assumed, and is thus usually very short.

Usually, my descriptions have a certain order, namely that used in identification: body size and shape; nuclear apparatus; contractile vacuole; cortex and extrusomes; cytoplasm and food; movement; somatic and oral ciliary pattern; occurrence and ecology; comparison with related species. Within the individual items, location of the structure comes first, followed by its shape and size.

Morphometry is indispensable for a good description of a ciliate and was performed on 10–20 randomly selected, well-impregnated specimens. The data is tabulated and thus repeated in the descriptions only if needed for clarity. Most observations are from material as obtained with the non-flooded Petri dish method, that is, not from clone cultures. Thus, I cannot exclude that similar but different species were sometimes confused, although this is unlikely because I excluded specimens which deviated in at least one prominent feature. Certainly, this can generate some bias in the data if used too uncritically. However, I usually excluded only such specimens which had, e.g., a different nuclear structure (likely often postconjugates), a distinctly deviating ciliary pattern (likely often injured, regenerating or malformed specimens), an unusually small size (likely often degenerating, just excysted or divided specimens), or a combination of deviating features. The inclusion of such individuals, which might sometimes belong to another species, would have artificially increased variability.

Illustrations of live specimens were based on free-hand sketches and/or micrographs and video prints. Generally, my main in vivo illustration of a certain species represents a summary of the observations, that is, shows a "representative" specimen composed on observations of live and prepared cells. Illustrations of prepared cells were made with a drawing device and show the

specimens as they are, smoothed only by removing obvious artifacts. Great care was taken to make these illustrations accurate and beautiful; usually, a "typical" specimen is shown with values near the arithmetic means.

Micrographs are an important supplement to any description, but often difficult to obtain because the cells are moving and/or out of the focal plane. Thus, much patience and skill is needed to produce meaningful micrographs. Even more helpful is scanning electron microscopy because it provides a three-dimensional view of the organism. Unfortunately, good scanning electron micrographs are difficult to obtain from soil ciliates for the reasons explained above. All these problems made it impossible to provide micrographs of all species described. If not stated otherwise, all figures are orientated with the anterior end of the organism directed to the top of the page.

2.5 Estimation of in vivo size

In vivo measurements of body size were performed at a magnification of ×100–250, while details such as extrusomes and food vacuoles were measured at ×1000, where a measuring unit of the ocular micrometer is 1 μm. Likewise, all measurements of prepared cells were made at a magnification of ×1000. Measuring body size in vivo provided only rough values because the cells were moving. I used these data mainly as a kind of control for shrinkage due to the preparation procedures.

Usually, I measure body length and width of a few contrasting specimens in vivo and without using a coverslip. As the organisms are moving, this provides only approximate values which are improved by the morphometric analysis of the preparations. The following percentages compensate preparation shrinkage; they were obtained from comparative analyses, such as shown in *Maryna* spp. (Foissner 2016): 15% (10–20%) for Foissner's protargol method, 20–30% when pure ethanol was used as a fixative; 5% for Chatton-Lwoff` silver nitrate preparations; and 0% for wet preparations fixed with osmium vapours or a minute drop of osmium acid (2%). Shrinkage is highly variable in SEM preparations, ranging from near zero to 100%[1] in very fragile structures, e.g., the lepidosomes of trachelophyllids (Foissner 2016). The same applies for protargol methods and silver carbonate preparations that do not fix specimens on a slide before bleaching and impregnation.

2.6 Species/subspecies concept

The species concept, of course, influences the number of species found and/or recognized as undescribed (Luckow 1995, McDade 1995, Turner 1999). I usually apply the phylogenetic species concept as defined by Nixon & Wheeler (1990): "A species is the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals (semaphoronts)". Basically, this is a morphospecies concept which is, according to Ehrendorfer (1984) and Finlay et al. (1996), as valid as any, and probably more pragmatic than any other; see Ehrendorfer (1984), Luckow (1995), McDade (1995), and Turner (1999) for detailed discussion.

I do not consider myself as splitter, that is, I classify species as undescribed (new) only if populations can be separated from their nearest relatives by at least one distinct (nonmorphometric) morphological feature, such as presence/absence of caudal cirri or rod-shaped vs. fusiform extrusomes, or if quantitative differences, such as body size and/or number of ciliary rows, are really conspicuous (>100%). Furthermore, I must have seen at least 10 individuals and studied the species in vivo and silver preparations, to provide reliable morphometrics, illustrations, and types. Certainly, the present monograph contains some exceptions because it is unlikely that the Australian soil ciliate fauna will be described or reinvestigated in the coming 30 years. However, it is my belief that careful

[1] Note by H. Berger: I suppose Willi meant 50%. A shrinkage of 100% would mean that a structure disappears.

live observation is usually indispensable for a good description. Thus, I rarely describe species seen only in silver slides.

Mayr (1963) defines a subspecies as "an aggregate of local populations of a species inhabiting a geographic subdivision of the range of the species, and differing taxonomically from other populations of the species". This concept, especially geographic isolation has been widely adopted, although there is still a lot of discussion (Böhme 1978, O'Neill 1982, Rolán-Alvarez & Rolán 1995). Unfortunately, biogeography of protozoa is still in its infancy, and thus Mayr's concept hardly can be applied. Nonetheless, subspecies are useful also in protists, when used restrictively and as a simple taxonomic tool.

In our monographs, we distinguish subspecies according to distinct morphometrical differences in important features (e.g., number of ciliary rows) and/or qualitative (morphological) characters whose taxonomic value is still doubtful or not known. It is the last mentioned feature which makes the subspecies concept so useful: the name can be easily withdrawn if later research proves the features used to be unreliable, and the discoverer does not lose priority to "armchair" taxonomists if the subspecies later gets species rank (ICZN 1999). Furthermore, subspecies "collect" the infraspecific variation, that is, data which tend to be lost (Zusi 1982), and enhance identification of species because of the broader concept; thus subspecies are especially useful for people and disciplines not specifically trained in taxonomy. In spite of the obvious advantages, protozoologists rarely used the subspecies/subgenus concept, although Kahl (1932) established some subgenera and varieties in ciliates and one third of the testate amoebae taxa are "variations" or "forms", most of which must be considered as subspecies according to the ICZN (Foissner & Korganova 2000). Further, subspecies are common in extant and fossil foraminifera (Boltovskoy 1954) and fossil tintinnids (Belokrys 1997). There is now a tendency to use them also in extant ciliates (Song & Wei 1998).

A further main factor influencing the number of species recognized as undescribed is the treatment of literature data. Many of the old protozoan species descriptions lack type material and are poor compared with the present standard because the pioneers did not have the advantages of modern methods. Clearly, there is a tendency to disrespect the efforts of our predecessors and to establish new taxa with new methods. My approach is to respect and reinterpret previous work and to neotypify species, provided that at least one main feature matches (see also chapter on neotypification). Representative examples for our way of making honest alpha-taxonomy can be found in the section on nassulids in the monograph on Namibian soil ciliates (Foissner et al. 2002).

2.7 Type material and neotypification

2.7.1 Type material

Most descriptions in this monograph are based on live observation and silver impregnation, which usually yield permanent slides. For the new and the neotypified species, at least one holotype (neotype) and one paratype slide have been selected. One or more slides have been selected for the species redescribed. All slides have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria. The slides usually contain several specimens, with about 10 relevant cells marked by a black ink circle on the coverslip.[1]

If appropriate, the type slides are accompanied by an equally-sized sheet of paper, which states the species and the kind of types contained (H – holotype specimen, N – neo(holo)type specimen,

[1] Note by H. Berger: Foissner planned a table showing the accession numbers of the type and voucher slides, like Table 1 in Foissner et al. (2002). This table was not prepared by W. Foissner. For details on type material and voucher slides, see paragraphs "Type material" or "Material" at individual descriptions in Chapter 4. For further details on slides of taxa described in Chapter 4, for example, accession numbers, see Chapter 5.

P – paratype specimen, V – voucher). The specimens which served as a basis for the illustrations are marked by the letter "D", for instance, PD = paratype used for illustration. The holotype specimen (H), of course, has been drawn. Note that some slides contain types of several species, which are distinguished by different colours or letters. Furthermore, several species occurring in Australia and described in this monograph have been found and studied previously in soil samples from other regions of the world. Thus, the type locality of some of the new taxa is not in Australia, that is, not contained in the site descriptions given above.[1] Brief site descriptions for these species and populations are provided in the respective occurrence and ecology sections. The samples were processed like those from Australia.

2.7.2 Neotypification

I broadly apply neotypification in this monograph.[2] As this poses a major problem (see below), I shall discuss it in detail. Furthermore, I published a paper in the Bulletin of Zoological Nomenclature discussing neotypification in protists (Foissner 2002).

Methods for preparing soft-bodied ciliates with a quality that provides meaningful types to be deposited have only been available for the past five decades. At present, most "modern" ciliate types are deposited in two centres: the Smithsonian Institution in the USA (Corliss 1972, Cole 1994) and the Museum of Natural History of Upper Austria in Linz (Aescht 1994, 2008).[3] However, more than 90% of all described ciliates lack type material at all, or the material hardly shows the species-specific features, or is difficult to obtain because deposited in private or University collections (see Foissner & Pfister 1997 for an example).

Certainly, the lack of types is one of the most difficult problems in ciliate and protozoan alpha-taxonomy in general. There are innumerable examples of poorly described species, doubtful identifications, and problematic redescriptions. Although my group usually recognizes a thorough redescription as "authoritative" (e.g. Foissner 1993), others do not.

Berger (1999), for instance, assigned *Onychodromopsis flexilis* Stokes, 1887, carefully redescribed and neotypified by Petz & Foissner (1996), to *Allotricha*, a genus and species never illustrated or carefully described. Obviously, no consensus can be reached, and ciliate identification and nomenclature remain a matter of choice.

Clearly, many of the existing problems could be solved by types. The present practice of using illustrations as types cannot solve the problem because these cannot be reinvestigated. Thus, neotypification is the only way to overcome these and related problems and to bring stability in ciliate taxonomy and identification. This was emphasized already in 1972 by Corliss, who established some neotypes for "difficult" ciliates in the sixties. Likewise, Medioli & Scott (1985) established neotypes for some testate amoebae. Generally, however, neotypification is exceedingly rare for protists. It was only recently that my group commenced using this valuable instrument on a large scale (Foissner 1997, 1999a, Foissner & Brozek 1996, Foissner & Dragesco 1996, Foissner & Kreutz 1996, Petz & Foissner 1996); several specialists followed (Agatha & Riedel-Lorje 1998, Petz et al. 1995, Song et al. 2001).

Neotypification is strictly regulated by article 75 of the Code (ICZN 1999). Because our neotypes deviate in an important aspect from the Code rulings, and protists have several peculiarities (Corliss

[1] Note by H. Berger: The detailed "site description" was not yet done by W. Foissner. I could not reconstruct these data within reasonable time. Thus, this section is not included in the present book. However, at each described species (see Chapter 4) a detailed site description has been provided by the author.

[2] Note by H. Berger: I checked the descriptions and found that none of the species redescribed in the present work is neotypified. Despite I did not change the text.

[3] Note by H. Berger: Many type slides of ciliates are also deposited in other repositories; for details, see Warren et al. (2017, p. 42).

1993), they need a detailed comment. We establish neotypes only if at least one of the following items applies:

1 No useable type material is available, and the identification appears reasonable.
2 The original description is so incomplete and/or based on so few specimens that any identification becomes arbitrary. Alternatively, such descriptions could be considered as species indeterminata. However, this would greatly increase the number of scientific names because many original descriptions of ciliates are very incomplete, at least from our present point of view. Thus, we prefer to identify our taxa with previous ones, even if these are poorly described, and to redefine them by detailed redescriptions; of course, identification requires matching of at least one main feature.
3 The species has one or more proposed subjective synonyms, that is, a questionable identity discussed in the literature. This is, in the absence of type material, a "classic" case for neotypification.
4 If there are several similar species whose identity is threatened by the species to be neotypified.
5 If there are competing redescriptions.
6 If the new preparations ("neotype slides") are of a quality allowing the specific features to be clearly recognizable.

Conditions as described above basically pose no problems for neotypification according to the Code. However, our neotypes usually do not comply with article 75.3.6 (ICZN 1999), that is, are not from or near the type locality. Thus, they might be considered as invalid. However, we defend our approach for the following reasons:

1 Most ciliates and protists are cosmopolitan, at least at morphospecies level (Finlay et al. 1996, Foissner 1999).
2 The existing chaos can be mastered only by types available to everyone. Certainly, the chaos produced by a few probably misidentified neotypes is much smaller than the existing one.
3 Considering the situation in alpha-taxonomy of ciliates, where only few regularly publishing taxonomists are left worldwide, we cannot wait for neotype material from or near to the type locality. If so, types will never be established! Furthermore, the chances of rediscovering such minute organisms at a certain locality are minimal because they may be in a dormant (cystic) stage most of their lives and cultivation is often not successful.

To sum up, I suggest that neotypes of protists, especially ciliates, should be freed from the type locality regulation of the International Code of Zoological Nomenclature (ICZN 1999; article 75.3.6.), provided that neotypification is based on a thorough redescription of the organism and useable neotype material has been deposited in an acknowledged repository.[1]

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[1] Note by H. Berger: I do not agree with W. Foissner in this respect, that is, I think the type locality regulation of the ICZN (1999) should be valid for all groups, including ciliates and other protists.

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