



An Atlas of Ciliated Protozoa commonly found in Aerobic Sewage-Treatment Processes.

An Aid to Monitor Treatment-Plant Performance

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This software is a beta release. We cannot be sure that it will operate cleanly under all circumstances, which is why you agreed to take this test version. Please report any issues, bugs, faults and usability issues through the project web site ciliateguide.myspecies.info.

1 Detailed System Requirements

The Program has been authored using Macromedia "Director". The resulting software runs on both Windows and Macintosh operating systems, but both require Quicktime™ to be installed in order to see the video clips. Quicktime™ is free and can be downloaded from: <http://www.apple.com/quicktime/download/>

The following operating systems have been successfully tested:

Windows:

Windows XP Pro with Quicktime™ version 7.0.3 or later

Windows XP Home with Quicktime™ version 7.1.3 (version 7.0.3 is incompatible)

Windows 2000 with 264 Kb RAM with Quicktime™ 7.0.3

Windows Vista (version unknown).

Macintosh:

OSX version 10.4 with 768Mb RAM. And Quicktime™ version 7.1.3

2 Installation Instructions

The software will require about 580 Mb free space available on the hard disc. A copy of Quicktime™ will be required (see above). If you run the application without Quicktime, you will get an error message from the application when you reach a page calling for a movie clip. It is OK to press "continue" at that point.

Windows XP Users*

Copy the folders "Casts" and "Video originals" and "Key. Exe" from the CD into a single folder on your hard drive.

NB Windows installations sometimes have trouble finding the "Cast" files (with .cst extension), which are all located in the folder called "Casts". On the first run it may be necessary to help Windows locate these files manually using the dialogue window that pops up.

Windows Vista Users

Copy the folders "Casts" and "Video originals" and "Key. Exe" from the CD into a single folder on your hard drive.

A shortcut to the newly installed program may be added to your desktop as follows. Go to the folder in which the Key Program resides. Right click on "Key" and choose "Create shortcut" from the list of options. A shortcut icon will appear on the desktop screen. Do not attempt to make a shortcut by dragging and dropping the "Key" icon direct from the folder to the desktop.

When you first run the application, if you find that you have a red square box where you expected to see a video clip then you have not have a copy of Quicktime on your machine. Download an up-to-date version from the internet and try again.

Mac OSX Users

Copy the folders "Casts" and "Video originals" and "Key.osx" from the CD into a single folder on your hard drive.

Screen Resolution

The main program window is 1000 x 720 pixels and you need to ensure that your screen is set to display at least that level of resolution.

3 Description of the Keys and their Use

a) Starting the Application

Start the application by double-clicking on the 'Key' icon.

The opening screen allows you to enter the specification of your sample. These data are stored and inserted each time the key is started or a new sample is to be examined. The data can be over-written for each sample. The current date is loaded automatically.

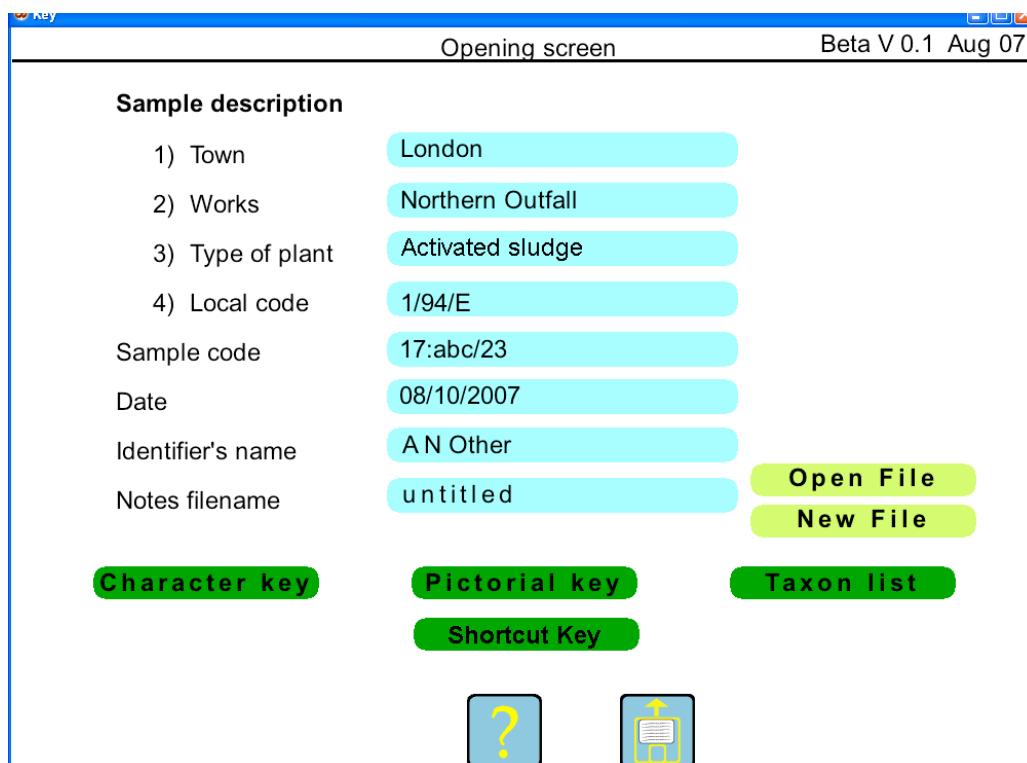


Fig. 1. Opening Screen

During use, there is always a button at the bottom of the screen containing a **yellow question mark**; **click and hold to display** a help box that tells you what the program expects you to do on the current page

b) Personal notes files

The program will keep Personal notes for you. A new “Notes” file may be opened by clicking on the “**New File**” button (light green on lower right of screen), the “**Notes filename**” box (lowest light blue box in central column) will be cleared and you can type in a name for the new notes file. If you already have a Personal notes file then this can be opened by clicking on the “**Open File**” button (light green on lower right of screen).

These personal notes are accessible to you at any page and are intended to help you make consistent decisions at any given point in the process, so to keep such aide-mémoire as you find useful. In the beta version the files generated also contain tracer data that records the path used to reach a particular screen and is used by the project team for debugging.

c) Results file.

The program will keep a file of your results. When you first open the program there will be a single icon of a floppy disc which when pressed enables you to load the results of an earlier sample which will be displayed on a **Profile Page**. The Profile Page will be described in detail later (see section 3g. on page 14). If necessary you can add further results to the file and save to disc.

If you do not open an existing file then information will be stored as you proceed until you wish to save the results. At that point you will be asked to name the new results file. Results will normally be saved after a sample has been completed from the Profile Page but you may save to file after each organism has been identified. A second blue floppy disc icon will appear at the bottom of the screen when you have sufficient data to save.

d) Routes for the identification of ciliates.

The main identification keys can be accessed by 4 main routes:

Route 1. Via the Pictorial Key Button

This gives the user access to a classical key that is strongly recommended for newcomers to ciliated protozoa and may be used as a tutorial introduction. The key asks specific questions in the order of ease of observation, so the first question asks whether the ciliate is attached or not, probably the simplest observation possible

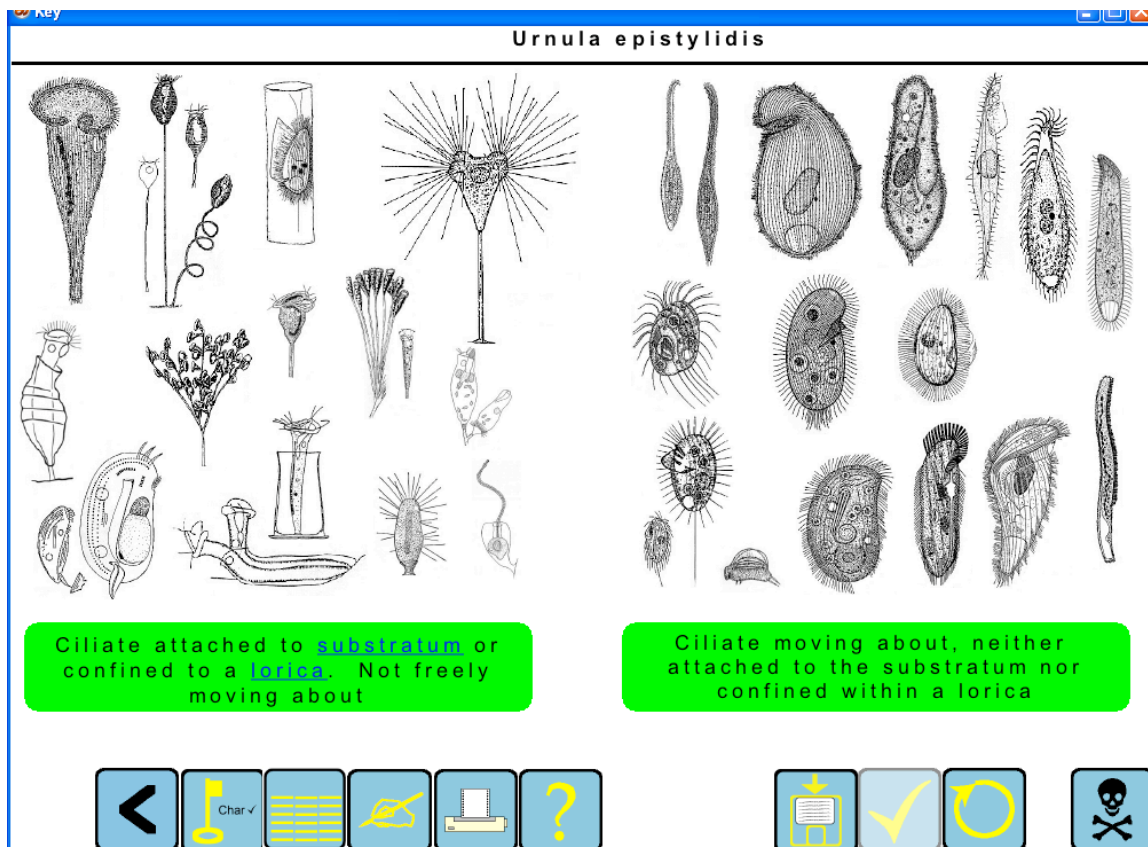


Fig 2. First Screen of Pictorial Key

At the bottom of the screen there is a row of icons. Putting the mouse pointer over an icon changes it from an arrow to a pointing finger (indicating that the system is ready to accept a mouse click) and the icon's name (and purpose) is displayed. The <Print> icon prints a screen-shot of the current window on the printer.

Between the icons and illustrations lie two green boxes containing brief descriptive statements. Clicking on the left-hand green box will take you to the next level dealing with attached ciliates, while clicking on the right hand box will take you to another page dealing with motile ciliates. The key allows for uncertainty, so that a species that can have either character (e.g. *Stentor* can be attached or free-swimming) can be identified by taking either route. Specimens can be identified by answering a series of these questions until a single species is recognized. Many of the terms used in the questions may be unfamiliar but those terms highlighted in blue are defined and explained, clicking on them will take you to an appropriate help page. For example the terms “substratum” and “lorica” in the page illustrated above will be defined by clicking on either of these terms. Further details concerning help pages are given on page 11.

Passing your mouse pointer over the diagrams will reveal the species name on the top left of the screen. In the example above the mouse pointer (not shown) rests upon the bottom right diagram of the left-hand diagram group (*Urnula epistylidis*). Clicking the mouse on a diagram will take you direct to that species. Not all of the genera or species are necessarily illustrated on these pages particularly at the beginning of the key where a large number of taxa are possible contenders. As you home in on the specimen's identity fewer and fewer available taxa will be left and so the proportion of total possible species illustrated will rise.

We can now go through the identification of a specimen using the pictorial key. The specimen concerned is assumed to live inside a membranous lorica (or shell), it is attached via a short stalk (in this case to another ciliate), has no cilia but has a long mobile trunk-like organelle at the opposite end to the stalk. Now select the description (on the first page of the pictorial key shown above) which best fits our specimen. Since it is attached and is confined within a lorica then you should select the left hand box by clicking on it. This will take you to the next screen shown below.

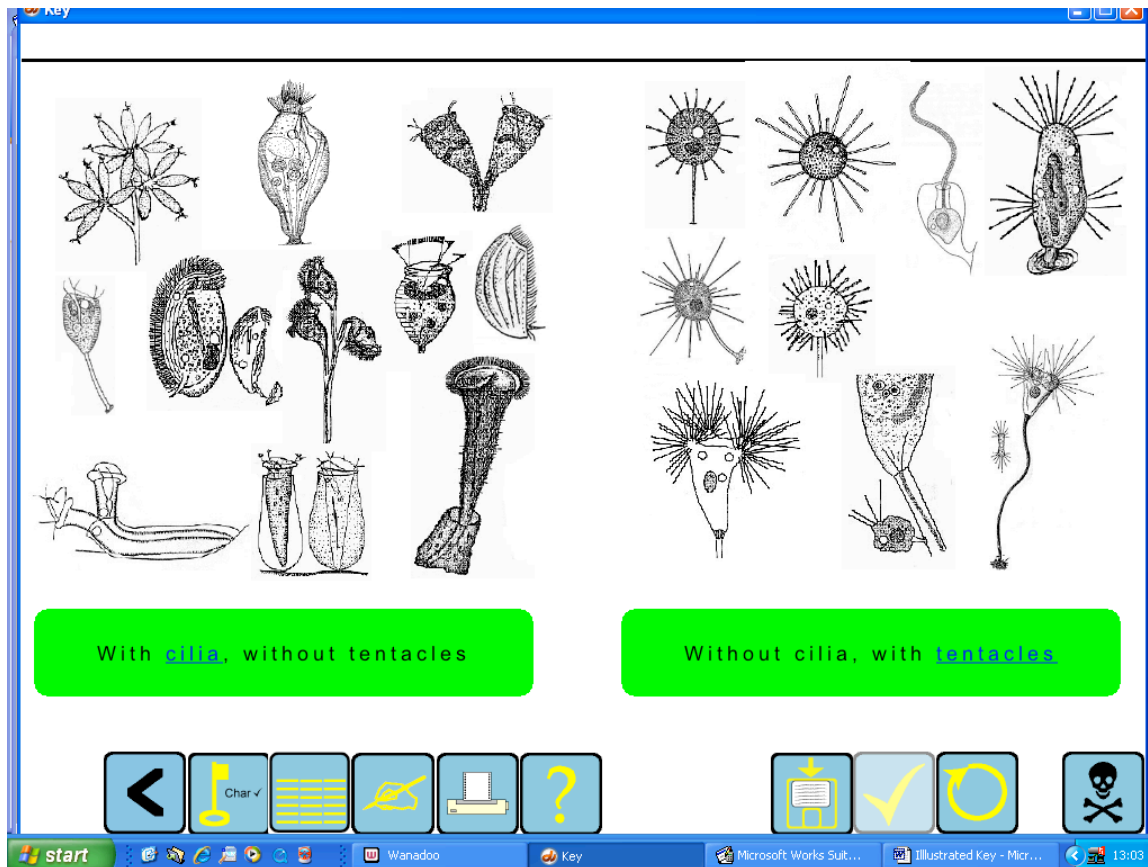


Fig 3. Next screen for identification of Urnula

Again, select the box which best describes the specimen. In this case it will be the right hand box since our specimen has no cilia but does have a trunk-like tentacle. Click on the right-hand box. **If at any time you make a mistake in the box selection you can move backwards through the pages one at a time by clicking on the blue arrow icon at the bottom on the extreme left-hand side.**

The next and final screen in the identification process appears as shown below. Select the right-hand box because the specimen in question has a mobile trunk-like tentacle. This will take you to the species page where you will find the specimen is described, there is a diagram and a video clip of a living specimen for you to compare with your own. An explanation of the species page will be found later (page 13).

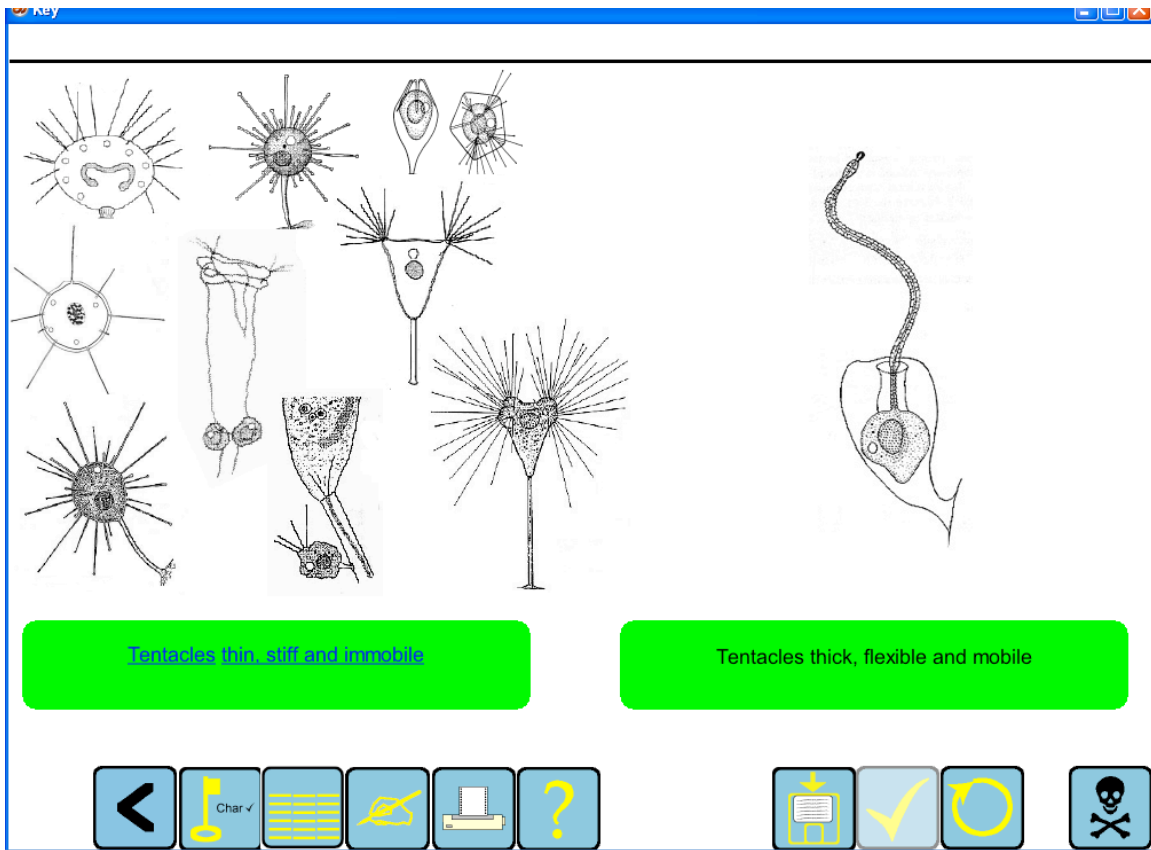


Fig. 4. Final screen in identification of Urnula

Route 2. Via the Shortcut key

The shortcut key on the opening screen (Fig. 1) gives access to the pictorial key part-way through the identification process. This should only be used by the more experienced, who can identify ciliates to at least the level of a broad descriptive group. Clicking and **holding down** on the green “Shortcut” button at the bottom of the opening screen will reveal a menu of nine groups (Fig. 5). Slide the mouse pointer up until your choice is highlighted. Releasing the button will take you part-way through the pictorial key. Note that the group labeled “Small whizzy ones” includes a wide ranging group of small (50 µm or less) often rapidly-moving ciliates (though they will slow or settle) from several taxonomic groups.

If the same previous specimen is used as in the pictorial key example (*Urnulla*) then the experienced user would know that the specimen was a suctorian. Selection of “Suctoria” from the menu would immediately bypass several pages going directly to that shown immediately above (Fig. 4).

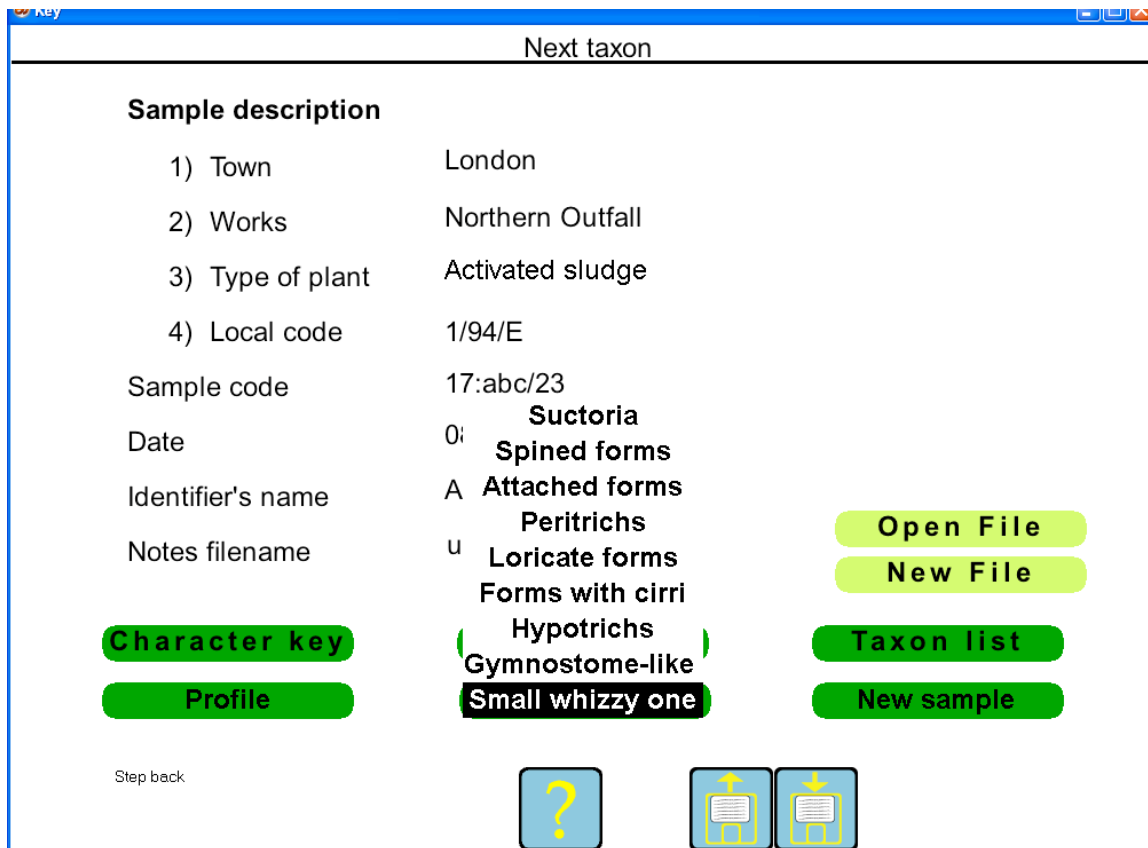


Fig. 5. The Shortcut Menu accessed from the Opening Screen

Route 3. Via the Taxon List key

Clicking on the Taxon list key on the opening page (Fig. 1) will result in a list of all those genera that may be identified following the keys provided. (Fig. 6) Clicking on any generic name will either take you to a species page, if only one species of the genus is known is sewage, or will open a menu of possible species names (Fig 7). Selection of a species in the menu will immediately go to a species description, usually illustrated with a diagram, movie clip or photomicrograph. Clicking again on the genus name will take you to the pictorial key where species of the genus selected may be identified. This allows the experienced operator who has the ability to recognize a specimen to generic level to quickly continue identification to the species level without having to proceed through several pages in the key.



Fig. 6. A full list of all Genera included is accessed from the List button on the Opening Screen

The list of genera accessed from the opening screen page is a single list of all genera included in the keys. Access from pages within the pictorial key reveals a shorter list that agree with the combined criteria (or character states) so far entered by answering the questions so far posed. As you answer questions in the pictorial key you will find that clicking on the Taxon List key will reveal shorter and shorter generic lists as you home in on the identity of the specimen. When the list of genera is short enough, then all possible species will be offered directly, rather than the genera themselves.

Acinera	Drepanomonas	Opercularia	Stentor
Acineta	Dysteria	Opisthonecta	Sterkiella
Amphileptus	helyomorpha	Oxytricha	Stylonychia
Aspidisca	helys	Paramecium	Supraspathidium
Blepharisma	archesium	Parastrongylidium	Tachysoma
Bursaria	Epistylis	Paraurostyla	Tetrahymena
Caenomorpha	Euplotes	Phascolodon	Thigmogaster
Calyptotricha	Glaucoma	Plagiocampa	Thuricola
Campanella	Halteria	Plagiopyla	Tokophrya
Carchesium	Heliophrya	Platycola	Trachelophyllum
Chaenea	Histiculus	Pleuronema	Trimyema
Chilodonella	Holophrya	Podophrya	Trithigmostoma
Cinetochilum	Holosticha	Prodiscophrya	Trochilia
Climacostomum	Kahillembus	Propygidium	Trochilopsis
Coleps	Lagynus	Pseudochilodonopsis	Urula
Colpidium	Leptopharynx	Pseudocohnilembus	Urocentrum
Colpoda	Litonotus	Pseudovorticella	Uroleptus
Cyclidium	Loxodes	Pyxicola	Uronema
Dexiostoma	Loxophyllum	Rhabdostyla	Urotricha
Dexiotricha	Manuelophrya	Saprodinium	Vaginicola
Didinium	Metacineta	Sathrophilus	Vorticella
Dileptus	Metacystis	Spathidium	Zoothamnium
Discophrya	Metopus	Spirostomum	

Fig. 7. Drop-down menu of the species of *Amphileptus* included.

Route 4. Via the Character key

Clicking on the Character Key allows the user to enter character states directly. This route should only be used by those experienced in ciliate taxonomy or if you have come to a point in the pictorial key where you cannot answer the next questions posed. This would usually be because the specimen is damaged or obscured so that a feature cannot be seen. In this case clicking on the Character Key button will reveal a screen filled with those characters used for the determination of the ciliate species included within the keys. You can enter the Character Key from a position part-way through the pictorial key as is the case illustrated in Fig. 8. In this example the Character Key was pressed at the position shown in Fig. 4, so the character states derived from your answers given in the pictorial key up to the Fig. 4 position have been already entered. Here, in Fig. 8, the “Attached” character state is “yes” (in blue) and the “Tentacles” character state is also “yes” since those questions were answered in the Pictorial Key earlier. These may be edited as you wish. At this point you should enter additional character state data which hopefully will enable you to bypass point at which you were stopped in the pictorial key. You are not expected to answer all character state questions: initially try something simple, for example add outline shape, size data, or number and position of contractile vacuoles. When further data have been entered either go to the Taxon List to see how many taxa share the characters you have entered or return to the Pictorial key by clicking on the button with a Key icon. The system will then find the most appropriate place in the key for you to continue. Hopefully further towards your goal. If not, return to the Character key and add more character state data and try again. You can monitor your progress by clicking on the “List” icon button (3rd from left). The list will display those genera remaining which agree with the character states entered. The aim is to reduce the list to as few as possible and then return to the Pictorial Key.

The screenshot shows a software window titled "Key" with a blue border. The main area is a form with several sections:

- Ecology:** Attached: yes, Attitude: , Attachment: , On ciliates: , Habit: , Substratum: , Loricata: , Stalk: .
- External features:** Tentacles: Tentacles: yes Type: , Spines: Spines: , Body plates: , Pliability: , Warts: , Longitudinal groove: , Body colour: , Body granular: , Body coat: .
- Body structure:** Macronucleus: Macronuclear number: , Shape: , Anterior: , Compact: , Transverse: , Micronuclei: , Contractile vacuole: CV: , Location: , Side: , Canals: , Terminal: .
- Ciliation:** Somatic cilia: , Cirri: , Transverse: , Caudals: , Length: , Oral cilia: Obvious oral cilia: , Apical: , Oral cilia: .
- Body shape:** Peristome: Disc: , Size: Smallest: , Biggest: , Small: , Size: , > 130: , > 200: , Shape: Shape code: , Widest point: , Outline 1: , Shape ratio: , Flattened: , Taper: , Reniform: , Left side: , Right side: , Symmetric: , Notch: , Anterior: Truncated: , Scallop: , Ridges: , Ant lat edge ribbed: , Anterior width: , Sharpness: , Direction: , Short nose: , Apical curl: , Beak: .
- Neck:** Neck: .
- Mouth:** Apical: , Direction: , Extrusome: , Posterior: , Furrow: , Trichites: , Basket coiled: .
- Posterior:** Posterior vacuole: , Tail: , Notch: , Shape: , Ridge: .

At the bottom, there is a navigation bar with icons: a left arrow, a key icon, a list icon, a question mark icon, a right arrow, a circular arrow icon, and a magnifying glass icon.

Fig. 8. Character state page with “Attached” and “Tentacles” character states already filled from previously answered questions in Pictorial Key.

If you position your mouse pointer over a character, the character question will appear in the top line of the screen.. For example if you place the mouse pointer over the “Loricata” character then the Question “Is a lorica around the cell? Some loricas are very thin and difficult to see” appears in the top line. (see Fig 9.). A single click on the loricata character reveals a small window containing a list of available states, together with 3 buttons, <Close> which closes the window and does nothing else, <Help> which takes you to a help page for this character, and <Clear> which deletes any value currently held for this character.

Some characters are dependent upon others. For example if a ciliate is not attached to a substratum then further questions concerning the type of attachment or substratum are meaningless. So characters concerning type of attachment are dependent upon the character state of “attached” being “yes” or positive. The Character Key table will not display dependent characters unless the primary character has been set to positive. In Fig. 9 below the “Attached” character is recorded as “yes” (i.e. positive) which results in the “Loricata” and “Stalk” characters being displayed

immediately below. Dependent characters will automatically appear or not so that the user does not have to add meaningless data.

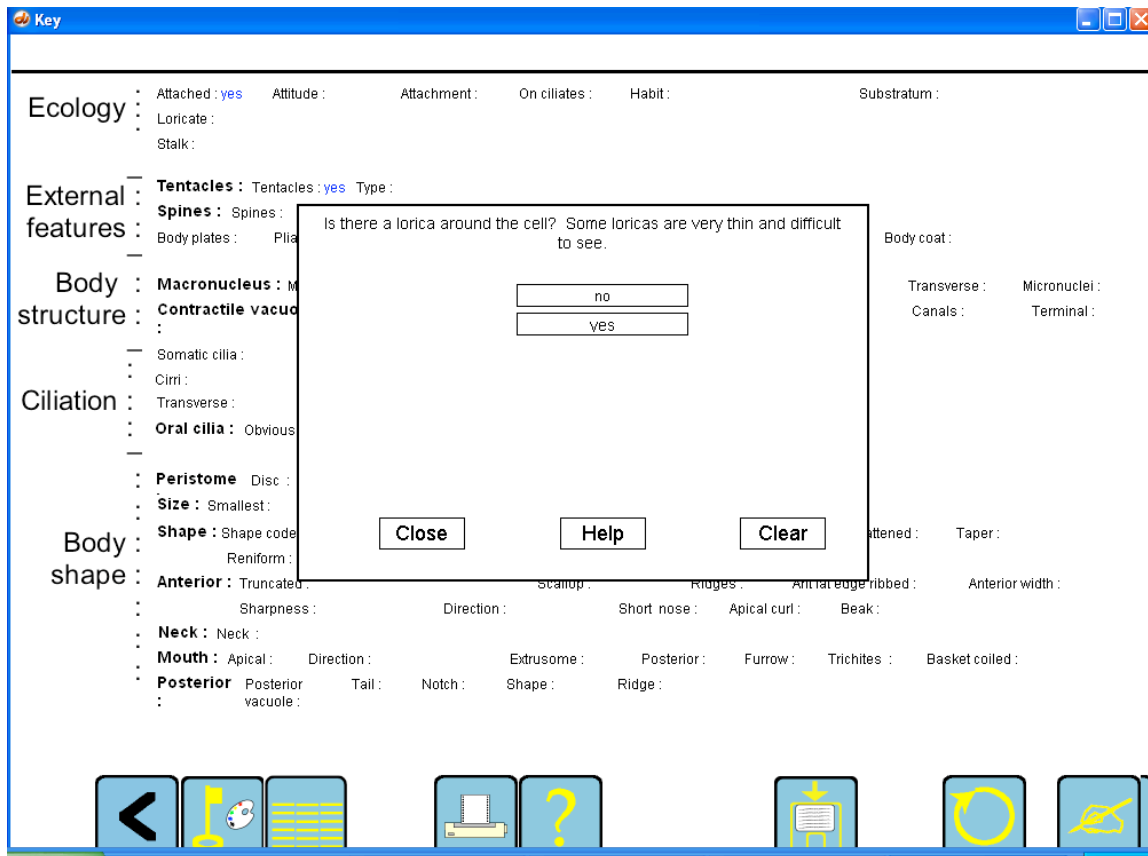


Fig. 9. The Table of Characters Screen with “Loricata” question being posed.

e). Help Pages

Help pages are provided from the Pictorial Key by clicking on the terms underlined in blue (see Fig. 2 Page 4) or from the Character Key by clicking on the <Help> button (see Fig 9 immediately above). The Help pages are arranged in chapters according to particular structures. For example clicking on the underlined term “lorica” in the Pictorial Key (Fig 2 Page 4) will lead to a Help page that defines a lorica. Clicking on the page forward button (2nd from left) at the bottom of that page leads you to a series of Help pages giving more information about loricas. In this example the sequence of Help pages is as follows:-

1. Lorica definition
2. Proportion of the body contained within the lorica (see Fig. 10 illustrated below)
3. Appearance of lorica
4. Closure structures found on loricas
5. Stalks on loricas
6. Stalk size on loricas

And then back again to 1. Lorica definition

The entry point into this sequence is determined by the program so that you will enter at the most appropriate point. You can advance through the sequence as you wish by clicking on the <step forward> button although the sequence order remains fixed.

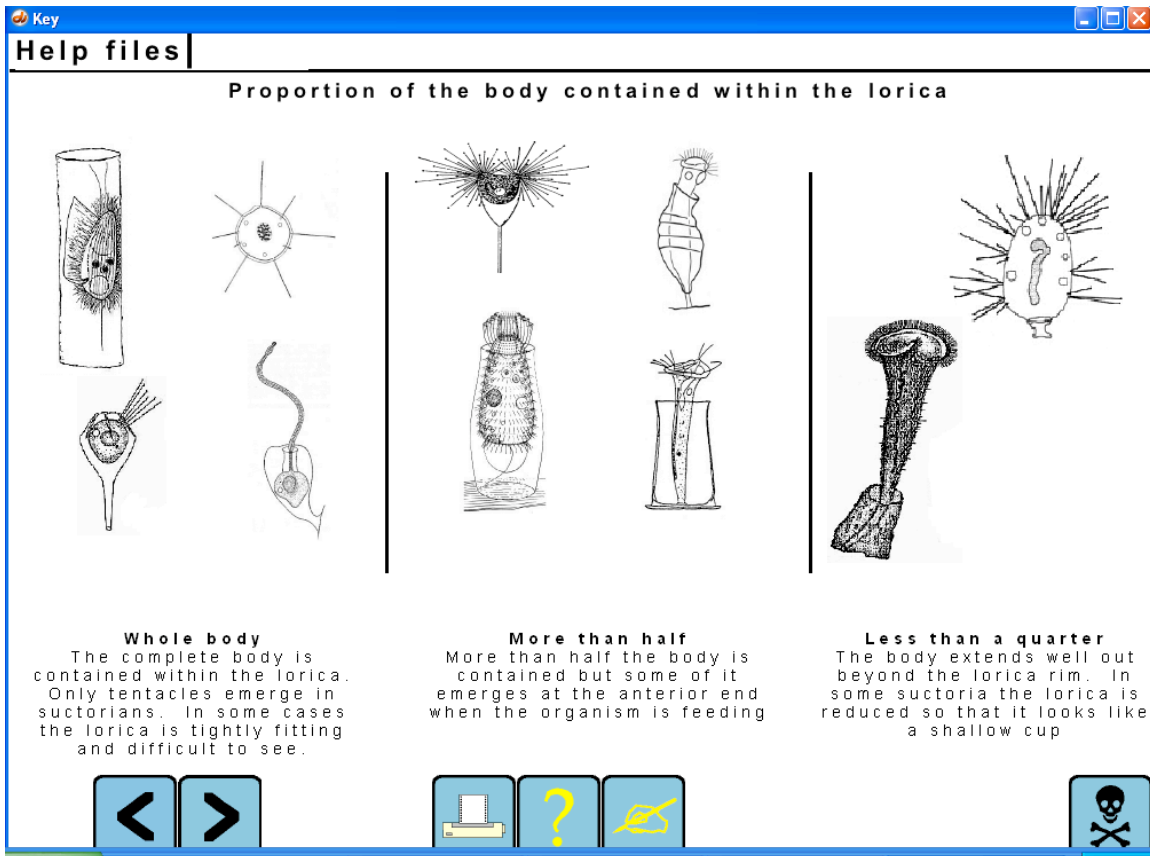


Fig. 10. A help page concerning the Lorica

You may return to your original entry point in Pictorial or Character Keys by clicking on the <Step Back> icon button (bottom left in Fig. 10)

f). Species Pages

Whatever route is taken eventually you will arrive at the Species page where your identified specimen is fully described and illustrated by means of a diagram and movie clip/or photomicrograph. Scale bars indicate 50 μm divided into 10 μm portions. The species is first described followed by a generalized description of the genus. The later is fully revealed by sliding the right-hand slide downwards.

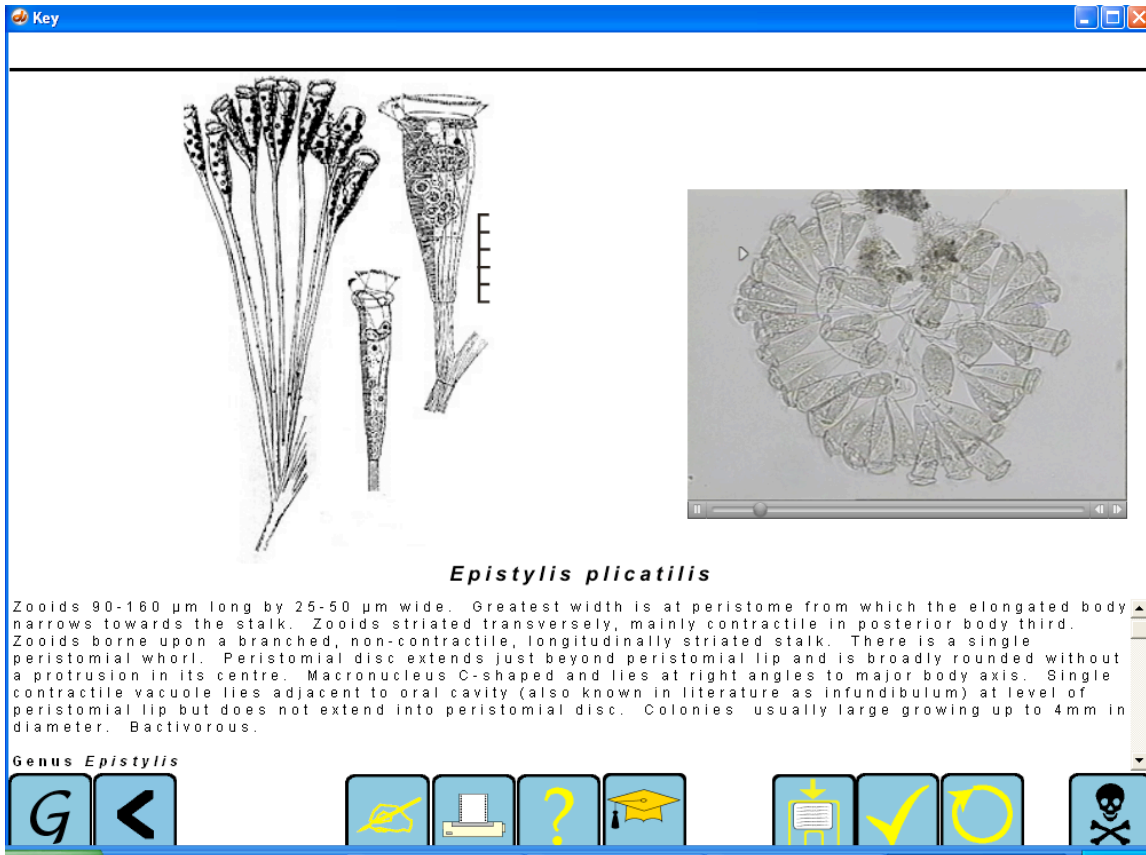


Fig. 11. The Species Page for *Epistylis plicatilis*

If the descriptions fit your specimen then you can confirm its presence in the sample by clicking on the <OK> icon represented by a tick (3rd from Right). Before this you may wish to compare the specimen with others of the same genus (if more than a single species is represented). This may be done by clicking on the Genus (G icon) on the extreme left. This will take you to the beginning of the key to species for the species concerned.

More information concerning the species identified may be obtained by clicking on the <Academic> icon (a mortar board). This will lead to a page containing an array of data concerning the nomenclature, taxonomy and ecology of the species together with a list of useful references as illustrated in Fig.12. There you will also find details of taxonomic authorities, synonyms, identification notes, comments on the taxonomic position, bibliographic details, including the sources for the diagrams, and ecological comments including saprobity information.

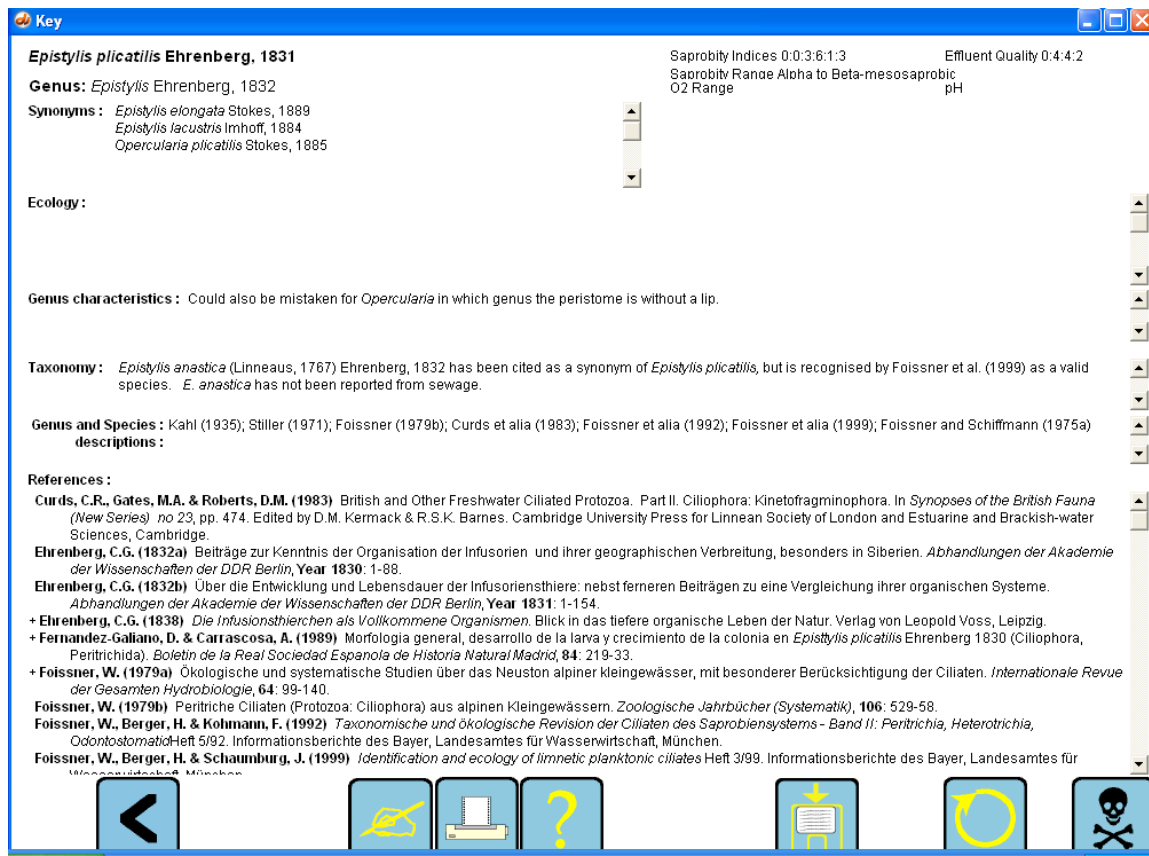


Fig 12. Contents of the "Academic" page for *Epistylis plicatilis*

At this point you should return to the Species page by clicking the <step back> button (extreme left) and if appropriate accept the identification as being correct by clicking on the <OK> button. If the identification is incorrect then you may go backwards through the pages by clicking on the <step back> button. Experienced users could also use the Character key route if they prefer. In any event eventually you will need to confirm the identity and record it in the sample list by clicking on the <OK> button. This will lead you to the Profile page (Fig. 13) where you will find that the species you have identified has now been listed as being present in the sample.

g). Profile Page and Effluent Prediction

Once a species has been added to the sample list then you are ready for another identification. Clicking on the <Next specimen> icon (a circular arrow) will take you back to the beginning of the program and you are ready to begin again. "Next Specimen" re-sets the key to the beginning but retains the profile for the current sample: note that the screen is not quite the same as the opening screen, because you can access the accumulating profile from here directly and you can wipe the profile by moving on to a new sample but remember to save the results before moving on to a new sample and results file.

After several specimens have been identified you will see that the Profile page has collected a list of the species you have identified in the current sample. At this point you may wish to add an estimate of the relative abundance of each species. This can be entered either by clicking and dragging on the red portion of the slider bar or by typing (after clicking) a value into the box. The values are percentages. This will be used to calculate a predicted value for the effluent based on the saprobity of the recorded taxa. Note that saprobity values are quite coarse-grained and the data are grouped, so that small differences in abundance values will not affect the outcome.

In the Profile page illustrated below note that there are two groups of numbers to the right of each species listed. The one next to the percentage box is the indicator value of that species based on Curds & Cockburn (1970) scheme. The six digit number to the extreme right of each species is the published Saprobic Index (Curds, 1992). This system is conceptually similar to the Curds index but divides the water quality assessment into 5 classes and adds an indicator value, essentially a measure of reliability for the taxon.

Weighted

Curds
Index

Curds
Index

Saprobic
Index

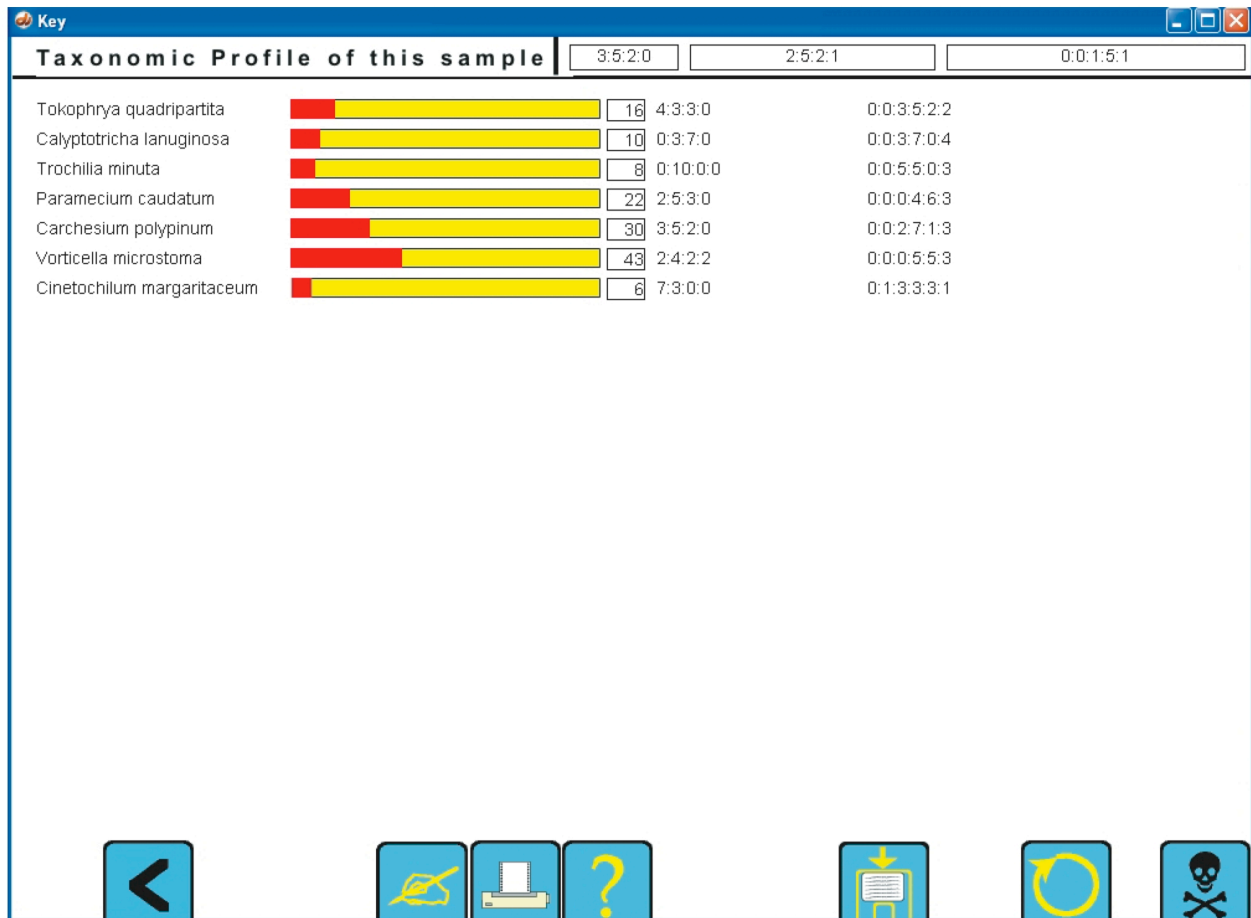


Fig. 13. The Profile Page.

Across the top of this page are three sets of numbers, being, from left to right, the Curds Index, the Weighted Curds Index and the Saprobity Index aggregated for the entire sample.

Curds Index

The individual numbers for each species have been condensed by simple average into a single group which is given in the left-hand box on the top row. In the example given this number group is 3:5:2:0 indicating that the population of ciliates identified in the sample indicate the the water or effluent quality from which the sample was derived is likely to be in the “Good to Very Good” range since most of the individual species normally occur in these conditions.

Weighted Curds Index

The second box on the top line is the same data set but where relative abundance of each species has been taken into account by a simple multiplicative addition followed by normalisation.

Saprobity Index

The right-hand box contains the simple average of the saprobity indices. The index is organised xenosaprobic; oligosaprobic; betamesosaprobic; alpha mesosaprobic; polysaprobic; Indicator value (1-5). This last is a measure of reliability of the taxon for prediction. A high value (5) indicates that the species has a restricted saprobity range and is thus of high value as a predictor.

Interpretation

In this beta version these figures are presented raw intending that beta-testers can assess which system is most satisfactory as a predictor of water quality. To interpret these figures consider, for example in Fig 13 *Tokophrya quadripartita* has indicator values of 4:3:3:0. Note that the total of this group of numbers is always equal to 10.

Individual numbers in the sequence refer to the frequency of occurrence of that species in environments where the water (or effluent) quality is “Very good”, “Good”, “Fair” or “Bad”. Thus the example species is more likely to appear in the range of “Very good to Fair” range but not in the “Bad” range. A clearer cut example may be seen in the case of *Trochilia minuta* which is only found in waters or effluents in the “Good” range.

In the current version the Indicator Value has not been used to bias the saprobity readings. The rationale behind this was that as the number of observed taxa increases then the aggregate profile should get smoother and less prone to stochastic observational effects. Later releases of the key could easily modify this calculation structure if there is sufficient user demand.

Thus, the aggregate values on the top of the screen are like a histogram and the user needs to estimate where the peak of a curve drawn through the blocks would lie. In the final version we would simplify this presentation.

References

Curds, C. R. & Cockburn, A. (1970). Protozoa in biological sewage-treatment processes - II. protozoa as indicators in the activated-sludge process. *Water Research* 4, 237-249.

Curds, C. R. (1992). Protozoa and the water industry. Cambridge: Cambridge University Press.

4 An Introduction to Aerobic Sewage-Treatment Processes

When sewage arrives at a treatment works it invariably receives some form of primary treatment to remove gross solids. All of these primary methods are physical and are not considered any further herein. However the supernatant sewage (commonly called settled sewage) flows from a sedimentation tank and is ready for some form of secondary treatment.

All forms of secondary treatment are biological and rely on the growth of micro-organisms to remove dissolved and suspended materials or to convert them into more acceptable compounds. These processes are aerobic and all involve some means of introducing atmospheric oxygen into the sewage and associated microbial populations.

Many methods have been devised over the past 150 years but the two most commonly used methods are biological filters (also known as percolating or trickling filters) and the activated-sludge process. Other methods covered here include Rotating Biological Contactors, Constructed Wetlands and Oxidation Ponds. It is important to broadly understand the construction and operation of these treatment systems to be able to set up appropriate sampling routines and appreciate how operational differences affect microbial populations.

a). Percolating Filters

Biological filtration is the oldest and one of the successful of the processes available today. Most users will already be familiar with its appearance. Typically there is a circular bed of mineral aggregate (clinker or stones) over which a slowly rotating distributor arm discharges settled sewage. When a new filter is brought into operation there is some initial reduction in the BOD₅ of the waste water, the removal efficiency is very poor. A filter needs to mature through the establishment of a wide range of microbial and animal life. Maturation takes several weeks for the microbial populations to become established but it is several months before the macro-invertebrates colonize. Initially it is the bacterial and fungal populations that develop on those areas of the clinker that are wetted by the sewage but as time goes on they grow towards other areas not in direct contact with the waste water. The protozoan populations are next to develop and eventually all surfaces of the clinker are covered in a dense biological film which is responsible for the purification processes that occur throughout the filter depth.

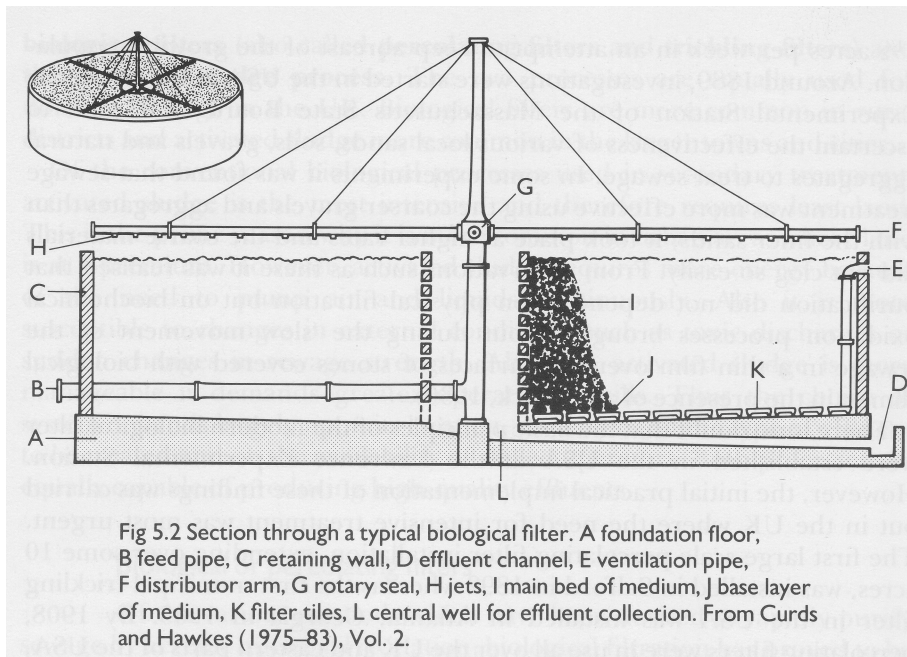


Fig 14. Section through a Biological Filter

A definite sequence of environmental changes occurs throughout the filter's depth otherwise the quality of the effluent leaving would be the same as the sewage entering. In filters where the microbial film is held in a static position there is a vertical stratification of environmental conditions. At the top there may be some surfaces that become anaerobic but as the purified water trickles down over the microbial film the dissolved and suspended organic are removed and conditions and microbes change. This is important when devising a sampling strategy since those organisms at the top will be exposed to sewage while those at the bottom to treated effluent.

b). The Activated-Sludge Process

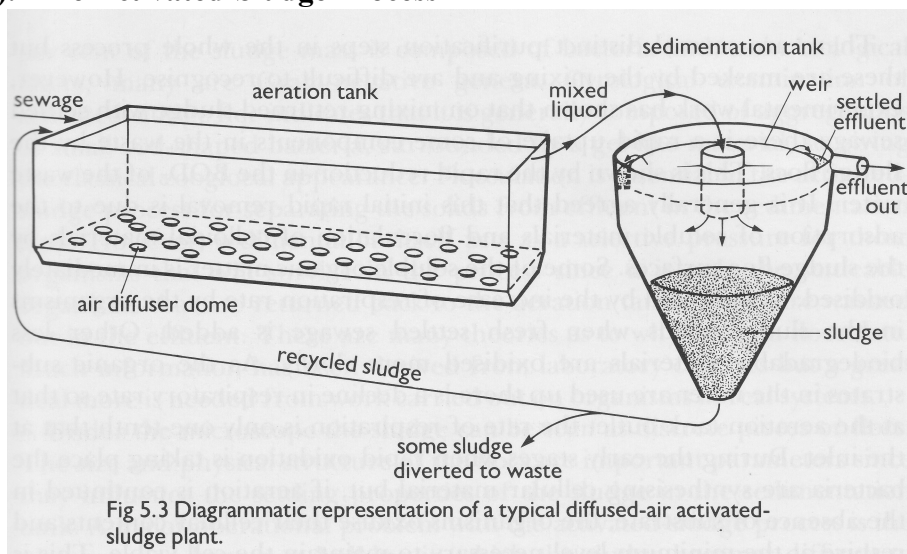


Fig. 15. The activated-sludge process

An outline of the most common type of activated-sludge process is illustrated above in Fig. 15, Settled sewage enters a tank where it is aerated with activated sludge for a period of between 3 and 8 hours. Mixing and aeration are usually achieved simultaneously by pumping air through porous ceramic diffuser tiles (or domes) spaced at frequent intervals along the base of the aeration tank. A concentration of dissolved oxygen of at least 1 mg l-1 must be maintained to achieve carbonaceous oxidation by heterotrophic bacteria and about 2 mg l-1 is necessary for nitrification to occur. After aeration the mixed liquor (sludge and effluent) overflows into a sedimentation tank where under quiescent conditions any solids capable of doing so will settle to the bottom whilst the purified effluent overflows at the surface via a weir. Some of the sludge solids are diverted to waste but the majority is returned back to the aeration tank to be mixed again with incoming settled sewage. Most activated-sludge plants operate at a level of between 2000-6000 mg l-1 dry weight of suspended solids in the aeration tank.

The aeration tanks are long, deep and narrow operating on a “plug or piston-flow” principle. Thus environmental conditions change radically along the length of the tank. At the input end the dissolved oxygen concentration will be low due to the presence of the greatest concentration of organic matter and the consequent high rates of microbial activity. At the effluent output end the organic content will have fallen and the oxygen concentration risen. Samples should therefore be taken from the outlet end of the tank since they will remain aerobic for the longest period of time.

c). Rotating Biological Contactors

In many ways RBC’s resemble a mixture of features found in biological filters and activated-sludge plants. As with filters the microbial biomass is attached to the surfaces of the reactor, that is to say it is a fixed-film reactor, but it differs significantly in that the surfaces take the form of discs which rotate at axle depth in a flow of sewage. As each part of the disc spends half its time submerged in sewage and half its time in the air, the film is kept both aerobic and in contact with the sewage. Like the activated-sludge process it is completely aquatic with no dry places as are found in biological filters.

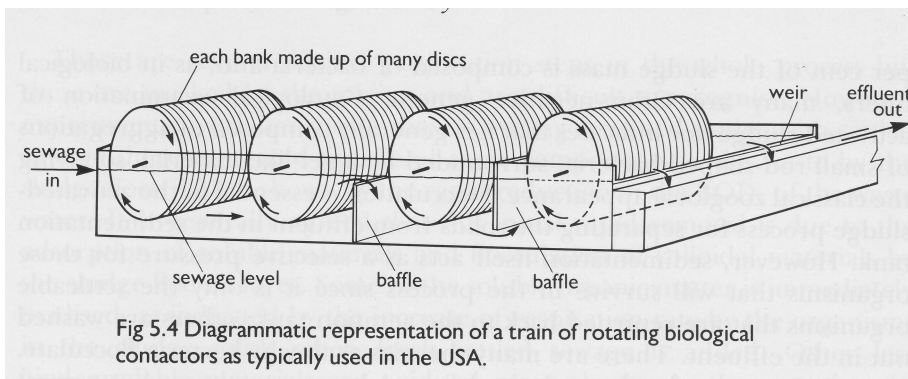


Fig. 16. A train of Rotating Biological Contactors

An outline of the process is illustrated above. Settled sewage travels along a channel fitted with baffles to prevent short-circuiting and ensure plug-flow conditions. Many large discs (up to about 4 m in diameter) of either plastic or metal mesh are mounted on common axle, rotate at about 1 r.p.m. partially submerged in sewage. These form a bank of discs and there are several banks of discs spaced at regular intervals along the length of the channel. Since plug-flow conditions are encouraged and access to the microbial film along the complete length of the sewage flow then RBC’s make an ideal source of material for looking at protozoa living under different environmental conditions. Sampling along the channel length reveals that a definite sequence of species occurs.

d). Constructed Wetlands

Probably the most common type of waste-water treatment constructed wetlands is that which employs horizontal subsurface flow through a system of emergent hydrophytes and are often referred to as a reed bed. The key feature of reed is that of rhizomes of hydrophytic plants, usually the common reed *Phragmites* provides an hydraulic pathway through which the waste water flows. This pathway is the annular space between rhizomes, roots and the planting medium usually pea gravel or soil. The movement of the mesh of roots and rhizomes within the planting medium prevents clogging and the hydrophytes provide atmospheric oxygen to the rhizosphere via the leaves, stems, roots and rhizomes. The wastewater is therefore treated aerobically by bacterial activity but also anaerobically in the surrounding planting medium.

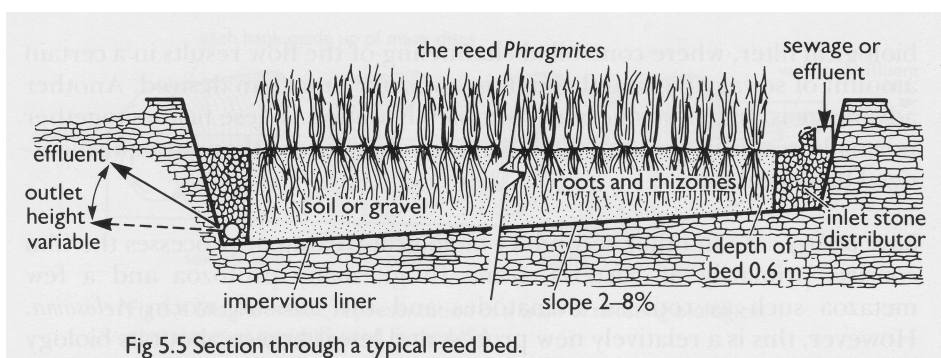


Fig. 17. A typical reed bed

The illustration above shows the basic features of a reed bed. Existing soil is removed from the site to a depth of about 10.6 m and sealed to retain water and prevent contamination of the groundwater. The excavated area is then back-filled, usually with pea gravel or soil, and the hydrophytes are planted. It is essential that the bed is kept water-logged for the successful growth of the hydrophytes, the liquid level being maintained at a depth of a few centimeters

below the bed surface. Samples may be taken easily and like RBC's make a good process for the study of protozoa growing in different environmental conditions.

e). Oxidation Ponds

Oxidation ponds rely upon algae as their source of oxygen. There are three basic types: firstly the facultative pond which is aerobic in the upper layers where light penetrates but is anaerobic at the bottom; secondly the high-rate aerobic lagoon which is shallow, is kept mixed and there is a high concentration of algae keeping the entire contents aerobic and thirdly the maturation pond or lagoon which is used for tertiary treatment when a high-quality effluent is required. Most waste stabilization ponds are facultative oxidation ponds and are used for the full treatment of crude sewage without primary sedimentation. They are characterized by having upper aerobic and lower anaerobic zones so that when sampling the distance from where the sewage enters is as important as the sampling depth.. Oxidation ponds are very different from the other processes described above in that they rely upon light as a source of energy in order to maintain oxygenation via the photosynthetic activities of the abundant green algae that are present. They have the advantage of being very cheap to run and are found principally in those parts of the world with sunny climates, a lot of land and cheap unskilled labour.

5 Effluent Quality Prediction

a). Taking a sample

i) For demonstration purposes.

If the samples are to be used simply to demonstrate the presence and variety of ciliated protozoa present in the processes then one should take samples from as many different places in the process as is feasible. In percolating filters the species in upper regions will be different to those in lower regions. Similarly, in rotating biological contactors, samples taken from discs close to the inflowing sewage will be different to those found in samples taken close to where the effluent leaves. The ciliate fauna changes little throughout the length of an activated-sludge aeration tank due to mixing, however faunal variations can be demonstrated if some form of artificial substrate is immersed into the aeration tank at different distances from influent and effluent ends. Samples should then be scraped from the substrates after 3-7 days immersion and the actual time determined experimentally for your situation. Reed beds should be sampled from all areas. Similarly samples should be taken from oxidation ponds at different depths since anaerobic zones may be found at the bottom of the tanks.

ii) For effluent quality prediction

If the samples are to be used for effluent quality prediction then they should be taken from zones in closest contact with the final effluent. In activated-sludge and RBC tanks these should be taken from the far end of the system. In activated-sludge plants the organisms which thrive are those which survive continually changing conditions. For example, the dissolved oxygen concentration at the input end is usually lower than at the output end. The organisms then overflow into a sedimentation tank where they will encounter rather low concentrations of oxygen (and may even be anaerobic) for several hours. The organisms in the mixed liquor therefore represent those which survive well under a variety of environmental conditions. However, immersion of artificial substrates in activated-sludge aeration tanks close to the settling tanks may develop a different ciliate fauna which is more closely representative of those which thrive in more stable conditions close to where the effluent leaves. Percolating filters are much more difficult to sample because of their construction, in most cases it is not possible to sample the lower regions of the filter which would be the ideal place. In these cases samples should be obtained by filtration of outflowing effluents through a finely-meshed plankton net.

iii) Size of Sample to be taken

Samples only need to be small in volume. It is vital that samples should be kept aerobic. A 5 ml sample is usually sufficient and this should be placed in a 25-50 ml tube for transport. A small sample in a comparatively large container is likely to remain aerobic for longer periods than larger samples. Normally, samples should be examined as quickly as possible although it has been shown that small samples can be sent through the post and examined the following day without significant change in the ciliate species content.

iv) Size of sample to be examined

Small samples of the primary sample should be put on a microscope slide and covered with a glass cover slip. This and subsequent slides should be scanned until no further species appear. Usually 3-5 slides are sufficient. Normally you are only attempting to identify the most common species in the sample as these will be those making up the majority of the ciliates present.

If you are carrying out quantitative studies then you will have to use a glass cytometer similar to those used to count blood cells (haemocytometer). There are several different types available and you should read the research literature for

further advice. However, Augustin et alia (1989) calculated that counting five 10 microlitre samples of mixed liquor was sufficient to recover 85% of the ciliates present. Significant further effort was required to detect 94% of ciliate species. We would recommend that their methods be applied for quantitative examinations.

b). Effluent quality prediction methods

Proper use of the identification program will result in a list of ciliate species present in the sample. Each species has an effluent quality rating associated with it so that effluent prediction can be carried out automatically. Ratings based on previously published experience are supplied: the developers are considering how these data can be supplemented to the user’s local conditions by input of the actual 5-day Biochemical Oxygen Demand value on the results data base. For instance, from time to time your results might be used to update the effluent quality ratings so that, after some time, your program will become more and more tuned to your own operating conditions and you could share and compare data with other operators at other sewage works.

Full-scale sewage treatment works are usually designed and operated to produce an effluent quality to meet locally imposed standards and your plants should not normally vary too much. This means that you will accumulate a lot of data within narrow quality limits. In order to obtain data which could be of value for identifying potential problems then the following methods could be used.

If you have access to an RBC plant then sampling from discs along the disc will reveal ciliate populations that change in a predictable manner. Remember to take samples of the sewage flow immediately below the discs sampled. These should be allowed to settle and the supernatant analyzed for BOD. You will find that the BODs of these samples will improve from input to output and you will be able to associate the ciliate populations identified with different BOD’s. Figure 18 represents a sequence different ciliate populations that may be identified down the length of a RBC with a series of seven banks of discs. The height of the cones shown represents the relative population density of each species on each of the discs. In this case, *Colpidium* and *Colpoda* are found mainly in the sewage input end of the disc train whereas *Coleps* is found at the effluent output end. Other species are more common in the mid-regions. Once you have established the normal sequence of ciliates in your own treatment plant then you will be in a position to monitor it. For example, if the baffles are not correctly positioned plug-flow conditions will not be achieved due to back-mixing and this will be reflected by the ciliates species being out of sequence. Similarly if the organic loading increases then the sequence of ciliates will react by moving down towards the effluent end. Species which live under adverse conditions will appear at the input end, and species that thrive under better conditions either move further down or are washed out. If you do not have access to an RBC then you can immerse artificial substrates in the mixed-liquor of an activated-sludge plant and carry out the same procedures as above.

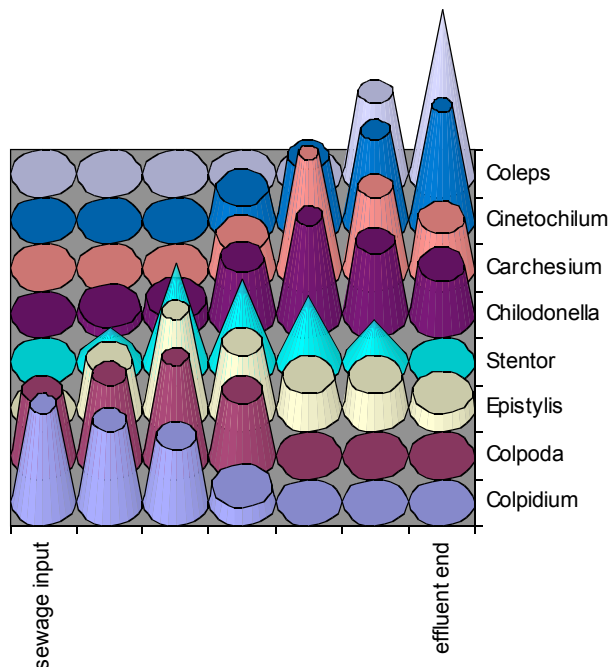


Fig 18. a sequence different ciliate populations that may be identified down the length of a RBC with a series of seven banks of discs.

6 Additional rare species not included in keys

We have attempted to include all species most commonly found in aerobic sewage-treatment processes in the keys. However, we are aware that other species have also been described that have not been included in the main body of our work. This is inevitable since some species are newly reported to occur in sewage treatment processes, some misidentifications have been corrected and some species new to science have been described we originally compiled the species list. To keep the work as comprehensive as possible we have included a list of other species reliably known to occur in these processes below together with diagrams, sizes and references. It should be pointed out however that all of these are likely to be comparative rarities and in many cases their saprobity value and significance are unknown.

***Anteholosticha mancoidea* (Hemberger, 1985) Berger, 2003**

Body very flexible, elongate with parallel margins, 120 x 20 – 30 µm, usually with eight macronuclear nodules that form an irregular longitudinal row.

Hemberger, H. (1985). Neue Gattungen und Arten hypotricher Ciliaten. *Archiv für Protistenkunde*, **130**: 397-417.

Berger, H. (2003). Redefinition of *Holosticha* Wrzesniowski, 1877 (Ciliophora, Hypotricha). *European Journal of Protistology*, **39**: 373-379.

Martin-Cereceda, M., Pérez-Uz, B., Serrano, S. and Guinea, A. (2002). An integrated approach to analyse biofilms of a full scale wastewater treatment plant. *Water Science and Technology*, **46**: 199-206.

Berger, H. (2006). *Monograph of the Urostyloidea (Ciliophora, Hypotrichia)*. Monographiae Biologicae, Vol. 85, Kluwer Academic Press, Dordrecht

***Colpidium kleini* Foissner, 1969**

This species closely resembles *Colpidium colpoda* which is included within the keys. The diagram in Foissner and Berger (1996), illustrates the differences between the two species. *Colpidium kleini* tends to be smaller and slimmer than *Colpidium colpoda*. Other illustrations can be found in Ganner and Foissner (1989).

Foissner, W. (1969). Ein neue Art aus der Gattung *Colpidium* (Stein, 1860): *Colpidium kleini* sp. n. (Hymenostomatida, Tetrahymenidae). *Acta Protozoologica*, **7**: 17-23

Foissner and Berger (1996). A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater Biology*, **35**: 375-482.

Ganner, B. and Foissner, F. (1989). Taxonomy and Ecology of some ciliates (Protozoa, Ciliophora) of the saprobic system. III. Revision of the genera *Colpidium* and *Dexiostoma*, and establishment of a new genus, *Paracolpidium* nov. gen.. *Hydrobiologia*, **182**: 181-218.

***Chaetospira muelleri* Lachmann, 1856**

This the only loricate ciliate with cirri (hypotrich) found in sewage-treatment processes. It is unlike any other loricate ciliate included in the keys. It is quite large attaining a length of 200- 300 µm.

Lachmann, C.F.G. (1856). Ueber die Organisation der Infusorien, besonders der Vorticellen. *Archiv für Anatomie und Physiologie und Wissenschaftliche Medicin. Leipzig: Jahr 1856*: 340-398.

Song, W. and Wilbert, N. (1989). Taxonomische Untersuchungen an Aufwuchsciliaten (Protozoa, Ciliophora) im Poppelsdorfer Weiher, Bonn. *Lauterbornia*, **3**: 2-221.

***Cyrtolophosis elongata* (Schewiakoff, 1892) Kahl, 1931**

Small (about 25µm long), elongated oval in outline shape. Not associated with a lorica and may be distinguished from *C. mucicola* by the terminal position of the contractile vacuole.

Foissner, W. (1993). Class Colpodea (Ciliophora). *Protozoenfauna*. (Ed. Matthes, D). G. Fischer, Stuttgart, Vol4/1, 798 pp.

Kahl, A. (1931). Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha ausser den im 1. Teil behandelten Prostomata. *Die Tierwelt Deutschlands*, **21**: 181-398.

Schewiakoff, W. (1892) Ueber die geographische Verbreitung der Susswasser-Protozoen. *Verhandlungen des Naturhistorisch-Medizinischen Vereins zu Heidelberg, (N.S)*, **4**: 544-567.

***Cyrtolophosis mucicola* Stokes, 1885**

Small (20-30 µm long) oval, loricate form. The lorica is gelatinous. Note the subterminal position of the contractile vacuole, not terminal as in *C. elongata*.

- Foissner, W. (1987). Neue und wenig bekannte hypotriche und colpodide Ciliaten (Protozoa, Ciliophora) aus Boden und Moosen. *Zool. Beitr. (N.F.)*, **31**: 187-282
- Foissner, W. (1993). Class Colpodea (Ciliophora). *Protozoenfauna*. (Ed. Matthes, D). G. Fischer, Stuttgart, Vol4/1, 798 pp.
- Stokes, A.C. (1885). Some new infusoria. *American Naturalist*, **19**: 433-443

***Dexiotricha granulosa* (Kent, 1881) Foissner et al. 1994**

This species closely resembles *Dexiotricha tranquilla* which is included in the keys. It is about the same size (50-60 µm long) but uniquely is full of dense inclusions. Careful examination shows these to be ring-like in shape. It has been reported from activated sludge and RBC's (Salvado et al. 2004)

- Foissner, W., Berger, H. and Kohmann, F. (1994). *Taxonomische und Ökologische Revision der Ciliaten des Saprobiensystems. Band III: Hymenostomata, Prostomatida, Nassulida.* Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft. **Band III**: 1- 483
- Kent, W.S. (1881). *A manual of the Infusoria: including a description of all known flagellate, ciliate, and tentaculiferous protozoa British and foreign, and an account of the organization and affinities of the sponges.* David Bogue, London, **Vol II**: 433-720
- Salvado, H., Palomo, A., Mas, M., Puigagut, J. & Gracia, M. D. (2004). Dynamics of nematodes in a high organic loading rotating biological contactors. *Water Research*, **38**: 2571-2578.

***Endosphaera engelmanni* (Engelmann, 1876) Dovgal, ??**

Endosphaera engelmanni (syn.*tenebrans*) – Igor Dovgal made *tenebrans* a synonym of *E.engelmanni*

Lives intracellularly within sessile peritrichs such as *Vorticella*, *Epistylis*, *Opercularia*. Intracellular stage 5 – 45 x 9 – 35 µm, sac-like with constriction at anterior end and rounded posterior end, with very large, spherical macronucleus. Swarmer stage spherical, 15 – 17 µm in diameter, with a needle-like projection that is 7 – 8 µm long.

- Engelmann, T.W. (1876). Ueber Entwicklung und Fortplanzung von Infusorien. *Morphologisches Jahrbuch*, **1**: 573-634.
- Esteban, G., Téllez, C. and Muñoz, A. (1991). Infraciliature, morphogenesis and life cycle of *Endosphaera terebrans* (Suctoria, Tokophoridae). *Journal of Protozoology*, **38**: 483-488.
- Dovgal, I. ??

***Epistylis bimarginata* Nenninger, 1948**

Zooid 66 – 93 x 30 – 42 µm with a C-shaped macronucleus that lies transversely in the upper half of the cell. The CV is dorsally located. The main distinguishing character is the double peristomial lip, the upper fold being slightly narrower than the lower fold.

- Nenninger, U. (1948). Die Peritrichen der Umgebung von Erlangen mit besonderer Berücksichtigung ihrer Wirtsspezifität. *Zoologische Jahrbücher Systematik*, **77**: 169-266
- Konstanynenko, L.A. (2007). The Peritrichia (Ciliophora, Peritrichia) in the activated sludge tank of a sewage treatment plant from *Zhytomyr*. *Vestnik Zoologii*, **41**: 169-174 (in Ukrainian with English summary)

***Epistylis thienemanni* (Nenninger, 1948) Konstanynenko, 2007**

Although originally described by Nenninger (1948) as *Rhabdostyla thienemanni*, Konstanynenko (2007) noted that this species is colonial and transferred it to the genus *Epistylis* (although it was mis-spelt as “*thinemanni*”). Zooid slightly constricted below the peristomial lip, 60 – 105 x 21 – 45 µm with a C-shaped macronucleus that lies transversely in the upper half of the cell. The CV is dorsally located just below the umbilicate peristomial disc.

- Nenninger, U. (1948). Die Peritrichen der Umgebung von Erlangen mit besonderer Berücksichtigung ihrer Wirtsspezifität. *Zoologische Jahrbücher Systematik*, **77**: 169-266
- Foissner, W., Berger, H. and Kohmann, F. (1992). *Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band II: Peritrichia, Heterotrichida, Odontostomatida.* Informationsbereiche des Bayer. Landestamtes für Wasserwirtschaft, 1-502.
- Konstanynenko, L.A. (2007). The Peritrichia (Ciliophora, Peritrichia) in the activated sludge tank of a sewage treatment plant from *Zhytomyr*. *Vestnik Zoologii*, **41**: 169-174 (in Ukrainian with English summary)

***Gastronauta aloisi* Oberschmidleitner and Aeschl, 1996**

Body elliptical in outline, dorsoventrally flattened with a domed dorsal surface, 50-70 x 40 µm. Single macronucleus, globular to ovoid in shape.

Oberschmidleitner, R. and Aescht, E. (1996). Taxonomische Untersuchungen über einige Ciliaten (Ciliophora, Protozoa) aus Belebtschlämmen oberösterreichischer Kläranlagen. *Beitrage zur Naturkunde Oberösterreich*, **4**: 3-30.

***Gastronauta membranaceous* Buetschli, 1899**

This is a broadly oval, dorso-ventrally flattened ciliate. About 50-70 µm long.

Blochmann, F. (1895). *Die mikroskopische Thierwelt des Süßwassers. Abteilung I: Protozoa*. 2. Aufl. Lucas Grafé & Sillem, Hamburg. XV + 134 pp.

Buestchli, O (1887-1889). *Protozoa Abt III. Infusoria und der Radiolaria*. In. Bronn, H.G. (ed). *Klassen und Ordnung des Thiers-Reichs*,. C.F. Winter, Leipzig. **Vol I**, pp. 1098-2035.

Kahl, A. (1931). *Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha ausser den im 1. Teil behandelten Prostomata*. *Die Tierwelt Deutschlands*: **21**: 181-398.

Patsch, B. (1974). Die Aufwuchsciliaten des Naturlehrparks Haus Wildenrath. Monographische Bearbeitung der Morphologie und Ökologie. *Arbeiten aus der Institut der Landwirtschaft. Zoologie. Bienenkunde* : **1**: 1-82.

***Heliophrya rotunda* (Hentschel, 1916) Matthes, 1954**

Heliophrya minima has been included in the key and differs from *Heliophrya rotunda* in the arrangement of its tentacles. The tentacles of *Heliophrya rotunda* are arranged in groups or bundles (fascicles) whereas those of *H. minima* are arranged singly around the edge of the flattened body. About 30-80 µm in diameter.

Hentschel, E. (1916). Biologische Untersuchungen über den tierischen und pflanzlichen Bewuchs im Hamburger Hafen. *Mitt. Zool. Mus. Hamb.*, **33**: 1-172

Matthes, D. (1954). Die Gattung *Heliophrya* Saedeleer & Tellier 1929. *Archiv für Protistenkunde*, **100**: 143-152.

Saedeleer, H. De., and Tellier, L.(1930). *Heliophrya collini* n. g., n. sp., acinetien d'eau douce. *Annals Soc Royal Zoolog Belgique*, **60** (yr. 1929): 12-15.

Holosticha mancoidea

Oberschmidleitner, R. and Aescht, E. (1996). Taxonomische Untersuchungen über einige Ciliaten (Ciliophora, Protozoa) aus Belebtschlämmen oberösterreichischer Kläranlagen. *Beitrage zur Naturkunde Oberösterreich*, **4**: 3-30.

***Litonotus varsaviensis* (Wrzesniowski, 1866) Wrzesniowski, 1870**

About 110 µm long. As in *Litonotus fusidens* the extrusomes (trichocysts) are found only in the neck region and the posterior end is broadly rounded without a tail-like region. Unlike *L. fusidens* however, *L. varsaviensis* has two lateral contractile vacuoles not just one.

Foissner, W. (1984). Taxonomie und Ökologie einiger Ciliaten (Protozoa: Ciliophora) des Saprobien-systems. I: Genera *Litonotus*, *Amphileptus*, *Opisthodon*. *Hydrobiologia*, **119**: 193-208.

Wrzesniowski, A. (1866) Verzeichnis der Infusorien, welche in Warschau und seiner Umgebungen von 1861-65 gesammelt wurden.. *Wykaz Szkoły Główniej Warszawskiej*, **No. 5**: 15-28.

Wrzesniowski, A. (1870). Beobachtungen über Infusorien aus der Umgebung von Warschau. *Zeitschrift für Wissenschaftliche Zoologie*. **20**: 467-511.

Metopus setifer

***Metopus setosus* (Kahl, 1927) Esteban et al., 1995**

Body 60 – 90 µm long, wide at anterior end, narrowing posteriorly. Single ovoid macronucleus in mid-body region and a terminal CV.

Kahl, A. (1927). Neue und ergänzende Beobachtungen heterotricher Ciliaten. *Archiv für Protistenkunde*, **57**: 121-203.

Esteban, G., Fenchel, T. and Finlay, B. (1995). Diversity of free-living morphospecies in the ciliate genus *Metopus*. *Archiv für Protistenkunde*, **146**: 137-164.

***Microthorax pusillus* Engelmann, 1862**

Laterally compressed, dorsal surface (edge) strongly convex, ventral edge slightly concave. Size 20-35 µm long.

Engelmann, T. W. (1862). Zur Naturgeschichte der Infusionsthier. *Zeitschrift für Wissenschaftliche Zoologie*. :11: 347-393.

Foissner, W., Berger, H., and Kohmann, F. (1994). *Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band III: Hymenostomatida, Prostomatida, Nassulida.* Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft. 548 pp.

Kahl, A. (1931). *Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha ausser den im 1. Teil behandelten Prostomata. Die Tierwelt Deutschlands: 21:* 181-398.

Leitner, A. R. and Foissner, W. (1997). Morphology and infraciliature of *Microthorax pusillus* Engelmann 1862 and *Spathidium deforme* Kahl 1928, two ciliates (Protozoa, Ciliophora) from activated sludge. *Linzer Biologische Beiträge*, **29**: 349-368.

***Opisthnecta matiensis* Martin-Cereceda et al., 1999**

Body widest in equatorial region, 45 – 72 x 25 – 40 µm with sausage-shaped macronucleus lying parallel to longitudinal axis of cell.

Martin-Cereceda, M., Serano, S and Guinea, A. (1999). Description of *Opisthnecta matiensis* n. sp. (Protozoa, Ciliophora), a new peritrich ciliate from wastewater. *Journal of Eukaryotic Microbiology*, **46**: 283-289

***Oxytricha lanceolata* Shibuya, 1930**

Body 90 – 100 x 30 – 50 µm, elliptical in outline, rounded at both ends, with two ellipsoidal macronuclear nodules.

Shibuya, M. (1930). Ciliates found in soils from some parts of Japan. Journal of the Imperial Agricultural Experimental Station. *Nishigahara*, **1**: 199-214 (in Japanese with English summary)

Berger, H. (1999). Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). *Monographiae Biologicae*, Vol. 78, Kluwer Academic Press, Dordrecht

Martin-Cereceda M., Perez-Uz B., Serrano S. and Guinea A. (2001). Dynamics of protozoan and metazoan communities in a full scale wastewater treatment plant by rotating biological contactors. *Microbiological Research*, **156**: 225-238.

***Parentocirrus brasiliensis* Paiva and da Silva-Neto, 2004**

Body elliptical in outline, 110 x 75 µm, with conspicuous AZM, two ventral and two marginal rows of rows, a single CV near the left margin and 4-6 macronuclear nodules.

Paiva, T.D. and da Silva-Neto, I.D. (2004). Description of *Parentocirrus brasiliensis* sp. n. (Ciliophora: Spirotrichea), a new ciliate protist present in activated sludge. *Zootaxa*, **504**: 1-10.

***Pseudoblepharisma tenue* (Kahl, 1926) Kahl, 1927**

Elongate ciliate with short anterior adoral zone of membranelles. About 100-200 µm long.

Kahl, A. (1926). Neue und wenig bekannte Formen der holotrichen und heterotrichen Ciliaten. *Archiv für Protistenkunde*: **55**: 197-438.

Kahl, A. (1927). Neue und ergänzende Beobachtungen holotricher Ciliaten. *Archiv für Protistenkunde*: **60**: 34-129.

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***Pseudomicrothorax agilis* Mermod, 1914**

Oval outline shape, dorso-ventrally flattened. with wide curved longitudinal stripes. About 30-70 µm long.

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Penard, E. (1922). *Etudes sur les infusoires d'eaux douces*. Georg et Cie, Geneve. 331pp.

***Pyxicola carteri* Kent, 1882**

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Kent, W.S. (1882). *A manual of the Infusoria: including a description of all known flagellate, ciliate, and tentaculiferous protozoa British and foreign, and an account of the organization and affinities of the sponges*. David Bogue, London, **Vol III**: 72-913.

***Spathidium deforme* Kahl, 1928**

Barrel-shaped to inversely pyriform, about 95 x 40 µm. Macronucleus near centre of body, variable in shape but usually oblong. CV large, terminally located.

Kahl, A. (1928) Die Infusorien (Ciliata) der Oldesloer Salzwasserstellen. *Archiv für Protistenkunde*, **19**: 50-123

Leitner, A.R. and Foissner, W. (1997). Morphology and infraciliature of *Microthorax pusillus* Engelmann 1862 and *Spathidium deforme* Kahl 1928, two ciliates (Protozoa, Ciliophora) from activated sludge. *Linzer biologische Beiträge*, **29**: 349-368

***Stentor muelleri* Ehrenberg, 1831**

Body usually 500 – 1,000 µm long, but can reach up to 3 mm, trumpet-shaped and often contained within a tube-like lorica. Macronucleus moniliform with 10-20 nodules. Lacks pigmented cortical granules.

Ehrenberg, C.G. (1831). Über die Entwicklung und Lebensdauer der Infusionsthier; nebst ferneren Beiträgen zu einer vergleichung ihrer organischen Systeme. *Abh. Dt. Akad. Wiss. Berlin*: year 1831: 1-154.

Foissner, W., Berger, H. and Kohmann, F. (1992). *Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band II: Peritrichia, Heterotrichida, Odontostomatida*. Informationsbereiche des Bayer. Landestamtes für Wasserwirtschaft, 1-502.

Foissner, W. and Wölfl, S. (1994). Revision of the genus *Stentor* Oken (Protozoa, Ciliophora) and description of *S. araucanus* nov. spec. from South American lakes. *Journal of Plankton Research*: 16, 255-289

***Thuricola follicata* Kent, 1881**

This is a large (240-420 µm long), loricate peritrich.. Another species of this genus, *Thuricola kellicottiana*, has already been included in the keys. They may be distinguished from each other by the presence of a long thin posterior stalk in *T. kellicottiana* (short and wide in *T. folliculata*), and by the presence of zoochlorellae in *T. folliculata* but not in *T. kellicottiana*.

Kent, W.S. (1881). *A manual of the Infusoria: including a description of all known flagellate, ciliate, and tentaculiferous protozoa British and foreign, and an account of the organization and affinities of the sponges*. David Bogue, London, **Vol II**: 433-720

Sommer, G. (1951). Die peritrichen Ciliaten des Grossen Ploner Sees. *Archiv für Hydrobiologie*, **44**: 349-440.

***Thuricola similis* Bock, 1963**

Lorica 158 – 248 µm long (mean 183 µm), cylindrical in shape and with somewhat irregular surface. Two zooids, one somewhat longer than the other with 30% - 50% of the zooid length extending beyond the aperture when feeding. Macronucleus vermiform.

Bock, K.J. (1963). Über das Auftereten einer *Thuricola* (Ciliaten, Peritricha) in Belebtschlamm. *Zoologica Anzeiger*, **171**: 91-96.

Trueba, F.J. (1980). A taxonomic revision of the peritrich ciliate genera *Thuricola* and *Pseudothuricola*. *Beaufortia* **30**: 125-138

Konstanynenko, L.A. (2007). The Peritrichia (Ciliophora, Peritrichia) in the activated sludge tank of a sewage treatment plant from Zhytomyr. *Vestnik Zoologii*, **41**: 169-174 (in Ukrainian with English summary)

***Urosomoides agiliformis* Foissner, 1982**

Body 80 - 100 x 20 – 30 µm, margins almost parallel, with two ellipsoidal macronuclear nodules.

Foissner, W. (1982). Ecology and taxonomy of the Hypotrichida (Protozoa: Ciliophora) of some Austrian soils. *Archiv für Protistenkunde*, **126**: 19-143

Berger, H. (1999). Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). *Monographiae Biologicae*, Vol. 78, Kluwer Academic Press, Dordrecht

Olmo, J.L. and Pérez-Uz, B. (2000). Morphology and morphogenesis of a Spanish population of *Urosomoida agiliformis* (Ciliophora, Hypotrichida) from a wastewater treatment plant. *Acta Protozoologica*, **39**: 117-123

***Vorticella peterhoffi* Banina, 1983**

Zooid inverted bell-shaped, 62 – 75 x 35 – 42 µm with a C-shaped macronucleus that lies in the upper half of the cell parallel with the longitudinal axis. The CV is dorsally located just beneath the thick peristomial lip.

Banina, N.N. (1983). Peritricha Sessilida fauna in activated sludge biocenosis. *Protozoologiy*, **a 8**: 87-116

Konstanynenko, L.A. (2007). The Peritrichia (Ciliophora, Peritrichia) in the activated sludge tank of a sewage treatment plant from Zhytomyr. *Vestnik Zoologii*, **41**: 169-174 (in Ukrainian with English summary)