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**STUDIES ON THE BIOSYSTEMATICS OF TRICHODINID  
CILIATES PARASITIC ON BRITISH FRESHWATER FISH**

A thesis presented for the degree  
of Doctor of Philosophy to the University of Stirling

By

William Hugo Gaze

Institute of Aquaculture  
University of Stirling  
Stirling, Scotland  
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## DECLARATION

I hereby declare that this thesis has been composed by myself and is the result of my own investigations. It has neither been accepted, nor has been submitted for any other degrees. All the sources of information have been duly acknowledged.

*William Gray*

## ABSTRACT

Of the numerous reports of trichodinids from British freshwater fish only four have been to species level, representing three *Trichodina* species and one *Trichodinella* species.

Twenty freshwater fish species were sampled from approximately sixty sites in Scotland, England and Wales. Skin and gill smears were prepared using Klein's silver staining technique, and morphometric measurements made from features of the adhesive disc taken from enlarged photomicrographs according to Lom's (1958) taxonomic criteria. The adoral cilia, used as a feature of generic classification were described for each trichodinid population from live preparations. Species discrimination and investigation of intraspecific morphological variation was aided by the use of Principal Components and Cluster analysis.

Thirteen trichodinid species were identified from British freshwater fish during this study including: *Trichodina acuta* Lom, 1961 from *Cyprinus carpio*, *Carassius auratus*, *Oncorhynchus mykiss*, and *Phoxinus phoxinus*; *Trichodina domerguei* Wallengren, 1897 from *Gasterosteus aculeatus*; *Trichodina tenuidens* Faure-Fremiet, 1944 from *Gasterosteus aculeatus*; *Trichodina pediculus* Ehrenberg, 1838 from *Gasterosteus aculeatus*; *Trichodina modesta* Lom, 1970 from *Abramis brama*; *Trichodina nigra* Lom, 1960 from *Cyprinus carpio*, *Salmo trutta* and *Oncorhynchus mykiss*; *Trichodina mutabilis* Kazubski & Migala, 1968 from *Phoxinus phoxinus*; *Trichodina rostrata* Kulemina, 1968 from *Phoxinus phoxinus*; *Trichodina intermedia* Lom, 1960 from *Phoxinus phoxinus*; *Trichodinella epizootica* Raabe, 1950 from *Perca fluviatilis* and *Oncorhynchus mykiss*; *Tripartiella lata* Lom, 1963 from *Phoxinus phoxinus*; *Tripartiella copiosa* Lom, 1959 from *Phoxinus phoxinus* and *Paratrachodina*

*incisa* Lom, 1959 from *Phoxinus phoxinus*, *Abramis brama* and *Rutilus rutilus*. Many of the previous species demonstrated considerable inter and intrapopulational morphological variation.

Immature specimens of *Trichodina acuta*, *Trichodina domerguei*, *Trichodina tenuidens* and *Trichodina intermedia* were examined, elucidating developmental morphology and its taxonomic relevance.

A study of intraspecific morphological variation was undertaken by sampling trichodinids from a population of *Gasterosteus aculeatus* over a twelve month period. Regression analysis indicated statistically significant negative correlations between water temperature and measurements of the adhesive disc in *Trichodina domerguei* and *Trichodina tenuidens*. Principal Components Analysis was utilised to differentiate extreme morphological variants of the two previous species.

A sonication technique modified from Shinn *et al.* (1993) was used to isolate skeletal structures of the adhesive disc from *Trichodina domerguei*, *Trichodina intermedia*, *Paratrichodina incisa* and *Trichodinella epizootica* for scanning electron microscopy. New structures which were previously undescribed are discussed, including denticle apophyses, denticular pins and a small peg-like structure on the central parts of the denticles. Actual denticle structure is compared to that visualised by silver staining using light microscopy, and its implications for generic classification of the trichodinids is commented upon.



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## CHAPTER 1. GENERAL INTRODUCTION

Trichodinids belong to a group of unicellular organisms (protozoa) known as the ciliates which are distinguished by a variety of characteristics including the possession of two types of nuclei, sexual conjugation, the possession of a ciliated pellicle and binary fission (Sleigh, 1991). Ciliate classification has been revised several times this century.

Corliss (1961) revised the classification of the ciliates, embracing the whole group within a single class (Ciliata Perty, 1852) for the first time. The suborder Mobilis containing the trichodinids was replaced with Mobilina Kahl, 1933; and the order Peritricha was redesignated Peritrichida Stein, 1859; which was placed within the subclass Holotricha Stein, 1859. This revision was incorporated in the Society of Protozoologists revised classification of the phylum Protozoa (Honigberg *et al.*, 1964 in: Cox, 1991).

A further revision of the protozoans sanctioned by the Society of Protozoologists was published in 1980 by Levine *et al.*, which recognised seven phyla within a subkingdom Protozoa raising the ciliates (Ciliophora Doflein, 1901) to a separate phylum. This system used by Lom & Dykova (1992), places trichodinids in the suborder Mobilina; order Peritrichida; subclass Peritrichia; class Oligohymenophora de Puytorac *et al.*, 1974. Subsequent revisions of protozoan classification have resulted in single celled eukaryotic organisms being regarded as a distinct Kingdom, the Protista (Margulis & Schwartz, 1988). Other authors have distributed them among new Kingdoms (e.g., Leedale, 1974; Lipscom, 1989 in: Cox, 1991). Cox (1991) considers Levine's classification to be obsolete, preferring the classification of Sleigh (1989) based on the work of Small & Lynn (1985). In this system trichodinids belong to the order

Mobilida; subclass Peritrichia; class Oligohymenophorea; subphylum Cryptophora.

Corliss (1961) placed all the mobiline peritrichs in one family, the Urceolariidae Dujardin, 1841 and considered the family Trichodinidae synonymous with the latter. Thus, many authors have referred to trichodinids as urceolarids. The Mobilina was later (Corliss, 1979 in: Sirgel, 1983) divided into five families: Urceolariidae, Leiotrochidae Johnston, 1938; Polycyclidae Poljansky, 1951; Trichodinopsidae Kent, 1881; and Trichodinidae Claus, 1874. Only the Trichodinidae possess complex denticles with blades and rays. Lom & Dykova (1992) give the authority for the family Trichodinidae as Raabe, 1959 as this author defined the family as it is now understood.

Six trichodinid genera have been reported ecto-commensal or parasitic on fish.

Key to trichodinid genera occurring in fish (from Lom & Dykova, 1992):

- 1.a. The adoral ciliary spiral makes two and a half to three turns.....*Vauchomia*
  - b. The adoral spiral makes approximately one turn (330°-540°).....2
  - c. The adoral spiral makes one half to three quarters of a turn (150°-290°).....3
- 2.a. The denticles have well developed rays and blades.....*Trichodina*
  - b. The blades of the denticles are stunted.....*Hemitrichodina*
- 3.a. The denticles have well developed rays.....4
  - b. The rays are stunted to form short crooks or platelets.....*Trichodinella*
  - c. No rays at all, central part indistinct, blades triangular.....*Dipartiella*
- 4.a. The centrifugal projections of denticles (blades) are attached to the central part almost perpendicularly and the denticles are inter-locked only by their central conical parts.....*Paratrachodina*
  - b. Blades extend from the central part obliquely backwards; denticles are

interlocked by central parts and by anterior projections of blades fitting into corresponding notches in blades of the preceding denticles.....*Tripartiella*

One of the most recent summaries of trichodinid species (Bradbury, 1994) follows the classification of Small & Lynn (1985). Only the genera *Trichodina* and *Vauchomia* are recognised, with the other genera in Lom's key amalgamated with *Trichodina*.

During this study the classification of Levine *et al.* (1980) will be adhered to, because this revision is the most recent undertaken and published by the Society of Protozoologists. It is also advantageous to follow this system for reasons of compatibility with recent publications in the area of trichodinid research. Under the system of Levine *et al.* trichodinids are classified within the subphylum Ciliophora as follows:

#### Class OLIGOHYMENOPHOREA:

The oral and somatic cilia are distinct, and consist of the paroral membrane (haplokinety) to the right side of the opening of the buccal cavity and usually three membranelles or peniculi (polykinety) to the left. The cytostome at the bottom of the buccal cavity opens into an inconspicuous cytopharynx. The body is either uniformly covered with cilia or may be localised. Some species are cyst forming, some loricate and others colonial.

#### Subclass PERITRICHIA

Body bell-shaped, conical or cylindrical. Prominent buccal ciliature dipping into the infundibulum. Somatic ciliature is reduced to one temporary ring of locomotor cilia

(tetroch larvae of sessilinids) or three permanent rings (mobilinids). Many species stalked or sedentary, others mobile, all with aboral scopula or holdfast organelle. Myonemes associated with strong contractile properties of stalk or part of body. Conjugation total, involving fusion of micro- and macro- conjugants.

#### Order PERITRICHIDA

With characters of subclass.

#### Suborder MOBILINA

Mobile organisms usually in the form of a flattened disc or hemisphere, similar to the migratory tetroch larvae of sessilinids. Complex attachment apparatus at aboral end, often with highly distinctive denticulate ring. Conjugation of unequally sized specimens rare. No cyst formation, transmission direct. Epizoic or endozoic on a variety of aquatic animals (some terrestrial with aquatic ancestry), fish are infected by a single family the Trichodinidae (discussed in Chapters 2 and 3).

Trichodinids have a saucer to bell shaped body, ranging in diameter from 140 $\mu$ m in *Trichodina truttae* Mueller, 1937 (Wellborn, 1967) to 22 $\mu$ m in *Trichodinella myakkae* Mueller, 1937 (Hoffman, 1978). The adhesive disc is the main taxonomic feature of trichodinids, and has been the focus of many detailed studies (see Chapters 2 and 3). The main component of the adhesive disc is the denticle ring, made up of a species specific number of denticles. This remarkable structure has been likened to the spinal column in vertebrates (Van As and Basson, 1990) giving strength and flexibility to the adhesive disc. The fine structure of the adhesive disc is discussed in Chapter 4. The

locomotor and oral cilia are located in two areas of the cell. The adoral ciliary spiral winds in an anti-clockwise direction around the adoral end of the organism and terminates in the buccal cavity. The number of degrees this turns constitutes an important taxonomic feature. In addition, trichodinids possess an aboral wreath of cilia known as the locomotor fringe or trochal band (Lom, 1958).

Trichodinid morphology is subject to variation within species, populations and even direct descendants. This subject is one of the main problems in trichodinid systematics, and is discussed in detail in Chapters 2, 3, 4 and 6. Morphological variation in protozoa due to environmental factors has long been a matter of interest. Kazubski (1982a) refers to work on this subject by Reynoldson (1950), Kazubski & Migala (1968), and Gold & Morales (1975). In all cases the observed variation was related to seasonal temperature changes. Lom & Stein (1966) speculate whether the host can have a direct effect upon trichodinid morphology. "Different features of *Trichodina tenuidens* Faure-Fremiet, 1944 populations from individual sticklebacks are certainly environment induced, probably resulting from the interaction between the influence of the host (chemical action) upon the ciliate and the ability of the ciliate to react sensitively to fine changes of its microbiotope".

The first recognisable description of a trichodinid was contained in letters sent to the Royal Society of London by Anthony Van Leeuwenhoek between 1673 and 1723 (Finley, 1969). Since Ehrenberg's first description of *Trichodina pediculus* in 1838 approximately 150 trichodinid species parasitic on fish have been adequately described (Lom & Dykova, 1992). Many more inadequate descriptions or synonyms of existing species are present in the literature. Unfortunately the discovery of a new parasite on a new host and recognition of a slight morphological deviation from other species

known to the researcher, have often been considered sufficient reasons for erecting a new species (Lom, 1970a). The inexact and insufficient descriptions of most early authors are so general that they could apply to any number of different currently recognised species. Dogel (syn. Dogiel) (1940 in: Lom, 1958) was the first author to use a set of principal characters for determination of a new species. Lom (1958) proposed additions and revisions to Dogel's criteria, which remain in use today. While the systematics of the Trichodinidae has been significantly advanced since the late 1950's, with many new species being recognised the problem remains as to the degree of intra-specific variation present. New species are still being created on the grounds of tiny morphological deviations from existing species, when these variations may be well within the normal range of morphological variation. Trichodinid taxonomy is discussed in detail in Chapters 2 and 3.

Trichodinids reproduce asexually and sexually, by binary fission and conjugation respectively (Lom & Dykova, 1992). Adhesive disc morphology associated with reproduction is discussed in Chapter 5. The two daughter cells resulting from binary fission are identical to the parent, except that they are half the size and possess half the number of skeletal elements. Ahmed (1977) states that Diller (1928) described the nuclear changes in a *Trichodina* species as endomixis (the development of a new micronucleus without karyogamy). However, the endomictic stages described by Diller conform closely to the exconjugative stages in *Trichodina reticulata* Hirschmann & Partsch, 1955 observed by Ahmed (1977) and it was therefore postulated that Diller misinterpreted exconjugation as endomixis.

Transmission in ectoparasitic trichodinids inhabiting the skin and gills is via free swimming individuals or by direct contact of host species (Lom & Dykova, 1992). Due

to the fact that many trichodinids have several hosts, different fish species can act as vectors between separate populations of the same parasite species. Bykhovskaya-Pavlovskaya *et al.* (1962) records sticklebacks acting as vectors for *Trichodina latispina* (syn. *Trichodina domerguei* Wallengren, 1897) between young salmon. Endoparasites of the urinary bladder are probably dispersed via the flow of urine, when settled on a new host they gain access via the exterior urinary aperture (Hoffman, 1978). One interesting trichodinid species, *Trichodina oviducti* Poljansky, 1951 of the thorny skate, *Raja radiata* Donovan, 1807, is thought to be venereally transmitted (Khan, 1972). In the male, infections occur along the seminal groove of the claspers, urogenital sinus, rectum and rectal sac. In females infections occur in the copulatory sac, rectum, rectal gland and urinary sinuses. Infections only occur in sexually mature individuals, which is a strong indication of a venereal mode of transmission.

There is a limited amount of literature regarding the behaviour of trichodinids. To date, only locomotory, attachment and possibly feeding behaviours have been observed. When swimming freely (Ahmed, 1977) trichodinids are orientated with their aboral surface forward. This broadside method of swimming enables the ciliate to affix itself to any new host without delay; the structure used whilst moving in this way is likely to be the locomotor fringe (ciliary wreath). Trichodinids are very active organisms, on the surface of the host they can be seen constantly rotating in an anti-clockwise direction whilst moving and when stationary. Salas (1991) observed that in *Trichodina acuta* Lom, 1961 rotation took 2.3-12.5 (mean=8.3±2.94) seconds. The anti-clockwise rotation observed is in the same direction as the adoral ciliary spiral, for this reason the direction of rotation could enhance the feeding current produced by the adoral cilia. When a trichodinid rotates on the spot the surrounding mucus covering the



hosts epithelium can be seen to move as well, following the direction of the metachronal movement of the cilia (Salas, 1991). This would support the idea that mucus is part of the ciliate's diet. Trichodinids must almost certainly show chemotaxis in order to distinguish areas on the host, host species or even host phyla. Smith (1929 in: Lom, 1969) stated that *Trichodinella* may be attracted to the gills by urea and other nitrogenous products diffusing through the gill platelets. O'Rourke (1961) reported that gill and skin mucus have a different composition of blood antigens which might enable parasites to distinguish between these sites. It was also suggested that each species of fish appears to secrete its own specific mucus.

The question of the site of infection of trichodinids on the host is interesting. Van As & Basson (1987) categorised trichodinids into four different groups based on site preference. The first group are opportunistic by nature, and were always found on the skin and never the gills. The second group parasitises a wide range of fish hosts, where they are found predominantly on the skin, but also on the gills. The third group are predominantly gill parasites, but were occasionally found on the skin. The last group are found only on the gills of their hosts, displaying a varying degree of host specificity, with some species restricted to certain genera or even to a single host species. Host specificity in trichodinids appears highly variable, with species such as *Trichodina acuta*, *Trichodina fultoni* Davis, 1947, *Trichodina pediculus* and *Trichodina nigra* Lom, 1961 infecting a large number of host species (Lom, 1970a) and species such as *Trichodina anguilli* Wu, 1961, *Trichodina intermedia* Lom, 1961, *Trichodina tenuidens* and *Trichodina janovice* Lom, 1961 parasitising only one or two host species (Lom, 1970a).

Trichodinids are a widely geographically dispersed group. During the early years

of research into trichodinids, the observation of a population in a new geographical location was enough for a new species name to be created (Lom & Hoffman, 1964). As research in the field progressed and means of species identification were enhanced (Lom, 1958), it became obvious that the same species were occurring throughout continents. Arthur & Lom (1984) surveyed the trichodinids of the Rybinsk Reservoir, USSR; "the investigation confirmed the widespread distribution of several ectozoic trichodinid species throughout the European region". Trichodinid species also occur on different continents (Lom & Hoffman 1964), *Trichodina fultoni* and *Trichodina nigra* have been found on different hosts in North America and Europe. *Trichodina pediculus* has been observed on diverse hosts from Asia, Europe and North America (Lom, 1970a). *Trichodina nigra*, *Trichodina mutabilis* Kazubski, 1968, *Trichodina acuta*, *Trichodina fultoni*, *Trichodina heterodentata* Duncan, 1977 and *Trichodinella epizootica* Raabe, 1950 can be found in Europe, Asia and Africa (Lom & Dykova, 1992). *Trichodina californica* Davis, 1947 and *Trichodina truttiae* have been observed in the U.S.A and U.S.S.R (Wellborn, 1967). However, populations of the same species in different geographical areas may display considerable interpopulational morphological variation (Kazubski, 1982b). The transportation of fish species with importance to aquaculture or the ornamental fish trade has undoubtedly contributed to the distribution of trichodinid species (Albaladejo & Arthur, 1989; Van As & Basson, 1989). The latter authors reported that the translocation of cichlids from southern Africa to the Middle and Far East resulted in at least one confirmed case of trichodinid translocation, ie. *Trichodina centrostrigata* Basson, Van As & Paperna, 1983. Lom & Hoffman (1964) also reported that *Trichodina reticulata* has been transported along with its goldfish host from Asia, to both Czechoslovakia and North America. Albaladejo & Arthur (1989)

surveyed cyprinid fish species imported into the Philippines for trichodinids of which ten species were found. Of these, nine were previously found in the Philippines, three had been previously recorded as imports, and one species was previously unrecorded. Host translocation cannot be blamed for all cases of wide geographic distribution, as trichodinid species were recorded from different continents before any fish imports or exports occurred (Lom & Hoffman, 1964).

The relationship between host, parasite and environment is a delicate one (Sanmartin Duran *et al.*, 1991), a balanced relationship leads to good health and growth of the host and a stable parasite population. A marginal relationship leads to chronic disease of the host and proliferation of parasites, which can often occur during intensive cultivation resulting in damaging epizootics. The ability of trichodinid populations to undergo rapid proliferation is well known. Lom (1959) found that if a perch, *Perca fluviatilis*, was left in a small container at room temperature for 7-10 days, the intensity of infection of *Trichodinella epizootica* on the gills increased enormously. Lom (1973) carried out another experiment where fish were kept at a high population density with insufficient food. The fish, concomitantly infected with *Oodinium pillularis*, were covered by a thick layer of trichodinids. After two months the fish became emaciated and were covered by a thick layer of whitish mucus, disintegrated epithelial cells and protozoa causing significant mortalities. Half of the fish were transferred into a tank with "model" conditions. Within a short time the invasion disappeared, six weeks later the fish were healthy again. Thus, proliferation was caused by a change in environmental parameters, or in host physiology caused by such changes.

Seasonal fluctuations of trichodinid populations are known to occur. Sanmartin Duran *et al.* (1991) recorded a sharp increase in the numbers of a *Trichodina* sp. on

farmed turbot, *Scophthalmus maximus* L., from April to August. Calenius (1980) recorded a dramatic increase in the number of *Esox lucius*, *Lota lota* and *Gymnocephalus cernua* infected with *Trichodinella epizootica* during the period March to June in Finland. This could be due to an increase in water temperature associated with the time of year. The protozoan invasion of fish is far more dependent on ecological conditions (temperature, oxygen concentration, feeding habits etc) than in terrestrial animals (Lom, 1969). Changes in these conditions may lead to an increased degree of infection by changing the rate of the parasite's development, as in infections of *Ichthyophthirius multifiliis* Fouquet, 1876. At the same time the physiological condition of the fish may afford trichodinid proliferation, for example during the winter months in temperate climates fish feed little, or not at all, and their tissue reserves become depleted (Snieszko, 1974).

Johansen & Svenson (1977) investigated the effect of water velocity on trichodinid infection of hatchery reared Atlantic salmon, *Salmo salar*. The results showed that percentage mortality of fish was reduced significantly with increased water velocity. They concluded that increasing the water velocity decreased the possibility for a sufficient number of parasites to infect the fish, perhaps due to better flushing of infectious material from the tanks. In addition Johansen & Svenson (1977) also suggest that the physical training produced by an increased swimming speed could benefit the fish's resistance to infection by decreasing the stress and fatigue caused by scratching themselves along the bottom of the tanks.

Hydrocarbon pollution also induces trichodinid population proliferation (Khan, 1990). Trichodinid infections on the gills of oil treated fish increased significantly when compared to control groups. In longhorn sculpins, *Myoxocephalus octodecimspinosus*,

the mean number of trichodinids on oil treated fish was seventeen times higher than the controls. The increased prevalence and intensity of trichodinid infection is probably associated with suppression of the immune system and conditions on the gills which were conducive to growth and reproduction (Khan, 1990).

Concomitant infections with parasites other than trichodinids may increase the intensity of infection. Das & Pal (1987) found that in mixed infections of *Dactylogyrus*, *Tripartiella* and *Trichodina*, it was apparent that numbers of trichodinids were greater than in single infections. Noble (1963 in: Lom, 1969) also found a greater prevalence of *Trichodina* in fish infected with monogeneans. The monogeneans provided a congenial environment for trichodinid population proliferation due to the initiation of tissue damage. Thus, it could be said that monogeneans have a synergistic effect on trichodinids.

Skin and gill mucus is thought to have a protective effect against ectoparasites. Urawa (1992) suggested that an increase in periodic acid-Schiff's reagent (PAS) positive mucus cells may be an effective defence mechanism against trichodinid infections. Balakhnin & Vavydov (1988) state that natural antibodies, hetero-haemagglutinins, have a protective action against *Gyrodactylus* in carp fingerlings. Lom (1969) refers to the work of Nigrelli (1935) and Jakowska (1963), the former suggesting that the action of fish mucus on ectoparasites could be compared to the effect of blood serum; whilst the latter found antibodies against *Staphylococcus aureus* in platyfish, *Xiphophorus maculatus* Guenther, mucus. The immune status of an animal is known to be connected with stress, and general physiological condition. Lom (1969) states that trichodinids are never abundant on a host in a good physiological state; this supports the hypothesis that natural immunity controls trichodinid infection. Khan (1991) suggests that stress has a

suppressive effect on the immune system mediated through raised cortisol levels. Another factor important in the protective properties of fish mucus is the age of the host (Lom, 1969). The general rule that the older the animal, the more developed is its innate immunity to a parasite is valid. Young fish under normal environmental conditions, with no detectable signs of stress have trichodinid infections, older fish living in the same conditions have no ectoparasites. Ciliates of the genera *Tripartiella* and *Trichodinella* can also be found on the skin of young fish, whereas on adults they occur exclusively on the gills (Lom, 1969).

In some situations environmental factors may prevail against trichodinids (Lom, 1969). In such a case there would be external protection as well as the host's non-specific defences acting against the parasites. As described by Dubinin (1948 in: Lom, 1969), increased salinity, caused by evaporation in summer pools, eliminated the surface protozoa (*Ichthyobodo*, *Trichodina*). Where a trichodinid population fails to establish itself, or remains at a very low level, it is often difficult to decide whether it is because of unsuitable environmental factors, or as suggested by Sergent (1963 in: Lom, 1969), because of an innate immunity.

Lom (1973) argued that the pathogenicity of trichodinids depends on the number of ciliates present on the fish. A healthy fish supports only a minimal number of trichodinids, sparsely distributed over the surface. Firmly attached ciliates are always a minority; most of them glide over the surface, the degree of irritation being quite negligible. In this situation the ciliates are said to be true ectocommensals, or symphorionts. When adverse conditions prevail against the fish, the suitability of the fish for trichodinids increases. What ever the reason, be it the lack of inhibiting substances or the presence of stimulating ones on the surface of the fish, the trichodinids

start to proliferate massively. A mass of trichodinids causes severe irritation, which may result in injury and disintegration of cells; the ciliates start to feed on cell debris and on the increased number of bacteria. Under these circumstances trichodinids may become real ectoparasites; i.e. they do not just damage the host but actually feed on it. In a trichodinid firmly attached to the host epithelium, the sharp border membrane bites into it and the host tissue is sucked into the vaulted adhesive disc (Lom & Dykova, 1992). Well defined swellings on the mucosa in the buccal cavity of the river lamprey, *Lampetra japonica* Martens, were observed using scanning electron microscopy, where specimens of an unidentified *Trichodina* species had become detached (Honma, Yoshie & Susuki, 1982). This was cited as evidence of the powerful suction exerted by the parasite.

Species which occur mainly on the skin (Hoffman, 1978) such as *Trichodina fultoni*, can cause extensive dermal lesions including extensive mucus production, irregular white blotches on the head and dorsal surface of the body, loosened scales, frayed fins and epithelial hyperplasia, anorexia, and listlessness, which is followed by death. The lesions caused by *Trichodina domerguei* are similar, and include sloughing of the epidermis and cutaneous haemorrhages (Hoffman, 1978). Khan (1991) reported whitish pustules on the body, haemorrhagic lesions, epithelial hyperplasia and sloughing on Atlantic salmon. These clinical signs were caused by *Trichodina truttae* under experimental conditions, and were recorded at the necropsy of the fish. Species which occur on the gills may cause hyperplasia of the gill epithelium, dyspnoea, and eventual death (Hoffman, 1978). Das & Pal (1987) reported hypertrophy, hyperplasia, and fusion of gill lamellae in cultured carp caused by simultaneous infections of gyrodactylids and trichodinids. The trichodinids were thought to have the major pathological effect, whilst

the gyrodactylids had a synergistic effect on the trichodinid population.

Hoffman (1978) states that, because the oral end of a trichodinid is directed away from the fish, it is unlikely that the parasite consumes living fish tissue. It may, however, feed on dislodged fish mucus resulting from irritation produced by the attachment disc. This reference to the commonly held belief that trichodinid infection stimulates mucus production (Bauer *et al.*, 1969 in: Pottinger *et al.*, 1984); is contrary to the findings of Ahmed, (1976) that trichodinid infection reduces the number of epidermal goblet cells, which would lead to epidermal demucification. It is possible that the primary reaction to trichodinid infection is an increase in mucus production, followed by exhaustion of goblet cells. However, if cutaneous haemorrhages occur as previously suggested, and Ahmed (1976) is correct that host erythrocytes can be found in food vacuoles, then it is possible that trichodinids feed on living tissue at times.

It has occasionally been reported that trichodinids are capable of inflicting more than just superficial damage (Frank, 1962). This author shows photomicrographs of *Trichodina domerguei* embedded deep in gill tissue of *Carassius carassius*, and suggests that they use enzymes to digest the surrounding tissue. Frank also reports perforation of capillaries in gill tissue, which would agree with Ahmed's findings of erythrocytes in food vacuoles. However, no subsequent work has corroborated these findings. The fish used in Frank's research were pond fish which were heavily infested when caught, and it is not certain if other factors could have caused the damage observed. Reichenbach-Klinke (1957 in: Frank, 1962) observed mass infections of trichodinids leading to "real" holes in the skin of the host. In nearly all cases it is agreed that mortalities occur due to secondary infections by opportunistic bacteria, not by the direct action of trichodinids. For example, Khan (1991) suggested that the presence of ulcers



and subcutaneous bacteria supported the view that unidentified opportunistic bacteria might have been the underlying cause of mortality in Atlantic salmon. Subasinghe (1993) suggests that trichodinids may accelerate the onset of epizootic ulcerative syndrome in snakeheads which causes significant mortalities in southern Asia.

Trichodinids have been implicated in severe diseases and mortalities of fish, causing serious economic losses in various parts of the world (Van As & Basson, 1987). Heavy trichodiniasis may cause losses of up to 50% of fish stocks (Lom & Dykova, 1992). *Trichodina fultoni* caused significant mortalities in young eels, *Anguilla anguilla*, under aquarium conditions (Markiewicz & Migala, 1980). Severe losses have been reported in chum salmon fry caused by *Trichodina truttiae* (Urawa & Arthur, 1991; Urawa, 1992). Khan (1991) reported deaths in Atlantic salmon kelts due to the same species. McArdle (1984) reported losses of up to 20% in sea cage reared *Oncorhynchus mykiss* and Atlantic salmon caused by an unidentified *Trichodina* species, associated with unusually high water temperatures. Sanmartin Duran *et al.* (1991) regarded trichodinids as one of the most important parasites of farmed flatfish, such as turbot. Serious infestation, corresponding to massive infection and/or secondary infections caused by other pathogens leading to wounds, can result in serious pathologies resulting in death. Mortalities of post-metamorphosed marine flatfish associated with trichodinid infection have also been reported by Pearse (1972) and Purdom & Howard (1971 in: Pearse, 1972). Even if trichodinid infection does not cause heavy mortalities economic losses can still be considerable. Sanmartin Duran *et al.* (1991) investigated the effects of trichodinid infection at sub-epizootic levels, where no clinical signs of illness were evident. Two groups of turbot were kept for 12 months, the first as controls being treated every three months, the second with the trichodinid population left unchecked.

After one year the control group had grown from an average of 16g to 343g, while the parasitised group had only increased in weight from 16g to 253.6g. So, even when the fish appeared healthy the economic losses due to retarded growth were considerable.

Trichodinids can be treated chemically using formalin, malachite green, pyridylmercuric acetate, acriflavine, methylene blue and potassium permanganate (Hoffman, 1978). However, it is preferable to avoid the conditions required for parasite proliferation, by ensuring optimal water quality and stocking density.

This study was undertaken to determine the trichodinid species present in Great Britain, by sampling populations of farmed and wild freshwater fish. Species identification was primarily based on Klein's silver stained material following the guidelines proposed by Lom (1958). Morphometric data were analysed using multivariate techniques to aid species discrimination and investigate intra-specific morphological variation. Immature specimens were examined to elucidate adhesive disc developmental morphology, and an in depth investigation of intra-population morphological variation was designed to elucidate seasonal trends. In addition, scanning electron microscopical techniques, previously unused in trichodinid research, aimed to illustrate generic and species specific characteristics of the adhesive disc structure.

## CHAPTER 2. TAXONOMY OF THE GENUS *TRICHODINA*, WITH SPECIES DESCRIPTIONS.

### INTRODUCTION

The genus *Trichodina* Ehrenberg, 1830 (Lom, 1958) is the largest within the family Trichodinidae Raabe, 1959. Over 100 species have been described from fish, most by means of Klein's silver impregnation technique (Klein, 1958). A further 69 species have been inadequately described (Lom & Dykova, 1992).

Dogel (1940, 1948 in: Lom, 1958) pioneered the use of uniform characteristics when describing a new species of trichodinid. He was careful to avoid the description of new species on the basis of small variations in morphology. Lom (1958) produced an historical overview of early work in the field, together with a proposal of uniform specific characteristics. Principal characteristics used by Dogel for determination of a new species include the following:

1. Position of micronucleus (mi) with regard to macronucleus (ma).
2. Diameter of ma (Figure 2.1) vertical to the plane of bilateral symmetry of the horseshoe.
3. Length of sector between terminations of ma.
4. Diameter of adhesive disc (Figure 2.3), variable according to the state of the animal and fixation used.
5. Number of denticles on denticulate ring.
6. Shape of denticles is considered to be characteristic of species.
7. Diameter of denticulate ring measured from the centre of intervening middle parts (Figure 2.3).

8. Number of radial pins on the striated membrane between two denticles. The number is considered to be an invariable, specific character.

9. Diameter of body width above the adhesive disc.

10. Situation of the contractile vacuole, central or exocentric; according to Dogel, this is very constant.

11. For control and exactitude the proportion between various measurements is determined.

12. Dimensions of individual denticles (Figure 2.2), proportion of b:t is greater than 1.

13. Taxonomic criteria to be taken from at least 25 specimens.

Lom (1958) criticised Dogel's use of ratios between individual measurements because of its intraspecific variability, and the position of  $m_i$  relative to  $m_a$  as dependent on the state of preparations caused by deformation during drying.

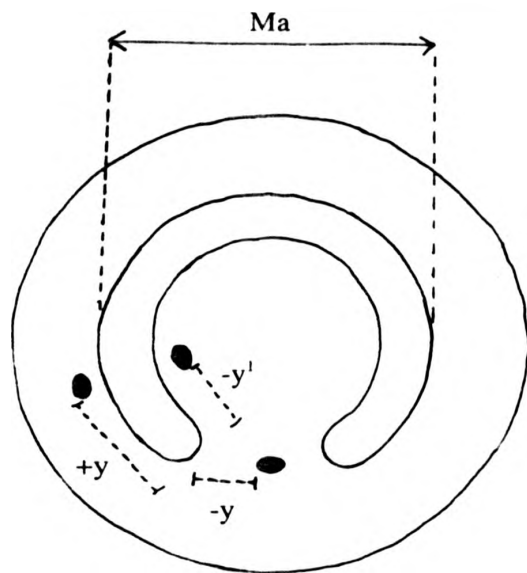
Faure-Fremiet (1943 in: Lom, 1958) took the most important specific characteristic to be denticle number, counted in 100 specimens.

Lom (1958) provided a revision of Dogel's original scheme of specific characteristics:

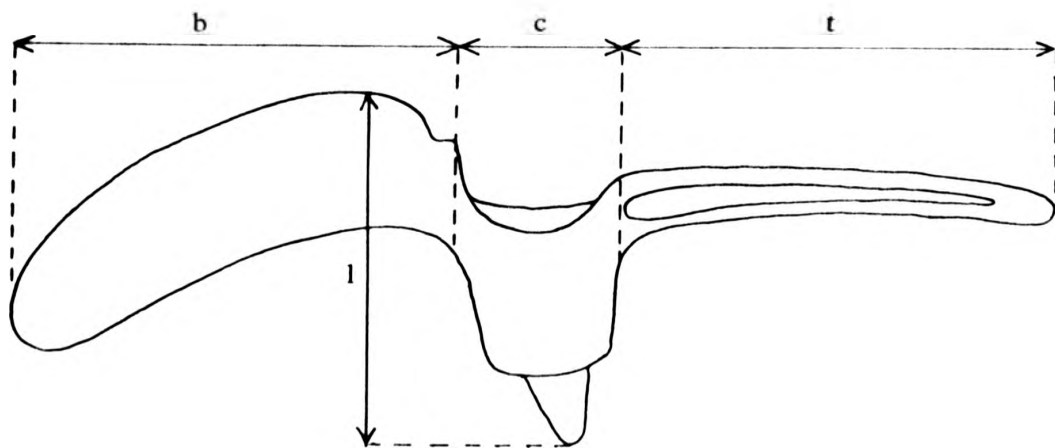
a) Shape of the body: the bottom surface with adhesive disc is called aboral; the opposite surface, oral. The height, maximum diameter, and maximum width of projection in obliquely prolonged species.

b) Structure of the adhesive disc. To include diameter, taken from silver stained specimens using Klein's technique (Klein, 1958) first used for *Trichodina* by Raabe (1950 in: Lom, 1958).

c) Shape of denticles, described from silver stained material. Staining with



**Figure 2.1.** Measurement of macronucleus and micronucleus:  $Ma$  = diameter of the horseshoe shaped macronucleus;  $-y^1$ ,  $+y$ ,  $-y$  = different positions of micronucleus in relation to  $Ma$ .

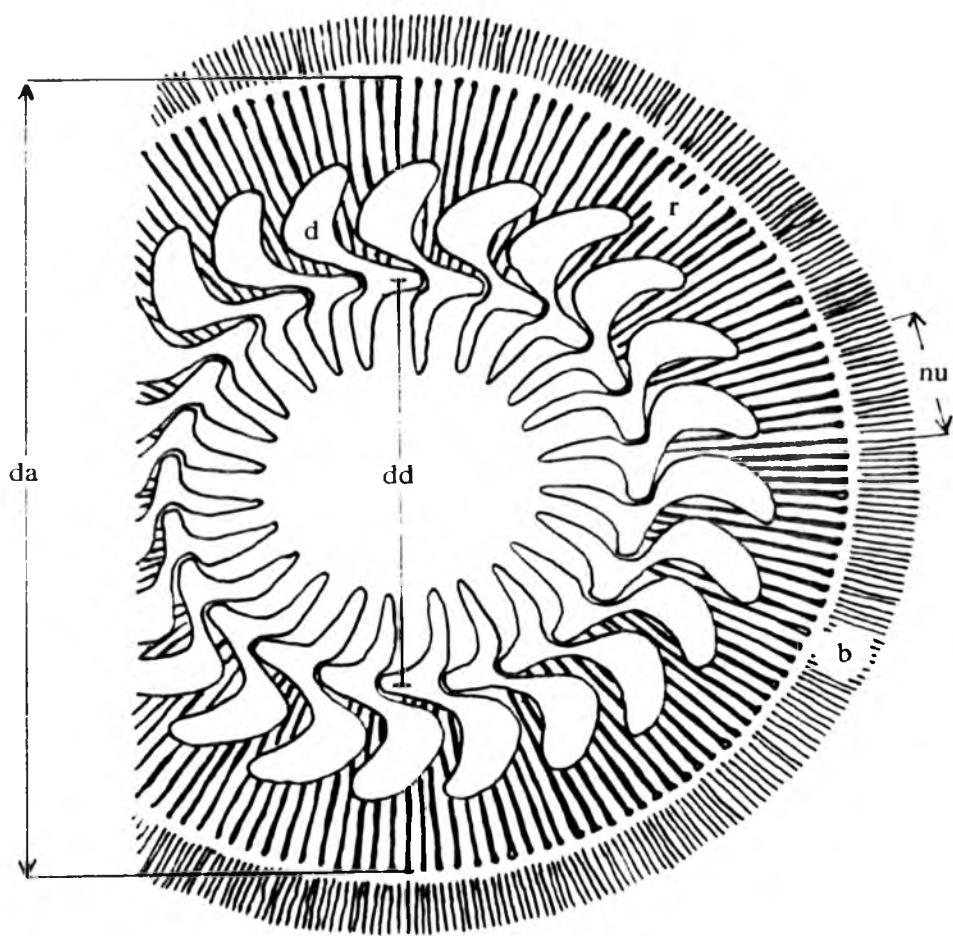


**Figure 2.2.** A denticle of *Trichodina*:  $t$ , length of thorn (ray);  $c$ , width of the central part;  $b$ , length of blade;  $l$ , length of denticle. (Redrawn from Lom, 1992).

saponin and Mallory's used by Faure-Fremiet, and Dogel's iron haematoxylin is said to fail to demonstrate the denticle blades.

- d) Dimensions of denticles (Figure 2.2).
- e) Number of denticles, although Lom points out that the number is not always constant for populations of the same species.
- f) Diameter of denticulate ring (Figure 2.3).
- g) Number of radial pins, on the striated membrane between neighbouring denticles. This is said to be constant, or of limited variation.
- h) Border membrane, consisting of a narrow finely striated band around the adhesive disc.
- i) Velum, a pellicular border or fold above the marginal cilia.
- j) Aboral cilia, consisting of one short and one long locomotory ring.
- k) Nuclear apparatus, after Dogel (1940, 1948).
- l) Course of the adoral cilia, particularly useful in generic differentiation, performing a turn of 360-450° in *Trichodina*.
- m) Situation of contractile vacuole, after Dogel (1940, 1948).
- n) Any additional specific characteristics, such as the presence of "zoochlorellae".
- o) Host specificity.

The specific characteristics proposed by Dogel, subsequently revised by Lom have remained as the basis of all trichodinid taxonomy. The emphasis placed upon various characteristics has changed with time, to the extent that recent authors refer only to the structure of the adhesive disc. Lom (1961) concludes that; "In distinguishing



**Figure 2.3.** Skeletal parts of the adhesive disc: b, peripheral pins in the border membrane; r, radial pins; nu, number of radial pins per denticle; d, denticles; dd, diameter of denticle ring; da, diameter of adhesive disc. (Redrawn from Lom, 1992).

individual species, the study of the mutual relation of the diameter of the body: denticulated ring: macronucleus, and other relations suggested by Dogel 1948, is according to our findings of no substantial value, and can be fully replaced by the study of the impregnated adhesive disc". However, details of characteristics such as body diameter, details of the nuclear apparatus and adoral cilia are often given in species descriptions.

Body diameter must be measured from live specimens, due to distortion which occurs during the drying of smears taken for silver staining. This leads to the complication that measurements for this variable must be taken from specimens which have not been accurately identified as a specific species. It is also probable that the osmoregulatory and contractile state of the specimen may have a profound effect on body size and shape.

Details of the nuclear apparatus were of greater significance in Dogel's (1948 in: Lom, 1958) set of uniform characteristics, because silver staining was not employed to allow accurate observations of adhesive disc morphology. The staining procedures for illustrating the nuclei such as haematoxylin and Robinow-Piekarski (Lom, 1970a), dictate that adhesive disc morphology is obscured. This leads to the situation where specimens from which nuclear measurements are made, must be assumed to belong to the same species as those from which measurements of the adhesive disc are taken. Trichodinids were commonly found in mixed populations of at least three species during this study (see Chapter 7), making the assessment of nuclear morphology practically impossible because of the lack of adhesive disc morphology needed to discriminate between specimens of species with a similar mean body diameter.

The adoral cilia are an important generic indicator, expressing a turn of 360-540°



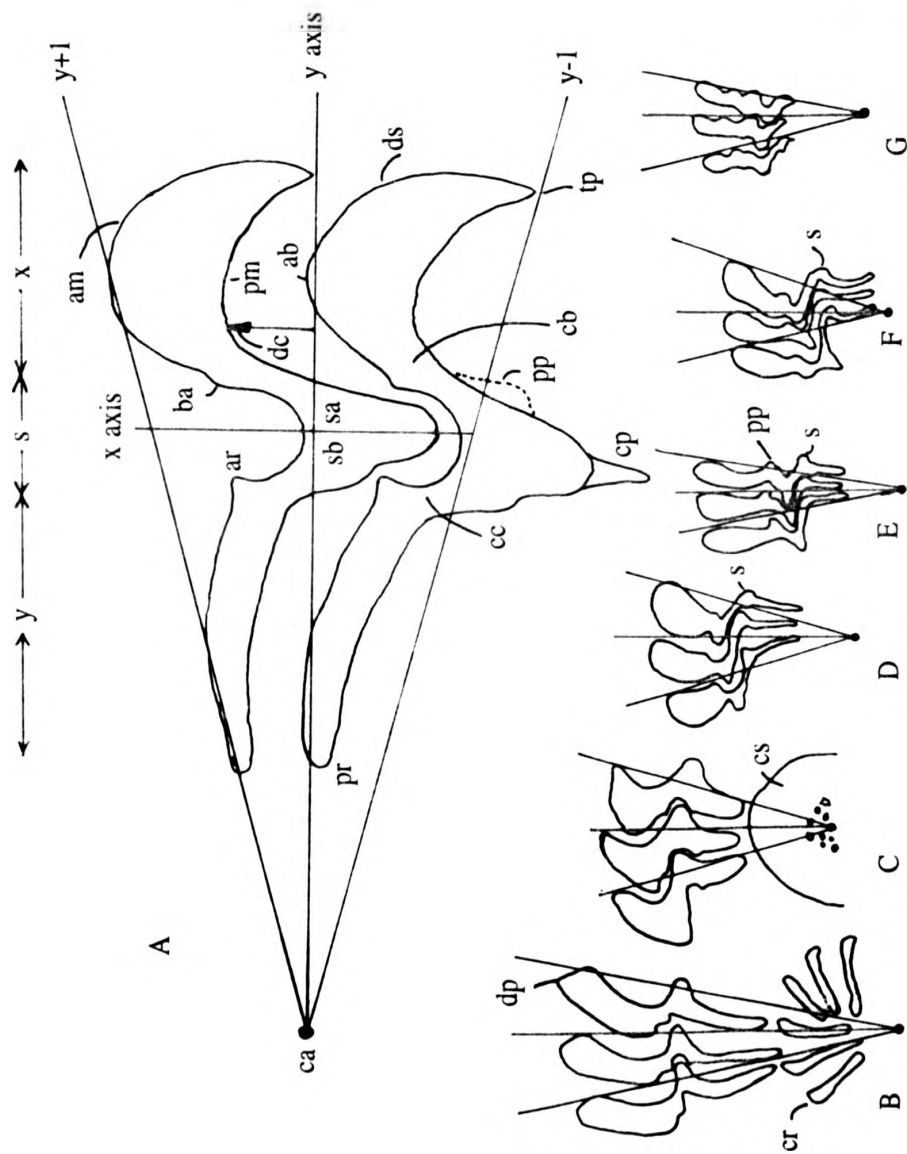
in *Trichodina*, although the effectiveness of this characteristic has become less clear with the description of species such as *Trichodina jiroveci* Grupcheva, 1980 (Grupcheva & Lom, 1980), the adoral spiral of which describes a turn of 210° (previously regarded as specific to *Trichodinella*, *Tripartiella* and *Paratrachodina*). The trend in recent literature is for this feature to be given less weight, in favour of the morphology of the adhesive disc which is receiving more detailed scrutiny.

Kazubski utilised Lom's (1958) criteria, significantly increasing our understanding of the silver stained adhesive disc as a taxonomic structure. Firstly, with a study on the growth of skeletal elements in *Trichodina pediculus*. (Kazubski, 1967), and secondly with a succession of papers investigating morphological variation of the adhesive disc (Kazubski, 1971, 1976, 1979, 1980, 1981, 1982a, 1982b, 1991 (parts a, b and c), Kazubski & Migala, 1968; Kazubski & Piecka-Rapacz, 1981)). The growth of skeletal elements (Kazubski, 1967) is important in determining the maturity of individual specimens (see Chapter 5). Trichodinids reproduce predominantly via asexual, binary fission. Thus, individuals with half the number of skeletal components are present in any population. Therefore, it is important that taxonomic measurements are only made from mature specimens. Kazubski & Migala (1968) introduced a measurement from the tip of the denticle blade to the tip of the ray. This was designated as denticle length, being synonymous with a different measurement proposed by Lom (1958). Kazubski's additional measurement has been universally adopted, but redesignated denticle span in later publications.

The most recent attempt to improve the criteria for description of the adhesive disc was by Van As & Basson (1989). They proposed, "a method to describe the shape of the denticles by constructing lines from the centre of the adhesive disc to the tip of

the denticles, which provide fixed points of reference that can aid in an accurate description of the denticle elements". This method requires an enlarged photomicrograph or drawing, and can also be applied to existing published work. The system of lines proposed is illustrated in Figure 2.4, along with descriptive terminology relating to different features of the denticle. During this study a high degree of morphological variation was observed in denticle form (Chapters 2, 3 and 6). However, the system proposed by Van As & Basson encourages the detailed study of a small number of trichodinids, or even a single specimen, producing species discrimination and the erection of new species on the basis of individual comparisons (Van As & Basson, 1989), which is a potentially dangerous procedure. If a large number of good quality photomicrographs illustrating the range of intra and interpopulational variation are available, an unknown population can be identified with greater accuracy. It is often necessary to take a 'step back' and compare populations rather than individuals, which may appear significantly different taken out of the wider context. For this reason it is suggested that Van As & Basson's system is not adopted in entirety. However, the nomenclature used in their denticle descriptions is very useful, as no other standardised criteria exist in the literature.

Van As & Basson (1989) suggested that body diameter be excluded from descriptions due to its high degree of variability and deformation, and that the former term be used to represent the diameter of the adhesive disc plus border membrane. As previously discussed, body diameter is of little or no taxonomic value and should therefore be disregarded. Using the term body diameter to represent adhesive disc diameter plus border membrane width is ambiguous and of no value as they are already measured separately. The final proposal of Van As & Basson (1989), is that



**Figure 2.4.** A. Diagram of trichodinid denticles illustrating the construction of x and y axes as fixed references for description of denticles. B-G. Diagram of trichodinid denticles to illustrate variation in denticle form. B. *Trichodina centrostrigata* Basson *et al.*, (1983); C. *T. compacta* n. sp. D. *Tripurittella nana* Basson & Van As, 1987. E. *T. orthodens* Basson & Van As, 1987. F. *T. macrosoma* Basson & Van As, 1987. G. *Trichodinella crenulata* Basson & Van As, 1987. Abbreviations: ab, apex of blade; am, anterior margin of blade; ar, apophysis of ray; ba, apophysis of blade; ca, centre of adhesive disc; cb, section connecting blade and central part; cc, section connecting central part and ray; cp, central conical part; cr, central ray; cs, central circle; dc, deepest point of curve; dp, distal part of blade; ds, distal surface of blade; ds, distal curve; pr, posterior projection; pp, posterior part; sa, section of ray; sb, section of ray above x axis; sb, section of central part below x axis; tp, tangent point; y, ray; x, blade. (Redrawn from Van As & Basson, 1989).

measurements be presented in a uniform way, consisting of minimum and maximum values, followed in parenthesis by the arithmetic mean, standard deviation and number of specimens measured. In the case of meristic data such as denticle and radial pin number, the mode should be substituted for the mean. This form of presentation has been used during this study, using mean values for all characteristics. This allows details of sample size, population structure of each characteristic as well as the mean value to be presented succinctly. When presenting morphological data it is also useful to give the sample date, to aid comparisons of data (see Chapter 6).

In summary, the taxonomic criteria used during this study are as follows; using enlarged silver stained photomicrographs of adult specimens, with the range, mean, standard deviation and sample size given for each measurement:

1. Adhesive disc diameter (A. d. diam.), mean value of 2 measurements taken at right angles to account for any distortion during drying.
2. Border membrane width (B. m. width).
3. Denticle ring diameter (D. r. diam.), mean value of 2 measurements taken at right angles.
4. Radial pins per denticle (R. p. / d.).
5. Denticle number (Dent. no).
6. Denticle length (D. length), from anterior border to central conical part (minus interlocking point, not visible in silver stained specimens), taken at right angles to the orientation of the central part of the denticle. Mean value of 3 randomly selected denticles.
7. Blade length (B. length), mean value of 3.
8. Ray length (R. length), mean value of 3.

9. Central part width (C. p. width), mean value of 3.
10. Central circle diameter (C. c. diam.) where present, mean of 2 measurements taken at right angles.
11. Denticle span (Dent. span), mean value of 3.
12. Course of the adoral cilia, observed in live material.
13. Host specificity
14. Site (Skin, gills, etc.).
15. Sample date.

The *Trichodina* species found during this study are described and discussed individually in this chapter.

#### **MATERIALS AND METHODS**

Fish from approximately sixty sites were sampled for trichodinids within Britain (Figures 2.5 and 2.6) between October 1991 and September 1994. Farmed and wild salmonoids were studied, in addition to wild coarse fish populations. Populations of farmed salmonids are readily available, and were regularly sampled from different sites. Sampling of wild fish populations was more opportunistic in nature. Species sampled included: Atlantic salmon, *Salmo salar* L., brown trout, *Salmo trutta* L., rainbow trout, *Oncorhynchus mykiss* Walbaum, Arctic charr, *Salvelinus alpinus* L., brook trout, *Salvelinus fontinalis* (Mitchill), whitefish, *Coregonus lavaretus* L., grayling, *Thymallus thymallus* L., pike, *Esox lucius* L., carp, *Cyprinus carpio* L., goldfish, *Carassius auratus* L., bream, *Abramis brama* L., minnow, *Phoxinus phoxinus* L., rudd, *Scardinius erythrophthalmus* L., roach, *Rutilus rutilus*, L., dace, *Leuciscus leuciscus* L., stone loach,

*Barbatula (Nemacheilus) barbatulus* L., eel, *Anguilla anguilla* L., three-spined stickleback, *Gasterosteus aculeatus* L., perch, *Perca fluviatilis* L., and ruffe, *Gymnocephalus cernua* L. Where possible a minimum of ten fish per population were sampled from each site. Fish were collected from farm cages and small streams using a hand net. Electro fishing, gill netting, seine netting, trawling and rod fishing techniques were also employed for the collection of fish. Fish were transported in oxygen enriched water from the sample site, in which they were subsequently maintained until processing.

Fish were killed by a sharp blow to the head. Small fish were examined in water from the sample site using an Olympus binocular microscope. Skin, fin and gill smears were studied from larger specimens using an Olympus CH2 compound microscope. The smears from infected fish were processed using Klein's silver impregnation technique (Klein, 1958). Skin and gill smears were air dried, impregnated with 2% aqueous solution of  $\text{AgNO}_3$  for 7-8 minutes and rinsed in distilled water. The  $\text{AgNO}_3$  was reduced to metallic silver using ultra violet light (254 nm) for 2 minutes using a multi-band U.V lamp (Mineralight, UV GL-50). Subsequently the slides were washed in tap water for 30 minutes, air dried and mounted in Canada Balsam diluted in xylene. For marine or brackish water trichodinid populations a modification of Klein's technique was utilised, consisting of fixing the smears with 6% formalin for 10 minutes followed by copious rinsing with distilled water before silver staining in the usual way. Where possible a minimum of forty specimens from each trichodinid population (Table 2.1) were photographed on Ilford FP4 50 ASA black and white film, using a BH2 Olympus compound microscope at 1000 times magnification with phase contrast. Enlarged photomicrographs (1800 $\times$  magnification) were measured using Camlab callipers,



**Figure 2.5.** Geographical distribution of salmonoid sample sites (black circles positive for trichodinids, white circles negative).



**Figure 2.6.** Geographical distribution of coarse fish sample sites (black circles positive for trichodinids, white circles negative).



**Table 2.1.** Sample sites positive for trichodinid populations.

Locality	Sample site	Habitat	Host Species
Highlands	Allt Loin (5/92)	Wild	<i>Phoxinus phoxinus</i>
	Ardtoe (8/94)	Wild	<i>Gasterosteus aculeatus</i>
Tayside	College Mill (8/93) (8/93, 10/93, 5/94)	Wild	<i>Salmo salar</i> <i>Phoxinus phoxinus</i>
	Almond Bank, SOAFD (8/93)	Farmed	<i>Salmo trutta</i>
	Airthrey Loch (5/92)	Wild	<i>Gasterosteus aculeatus</i>
Central	Airthrey burn (7/92)	Wild	<i>Salmo trutta</i>
	Pond, Institute of Aquaculture (4/94)	Ornamental	<i>Carassius auratus</i>
	Buckieburn (11/91, 1/92)	Farmed	<i>Salmo Salar</i>
	River Devon, Dollar (8/95)	Farmed	<i>Oncorhynchus mykiss</i>
	Strathclyde	Castle Semple Water (6/92, 9/92)	Wild
Strathclyde	Loch Fad, Bute (6/93)	Farmed	<i>Oncorhynchus mykiss</i>
	River Doon (9/92)	Farmed	<i>Oncorhynchus mykiss</i>
	Dumfries	Moffat (3/93)	Farmed
Clwyd	Lake Bala (9/93)	Wild	<i>Phoxinus phoxinus</i>
Yorkshire	Coarse fish dealer (12/92)	Wild	<i>Rutilus rutilus</i>
			<i>Abramis brama</i>
Norfolk	Coarse fish dealer (11/92)	Wild	<i>Cyprinus carpio</i>
Cheshire	Chorlton, coarse fish dealer (12/92)	Wild	<i>Scardinius erythrophthalmus</i>
Hampshire	River Test (8/94)	Farmed	<i>Oncorhynchus mykiss</i>

accurate to 0.05 mm. Measurements taken using an eye-piece graticule (100×0.01 mm) at the maximum magnification attainable (1250 times) gave a resolution of 0.62µm per eye-piece unit, measurements taken from photomicrographs gave 0.028µm per

graduation. Thus, the technique using measurements made from photomicrographs (1800 times) gives 22.32 times greater accuracy than was directly attainable using an eye-piece graticule at 1250 times.

### **Principal Components Analysis**

Principal Components Analysis (PCA) was undertaken to assess intraspecific variation of morphometric measurements using Systat 5.3 (1991). The goal of PCA is to summarise the variability between specimens in a multivariate data set as accurately as possible using a few components. PCA decomposes a correlation matrix, the component loadings that result are the correlation of each component with each original variable. Each component or factor is ordered by the percentage variance it explains. The component which explains the most variation (Factor 1) is plotted on the x-axis, the next component (Factor 2) is plotted at right angles on the y-axis (information normally presented in a 2 D plot). Factor 3 is placed at right angles with Factor 2 and Factor 4 at right angles to the resultant of the first three etc. Component loadings of each variable summarise its contribution to the total variation expressed by the component. Thus, the relative variability of each taxonomic variable can be assessed. The variance expressed by the most important components (1, 2 and 3) is quoted, to indicate the percentage of total variation explained by a plot. The data was log transformed, in an attempt to normalise the distribution of each variable.

## SPECIES DESCRIPTIONS

### *TRICHODINA ACUTA* LOM, 1961

(syn. *Trichodina domerguei* f. *latispina* Dogel, 1940;  
*Trichodina domerguei* var. *diaptomi* Sramek-Husek, 1953;  
*Trichodina domerguei* f. *acuta* Lom, 1961)

#### Introduction

*Trichodina acuta* was first described as *Trichodina domerguei* f. *latispina* from *Diaptomus vulgaris* (Dogel, 1940 in: Lom, 1960). *Trichodina* species with a clear central circle or disc were thought to be closely related (Lom, 1961), thus *Trichodina acuta* was classified as a form of *Trichodina domerguei*. Lom considered the name *Trichodina domerguei* var. *diaptomi* Sramek-Husek 1953 a synonym of *Trichodina domerguei* f. *latispina* (Lom, 1960). Lom (1961) reclassified diaptomus trichodinid populations as *Trichodina domerguei* f. *acuta*, distinct from earlier descriptions by Dogel (1940) and Raabe (1959). This form was also recorded from *Cyprinus carpio*, *Perca fluviatilis*, *Lucioperca lucioperca* Heckel, sunbleak *Leucaspis delineatus* Heckel, zander *Stizostedion lucioperca* L., bitterling *Rhodeus sericeus* Pallas and on the skin of several species of frog tadpoles in Czechoslovakia (Lom, 1961). *Trichodina domerguei* f. *acuta*, again named as *Trichodina domerguei* f. *latispina*, was described from the skin and rarely gills, of *Cyprinus carpio*, silver carp *Hypophthalmichthys molitrix* Val., grass carp *Ctenopharyngodon idella* Val., various tadpoles and on the surface of the copepods *Sinodiaptomus sarsi* and *Neodiaptomus handeli* in China (Chen Chih-Leu, 1963). Seasonal variation in size was noted in *Trichodina domerguei* f. *acuta* (Kazubski & Migala, 1968) parasitising *Cyprinus carpio* in Poland.

*Trichodina domerguei* f. *acuta* was raised to species level as *Trichodina acuta* (Lom, 1970a). This paper summarises the occurrence of this species as of March 1969: from large-mouth bass, *Micropterus salmoides* Lacepede, Alabama, USA (Lom, 1970a); from some of the hosts previously mentioned from the European part of the USSR by Kaskovsky (1965), Ivanova (1966) and Kulemina (1968); from *Carassius auratus* from the USSR (Stein, 1968) and from *Lucioperca lucioperca* in Azerbaidzhan. Further records of *Trichodina acuta* include: *Lucioperca lucioperca* from the Soviet Danube (Kostenko, 1972), *Tilapia zillii* Gervais and *Oreochromis mossambicus* Peters in the Philippines (Duncan, 1977), *Oncorhynchus mykiss* in Bulgaria (Grupcheva, 1975b), *Oreochromis niloticus* Trewavas, throughout the Philippines (Bondad-Reantaso & Arthur, 1989), *Cyprinus carpio* and grass carp, *Ctenopharyngodon idella* Val. from the Philippines (Albaladejo & Arthur, 1989) and *Cyprinus carpio* from eastern Germany (Grupcheva & Sedlaczek, 1993).

Van As & Basson have produced a large number of papers detailing the trichodinid fauna of South Africa and Israel. *Trichodina acuta* has been reported from South Africa (Basson, Van As & Paperna, 1983) parasitising *Oreochromis mossambicus*, *Pseudocrenilabrus philander* Weber, congo tilapia *Tilapia rendalli* Boulenger, *Tilapia sparrmanii*, *Barbus trimaculatus* Peters and *Cyprinus carpio* and additionally, from blue tilapia *Tilapia aurea* Steindachner, *Ctenopharyngodon idella* and *Cyprinus carpio* in Israel. Specimens from Israel were considerably larger in size than those obtained from South Africa with the denticles described as being stouter in the South African specimens. Israeli specimens are said to be similar to a European population parasitising *Cyprinus carpio* (Kazubski & Migala, 1968). The Philippino population (Duncan, 1977) is described as being intermediate between South African and Israeli forms in terms of

denticle shape and dimensions (Van As & Basson, 1989). On the basis of these differences the *Trichodina acuta* populations found on 20 fish species in South Africa and Israel that were smaller with stouter denticle blades, were later redescribed as *Trichodina compacta* (Van As & Basson, 1989; 1992). It was stated that both *Trichodina acuta* and *Trichodina compacta* could be found together in some populations from Israel. *Trichodina acuta* is described as a "European import" to South Africa (Basson & Van As, 1993) parasitising farmed *Oncorhynchus mykiss*.

Basson & Van As (1991) suggested that all copepod trichodinids belong to the same species i. e. *Trichodina diaptomi*. South African specimens are compared to other authors data and photomicrographs, including those of Lom (1961) and Chen-Chih Lieu (1963), both of whom originally described their specimens as synonyms of *Trichodina acuta*. Basson & Van As (1991) stated that all copepod populations include the presence of a central circle, a feature possessed by all fish populations of *Trichodina acuta* (Lom, 1961). *Trichodina diaptomi* are said to be smaller than *Trichodina acuta* (Basson & Van As, 1991). However, specimens identified as *Trichodina domerguei* f. *latispina* from copepods (Lom, 1960) were smaller than populations of the same species found on fish; Chen-Chih Leu (1963) reported an increase in size when copepod specimens were transferred to fish and *visa versa*. Lastly, Van As & Basson state that "they have not yet encountered any specimens of trichodinids resembling *Trichodina diaptomi* on fish hosts"; however the specimens they re-identified so closely resembled those found on fish hosts that they were classified by Lom (1960) as *Trichodina domerguei* f. *acuta*! This hypothesis based on comparisons of individual specimens from the literature, is rather tenuous and should be regarded with circumspection. If *Trichodina diaptomi* is indeed synonymous with *Trichodina acuta* it would suggest that the latter species is indigenous to South Africa, contradictory to the opinion of Basson & Van As (1993).

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## Results

Seven populations of a skin trichodinid species from a range of hosts were found during the course of this study, all adhering to Lom's (1961) original description of *Trichodina domerguei* f. *acuta*. Details of the seven populations, together with morphometric and meristic data are presented in Table 2.2. Populations of *Salmo trutta*, *Oncorhynchus mykiss*, *Carassius auratus* and *Phoxinus phoxinus* constitute new British host records for *Trichodina acuta*. Individual specimens of trichodinids identified tentatively as *Trichodina acuta*, were obtained from *Gasterosteus aculeatus* (Airthrey Loch), *Oncorhynchus mykiss* (River Test) and *Scardinius erythrophthalmus* (Chorlton, Nr. Chester). The latter specimen was significant in that it was larger than any others observed (a. d. diam=65.15µm).

*Trichodina acuta* is of medium size and adhesive disc diameter ranged from 45.3 to 64.0µm in the seven populations sampled, whilst denticle number ranged from 16-25. It is characterised by a small central circle which stains a similar colour to the denticles. The denticle blades are broad 'sickle like' structures, often coming to a point. The apophysis of the blade is unusually pronounced, as is the posterior projection of the central part. The inner denticle rays are straight or slightly curved, and either rounded or pointed at the end. The rays are often angled backwards to the anterior of the denticle although this is variable. The number of radial pins per denticle are cited in the literature as being approximately 8-9. However, the specimens observed during this study seemed to have very thick pins which often looked double, or alternately single then double, in well stained specimens. Specimens observed illustrated an adoral ciliary spiral of 360-380°. Photomicrographs of specimens from each population are illustrated in Figures 2.7, 2.8, 2.9 and 2.10. The range in denticle morphology for all the populations

**Table 2.2. Morphometric and meristic data for *Trichodina acuta*.**

Author	Lom, 1961	This study (a)	This study (b)	This study (c)	This study (d)	This study (e)	This study (f)	This study (g)
Host	Various	<i>Salmo trutta</i>	<i>Cyprinus carpio</i>	<i>Phoxinus phoxinus</i>	<i>Oncorhynchus mykiss</i>	<i>Oncorhynchus mykiss</i>	<i>Carassius auratus</i>	<i>Oncorhynchus mykiss</i>
Localisation	Skin, gills	Skin	Skin	Skin	Skin	Skin	Skin	Skin
Locality	Bohemia	Airthrey Loch 11/91	Norfolk 17/11/92	Castle Semple Water 20/9/92*	Moffat 18/3/93	Loch Fad 6/93	Pond, Institute of Aquaculture 4/4/94	Dollar 14/8/94
A. d. diam.	42-53**	38.4-51.8 (44.7±3.6, 33)	42.6-55.9 (50.0±4.7, 8)	46.4-64.0 (53.5±4.4, 25)	44.5-58.6 (51.0±3.8, 23)	39.9-53.6 (45.3±3.9, 17)	41.1-60.9 (52.3±4.7, 42)	39.7-52.4 (47.2±3.7, 26)
B. m. width	3.5-5	3.4-4.9 (4.0±0.3, 33)	3.7-5.1 (4.5±0.5, 8)	4.2-5.6 (4.8±0.4, 25)	3.9-4.9 (4.4±0.2, 23)	4.0-5.1 (4.6±0.3, 17)	4.4-5.3 (4.9±0.2, 42)	4.1-5.0 (4.4±0.5, 26)
D. r. diam.	23-32	21.8-31.0 (26.4±2.5, 33)	25.6-32.4 (28.8±2.5, 8)	21.0-29.3 (25.2±2.3, 25)	25.6-32.8 (29.4±2.0, 23)	22.1-32.7 (26.2±2.7, 17)	25.4-37.5 (31.1±3.0, 42)	22.6-31.6 (27.2±2.6, 26)
Dent. no.	18-21	17-22 (19.4±1.1, 33)	18-20 (18.8±0.7, 8)	18-21 (19.2±0.7, 25)	19-23 (20.5±1.1, 23)	17-20 (18.5±1.0, 17)	16-25 (20.5±1.8, 42)	17-20 (18.6±0.9, 26)
R. p/ d.	8	8-11 (9.4±1.0, 33)	8-11 (9.75±1.0, 8)	7-9 (8.5±0.7, 24)	8-10 (9.5±0.7, 22)	9-12 (10.0±1.0, 17)	8-12 (9.6±1.1, 42)	8-10 (9.2±0.5, 26)
D. length	10-11	7.2-9.7 (8.4±0.6, 33)	8.3-10.4 (9.3±0.3, 8)	6.4-8.6 (7.7±0.5, 25)	7.9-11.3 (9.3±0.8, 23)	8.0-9.7 (8.6±0.5, 17)	6.7-11.3 (8.8±1.0, 42)	7.7-9.6 (8.7±0.5, 26)
B. length	4.5-6	3.4-5.8 (4.8±0.5, 33)	4.3-6.3 (5.3±0.6, 8)	3.4-5.6 (4.9±0.4, 25)	4.6-7.9 (5.7±0.6, 23)	4.1-5.9 (4.9±0.5, 17)	4.3-6.7 (5.7±0.6, 42)	4.1-6.0 (5.1±0.5, 26)
R. length	4-7	4.9-6.8 (5.8±0.5, 33)	5.0-7.8 (6.3±0.9, 8)	4.9-6.7 (5.6±0.5, 25)	5.5-8.2 (6.7±0.7, 23)	5.6-7.2 (6.3±0.5, 17)	5.2-8.9 (7.1±0.9, 42)	4.6-7.2 (5.6±0.6, 26)
C. p. width	3-4	2.2-3.0 (2.6±0.2, 33)	2.5-3.6 (3.0±0.3, 8)	2.0-2.8 (2.4±0.2, 25)	2.4-3.4 (2.8±0.2, 23)	2.1-3.1 (2.6±0.3, 17)	2.3-3.8 (3.1±0.4, 42)	2.4-3.8 (2.8±0.4, 26)
C. c. diam.	9-12	6.7-11.8 (9.8±1.2, 25)	8.6-10.0 (9.4±0.8, 3)	4.3-10.9 (8.8±1.2, 25)	8.9-17.3 (11.2±1.9, 17)	7.3-11.4 (8.8±0.9, 16)	8.0-12.7 (10.2±1.4, 18)	7.6-11.5 (10.1±0.9, 26)
Dent. span	-	11.3-14.8 (13.1±1.0, 33)	12.9-16.2 (14.4±1.2, 8)	11.4-14.6 (12.7±0.9, 25)	12.9-19.1 (15.1±1.3, 23)	12.0-15.2 (13.6±0.9, 17)	11.7-18.4 (15.9±1.4, 42)	11.8-15.8 (13.6±1.1, 26)

\* kept at room temperature for two weeks \*\* range of the mean for different populations and hosts



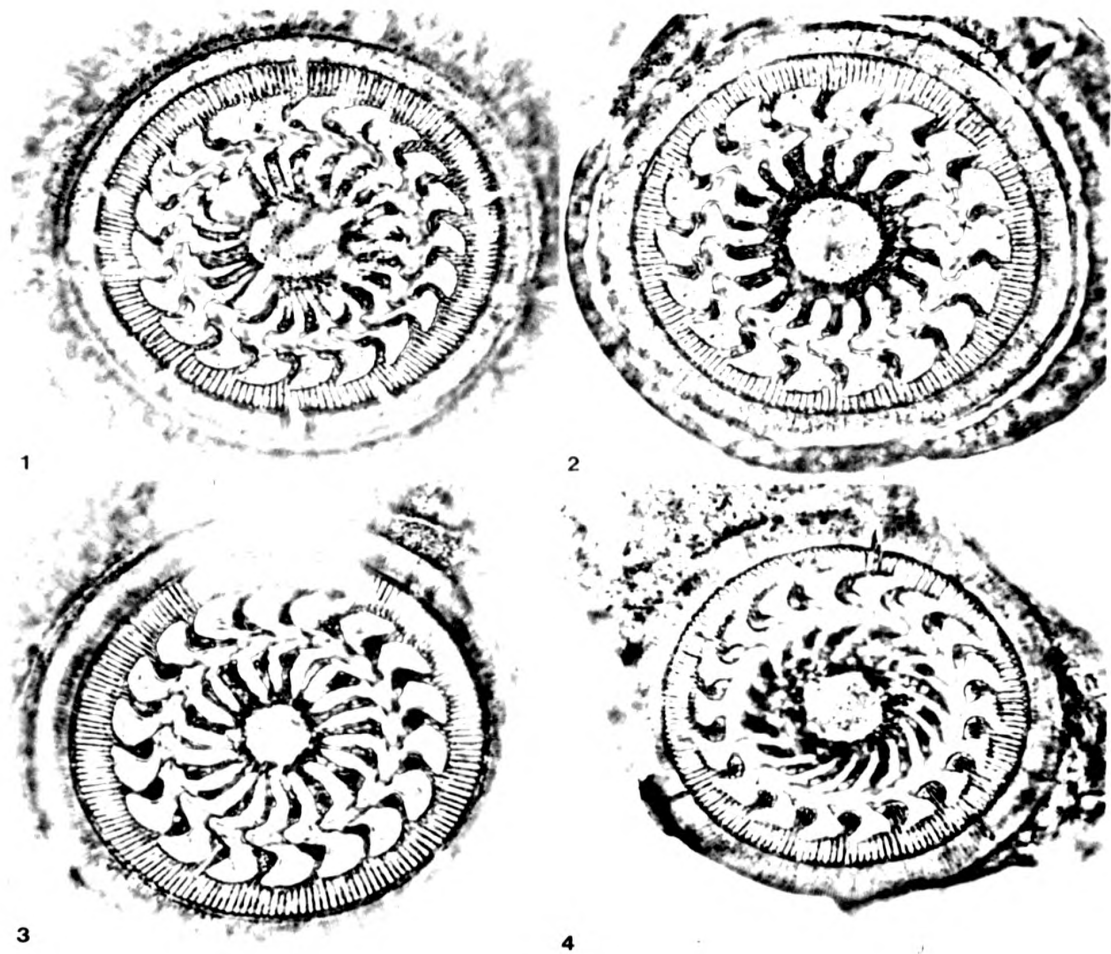
sampled is illustrated in Figure 2.11.

Morphological variation within each population is considerable, some specimens have much broader denticles with a pronounced distal surface of the blade rather than the normal continuous curve of the anterior margin. Other variations of blade morphology include: a far less curved posterior margin or 'spatulate' appearance than the normal 'sickle' shape, a reduction in size relative to denticle span and a reduced appearance of the apophysis of the blade. No consistent marked variation in morphology could be differentiated between populations.

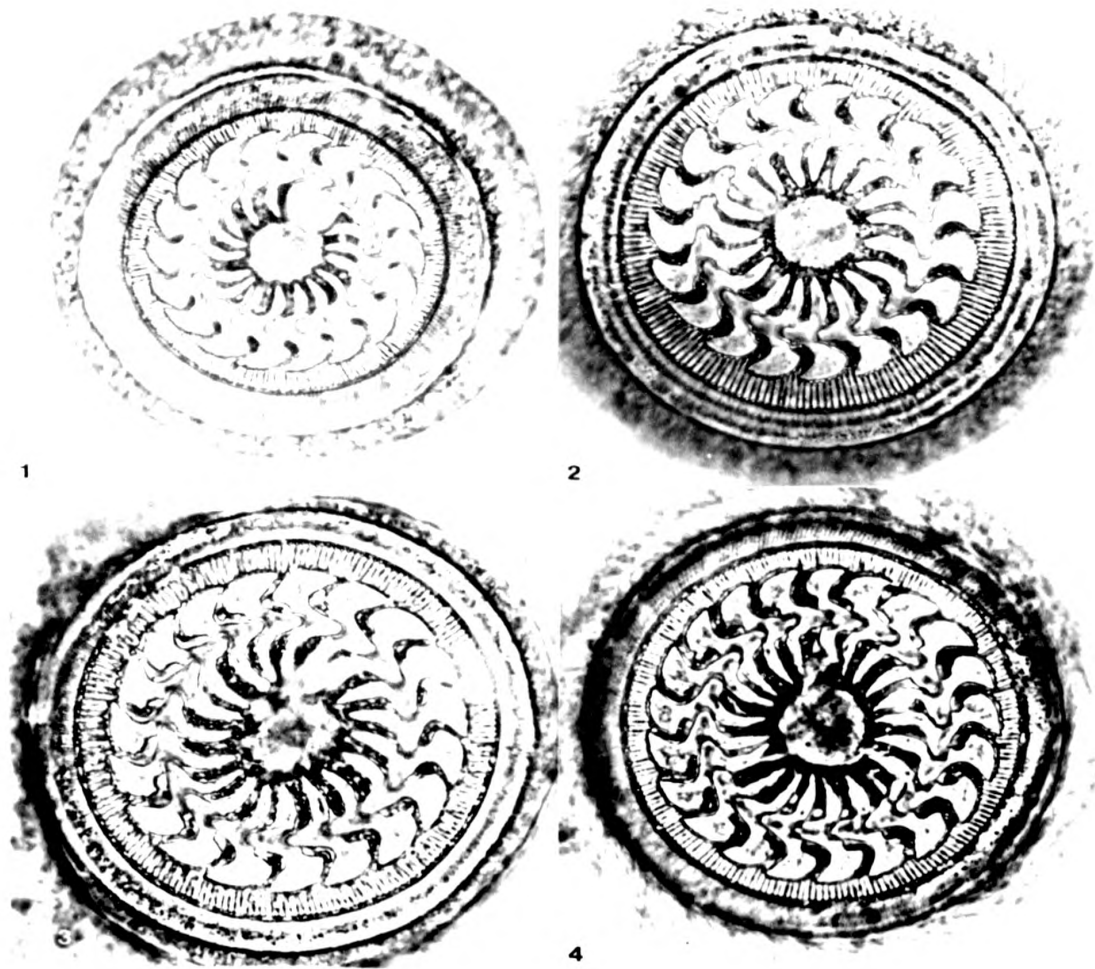
There are no obvious relationships between host species and trichodinid morphology, or sample date and trichodinid size. It is notable that the population with the largest mean adhesive disc diameter from *Phoxinus phoxinus* was maintained at room temperature (approximately 20°C) for two weeks before sampling.

To analyse any variation suggested by the morphometric and meristic data obtained, Principal Components Analysis was used to compare the seven populations in detail. The component loadings of the first three factors are given in Table 2.3. This illustrates that denticle ring diameter and denticle span are the main variables acting in Factor 1. Denticle number and denticle length are the dominant variables acting against each other in Factor 2. In Factor 3 border membrane width and in the opposite direction denticle number are the key variables. The percentage variance explained by each component is given in Table 2.4

The PCA plot of Factor one against Factor two, with each population surrounded by a 60% ellipse is presented in Figure 2.12. This illustrates that whilst most of the populations show a high degree of overlap, population (c) from *Phoxinus phoxinus* (Castle Semple Water), and to a lesser extent population seven from *Carassius auratus*



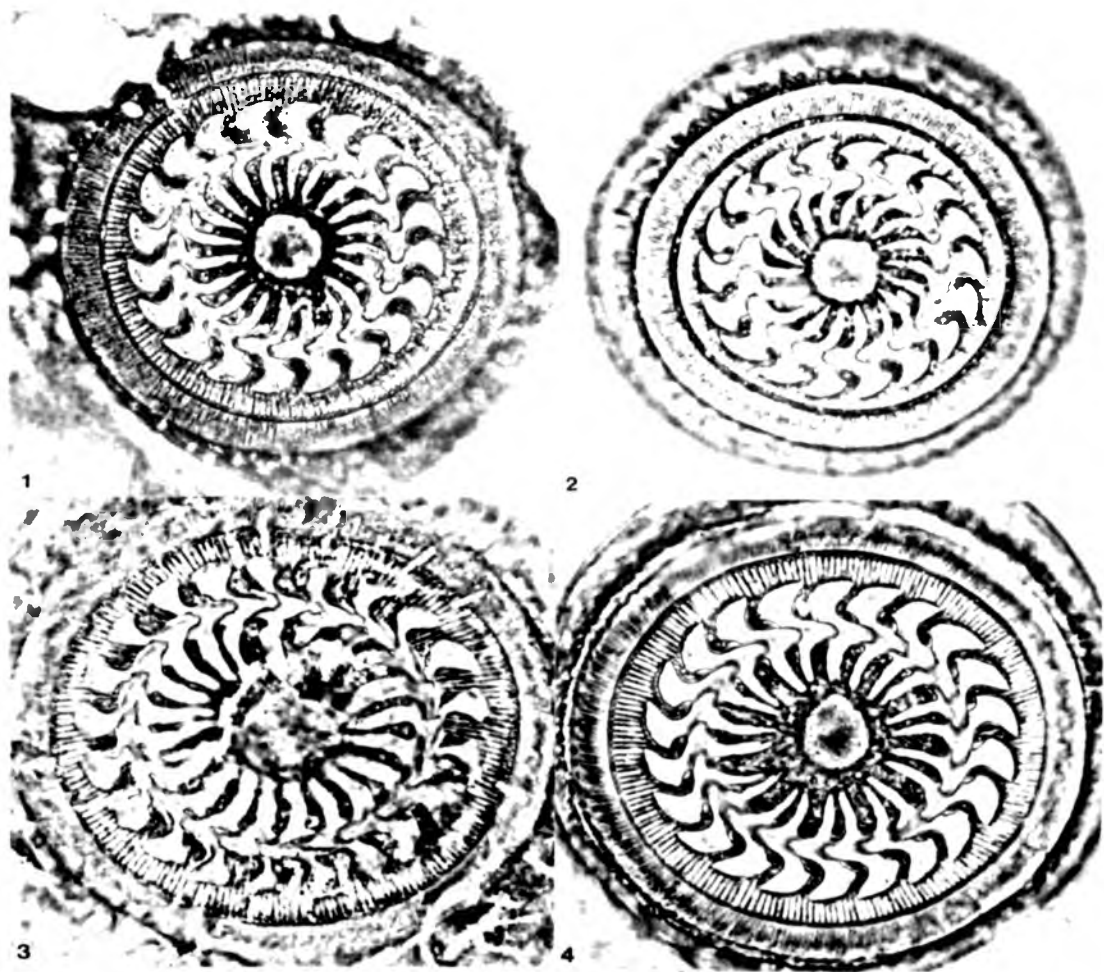
**Figure 2.7.** Silver stained adhesive disc morphology in specimens of *Trichodina acuta*, populations a and b:  
1, 2. Population (a), *Salmo trutta* (Airthrey Loch). (1200×).  
3, 4. Population (b), *Cyprinus carpio* (Norfolk). (1200×).



**Figure 2.8.** Silver stained adhesive disc morphology in specimens of *Trichodina acuta*, populations c and d:

1, 2. Population (c) from *Phoxinus phoxinus* (Castle Semple Water). (1200 $\times$ ).

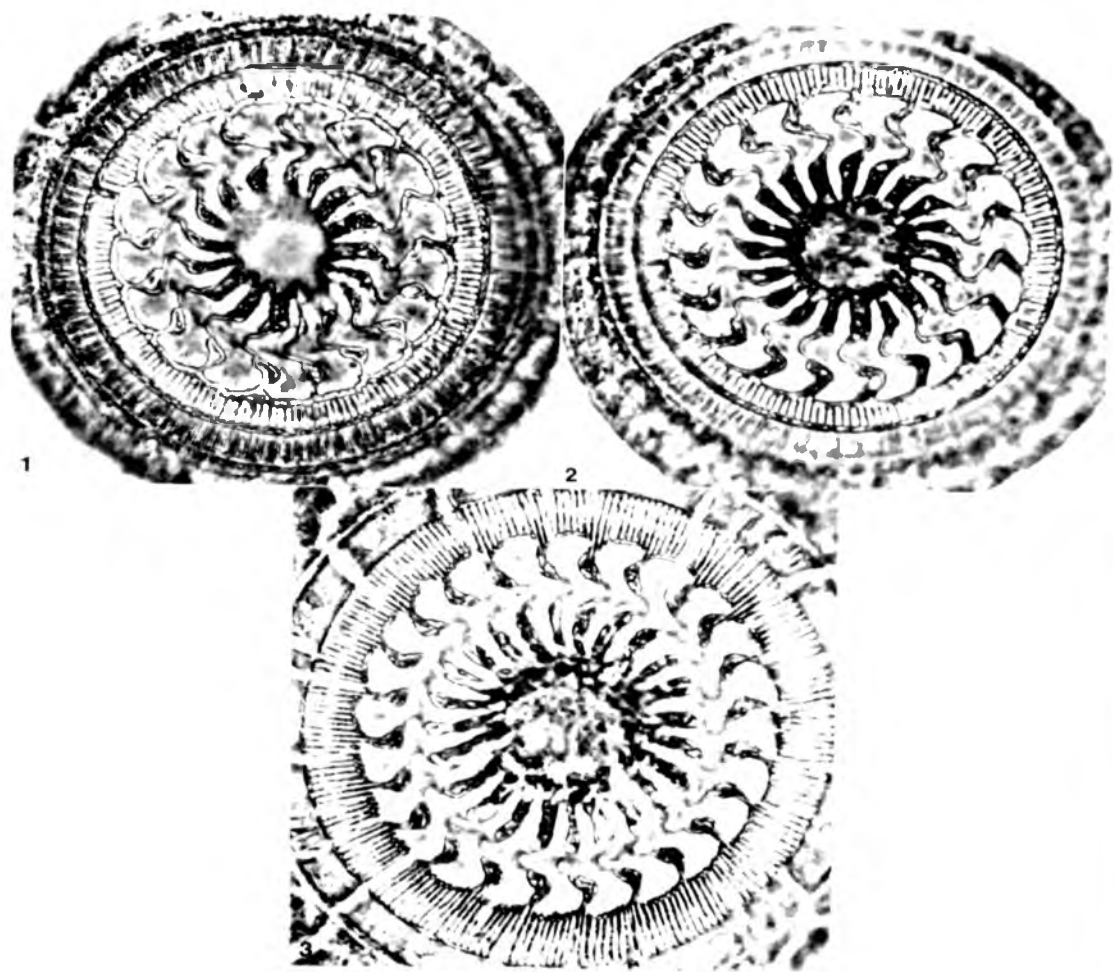
3, 4. Population (d) from *Oncorhynchus mykiss* (Moffat). (1200 $\times$ ).



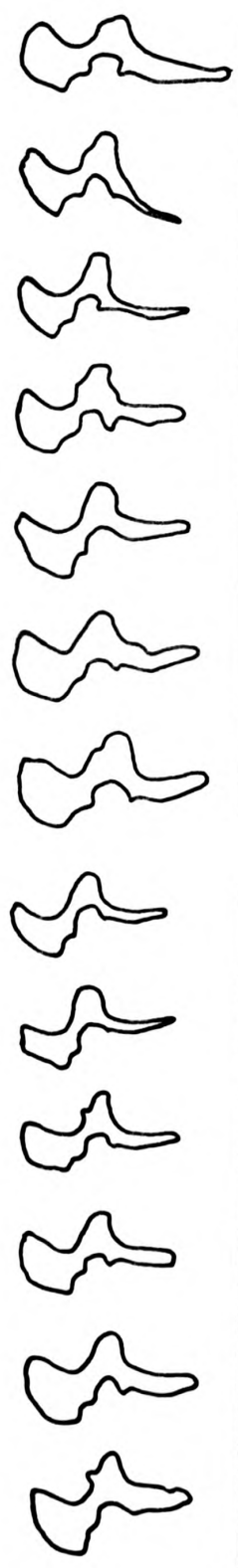
**Figure 2.9.** Silver stained adhesive disc morphology in specimens of *Trichodina acuta*, populations e and f:

1, 2. Population (e) from *Oncorhynchus mykiss* (Loch Fad). (1200 $\times$ ).

3, 4. Population (f) from *Carassius auratus* (Pond, Stirling). (1200 $\times$ ).

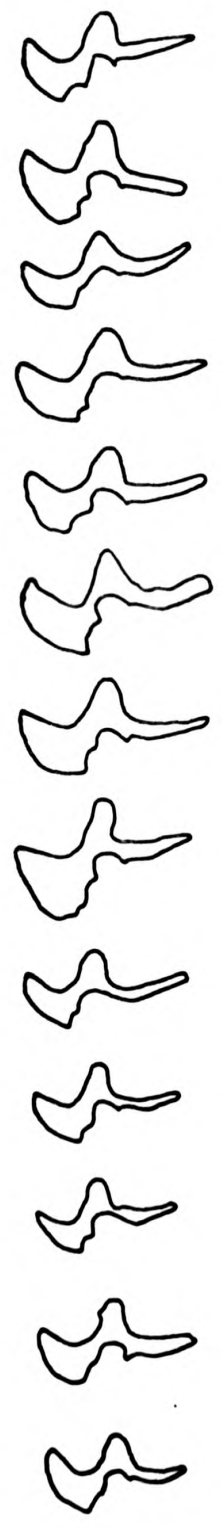


**Figure 2.10.** Silver stained adhesive disc morphology in specimens of *Trichodina acuta*, population g (1-2), and a single specimen from *Scardinius erythrophthalmus* (3).  
1, 2. Population (g) from *Oncorhynchus mykiss* (Dollar). (1200 $\times$ ).  
3. Specimen from *Scardinius erythrophthalmus* (Chorlton). (1200 $\times$ ).



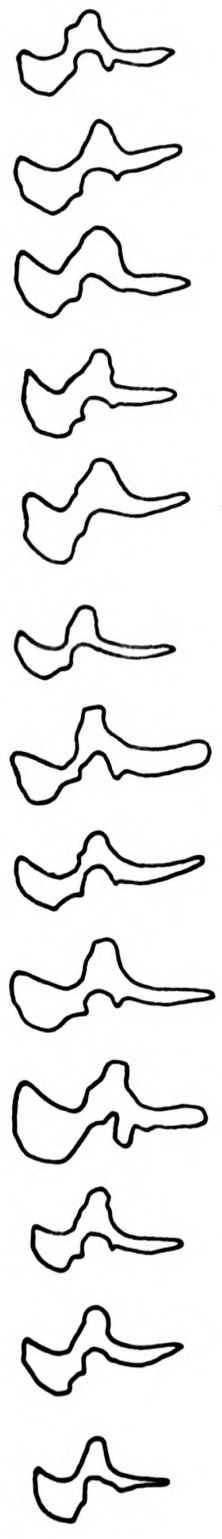
From  
*S. erythrophthalmus*

b



e

d

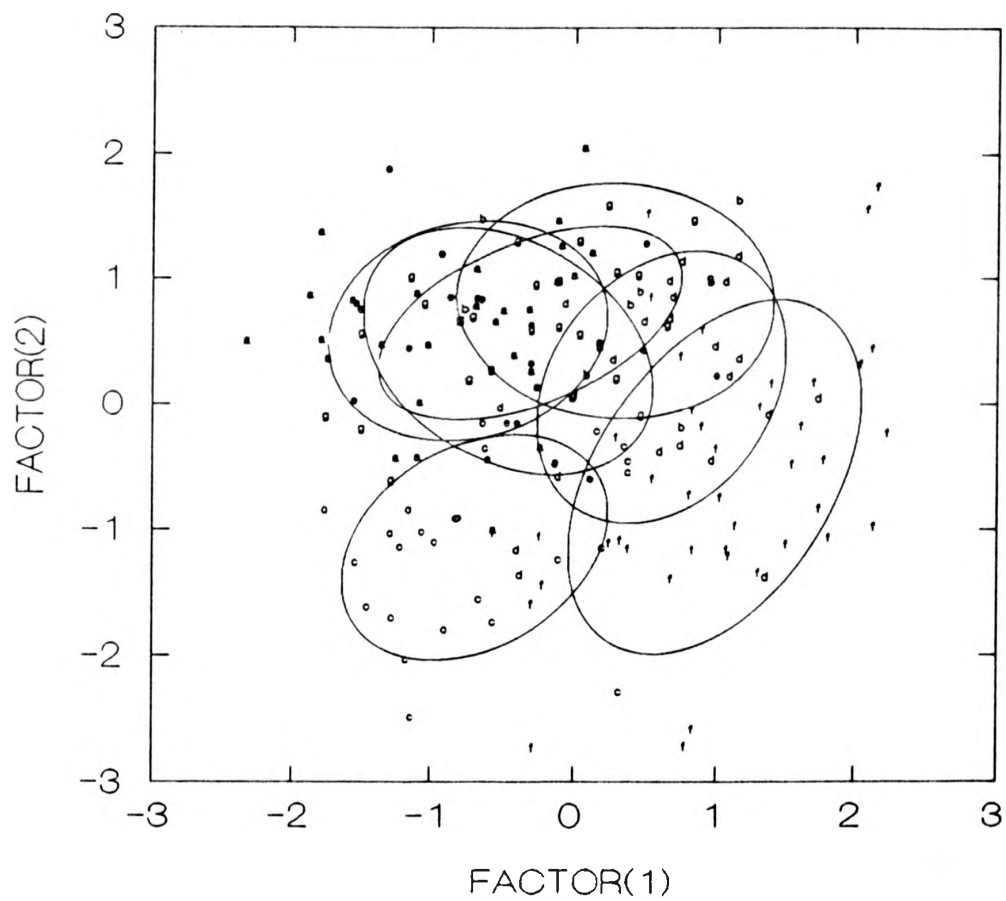


e

f

b

Figure 2.11. Range of denticle morphological variation in *Trichodina acuta* (a-g).



**Figure 2.12.** PCA plot of *Trichodina acuta* populations (a-g), enclosed by 60% ellipses.



(pond, Institute of Aquaculture), are separated from the main group. Population (c) is separated from the main group along Factor 2 where denticle number and denticle

**Table 2.3.** Component loadings of each variable in the first three principal components.

	Factor 1	Factor 2	Factor 3
A. d. diam.	0.759	-0.296	-0.232
B. m. width	0.369	-0.548	-0.692
D. r. diam.	0.937	0.027	0.112
Dent. no.	0.311	-0.675	0.598
D. length	0.649	0.598	-0.091
B. length	0.821	0.066	0.037
R. length	0.739	-0.026	0.122
C. p. width	0.744	0.161	0.013
Dent. span	0.938	0.072	0.073

length are the dominant variables, mean denticle length in this population is the smallest of the seven populations analysed. Population (f) is separated along both axes, having the largest mean denticle ring diameter and denticle number.

**Table 2.4.** Percentage of variation explained by the first three principal components.

	Variance explained by components	% of total variance	Cumulative percentage
Factor 1	4.762	52.915	52.915
Factor 2	1.238	13.759	66.674
Factor 3	0.933	10.365	77.039

## Discussion

The separation expressed in Figure 2.12 by the populations from *Phoxinus phoxinus* and *Carassius auratus* is a result of intra-specific morphological variation. This variation is



probably caused by the contribution of four basic factors; temperature, host species, geographic separation and sampling error.

The two populations previously mentioned were taken from environments with a relatively high water temperature, *Phoxinus phoxinus* from a tank at room temperature and *Carassius auratus* from a very small shallow ornamental pond during warm weather. The adhesive disc diameter of *Trichodina acuta* decreases with increasing temperature (Kazubski & Migala, 1968). Therefore, as the two populations in question have the highest mean adhesive disc diameters temperature dependent morphological variation seems unlikely.

Host induced variation is perhaps also unlikely, because although four out of five of the main group of populations are from salmonids the fifth is from a cyprinid host (b, carp), which is distinct from the other cyprinid populations (c and f).

Geographic separation would usually indicate the time available for genetic drift to have occurred since a 'founder' population diverged as the host species range increased. Therefore, it would be expected that the greater the geographic separation of two trichodinid populations the greater the inter-population morphological variation will be. However, when comparing parasite populations from a range of farmed, introduced and indigenous host species the basis for the theory behind geographic variation becomes confused because of artificial relocation of fish populations.

Sampling error is caused by the sample size being too small to represent the actual variation within a population. (Because of probability theory, samples may not represent total variation, thus samples from the same population may illustrate natural variation).

Thus, in the case of *Trichodina acuta* no single factor can be identified to

explain the observed variation.

The high degree of intraspecific variation observed in *Trichodina acuta* lends weight to the idea that *Trichodina diaptomi* (Basson & Van As, 1991) may be synonymous with *Trichodina acuta*, as one of the main characteristics of *Trichodina diaptomi* cited is its small mean adhesive disc diameter. The mean adhesive disc diameter varies by 20% from the smallest to the largest population of *Trichodina acuta* observed in this study. The danger of using only mean values when comparing populations must be emphasised, as this gives no indication of the distribution about the mean and whether the mean is skewed by a small number of extremely low or high values.

Specimens of *Trichodina acuta* were found with a denticle form almost identical to that found in *Trichodina compacta* (Van As & Basson, 1989), being more robust with a defined distal surface to the blade; the only discrepancy being in adhesive disc diameter and relative central circle diameter. The population described as *Trichodina compacta* is said to be smaller than *Trichodina acuta*. However, its mean adhesive disc diameter is nearer to that given for the population with the smallest specimen size in this study, than the smallest population found here is to the largest. The additional fact that the Philippino population of *Trichodina acuta* (Duncan, 1977) is described as being intermediate in size and morphology between European forms (Kazubski & Migala, 1968), and *Trichodina compacta*, suggests that all these populations may represent different forms within *Trichodina acuta*.

Depending on the morphology of specimens in any 'founder' population, populations of any organism relying partly or completely on binary fission could quickly become morphologically distinct whilst still belonging to the same species. Two separate

populations could be produced from single atypical specimens, which by reproducing asexually to form clonal populations could give an impression of morphological dissimilarity whilst retaining genetic similarity and the ability to interbreed sexually.

The literature illustrates the low host specificity of *Trichodina acuta*, having been described from approximately 20 fish species, diaptomus and frog tadpoles as previously discussed. This low host specificity is reinforced by the fact that it was the most common species found during this study. Photomicrographs and morphometric data from 177 specimens contributed to the identification of *Trichodina acuta* from seven host species, *Salmo trutta*, *Oncorhynchus mykiss*, *Cyprinus carpio*, *Carassius auratus*, *Phoxinus phoxinus*, *Scardinius erythrophthalmus* and *Gasterosteus aculeatus*. *Salmo trutta* and *Phoxinus phoxinus* constitute new host records for this species.

#### **TRICHODINA DOMERGUEI WALLENGREN, 1897**

##### **Introduction**

Lom & Stein (1966) discuss the history of *Trichodina domerguei* (Wallengren, 1897) in the literature; *Cyclochaeta domerguei* Wallengren (1897) was the first record of trichodinids from the surface of sticklebacks; subsequently Wetzel (1927) and Zick (1928) recognised that the genus *Cyclochaeta* was in fact synonymous with *Trichodina*. Dogel (1940 in: Lom & Stein, 1966) considered that all trichodinids from fresh water fish belonged to the species *Trichodina domerguei* Wall., he described five "forms" from different hosts one of which was *Trichodina domerguei* f. *latispina* from *Pungitius pungitius*. Faure-Fremiet (1943) on analysing trichodinid populations from the surface of *Gasterosteus aculeatus* divided them into two species, *Trichodina domerguei* f.

*latispina* and a new species *Trichodina tenuidens* (Lom & Stein, 1966). In 1959 Raabe, using silver stained material designated the typical *Gasterosteus* trichodinid form as *Trichodina domerguei* f. *latispina*. Presumably, Raabe's typical *Gasterosteus* trichodinid was sampled from skin smears, as the occurrence of *Trichodina tenuidens* typically found on the gills is not mentioned.

The many forms and subspecies of *Trichodina domerguei* described by various authors have been revised as follows (Lom & Hoffman, 1964; Lom & Stein, 1966; Lom, 1970a): *Trichodina domerguei* f. *magna* Lom, 1961 → *Trichodina fultoni* Lom & Hoffman, 1964. *Trichodina domerguei* f. *megamicronucleata* Dogel, 1940 → *Trichodina reticulata* Hirschmann & Partsch, 1955. *Trichodina domerguei* f. *borealis* Dogel, 1940 → *Trichodina borealis* (Shulman & Shulman-Albowa, 1953). *Trichodina domerguei* f. *percarum* Dogel, 1940 → *Trichodinella epizootica* (Raabe, 1950). *Trichodina domerguei* f. *caspialosae* Dogel, 1940 → *Trichodina caspialosae* (Lom, 1962). *Trichodina domerguei* f. *meridionalis* Dogel, 1940 → nomen dubium (conglomerate of several species). *Trichodina domerguei* subsp. *jadranica* Raabe, 1959 → *Trichodina jadranica* (Haider, 1964). *Trichodina domerguei* subsp. *cardii* → nomen dubium. *Trichodina domerguei* subsp. *acuta* Lom, 1961 → *Trichodina acuta* (Lom, 1970). *Trichodina domerguei* subsp. *esocis* Lom, 1961 → *Trichodina esocis* (Lom, 1970). *Trichodina domerguei* subsp. *partidisci* Lom, 1961 → nomen dubium.

Lom (1970a) mentions *Trichodina domerguei* subsp. *gobii* (Haider, 1964) which he suggests merits raising to a separate species. However, later references to *Trichodina gobii* Lom, 1970 (Arthur & Lom, 1984) refer to a species previously known as *Trichodina nigra* subsp. *gobii* Lom, 1961. Therefore, the status of *Trichodina domerguei* subsp. *gobii* is uncertain.

*Trichodina domerguei* has subsequently been recorded from *Gasterosteus aculeatus* and *Pungitius pungitius*, in France, Poland, USSR and the Japanese coast; straight-nosed pipefish *Nerophis ophidion* L., *Gobius minutus* L., black goby *Gobius niger* L. and *Cottus (Myoxocephalus) scorpius* from the Baltic Sea (Lom & Stein, 1966). *Trichodina domerguei* has been recorded in Britain on *Gasterosteus aculeatus* from Llyn Padarn (Chubb, 1970b), the Norfolk Broads (Dartnall *et al.*, 1972) and from *Pungitius pungitius* in the River Roding, Essex (Dartnall *et al.*, 1973). Further records include those of Calenius (1980), from *Gasterosteus aculeatus* and four-horned sculpins *Myoxocephalus quadricornis* L. in Finland and Grupcheva & Sedlacek (1993) from *Gasterosteus aculeatus* from small ponds in eastern Germany.

Isakova (1970) transferred populations of *Trichodina domerguei* and *Trichodina tenuidens* parasitising sticklebacks from fresh to sea water, and noted that only when done gradually did the ciliates survive. It was suggested that gill protozoans i.e. *Trichodina tenuidens* were less resistant to changes in salinity, because of their exposure to excess salts excreted by the host.

## Results

During this study *Trichodina domerguei* was recorded from *Gasterosteus aculeatus* from Airthrey Loch. A second population was identified from Ardtoe on the west coast of Scotland inhabiting water at a salinity of 33‰. Due to the reduced quality of stain produced with marine trichodinids, only a few good quality specimens were obtained from this population. Morphometric and meristic data for the two populations are given in Table 2.5.

In addition to the previous records, three specimens of *Trichodina domerguei*

were found on *Oncorhynchus mykiss* from Moffat.

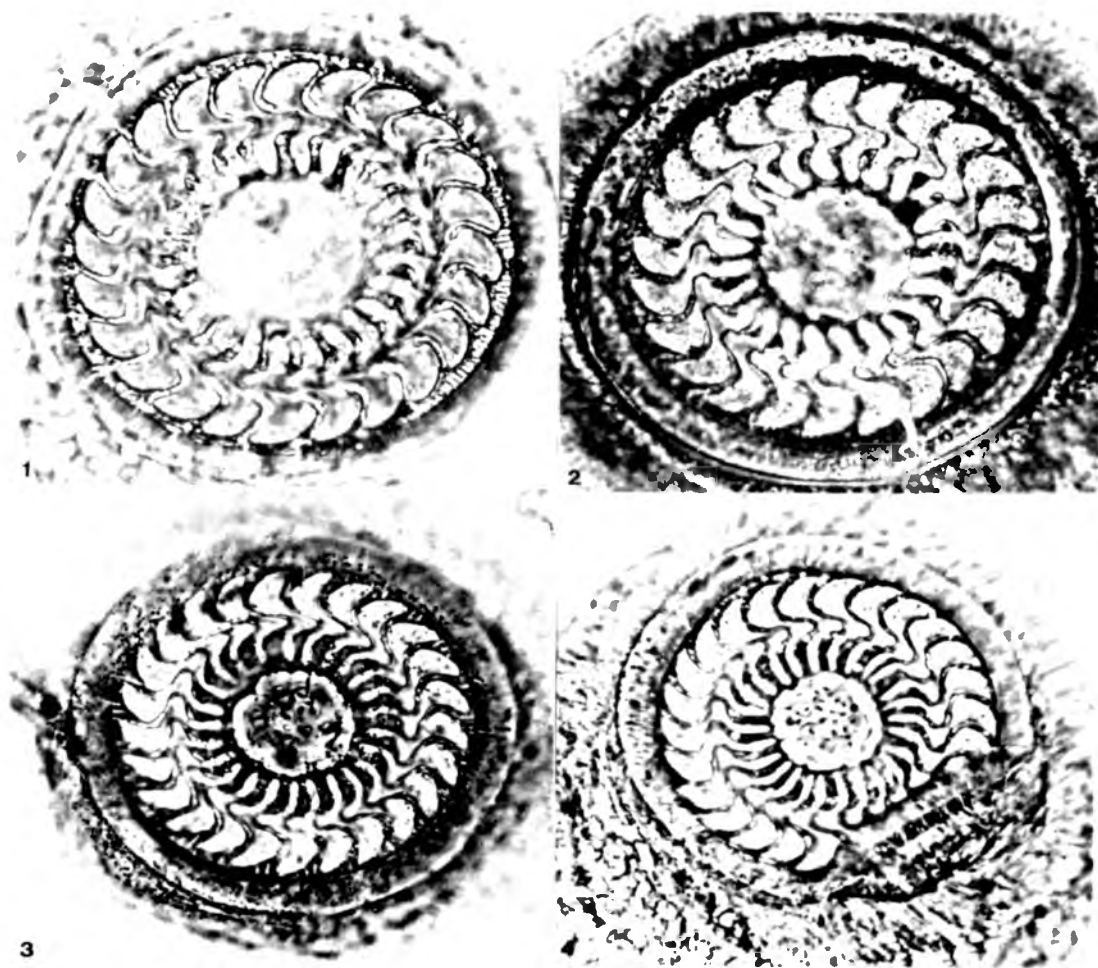
*Trichodina domerguei* has an adoral disc diameter of 39.8-68.3 $\mu$ m (Airthrey Loch & Ardtoe) with broad rounded denticle blades and slightly curved, relatively short, broad rays, often with a well defined anterior ray apophysis (see Chapter 4). An unusual feature of the denticle rays is that they are often broader at the end than where they join the central part of the denticle. This species has a well defined central circle, interspersed with granules or vacuoles. The adoral cilia described a turn of approximately 390°.

**Table 2.5.** Morphometric and meristic data for *Trichodina domerguei*.

Author	Lom & Stein, (1966)	This study	This study
Host	<i>Pungitius pungitius</i> and <i>Gasterosteus aculeatus</i>	<i>Gasterosteus aculeatus</i>	<i>Gasterosteus aculeatus</i>
Localisation	Skin, rarely gills	Skin, very rarely gills	Skin
Locality	Near Leningrad	Airthrey Loch 2/5/92	Ardtoe 11/8/94
A. d. diam.	43-61 (51)	51.6-68.3 (59.8 $\pm$ 4.3, 24)	39.8-46.5 (43.7 $\pm$ 2.5, 6)
B. m. width	3.5-5	4.0-5.6 (4.8 $\pm$ 0.4, 24)	4.6-5.4 (4.8 $\pm$ 0.3, 6)
D. r. diam.	28-33 (31)	27.8-40.8 (32.3 $\pm$ 3.3, 24)	25.6-29.8 (27.9 $\pm$ 1.5, 6)
Dent. no.	22-28 (24)	23-31 (25.3 $\pm$ 2.0, 24)	23-27 (25.2 $\pm$ 1.8, 6)
R. p / d.	9-10	8-11 (9.75 $\pm$ 0.8, 6)	8-11 (9.2 $\pm$ 1.2, 6)
D. length	11-12	7.8-9.6 (8.7 $\pm$ 0.6, 24)	7.6-9.6 (8.2 $\pm$ 0.7, 6)
B. length	3.5-7	5.0-9.3 (6.5 $\pm$ 0.9, 24)	4.9-6.1 (5.7 $\pm$ 0.5, 6)
R. length	4-5	4.2-5.8 (4.9 $\pm$ 0.4, 24)	4.8-5.6 (5.0 $\pm$ 0.3, 6)
C. p. width	3	2.3-3.1 (2.6 $\pm$ 0.2, 24)	2.2-2.6 (2.4 $\pm$ 0.1, 6)
C. c. diam.	-	15.1-26.8 (19.1 $\pm$ 2.9, 23)	12.3-16.1 (14.5 $\pm$ 1.3, 6)
Dent. span	-	11.9-15.6 (13.8 $\pm$ 1.1, 24)	11.9-13.8 (13.2 $\pm$ 0.7, 6)

Photomicrographs of both populations are illustrated in Figure 2.13.

The marine specimens are substantially smaller than those from fresh water, with shorter denticle blades but similar ray length.



**Figure 2.13.** Silver stained adhesive disc morphology in specimens of *Trichodina domerguei* from *Gasterosteus aculeatus*.

**1, 2.** Specimens from Airthrey Loch (freshwater). (1200 $\times$ ).  
**3, 4.** Specimens from Ardtoe (marine). (1200 $\times$ ).

## Discussion

*Trichodina domerguei* is unusual in being one of the few euryhaline trichodinid species recorded to date. It is similar to *Trichodina jadratica*, *Trichodina cottidarum* Lom, 1970 (Lom, 1970b) and *Trichodina murmanica* Polyanski, 1955 (Lom & Dykova, 1992). These species are also euryhaline or marine trichodinids which may suggest that *Trichodina domerguei* is of marine origin. This idea is supported by the high degree of host specificity (euryhaline sticklebacks) displayed in the freshwater environment and the comparatively large number of marine host species. The smaller dimensions of the marine specimens described in this study agree with the findings of Lom (1970b) who remarks on the small specimen size in marine populations. This is possibly further evidence of environmentally induced morphological variation. Morphological variation of *Trichodina domerguei* is discussed in greater detail in Chapter 6.

A fresh water species *Trichodina fultoni* Davis, 1947 (Stein, 1984), is of also of similar appearance being originally considered a subspecies of *Trichodina domerguei*. However, it has a higher number of more delicate denticles with a greater curvature of the denticle blades.

The small number of specimens identified from the skin of *Oncorhynchus mykiss* is undoubtedly an accidental infection from neighbouring sticklebacks, and does not represent a natural host.

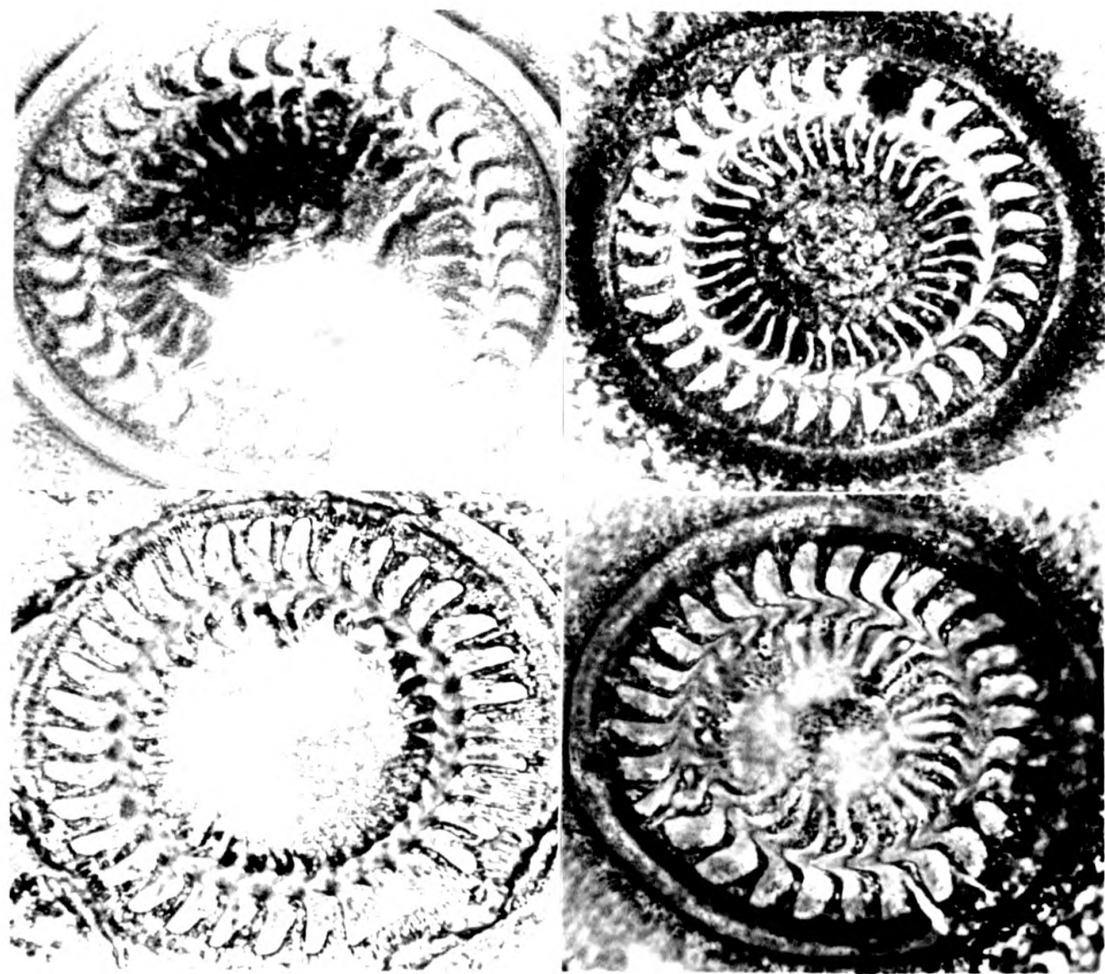


## **TRICHODINA TENUIDENS FAURE-FREMIET, 1944**

### **Introduction**

*Trichodina tenuidens* Faure-Fremiet, 1944 is commonly found alongside *Trichodina domerguei* parasitising *Gasterosteus aculeatus* and *Pungitius pungitius*. The first description of *Trichodina tenuidens* occurred when Faure-Fremiet found a bimodal curve on a graph of denticle number from trichodinids parasitising *Gasterosteus aculeatus* (Lom & Stein, 1966). One peak corresponded to *Trichodina domerguei* (23 denticles), the other (31-34 denticles), he considered a different species and designated as *Trichodina tenuidens*. Descriptions of trichodinids considered synonymous with *Trichodina tenuidens* (Lom & Stein, 1966) include: *Cyclochaeta domerguei* Wall., pro parte, *Trichodina pediculus* f. *latispina* (Dogel, 1940) Stryjecka-Trembaczowska, 1953; and *Trichodina gracilis* Poljansky, 1955.

*Trichodina tenuidens* has been recorded on the gills, and rarely on the skin of *Gasterosteus aculeatus* and *Pungitius pungitius* (Lom & Stein, 1966) from France, Poland and the USSR (Baltic, White and Barents Sea coasts). British records include *Gasterosteus aculeatus* from Llyn Padarn (Chubb, 1970b), the Norfolk Broads (Dartnall *et al.*, 1972) and *Pungitius pungitius* in the River Roding, Essex (Dartnall *et al.*, 1973). Further records include those of Calenius (1980), from *Gasterosteus aculeatus* in Finland and Grupcheva & Sedlaczeck (1993) on *Gasterosteus aculeatus* from small ponds in eastern Germany. The morphological variation of denticle form and appearance of the central circle in *Trichodina tenuidens* is said to be one of the greatest amongst trichodinid species (Lom & Stein, 1966).



**Figure 2.14.** Silver stained adhesive disc morphology in specimens of *Trichodina tenuidens* from *Gasterosteus aculeatus* (Airthrey Loch). (1200 $\times$ ).

## TRICHODINA TENUIDENS FAURE-FREMIET, 1944

### Introduction

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*Trichodina tenuidens* has been recorded on the gills, and rarely on the skin of *Gasterosteus aculeatus* and *Pungitius pungitius* (Lom & Stein, 1966) from France, Poland and the USSR (Baltic, White and Barents Sea coasts). British records include *Gasterosteus aculeatus* from Llyn Padarn (Chubb, 1970b), the Norfolk Broads (Dartnall *et al.*, 1972) and *Pungitius pungitius* in the River Roding, Essex (Dartnall *et al.*, 1973). Further records include those of Calenius (1980), from *Gasterosteus aculeatus* in Finland and Grupcheva & Sedlacek (1993) on *Gasterosteus aculeatus* from small ponds in eastern Germany. The morphological variation of denticle form and appearance of the central circle in *Trichodina tenuidens* is said to be one of the greatest amongst trichodinid species (Lom & Stein, 1966).

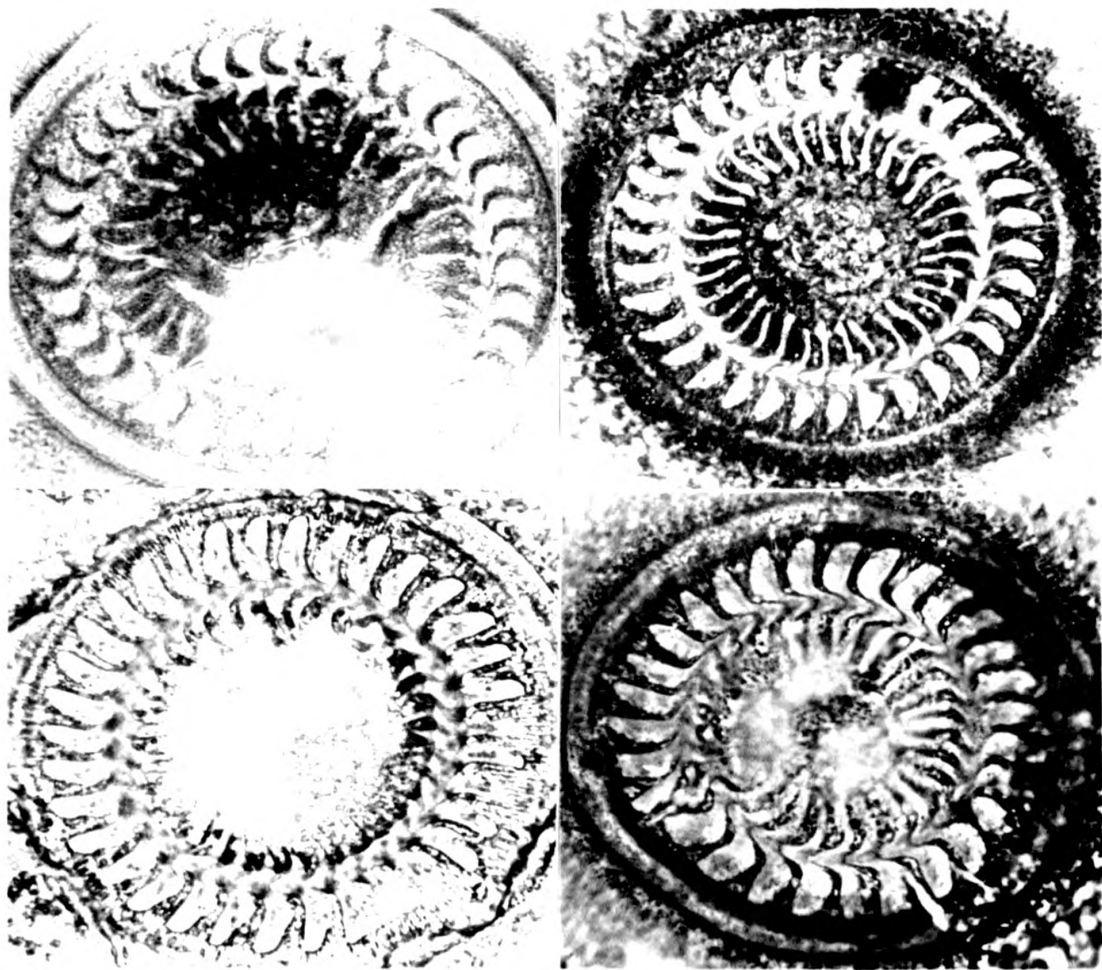
## Results

*Trichodina tenuidens* is characterised by the morphology of the adhesive disc (Fig 2.14). It is a medium sized *Trichodina* species (a. d. diam=52.3-78.6 $\mu$ m) with a relatively high number of denticles, which are finer and more elongated than in *Trichodina domerguei*. The denticle blade has an almost straight or slightly curved posterior margin, and the blade itself is highly variable in form. The inner rays are longer than those of *Trichodina domerguei*, but share the trait of widening towards the tip. The central part of the disc ranges from an argentophilic circular structure, to a few large granules. The adoral cilia described a turn of approximately 380-400°. Morphometric and meristic data for a population of *Trichodina tenuidens* (Airthrey Loch) are given in Table 2.6.

As in the case of *Trichodina domerguei*, *Trichodina tenuidens* is a euryhaline

**Table 2.6.** Morphometric and meristic data for *Trichodina tenuidens*.

Author	Lom & Stein, 1966	This study
Host	<i>Gasterosteus aculeatus</i>	<i>Gasterosteus aculeatus</i>
Localisation	Gills, rarely skin	Gills, rarely skin
Locality	Lake Mamry, Poland	Airthrey Loch 2/5/92
A. d. diam.	45-69	52.3-78.6 (70.8 $\pm$ 6.3, 16)
B. m. width	4.5-5	4.1-5.8 (4.7 $\pm$ 0.4, 16)
D. r. diam.	25-40 (31)	30.8-45.6 (40.7 $\pm$ 3.9, 16)
Dent. no.	25-33 (28)	29-38 (34.7 $\pm$ 2.8, 16)
R. p / d.	8-9	9-12 (9.5 $\pm$ 0.1, 15)
D. length	7-9	6.4-8.2 (7.3 $\pm$ 0.4, 16)
B. length	4.5-7	5.6-8.6 (7.2 $\pm$ 0.9, 16)
R. length	5-7	6.1-9.8 (7.4 $\pm$ 0.8, 16)
C. p. width	2-2.5	1.8-2.7 (2.3 $\pm$ 0.2, 16)
Dent. span	-	14.2-19.4 (16.7 $\pm$ 1.5, 16)



**Figure 2.14.** Silver stained adhesive disc morphology in specimens of *Trichodina tenuidens* from *Gasterosteus aculeatus* (Airthrey Loch). (1200 $\times$ ).

species. It was identified during this study from a marine population of *Gasterosteus aculeatus* from Ardtoe on the west coast of Scotland, although insufficient numbers were available to enable morphometric comparison with freshwater specimens. The previous sample from Airthrey Loch has a larger mean specimen size than that given by Lom & Stein.

### Discussion

The Airthrey Loch sample (2/5/92) of *Trichodina tenuidens* is composed of unusually large specimens, (this is possibly due to the small sample size). Large specimens are usually associated with low water temperatures during the winter (see Chapter 6).

*Trichodina tenuidens* is very closely related to *Trichodina domerguei*, being very similar in size and appearance. They are separated mainly by denticle number and finer denticle morphology in *Trichodina tenuidens*. Lom & Stein (1966) comment on the presence of specimens which, if considered in isolation, cannot be distinguished as one species or the other. These 'intermediate' specimens are thought (Lom & Stein, 1966) to represent the extremes in variation of either species, which is discussed in detail in Chapter 6. The high degree of host specificity exhibited by both species, especially *Trichodina tenuidens*, and their presence in marine and freshwater environments, reinforces the close links between the two species.

The fact that *Trichodina tenuidens* is found almost exclusively on the gills is another unusual characteristic. During this study specimens were occasionally found in skin smears, originating from the opercular region. There are few obligate gill trichodinid species in the genus *Trichodina*, most gill species belong to the genera *Tripartiella*, *Trichodinella* and *Paratrachodina*.

## TRICHODINA PEDICULUS EHRENBERG, 1838

### Introduction

*Trichodina pediculus* Ehrenberg, 1838 is a widely distributed species occurring mainly on hydras, but also on tadpoles and various species of fish (Kazubski, 1991 a, b and c). Many early synonymous populations of this species are described in the literature (Lom, 1970a) including: *Cyclidium pediculus* Muller, 1786; *Urceolaria discina* Lamark, 1816; *Bursaria pediculus* Bory de St. Vincent, 1822; *Numulella conchyliospermatica* Carus, 1832; *Urceolaria stellina* Dujardin, 1841; *Trichodina fultoni* Davis, 1947 pro partum and *Trichodina hydrae* Susuki, 1950. *Trichodina pediculus* has been reported from black carp *Mylopharyngodon piceus* Richardson, *Ctenopharyngodon idella*, bighead carp *Aristichthys nobilis* Richardson and *Hypophthalmichthys molitrix* in China (Chen, 1956 in: Lom, 1970a); from *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* in the USSR (Ivanova, 1967; and Musselius, 1967; in Lom, 1970a); and from fry of many species of cyprinids (Kulemina, 1968 and Kazubski, 1965 in: Lom, 1970a). Wellborn (1967) reported *Trichodina pediculus* from the southern United States on *Micropterus salmoides*, however this population differs in denticle number and ray morphology from other descriptions. Further descriptions of *Trichodina pediculus* mainly from Czechoslovakia (Lom, 1970a), are from hydra, the nasal pits of *Ctenopharyngodon idella*, the skin of adult *Leuciscus cephalus*, *Abramis brama*, *Rutilus rutilus*, *Cyprinus carpio*, *Carassius auratus f. gibelio*, *Alburnus alburnus*, *Scardinius erythrophthalmus*, *Stizostedion lucioperca* and the surface of *Hydra vulgaris attenuata*. Other reports are from *Esox lucius* in Finland (Calenius, 1980), and the USSR (Arthur & Lom, 1984). Basson, Van As & Paperna (1983) reported *Trichodina pediculus* from South Africa and

Israel, however morphometric measurements and photomicrographs suggested this to be a misidentification, which Van As & Basson (1989) rectified in a later publication.

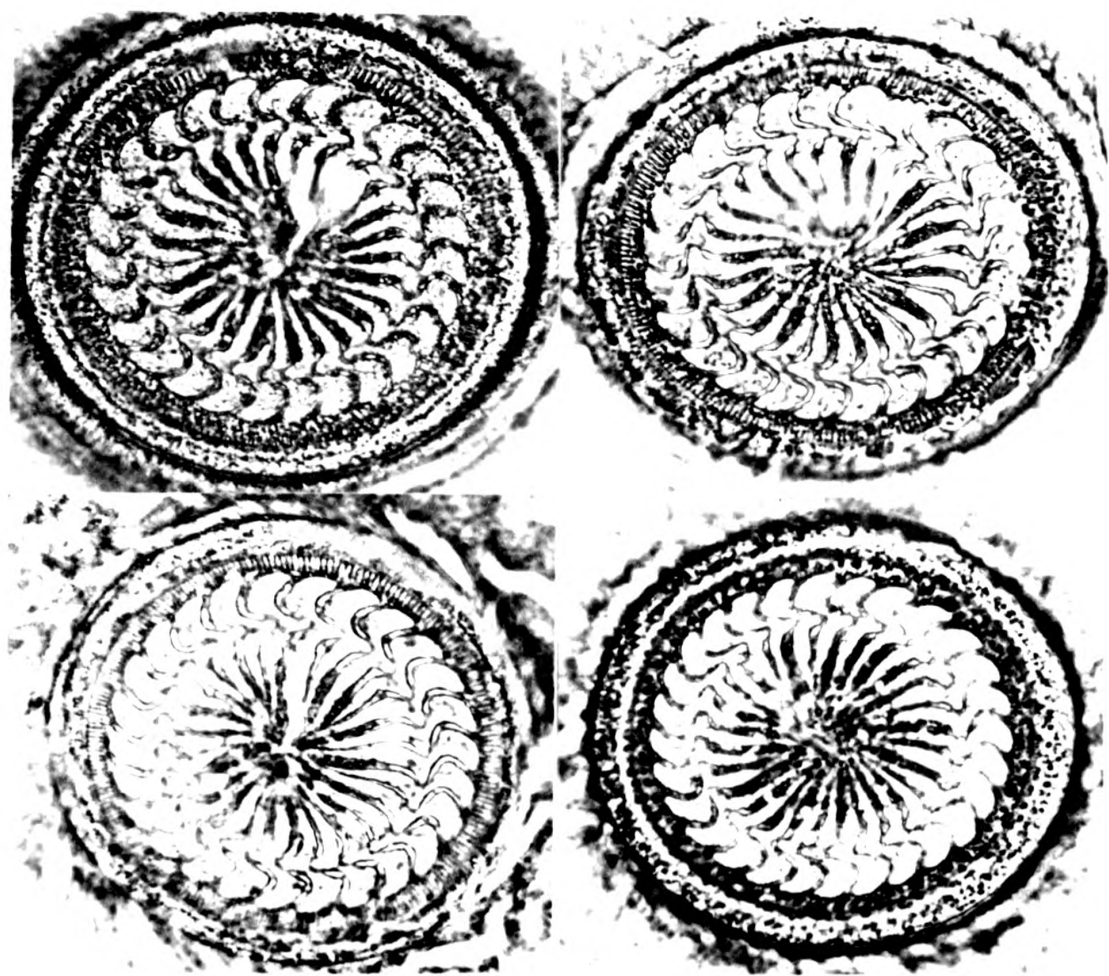
## Results

Small numbers of *Trichodina pediculus* were found on the skin of *Gasterosteus aculeatus*, photomicrographs, morphometric and meristic data are presented in Figure 2.15 and Table 2.7. Denticle morphology is notable for the short, broad, curved blades almost forming a 'crook' at the apex of the anterior margin. In some specimens the anterior blade margin appears to form a continuous radius, in others a pronounced distal surface can be discerned parallel to the edge of the border membrane. The distinguishing features of *Trichodina pediculus* are the denticle rays, which are extremely long and tapering. The centre of the adhesive disc appears granular, with no central circle visible.

**Table 2.7.** Morphometric and meristic data for *Trichodina pediculus*.

Author	Kazubski, 1991	This study
Host	<i>Carassius carassius</i>	<i>Gasterosteus aculeatus</i> 1992-1993
Localisation	-	skin
Locality	Kortowo, Poland	Airthrey Loch
A. d diam.	(54.96±4.52)	46.0-57.2 (50.1±3.6, 12)
B. m. width	(3.9)	3.4-5.0 (4.4±0.4, 12)
D. r. diam.	(35.70±2.83)	29.3-34.0 (32.0±1.6, 12)
Dent. no.	(28.29±1.54)	26-29 (27±1.0, 12)
R. p. / d.	-	6-8 (7.5±0.8, 9)
D. length	-	6.6-8.3 (7.5±0.6, 12)
B. length	-	4.9-6.2 (5.6±0.3, 12)
R. length	-	9.2-13.6 (11.6±1.3, 12)
C. p. width	-	1.3-2.5 (1.9±0.3, 12)
Dent. span	(19.12±2.38)	16.6-21.8 (18.9±1.6, 12)





**Figure 2.15.** Silver stained adhesive disc morphology in specimens of *Trichodina pediculus* from *Gasterosteus aculeatus* (Airthrey Loch). (1200 $\times$ ).

## Discussion

The specimens of *Trichodina pediculus* were very similar morphologically and morphometrically to those described by Kazubski (1991a, b and c). In view of the low numbers of *Trichodina pediculus* found on sticklebacks in Airthrey Loch (N=12) over the course of a years sampling, it may be that these findings represent accidental infections from a population parasitising hydra or other fish species.

Lom (1970a) found a heavy infection in the nasal pits of *Ctenopharyngodon idella*, but virtually none on the skin. The specimens identified during this study were from skin smears taken during a years sampling of *Trichodina domerguei* and *Trichodina tenuidens*. At no time were the nasal pits of sticlebacks investigated.

This sample of *Trichodina pediculus* constitutes a British record and *Gasterosteus aculeatus* a new host record.

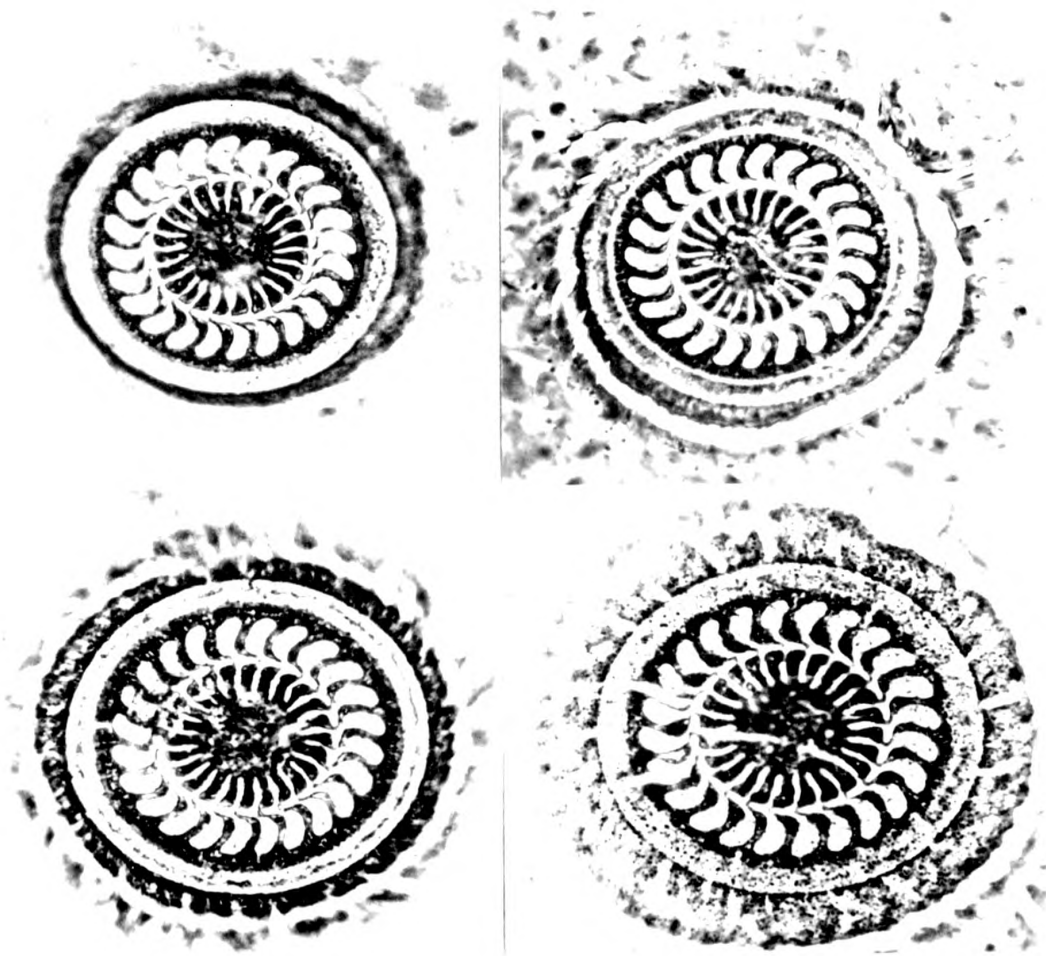
## TRICHODINA MODESTA LOM, 1960

### Introduction

*Trichodina modesta* Lom, 1970 was first reported from the gills of *Vimba vimba* L., in South Bohemia, and *Abramis brama* from the Tisza River in Hungary (Lom, 1970a). Stein (1984) reported the same trichodinid species parasitising *Abramis brama* from the north eastern Soviet Union. Arthur & Lom (1984) subsequently reported the species from the gills of white bream, *Blicca bjoerkna* L. collected from the Rybinsk reservoir (USSR), constituting only the third report of this species.

### Results

A single population of *Trichodina modesta* on the gills of *Abramis brama* was found



**Figure 2.16.** Silver stained adhesive disc morphology in *Trichodina modesta* from *Abramis brama* (Yorkshire). (1200 $\times$ ).

**Table 2.8.** Morphometric and meristic data for *Trichodina modesta*.

Author	Lom, 1970a	This study
Host	<i>Abramis brama</i>	<i>Abramis brama</i>
Localisation	Gills	Gills
Locality	River Tisza, Hungary	Yorkshire 20/2/93
A. d. diam.	28-43(33)	31.3-42.6 (35.1±3.0, 21)
B. m. width	3-3.5	3.4-4.3 (3.9±0.3, 21)
D. r. diam.	15-20 (18)	18.5-23.9 (20.8±1.6, 21)
Dent. no.	22-25 (23)	22-26 (24.0±1.0, 21)
R. p. / d.	7-8	6-10 (8.3±0.9, 17)
D. length	3-3.5	4.5-5.9 (5.2±0.4, 21)
B. length	4-4.5	4.5-5.8 (5.1±0.4, 21)
R. length	3.5-4	3.8-5.1 (4.5±0.4, 21)
C. p. width	1.5	1.1-1.6 (1.3±0.1, 21)
Dent. span	-	9.3-12.1 (10.8±0.7, 21)

during this study. It is relatively small (a. d. diam = 31.1-42.6µm) and the denticles have relatively delicate inner rays, straight or slightly curved posteriorly. The blades are elongated (Lom, 1970a), and gently curved with a noticeable thickening of the posterior border. The denticle blades usually terminate with a rounded anterior margin, but sometimes form a flattened distal surface. The oral cilia express a turn of approximately 360-400°, similar to most other *Trichodina species*. Photomicrographs, morphometric and meristic data are given in Figure 2.16 and Table 2.8.

### Discussion

The population of *Trichodina modesta* found in this study agrees closely with Lom's (1970a) nominate population from *Abramis brama* in form and size. The small differential in size may be due to the low water temperature during February, when the population in this study was sampled. As discussed in detail in Chapter 6, trichodinid

size universally increases with decrease in temperature. Lom (1970a) gives no details of the sampling date or water temperature at the time his description was made. The description of *Trichodina modesta* from *Blicca bjoerkna* (Arthur & Lom, 1984) collected during the spring, also presents a smaller mean specimen size (mean a. d. diam = 27.0µm). However, the different host species and geographic separation may account for this discrepancy. Due to the small number of observed populations, the extent of morphological variation in this species is unknown.

*Trichodina modesta* bears some resemblance to summer forms of *Trichodina mutabilis* Kazubski, 1968 (Grupcheva, 1975a; Lom, Golemansky & Grupcheva, 1976; Albaladejo & Arthur, 1989) and *Trichodina rostrata* Kulemina, 1968 (Lom, 1970a; Kashkovsky, 1974; Grupcheva & Sedlacek, 1993). However, both these species differ in denticle form and are considerably larger in size.

*Trichodina spathulata* Kulemina, 1968 from *Abramis brama* fry, is cited as being significantly smaller than *Trichodina modesta* (Lom, 1970a) with wider blades. However, Kashkovsky's (1974) photomicrograph and morphometric measurements for *Trichodina spathulata* from *Abramis brama* (mean a. d. diam = 26µm) do not appear to vary significantly from previous descriptions of *Trichodina modesta*. The specimens of *Trichodina modesta* found during this study vary in denticle blade form; some specimens conforming to Lom's (1970a) original description, while others display slightly broader denticles. Considering that all the descriptions of *Trichodina modesta* and *Trichodina spathulata* are from closely related "bream" host species, the small discrepancy in form and size may suggest that the two species are synonymous. In this case the populations of *Trichodina modesta* described so far, would revert to *Trichodina spathulata*.

## TRICHODINA NIGRA LOM, 1960

### Introduction

The first description of *Trichodina nigra* Lom, 1960 was from the skin and more rarely the gills of *Cyprinus carpio*, *Scardinius erythrophthalmus*, *Rutilus rutilus*, *Abramis brama*, *Perca fluviatilis*, tench *Tinca tinca* L., bleak *Alburnus alburnus* L., *Leuciscus cephalus* and on the skin of different frog tadpoles in Czechoslovakia. Lom (1961) states that in the hosts previously mentioned "the individuals of *Trichodina nigra* agree in their principal characters, so that there is no substantial diversity among them". However, it was noted in certain other hosts that specimens deviated from the 'norm', and were designated as separate forms.

*Trichodina nigra* f. *gobii* Lom, 1960 (Lom, 1961) was described from the gills of gudgeon *Gobio gobio* L., this form closely resembles photomicrographs of *Trichodina nigra* f. *nigra* given by Kazubski & Migala (1968). The measurements given for the 'form' *gobii*, are somewhat smaller than those given by Kazubski. However, comparisons are made difficult by the lack of sample size being quoted in Lom's work.

*Trichodina nigra* f. *nemachili* Lom, 1960 (Lom, 1961) was described from the gills of *Barbatula (Nemachilus) barbatulus*. This form was later described by Calenius (1980) from *Barbatula barbatulus* in Finland, although this closely resembled another form, *Trichodina nigra* f. *cobitis* Lom, 1960 (Lom, 1961). *Trichodina nigra* f. *cobitis* was described from spined loach *Cobitis taenia* L. (Lom, 1961), it was subsequently described by Calenius (1980) (incorrectly identified as f. *nemachili*), and Stein (1984). All reports of *Trichodina nigra* f. *cobitis* illustrate a high degree of homogeneity, and are distinct from any subsequent reports of *Trichodina nigra*.

Stein (1967) followed Lom's precedent by describing a *Trichodina* population from *Salvelinus alpinus*, *Oncorhynchus mykiss* and arctic grayling *Thymallus arcticus* (Pallas) in the USSR, which he designated as *Trichodina nigra* f. *kamchatika*.

The known geographic range of *Trichodina nigra* was extended considerably when it was reported from the mud sunfish *Acantharchus pomotis* in North America (Hoffman & Lom, 1967).

A detailed description of *Trichodina nigra* from *Cyprinus carpio* in Poland (Kazubski & Migala, 1968), closely conformed to Lom's original description (Lom, 1961). Kazubski re-examined Lom's original material and discovered that the morphometric data given for the species by Lom (1961), was confused by the inclusion of data from specimens of *Trichodina pediculus*. Lom (1970a), described additional populations of *Trichodina nigra* from the gills of *Gobio gobio*, described as *Trichodina nigra* subsp. *nigra*.

A new subspecies *Trichodina nigra* subsp. *luciopercae* from the gills of *Stizostedion luciopercae* in Hungary was described by Lom (1970a) and subsequently Stein (1984); this species is distinguished from *Trichodina nigra* by triangular denticle blades and finer rays. Lom (1970a) described a second new subspecies, *Trichodina nigra* subsp. *siluri* from the gills of wels, *Siluris glanis* L. in Hungary and Bohemia.

Lom (1970a) concluded that four subspecies could be identified: *Trichodina nigra* subsp. *nigra*, *Trichodina nigra* subsp. *gobii*, *Trichodina nigra* subsp. *luciopercae*, and *Trichodina nigra* subsp. *siluri*. The status of *Trichodina nigra* f. *cobitis* and *Trichodina nigra* f. *nemachili* being raised to species level, and *Trichodina nigra* f. *kamchatika* classified as a synonym of *Trichodina strelkovi* Chan, 1961. It should be noted that explanations of plates in Lom (1970a) may be confused, with specimens

closely resembling *Trichodina nigra* labelled as *Trichodina mutabilis*.

Further reports of *Trichodina nigra* include: Kostenko (1972) on *Asporo zingel* from the Danube; Kashkovsky (1974) from *Cyprinus carpio*, *Carassius carassius*, *Abramis brama* and *Rutilus rutilus* in the USSR; Grupcheva (1975b) on *Oncorhynchus mykiss* from Bulgaria; Kostenko & Koraev (1976) from the USSR; Stein (1979) from Lake Baikal; Kazubski & Pilecka-Rapacz (1981) parasitising *Lucioperca lucioperca* from the Szczecin Gulf; Stein (1982) from the Kurish Gulf of the Baltic Sea and Stein (1984) from the USSR. Basson, Van As & Paperna (1983) reported *Trichodina nigra* from *Oreochromis mossambicus*, *Pseudocrenilabrus philander*, and *Tilapia sparrmanii* in South Africa and *Cyprinus carpio* in Israel. In a later paper Basson & Van As (1993) reconsidered their previous identification stating that the South African specimens probably represent another species.

Arthur & Lom (1984) described *Trichodina nigra* on the gills of *Rutilus rutilus* and blue bream *Abramis ballerus* L. from the Rybinsk Reservoir in the USSR. They suggested that the concept of subspecies had become obsolete, due to the large number of new trichodinid species obscuring the level of distinctness between the subspecies of *Trichodina nigra*. Thus, the taxon of *Trichodina nigra* was retained for *Trichodina nigra nigra* Lom, 1960 and the remaining subspecies regarded separately as *Trichodina gobii* Lom, 1970; *Trichodina lucioperca* Lom, 1970; and *Trichodina siluri* Lom, 1970.

Additional reports of *Trichodina nigra*, after Arthur & Lom's (1984) revision of the species include; Van Than & Margaritov (1986) from phytophagous fish in Bulgaria, Albaladejo & Arthur (1989) from *Cyprinus carpio* in the Philippines, Halmetoja *et al.* from *Perca fluviatilis* and *Rutilus rutilus* from Finland and Pojmanska (1995) from *Cyprinus carpio* in Poland.



## Results

Seven populations of a *Trichodina* species were found on the skin of *Cyprinus carpio*, *Salmo trutta* and *Oncorhynchus mykiss* resembling *Trichodina nigra* as described in the literature, illustrating an adoral ciliary spiral of 390-430°. Morphometric and meristic data for all the populations are given in Table 2.9.

Photomicrographs of specimens from all the populations are illustrated in Figures 2.17, 2.18, 2.19 and 2.20. The populations were compared statistically using PCA analysis. The component loadings of the first three factors are given in Table 2.10. The component loadings indicate that in Factor 1, denticle span, blade length, ray length and adhesive disc diameter are the main contributing variables. Denticle number is the main variable in Factor 2 and border membrane width in Factor 3. The percentage variance explained by the first three factors is given in Table 2.11. The PCA plot of Factor 1 against Factor 2 is illustrated in Figure 2.21. The seven populations circled by 60% ellipses, are separated into two groups along Factor 1, populations (b), (e) and (f) having a significantly smaller mean denticle span. These populations are separated along Factor 2, population (b) having a lower mean denticle number than (e) or (f). The group containing population (a), (c), (d) and (g) are clustered closely together along Factor 1, but are slightly separated along Factor 2 illustrating slight variation in denticle number.

Populations (a) from *Salmo trutta* (Buckieburn), (c), (d) and (g) from *Oncorhynchus mykiss* are very similar morphologically. They are characterised by massive denticles with broad curving blades, which display considerable inter and intra populational variation. Some blades end in a sharp point, whilst others are more rounded; some specimens display a continuous curving anterior border and others a well defined distal surface. The denticle rays are generally longer than the blades and are

**Table 2.9.** Morphometric and meristic data for *Trichodina nigra*.

Author	Kazubski & Migala, 1968	This study (a)	This study (b)	This study (c)	This study (d)	This study (e)	This study (f)	This study (g)
Host	<i>Cyprinus carpio</i> 20/4/64- 27/10/65	<i>Salmo trutta</i> 11/91	<i>Cyprinus carpio</i> 17/11/92	<i>Oncorhynchus mykiss</i> 18/3/93	<i>Oncorhynchus mykiss</i> 6/93	<i>Salmo trutta</i> 19/8/93	<i>Salmo trutta</i> 23/8/93	<i>Oncorhynchus mykiss</i> 14/8/94
Localisation	Skin	Skin	Skin	Skin	Skin	Skin	Skin	Skin
Locality	Poland	Buckieburn	Norfolk	Moffat	Loch Fad	College Mill	Almond Bank	Dollar
A. d. diam.	33.9-61.5 (47.2, 153)	47.2-70.6 (57.4±5.2, 22)	35.2-52.1 (43.1±4.5, 37)	42.4-57.2 (49.4±4.2, 17)	44.4-47.5 (45.7±1.6, 3)	36.5-47.7 (43.0±3.5, 17)	40.6-48.8 (44.6±2.3, 22)	41.2-53.6 (46.5±3.9, 22)
B. m. width	(5.0)	2.8-7.2 (4.8±0.8, 22)	3.8-5.9 (4.7±0.4, 37)	3.9-6.1 (5.0±0.5, 17)	4.8-5.3 (5.0±0.1, 3)	4.2-5.9 (5.0±0.4, 17)	4.2-5.4 (4.8±0.3, 22)	4.0-9.4 (5.0±0.5, 22)
D. r. diam.	22.8-34.0 (28.5, 153)	22.8-33.3 (28.4±2.2, 22)	20.5-31.8 (25.0±3.0, 37)	24.4-34.4 (29.3±2.7, 17)	24.8-27.4 (25.7±1.4, 3)	22.6-30.4 (26.1±2.2, 17)	24.7-31.3 (27.6±1.6, 22)	24.1-32.2 (27.9±2.3, 22)
Dent. no.	17-28 (22.5, 153)	16-21 (19.9±1.2, 22)	19-27 (21.5±1.7, 37)	20-24 (21.8±1.2, 17)	18	23-25 (24.1±0.9, 17)	23-26 (24.1±0.8, 22)	18-24 (21.9±1.5, 22)
R. p. / d.	9-11	9-11 (9.9±0.7, 22)	8-10 (9.4±0.5, 36)	8-12 (10.3±0.9, 16)	10-11	9-12 (10.3±0.9, 16)	9-12 (10.7±0.8, 22)	8-11 (9.6±0.9, 22)
D. length	-	8.3-10.6 (9.1±0.7, 22)	6.3-9.7 (7.8±0.7, 37)	7.9-10.2 (8.9±0.7, 17)	8.4-9.4 (8.9±0.4, 3)	5.8-7.8 (7.1±0.6, 17)	6.0-8.5 (7.5±0.6, 22)	7.0-9.8 (7.9±0.6, 22)
B. length	-	5.6-7.8 (7.1±0.5, 22)	4.9-6.7 (5.9±0.5, 37)	6.3-7.9 (7.2±0.1, 17)	6.8-7.4 (7.0±0.3, 3)	4.4-5.8 (5.3±0.4, 17)	4.8-5.9 (5.4±0.3, 22)	5.4-8.3 (6.9±0.6, 22)
R. length	-	6.1-8.6 (7.9±0.6, 22)	4.0-7.0 (5.3±0.6, 37)	6.5-11.1 (8.4±1.1, 17)	7.1-9.2 (8.0±1.0, 3)	4.8-7.2 (5.6±0.6, 17)	4.2-6.4 (5.4±0.5, 22)	6.0-9.3 (7.5±0.9, 22)
C. p. width	-	1.7-2.8 (2.2±0.3, 22)	1.7-2.8 (2.2±0.3, 37)	2.2-3.6 (2.7±0.3, 17)	2.1-2.6 (2.4±0.1, 3)	1.8-3.2 (2.5±0.3, 17)	2.1-3.6 (2.7±0.4, 22)	2.3-3.4 (2.9±0.3, 22)
Dent. span	11.1-17.5 (14.8, 153)	14.4-18.3 (17.2±1.1, 22)	11.6-16.0 (13.3±1.0, 37)	15.2-22.2 (18.4±1.7, 17)	16.9-17.5 (17.1±0.3, 3)	10.7-15.6 (13.4±1.1, 17)	11.5-14.6 (13.5±0.9, 22)	13.1-19.3 (17.0±1.5, 22)

angled straight down or to the posterior side of the denticle with a pronounced apophysis. They taper from a broad or very broad junction with the central part to a rounded tip.

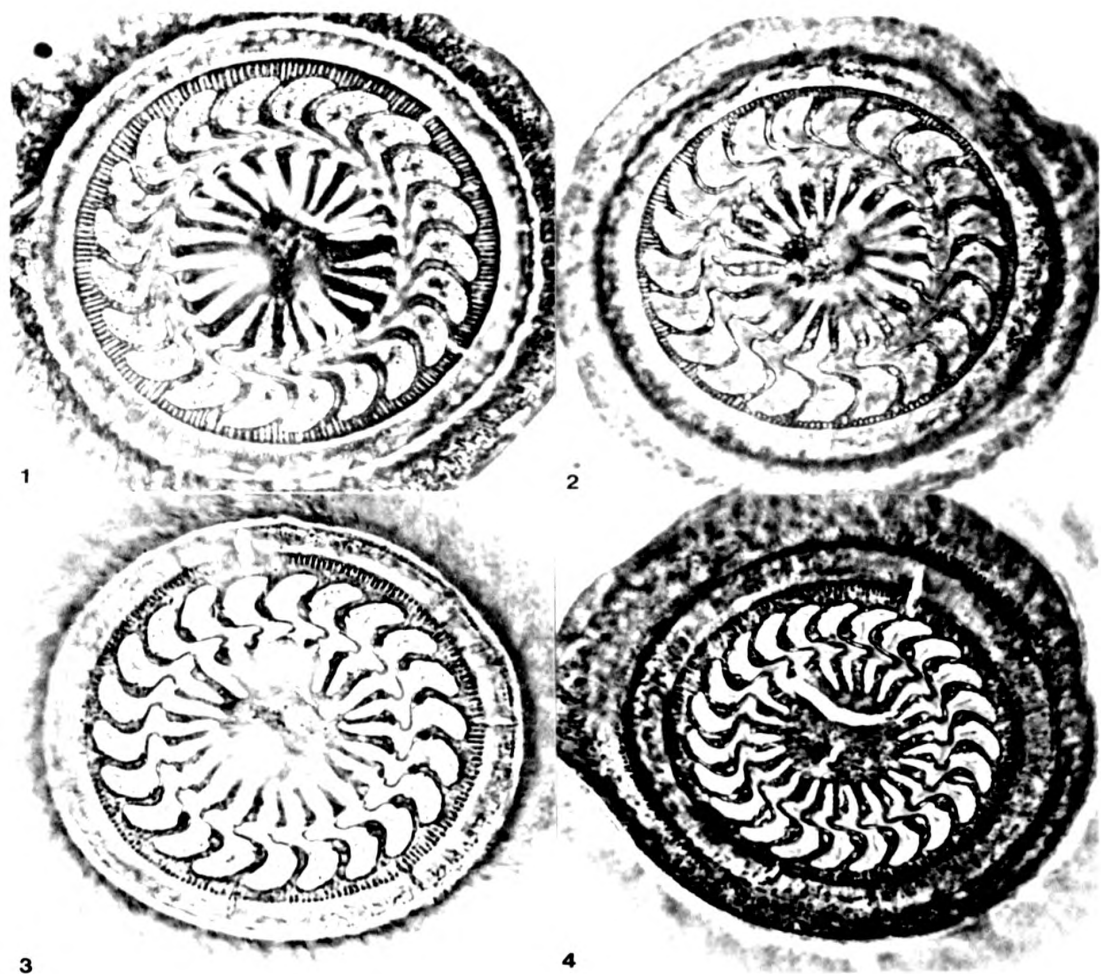
**Table 2.10.** Component loadings of each variable in the first three principal components.

	Factor 1	Factor 2	Factor 3
A. d. diam.	0.858	0.067	-0.026
B. m. width	0.267	0.368	-0.886
D. r. diam.	0.759	0.505	0.147
Dent. no.	-0.325	0.855	0.150
D. length	0.808	-0.355	0.051
B. length	0.900	-0.145	-0.086
R. length	0.880	-0.094	0.092
C. p. width	0.367	0.686	0.204
Dent. span	0.956	-0.013	0.080

**Table 2.11.** Percentage of variation explained by the first three principal components.

	Variance explained by components	% of total variance	Cumulative percentage
Factor 1	4.775	53.050	53.050
Factor 2	1.753	19.473	72.523
Factor 3	0.896	9.951	82.474

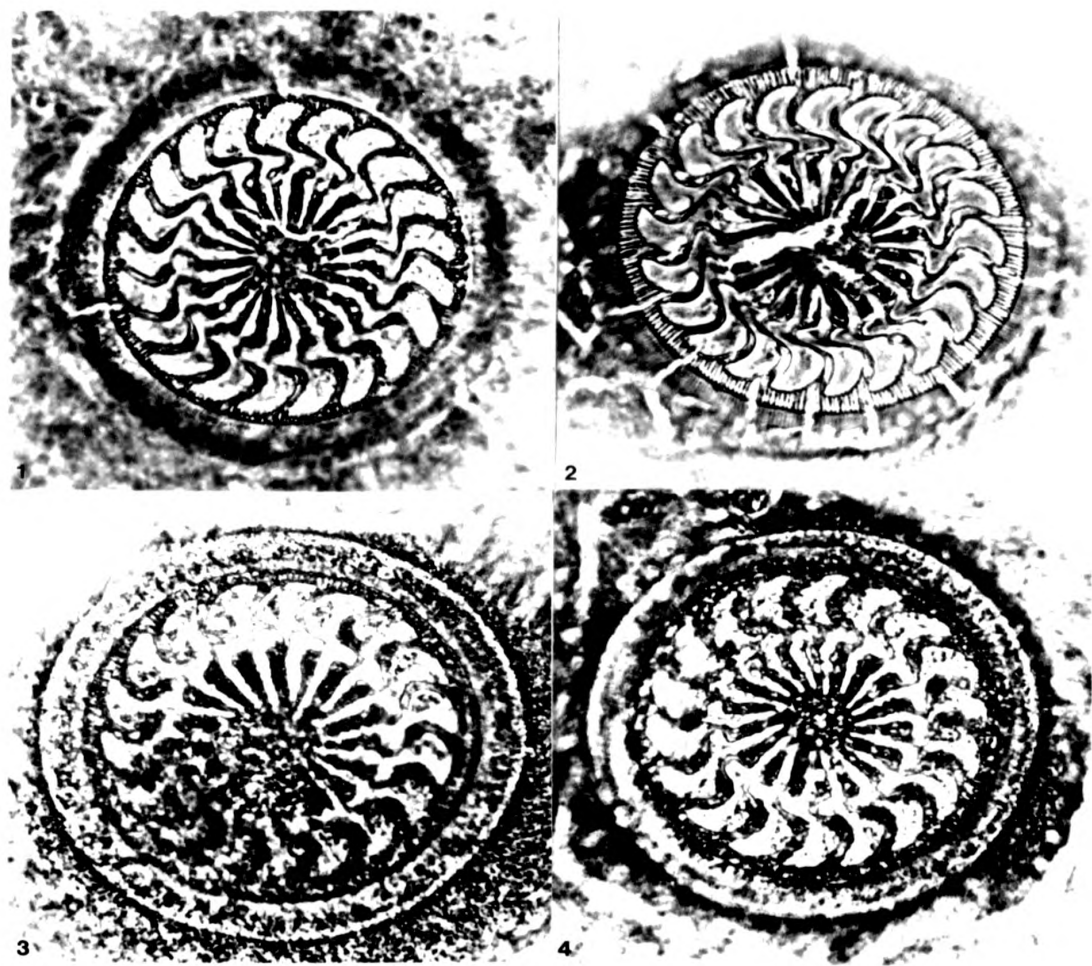
Population (b) from *Cyprinus carpio*, resembles the previous group in many ways, the exception being slightly more rounded points to the blades and slightly shorter denticle rays which are approximately equal in length to the blades. Some specimens of population (b) are identical with specimens from the previous group, except for slightly shorter rays.



**Figure 2.17.** Silver stained adhesive disc morphology in specimens of *Trichodina nigra* (a and b).

**1, 2.** Specimens from *Salmo trutta* (Buckieburn). (1200 $\times$ ).

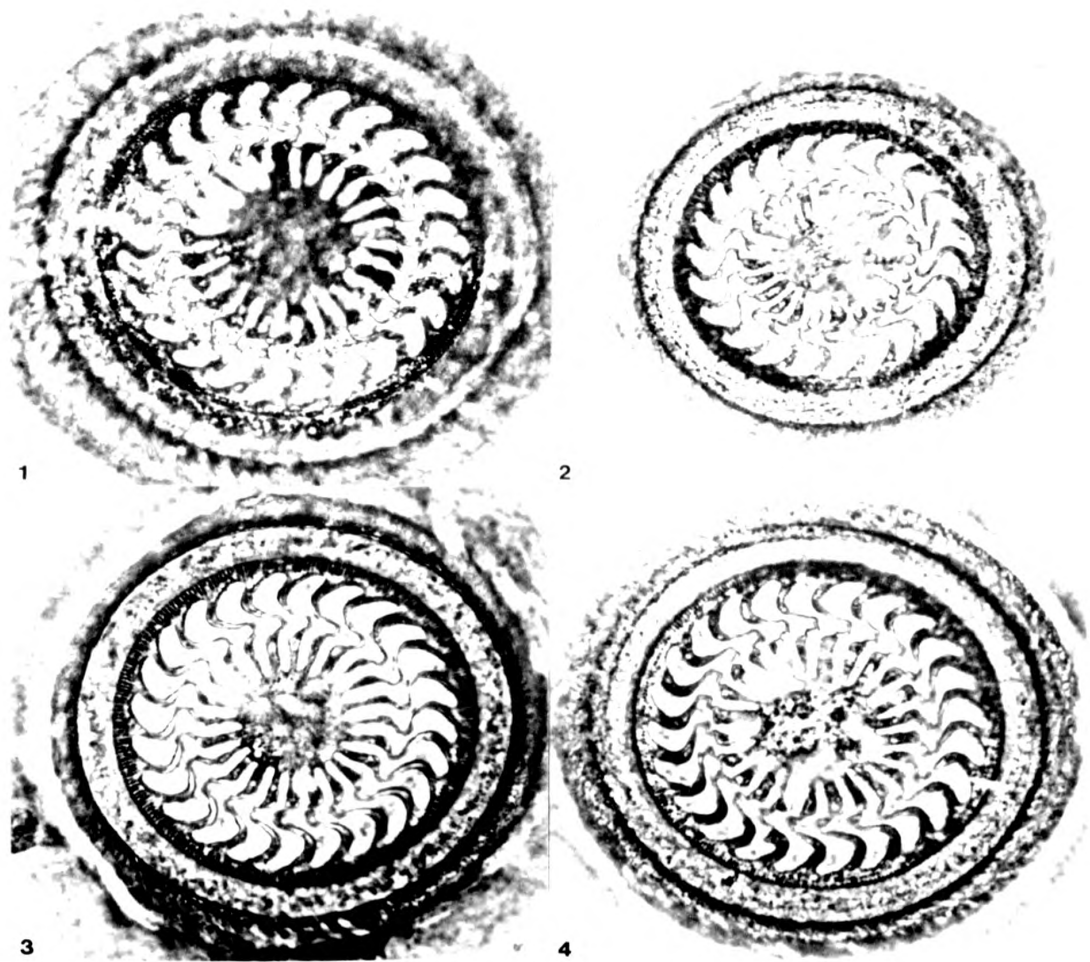
**3, 4.** Specimens of *Cyprinus carpio* (Norfolk). (1200 $\times$ ).



**Figure 2.18.** Silver stained adhesive disc morphology in specimens of *Trichodina nigra* (c and d).

1, 2. Specimens from *Oncorhynchus mykiss* (Moffat). (1200 $\times$ ).

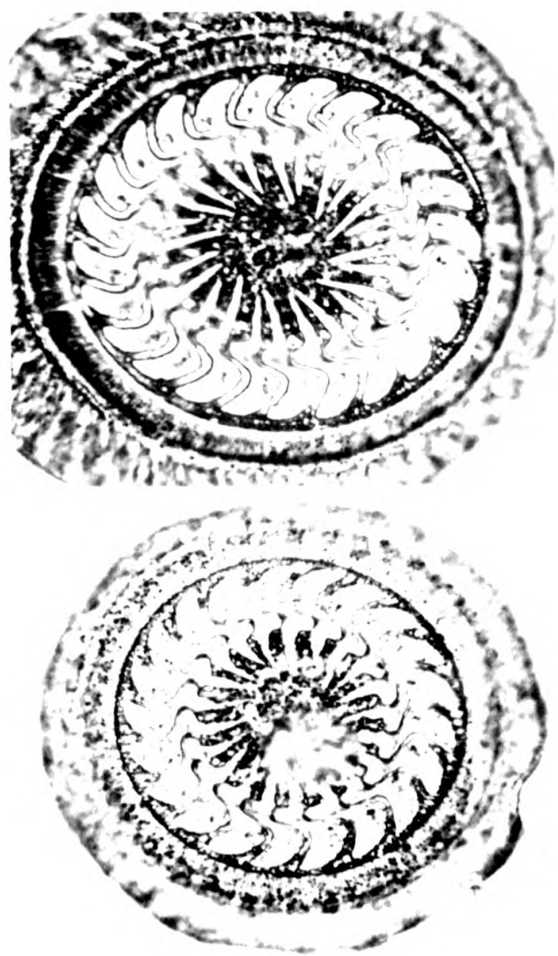
3, 4. Specimens from *Oncorhynchus mykiss* (Loch Fad). (1200 $\times$ ).



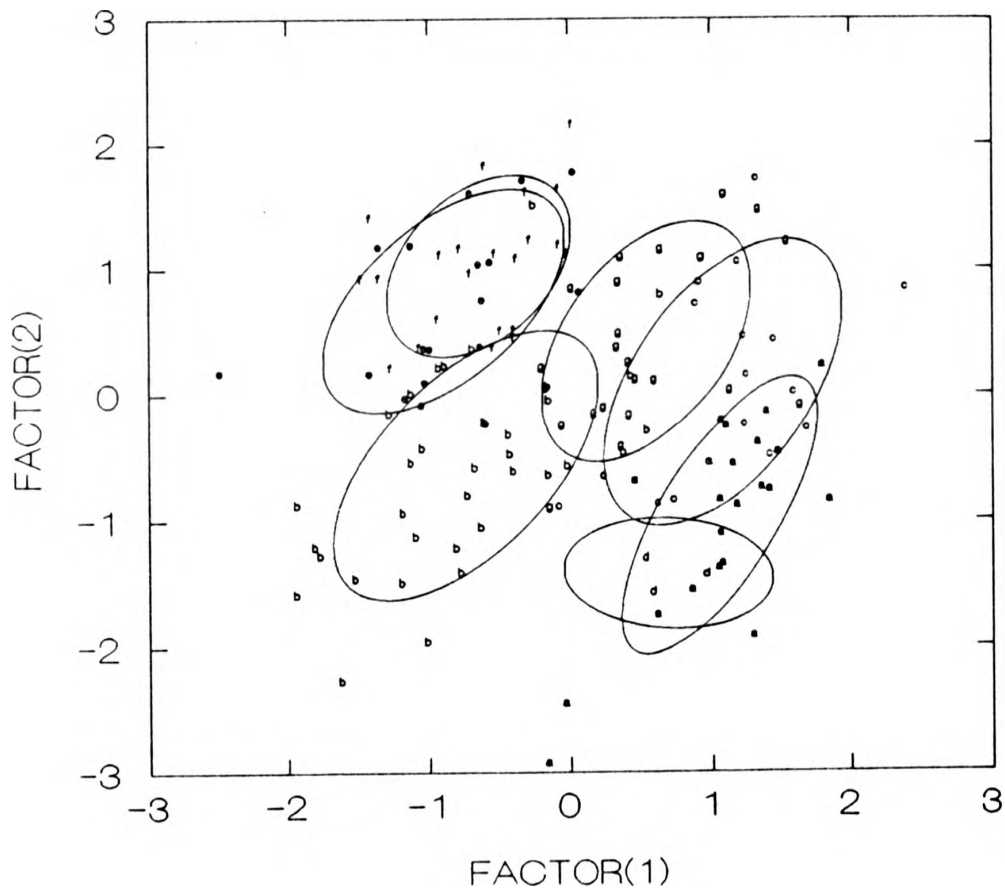
**Figure 2.19.** Silver stained adhesive disc morphology in specimens of *Trichodina nigra* from the skin of *Salmo trutta* (e and f).

**1, 2.** Specimens from College Mill. (1200 $\times$ ).

**3, 4.** Specimens from Almond Bank. (1200 $\times$ ).



**Figure 2.20.** Silver stained adhesive disc morphology in specimens of *Trichodina nigra* (g) from *Oncorhynchus mykiss* (Dollar). (1200 $\times$ ).



**Figure 2.21.** PCA plot of *Trichodina nigra*, populations (a-g) enclosed by 60% ellipses.



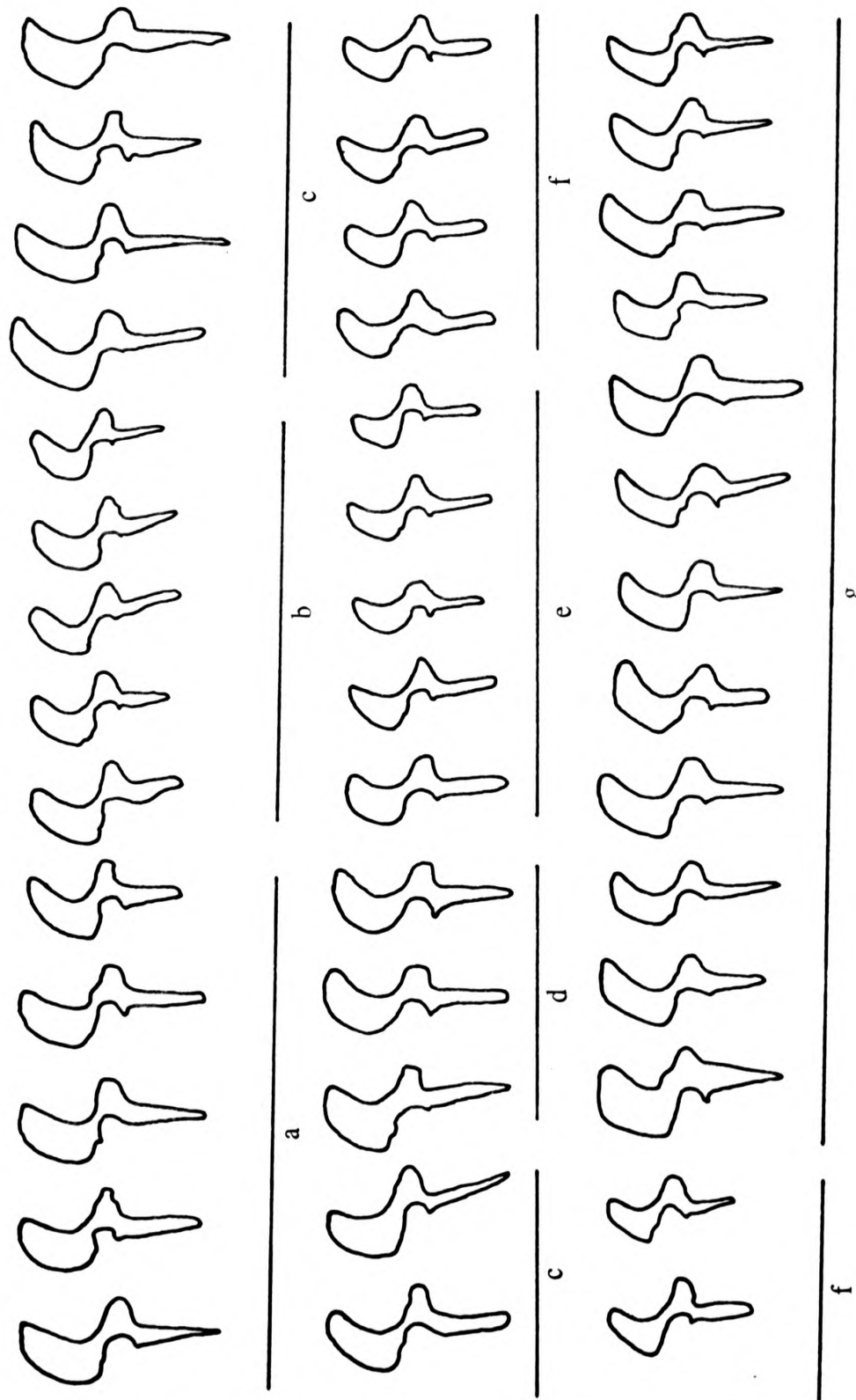


Figure 2.22. Range of denticle variation in *Trichodina nigra* (a-g).

Populations (e) and (f) *Salmo trutta* (College Mill and Almond Bank) have similar proportions to population (b), but have a higher mean denticle number. The denticle blades are slightly shorter, sometimes with a defined "elbow" in the lower anterior margin.

When all the specimens are considered together, at least one specimen in each population bears a close affinity to one specimen in each of the others. Population (b) appears to be intermediate in form, with some specimens resembling those in populations (a), (c), (d) and (g); and others those in populations (e) and (f). This suggests that the seven populations represent a range of morphological variants in one homogeneous group. Figure 2.22. gives a diagrammatic representation of denticle form in *Trichodina nigra* populations found during this study.

### Discussion

All the populations described in this study appear to represent a gradient of morphological variability within one closely related group.

Population (a) from *Salmo trutta* (Buckieburn, 11/91) is almost identical with some of the winter specimens of *Trichodina nigra* as described by Kazubski & Migala (1968). The specimens in (a) are very large, but were sampled during late November when water temperature was very low. Some specimens in (a) bear a very close resemblance to *Trichodina cobitis* (Lom, 1961), but were considerably larger in size.

Population (b) from *Cyprinus carpio* (Norfolk, 17/11/92) was readily identifiable with the findings of Kazubski & Migala (1968), and only varied slightly in mean denticle span. Certain specimens from population (b) also bear some resemblance to *Trichodina nigra* f. *kamchatika* (Stein, 1967).

Population (c) from *Oncorhynchus mykiss* (Moffat, 18/3/94) consists of specimens agreeing with the descriptions of Kazubski & Migala (1968), although some specimens differ in blade form and more closely resemble *Trichodina cobitis* (Lom, 1961).

Population (d) from *Oncorhynchus mykiss* consisted of only three specimens, which were very similar to some of Kazubski & Migala's winter specimens of *Trichodina nigra*.

Populations (e) and (f) from *Salmo trutta* (College Mill and Almond Bank, 8/93) were almost identical, and were most distinct from the normal form of *Trichodina nigra* being smaller in diameter and possessing more angular denticle blades. Various specimens of populations (e) and (f) illustrated similar blade form to specimens of *Trichodina nigra* (Kazubski & Migala, 1968), *Trichodina nigra* f. *gobii* (Lom, 1961) and *Trichodina nigra lucioperca* (Lom, 1970a) described in the literature.

Population (g) from *Oncorhynchus mykiss* (Dollar, 14/8/94) consisted of some 'normal' forms (Kazubski & Migala, 1968), but also had some specimens which displayed massive denticles. These specimens illustrated many of the typical *Trichodina nigra* features, and were very similar to a specimen of this species described by Albaladejo & Arthur (1989) from the Philippines.

Thus, three basic morphological variants could be discerned in this study, the first from *Salmo trutta* and *Oncorhynchus mykiss* (a, c, d and g), the second from *Cyprinus carpio* (b) and the third from *Salmo trutta* (e and f). The effect of host species on morphology is unclear, with no obvious trend being apparent.

Many of the species descriptions in the literature lack good quality photomicrographs illustrating the range of morphological variation in that population.

Kazubski & Migala (1968) provide twelve photographs showing different variants, and is by far the most useful paper concerning this species. The populations of *Trichodina nigra* described from *Salmo trutta* constitute a new host record.

In conclusion it is tentatively proposed that all the populations described here belong to the species *Trichodina nigra* constituting a new British record. However, some specimens observed during this study resembled specimens of *Trichodina nigra* f. *kamchatika* Stein, 1967; *Trichodina cobitis* Lom, 1970; *Trichodina gobii* Lom, 1970 and *Trichodina lucioperca*. Ideally, to clarify this situation, the populations from which the previous species were first described, must be studied to determine the range of morphological variation within them. *Trichodina nigra* remains one of the most difficult species to identify because of confusion in the literature and the considerable morphological variation displayed.

### ***TRICHODINA SP.***

#### **Introduction**

Three *Trichodina* species were observed from the gills of *Phoxinus phoxinus* (College mill) . However the low number of specimens recovered made certain identification to species level impossible. The specimens were unlike any encountered in other sites, so a brief investigation of their morphology and affinities to known species was undertaken.

#### **Results**

Morphometric and meristic data for *Trichodina sp.* 1, 2 and 3 are presented in Table

2.12. Photomicrographs for the three species are given in Figure 2.23. Species 1 is characterised by a relatively large adhesive disc diameter, with a high denticle number (mean=29.3). The denticles are long, with curving blades slightly flattened on the distal surface. The posterior margin of the blade is visibly thickened, this 'ridge' becomes broader towards the end of the blade. The rays are long, and either straight or curving to the posterior side of the denticle.

**Table 2.12.** Morphometric and meristic data for *Trichodina* species from the gills of *Phoxinus phoxinus*, and comparative data for known similar species.

Author	Kazubski & Migala, 1968	This study	Lom, 1970a	This study	This study
Species	<i>Trichodina mutabilis</i>	Sp. 1 n=3	<i>Trichodina rostrata</i>	Sp. 2 n=3	Sp. 3 n=1
Host	<i>Cyprinus carpio</i>	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i>
Localisation	Gills	Gills	Gills	Gills	Gills
Locality	Warsaw	College Mill	Prague	College Mill	College Mill
A. d. diam	40-67	52.8-55.9 (55.2)	44-55 (51)	52.5-62.2 (57.4)	52.6
B. m. width	6	6.1-6.3 (6.2)	5-6	(11.2)	6.2
D. r. diam	25-42	31.4-32.1 (31.8)	28-41 (34)	32.3-39.1 (34.8)	33.1
Dent. no	22-33	29-30 (29.3)	26-28 (27)	26	29
R. p. / d.	9-10	10	9-10	11	10
D. length	-	6.8- 7.7 (7.1)	10	7.0-9.3 (8.1)	7.1
B. length	-	7.7-8.1 (7.8)	8	6.2-8.8 (7.4)	7.9
R. length	-	8.5-9.7 (8.8)	7	5.3-8.2 (7.1)	7.9
C. p. width	-	1.8-2.3 (2.1)	3	(2.9)	1.7
Dent. span	14.8-20.7	18.0-19.3 (18.7)	-	15.7-19.6 (17.8)	18.4

Species 2 is quite large, with a denticle number of 26. It has broad curving denticle blades which are noticeably thickened along the posterior margin, as well as a displaying a thickened projection directed to the anterior of the blade. The ray possesses

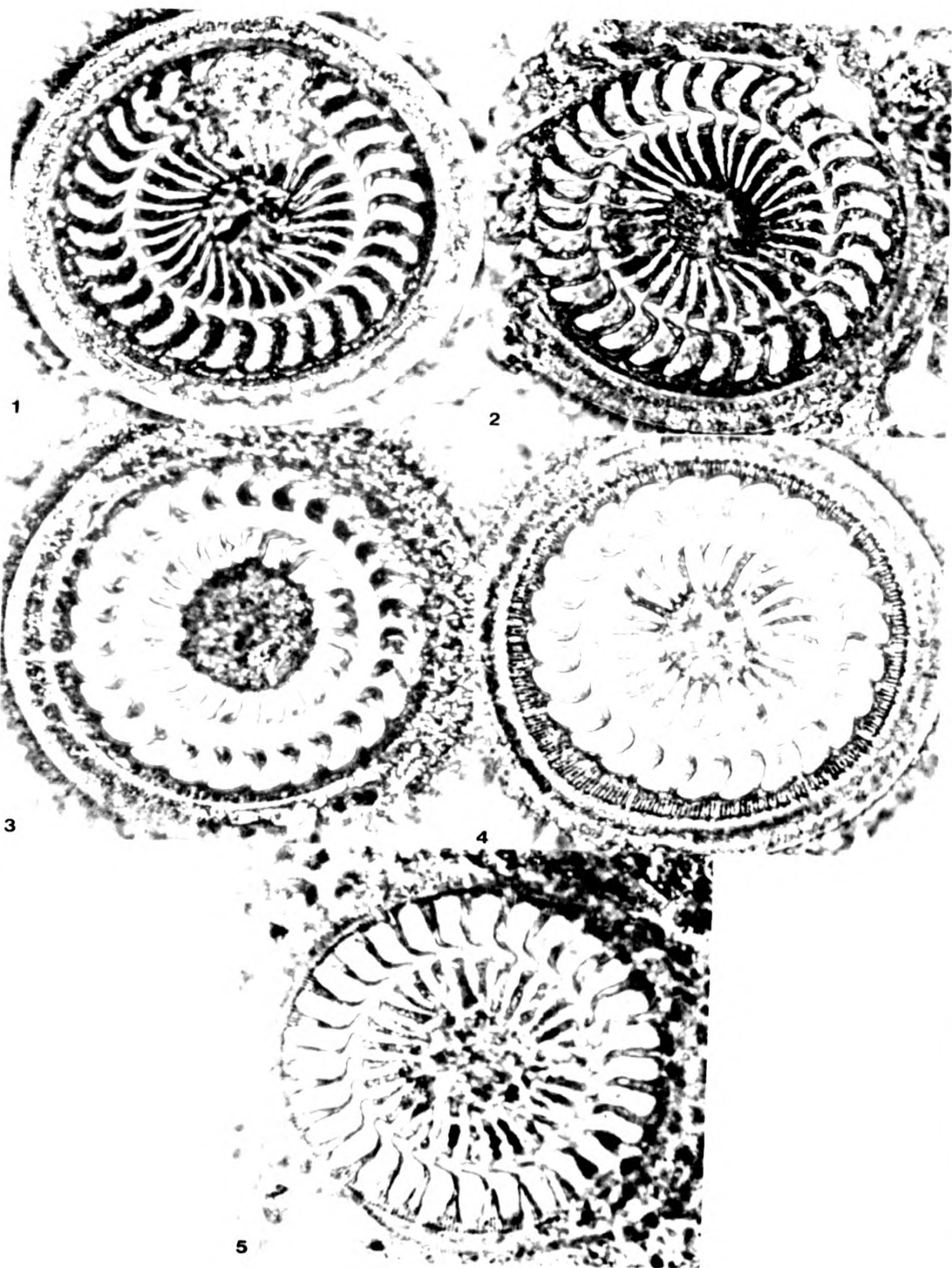
**Figure 2.23.** Silver stained adhesive disc morphology of Sp.1, Sp.2 and Sp.3 from *Phoxinus phoxinus* (College Mill).

**1, 2.** Sp.1 (*Trichodina mutabilis*). (1200×).

**3, 4.** Sp.2\* (*Trichodina rostrata*). (1200×)

**5.** Sp.3. (1200×).

nd Sp.3 from



a thickened part resembling a 'normal' structure, with a thinner part to the anterior side. This produces an unusually broad denticle ray, which is only fully visible in one specimen.

Species 3 also has a relatively high denticle number (29), and the blades are straight with a rounded distal surface. The inner rays are quite broad, and taper towards the tip.

### Discussion

Sp. 1 closely resembles *Trichodina mutabilis* Kazubski & Migala, 1968 from *Cyprinus carpio*. The measurements given by Kazubski & Migala (1968) for their "winter" specimens correspond almost exactly to those given in Table 2.12 for Sp.1. *Trichodina mutabilis* is noted for its particularly high seasonal variability by the previous authors. This species has subsequently been reported as parasitising *Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix* (Chen, 1963 in: Lom, 1970a); *Leucapsis delineatus*, *Carassius auratus gibelio*, *Rutilus rutilus*, *Rhodeus sericeus* (Lom, 1970a) from Bohemia; *Tinca tinca* from the Danube (Kostenko, 1972); freshwater fish in the USSR (Kashkovsky, 1974; Stein, 1984); *Oncorhynchus mykiss* (Grupcheva, 1975b) from Bulgaria and *Cyprinus carpio* (Albaladejo & Arthur, 1989) from the Philippines.

*Trichodina izyumovae* Arthur & Lom, 1984 bears a close resemblance to *Trichodina mutabilis* and sp.1. The similarity between the two 'species' is noted (Arthur & Lom, 1984), but the lack of variation observed in *Trichodina izyumova* is used to differentiate the two. Kazubski & Migala (1968) observed variation in *Trichodina mutabilis* over a two year period with a considerable sample size. The lack of variation observed by Arthur & Lom (1984) in a sample of 25 specimens does not seem sufficient



to warrant the proposal of a new species. Therefore, it is possible that *Trichodina izyumovae* is a synonym of *Trichodina mutabilis*.

Sp. 2 is comparable in form and morphometrics with *Trichodina rostrata* Kulemina, 1968 reported from *Rutilus rutilus* and *Abramis brama* in the USSR (Kulemina, 1968); *Rhodeus sericeus* (Lom, 1970a) from Bohemia; *Rutilus rutilus* (Arthur & Lom, 1984) from the USSR and *Cyprinus carpio* (Grupcheva & Sedlaczek, 1993) from eastern Germany.

The single specimen of Sp. 3 does not readily associate with any species descriptions in the literature. The denticle rays are reminiscent of *Trichodina nigra* (Lom, 1960), with a broad tapering form. The denticle number and blade form are similar to those found in *Trichodina tenuidens*, but there is no central circle in sp. 3 which would be expected in the previous species. Until a representative sample of this population is studied no realistic species identification can be achieved.

### SUMMARY AND CONCLUSIONS

**Table 2.13.** Summary of *Trichodina* species identified during this study.

	Host species	New British host records	New Host records
<i>Trichodina acuta</i>	<i>Salmo trutta</i> <i>Oncorhynchus mykiss</i> <i>Carassius auratus</i> <i>Phoxinus phoxinus</i>	<i>Salmo trutta</i> <i>Oncorhynchus mykiss</i> <i>Carassius auratus</i> <i>Phoxinus phoxinus</i>	<i>Salmo trutta</i> , <i>Phoxinus phoxinus</i>
<i>Trichodina domerguei</i>	<i>Gasterosteus aculeatus</i>		
<i>Trichodina tenuidens</i>	<i>Gasterosteus aculeatus</i>		
<i>Trichodina pediculus</i> (New British record)	<i>Gasterosteus aculeatus</i>	<i>Gasterosteus aculeatus</i>	<i>Gasterosteus aculeatus</i>
<i>Trichodina modesta</i> (New British record)	<i>Abramis brama</i>	<i>Abramis brama</i>	
<i>Trichodina nigra</i> (New British record)	<i>Salmo trutta</i> <i>Oncorhynchus mykiss</i> <i>Cyprinus carpio</i>	<i>Salmo trutta</i> <i>Oncorhynchus mykiss</i> <i>Cyprinus carpio</i>	<i>Salmo trutta</i>

Table 2.13 illustrates the populations of *Trichodina* identified during this study. The

three species which have been previously reported from this country, *Trichodina acuta*, *Trichodina domerguei* and *Trichodina tenuidens* were redescribed. Three additional species, *Trichodina pediculus*, *Trichodina modesta* and *Trichodina nigra* were described for the first time from British fish. Small numbers of specimens tentatively identified as *Trichodina mutabilis* and *Trichodina rostrata* were also previously un-described from this country.

From the sampling undertaken in this study *Trichodina acuta* and *Trichodina nigra* were by far the most common *Trichodina* species encountered. This is in part due to the fact that they are found on salmonids, which were sampled most regularly. *Trichodina acuta* and *Trichodina nigra* also displayed the least host specificity, infecting salmonids and cyprinids.

*Trichodina domerguei* and *Trichodina tenuidens* were described from *Gasterosteus aculeatus* in fresh and marine water, and they illustrated a high degree of host specificity; single specimens of *Trichodina domerguei* occurred occasionally on *Phoxinus phoxinus* and *Oncorhynchus mykiss* in the same water body as sticklebacks.

*Trichodina pediculus*, previously un-described from Britain, is particularly common on the skin of cyprinids in mainland Europe (Kazubski & Migala, 1968; Kazubski, 1991c), thus it was surprising to find the only specimens on the skin of *Gasterosteus aculeatus*. Only 12 well stained specimens were observed during 25 sample dates over one year. This suggests that these specimens probably represent an accidental infection from other fish hosts or hydra.

One population of *Trichodina modesta* was discovered for the first time in this country on the gills of *Abramis brama*. This *Trichodina* species is specific to bream (Lom, 1970a; Stein, 1984; Arthur & Lom, 1984), and was found in the single population

of *Abramis brama* sampled during this study.

**CHAPTER 3. TAXONOMY OF THE GENERA, *TRICHODINELLA*,  
*TRIPARTIELLA* AND *PARATRICHODINA*; WITH DESCRIPTIONS  
OF SPECIES FOUND DURING THIS STUDY, INCLUDING  
*TRICHODINA INTERMEDIA*.**

**INTRODUCTION**

The genus *Trichodinella* Sramek-Husek, 1963 was initially proposed by Raabe in 1950. This was originally designated *Brachyspira*, but this name was already in use in molluscan systematics (Lom, 1959). According to Raabe's original description (Lom, 1959), members of the genus are characterised by a short adoral ciliary spiral, describing a turn of approximately 180°. The type species representing the genus is *Trichodinella epizootica*, reported from a variety of fish hosts (Lom, 1959).

The genus *Trichodinella* was further divided into three subgenera by Lom, (1959): *T. (Trichodinella)*, *T. (Tripartiella)* and *T. (Foliella)*. The subgenus *Trichodinella* was characterised by greatly reduced denticle rays and straight rather than curved blades as observed in *Trichodina*, and with a "spike" like anterior projection above the central part of the denticle. The subgenus *Tripartiella* was described as possessing "*Trichodina*-like" denticle rays (Lom, 1959), with an anterior projection above the central part interlocking with the preceding denticle. The subgenus *Foliella* was described as having denticle rays in the form of a "rounded little plate", but otherwise similar to *Trichodinella*. The "complex" is also characterised by the exceptionally small specimen size when compared to the genus *Trichodina*.

A fourth subgenus *Dogielina* Raabe 1959, in which the denticles lack inner rays

and possess rather indistinct central parts was described from the gills of *Gobius niger* (Raabe, 1959 in: Lom, 1959). This genus was subsequently renamed *Dipartiella* Stein, 1961 (Lom & Dykova, 1992), and has not been reported since its original description. Lom & Dykova (1992) suggest that it may have been a "misinterpretation of a *Trichodinella*".

The genus *Semitrichodina* Kazubski, 1958 (found in terrestrial molluscs) was discussed as a possible synonym of the subgenus *Tripartiella* by Lom (1959). However, Lom asserts that this genus is quite distinct, since the adoral ciliary spiral is less than 180°, the denticles are very "*Trichodina* -like" in their blade and ray structure, the large sized body is regularly furrowed with oblique grooves and the genus inhabits a completely different "biotope".

*Tripartiella* was raised to genus level and was subdivided into *Tripartiella* (*Tripartiella*) and *Tripartiella* (*Paratrichodina*) by Lom (1963a). This new subgenus was characterised by the adoral cilia defining a turn well below that of the *Trichodina* (minimum 360°), but with denticles approaching the form of those found in *Trichodina* without the anterior blade projection apparent in *Trichodinella* and *Tripartiella*.

The classification of the *Trichodinella* "complex" was subsequently rationalised by Lom & Haldar (1977) to include three separate genera, described as follows: (a diagrammatic representation of the denticle structure of the genera *Trichodinella*, *Tripartiella* and *Paratrichodina* is illustrated in Figure 3.1 (Lom & Haldar, 1977)).

*Trichodinella* Sramek-Husek, 1953; syn. *Brachyspira* Raabe, 1950. Adoral spiral 180-270°. Denticles with a delicate central part, with an anterior projection fitting into a notch between the central part and the blade of the preceding denticle. The inner ray forms a delicate, curved hook, which is difficult to impregnate due to its extremely

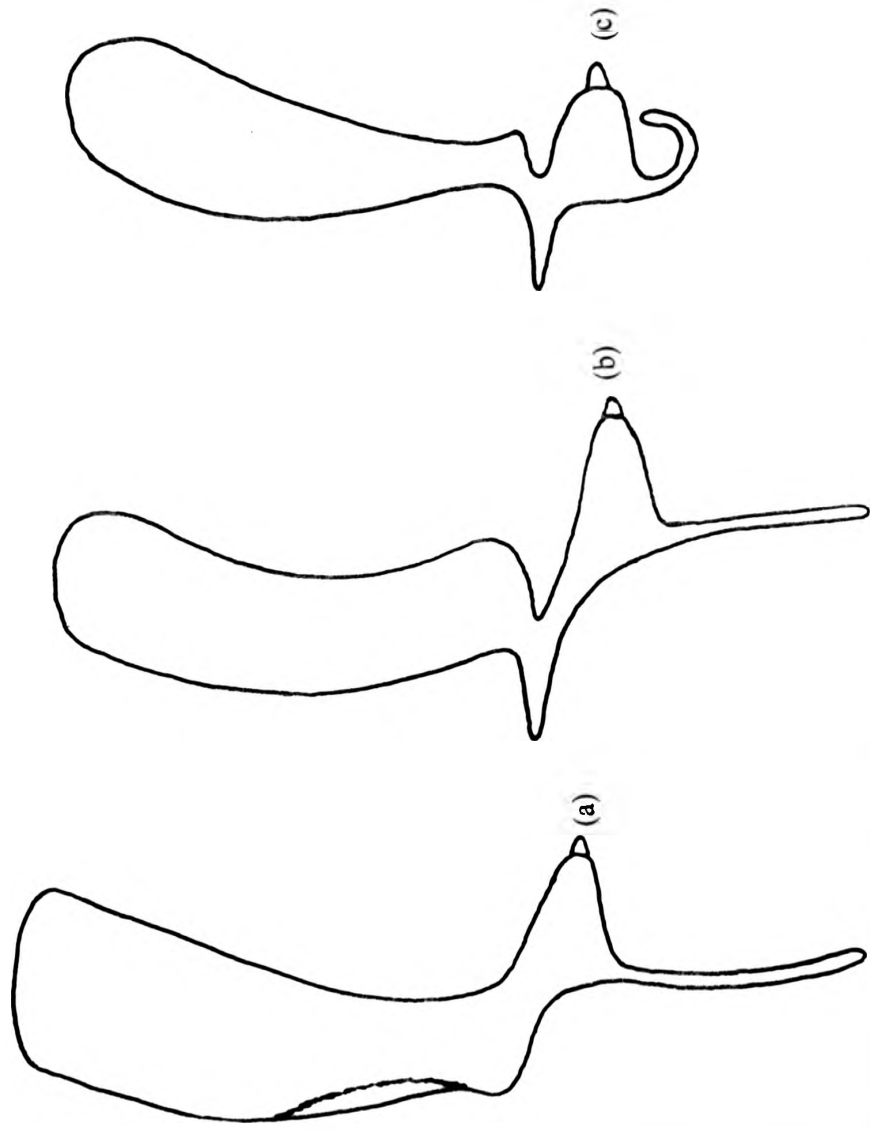


Figure 3.1. A diagrammatic representation of denticle form in: (a) *Paratrichodina*, (b) *Tripartiella* and (c) *Trichodinella*.

small size. It is found exclusively on fish gills, and consists of eleven species (some probably synonymous with *Trichodinella epizootica*) predominantly on freshwater hosts (Lom & Dykova, 1992). The subgenera *Foliella* is abolished, and species included in the genus *Trichodinella* (Lom & Haldar, 1977).

*Tripartiella* Lom, 1959 has an adoral spiral described as making a turn of 180-290°. The blades are slanted obliquely to the posterior side of the denticle, they join the central part by a narrow base and extend anteriorly in a "short and thin or wide and knee like projection". These correspond with a notch in the preceding denticle. They are found exclusively on the gills of freshwater fish, consisting of twenty four species (Lom & Dykova, 1992).

*Paratrichodina* Lom, 1963 has an adoral spiral of 150-180°. The denticles are similar to those of the genus *Trichodina*, being wedged together only by the central parts. If an anterior projection of the central part is present it does not locate within a notch in the preceding denticle. Seven species are found on the gills of freshwater and marine fish, with three additional species inhabiting the urinary tract (Lom & Dykova, 1992).

*Dipartiella* Stein, 1961 (*Dogielina* Raabe, 1959) is concluded to be a misinterpretation of a *Tripartiella* species, with which Stein agrees (1976, personal communication, in: Lom & Haldar, 1977).

It is concluded (Lom & Haldar, 1977) that "the genera *Tripartiella* and *Trichodinella* are closely related to each other by having denticles wedged together by a double system of central conical parts and anterior projections of the blades". These authors considered this to be evolutionarily more advanced than the more "*Trichodina*-like" genus *Paratrichodina*.

The previous system of classification has been universally accepted by authors studying trichodinid taxonomy. However, Guhl (1990) is isolated in the opinion that trichodinids cannot be differentiated using the structure of the adhesive disc, because of intraspecific variation in denticle morphology. Guhl suggested that members of the genera *Tripartiella* and *Paratrichodina* belong to the genus *Semitrichodina*. For example, Guhl states that populations described as *Tripartiella lata* Lom, 1963 and *Paratrichodina incisa* Lom, 1963 belong to the species *Semitrichodina incisa*. This is extremely unlikely if not impossible, because of the vast differences in adhesive disc morphology, and is contrary to all current thinking in the field.

The taxonomic criteria for differentiation of the three genera constituting the *Trichodinella* "complex" is identical to that of the genus *Trichodina*, as discussed in the previous chapter. The only additional consideration needed is the recognition that because of their extremely small size, these genera are more vulnerable to staining artifacts (see Chapter 4).

## **MATERIALS AND METHODS**

The sampling methods and analytical techniques utilised during this chapter are given in Chapter 2.

### **Cluster analysis**

Cluster analysis was undertaken using Systat 5.3, in an attempt to identify subpopulations within a particular sample. This technique is a multivariate procedure for detecting natural groupings within data. Clusters are split by maximising between-cluster variation and minimising within-cluster variation. Therefore, cluster analysis



seeks to classify a set of objects into subgroups although neither the number or members of the subgroup are known. Cluster analysis is designed to look for the number of clusters which are specified. Successive analyses from 2-15 clusters are performed, and the "optimal" number of groupings is indicated by the F-ratio and probability.

## SPECIES DESCRIPTIONS

### *TRICHODINELLA EPIZOOTICA* RAABE, 1950

(syn. *Brachyspira epizootica* Raabe, 1950;  
*Trichodinella epizootica* f. *percarum* Lom, 1959;  
*Trichodinella maior* Reichenbach-Klinke, 1962;  
*Trichodinella percarum* (Dogiel, 1940) Chan, 1961);  
*Trichodinella percarum* f. *lotae* Chan, 1961;  
*Trichodinella lotae* (Chan, 1961) Stein, 1962;  
*Trichodinella carassii* (Dogiel, 1940) Kostenko, 1969)

#### Introduction

*Trichodinella epizootica* (Raabe, 1950) was first described using Klein's silver staining technique by Lom (1956 in: Lom, 1959) from *Tinca tinca*. Lom (1959) describes populations from *Perca fluviatilis* and *Gymnocephalus cernua*, designated *Trichodinella epizootica* f. *percarum*, having more "obliquely slanting" denticle blades. Further reports of *Trichodinella epizootica* by Lom (1963a) from different hosts include *Esox lucius*, *Lota lota*, *Lucioperca lucioperca*, *Salmo trutta*, barbel *Barbus barbus* L., loach *Misgurnus fossilis* L. and *Rhodeus sericeus*. Lom points out the difficulty in successfully impregnating the adhesive disc of this species, and states that a number of individuals are required to "reconstruct" the true denticle structure.

Kostenko (1972) reported *Trichodinella carassii* (syn. *Trichodinella epizootica*,

Lom & Haldar, 1977) from the round-goby *Neogobius melanostomus* Pallas and *Tinca tinca* from the River Danube. Kashkovsky (1974) reported *Trichodinella epizootica* as three different synonymous species from the USSR; *Trichodinella epizootica* from *Gymnocephalus cernua* and *Esox lucius*, *Trichodinella carassi* from *Cyprinus carpio* and *Carassius carassius*, and *Trichodinella percarum* from *Perca fluviatilis*. This author differentiated the previous populations on small differences in blade morphology and host preference. Grupcheva (1975a) reported *Trichodinella epizootica* on freshwater fish from Bulgaria.

Lom & Haldar (1977) summarised previous work on *Trichodinella epizootica*, and cited additional host records including *Asporo zingel*, *Cyprinus carpio*, silver bream *Blicca bjoerkna* L., *Carassius carassius*, *Carassius auratus*, *Stizostedion lucioperca*, *Oncorhynchus nerka*, peled *Coregonus peled* Gmel., razor fish *Pelecus cultratus* L. and *Scardinius erythrophthalmus*. Lom remarks on the variation in morphology unrelated to host species illustrated by various populations, and concludes that they cannot be separated into independent species. Kashkovsky and Stein both independently recorded populations of *Trichodinella epizootica* with the unusual characteristic of the adoral spiral describing a turn of 180-270° (Lom, 1977). The most highly variable morphological characteristics described by Lom & Haldar (1977) include:

"spoon-like or board-like" denticles depending on the evenness of the blade margins, the degree of development of the anterior spike and posterior notch. The posterior projection of the blade may be more or less pronounced, being more pronounced in populations from *Esox lucius*, *Coregonus peled* and *Gymnocephalus cernua*. The inner ray or hook may be difficult to stain completely, altering the appearance of the denticles. The distance between the blades and the border membrane

is also variable, which may depend on the state of contraction of the adhesive disc.

Additional records of *Trichodinella epizootica* include: Stein (1979) from Lake Baikal; Calenius (1980) on *Gymnocephalus cernua*, *Esox lucius* and *Lota lota* in brackish and freshwater from Finland; Stein (1982, 1984) from the USSR; Basson, Van As & Paperna (1983) on *Oreochromis mossambicus*, *Tilapia rendalli* and *Cyprinus carpio* from South Africa, and *Carassius auratus*, *Cyprinus carpio* and *Hypophthalmichthys molitrix* from Israel; Basson & Van As (1987) give further host records from South Africa from *Pseudocrenilabrus philander*, *Barbus trimaculatus*, and *Barbus paludinosus*, Basson & Van As (1993) speculate that their previous identifications of *Trichodinella epizootica* may be incorrect and therefore represent a new species. Arthur & Lom (1984) reported *Trichodinella epizootica* from *Perca fluviatilis*, burbot *Lota lota* L., *Esox lucius* and *Abramis ballerus* in the USSR; Van Than & Margaritov (1986) from Bulgaria; Albaladejo & Arthur (1989) from *Cyprinus carpio* in Indonesia, *Ctenopharyngodon idella* and *Aristichthys nobilis* in Taiwan and *Carassius auratus* in Hongkong.

*Trichodinella epizootica* has been reported from the gills of *Perca fluviatilis* in Britain by Abolarin (1966 in: Chubb, 1977), this constitutes the only record of a trichodinellid in this country.

## Results

Three populations of a species resembling *Trichodinella epizootica* were found during this study. The morphometric and meristic data for these populations are given in Table 3.1. In addition to the populations described, specimens of a species resembling *Trichodinella epizootica* were observed from populations of *Oncorhynchus mykiss* (River

Test, Hampshire) and *Salvelinus fontinalis* (Moffat). However, no silver stained material was recovered.

*Trichodinella epizootica* is characterised by its extremely small size, adhesive

**Table 3.1.** Morphometric and meristic data for *Trichodinella epizootica*.

Author	Lom & Haldar, 1977	This study (a)	This study (b)	This study (c)
Host	<i>Perca fluviatilis</i>	<i>Perca fluviatilis</i>	<i>Perca fluviatilis</i>	<i>Oncorhynchus mykiss</i>
Localisation	Gills	Gills	Gills	Gills
Locality	Pooled data from literature and author's records	Castle Semple Water 30/6/92	Humberside (fish dealer) 18/1/93	Dollar 17/8/94
A. d. diam.	13-30	17.7-24.3 (20.6±1.8, 32)	17.6-29.1 (23.4±3.2, 28)	16.2-25.1 (20.8±2.2, 29)
B. m. width	1.8-3.3	1.8-2.6 (2.2±0.2, 32)	1.4-2.6 (2.2±0.3, 28)	1.3-2.3 (1.9±0.2, 29)
D. r. diam	8-27	8.2-12.8 (10.7±1.2, 32)	9.4-16.3 (12.7±2.0, 28)	7.1-11.5 (10.0±1.1, 29)
Dent. no.	16-28	20-24 (22.4±1.1, 32)	24-29 (26.4±1.1, 28)	20-25 (22.5±1.5, 29)
R. p. / d.	4-6	5-6 (5.3±0.5, 25)	5 (5, 25)	4-6 (4.9±0.6, 26)
D.length	-	2.3-3.6 (2.9±0.3, 32)	1.9-3.4 (2.8±0.3, 28)	2.1-2.9 (2.5±0.2, 29)
B. length	2.2-3.6	2.9-4.3 (3.6±0.3, 32)	2.3-4.3 (3.7±0.5, 28)	2.9-4.1 (3.6±0.3, 29)
R. length	-	-	-	-
C. p. width	0.6-2.2	0.6-1.1 (0.9±0.1, 32)	0.5-1.2 (0.9±0.2, 28)	0.6-1.3 (0.9±0.2, 29)
Dent. span	-	3.9-5.4 (4.6±0.4, 32)	2.9-5.3 (4.6±0.6, 28)	3.5-5.1 (4.5±0.4, 29)

disc diameter of the specimens measured in this study ranged from 16.2-29.1µm. The adoral cilia expresses a turn of approximately 180°. The denticles are almost triangular in shape, widening from the central part to a wide flattened distal surface parallel with the border membrane. The central parts are relatively robust, with an anterior projection where they join the blade, locating with a notch in the posterior margin of the

preceding blade. The inner ray forms a small hook directed posteriorly, which is often poorly stained giving the impression of a massive central part of the denticle. The centre of the adhesive disc illustrates a central circle of varying argentophilic property, being more uneven and less defined than in *Trichodina domerguei* (Lom & Stein, 1966). All parts of the adhesive disc are subject to staining artifact (Lom, 1963a; Lom & Haldar, 1977). The quality of stain is so unreliable that in some populations found during this study, the majority of specimens appeared as opaque discs. This problem means that a very large number of specimens are needed in order to gain a sample of adequately stained specimens.

Principal Components Analysis was undertaken to observe the relationships between the three populations found. The component loadings on the first three principal components are given in Table 3.2. This illustrates that adhesive disc diameter, blade

**Table 3.2.** Component loadings on the first three principal components, in the analysis of *Trichodinella epizootica*.

	Factor 1	Factor 2	Factor 3
A. d. diam.	0.900	-0.247	0.141
B. m. width	0.510	0.097	-0.709
D. r. diam.	0.861	-0.408	-0.015
Dent. no.	0.399	-0.832	0.138
D. length	0.701	0.137	-0.362
B. length	0.870	0.273	0.157
C. p. width	0.477	0.404	0.417
Dent. span	0.857	0.386	0.130

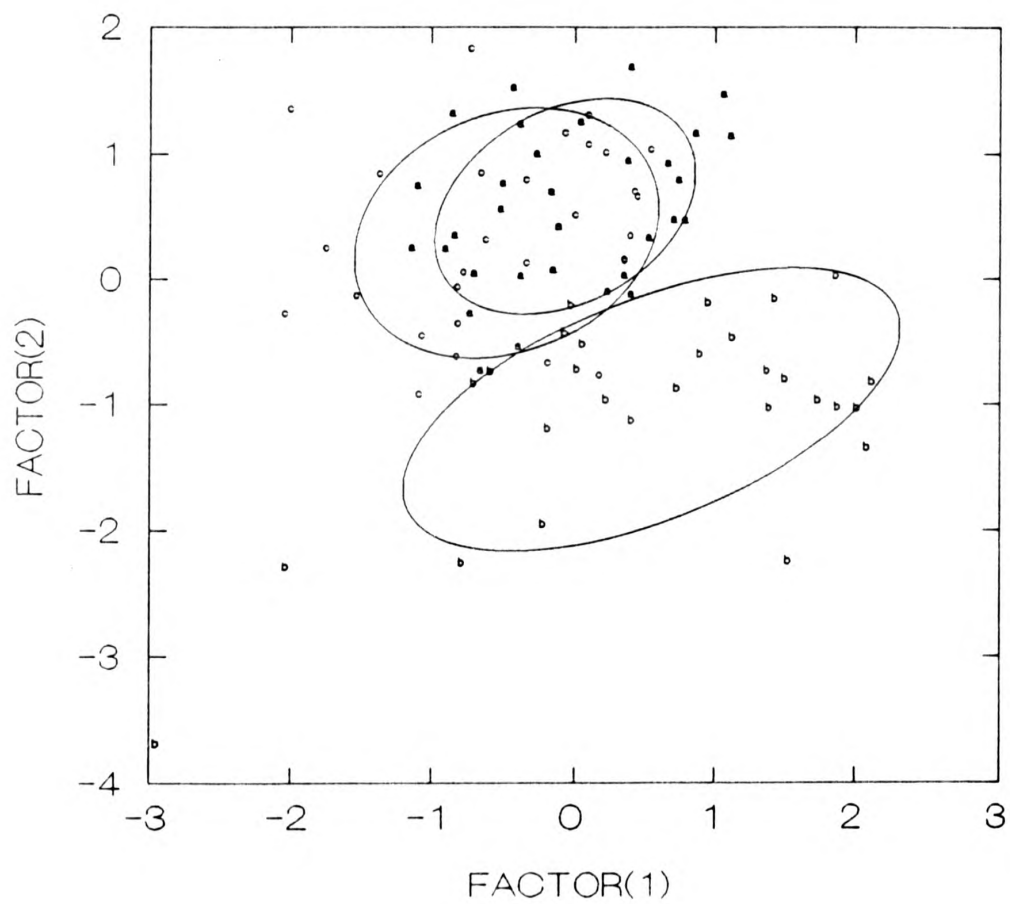
length and denticle ring diameter are the main variables acting in Factor 1. The primary variable acting in Factor 2 is denticle number, and border membrane width in Factor 3. The percentage variance explained by the first three principal components is illustrated

in Table 3.3. Nearly 70% of the variation is expressed in the first two components. A plot of Factor 1 against Factor 2 in the analysis of *Trichodinella epizootica* is illustrated in Figure 3.2. The three populations in Figure 3.2. are encircled by 60% ellipses. Populations (a) and (c) from *Perca fluviatilis* (Castle Semple Water) and *Oncorhynchus mykiss* (Dollar) are separated from population (b) *Perca fluviatilis* (Humberside). The separation observed is mainly along Factor 2 (main variable denticle number), and to a lesser extent along Factor 1 (main variable adhesive disc diameter). Population (b) also has a higher mean denticle number, 26.4 apposed to 22.4 (a) and 22.5 (c). Population (b) also has a slightly greater mean adhesive disc diameter of 23.4 $\mu$ m, than (a) 20.6 $\mu$ m and (c) 20.8 $\mu$ m.

**Table 3.3.** Percentage of variance explained for the first three principal components.

	Variance explained by components	% of total variance	Cumulative percentage
Factor 1	4.183	52.283	52.283
Factor 2	1.336	16.696	68.979
Factor 3	0.889	11.106	80.085

Denticle form in the three populations illustrates different affinities than suggested by the PCA analysis. Morphometrically populations (a) and (c) show a high degree of similarity, whereas denticle form in populations (a) and (b) from *Perca fluviatilis* can be differentiated from population (c) parasitising *Oncorhynchus mykiss*. Specimens from *Perca fluviatilis* show typical *Trichodinella epizootica* denticle morphology, as illustrated in Lom & Haldar (1977). Specimens from *Oncorhynchus mykiss* have a slightly reduced anterior projection, narrower, more obliquely slanting blades, and in some specimens a suggestion of a second anterior projection similar to that displayed in *Trichodinella subtilis*. Photomicrographs of specimens from the three populations are illustrated in Figure 3.3.



**Figure 3.2.** Plot of Factor 1 against Factor 2 in the Principal Components Analysis of *Trichodinella epizootica* populations (a-c).

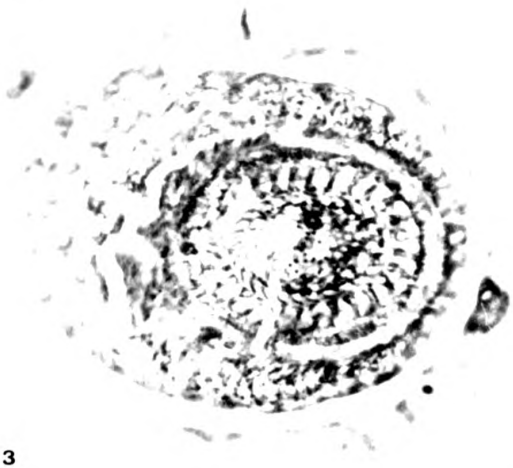
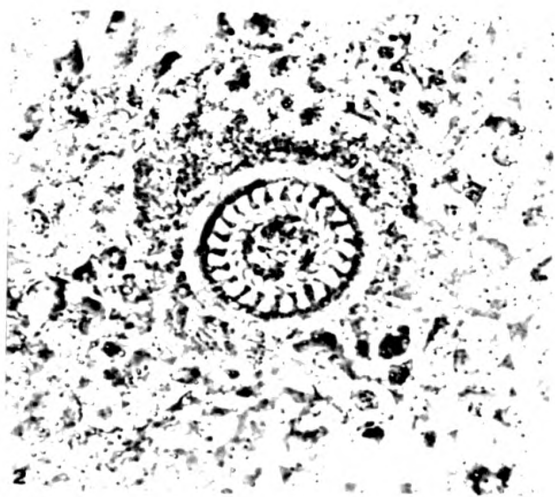
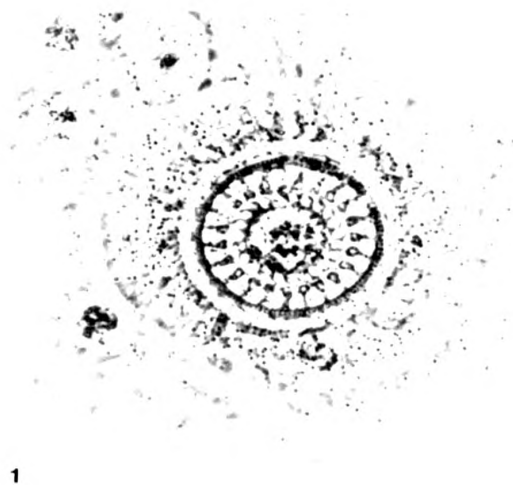
**Figure 3.3.** Silver stained adhesive disc morphology in populations (a-c) of *Trichodinella epizootica*.

**1, 2.** Specimens from *Perca fluviatilis* (Castle Semple Water). (1200×).

**3, 4.** Specimens from *Perca fluviatilis* (Humber side). (1200×).

**5, 6.** Specimens from *Oncorhynchus mykiss* (Dollar). (1200×).





## Discussion

A species closely related to *Trichodinella epizootica* is *Trichodinella subtilis* Lom, 1959. This species was first described from the gills of *Carassius carassius*. (Further reports include: Lom (1963a) from *Cyprinus carpio*, *Tinca tinca*, *Rhodeus sericeus* and *Blicca bjorkna*; Lom & Hoffman (1964) from *Carassius auratus* in North America; Kazubski & Migala (1968) from *Cyprinus carpio* in Poland; Lom & Haldar (1977) from *Pelecus cultratus* and *Oncorhynchus mykiss*; Grupcheva (1975a) from *Scardinius erythrophthalmus* and *Cyprinus carpio* in Bulgaria; and Stein (1984) in the USSR.

This species has almost exactly the same range of measurements for components of its adhesive disc as *Trichodinella epizootica* (Lom & Haldar, 1977); its adoral cilia also describes a turn of 180°. The only differences are that *Trichodinella subtilis* has a second smaller anterior projection above the first. It illustrates three oblique furrows on the surface of the body, and some populations illustrate a slightly higher number of denticles (Lom & Haldar, 1977). Kazubski & Migala (1968) comment on the extreme similarity of the two species, but maintain that if comparable material is available the species can be easily differentiated. However, some of the specimens illustrated by Kazubski & Migala (1968) resemble typical *Trichodinella epizootica*, and others illustrate the characteristic double anterior projection typical of *Trichodinella subtilis*. In consideration of the variation illustrated by *Trichodinella epizootica* (Lom & Haldar, 1977) and the difficulty of impregnation (Lom, 1959), it would seem that the two species may be difficult to discriminate.

The populations of *Trichodinella epizootica* found during this study fall within the range of measurements given by Lom & Haldar (1977). Population (b) has noticeably larger mean values for the morphometric variables than (a) and (c), at the upper end of

the ranges given by Lom & Haldar (1977). The disparity in size is probably due to seasonal variation. Population (b) being sampled in January, (a) and (c) in late June and August. The effect of temperature on trichodinid morphology has been investigated by Kazubski (1981, 1982a, 1991a, b and c) and Kazubski & Migala, 1968), in all cases specimen size was found to increase with a decrease in temperature. Denticle number is positively correlated with adhesive disc diameter, giving a higher denticle number in "winter" specimens with a larger mean adhesive disc diameter (see Chapter 6). Kazubski & Migala (1968) describe an increase in size and denticle number in *Trichodinella subtilis*, starting in summer and lasting until late autumn. They noted that the variability is not as marked as observed in *Trichodina* species. However, this is thought to be due to measuring error, caused by the small size of the specimens (Kazubski & Migala, 1968).

Discerning "actual" variation in denticle form of species belonging to the genus *Trichodinella* is extremely difficult, due to their small size, intraspecific variation and imperfect impregnation (Lom, 1963a). Figure 3.4 illustrates blade form in *Trichodinella* specimens reported as *Trichodinella epizootica* (1-3) (Lom, 1963a) and *Trichodinella subtilis* (4-6) (Lom, 1963a) and (7-13) (Kazubski & Migala, 1968). Populations found during this study are illustrated by denticles (14-17) from population (a), (18-21) from population (b) and (22-25) from population (c).

Denticle (1): *Trichodinella epizootica* (Lom, 1963a) from *Lota lota* represents the typical form with a narrow junction of blade and central part of the denticle, and with part of the curved inner ray visible.

Denticle (2): *Trichodinella epizootica* (Lom, 1963a) from *Esox lucius* possesses a pronounced posterior projection also visible in denticle (15) from *Perca fluviatilis* (this study), and an anterior blade margin perpendicular to the central part rather than angled

anteriorly. This well stained specimen represents rectangular blade form, which may be closer to actual form than the more common "triangular" form narrowing at its junction with the central part. In lightly stained specimens found during this study, the blades were more clearly illustrated; appearing broader and in closer proximity to adjacent denticles than in heavily impregnated specimens.

Denticle (3): *Trichodinella epizootica* (Lom, 1963a), the blade appears more obliquely angled, and has a poorly impregnated anterior blade margin giving an impression of a small secondary anterior projection. Figure 3.5 (A) gives the observed form of this denticle with the suggested form illustrated by the dotted lines. A second anterior projection is illustrated in Figure 3.5 (D), which represent denticle form in *Trichodinella subtilis* from the gills of *Rhodeus sericeus* (Lom, 1963a).

Figure 3.4 (7-13) represents denticle form in *Trichodinella subtilis* (Kazubski & Migala, 1968), from *Cyprinus carpio* in Poland. Denticle appearance varies from that of *Trichodinella epizootica* like form illustrated in (11), to typical *Trichodinella subtilis* form (12) with a secondary anterior projection and obliquely slanting blade. The observed and proposed "actual" form (based on a composite "picture" of many specimens) of denticle (12) is illustrated in Figure 3.5 (B). As in many trichodinid species (see Chapter 4) parts of the blade are so thin that they are almost impossible to visualise with silver staining, often only appearing as an extremely faint shadow. Denticle (13) is notable for an extremely long posterior projection (posterior portion of blade imperfectly stained), which meets the adjacent denticle at exactly the same point as the second anterior projection is usually located.

Population (a) from *Perca fluviatilis* found during this study, contains specimens which illustrate a range of denticle form, see Figure 3.3 and 3.4 (14-17). Denticle (14) displays

**Figure 3.4.** Denticle form in *Trichodinella epizootica* and *Trichodinella subtilis*.

**A).** Denticle form in *Trichodinella epizootica* (1-3) and *Trichodinella subtilis* (4-6), redrawn from Lom (1963a).

**B).** Denticle form in *Trichodinella subtilis* redrawn from Kazubski & Migala (1968).

**C).** Denticle form of specimens from population (a) on the gills of *Perca fluviatilis* (Castle Semple Water) found during this study.

**D).** Denticle form of specimens from population (b) on the gills of *Perca fluviatilis* (Humberside) found during this study.

**E).** Denticle form of specimens from population (c) on the gills of *Oncorhynchus mykiss* (Dollar) found during this study.

*subtilis*.

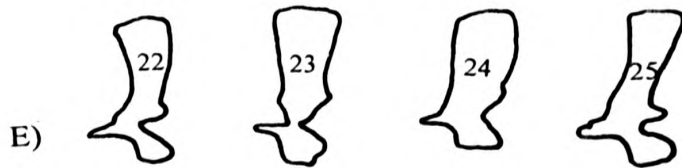
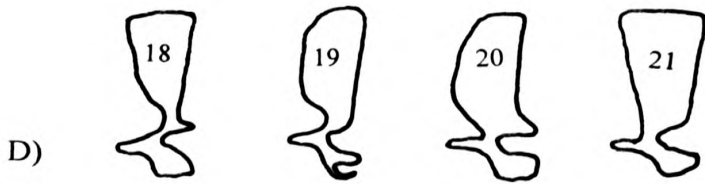
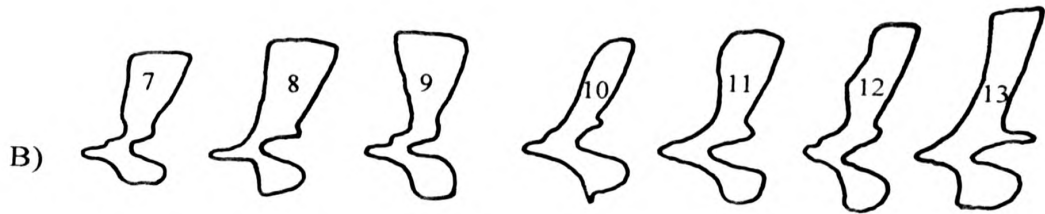
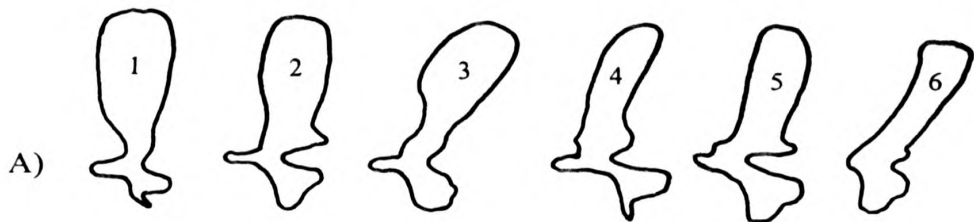
*subtilis* (4-6).

ala (1968).

*a fluviatilis*

*a fluviatilis*

*hus mykiss*



**Figure 3.5.** "Observed" and proposed "actual" denticle morphology produced by imperfect silver impregnation in specimens from populations of *Trichodinella epizootica* and *Trichodina subtilis*.

**A).** Diagrammatic representation of a *Trichodinella epizootica* denticle (Lom, 1963a) (Figure 3.4, 3), solid line illustrates "observed" form, broken line illustrates proposed "actual" form.

**B).** Diagrammatic representation of a *Trichodinella subtilis* denticle (Kazubski & Migala, 1968) (Figure 3.4, 12), solid line illustrates "observed" form, broken line proposed "actual" form.

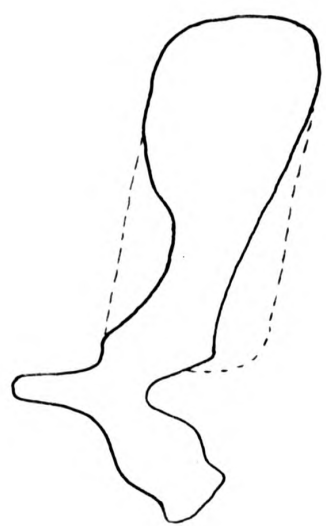
**C), D).** Diagrammatic representation of "observed" denticle form (Figure 3.4, 15 and 16) in specimens from population (a), solid line illustrates "observed" form, broken line "actual" form, hatching represents unstained background, crosshatching represents portion of preceding denticle apparently incorporated into the adjacent one.

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*ta epizootica*

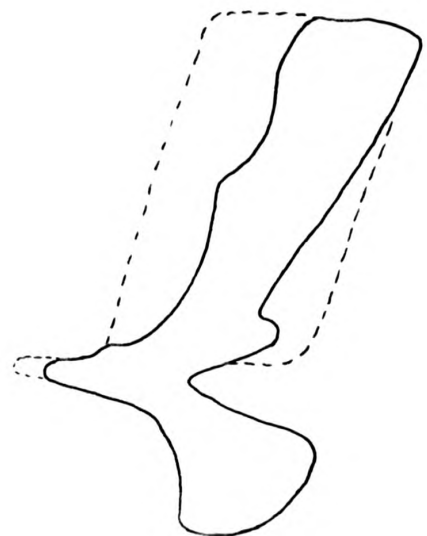
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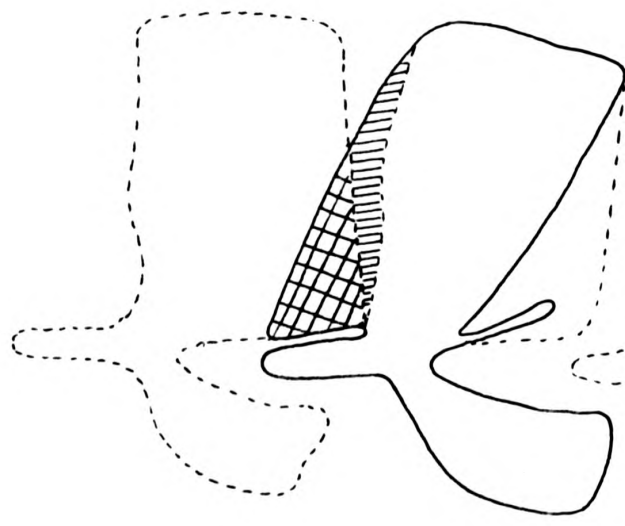
4, 15 and  
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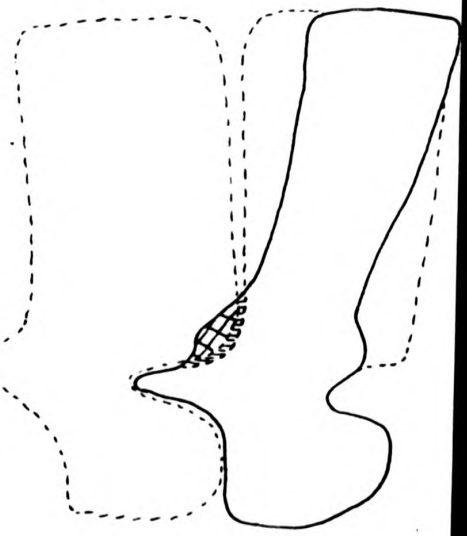
(A)



(B)



(C)



(D)



a relatively well impregnated posterior margin of the blade. Denticle (15) illustrates a form which appears to have an unusually shaped anterior margin to the blade. This is part of the preceding blade's posterior margin, which due to staining artifacts appears to be incorporated into the anterior margin of the blade adjacent to it (see Figure 3.5 (C)). This is exemplified in Figure 3.4 (16) and 3.5 (D), where it is possible that only the very tip of the lower posterior margin is incorporated into the adjacent denticle, giving the appearance of a large secondary posterior projection.

Specimens from population (b) from *Perca fluviatilis* illustrate typical *Trichodinella epizootica* form, Figures 3.3 and 3.4 (18-21). Denticle morphology is very similar to that observed in population (a).

Population (c) from *Oncorhynchus mykiss* illustrates rather different denticle form (Figures 3.3 and 3.4 (22-25)) when compared to populations (a) and (b). The denticles are slightly finer, and more obliquely angled than in typical *Trichodinella epizootica*. A small anterior projection is present in some specimens (Figure 3.4 (22 and 25)), although not so pronounced as in some specimens of *Trichodinella subtilis* (Lom, 1963a). Denticle (24) illustrates typical *Trichodinella epizootica* form.

The difference in denticle form found during this study between specimens from *Perca fluviatilis* and *Oncorhynchus mykiss* are probably due to a combination of intraspecific morphological variation, and variation in staining. The specimens from *Oncorhynchus mykiss* have slightly smaller denticles which may be more prone to unreliable staining characteristics. The anterior projection observed in some of the specimens from population (c) is undoubtedly an artifact, being part of the anterior margin. In Chapter 4, scanning electron micrographs illustrate the denticles of a specimen from population (c). It appears that the lower part of the anterior blade border

is thickened, giving more consistent staining characteristics than the thinner portion. The angled blades of the denticles in population (c) is also likely to be exaggerated by incomplete staining of the denticle (see Figure 3.5 (B)).

A high degree of intraspecific variation was observed in *Trichodina* species, as discussed in the previous chapter. The differences between the *Trichodinella* populations found during this study were relatively insignificant. The almost identical morphometric measurements of population (a) and (c) would strongly suggest that they are the same species. Therefore, it is suggested that the populations found during this study belong to a single species, *Trichodinella epizootica*.

In view of the similarity between *Trichodinella epizootica* and *Trichodinella subtilis*, it is possible that they are part of the same species. Lom (1963a) states that the anterior projections of *Trichodinella subtilis* are most prominent in live specimens, when the disparity between thick and thin parts of the denticle are most apparent. The aboral furrows on the body surface have only been mentioned once (Lom, 1963a), with no records from other authors. As this characteristic is often overlooked, its presence or absence in other populations of the two species is uncertain.

### **TRIPARTIELLA LATA LOM, 1963**

#### **Introduction**

*Tripartiella lata* Lom, 1963 was first reported from the gills of *Phoxinus laevis* in Czechoslovakia, and was subsequently described from *Phoxinus phoxinus* in the same locality (Lom, 1963a). Additional records include the gills of *Oncorhynchus nerka* in Kamchatka (Konovalov, 1971 in: Lom & Haldar 1977), *Oncorhynchus mykiss* in

Bulgaria (Grupcheva, 1975b), the gills of *Pimephales vigilax* from the USA (Lom & Haldar, 1977), smelt *Osmerus eperlanus* L. inhabiting freshwater in Finland (Calenius, 1980), and *Pimephales vigilax* from the USSR (Stein, 1984). The specimens reported from the USA (Lom & Haldar, 1977), were described as being "distinguishable" from the Czechoslovakian populations.

*Tripartiella lata* is said to be closely related to *Tripartiella bursiformis* and *Tripartiella bulbosa*, the difference being the presence of a wide "knee-like" anterior projection of the denticle blade (Lom & Haldar, 1977). The adoral ciliary spiral typically describes a turn of 270°.

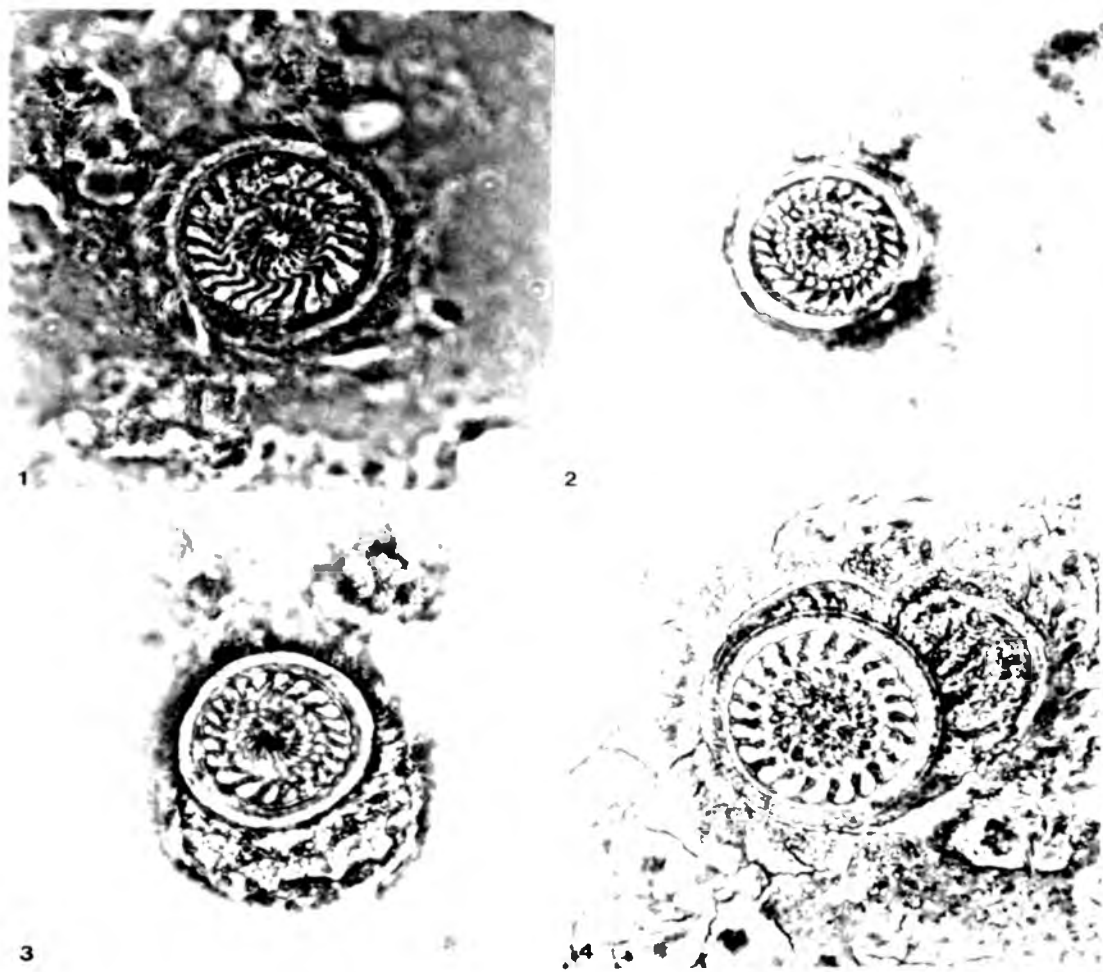
### Results

Eleven specimens of *Tripartiella lata* were found on *Phoxinus phoxinus* from four different localities, the morphometric and meristic data for these specimens are given in Table 3.4. The specimens of *Tripartiella lata* found during this study were similar in size and morphology from the four host populations. In common with *Trichodinella epizootica* discussed previously, *Tripartiella lata* illustrated comparatively poor silver impregnation contributing to the small sample size presented in Table 3.4.

The adhesive disc morphology (Figure 3.6, 1-3) was characterised by a small adhesive disc diameter (16.8-23.6µm), and an extremely small denticle ring diameter (6.5-10µm). The relatively small denticle ring diameter is the result of the denticle blade to inner ray ratio. The blades are long "boomerang" shaped structures with the characteristic "knee-like" anterior projection, just above the central part of the denticle. The denticles widen from the "knee" into a petal shape, with a rounded distal surface. The inner rays are perpendicular to the central parts, and are very small.

**Table 3.4.** Morphometric and meristic data for *Tripartiella lata*.

Author	Lom, 1963a	This study	This study	This study	This study
Host	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i> (fry)	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i>
Localisation	Gills	Gills	Skin	Gills	Gills
Locality	Bohemia	Allt Loin 25/5/92	Castle Semple Water 1/10/92	College Mill 3/8/93	Lake Bala 30/9/93
A. d. diam.	17-25 (22)	(23.6, 1)	18.7-22.2 (20.6±1.3, 7)	(19.3, 1)	16.8-18.9 (17.9±1.4, 2)
B. m. width	1.3	(2.5, 1)	1.9-2.1 (2.0±0.1, 7)	(1.9, 1)	1.8-2.3 (2.1±0.4, 2)
D. r. diam	6-9 (8)	(10.0, 1)	8.0-9.9 (8.7±0.6, 7)	(7.6, 1)	6.5-7.7 (7.1±0.8, 2)
Dent. no.	20-24 (22)	(24, 1)	23-25 (23.9±0.9, 7)	(22, 1)	22-23 (22.5±0.7, 2)
R. p. / d.	4-5	(6, 1)	-	-	(8, 1)
D.length	4.7	(3.7, 1)	3.2-3.7 (3.5±0.2, 7)	(3.2, 1)	3.5-3.8 (3.6±0.2, 2)
B. length	4.2	(5.7, 1)	4.2-5.9 (4.8±0.7, 7)	(5.4, 1)	4.5-4.9 (4.7±0.3, 2)
R. length	1.5	(2.1, 1)	1.5-2.7 (2.0±0.4, 7)	(1.9, 1)	1.7-2.0 (1.9±0.2, 2)
C. p. width	1.1	(0.7, 1)	0.5-1.0 (0.7±0.2, 7)	(0.7, 1)	0.8-1.0 (0.9±0.1, 2)
Dent. span	-	(8.4, 1)	6.4-8.2 (7.2±0.6, 7)	(7.7, 1)	7.0-7.3 (7.1±0.2, 2)



**Figure 3.6.** Silver stained adhesive disc morphology in specimens of *Tripartiella lata* and *Tripartiella copiosa*.

1. Specimen of *Tripartiella lata* from *Phoxinus phoxinus* (Allt Loin). (1200×).
2. Specimen of *Tripartiella lata* from *Phoxinus phoxinus* (Castle Semple Water). (1200×).
3. Specimen of *Tripartiella lata* from *Phoxinus phoxinus* (Bala). (1200×).
4. Specimen of *Tripartiella copiosa* from *Rutilus rutilus* (Yorkshire). (1200×)

### **Discussion**

The specimens of *Trichodina lata* in this study conform morphologically and morphometrically to those of Lom's (1963a) original description. This description of *Tripartiella lata* from *Phoxinus phoxinus* constitutes a new British record and is also the first report of a member of the *Tripartiella* genus from Britain. A range of hosts are reported in the literature, but this report reinforces the view that minnows (*Phoxinus phoxinus* and *Phoxinus laevis*) (Lom, 1963a) are the most common.

The occurrence of *Tripartiella lata* on the skin of *Phoxinus phoxinus* from Castle Semple Water, rather than on the gills is probably due to the immaturity of the host specimens. Lom (1963a) suggests that the properties of skin in fry are comparable to the gills of adult fish.

Given the report of *Tripartiella lata* from *Oncorhynchus mykiss* in Bulgaria (Grupcheva, 1975b) and the wide geographic distribution displayed by *Tripartiella lata* on *Phoxinus phoxinus* in Britain, it was surprising that this species was not recorded from any of the numerous populations of *Oncorhynchus mykiss* sampled during this study.

### **TRIPARTIELLA SP.**

#### **Introduction**

A single specimen of a *Tripartiella* species was found on the gills of *Rutilus rutilus* from Yorkshire. A brief morphological description, and tentative identification to species level was undertaken.

## Results

The *Tripartiella* specimen (Figure 3.6, 4) has a small adhesive disc diameter (18.5 $\mu$ m), and a denticle ring diameter of 13.9 $\mu$ m. The denticles extends anteriorly in a fine projection characteristic of the genus, contributing to a denticle length of 4.6 $\mu$ m. The blades (b. length=4.4 $\mu$ m) are obliquely angled, widening from the central part to a flat distal surface parallel with the border membrane (b. m. width=2.7 $\mu$ m). The inner rays are fine slightly curved structures, angled to the posterior of the denticle (r. length=2.6 $\mu$ m). The central part of the denticle is narrow (0.8 $\mu$ m), and denticle span is 7.6 $\mu$ m.

## Discussion

The specimen previously described corresponds closely with photomicrographs and morphometric data given by Lom (1963a) for *Tripartiella copiosa* Lom, 1959 from *Rutilus rutilus*. *Tripartiella copiosa* differs considerably from *Tripartiella lata*, having a fine anterior projection rather than the "knee-like" anterior projection displayed by *Tripartiella lata*. This species was first described from the gills of *Rhodeus sericeus* in Czechoslovakia (Lom, 1959). It is subject to variable silver impregnation (Lom, 1963a), similar to that described previously in *Trichodinella epizootica*. *Tripartiella copiosa* was reported on the gills of *Alburnus alburnus*, *Leuciscus leuciscus*, *Rhodeus sericeus*, *Blicca bjoerkna*, *Leuciscus cephalus*, *Leucaspis delineatus* and *Cobitis taenia* from Czechoslovakia (Lom, 1963a). Populations inhabiting the gills of *Gobio gobio* (Lom, 1963a), described as similar to *Tripartiella copiosa* with the exception of a triangular anterior projection were designated as *Tripartiella obtusa* Ergens & Lom, 1970 (Lom & Haldar, 1977).

Additional reports of *Tripartiella copiosa* include: Kashkovsky (1974) from the gills of *Rutilus rutilus* in the USSR, Grupcheva (1975a) from danube bleak *Chalcalburnus chalcoides* in Bulgaria, Stein (1979, 1984) in the USSR and Calenius (1980) from the gills of *Esox lucius*, *Coreogonus albula* and *Thymallus thymallus* in Finland.

The *Tripartiella* specimen from *Rutilus rutilus* found during this study is tentatively identified as *Tripartiella copiosa*, which has not been previously recorded from Britain.

#### **PARATRICHODINA INCISA LOM, 1959**

(Syn. *Trichodinella (Tripartiella) incisa* Lom, 1959  
*Tripartiella (Paratrachodina) incisa* Lom, 1963)

#### **Introduction**

*Paratrachodina incisa* was initially reported as *Trichodinella (Paratrachodina) incisa* from *Barbatula (Nemacheilus) barbatulus* by Lom (1959). It was differentiated from previously described species by a deep notch in the anterior margin of the denticle blade. The blades were described as joining the central part at the same level as the junction with the inner ray, whereas in *Trichodinella* and *Tripartiella* species the blade is off set anteriorly to the inner ray.

Populations were later described as *Tripartiella (Paratrachodina) incisa* from *Phoxinus laevis*, *Rutilus rutilus*, *Gobio gobio*, *Scardinius erythrophthalmus* and the tadpoles of *Rana esculenta* by Lom (1963a). Stein (1967) reported this species under the same name from *Thymallus arcticus* in Kamchatka, and Kashkovsky (1974) from *Rutilus rutilus* and *Phoxinus phoxinus* in the USSR.



The subgenus *Paratrichodina* was raised to genus level by Lom & Haldar (1977) giving the current designation of *Paratrichodina incisa*. Lom & Haldar (1977) differentiated populations previously described from *Gobio gobio* (Lom, 1963a) as a separate species, *Paratrichodina corlissi*. The main feature discriminating *Paratrichodina corlissi* from *Paratrichodina incisa* was the lack of a notch in the anterior blade margin. The measurements of the two species fall within the same range. The adoral cilia display similarities ranging from 150-210° in observed populations of *Paratrichodina incisa* and 180-240° in *Paratrichodina corlissi*. Lom & Haldar (1977) also remark that some populations of *Paratrichodina incisa* from *Barbatula (Nemacheilus) barbatulus* and *Phoxinus phoxinus* illustrate a smaller notch in the denticle blades, which may be reduced to a narrow slit in some specimens. Populations were also reported from *Leuciscus leuciscus* and *Leuciscus idus* (Lom & Haldar, 1977).

Additional reports of *Paratrichodina incisa* include: Stein (1979, 1984) from the USSR, Calenius (1980) from the gills of *Phoxinus phoxinus* in Finland, and Arthur & Lom (1984) from *Leuciscus idus* and *Abramis ballerus* from the Rybinsk Reservoir (USSR).

### Results

Six populations of a species resembling *Paratrichodina incisa* were found during this study from *Phoxinus phoxinus*, *Abramis brama* and *Rutilus rutilus*. Morphometric and meristic data for these populations are presented in Table 3.5. The populations of this species were characterised by their small size (a. d. diam=15.7-29.2µm) in common with many *Trichodinella*, *Tripartiella* and other *Paratrichodina* species. The number of denticles ranged from 21-28, with a notch in the anterior blade margin characteristic of

**Table 3.5.** Morphometric and meristic data for *Paratrichodina incisa*.

Author	Lom & Haldar, 1977	Our findings (a)	Our findings (b)	Our findings (c)	Our findings (d)	Our findings (e)	Our findings (f)
Host	Pooled data from all hosts	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i> (fry)*	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i>	<i>Abramis brama</i>	<i>Rutilus rutilus</i>
Localisation	Gills	Gills	Skin	Gills	Gills	Gill	Gill
Locality	-	Allt Loin 25/5/92	Castle Semple Water 1/10/92	College Mill 3/8/93	Lake Bala 30/9/93	Yorkshire 20/2/93	Yorkshire 1/4/93
A. d. diam.	16-31	17.8-23.7 (21.2±3.0, 3)	15.9-23.2 (20.6±2.2, 9)	22.7-29.2 (25.3±3.0, 4)	17.6-27.7(21.7±2.9, 17)	19.2-26.7 (22.4±1.9, 29)	17.0-25.6 (21.4±1.9, 28)
B. m. width	1.3-3.3	1.6-2.4 (2.0±0.4, 3)	1.4-2.3 (1.9±0.3, 9)	2.3-2.5 (2.4±0.1, 4)	1.8-2.6 (2.1±0.3, 17)	1.9-2.6, (2.2±0.1, 29)	1.8-2.4 (2.2±0.2, 28)
D. r. diam.	9-30	13.2-14.8 (14.1±0.8, 3)	9.0-14.5 (12.1±1.7, 9)	14.1-17.7 (15.4±1.7, 4)	11.1-16.6 (13.3±1.7, 17)	11.1-16.2 (13.3±1.4, 29)	9.7-14.4 (12.1±1.2, 28)
Dent. no.	19-30	22-24 (23.0±1.0, 3)	21-24 (22.7±1.1, 9)	24-25 (24.5±0.6, 4)	22-28 (24.8±1.8, 17)	21-25 (23.6±1.0, 29)	21-25 (23.6±0.9, 28)
R. p. / d.	4-8	5-6 (5.5±0.7, 2)	5-6 (5.6±0.5, 4)	(6, 1)	5-6 (5.5±0.4, 9)	5-6 (5.4±0.5, 23)	4-6 (4.7±0.6, 22)
D. length	-	2.3-2.7 (2.5±0.2, 3)	2.0-3.2 (2.5±0.3, 9)	2.4-3.2 (2.9±0.4, 4)	1.9-2.7 (2.3±0.2, 17)	2.0-3.1 (2.5±0.3, 29)	2.1-3.1 (2.4±0.3, 28)
B. length	2.3-4.8	2.5-3.5 (3.1±0.5, 3)	2.3-3.6 (3.0±0.5, 9)	3.5-4.3 (3.9±0.4, 4)	2.8-4.1 (3.3±0.4, 17)	3.1-4.0 (3.5±0.3, 29)	2.6-4.0 (3.5±0.4, 28)
R. length	1.2-4.3	1.5-2.4 (2.1±0.5, 3)	1.5-2.5 (2.0±0.4, 9)	2.2-3.6 (2.7±0.6, 4)	1.6-3.3 (2.2±0.4, 17)	1.3-3.7 (2.4±0.4, 29)	1.3-2.8 (2.2±0.4, 28)
C. p. width	0.6-2.2	0.6-1.2 (0.9±0.3, 3)	0.6-1.1 (0.9±0.2, 9)	0.6-2.6 (2.0±0.9, 4)	0.7-1.1 (0.8±0.1, 17)	0.6-1.0 (0.8±0.1, 29)	0.7-1.0 (0.8±0.1, 28)
Dent. span	-	5.3-6.7 (6.1±0.7, 3)	5.1-7.2 (6.0±0.7, 9)	6.5-8.5 (7.3±0.9, 4)	5.3-7.3 (6.1±0.6, 17)	5.1-7.7 (6.7±0.5, 29)	5.0-7.7 (6.6±0.7, 28)

\* Kept at room temperature (approx. 20° C) for 2 weeks

the species. This gives the impression of an anterior projection, which does not locate with a notch in the posterior blade margin of the adjacent denticle as in *Trichodinella* and *Tripartiella*. The denticle blades (2.3-4.3 $\mu$ m) are perpendicular to the central parts of the denticles with a flattened distal surface parallel with the border membrane. The inner rays (1.3-3.7 $\mu$ m) are slightly smaller than the blades and are straight or slightly curved, angled slightly to the anterior or posterior of the denticle. The adoral ciliary spiral ranged from 190-230° in observed specimens.

Principal Components Analysis was employed to help visualise morphometric variation between populations. The component loadings of the first three principal components are given in Table 3.6, demonstrating that adhesive disc diameter, denticle span and denticle ring diameter are the main variables acting in Factor 1. Denticle number is the major variable in Factor 2, and central part width in Factor 3.

The percentage variance explained by the first three principal components is demonstrated in Table 3.7, 62.6% of variation being explained by the first two factors.

Figure 3.7 illustrates a PCA plot of Factor 1 against Factor 2, with populations (b), (d), (e) and (f) encircled by 60% ellipses. The sample size in populations (a) n=3 and (c) n=4 is too small for Systat to accurately interpret, and are therefore represented by the appropriate letter without being enclosed in an ellipse. Populations (e) from *Abramis brama* and (f) from *Rutilus rutilus* show a high degree of homogeneity. Population (b) from *Phoxinus phoxinus* fry (Castle Semple Water) overlaps populations (e) and (f), but illustrates greater variation along Factor 1 and 2. Population (b) has the lowest mean adhesive disc diameter and denticle number of the six observed. Population (d) overlaps (b), (e) and (f) but is separated mainly along Factor 1, having a significantly higher mean denticle number than the others. Photomicrographs of

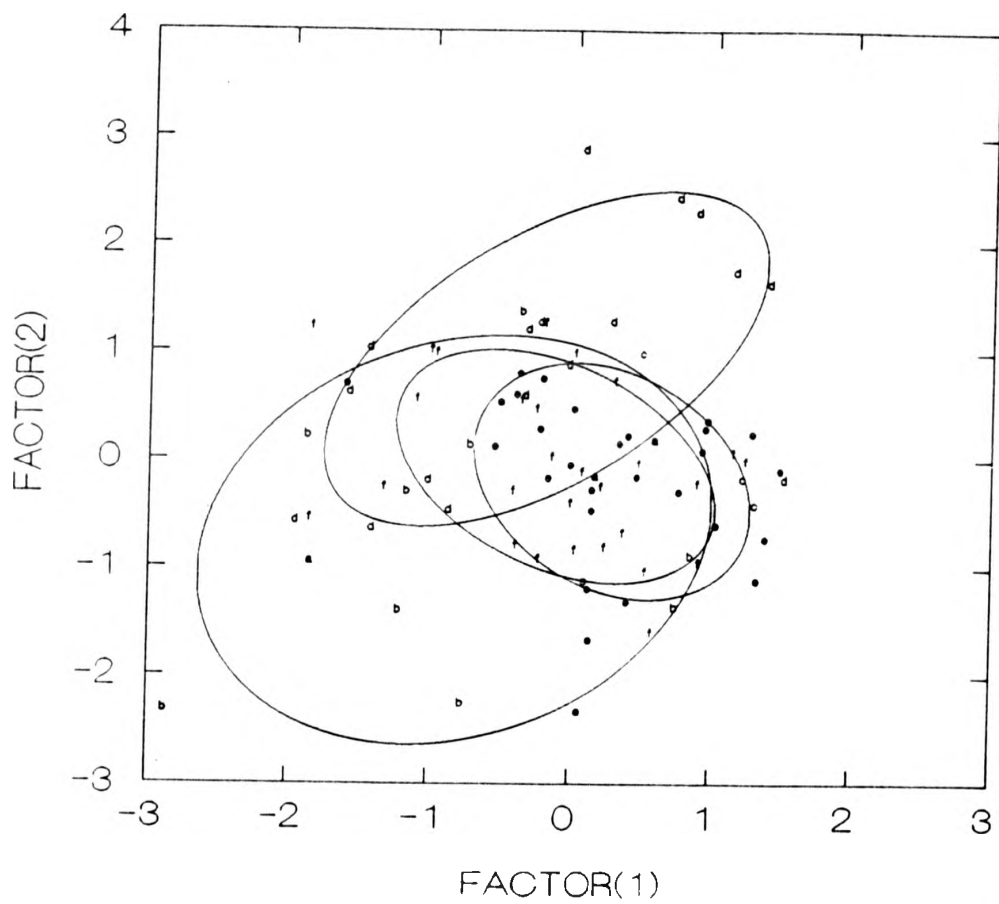
**Table 3.6.** Component loadings on the first three principal components, in the analysis of *Paratrichodina incisa*.

	Factor 1	Factor 2	Factor 3
A. d. diam.	0.901	0.140	-0.063
B. m. width	0.564	0.324	0.320
D. r. diam.	0.874	0.103	-0.169
Dent. no.	0.479	0.763	-0.165
D. length	0.662	-0.283	0.082
B. length	0.768	0.001	0.246
R. length	0.712	-0.343	0.161
C. p. width	0.387	-0.178	-0.826
Dent. span	0.892	-0.296	0.072

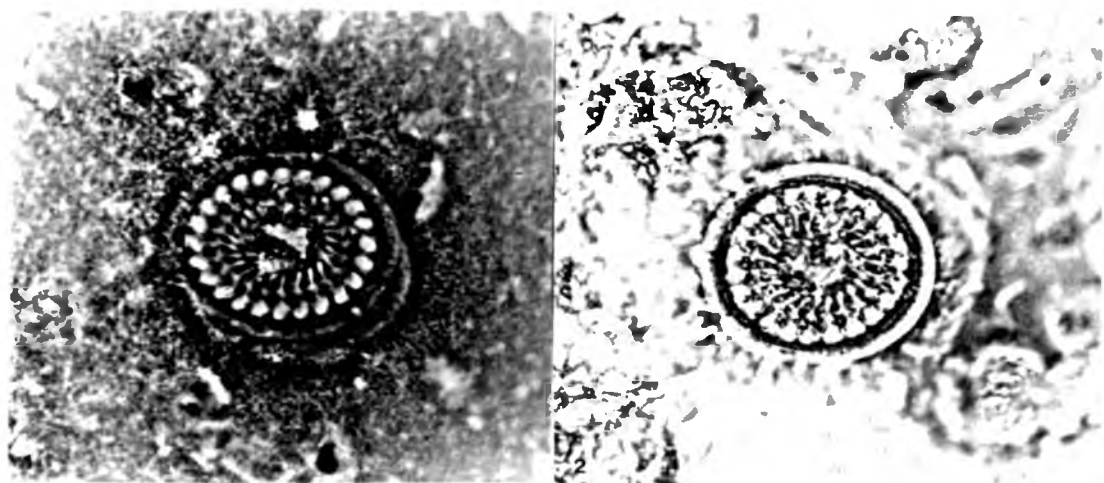
**Table 3.7.** Percentage of variance explained by the first three principal components, in the analysis of *Paratrichodina incisa*.

	variance explained by components	% of total variance	Cumulative percentage
Factor 1	4.603	51.147	51.147
Factor 2	1.034	11.491	62.638
Factor 3	0.942	10.468	73.106

specimens from the six populations found during this study are illustrated in Figures 3.8, 3.9 and 3.10. Denticle morphology in the six populations was very similar. Populations (a), (c), (e) and (f) displayed typical form with a pronounced notch in the blades present in all specimens. Populations (b) and (d) contained some specimens with a reduced or slit like notch, which was completely absent in individual denticles. This was particularly noticeable in the smaller specimens (Figure 3.8, 4). Population (d) contained one specimen with a particularly strange appearance of the central parts of the denticle (Figure 3.9, 4).



**Figure 3.7.** Plot of Factor 1 against Factor 2 in the Principal Components Analysis of *Tripartiella incisa* populations (a-f).

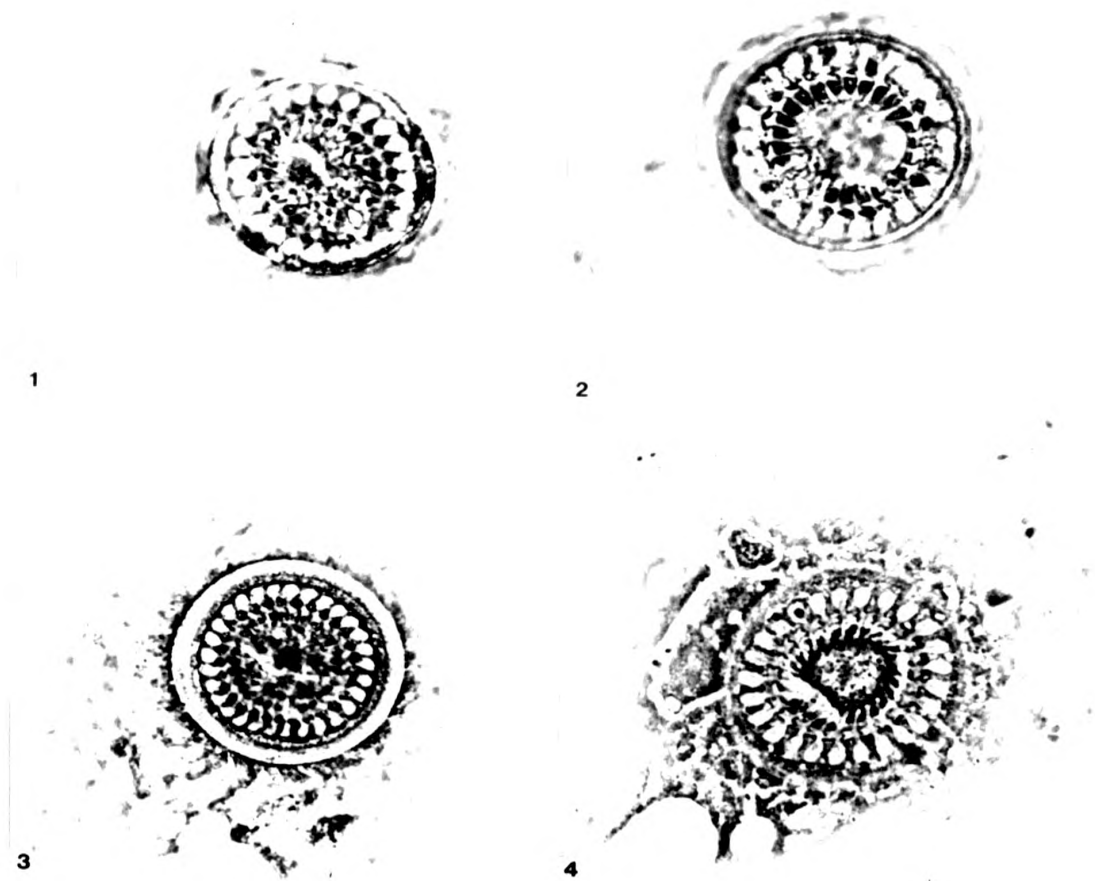


3



4

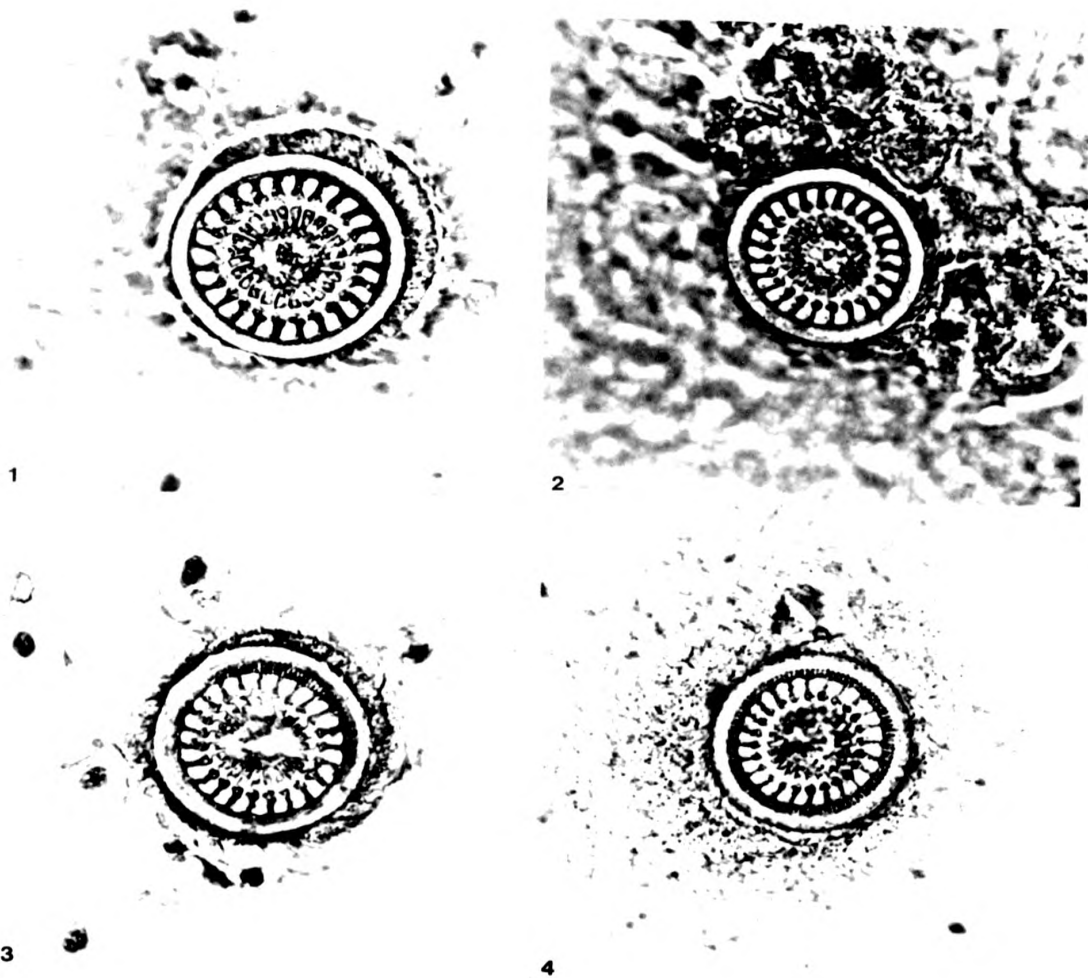
**Figure 3.8.** Silver stained adhesive disc morphology in specimens of *Paratrichodina incisa* from *Phoxinus phoxinus*.  
1, 2. Specimens from Allt Loin. (1200 $\times$ ).  
3, 4. Specimens from Castle Semple Water. (1200 $\times$ ).



**Figure 3.9.** Silver stained adhesive disc morphology in specimens of *Paratrichodina incisa* from *Phoxinus phoxinus*.

**1, 2.** Specimens from College Mill. (1200 $\times$ ).

**3, 4.** Specimens from Lake Bala. (1200 $\times$ ).



**Figure 3.10.** Silver stained adhesive disc morphology in specimens of *Paratrichodina incisa*.

1, 2. Specimens from *Abramis brama* (Yorkshire). (1200 $\times$ ).

3, 4. Specimens from *Rutilus rutilus* (Yorkshire). (1200 $\times$ ).



## Discussion

The six populations of *Paratrichodina incisa* observed in this study exhibited limited variation. Populations (e) and (f) were almost identical, this may be due to the fact that the host populations were transported and subsequently kept in the same water. Therefore, these two populations may represent one "real" population, having occurred as a result of artificial infection due to the close proximity of the hosts. However, both *Abramis brama* and *Rutilus rutilus* illustrated a similar intensity of infection. In addition, populations of *Paratrichodina incisa* have been previously recorded from *Rutilus rutilus* (Lom, 1963a) and *Abramis ballerus* (Arthur & Lom, 1984) indicating that both bream and roach are likely hosts. As both host populations were from the same water body, and both are susceptible to infection it is equally possible that two natural populations are represented.

Population (b) from *Phoxinus phoxinus* (Castle Semple Water) was kept at approximately 20° C, before sampling. This may account for the smaller mean diameter of this population, due to the negative correlation between water temperature and size (see Chapter 6).

The separation exhibited by population (d) cannot be explained by seasonal or host induced variation, and is probably due to genetic differences.

It is worth noting that when sample sizes are unequal, the size of ellipses displayed in Principal Components Analysis can be effected. In small samples, extremely small or large specimens become more significant accounting for a higher percentage of total variation than in a larger sample. This has the effect of producing enormous ellipses, which are misleading. Thus, populations (a) and (c) were effectively excluded from the analysis.

The range of denticle morphology apparent in the populations of *Paratrichodina incisa* found during this study was similar to that described by Lom & Haldar (1977). The reduced anterior notch in the denticle blades exhibited in some specimens gives an appearance approaching that of *Paratrichodina corlissi* (Lom & Haldar, 1977). However, in the latter species the anterior notch was lacking in all specimens.

*Paratrichodina voikarensis* Kashkovsky & Lom, 1979 from *Coregonus nasus* and *Coregonus peled* (Kashkovsky & Lom, 1979), considered a synonym of *Paratrichodina corlissi* by Stein (1984), is similar in size and morphology to some specimens found during this study. *Paratrichodina voikarensis* is reported to have an adoral ciliary spiral of 240-260° as opposed to 150-210° in *Paratrichodina incisa*, although it reached 230° in specimens of the latter species observed during this study.

A single specimen (Figure 3.9, 4) of population (d) from *Phoxinus phoxinus* (Lake Bala) resembles *Paratrichodina uralensis* Kashkovsky & Lom, 1979 from *Acipenser ruthenus* (Kashkovsky & Lom, 1979). A small posterior projection on the same level as the anterior projection of the denticle blade in *Paratrichodina incisa* is apparent. The size and adoral cilia in *Paratrichodina uralensis* falls within the same range as in *Paratrichodina incisa*.

A further species, *Paratrichodina erectispina* Lom & Haldar, 1977 (Lom & Haldar, 1977) from *Pimephales vigilax* in the USA, closely resembles *Paratrichodina incisa*, the main differentiating characteristic being an aboral ciliary spiral of 230-280°.

*Paratrichodina voikarensis*, *Paratrichodina uralensis*, *Paratrichodina erectispina* and *Paratrichodina corlissi* illustrate similarities to specimens found during this study (see Table 3.8). These species are very closely related to *Paratrichodina incisa*, but have only been described from one or two populations. With more populations sampled

**Table 3.8.** Comparison of morphometric measurements in *Paratrichodina incisa*, *Paratrichodina voikarensis*, *Paratrichodina uralensis*, *Paratrichodina erectispina*, and *Paratrichodina corlissi*.

Author	This study	Kaskovsky & Lom, 1979	Kashkovsky & Lom, 1979	Lom & Haldar, 1977	Lom & Haldar, 1977
Host	Pooled data	<i>Coregonus nasus</i>	<i>Acipenser ruthenus</i>	<i>Pimephales vigilae</i>	<i>Gobio kessleri</i>
Species	<i>Paratrichodina incisa</i>	<i>Paratrichodina voikarensis</i>	<i>Paratrichodina uralensis</i>	<i>Paratrichodina erectispina</i>	<i>Paratrichodina corlissi</i>
A. d. diam.	15.7-29.2 (21.9±2.4, 90)	18-26 (24)	20-26 (24)	20-31 (25)	19-25 (22)
B. m. width	1.4-2.6 (2.2±0.2, 90)	2-3	1-2	2	1.8-2.2
D. r. diam.	9.0-17.7 (12.9±1.6, 90)	15-16 (15)	21-16 (14)	12-19 (15)	10-15 (12)
Dent. no.	21-28 (23.7±1.3, 90)	23-25	18-25 (22)	24-27 (25)	18-24 (21)
R. p. / d.	4-6	6	6	4-5	5-6
D. length	1.9-3.2 (2.5±0.3, 90)	-	-	-	-
B. length	2.3-4.3 (3.4±0.4, 90)	3-4	2.4-4.8 (4)	3-3.5	3.3-3.8
R. length	1.3-3.7 (2.3±0.4, 90)	2-4 (3)	1.8-3.6 (2.4)	2-2.5	2.2-3.3
C. p. width	0.6-2.6 (0.9±0.2, 90)	1-2	1-2	1.2-1.4	1-2
Dent. span	5.0-8.5 (6.5±0.7, 90)	-	-	-	-
Aadoral cilia	190-230°	240-260°	180-230°	230-280°	180-240°

the distinction between species may be corroborated, or the range of morphological variation may "blur" the apparent distinctions observed. The blade morphology upon which many of the previous *Paratrichodina* species are distinguished is very prone to staining artifacts (see Chapter 4).

This report of *Paratrichodina incisa* constitutes a new British record and the first record of a *Paratrichodina* species in this country.

## TRICHODINA INTERMEDIA LOM, 1960

### Introduction

*Trichodina intermedia* Lom, 1960 does not fall within the characteristics of any single genus (Lom, 1961), as suggested by its name. It is dealt with in this chapter because it appeared to show a closer affinity to previously described *Paratrichodina* species, than those of *Trichodina*.

Only two reports of *Trichodina intermedia* were found in the literature, Lom (1961) from *Phoxinus laevis* in Czechoslovakia and Stein (1979, 1984) from various fish species in the USSR.

The population described as *Trichodina intermedia* (Lom, 1961) displayed an adoral ciliary spiral of 310-340°, this being its most peculiar feature. This was described as being unique in approaching the appearance of the oral cilia found in *Tripartiella*. The denticles were described as being similar to *Paratrichodina incisa* in blade form, the central parts slender with a straight, narrow and short inner ray. A notch in the anterior margin of the blade was apparent in some specimens, which was assumed to be a staining artifact caused by the blade being extremely thin (Lom, 1961). Lom (1961) concluded that this species demonstrated the need for a revision of the Trichodinidae.

A description of a second species, *Trichodina janovice* Lom, 1960, was also reported from *Phoxinus laevis* (Lom, 1961). This resembled *Trichodina intermedia* in denticle structure, but was much larger with an adhesive disc diameter of 57-69µm. Denticle number was 41-43 and the aboral cilia displayed a turn of approximately 380°. Although *Trichodina janovice* is obviously very closely related to *Trichodina intermedia*, its aboral cilia are within the normal range of the genus *Trichodina*.

Both these species seem to have been ignored in subsequent literature discussing generic characteristics and inter-relationships (Lom & Haldar, 1977; Lom & Dykova, 1992), possibly because of their "difficult" taxonomic status.

### Results

Four populations of a species resembling *Trichodina intermedia* were found inhabiting the gills (also skin in fry) of *Phoxinus phoxinus* during this study. Morphometric and meristic data are given in Table 3.9. Specimens from the four populations described in Table 3.9 were characterised by an adhesive disc diameter of 26.7-46.2 $\mu$ m and a denticle number of 25-37. The denticles have rectangular blades which often appear to widen towards a flattened distal surface. Most specimens display straight blades with parallel margins, but some illustrate a slight posterior curvature. The inner rays are slightly shorter than the blades and are stouter than in *Paratrachodina incisa*. They are straight or very slightly curved anteriorly, usually being angled straight down. The adoral ciliary spiral illustrates a turn of 280-340°.

To elucidate any morphometric variation between the four populations, Principal Components Analysis was undertaken. The component loadings of the first three principal components are given in Table 3.10.

Adhesive disc diameter, denticle ring diameter and denticle span are the main variables acting in Factor 1. Denticle number is the main variable acting in Factor 2 and central part width in Factor 3.

The percentage variance explained by the first three factors is illustrated in Table 3.11. The first two accounting for 72.558% of the total.

Figure 3.11 illustrates a plot of Factor 1 against Factor 2, with the four

**Table 3.9.** Morphometric and meristic data for *Trichodina intermedia*.

Author	Lom, 1961	Our findings (a)*	Our findings (b)	Our findings (c)	Our findings (d)
Host	<i>Phoxinus laevis</i>	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i> (fry)	<i>Phoxinus phoxinus</i>	<i>Phoxinus phoxinus</i>
Localisation	Gills, rarely skin	Gills	Skin	Gills	Gills
Locality	Czechoslovakia	Allt Loin 25/5/92	Castle Semple Water 1/10/92	College Mill 3/8/93	Lake Bala 30/9/93
A. d. diam.	31-41 (34)	26.7-45.0 (36.0±4.7, 52)	27.5-40.2 (34.5±4.3, 11)	29.9-46.2 (37.6±4.6, 11)	29.2-42.1 (36.3±3.9, 18)
B. m. width	3	2.3-3.4 (2.8±0.3, 52)	1.8-3.0 (2.5±0.4, 11)	2.0-3.1 (2.7±0.3, 32)	2.3-3.3 (2.8±0.3, 18)
D. r. diam	19-28 (22)	16.1-29.8 (22.9±3.5, 52)	16.6-28.8 (21.5±3.6, 11)	18.0-31.2 (24.2±3.5, 32)	19.3-27.1 (23.5±2.4, 18)
Dent. no.	27-36 (31)	25-37 (29.4±3.6, 52)	25-35 (28.9±2.8, 11)	26-34 (30.8±2.2, 32)	28-35 (31.7±2.1, 18)
R. p. / d.	6-7	6-10 (7.8±0.9, 44)	6.5-9 (7.3±1.0, 6)	5.5-7 (6.3±0.5, 19)	5.5-9 (7.1±1.0, 9)
D. length	5-6	3.0-5.7 (4.0±0.7, 52)	2.7-4.4 (3.6±0.6, 11)	2.7-5.2 (3.8±0.6, 32)	2.9-4.8 (3.6±0.5, 18)
B. length	4	4.0-6.7 (5.3±0.6, 52)	4.1-6.2 (5.0±0.8, 11)	4.3-7.7 (5.4±0.7, 32)	4.1-6.2 (5.2±0.6, 18)
R. length	3.3	2.5-5.7 (4.2±0.8, 52)	2.9-4.9 (4.0±0.7, 11)	3.4-6.0 (4.4±0.7, 32)	3.3-5.0 (4.1±0.5, 18)
C. p. width	1.5	0.8-1.8 (1.0±0.1, 52)	1.1-1.8 (1.4±0.2, 11)	0.9-2.7 (1.8±0.1, 32)	0.8-1.5 (1.2±0.2, 18)
Dent. span	-	8.2-16.3 (10.7±1.6, 52)	8.3-12.2 (10.2±1.4, 11)	8.8-14.1 (10.8±1.4, 32)	8.3-12.3 (10.5±1.2, 18)

\* Kept at room temperature (approximately 20° C) before sampling

populations encircled by 60% ellipses. Populations (a) from Allt Loin, (b) from Castle Semple Water, (c) College Mill and (d) from Lake Bala illustrate a high degree of overlap. Population (a) illustrates the greatest range in denticle number (25-37) which is probably due to the large sample size. Population (b) has the smallest mean adhesive disc diameter, which may be due to the artificially high water temperature from which they were sampled.

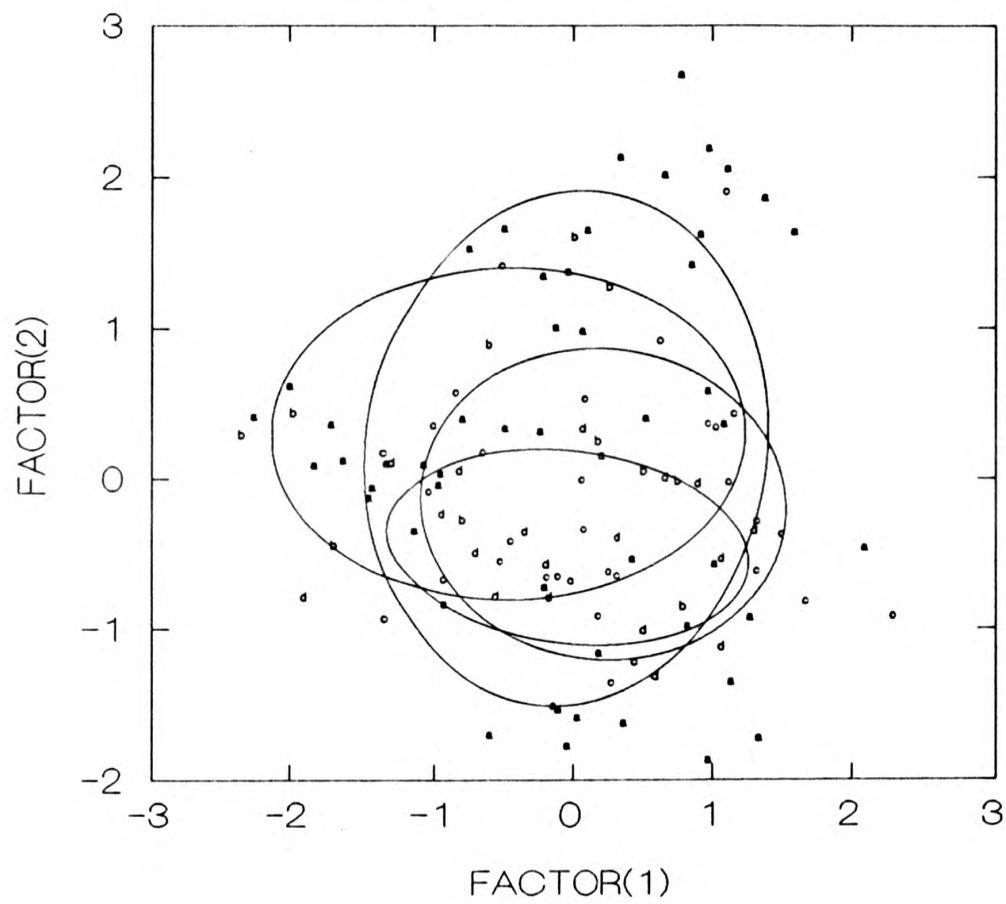
**Table 3.10.** Component loadings on the first three principal components, in the analysis of *Trichodina intermedia*.

	Factor 1	Factor 2	Factor 3
A. d. diam.	0.939	-0.246	0.130
B. m. with	0.496	0.297	-0.414
D. r. diam.	0.917	-0.279	0.113
Dent. no.	0.423	-0.837	0.159
D. length	0.711	0.453	0.089
B. length	0.853	-0.231	-0.186
R. length	0.787	0.305	-0.189
C. p. width	0.452	0.457	0.693
Dent. span	0.906	0.134	-0.173

**Table 3.11.** Percentage of variance explained by the first three principal components, in the analysis of *Trichodina intermedia*.

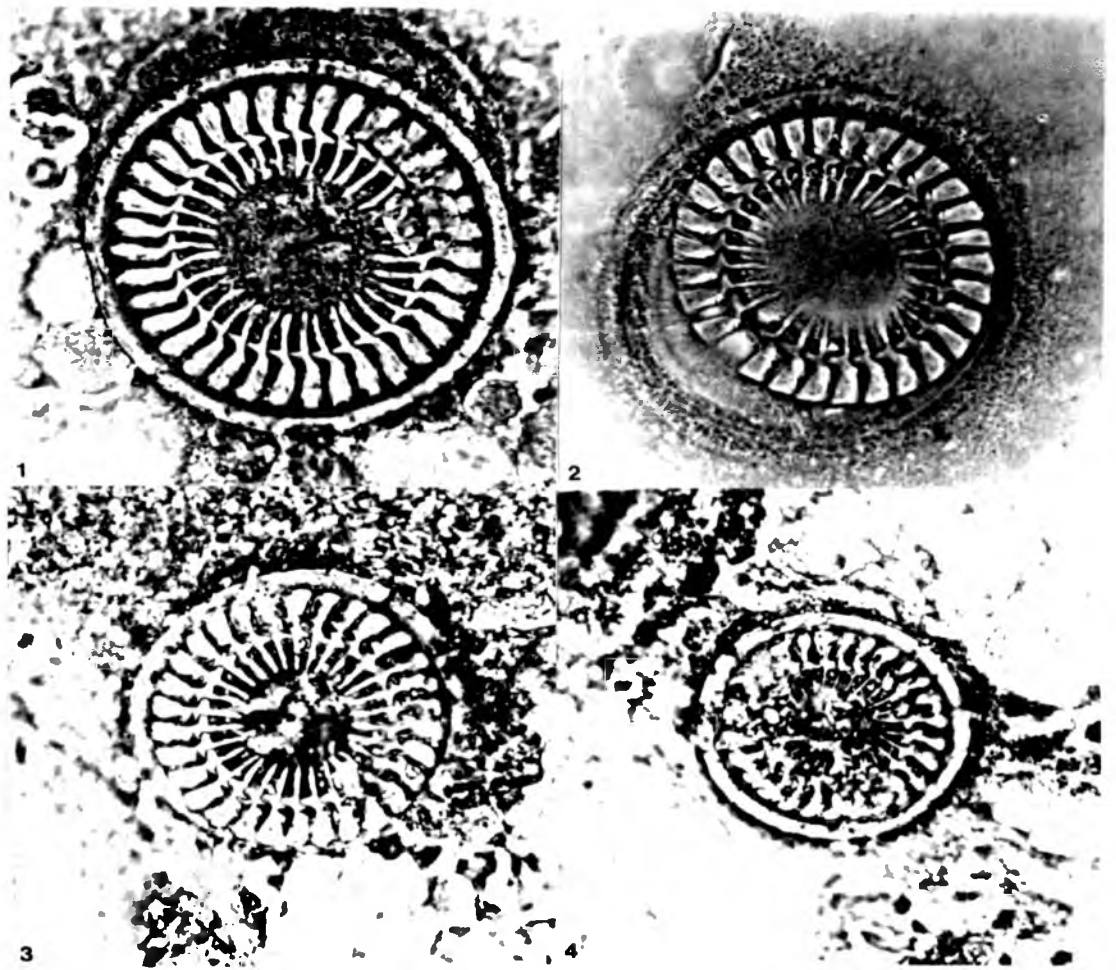
	Variance explained by components	% total variance explained	Cumulative percentage
Factor 1	5.025	55.830	55.830
Factor 2	1.506	16.728	72.558
Factor 3	0.815	9.054	81.612

The denticles varied considerably in size, with a denticle span ranging from 8.2-16.3 $\mu$ m within the four populations. Denticle form varied considerably with some specimens illustrating broader blades with a high degree of curvature in population (a). Population (b) illustrated uniform denticle appearance, with the exception of one specimen with prominent notches in the anterior blade margins. A small number of specimens in population (c) had unusually curved blades, and population (d) displayed typical denticle structure. Photomicrographs of specimens found during this study are illustrated in Figures 3.12 and 3.13.



**Figure 3.11.** Plot of Factor 1 against Factor 2 in the Principal Components Analysis of *Trichodina intermedia*.

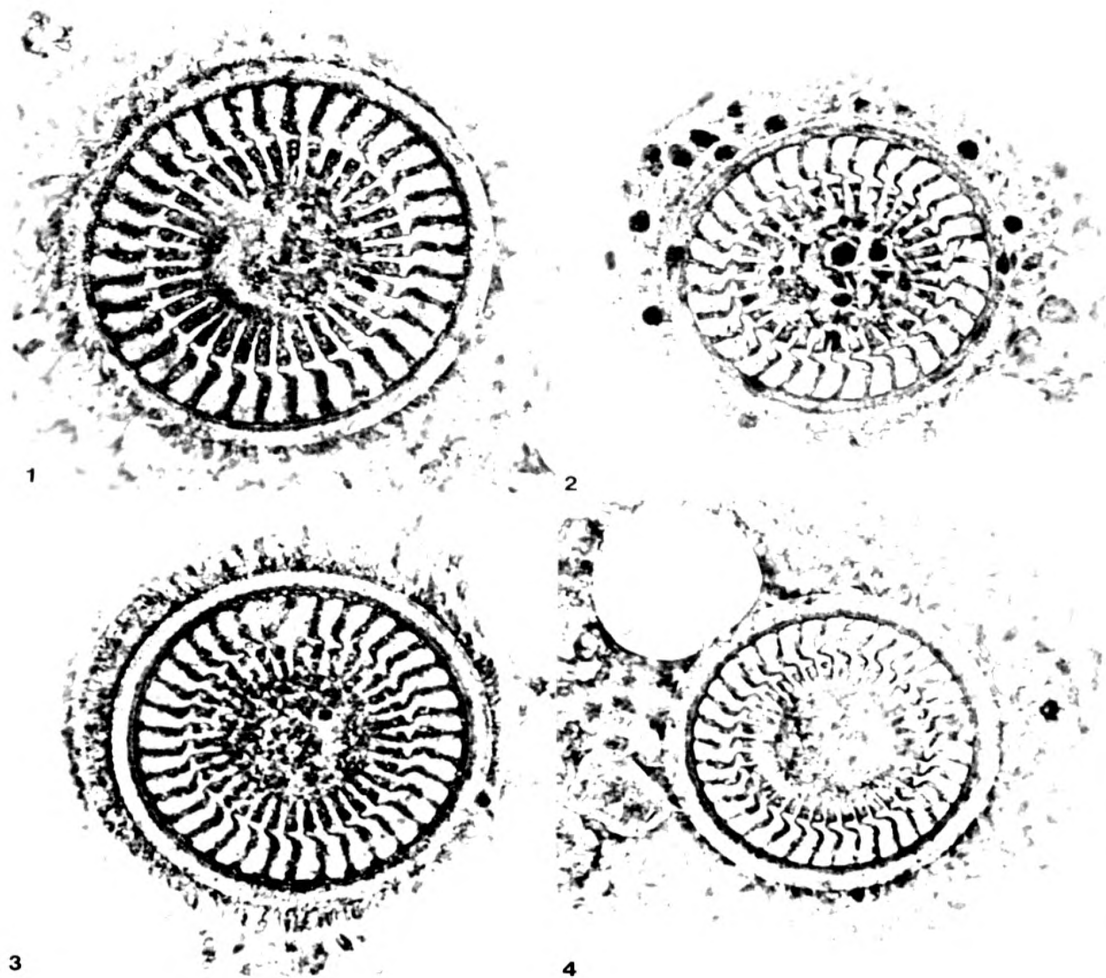




**Figure 3.12.** Silver stained adhesive disc morphology in specimens of *Trichodina intermedia* from *Phoxinus phoxinus*.

1, 2. Specimens from Allt Loin. (1200 $\times$ ).

3, 4. Specimens from Castle Semple Water. (1200 $\times$ ).



**Figure 3.13.** Silver stained adhesive disc morphology in specimens of *Trichodina intermedia* from *Phoxinus phoxinus*.

1, 2. Specimens from College Mill. (1200 $\times$ ).

3, 4. Specimens from Lake Bala. (1200 $\times$ ).

## Discussion

The majority of specimens found during this study closely resembled the population described by Lom (1961), and are therefore identified as *Trichodina intermedia*.

This species was found in mixed populations with *Paratrichodina incisa* in all cases. The mean adhesive disc diameter of the two species is considerably different, but the ranges overlap slightly. Some very small specimens of *Trichodina intermedia* closely resemble *Paratrichodina incisa*. They can only be differentiated in the context of a large number of photomicrographs of each species, enabling visual comparison. Blade form is usually different, with no apparent notch in *Trichodina intermedia* specimens. These small specimens of *Trichodina intermedia* are similar to *Paratrichodina corlissi* (Lom & Haldar, 1977). Cluster analysis was performed on the pooled data of *Paratrichodina incisa* and *Trichodina intermedia*. Cluster analysis detects natural groupings within a data set. This demonstrated that the highest probability was that two clusters existed within the data. When cross referenced with the original photomicrographs, the two clusters were found to contain the specimens already determined as *Trichodina intermedia* and *Paratrichodina incisa*.

Two endozoic species of the genus *Paratrichodina* have been reported from *Phoxinus phoxinus*, these are *Paratrichodina alburni* Vojtek, 1957 (Lom & Haldar, 1976), and *Paratrichodina phoxini* Lom, 1963 (Lom, 1963b). Both these species are very similar to the populations of *Trichodina intermedia* found during this study. However, *Paratrichodina phoxini* is almost identical in proportion and appearance. Table 3.12 illustrates measurements for the principal variables in recorded populations of *Trichodina intermedia* and *Paratrichodina phoxini*. Some specimens of *Trichodina intermedia* found during this study illustrated a greater affinity to the photomicrographs

of *Paratrichodina phoxini* (Lom, 1963b), than those of *Trichodina intermedia* (Lom, 1961).

**Table 3.12.** Morphometric and meristic data for recorded populations of *Trichodina intermedia* and *Paratrichodina phoxini*.

Species	<i>Trichodina intermedia</i>	<i>Trichodina intermedia</i>	<i>Trichodina intermedia</i>	<i>Paratrichodina phoxini</i>	<i>Paratrichodina phoxini</i>
Author	Lom, 1961	Stein, 1984	This study	Lom, 1963b	Stein, 1984
A. d. diam.	31-41 (34)	25.5-43.5	26.7-46.2 (36.3)	30-37 (33)	30-43
D. r. diam.	19-28 (22)	12-28	16.1-31.2 (23.3)	19-22 (21)	19-30
Dent. no	27-36 (31)	22-36	25-37 (30.1)	30-35 (32)	30-39
Adoral spiral	310-340°	310-340°	280-340°	180-270°	180-270°

The main difference between the reports of the two species is the smaller turn of the aboral cilia in *Paratrichodina phoxini*. However, in the light of the small number of populations described and the difficulty in distinguishing between specimens of *Paratrichodina incisa* and *Trichodina intermedia* *in vivo* during this study (ie. determining the lower range of the aboral ciliary spiral in *Trichodina intermedia*); this difference does not seem sufficient to determine two separate species. The morphological variation displayed in *Trichodina nigra* and *Trichodina mutabilis* (Kazubski & Migala, 1968) is far greater than that apparent between populations of *Trichodina intermedia* and *Paratrichodina phoxini*.

Lom (1963b) apparently erected the species *Paratrichodina phoxini* predominantly on the basis of its habitation in the urinary tract. Unfortunately this site was not examined during this study. However, it is possible that *Trichodina intermedia* could inhabit either the gills or the urinary tract.

*Trichodina intermedia* occupies a difficult position, illustrating affinities with *Paratrachodina* species whilst displaying aboral cilia with a spiral smaller than the lower limits of *Trichodina*. Lom & Haldar (1977) state that the upper limit of the adoral cilia in *Trichodinella*, *Tripartiella* and *Paratrachodina* is 290°, and the lower limit of *Trichodina* is 330°. Thus *Trichodina intermedia* with an adoral ciliary spiral of 280-340° (this study) straddles the divide.

Some other species of *Trichodina* have been found to have adoral ciliary spirals below 330°. *Trichodina oviducti* Polyanskii from the genital tract of *Raja radiata* a curious venereally transmitted species, has an adoral ciliary spiral of 220-380° (Khan, 1972). *Trichodina jiroveci* Grupcheva & Lom, 1980 has an aboral ciliary spiral of 210° (Grupcheva & Lom, 1980). This species has typical *Trichodina* denticles, reminiscent of those seen in *Trichodina nigra*. Another new species *Trichodina valkanovi* reported by Grupcheva & Lom (1980), has an aboral ciliary spiral of 220°.

Thus, the importance of the length of the adoral cilia as a generic characteristic appears to be less distinct than previously thought, and recently described species have been in effect classified on the basis of denticle morphology alone.

The description of *Trichodina intermedia* from *Phoxinus phoxinus* during this study constitutes a new British record.

#### CONCLUSIONS AND SUMMARY

Taxonomy of the three genera described in this chapter is more complex than that of *Trichodina*, where denticle structure is accurately represented by silver impregnation. Specimens of *Trichodinella*, *Tripartiella* and *Paratrachodina* are often subject to staining artifact (see Chapter 4), usually along the delicate denticle blade margins. Great care

must be taken not to designate new species on the grounds of differences in "observed" denticle structure which do not exist. The value of the adoral cilia as a generic characteristic is questionable, with new *Trichodina* species being described displaying a spiral of only 210° as in *Trichodina jiroveci* and 220° as in *Trichodina valkanovi* (Grupcheva & Lom, 1980). As a specific characteristic these cilia are not infallible, *Paratrichodina incisa* displays an adoral ciliary spiral of 150-230° (Lom & Haldar, 1977 and this study), a range of 80°. Lom & Haldar (1977) discriminated *Paratrichodina incisa* (150-230°) from *Paratrichodina erectispina* (230-280°) primarily by the adoral cilia. Intraspecific morphological variation of this feature is poorly documented. However, as illustrated in this study, additional descriptions may illustrate an increase in the range of spiral rotation compared to that previously documented.

The description of *Trichodinella epizootica* from this study represents the second report from Britain. *Perca fluviatilis* was the most common host of *Trichodinella epizootica* in this study, and is the type host for this species (Lom & Dykova, 1992). Population (c) of *Trichodinella epizootica* was found on the gills of *Oncorhynchus mykiss*, constituting a new British host record. *Oncorhynchus mykiss* is a recorded host of *Trichodinella subtilis*, which is distinguished from *Trichodinella epizootica* mainly on the basis of a second anterior denticle projection. Some specimens of population (c) resembled specimens identified as *Trichodinella subtilis* from *Cyprinus carpio* by Kazubski & Migala (1968). However, the small morphological differences between specimens of the populations described during this study were thought to result predominantly from staining inconsistencies and were therefore all classified as *Trichodinella epizootica*. This species has been recorded from approximately nine host species world wide (Lom & Dykova, 1992), but was only found on two of

approximately 20 fish species examined during this study.

*Tripartiella lata* was reported from *Phoxinus phoxinus* in small numbers during this study, constituting a new British record. This species displays a high degree of host specificity, especially in Britain, where numerous samples of a previously recorded host *Oncorhynchus mykiss* were found to be uninfected.

One specimen of a species tentatively identified as *Tripartiella copiosa* from the gills of *Rutilus rutilus* was found during this study. This constitutes a new British record.

*Paratrichodina incisa* was reported from the gills of *Phoxinus phoxinus*, *Rutilus rutilus* and *Abramis brama* during this study and is a new British record. *Abramis brama* constitutes a new host record, although *Paratrichodina incisa* has been previously reported from *Abramis ballerus*. This trichodinid species was found on all the populations of minnows sampled. Some specimens of *Paratrichodina incisa* closely resembled specimens of *Paratrichodina voikarensis* and *Paratrichodina uralensis* (Kashkovsky & Lom, 1979), and *Paratrichodina erectispina* and *Paratrichodina corlissi* (Lom & Haldar, 1977). Little is known of the morphological variation within these species as they have only been described from one or two populations. Combined with the inconsistent staining characteristics of these small trichodinids, future work may result in some species being recognised as synonyms of *Paratrichodina incisa*.

*Trichodina intermedia* was described from the gills of all the populations of *Phoxinus phoxinus* examined during this study constituting a new British record. This trichodinid species displayed a high degree of host specificity in Britain, although it has been recorded from several fish species in the USSR (Stein, 1984). An endoparasitic trichodinid population named as *Paratrichodina phoxini* has been reported from

*Phoxinus phoxinus* (Lom, 1963b), which is virtually indistinguishable from *Trichodina intermedia*. The examination of large numbers of *Trichodina intermedia* during this study suggests that *Paratrichodina phoxini* may be a possible synonym of the former species.

*Trichodinella epizootica*, *Paratrichodina incisa* and *Trichodina intermedia* (this study) all show affinities with different species described by other authors. When the high degree of intraspecific morphological variation displayed by *Trichodina acuta*, *Trichodina tenuidens* and *Trichodina nigra* (this study) is considered, the tiny morphological differences by which some of the species defined by other authors have been discriminated becomes apparent.



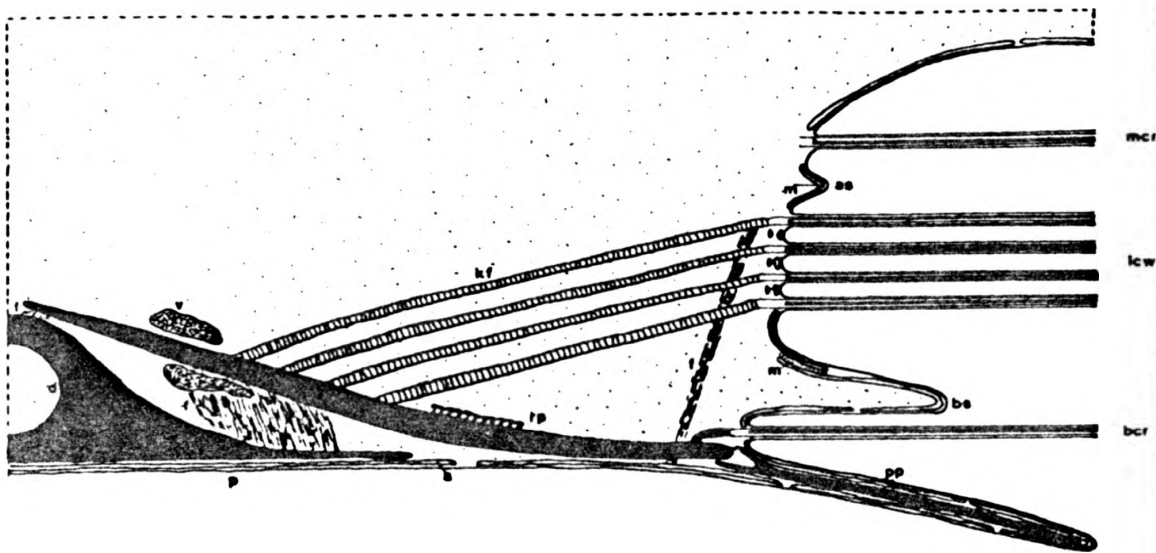
## CHAPTER 4. THE FINE STRUCTURE OF ADHESIVE DISC MORPHOLOGY.

### INTRODUCTION

The first ultrastructural study of trichodinids was a description of the endoparasitic species *Trichodina urinicola* using transmission electron microscopy (Favard, Carasso & Faure-Fremiet 1963 in: Lom, 1973). Lom (1973) completed the first ultrastructural study of an ectoparasitic species, *Trichodinella epizootica* from *Perca fluviatilis*, using scanning and transmission electron microscopy.

The most detailed study of trichodinid ultrastructure to date is that of Hausmann & Hausmann (1981 a, b) in which the locomotor fringe, oral apparatus and the adhesive disc of *Trichodina pediculus* are described.

The locomotor fringe is a complex structure made up of three distinct structures (Figure 4.1), the main component being the locomotor ciliary wreath made up of many oblique rows of 8 cilia (Hausmann & Hausmann, 1981a) (6 in *Trichodinella epizootica*, (Lom, 1973)). Either side of this wreath is a single row of cilia, separated from the wreath by a septum. The basal row of cilia is associated with the skeletal elements of the adhesive disc. The marginal ciliary ring is situated adorally to the wreath, although only a few of the kinetosomes are ciliated (Hausmann & Hausmann, 1981a). This row consists of barren kinetosomes in *Trichodinella epizootica* (Lom, 1973), and is therefore lacking cilia. In *Trichodina pediculus* the septa differ in structure, the basal septum being much larger with more reinforcing microtubules than the anterior septum (Hausmann & Hausmann, 1981a). *Trichodina nigra*, *Tripartiella copiosa* and *Tripartiella kashkovskiyi* were described by Maslin-Leny & Bohatier (1984) using



**Figure 4.1.** Diagram of the main structures of the adhesive disc and locomotor fringe from a longitudinal section through *Trichodina pediculus* (Redrawn from Hausmann & Hausmann, 1981b): mcr, marginal ciliary ring; lcw, locomotor ciliary wreath; bcr, basal ciliary ring; as, anterior septum; bs, basal septum; m, microtubules; d, denticle; rp, radial pin; pp, peripheral pin; p, pellicle; a, alveolus; v, vesicle; f, filaments; kf, kinetodesmal fibres.

transmission electron microscopy. The arrangement of the locomotor fringe structure was similar to that previously described for *Trichodina pediculus*.

The oral apparatus consists of an adoral ciliary spiral, a buccal cavity and a cytostome (Hausmann & Hausmann, 1981a). The adoral ciliary spiral follows an anticlockwise path around the adoral end of the cell ending in the buccal cavity. The adoral spiral is made up of two components, a haplokinety and a polykinety (3 rows of cilia), characteristic of the oligohymenophoran ciliates. As the spiral enters the buccal cavity the kinetys separate and spiral towards the cytostome (Hausmann & Hausmann, 1981a).

The adhesive disc of *Trichodina pediculus* appears more complex when viewed with transmission electron rather than light microscopy (Hausmann & Hausmann, 1981b). A pellicle covers the entire surface of the cell including the denticles. The centripetal ends of the radial pins are attached to the denticles by filamentous material. The denticles have been shown to be proteinaceous in nature in *Trichodina tenuidens* (Fremiet & Theraux, 1944). As the pins progress distally their appearance in cross-section changes from a thin bar to a I shape. In this region the pins are joined by four kinetodesmal fibres (Figure 4.1) originating from the basal bodies of the locomotor ciliary wreath (Hausmann & Hausmann, 1981b). These basal bodies also give rise to bundles of filaments which pass between the radial pins and affix to the epiplasm. Further along the pins the cross section resembles a T shape, with an extension to the right side of each pin overlapping the one adjacent. In cross-section the centrifugal ends of the pins resemble a ⊥ shape, and are attached to the basal bodies of the basal ring of cilia by means of a dense globular material (Hausmann & Hausmann, 1981b). Three peripheral pins are attached to each radial pin by a dense layer of material, forming a

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hinge when viewed laterally. Hausmann & Hausmann (1981b) suggest that the adhesive disc found in trichodinids is one of the most complex skeletal structures found within any eukaryotic cell. They assume that the fibres associated with the adhesive disc are contractile, and act on the hinged levers (radial pins) to allow vaulting of the disc. The filaments attached to the epiplasm are given as evidence for the structural role of the pellicle.

Various protozoa contain contractile fibres, including members of the Peritrichida (Sleigh, 1991). The filaments of these fibres appear to be formed from a single type of protein molecule known as spasmin, which contracts in the presence of calcium ions. Sleigh (1991) suggests that by controlling the free calcium ion concentrations the organism can regulate the contraction of the filaments.

Lom (1973) gives a more detailed description of the function of the adhesive disc in *Trichodinella epizootica*. The interconnected radial pins allow the border of the disc to follow any unevenness of the substrate, allowing purchase even as the ciliate glides over it. In a loosely attached ciliate the adhesive disc is relatively flat, the border membrane projecting in the same direction as the radial pins. In a ciliate moving over a rough surface the border membrane may even be angled upwards. Before attachment the ciliate stops moving and produces a strong water current with its locomotory cilia pushing itself onto the substrate. Then the border membrane bites into the substrate whilst angled downwards, and simultaneously the disc is vaulted drawing epithelial cells into the vaulted disc.

A number of studies have used scanning electron microscopy to illustrate the surface topography of trichodinids, the main features of which are the locomotor fringe and adoral cilia previously described. For example, Khan, Barber & McCann (1974)

described the ciliature and external appearance of *Trichodina oviducti*, parasitic in the reproductive tract of *Raja radiata*, whilst Arthur & Margolis (1984) and Urawa & Arthur (1991) documented the surface topography of *Trichodina truttae* from juvenile salmonids in British Columbia and Japan. This species was characterised by an oral surface covered in irregular ridges, radiating from its centre. The adoral cilia consisted of short stubby cilia, with only a small zone of the typical well developed long cilia seen in *Trichodina pediculus* (Hausmann & Hausmann, 1981a); this feature was considered species specific. The morphology of a *Trichodina* species from the buccal cavity of *Lampetra japonica* was described using scanning electron microscopy by Honma, Yoshie & Susuki (1982). A new species, *Trichodina japonica* was described from the gills of *Anguilla japonica* by Imai, Miyazaki & Nomura (1991). These authors concluded that species identification may be possible using scanning electron microscopy alone. However, in many species the denticles are obscured by the overlying pellicle to a greater extent than observed in *Trichodina japonica*, making identification impossible. *Trichodina labrisoma* (Rand, 1993) from *Labrisomas nuchipinnis* was also described using scanning electron microscopy.

Two new genera have been described using scanning electron microscopy, *Trichodoxa* (Sirgel, 1983) from the genital system of terrestrial pulmonates, and *Pallitrichodina* from the mantle cavity of giant African land snails (Van As & Basson, 1993).

The fine structure of the adhesive disc is difficult to ascertain using light microscopy, but scanning electron microscopy could be a useful tool if the skeletal structures can be liberated. A technique normally used for the softening of chitin was used to liberate the adhesive disc from the cell of *Trichodina dampanula* Van Der Bank,

Basson & Van As, 1989 (Van As & Basson, 1990). Photomicrographs shown by these authors illustrate that their technique produced relatively clean preparations of liberated denticle rings, although often overlying or overlain by cellular debris. Two distinct articulation surfaces on the adoral surface of the central part of the denticle were highlighted, the apophysis of the blade and ray. It was suggested that this allowed restricted movement between individual denticles.

Scanning electron microscopy was used in this study to observe the topography and adhesive disc structure of certain trichodinid species. A sonication technique was used to liberate the adhesive disc in species of *Trichodina*, *Paratrachodina* and *Trichodinella*.

## MATERIALS AND METHODS

### Sonication

Fine structure of skeletal elements in trichodinids was elucidated by scanning electron microscopy of liberated adhesive discs. A sonication technique modified from Shinn *et al.* (1993) used to liberate the skeletal structures of gyroductylid monogeneans was utilised. Unfixed trichodinids were collected by micropipette from a petri dish containing excised gills or fins, and transferred to a glass centrifuge tube containing 3ml of distilled water. Centrifuge tubes were located in a wire rack placed inside a sonic water bath (Kerry Pulsatron Pul 60) with a minimum power output of 100 W. Samples were sonicated for 5-10 minutes to give a range of cell disruption. The samples were then centrifuged at 6,000 rpm for 5 minutes. The supernatant was decanted and the pellet resuspended in distilled water. This procedure was repeated 5 times, and the final pellet

resuspended in 1 ml of distilled water. This suspension was pipetted onto round cover slips and air dried. The cover slips were viewed using a light microscope (Olympus BH2) and the positions of skeletal structures marked with small triangles of adhesive paper. Cover slips were subsequently glued onto aluminium stubs with araldite and sputter coated with gold (Edwards Sputter Coater 5150B). Specimens were examined using a (500 Philips) scanning electron microscope.

#### **Fixation for S.E.M.**

Specimens were fixed in situ on gills or fins for 1 hour, in 1% glutaraldehyde in 0.1M sodium cacodylate (PH 7.2-7.4) and transferred to 3% glutaraldehyde in 0.1M sodium cacodylate buffer for 1 day. The fixed material was vigorously shaken and the supernatant containing the trichodinid specimens was transferred to a 100ml syringe with a Swinnex adaptor containing a Whatman 0.4µm cyclopore track etch membrane and rinsed with sodium cacodylate buffer. The membrane (with specimens affixed) was then transferred into a watch glass containing 1% osmium tetroxide in 0.1M sodium cacodylate for 1 hour. This was followed by dehydration in 60-90-100% acetone for 30 minutes at each concentration. The membrane was cut into quarters and critical point dried (Biorad) at 1200 psi and 32°C. The membranes were mounted and sputter coated and examined as described previously.

## **RESULTS AND DISCUSSION**

### ***TRICHODINA DOMERGUEI* WALLENGREN, 1897**

The aboral surface of *Trichodina domerguei* from *Gasterosteus aculeatus* (Airthrey

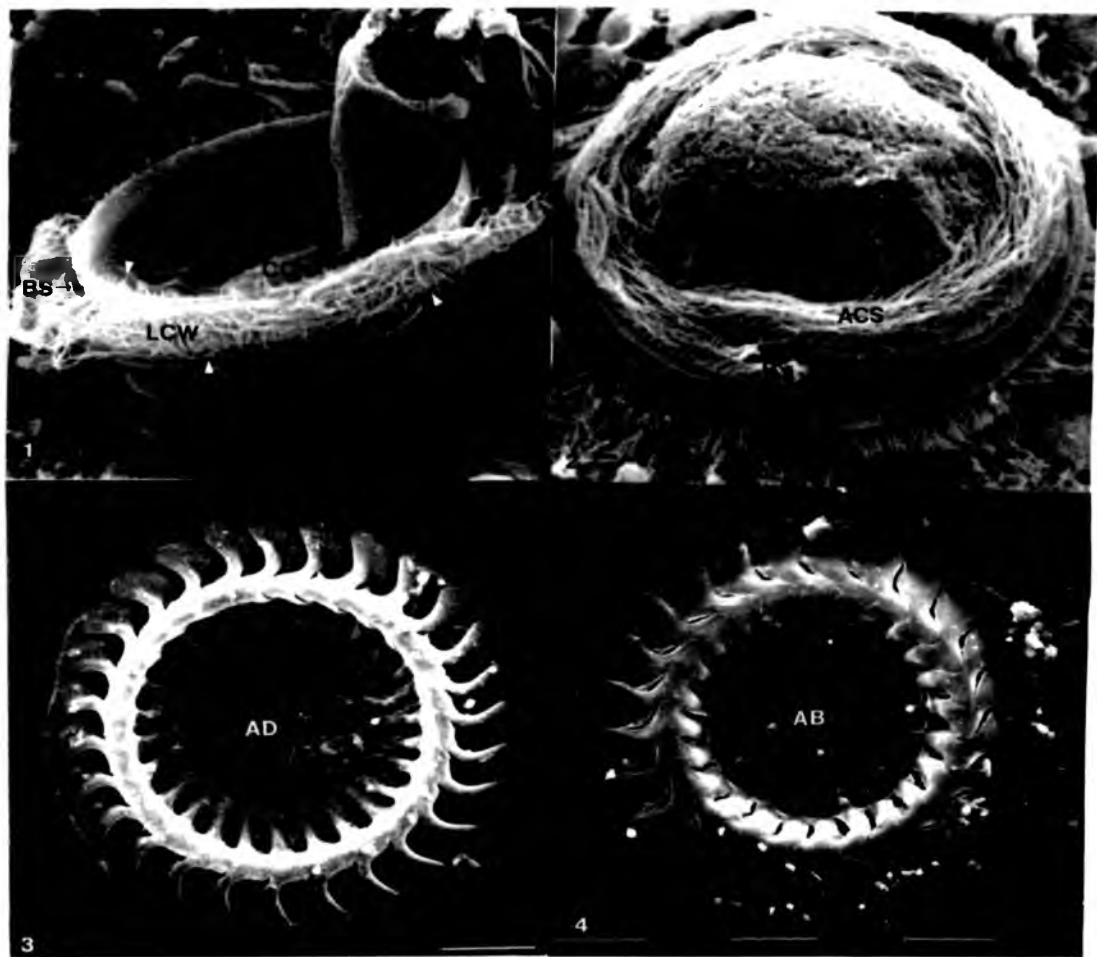


Loch) illustrates the adhesive disc structure using scanning electron microscopy (Figure 4.2.1). The disc is heavily vaulted, with the denticles, central circle and border membrane clearly visible. The aboral ciliary complex is similar to that of *Trichodina pediculus* (Hausmann & Hausmann, 1981a) containing three distinct bands. The main component consists of many rows of cilia making up the locomotor ciliary wreath, this is bordered by the basal ciliary ring and marginal ciliary ring. The three components of the aboral ciliary complex are separated by pellicular folds, the basal septum and anterior septum. The latter is greatly reduced being visible as a fine line between the marginal and locomotor cilia. The velum forms a broad ridge adorally to the marginal cilia (Figure 4.1.2).

Adorally, the dominant feature of *Trichodina domerguei* (Figure 4.2.2) is the ciliary spiral circling anticlockwise into the buccal cavity. The spiral expresses a turn in excess of 400°.

The adoral surface of a liberated denticle ring from *Trichodina domerguei* is illustrated in Figure 4.2.3, with the underlying pellicle still intact. The denticle blades are thickened along the posterior margins. The inner rays also display a more electron dense central ridge, with a much thinner region surrounding it. The blade and ray join the tubular central part of the denticle towards its aboral surface. This gives the impression of the central part being more massive when viewed adorally, in comparison to the view from its aboral surface (Figure 4.2.4) which is seen in silver stained material.

Figure 4.3.1 illustrates an enlargement of part of the adoral surface of the denticle ring displayed in Figure 4.2.3. A previously undescribed small peg like structure approximately 0.75µm in length with an opening towards its end, can be



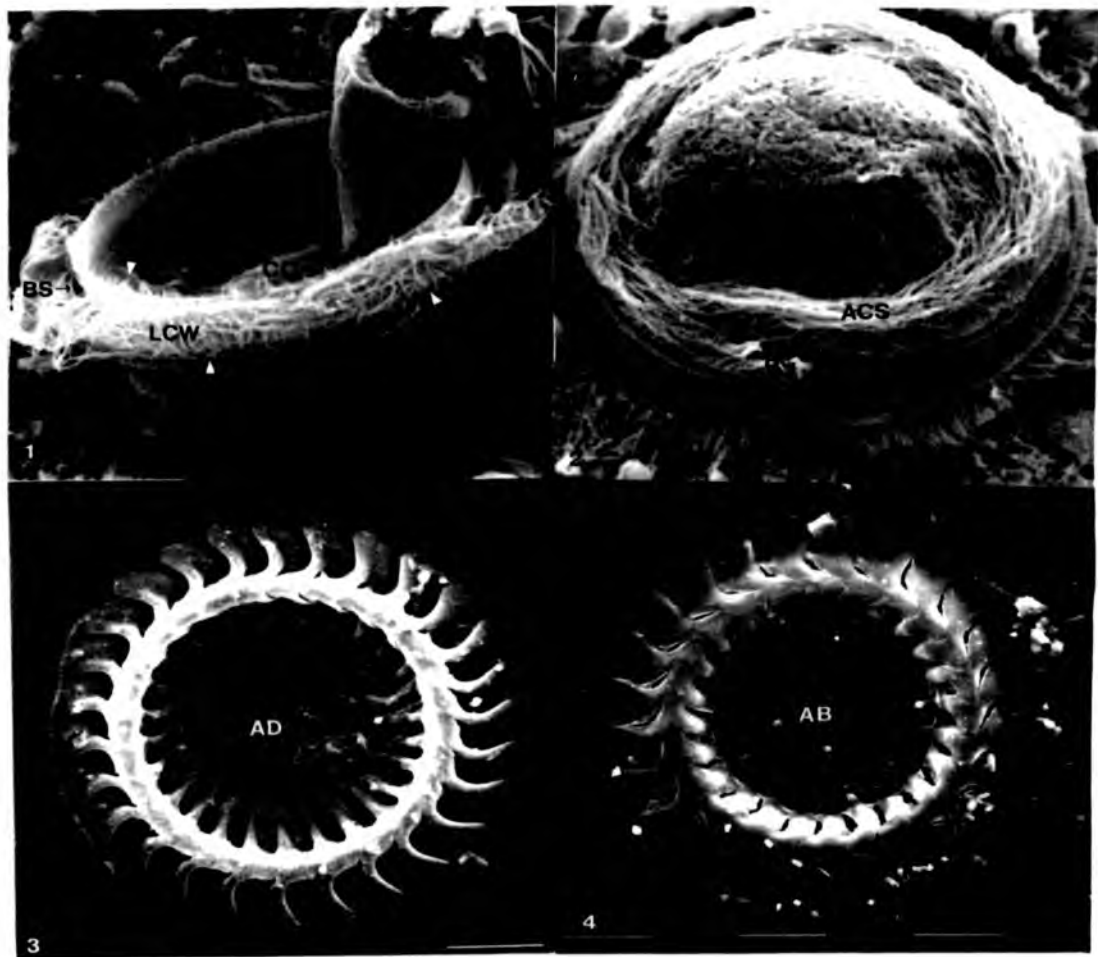
**Figure 4.2.** The surface topography and isolated adhesive disc structure of *Trichodina domerguei* revealed using scanning electron microscopy.

1. Aboral view of surface topography: BM, border membrane; D, denticle; CC, central circle; BCR, basal ciliary ring; LCW, locomotor ciliary wreath; MCR, marginal ciliary ring; BS, basal septum; AS, anterior septum. (Scale bar=10 $\mu$ m).

2. Adoral view of surface topography: ACS, adoral ciliary spiral; BC, buccal cavity; V, velum. (Scale bar=10 $\mu$ m).

3. Adoral (AD) view of denticle ring: (Scale bar=10 $\mu$ m).

4. Aboral (AB) view of denticle: (Scale bar=10 $\mu$ m).



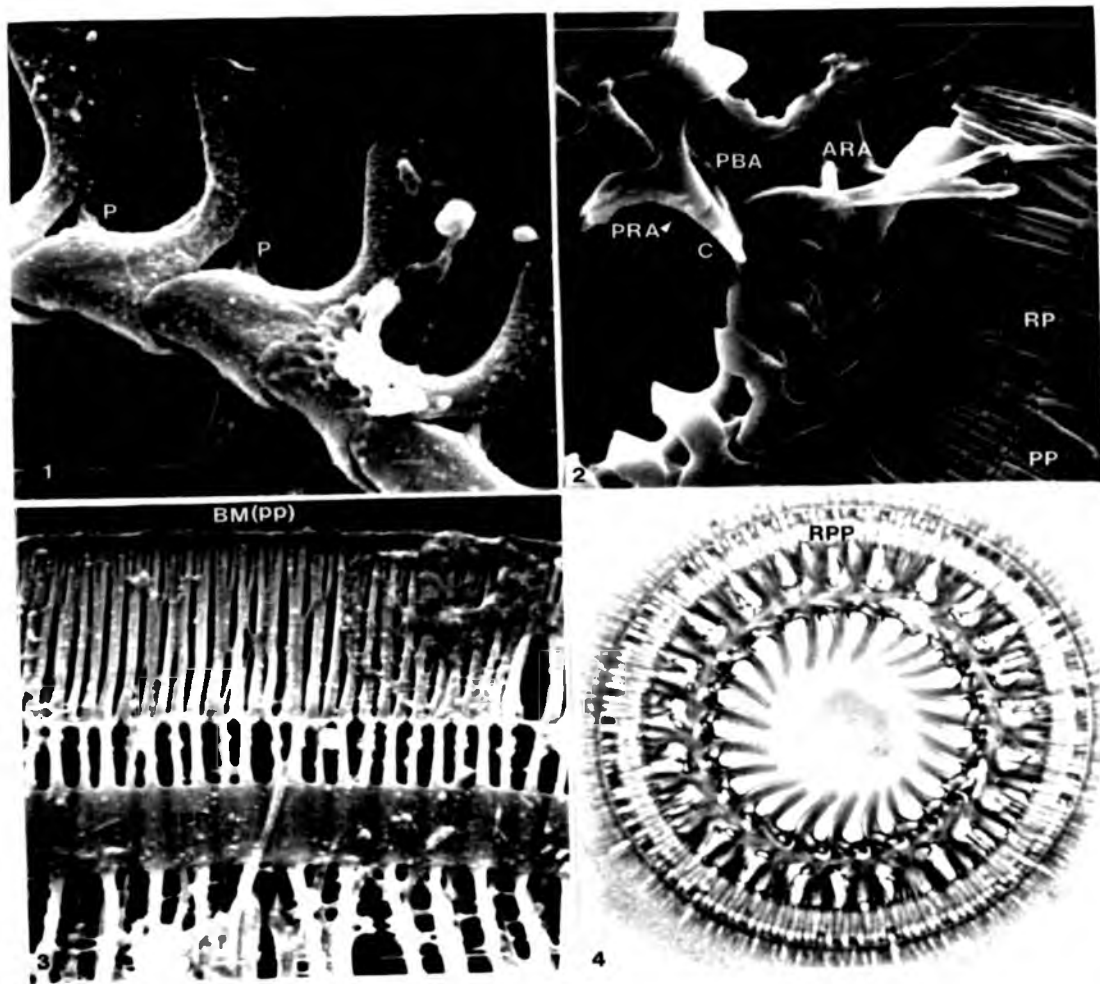
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3. Adoral (AD) view of denticle ring: (Scale bar=10 $\mu$ m).

4. Aboral (AB) view of denticle: (Scale bar=10 $\mu$ m).



**Figure 4.3.** The isolated denticle ring structure of *Trichodina domerguei* revealed by scanning electron microscopy (1-3), and silver stained adhesive disc morphology of *Trichodina acuta* using light microscopy (4).

1. Adoral view of denticle ring: p, peg like structures. (Scale bar=1 $\mu$ m).
2. Aboral view of denticle ring: C, central conical part; PRA, posterior ray apophysis; PBA, posterior blade apophysis; ARA, anterior ray apophysis; RP, radial pins; PP, peripheral pins. (Scale bar=10 $\mu$ m).
3. Part of adhesive disc: BM, border membrane; (PP) peripheral pins; RPP, radial pin projections. (Scale bar=1 $\mu$ m).
4. Adoral view of *Trichodina acuta*: RPP, radial pin projections. (Scale bar=10 $\mu$ m).

seen on the centrifugal surface of the central part of the denticles. These structures are only visible on a few denticles, in places where the ring appears to be slightly disrupted. It is possible that these pegs normally locate with the underside of the adjacent denticle blades, or are dislodged during sonication. No such structures are apparent from the aboral surface in electron or light microscopy. Some silver stained specimens of *Trichodina acuta* (see Chapter 2) appear to bear similar projections, although these were thought to be part of the posterior blade apophysis which acts as an articulation surface between denticles. The peg like structures may act to limit articulation, or act as additional articulation points reinforcing the integrity of the denticle ring.

The aboral surface of a broken denticle ring complete with radial and peripheral pins is illustrated in Figure 4.3.2. The radial pins are positioned adorally to the denticles, although they appear to be continuous with them.

Where the denticle ring has broken (Figure 4.3.2) the interlocking structures are revealed. The central conical part locates within the preceding denticle. Either side of this projection an articulation surface or apophysis is visible, the posterior blade apophysis and posterior ray apophysis. These locate with corresponding structures in the adjacent denticle. Van As & Basson (1990) noted an apophysis of the blade and ray from an adoral view of a broken denticle ring of *Trichodina dampanula*. However, their studies did not reveal any articulating surfaces associated with the conical central part of the preceding denticle. It is proposed that the structures described by Van As & Basson are designated anterior blade apophysis and anterior ray apophysis, to distinguish them from the new structures described in this study. In *Trichodina domerguei*, the anterior ray apophysis is much larger than in *Trichodina dampanula* (Van As & Basson, 1990), forming a defined projection (approx. 1.6 $\mu$ m) locating with the posterior ray

apophysis of the preceding denticle.

In some cases the intact structure of radial and peripheral pins is isolated during sonication (Figure 4.3.3). A continuous band can be seen running parallel with the border membrane, appearing to overlay the radial pins. These radial pin projections are occasionally observed in silver stained material, as in Figure 4.3.4, an adoral view of *Trichodina acuta*. The radial pins widen at their centrifugal ends, where they join approximately three peripheral pins. This number appears to be constant, being found in *Trichodinella epizootica* (Lom, 1973 and this study), *Trichodina pediculus* (Hausmann & Hausmann, 1981b) and *Trichodina domerguei* (this study).

#### **TRICHODINA INTERMEDIA LOM, 1960**

The adoral surface topography of *Trichodina intermedia* from *Phoxinus phoxinus* (College Mill) reveals the adoral ciliary spiral displaying an incomplete turn of approximately 340° (Figure 4.4.1). The aboral locomotor fringe of cilia is visible protruding from beneath the velum.

Denticle form viewed aborally in sonicated material is illustrated in Figures 4.4.2, 4.4.3 and 4.4.4. The denticle blades are typical of *Trichodina*, with a curved anterior margin forming a semi-lunar shape. This is quite unlike the more spatulate blade appearance of silver stained specimens, which bear a resemblance to *Paratrichodina incisa*. This disparity is due to the extreme thinness of the anterior portion of the blade. The posterior part of the blade is considerably thicker, and the anterior margin integral with the central part of the denticle gives the appearance of an anterior projection. Thus, the appearance of blade form in silver stained material is misleading, with the anterior margin obscured by silver deposition.

The fragility of the anterior region of the blades is demonstrated in Figure 4.4.2. Deep splits occur, which adds to the suggestion of an anterior projection in silver stained material. The fibrous nature is revealed where the blade has been pulled apart, leaving thin strands spanning the gap.

The posterior ray and blade apophysis illustrated in Figure 4.4.3 are larger and more defined than in *Trichodina domerguei*. The conical central part of the denticle differs in shape and size when compared to that of *Trichodina domerguei* (Figure 4.3.2). By measuring the length of the conical part of the denticle and comparing it to the length of the central part visible in a complete denticle ring, the extent of insertion of the conical part in the preceding denticle can be derived. In *Trichodina intermedia* the interlocking conical part of the denticle is so long that it must project into the interlocking conical part of the adjacent denticle. In *Trichodina domerguei* the relatively small size of this structure indicates that it only penetrates the central part of the denticle visible in the complete ring. The large relative size of the conical central part of the denticle in *Trichodina intermedia* may afford increased strength to the denticle ring, or decrease its lateral movement. The anterior ray apophysis (Figure 4.4.4) is very similar to that observed in *Trichodina domerguei* (Figure 4.3.2). The inner rays of *Trichodina intermedia* are wider than apparent in silver stained material, as the delicate ray margins may not be visualised.

A direct comparison of denticle ring morphology in *Trichodina intermedia* and *Paratrichodina incisa* is illustrated in Figure 4.4.5. This specimen of *Trichodina intermedia* illustrates actual blade form and blade form as apparent in silver stained material. The similarity between the latter and that expressed by *Paratrichodina incisa* is clearly displayed.

**Figure 4.4.** The surface topography and isolated denticle ring structure of *Trichodina intermedia* revealed by scanning electron microscopy.

1. Adoral view of surface topography: ACS, adoral ciliary spiral; V, velum; LF, locomotor fringe. (Scale bar=10 $\mu$ m).

2. Aboral view of sonicated denticle ring: A, thin anterior region of blade; p, thicker posterior region of blade; a, thickened region of central part / anterior blade margin producing "anterior projection" suggested in silver stained material; s, split or tear in the anterior blade margin. (Scale bar=1 $\mu$ m).

3. Aboral view of interrupted denticle ring: C, central conical part; PBA, posterior blade apophysis; PRA, posterior ray apophysis. (Scale bar=10 $\mu$ m).

4. Aboral view of interrupted denticle ring. ARA, anterior ray apophysis. (Scale bar=10 $\mu$ m)

5. Comparison of *Trichodina intermedia* and *Paratrachodina incisa* viewed aborally. A, *Trichodina intermedia*: a, denticle blade illustrating actual form; b, blade form similar to that observed in some silver stained specimens; B, *Paratrachodina incisa*. (Scale bar=10 $\mu$ m).



*Trichodina*

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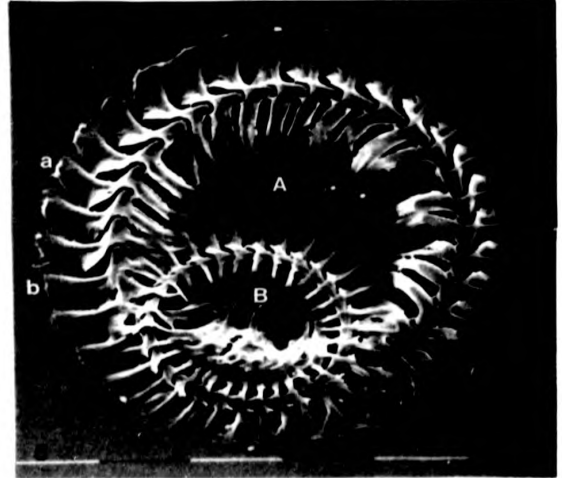
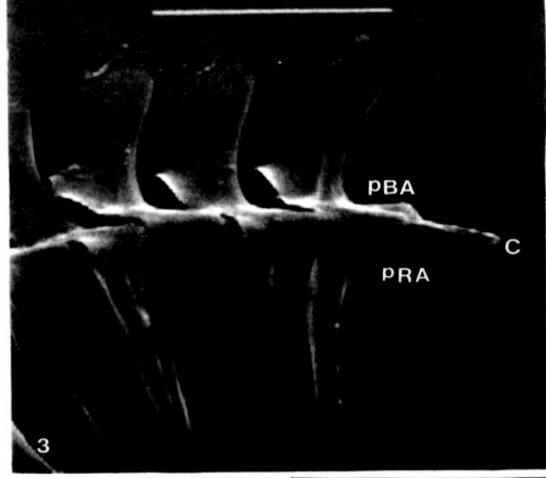
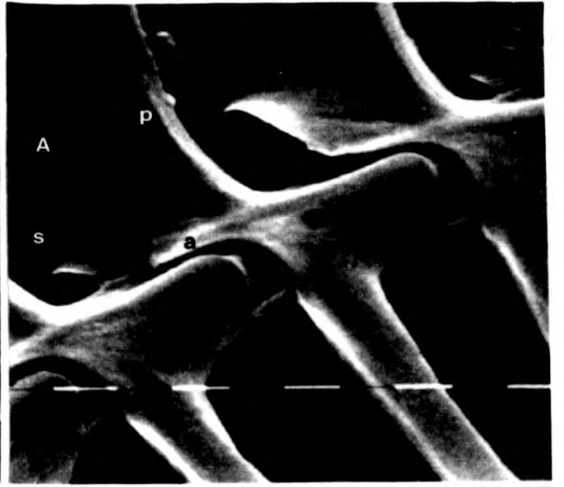
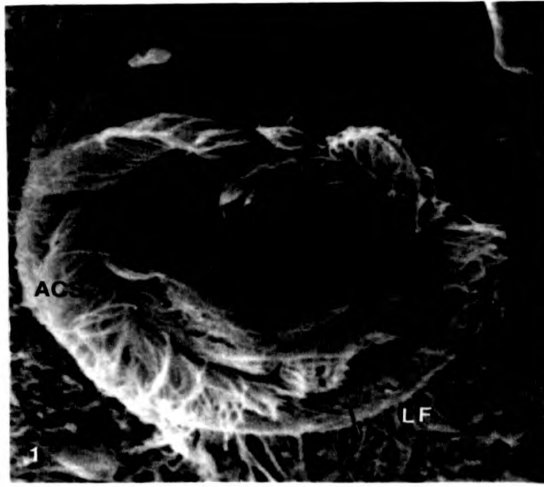
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### PARATRICHODINA INCISA LOM, 1959

Specimens of *Paratrichodina incisa* from *Rutilus rutilus* (Yorkshire) and *Phoxinus phoxinus* (College Mill) were studied using scanning electron microscopy.

The adoral surface topography of a specimen from *Phoxinus phoxinus* is illustrated in Figure 4.5.1. The adoral ciliary spiral describes a turn of approximately 220°. The first part of the spiral does not appear to bear cilia, resembling a narrow groove displaying barren kinetosomes or vestigial cilia (Figure 4.5.2). This arrangement may be present in other species, but is often obscured by overlapping cilia.

The adhesive disc structure of *Paratrichodina incisa* is displayed in Figure 4.5.3. The pellicle covering the disc has been disrupted due to poor fixation, illustrating the underlying structures. The radial and peripheral pins are revealed, but denticle form is masked by pellicular material.

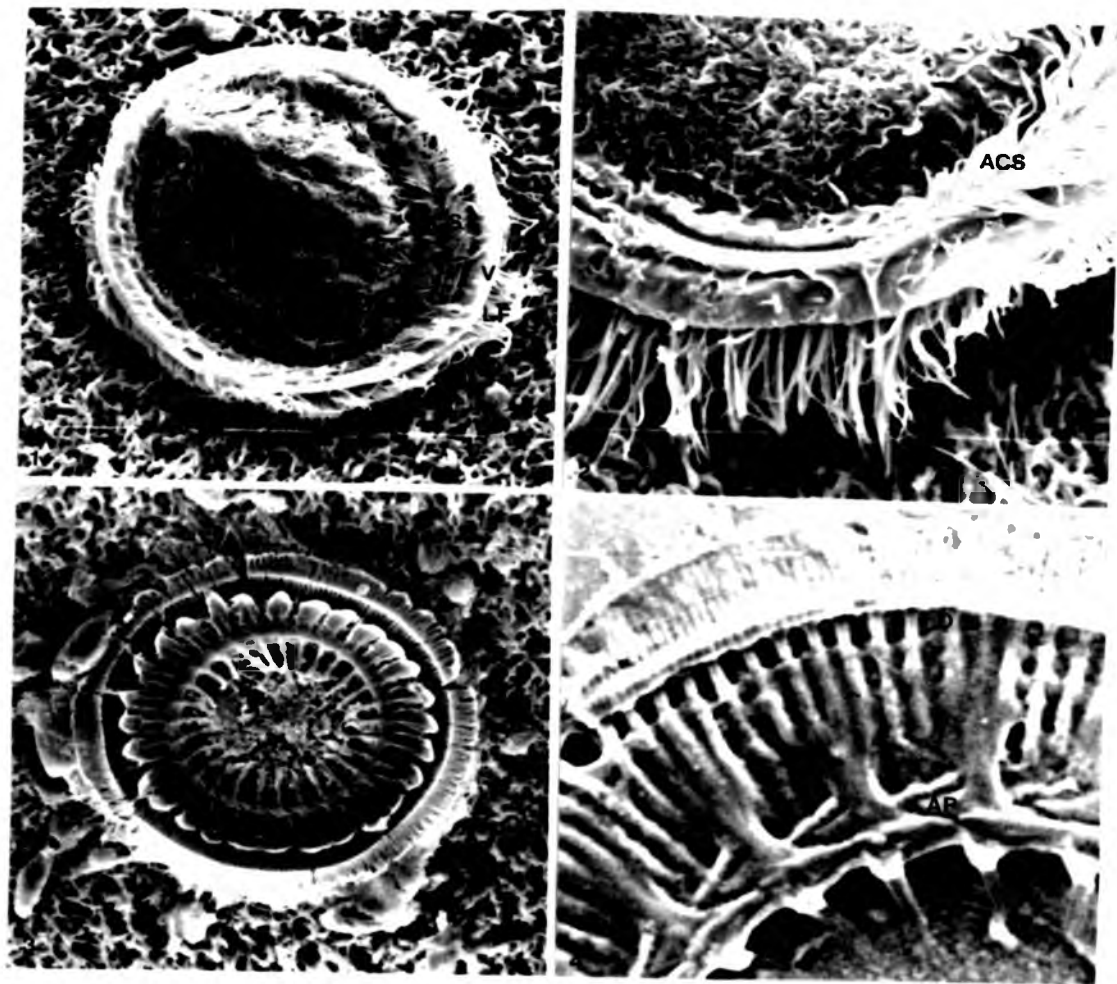
A partially sonicated specimen viewed aborally from *Rutilus rutilus* (Figure 4.5.4) illustrates the typical denticle form as seen in silver stained material, complete with the radial and peripheral pins. The denticle blades are extremely thin with poor definition of the anterior and posterior blade margins, resembling an inverted isosceles triangle. The blades possess a flat distal surface, parallel with the border membrane. An anterior projection is present at the central part / blade junction of the denticle. The denticle form of *Paratrichodina incisa* from *Phoxinus phoxinus* (Figure 4.6.1) closely resembles that from *Rutilus rutilus*. However, in one denticle the anterior blade margin deviates from the "normal" form, producing a continuous straight line from the distal surface to the tip of the anterior projection. This produces a rectangular appearance rather than the usual triangular form. Figure 4.6.2, shows a particularly well preserved specimen from *Phoxinus phoxinus*. The complete denticle structure was only clearly

apparent in this specimen. The lower anterior blade margins are intact, but display a varying degree of damage in the form of slanting splits, similar to those observed in *Trichodina intermedia*. In addition, a slanting ridge is perceptible on the anterior part of the blade describing the outline of the blade as visualised in silver impregnation. This line is also visible in the denticle illustrated in Figure 4.6.1.

Therefore, the denticles illustrated in sonicated specimens of *Paratrichodina incisa* graduate from the silver stained appearance through various intermediate forms to that of an uninterrupted rectangle. It can be surmised that the lower anterior blade margin remains unstained in silver impregnation, due to its extreme fragility.

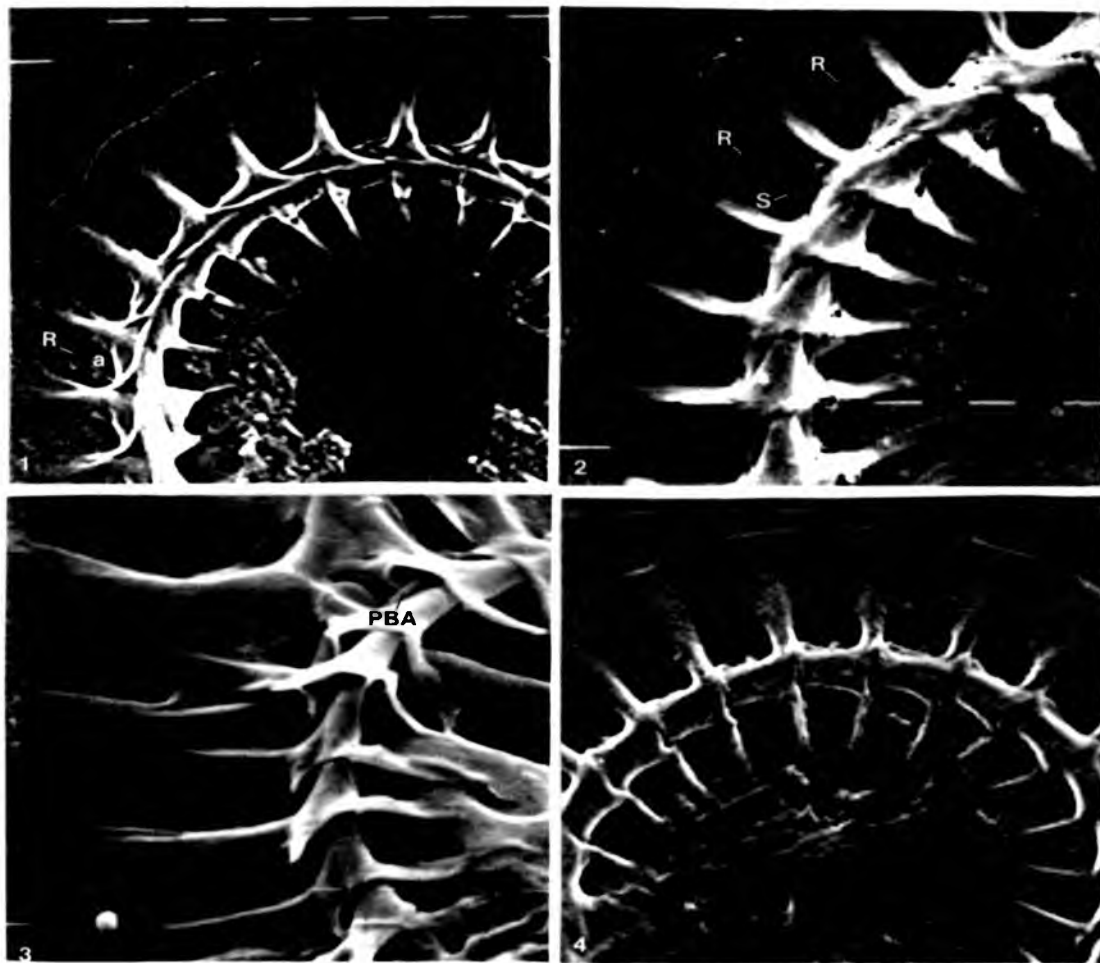
The anterior projections visible in Figure 4.6.2 appear to protrude slightly beyond the anterior blade margin, locating tightly against the preceding denticle. A posterior blade apophysis is present on the central part of the denticle (Figure 4.6.3), visible adorally (Figure 4.6.4) obscuring the anterior projections. However, the anterior ray apophysis and posterior ray apophysis appear to be absent (Figure 4.6.3). Thus, the anterior projection may restrict articulation in place of the peg like anterior ray apophysis present in *Trichodina* species.

Lom (1963a) described the notch in the anterior blade margin visible in silver stained specimens of *Paratrichodina incisa* as a staining artifact. It was suggested that this gave the "impression" of an anterior projection, similar to that in *Tripartiella*. Although the anterior projection described in this study is integral with the denticle blade, it is a well defined structure continuous with the central part. The thickness of the anterior projection allows it to function structurally, limiting articulation of the denticle ring. In this sense it can be described as a separate structure, rather than as part of the blade. Lom (1963a) considered *Paratrichodina* denticle form to be "similar to that



**Figure 4.5.** The surface topography and isolated denticle ring structure of *Paratrichodina incisa* revealed by scanning electron microscopy.

1. Surface topography of specimen from *Phoxinus phoxinus* (College Mill) viewed adorally: ACS, adoral ciliary spiral; V, velum; LF, locomotor fringe. (Scale bar=10 $\mu$ m).
2. Adoral surface of specimen from *Phoxinus phoxinus* (College Mill) showing origin of ACS which lacks cilia. (Scale bar=10 $\mu$ m).
3. Aboral view of adhesive disc structure in specimen from *Phoxinus phoxinus* (College Mill) revealed by poor fixation. (Scale bar=10 $\mu$ m).
4. Aboral view of a partially sonicated specimen from *Rutilus rutilus* (Yorkshire). AP, anterior projection; D, distal surface of blade. (Scale bar=1 $\mu$ m).



**Figure 4.6.** Isolated denticle ring structure of *Paratrichodina incisa* from *Phoxinus phoxinus* (College Mill) revealed using scanning electron microscopy.

1. Typical appearance of denticle ring viewed aborally: a, anterior region of denticle not normally apparent; R, ridge outlining denticle margin "apparent" in silver stained material. (Scale bar=1 $\mu$ m).
2. Aboral view of denticle ring with exceptionally well defined anterior blade margins: S, split in extremely thin anterior region of blade; R, ridge outlining denticle margin as seen in silver stained material. (Scale bar=1 $\mu$ m).
3. Aboral view of disrupted denticle ring: PBA, posterior blade apophysis. (Scale bar=1 $\mu$ m).
4. Adoral view of denticle ring. (Scale bar=1 $\mu$ m).

found in *Trichodina*". The blades of *Paratrichodina incisa* as revealed by electron microscopy, are dissimilar to any observed in *Trichodina*, lacking the anterior and posterior ray apophysis and illustrating a substantial anterior projection of the denticle blade. Both of the latter characteristics differentiate *Paratrichodina incisa* from *Trichodina domerguei* and *Trichodina intermedia*.

#### **TRICHODINELLA EPIZOOTICA RAABE, 1950**

Complete specimens of *Trichodinella epizootica* were not processed for scanning electron microscopy, as insufficient numbers were available. However, the fine structure of *Trichodinella epizootica* (Lom, 1973) was described previously. *Trichodinella* inhabiting the gills of *Oncorhynchus mykiss* from Dollar and the River Test were sonicated; only a few specimens were recovered, most being lost due to their small size in the repeated washes involved in the technique. Figures 4.7.1 and 4.7.2 illustrate the aboral surface of a partially sonicated Dollar specimen of *Trichodinella epizootica*, complete with radial and peripheral pins. The curved hook like inner rays characteristic of the genus are clearly visible. The central parts of the denticles are larger than is apparent in silver stained material, with a broader part positioned adorally to a defined aboral topography forming the typical silver stained appearance.

From the aboral side the denticle blades can barely be discerned, with only the thicker posterior part clearly visible. The radial pins underneath are clearly visible through the blades. This population identified as *Trichodinella epizootica* bore similarities to specimens of *Trichodinella subtilis* the denticle blades of which are said to be characterised by two anterior projections (Lom, 1963a). However, no evidence of a second projection can be seen in the present material, although the lower anterior

**Figure 4.7.** Isolated adhesive disc structure in *Trichodinella epizootica* from *Oncorhynchus mykiss* revealed by scanning electron microscopy.

1. Partially sonicated specimen illustrating components of the adhesive disc viewed aborally (Dollar). (Scale bar=1 $\mu$ m).

2. Enlargement of previous specimen: AP, anterior projection. (Scale bar=1 $\mu$ m).

3. Adoral view of denticle ring (River Test): IR, inner ray; 2nd AP?, possible secondary anterior projection described in populations identified as *Trichodinella subtilis* by other Lom (1963a) and Kazubski & Migala (1968). (Scale bar=1 $\mu$ m).

4. Enlargement of previous specimen: A, thin anterior region of denticle blade; p, thick posterior region. (Scale bar=1 $\mu$ m).

5. Radial and peripheral pins: RP, radial pins; PP, peripheral pins; a, fibrous material (attachment to denticle); b, nodule (attachment point of kinetosomes of the basal cilia). (Scale bar=1 $\mu$ m).

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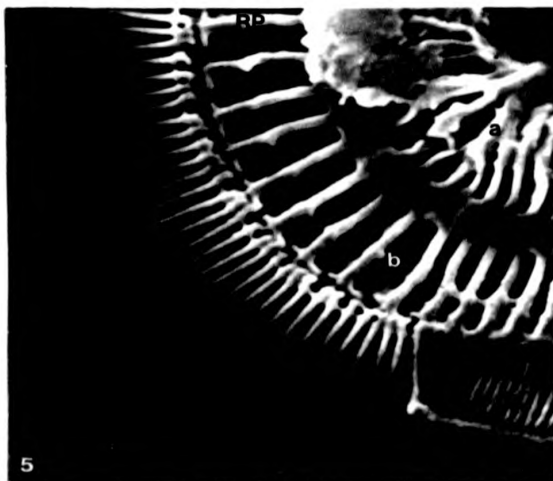
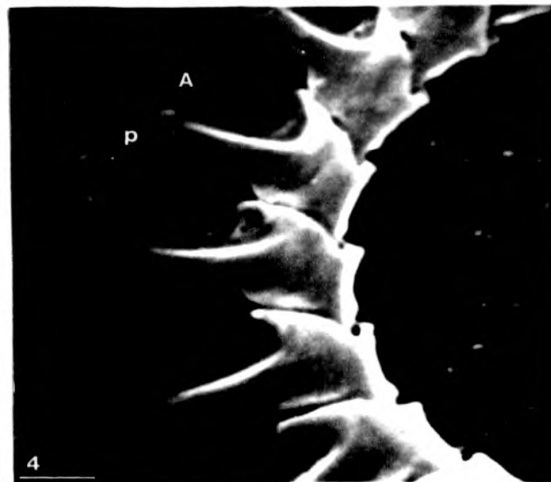
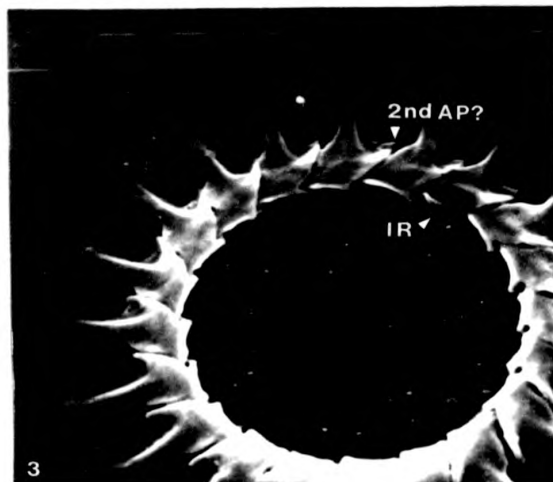
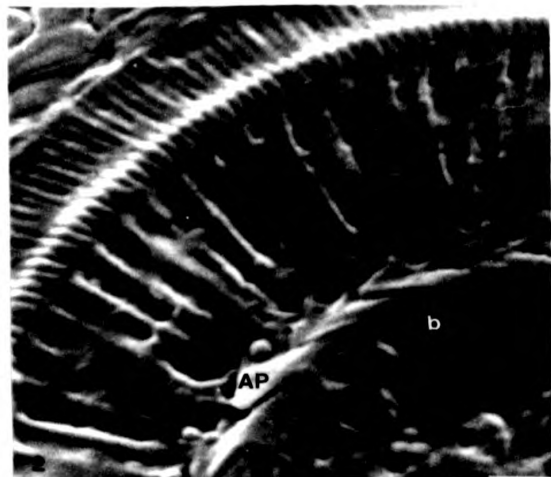
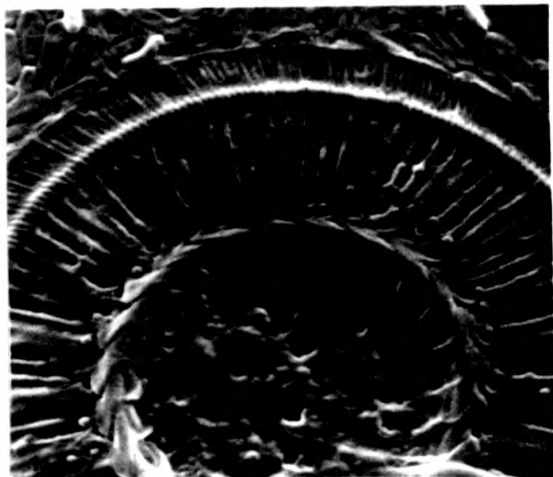
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margins of certain denticles appear to be slightly thickened. These may be revealed in silver impregnation whilst the remainder may be unstained, producing the appearance of a second smaller projection.

A River Test specimen viewed from the adoral surface is shown in Figures 4.7.3 and 4.7.4. The inner rays do not appear to project below the centripetal border of the central parts, although in one denticle (Figure 4.7.3) the inner ray may be visible curling under the central part. This considerable extension of the central parts below the point where the inner ray originates, may explain why most of the ray is obscured in silver stained specimens. The denticle blades clearly illustrate a thickened posterior part and an extremely thin anterior portion (Figure 4.7.4). Inconsistent staining of this region may account for the differences observed between populations of *Trichodinella epizootica* described in Chapter 3.

The adoral surface of the central parts of the denticles project to the anterior of the blades. Thus, the top right hand corner of the central part viewed adorally may appear as a second anterior projection when viewed from the aboral side. Unfortunately the aboral surface of specimens from the River Test population could not be described using scanning electron microscopy, as this was the only specimen recovered and the silver stained material was of poor quality. Therefore it is impossible to determine whether these specimens belong to the same species as those from Dollar, although they appeared to be identical in live preparations and are therefore assumed to belong to the species *Trichodinella epizootica*. However, the two surfaces of the denticle are on different planes which would be difficult to reveal with silver staining. Thus, the anterior corner of the central part of the denticle may be revealed in some silver stained preparations and not in others. An example of this variable staining occurs in certain

*Trichodina* species observed during this study, where specimens appear to possess a projection above the central part directed posteriorly. This is the posterior blade apophysis which in the majority of specimens is not visible.

Figure 4.7.5 illustrates the radial and peripheral pins of *Trichodinella epizootica*. The radial pins widen more dramatically at their centrifugal ends than in *Trichodina domerguei* (Figure 4.3.3), and the peripheral pins are shorter in relation to them. Part of the membrane surrounding the peripheral pins has been removed during sonication, this displays their highly tapered form. There appears to be fibrous material attached to the radial pins at their centripetal ends, this may be the remnants of the material used in attachment to the denticle. Towards the centripetal ends of the radial pins a small projection or nodule is visible, this may be the point where the kinetosomes of the basal cilia were attached.

#### SUMMARY AND CONCLUSIONS

Certain features of the complex adhesive disc structure present in trichodinids were revealed during this study of sonicated specimens using scanning electron microscopy.

Denticle form in *Trichodina domerguei* is more complex than previously indicated by light microscopical observations. Previously undescribed in any species were articulation structures either side of the central conical part of the denticle, visible in a broken ring. These structures were termed the posterior blade and ray apophysis, they probably limit rotation relative to the adjacent denticle and may also limit lateral flexing of the denticle ring (Figure 4.3.2). A peg-like anterior projection of the central part / ray junction (anterior ray apophysis) which is obscured in the complete denticle ring, interlocks with the adjacent posterior ray apophysis and the ray itself (Figure

4.3.2). This structure presumably also serves to restrict denticle rotation, lateral movement and flexing in an aboral / adoral plain. Van As & Basson (1990) were the first authors to describe denticular structures associated with articulation. They described a blade and ray apophysis from an adoral view of a disrupted denticle ring from *Trichodina dampanula*. It is suggested that these structures described by Van As & Basson be designated the anterior blade and ray apophysis to differentiate them from the posterior blade and ray apophysis newly described in this study. An adoral view of a disrupted denticle ring was not obtained in this study, so the presence of an anterior blade apophysis in *Trichodina domerguei* could not be corroborated.

Previously undescribed in any trichodinid was a peg like structure on the centrifugal surface of the central part of the denticle, observed in *Trichodina domerguei* (Figure 4.3.1). This structure was only seen in a few denticles of one sonicated specimen viewed adorally, where the denticles were slightly pulled apart. It is probable that these pegs locate in the underside of the adjacent blade restricting articulation, or strengthening the integrity of the denticle ring.

A posterior ray and blade apophysis as well as anterior ray apophysis were also present in *Trichodina intermedia*, but were more pronounced than in *Trichodina domerguei* (Figures 4.4.3 and 4.4.4).

*Paratrachodina incisa* possessed a posterior blade apophysis (Figure 4.6.3) which presumably interlocks with a corresponding anterior blade apophysis on the adoral surface of the adjacent denticle. The anterior ray apophysis and posterior ray apophysis previously described for *Trichodina domerguei* and *Trichodina intermedia* were not present in *Paratrachodina incisa*. It can be presumed that the thickened anterior blade projection (Figure 4.5.4) which corresponds with the posterior blade apophysis of the

adjacent denticle helps to limit denticle rotation. This arrangement may give greater lateral stiffness to the denticle ring, and also give additional resistance to flexure in an aboral / adoral plain.

The absence of a posterior blade apophysis and anterior ray apophysis in *Paratrichodina incisa* may be a discriminating feature of the genus. However, these structures were not constant in another genus, *Trichodina*. The posterior blade and ray apophysis present in *Trichodina domerguei* and *Trichodina intermedia* were not observed in *Trichodina dampanula* described by Van As & Basson (1990). The articulation surfaces in the genera *Trichodinella* and *Tripartiella* have not been described to date. Although it is likely they bear similarities to those of *Paratrichodina* as they fall into the same group of small species displaying an incomplete turn of the ciliary spiral, at present it cannot be determined whether these structures will prove taxonomically important.

This study has highlighted the marked differences in denticle form observed using light and electron microscopy in *Paratrichodina incisa*, *Trichodinella epizootica* and to a lesser extent *Trichodina intermedia*. The anterior blade border of *Paratrichodina* and *Trichodinella* species seems especially vulnerable to staining artifacts (this study and Lom, 1959, 1963a; Kazubski & Migala, 1968). Classification of smaller trichodinids of the genera *Paratrichodina*, *Tripartiella* and *Trichodinella* prone to variable silver impregnation would be aided by routine examination of sonicated specimens using scanning electron microscopy, especially when describing a new species. By sonicating material for variable durations, specimens can be observed in different states of disruption from complete adhesive discs to isolated denticles. This technique appears superior to the digestion technique used by Van As & Basson (1990),

giving much cleaner preparations.

## CHAPTER 5: OBSERVATIONS ON ADHESIVE DISC MORPHOLOGY DURING REPRODUCTION.

### INTRODUCTION

Trichodinids reproduce asexually by transverse binary fission, and, rarely, sexually by conjugation (Lom & Dykova, 1992). Asexual reproduction has been investigated by few authors, including Kazubski (1967), Ahmed (1977), Feng (1985) and Chardez (1985). Trichodinids divide into two daughter individuals, with half the original number of denticles and radial pins (Lom, 1961). Before cell division in *Trichodina reticulata* the precursors of the new denticle rings form a fine ring around the original denticle ring of the mother cell (Ahmed, 1977). The macronucleus enlarges and changes from the normal horseshoe shape to a sphere, which pulls apart into two; the micronucleus divides mitotically. The aboral and adoral cilia are retained, but the cytopharynx disappears during fission. In each daughter cell denticles derived from the parent are reabsorbed with simultaneous development of a new denticle ring with the adult complement of denticles (Ahmed, 1977). New radial pins develop between the original ones thus regaining the adult number. Feng's (1985) description of binary fission in *Trichodina nobilis* Chen, 1963 agrees with Ahmed (1977). At the "optimum" temperature of 22-29°C, the asexual cycle of reproduction takes 24 hours. Cellular reorganisation predivision taking from 0.5-1.0 hours, division 1-3 minutes, growth of daughter cell 1.5-3.0 hours and the adult form existing for 20.0-22.0 hours (Feng, 1985).

Kazubski (1967) conducted a study on the growth of skeletal elements in *Trichodina pediculus* from *Pelamatohydra oligactis*, *Rutilus rutilus* and *Coregonus albula*. The new denticle ring of the daughter cell was described as forming post

division, contrary to the findings of Ahmed (1977) and Feng (1985) where the new denticle ring was observed as a fine ring before division. The denticles in *Trichodina pediculus* developed from small "slats", with the central part, blade and ray developing in approximate succession (Kazubski, 1967). The new denticle ring enlarges during the period of development of the denticles. As the denticle ray is the last component formed, Kazubski suggested that they could be used as a measure of the relative age of a trichodinid. The radial pins attained adult number and appearance at the same time as the growth of denticle rays was completed. Kazubski concluded that radial pin growth may be a more convenient measure of age, especially in species where the denticle rays are less developed. Denticle number was found to be marginally higher in young specimens than in adults, and it was suggested that a tendency exists for an increase in denticle number from one generation to the next.

Sexual reproduction in trichodinids was most recently described by Ahmed (1977). Ahmed described isogamous and anisogamous conjugation in *Trichodina reticulata*. In the latter species as described by Ahmed (1977) the aboral surface of the microconjugant (in anisogamy) fits over the adoral surface of the macroconjugant. The micronuclei swell and move to the centre of each cell where they divide mitotically. Simultaneously the macronuclei break down into large fragments which continue to break up into minute spherical granules. As the macronuclei fragment the micronuclei undergo two further divisions. At this point the protoplasm of the two cells join, and the contents of the microconjugant pass into the macroconjugant. A new denticle ring forms in the macroconjugant, containing the same number of denticles as the original. The gametic nuclei are then assumed to combine to form a synkaryon, and the remaining nuclei are reabsorbed. The remains of the microconjugant disintegrate leaving an

exconjugant containing the synkaryon and fragmented macronuclei. The zygotic nucleus divides three times, forming eight micronuclei; the parental inner denticle ring is reabsorbed at approximately the same time as the second division is completed. Seven of the micronuclei form the macronuclear anlagen, and the remaining micronucleus divides during cell division. The macronuclear anlagen are distributed during subsequent cell division, until each daughter individual contains one micronucleus and macronuclear anlagen. Thus seven daughter cells are produced, the macronuclei then increase in size and assume the characteristic horse shoe shape. Ahmed (1977) does not give details of adhesive disc development during the successive divisions of the exconjugant to form the seven daughter cells, and it is assumed that this process is the same as that occurring during asexual binary fission. This chapter details developmental morphology of the adhesive disc associated with binary fission in four *Trichodina* species, observed using scanning electron and light microscopy.

## **MATERIALS AND METHODS**

Preparation of silver stained and sonicated specimens was undertaken as previously described in Chapter 2 and Chapter 4 respectively.

## **RESULTS**

### ***TRICHIODINA ACUTA* LOM, 1961**

The reproductive stages of *Trichodina acuta* illustrated in Figures 5.1 and 5.2 are compiled from the seven populations described in Chapter 2. Figure 5.1 and Figure 5.2 (1-3) present adhesive disc development viewed aborally during and after binary fission, which occurs during asexual reproduction and the later stages of conjugation. A



specimen of *Trichodina acuta* in the late stages of division (Figure 5.1.1) illustrates two daughter cells in which the denticle rings are still disrupted. A ring of fine "slats" surrounds each ring, being the precursor of the new adult denticle ring. The denticles are divided unequally between the two daughter cells, 11 and 9 respectively. Figures 5.1.2 and 5.1.3 illustrate two newly formed daughter cells, in which the new denticle rings illustrate no further development than seen in the dividing individual. The parental denticle rays of one daughter cell (5.1.3) are already being reabsorbed, in contrast to the other individual (5.1.2) where the parental denticles are still complete.

Figures 5.1.4-5.2.3 show adhesive disc development post division. The "slats" thicken and the new blades appear as small rectangular processes (5.1.4), at this stage the daughter cell's parental denticles remain intact. In this individual the developing ring contains 20 denticles, twice the number (10) of the original daughter cell. The denticle blades continue to grow, assuming their characteristic curved form (5.1.5). The central parts have developed almost to their mature state. The parental denticles have been partially reabsorbed, and the blades and rays are reduced while the central parts are left intact. In this individual there are 10 parental denticles, but only 18 new structures. In Figure 5.2.1 the denticle blades are nearly fully formed, and the inner rays can be seen as very small centripetal projections. The ratio of old to new denticles is 10:19. Figure 5.2.2 illustrates a specimen of *Trichodina acuta* approaching mature denticle form illustrated, with only the inner rays being slightly reduced. The parental ring is almost completely reabsorbed, with only small denticle fragments apparent. The final developmental form observed (5.2.3) has fully formed denticles, and the central circle of the adhesive disc is apparent. Only the alternate thick and thin radial pins reveal this individual as immature. New radial pins can be seen as fine lines between the original

**Figure 5.1.** Silver stained developmental stages of *Trichodina acuta* viewed aborally.

1. Specimen in a late stage of division with ring of fine "slats" (S) surrounding each daughter ring, constituting the precursor of the new ring. (1600×).
2. Newly formed daughter cell. (1200×).
3. Newly formed daughter cell, with parental denticle rays beginning to be reabsorbed. (1200×).
4. Daughter cell in which the blades of the new denticle ring are partially formed. (1200×).
5. Daughter cell in which the denticle blades are further developed, the central parts of the denticles are almost complete and the blades of the parental denticles are partially reabsorbed. (1200×).

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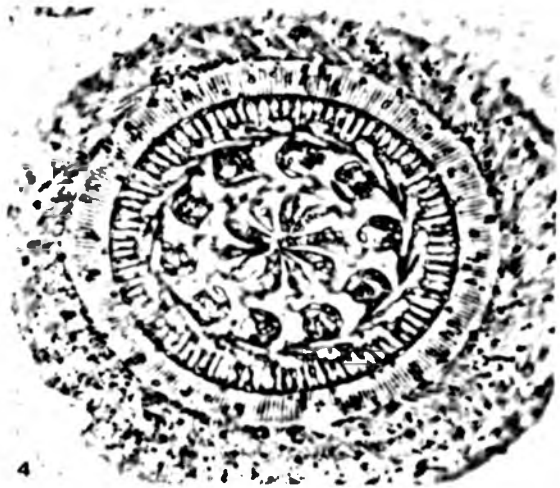
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4



5

**Figure 5.2.** Silver stained developmental stages of *Trichodina acuta*.

1. Daughter cell viewed aborally, in which the new denticle blades and central parts are almost fully formed. The denticle rays are present in the form of small centripetal projections. The parental denticle blades have nearly been fully reabsorbed. (1200×).
2. Daughter cell viewed aborally, in which the denticles are approaching adult form, with only the inner rays displaying signs of immaturity. Fragments of the parental denticle ring are still visible. (1200×).
3. Immature specimen viewed aborally, with fully formed denticles and central circle (CC) of the adhesive disc. New radial pins (np) are apparent as fine lines between the parental pins. (1200×).
4. Specimen viewed adorally, in which the adoral ciliary spiral has undergone division, before binary fission of the cell. (1200×).
5. Aboral view of an exconjugant during conjugation. Both denticle rings contain the same number of denticles. (1200×).

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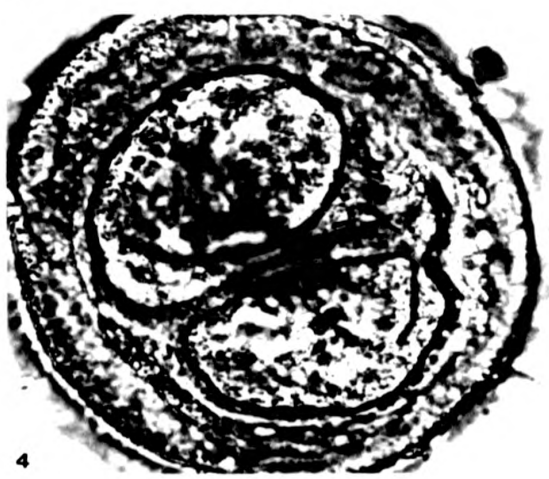
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pins of parental origin.

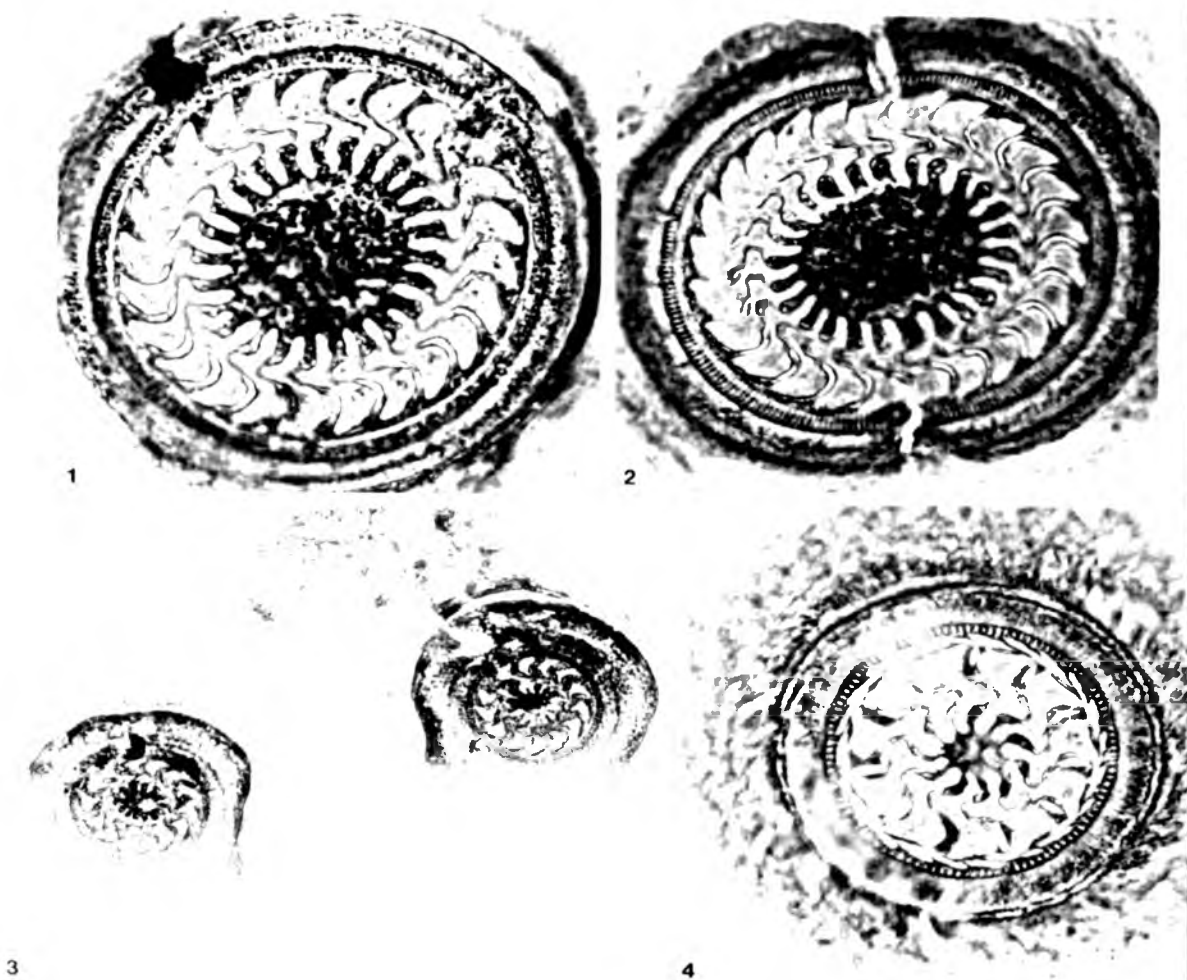
Figure 5.2.4 illustrates a specimen of *Trichodina acuta* viewed adorally before binary fission. The adoral ciliary spiral has already undergone division, each new spiral will be incorporated into a daughter cell during cell division.

Figure 5.2.5 represents a sexual stage with a double denticle ring, both containing the same number of denticles. The adult number of radial pins are present. These characteristics are demonstrated by the macroconjugant during sexual reproduction (Ahmed, 1977).

#### ***TRICHODINA DOMERGUEI* WALLENGREN, 1897**

The sequence of adhesive disc development displayed by *Trichodina domerguei* from *Gasterosteus aculeatus* (Airthrey Loch) (Figure 5.3 and 5.4) is very similar to that presented for *Trichodina acuta*. Figures 5.3.1 and 5.3.2 illustrate specimens in the very early stages of binary fission. The first individual, in predivision, exhibits a fine ring of "slat-like" denticle precursors surrounding the original ring. The second (5.3.2) is just starting to divide. Figure 5.3.3 illustrates two daughter cells post division. The daughter cell in Figure 5.3.4 possesses a fine outer ring of denticle precursors (old to new denticle ratio 11:22).

Figures 5.4.1-5.4.4 illustrates denticle growth starting with the blades, then the central parts, followed by the inner rays. Simultaneously the parental denticle ring is reabsorbed with the blades disappearing first, followed by the central parts and finally the inner rays. Thus development of the new denticle ring follows the same sequence as the reabsorption of the old one. The specimen displayed in Figure 5.4.2 is notable for the appearance of the old denticle blades. Each blade is apparently divided into two, with



**Figure 5.3.** Silver stained adhesive disc morphology of *Trichodina domerguei* from *Gasterosteus aculeatus* during binary fission and in the early developmental stages post division.

1. Adult individual demonstrating the outer ring of denticle precursors, which form new denticles in the daughter cells. (1200 $\times$ ).

2. Specimen in early stage of division. (1200 $\times$ ).

3. Two newly separated daughter cells. (1200 $\times$ ).

4. Daughter cell with new denticles in very early stage of development. (1200 $\times$ ).

**Figure 5.4.** Silver stained specimens of *Trichodina domerguei* from *Gasterosteus aculeatus* viewed aborally, in the later stages of development.

1. Specimen displaying early development of new denticle blades. (1200×).
2. Specimen illustrating further development of denticle blades and central parts. Old denticle blades appear divided, with the thinner anterior portions (a) illustrating a higher degree of reabsorption. (1200×).
3. Specimen illustrating mature denticle blade and central part form, with inner rays apparent as tiny centripetal projections (p). (1200×).
4. Specimen displaying further denticle ray development. Only the inner rays, and fragments (f) of the central parts of the parental denticles are visible. (1200×).
5. Immature specimen illustrating mature denticle form. (1200×).
6. Immature specimen displaying central circle, but thin/thick radial pins. (1200×).



*Gasterosteus*

(x).

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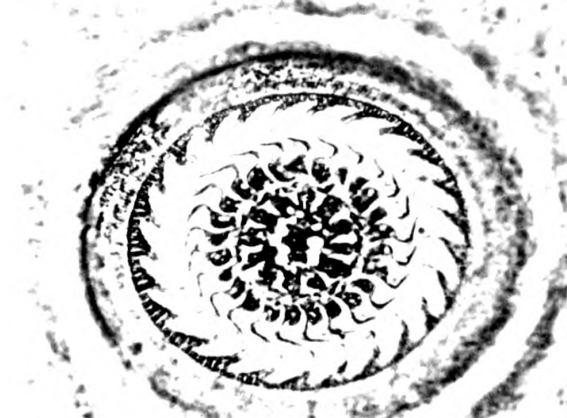
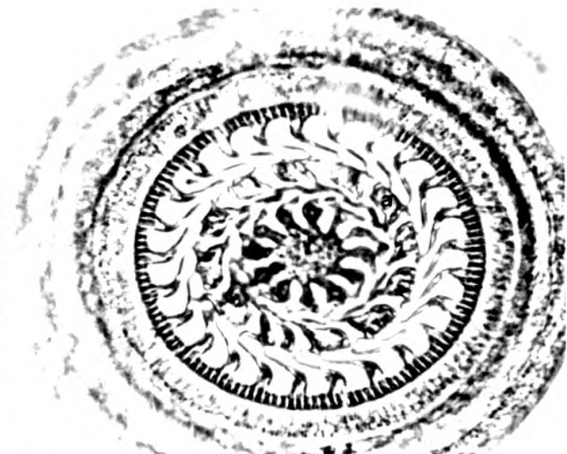
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the thinner anterior portion more heavily stained. The old to new denticle ratio for the previous four specimens is 12:25, 12:25, 13:25 and 12:25 respectively.

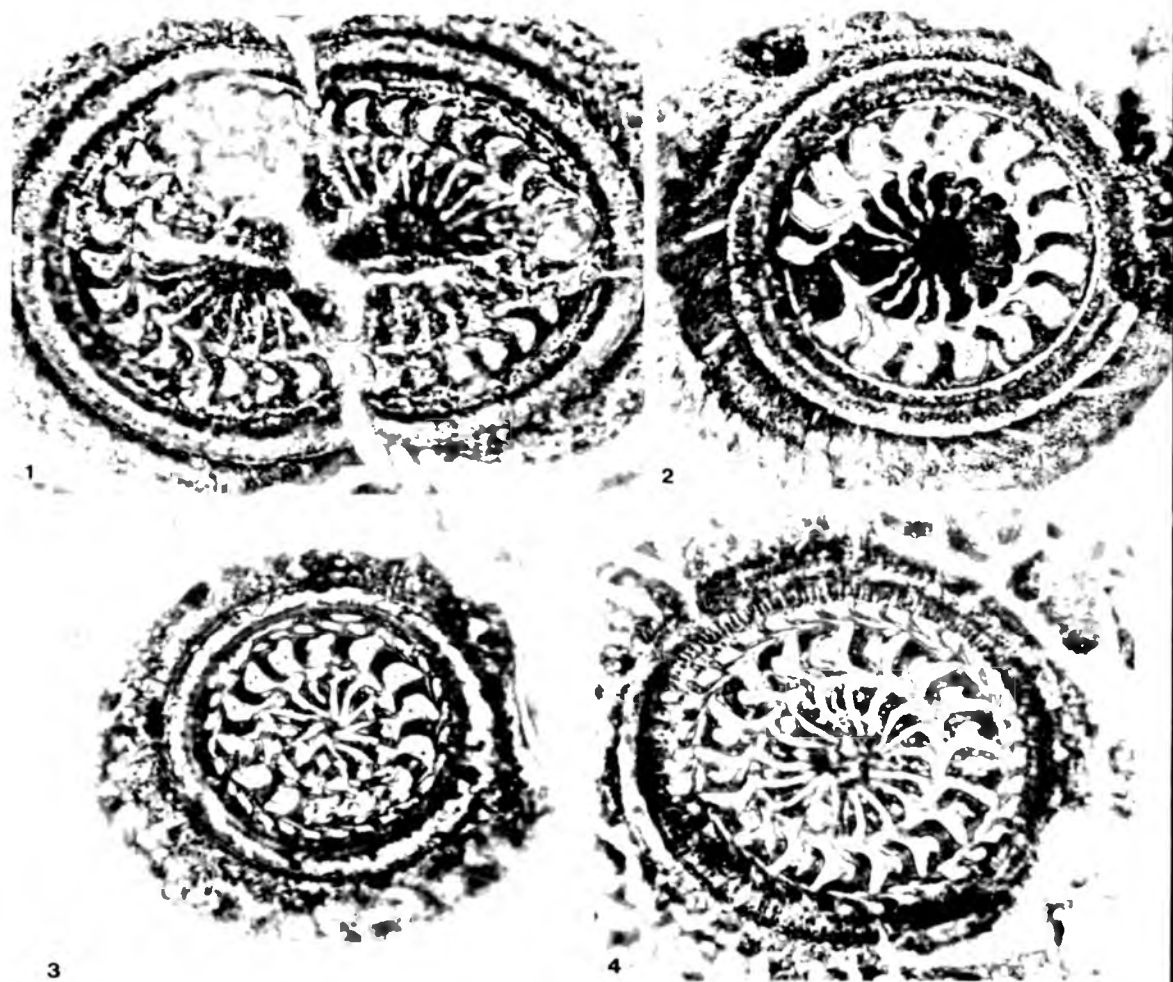
Figures 5.4.5 and 5.4.6 illustrates two specimens in the late stages of adhesive disc reorganisation. The first has mature denticles and in the second the central circle has appeared. Both specimens illustrate immature radial pin form, with only the parental pins easily observed.

#### ***TRICHODINA TENUIDENS* FAURE-FREMIET, 1944**

The development of *Trichodina tenuidens* from *Gasterosteus aculeatus* (Airthrey Loch) during binary fission and subsequent growth follows the same pattern as in the species described previously. A fine ring of denticle precursors is visible, surrounding those of the parent in an early stage of division (Figure 5.5.1). A newly divided daughter in which the denticle ring is still interrupted is illustrated in Figure 5.5.2.

Progressive developmental stages of immature specimens with double denticle rings are displayed in Figures 5.5.3-5.6.1. The typical pattern of new denticle growth and old denticle reabsorption as displayed by *Trichodina acuta* and *Trichodina domerguei*, is also apparent in *Trichodina tenuidens*. The outermost, anterior portion of the old denticle blades are reabsorbed first (Figure 5.5.4), followed by the rest of the blade leaving a small portion of the posterior blade margin (Figure 5.6.1). The old to new denticle ratios of the three previous individuals illustrated are 14:28, 17:34 and 15:31.

In Figure 5.6.2 only the old denticle rays, complete with the lower posterior margins of the central parts remain, with the new denticles approaching adult form. Irregular silver staining bodies can be seen associated with the denticle ray remnants in



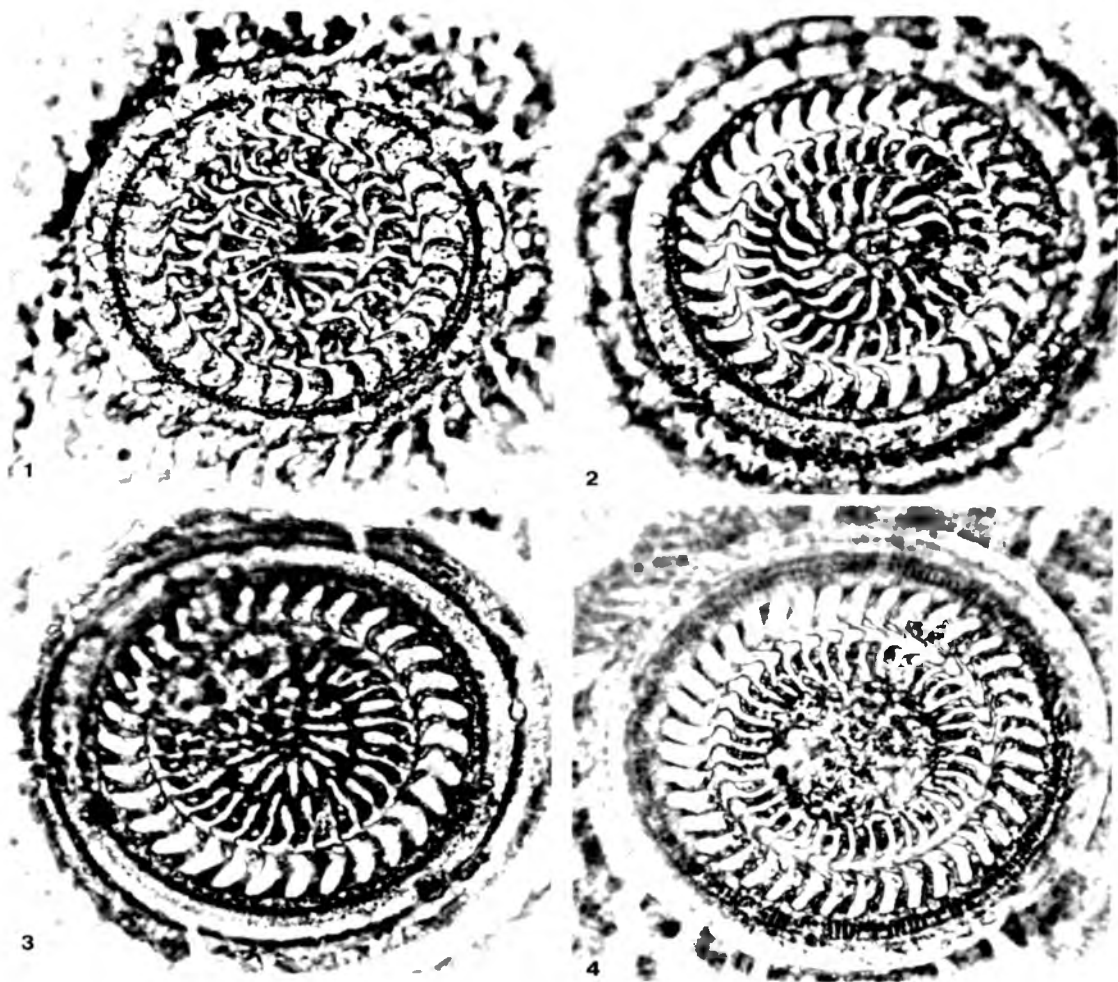
**Figure 5.5.** Silver stained adhesive disc morphology of immature specimens of *Trichodina tenuidens* from *Gasterosteus aculeatus*.

1. Individual in an early stage of division. (1200 $\times$ ).

2. Newly divided daughter cell. (1200 $\times$ ).

3. Daughter cell illustrating early stages of new denticle growth. (1200 $\times$ ).

4. Daughter cell with more advanced state of denticle blade development. (1200 $\times$ ).



**Figure 5.6.** Silver stained adhesive disc morphology of immature specimens of *Trichodina tenuidens* from *Gasterosteus aculeatus*.

1. Specimen in which new denticle blades are almost complete, and the inner rays are in an early stage of development. (1200 $\times$ ).

2. Immature specimen in which the inner rays of the old denticle ring are visible in the centre of the adhesive disc, associated with irregular silver staining bodies (b). (1200 $\times$ ).

3. Specimen in which the old denticle rays remain in the centre of the adhesive disc. (1200 $\times$ ).

4. Immature specimen in which the old denticle ring has been completely reabsorbed, but the radial pin appearance is still immature. (1200 $\times$ ).

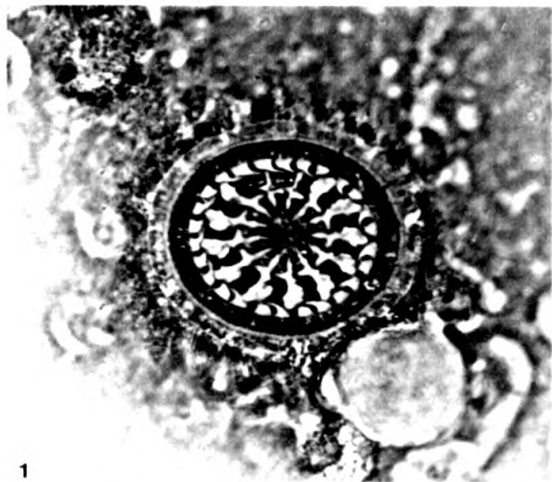
the latter specimen. Figure 5.6.3 illustrates another specimen with mature denticles and the old denticle rays still present in the centre of the adhesive disc. The immaturity of the final specimen (Figure 5.6.4) is indicated by the radial pins, with only the parentally derived pins visible. The denticles are of adult appearance.

#### ***TRICHODINA INTERMEDIA* LOM, 1960**

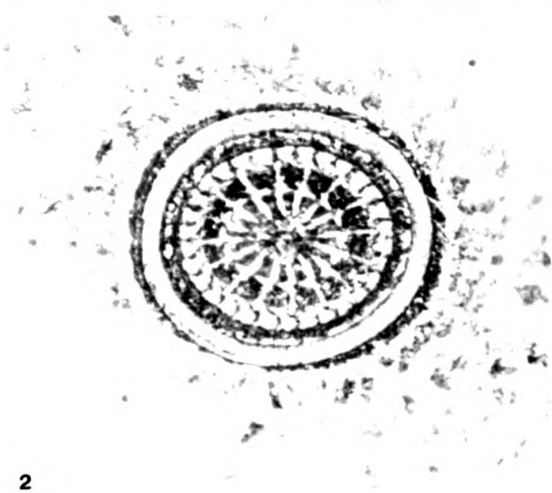
Developmental stages of *Trichodina intermedia* from *Phoxinus phoxinus* are illustrated in Figures 5.7 and 5.8. Immature specimens were observed from silver stained and sonicated material.

Figures 5.7.2 and 5.7.3 represent two silver stained daughter cells with the new denticle rings in an early stage of development. The sequence of new denticle development and old denticle reabsorption appears similar to that of the species previously described. The old to new denticle ratio for the two specimens are 13:26 and 16:30 respectively.

An aboral view of a sonicated specimen of *Trichodina intermedia* using scanning electron microscopy is illustrated in Figures 5.8.1 and 5.8.2. The new denticle ring shows central part and blade growth, but the inner rays are still lacking. The blades of the old denticles have been completely reabsorbed. The parental denticle rays and central parts have been partially reabsorbed, revealing the interlocking processes of each denticle. The old to new denticle ratio is 16:35. Centrifugal to the new denticle ring the number of radial pins per denticle is approximately seven. This resembles the mature form, with the new radial pins being slightly thinner than the old parental structures. Centripetal to the new denticle ring only the old radial pins are present. At higher magnification (Figure 5.8.2) the new peripheral pins can be seen as very thin lines

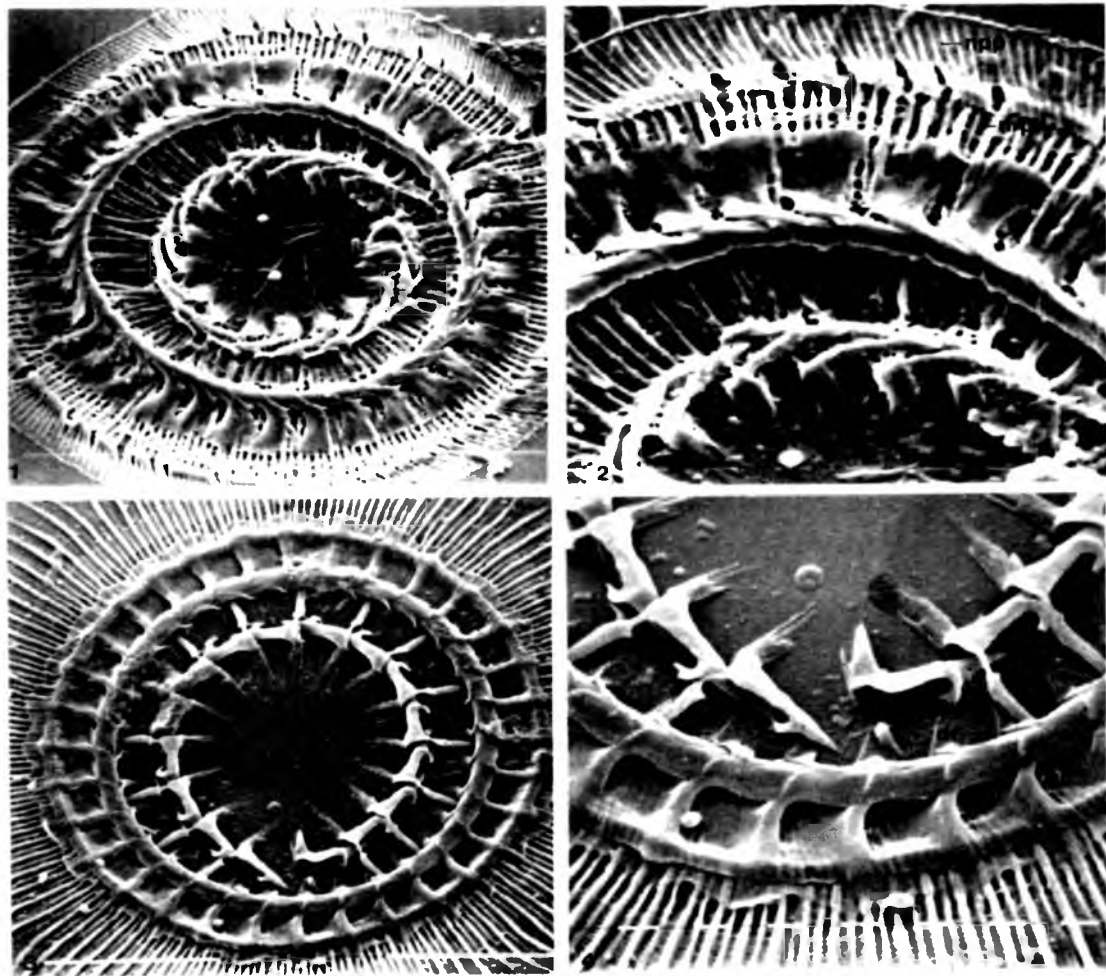


1



2

**Figure 5.7.** Aboral view of immature silver stained specimens of *Trichodina intermedia* from *Phoxinus phoxinus*. (1200 $\times$ ).



**Figure 5.8.** Scanning electron micrographs of sonicated immature specimens of *Trichodina intermedia* from *Phoxinus phoxinus*.

1. Aboral view of immature specimen. (Scale bar=10 $\mu$ m).
2. Enlargement of Figure 5.8.1. nrp, new radial pin; npp, new peripheral pin. (Scale bar=1 $\mu$ m).
3. Adoral view of immature specimen. (Scale bar=10 $\mu$ m).
4. Enlargement of Figure 5.8.3. dr, denticle ray; r, ridge-like junction of radial pins and finer pins attached to adoral surface of denticle blade. (Scale bar=1 $\mu$ m).



between the parentally derived pins.

Figures 5.8.3 and 5.8.4 illustrate an adoral view of a sonicated individual, in a slightly more advanced stage of development in comparison to the previous specimen. The new denticle rays are visible as small triangular structures. The old denticles have been partially reabsorbed, but remnants of the blades are still present. The old to new denticle ratio is 16:32. The radial pins observed centripetal to the new denticle ring in Figures 5.8.1 and 5.8.2 have been reabsorbed. The radial pins centrifugal to the new denticle ring in Figures 5.8.3 exhibit a considerable increase in length relative to the previous specimen. A previously undescribed feature in trichodinids is a defined ridge (Figure 5.8.4) at a junction between the radial pins and a slightly greater number of finer pins which terminate at the centripetal border of the denticle blades. This arrangement resembles the radial pin junction with the border membrane, present in all trichodinid species.

## DISCUSSION

In specimens of *Trichodina acuta*, *Trichodina domerguei* and *Trichodina tenuidens* observed during this study, the new denticle ring was present before (5.3.1) and during (5.1.1, 5.3.2 and 5.5.1) binary fission. This agrees with the findings of Ahmed (1977) and Feng (1985) for *Trichodina reticulata* and *Trichodina nobilis* respectively. Kazubski (1967) describes the growth of the new denticle ring commencing after cell division in *Trichodina pediculus* and Chardez (1985) illustrates the new denticle ring appearing post division in an unspecified *Trichodina* species. This discrepancy in the stage at which the new denticle ring appears may be due to interspecific variation in the process of binary fission. However, it is likely that Kazubski (1967) and Chardez (1985) assumed that the



new ring appeared post division, because they did not encounter stages immediately prior to or during division.

The growth of skeletal elements in the species observed during this study was very similar to that described for *Trichodina pediculus* (Kazubski, 1967). The denticle blades appear first, with simultaneous development of the central parts. By the time the denticle rays appear the denticle blades and central parts are approaching maturity. At this point *Trichodina intermedia*, characterised by its relatively long blades, displays comparatively immature denticle form. Reabsorption of the old denticle ring generally takes the same time as the new ring takes to develop. There is some variability in denticle reabsorption, with some specimens showing the same degree of reabsorption at different stages of new denticle growth.

In *Trichodina centrostrigata* (Basson, Van As and Paperna, 1983) the denticle rays of the daughter cell remain in the centre of the adhesive disc. These remnants must be reabsorbed at some point, otherwise additional rings would accumulate with subsequent divisions. *Trichodina pacifica* (syn: *Trichodina miranda* Stein) (Stein, 1984) illustrates the same retention of inner rays in the centre of the adhesive disc. This marine species closely resembles *Trichodina tenuidens* which is euryhaline. Several well developed specimens of *Trichodina pacifica* illustrated by Stein (1984) show mature denticles but still retain the daughter denticle rays as seen in *Trichodina tenuidens* during this study (Figures, 5.6.2 and 5.6.3).

In species with a central circle such as *Trichodina domerguei* and *Trichodina tenuidens*, this structure is the last to appear in developing specimens. The remaining denticle rays in Figure 5.6.2 appear to be associated with irregularly shaped, silver stained bodies similar to those apparent in mature specimens of *Trichodina tenuidens*.

This suggests that the central circles, which appear the same shade as denticles in silver stained material, might be produced by coalesced denticular material during reabsorption.

The specimens illustrated in this chapter constitute the majority of good quality double ringed immature specimens encountered during this study. Feng (1985) describes the cycle of asexual reproduction in *Trichodina nobilis* taking 24 hours, with division taking 1-3 minutes and subsequent growth 1.5-3.0 hours. The small number of specimens observed during division in this study correlate with the time span described by Feng (1985) for this process. A large number of immature specimens were observed during this study, the vast majority of which were only identifiable by the immature appearance of their radial pins. The percentage of these specimens in which a double denticle ring was apparent was relatively small. This would suggest that the period of new denticle ring development and old denticle reabsorption is comparatively short, followed by a longer period in which the new radial pins develop to resemble mature structures.

Kazubski (1967) stated that denticle number was slightly higher in immature than mature specimens, and suggested that denticle number may increase from one generation to the next. (Due to the very short life cycle of trichodinids, this would soon lead to specimens containing thousands of denticles). The number of immature specimens observed in this study precludes any statistical conclusions on variation in denticle number in successive generations. However, observations on denticle number in dividing and maturing specimens were made. Where a specimen contained an odd number of denticles its daughter cells will contain different numbers (eg. 13:14 in Figure 5.3.3). If double the number of denticles form in the new ring as usually occurs, then a parent

of 27 denticles will produce offspring of 26 and 28 denticles. In Figure 5.1.1 an adult with 20 denticles is dividing unequally to form daughter cells of 9 and 11, which would be expected to form adults with 18 and 22 denticles. Thus, the mean denticle number of the offspring is normally the same as the parent. However, in some specimens a slightly smaller or greater number than double the daughter cell denticle number occurs. This may be a random occurrence, or more probably a characteristic enabling the daughter cell to regain the parental complement of denticles.

The scanning electron micrographs of immature specimens of *Trichodina intermedia* illustrate the development of new structures and reabsorption of old ones more clearly. The ridge-like junction of radial pins and finer pins which can be designated as denticular pins, has not previously been described. This side of the adhesive disc is not clearly visualised in silver stained material. The adoral view of *Trichodina acuta* (Figure 4.3.4) is one of only three such specimens out of tens of thousands observed during this study. The fine denticular pins are barely perceptible towards the lower margins of the blades. Whether this junction allows additional articulation of the radial pins relative to the denticles remains unclear.

In all the specimens possessing double denticle rings, the old denticles and parts of the radial pins which are subsequently reabsorbed are in very close proximity to the developing denticles. It is possible that the skeletal material may be directly incorporated from the old to new structures, rather than being completely reabsorbed into the protoplasm and reincorporated into the daughter cell's skeletal structures.

The specimen illustrated in Figure 5.2.5 was the only specimen observed with the same number of denticles in each denticle ring. Ahmed (1977) describes this arrangement in the exconjugant of *Trichodina reticulata* during sexual reproduction.

That only one such specimen was observed during this study would suggest that conjugation is a rare occurrence, with this phase lasting a very short period of time. Ahmed (1977) is unclear about the exact sequence of adhesive disc development in the series of cell divisions following conjugation. If the same process occurs during binary fission in sexual and asexual reproduction as is likely, then the daughter cells illustrated in this chapter could represent derivatives of either asexual or sexual binary fission. The duration of existence of macro or exconjugants (Figure 5.2.5) is not known, although almost certainly short, the rarity with which these stages have been observed (this study and Ahmed, 1977) suggests that the daughter cells and immature specimens seen in this study are predominantly produced via asexual binary fission.

**CHAPTER 6: MORPHOLOGICAL VARIATION OF *TRICHODINA*  
*DOMERGUEI* AND *TRICHODINA TENUIDENS* PARASITISING  
*GASTEROSTEUS ACULEATUS*.**

**INTRODUCTION**

Intraspecific morphological variation within or between trichodinid populations has been commented on by many workers, but usually as an "aside" whilst describing a species from a relatively small number of individuals. Interpopulational variation is commonly described where samples of the same trichodinid species are compared non-statistically from different geographic regions, environments or host species. Interpopulational variation is extremely complex due to the large numbers of possible environmental and genetic factors acting on morphology. A major factor contributing to observed variation between populations is that samples being compared may not be representative of the population they are taken from. Therefore, it is necessary for the successful analysis of interpopulational variation that intrapopulational variation is clearly understood.

Specific research into morphological variation in trichodinids is limited. Growth of skeletal elements (Kazubski, 1967) can produce apparent variation within a population due to differences in the maturity of specimens (see Chapter 5). During asexual reproduction trichodinids undergo binary fission where the adhesive disc of the parent specimen breaks into two parts. These close to form two daughter cells with approximately half the skeletal components of the adult. Subsequently reorganisation of the adhesive disc occurs, which results in the formation of a new denticle ring characteristic of the species.

Studies on interpopulational variation (Kazubski, 1971, 1976, 1979, 1980, 1981

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**INTRODUCTION**

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Studies on interpopulational variation (Kazubski, 1971, 1976, 1979, 1980, 1981

and 1982b) concentrate on the morphological variation in trichodinids parasitising the internal cavities of terrestrial molluscs and amphibians. These provide a good model for such studies due to the limited interchange of trichodinids between host individuals, allowing the parasites of each host to be treated as distinct populations. *Semitrichodina sphaeronuclea* Lom, populations were studied from two different land snails, one species of the Limacidae and one of the Zontidae (Kazubski, 1971). Kazubski considered that the two host species harboured different trichodinid forms which could be caused by host factors, or the host's chosen niche; the Limacidae inhabit dry warm areas while the Zontidae inhabit the moist and shaded banks of mountain streams. Seasonal changes were noted, with a decrease in body dimensions of summer specimens compared with spring and autumn specimens. The variability of *Semitrichodina sphaeronuclea* according to altitude was studied in *Bielzia coeruleans* (Bielz) (Kazubski, 1976), this slug species illustrates restricted niche requirements and was sampled over a period of six days from one forest at altitudes from 760-1300m. A statistically significant increase in adhesive disc diameter and denticle number was observed with increasing altitude and therefore decreasing temperature. In a later paper (Kazubski, 1981) populations of *Semitrichodina sphaeronuclea* from the Limacidae and Zontidae were studied from a localised area, Kazubski concluded that significant differences between ciliates from the two hosts were predominantly genetic.

Measurements of *Trichodina vesicularum* Faure-Fremiet and *Trichodina faurefremietii* Kazubski, parasitising newts from Poland and France (Kazubski, 1979), were subjected to analysis of variance. Geographical variability was evident in *Trichodina vesicularum* between French and Polish specimens, but populations of *Trichodina vesicularum* from different host species, *Triturus vulgaris* L. and *Triturus*

*montandoni* Boulenger, illustrated no significant variation. However, the variability between different subpopulations (one host individual) was much greater than that caused by any other factor. Variation between subpopulations of *Trichodina ranae* da Cunha, 1950 parasitising the urinary bladder of *Rana esculenta* L., is significantly different, whilst variation due to environmental factors is statistically insignificant (Kazubski, 1980). Kazubski suggests that the endoparasitic way of life these ciliates lead limits exchange of parasites and thus particular populations of *Trichodina ranae* may correspond to a single or a few mixed clones.

Studies on trichodinid populations from freshwater fish and tadpoles (Kazubski & Migala, 1968; Kazubski, 1981, 1982a, 1991a, b and c) have concentrated on analysing individual host parasite populations and seasonal variation within discrete water bodies. Investigation into seasonal variation of the morphology of trichodinids parasitising *Cyprinus carpio* in Poland (Kazubski & Migala, 1968) illustrated a marked increase in the mean adhesive disc measurements of *Trichodina nigra*, *Trichodina acuta*, *Trichodina mutabilis* and *Trichodinella subtilis* during the winter. The change in mean size was most marked in the *Trichodina* species with corresponding changes in denticle form in *Trichodina mutabilis*, conversely *Trichodina acuta* displayed little or no variation in denticle form. *Trichodina nigra* parasitising *Lucioperca lucioperca* from the Szczecin Gulf (Kabuski & Pileckarapacz, 1981) appeared to follow the trend of larger specimens being present in winter samples, but this was not statistically significant when subjected to analysis of variance. Seasonal variability was much more evident in *Trichodina reticulata* (Kazubski, 1981) parasitising *Carassius carassius* from a small pond in Kortowo. This is explained by the large temperature fluctuation in small water bodies compared to relatively stable environments such as the Szczecin Gulf. Host induced



variation of *Trichodina pediculus* from hydras, tadpoles, and crucian carp (Kazubski, 1991a, b and c) from small ponds in Kortowo was shown to be insignificant, with only underlying seasonal variation present. In summary Kazubski (1982b) suggested that in "closed" populations with very little or no exchange of individuals the interpopulational variation, probably genetic in character, is dominant while the influence of any environmental factors is inconspicuous. In "open" populations with easy exchange of individuals the significance of interpopulational variation diminishes in favour of the influence of various environmental factors.

In this chapter the morphological variation of trichodinids parasitising *Gasterosteus aculeatus* is investigated, using material collected from a population of sticklebacks at regular intervals during 1993/1994. The aims of this survey are to elucidate the trichodinid fauna of the stickleback population, to investigate intraspecific variation and to clarify the relationship between *Trichodina domerguei* and *Trichodina tenuidens* which are commonly recorded from sticklebacks throughout Europe. The definitive work on trichodinids from sticklebacks by Lom & Stein (1966), gives a detailed review of the research undertaken on the two species. *Trichodina domerguei* is found predominantly on the skin of sticklebacks, whilst *Trichodina tenuidens* is usually confined to the gills. Lom & Stein concluded that *Trichodina domerguei* displays the normal range of variation expressed in other trichodinid species examined, but *Trichodina tenuidens* is conspicuous in its extreme variability. They explained this as the result of "chemical action of the host" combined with the ability of the ciliate to react sensitively to fine changes of its microbiotope. On sticklebacks intermediate forms can be found, which when considered separately cannot be accurately identified. In Lom & Stein's (1966) opinion these are the extremes of variation ranges of either *Trichodina*

*domerguei* or *Trichodina tenuidens* and cannot give rise to the assumption that there is one highly polymorphic species.

## MATERIALS AND METHODS

Ten specimens of *Gasterosteus aculeatus* were collected randomly using a hand net from a small area of Airthrey Loch (University of Stirling). This is a small, eutrophic, manmade lake with a maximum depth of approximately 2m. It has very little inflow of water and is susceptible to a large temperature range (Fig 6.11). Samples were taken at approximately two week intervals between September 1993 and September 1994 (Table 6.1).

**Table 6.1.** Sampling dates for *Gasterosteus aculeatus* from Airthrey Loch.

Day	Date	Day	Date
1	7/9/93	209	4/4/94
13	20/9/93	226	21/4/94
27	4/10/93	245	10/5/94
48	25/10/93	261	26/5/94
71	17/11/93	280	14/6/94
83	29/11/93	295	29/6/94
106	22/12/93	310	14/7/94
127	12/1/94	330	3/8/94
143	28/1/94	347	20/8/94
156	10/2/94	360	2/9/94
176	2/3/94	380	22/9/94
192	18/3/94		

Water temperature was recorded using a maximum-minimum thermometer at a depth of 1 m. Two smears were taken from both gill and skin for each fish and silver stained. Where possible a minimum of 40 well stained trichodinid specimens from both the skin and gills were randomly selected and photographed for each sampling date. (On the rare

occasion that specimens obviously belonging to *Trichodina domerguei* were found on the gills and specimens clearly belonging to *Trichodina tenuidens* were found on the skin, they were not included in the analysis. This was justified because the primary interest of this analysis was to investigate the morphometric overlap of the two species). The specimens were measured and analysed using PCA in the same way as described in Chapter 2. Pearson's correlation is used as a measure of dissimilarity (Shinn, 1993), which summarises the nature of relationships between morphometric variables.

## RESULTS

The morphometric and meristic data for the specimens of *Trichodina domerguei* and *Trichodina tenuidens* (N=1189) collected over a 12 month period from *Gasterosteus aculeatus* (Airthrey Loch) were subjected to Principal Components Analysis. The component loadings of the first three Factors are given in Table 6.2. This illustrates that adhesive disc diameter, denticle ring diameter and denticle span are the main variables acting in component one, with border membrane width, denticle length and central part width acting in the opposite direction to the other variables. Component two identifies denticle length and central part width as the key variables, with denticle number and ray length acting against the other variables. Component three only identifies border membrane width and to a lesser extent ray length as having any significant effect. The percentage variance explained by the first three factors is given in table 6.3.

One objective of this analysis was to investigate the relationship between the skin and gill trichodinids. The PCA plot is presented in Figure 6.1, where the skin specimens (represented by the letter a) and gill specimens (represented by the letter b) are enclosed by ellipses including seventy percent of each sub-set. The skin and gill

**Table 6.2.** Component loadings on the first three factors in the analysis of trichodinids from *Gasterosteus aculeatus*.

	1	2	3
A. d. diam	0.934	0.246	0.003
B. m. width	-0.375	0.307	0.864
D. r. diam	0.943	0.173	0.024
Dent. no	0.836	-0.403	0.020
D. length	-0.174	0.887	-0.098
B. length	0.642	0.647	-0.087
R. length	0.805	-0.345	0.234
C. p. width	-0.109	0.785	-0.079
Dent. span	0.909	0.177	0.136

populations show a clear separation with little or no overlap between them. When the skin specimens were examined in isolation it was apparent that there was one form

**Table 6.3.** Percentage of variance explained by the first three principal components.

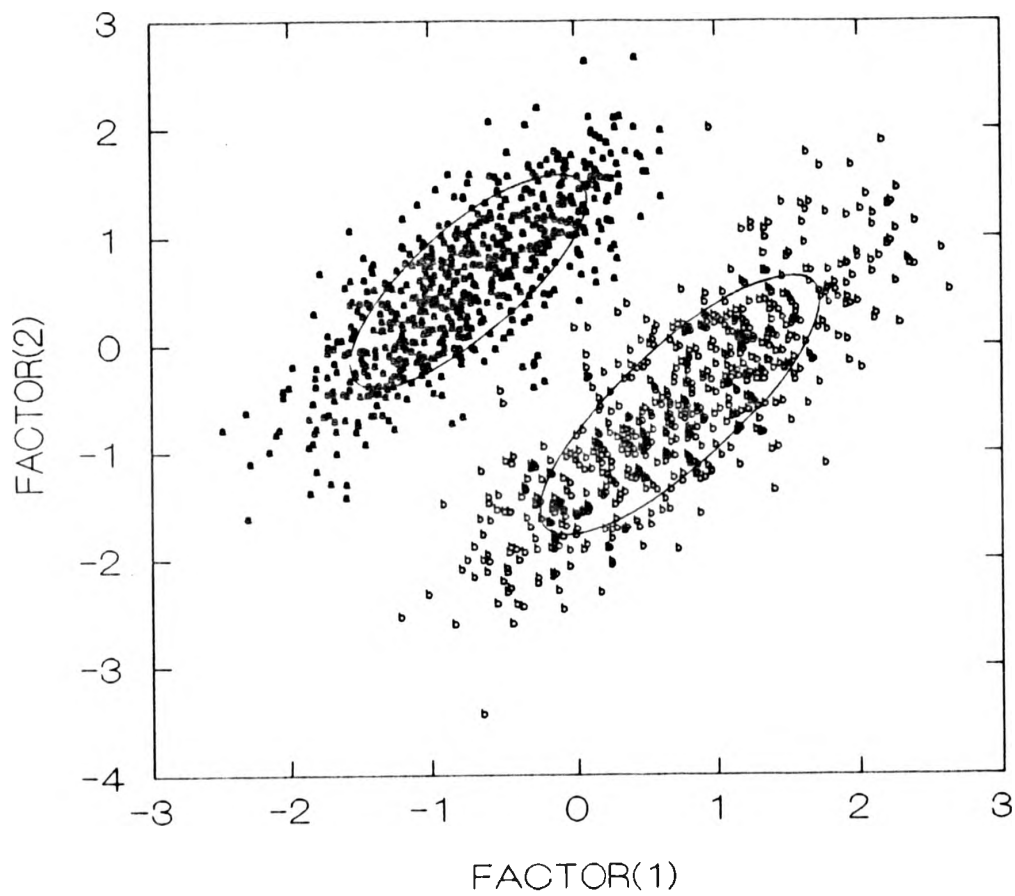
	Variance explained by components	% of total variance	Cumulative percentage
Factor 1	4.531	50.343	50.343
Factor 2	2.319	25.769	76.112
Factor 3	0.843	9.371	85.483

conforming closely to morphometric data and photomicrographs given by Lom & Stein (1966) for *Trichodina domerguei*. Details of the morphometric and meristic data for this species are given in Table 6.4.

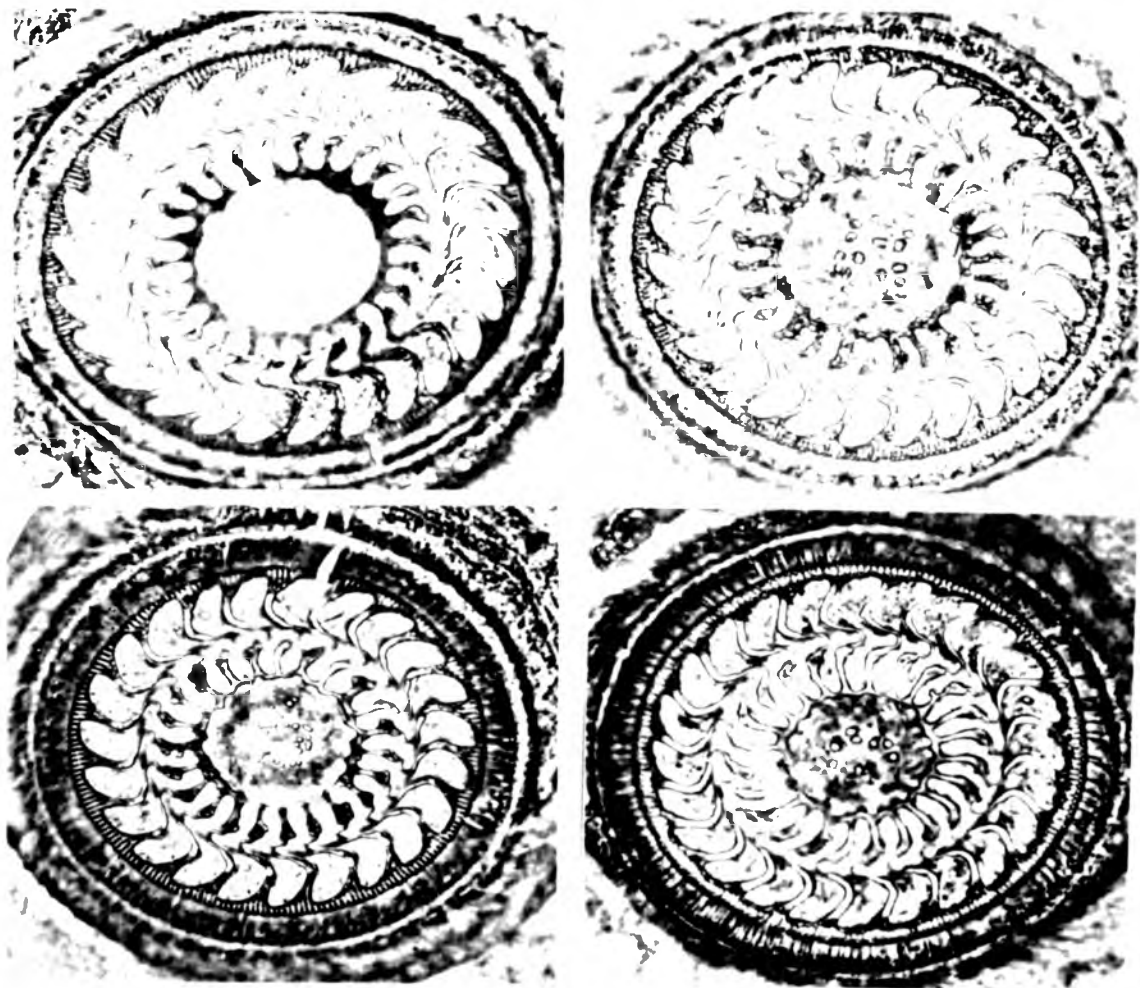
**Table 6.4.** Morphometric and meristic data for *Trichodina domerguei*.

Author of description	Lom & Stein (1965)	This study
Host	<i>Pungitius pungitius</i> and <i>Gasterosteus aculeatus</i>	<i>Gasterosteus aculeatus</i>
Localisation	skin, rarely gills	skin, rarely gills
Locality	Kristatalka-creek near Leningrad	Airthrey Loch, Stirling
A. d. diam	45.0-90.0 (51)	43.9-72.4 (56.6±5.6, 599)
B. m. width	3.5-5.0	3.1-6.0 (4.7±0.4, 599)
D. r. diam	28.0-33.0 (31)	22.2-38.4 (29.8±3.0, 599)
Dent. no	22-28 (24)	20-30 (24±1.6, 599)
D. length	11.0-12.0	6.6-10.4 (8.5±0.7, 599)
B. length	3.5-7.0	4.6-9.5 (6.5±0.8, 599)
R. length	4.0-5.0	3.4-6.5 (4.7±0.6, 599)
C. p. width	3.0	1.6-3.2 (2.3±0.2, 599)
Dent. span	-	10.4-17.2 (13.6±1.2, 599)

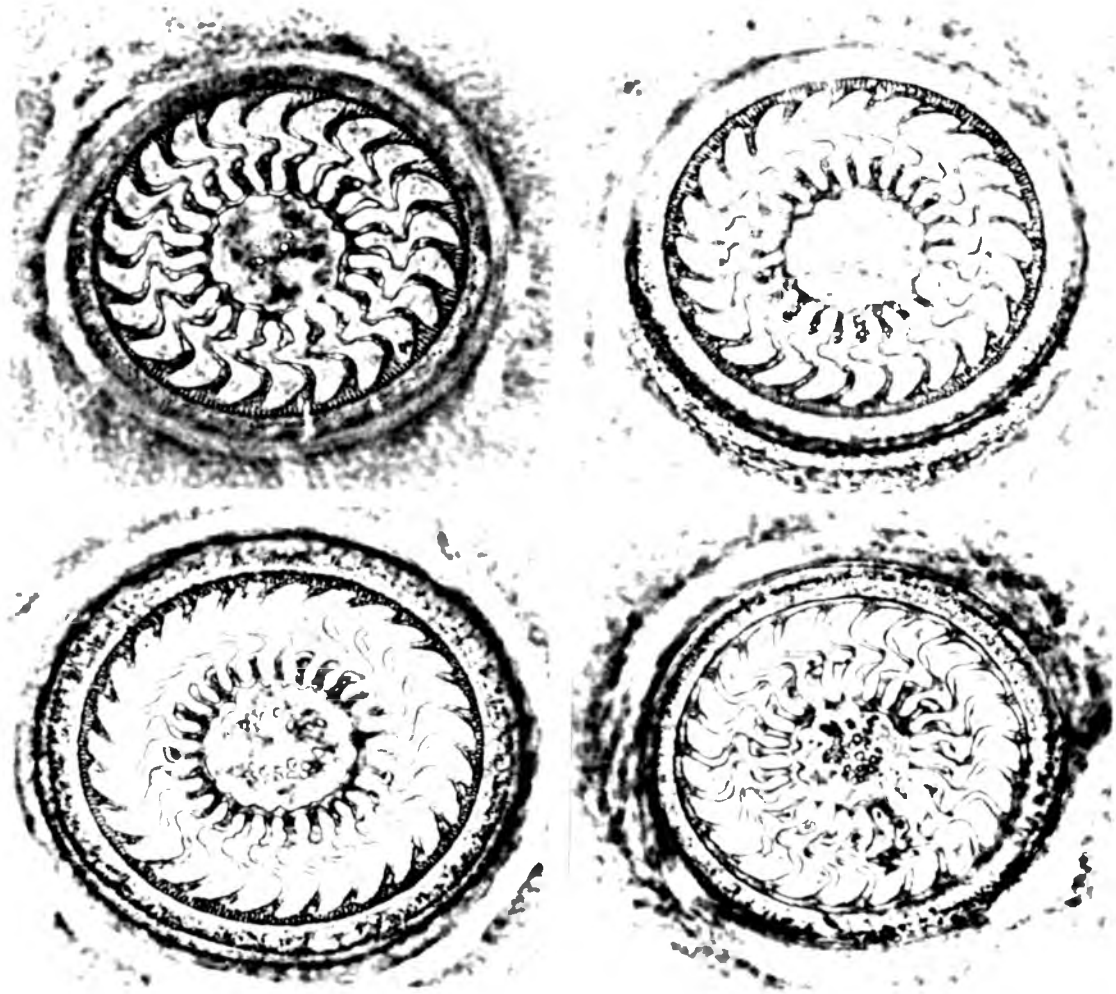
Photomicrographs of *Trichodina domerguei* are illustrated in Figures 6.2 and 6.3, with the range of denticle form shown in Figure 6.4. Denticle blade morphology varies considerably, in the degree of curvature displayed by the posterior margin, and the appearance of the anterior margin which is either continuous, or stepped forming a flattened or curved distal surface. The posterior point of the central part of the denticle varies from a flattened to a rounded appearance. Rays vary in width, the angle in which they project from the central part and in their curvature. Distance between the points of rays and border of the central circle varies from a significant gap to almost touching. Central circle appearance is relatively consistent between specimens, with limited variation in size and form. It must be again stressed that these descriptions are based



**Figure 6.1.** Plot of Factor 1 against Factor 2 in the PCA of skin and gill trichodinid populations from *Gasterosteus aculeatus*; a, gill specimens; b, skin specimens.



**Figure 6.2.** Silver stained adhesive disc morphology in specimens of *Trichodina domerguei* from the skin of *Gasterosteus aculeatus*. (1200 $\times$ ).



**Figure 6.3.** Silver stained adhesive disc morphology in specimens of *Trichodina domerguei* from the skin of *Gasterosteus aculeatus*. (1200x).



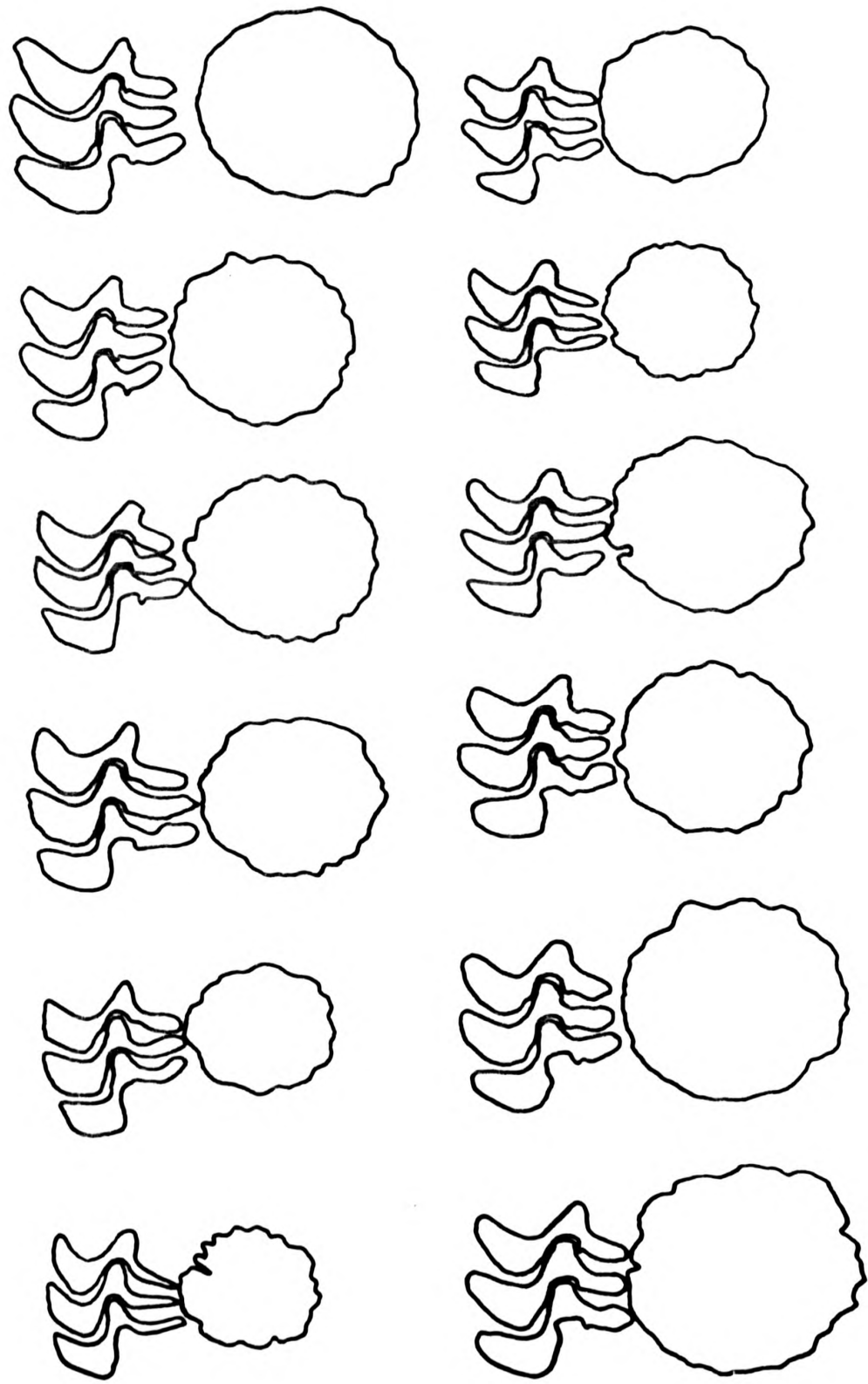
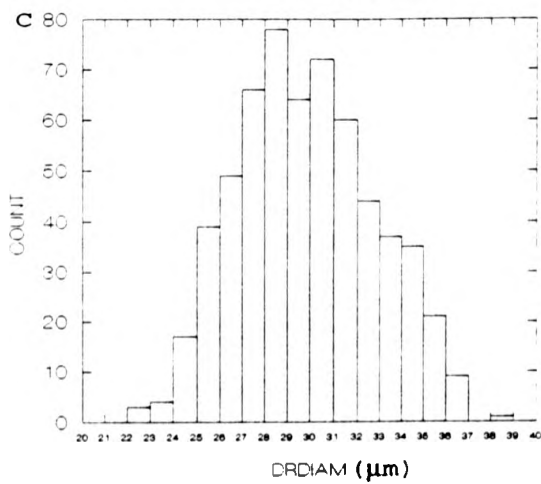
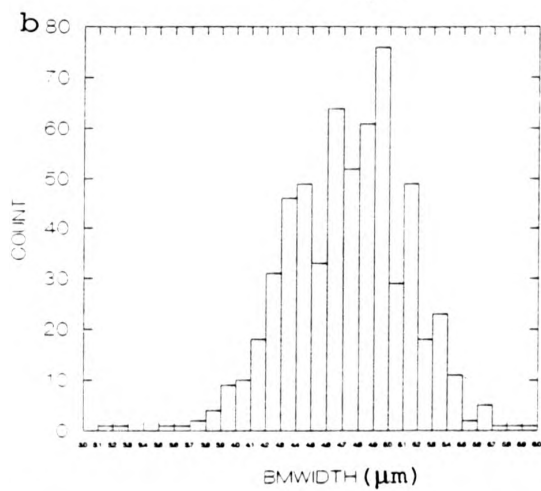
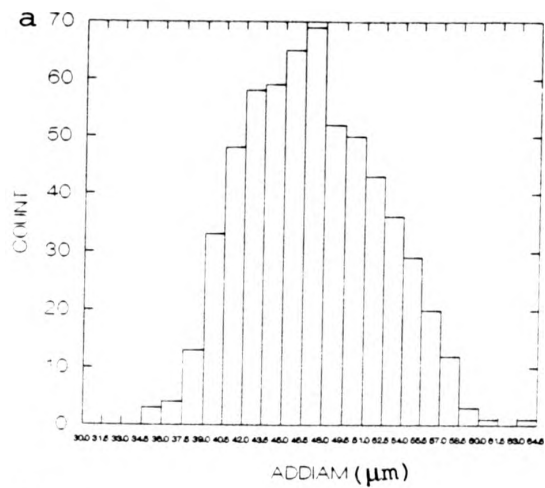


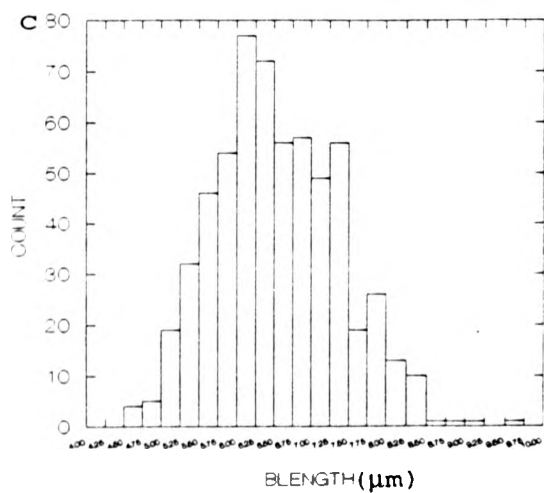
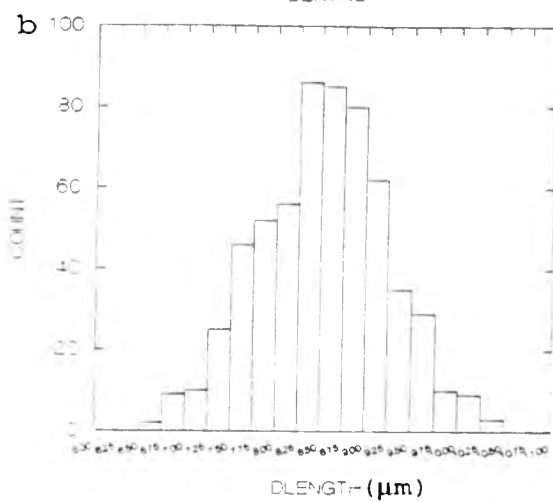
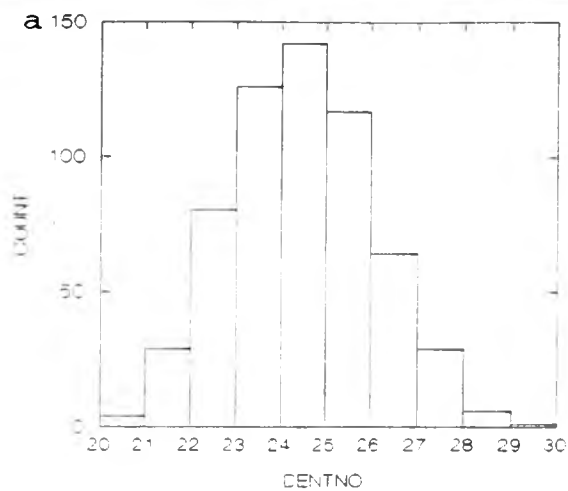
Figure 6.4. Range of morphological variation in denticle form of *Trichodina domerguei*

on silver stained specimens which are subject to artifact, and therefore may not completely represent actual morphology.

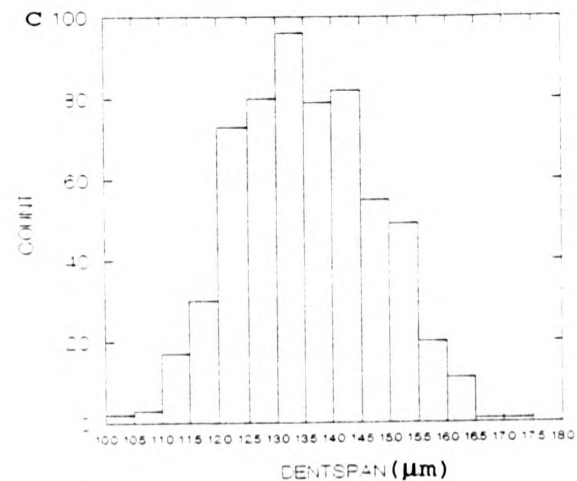
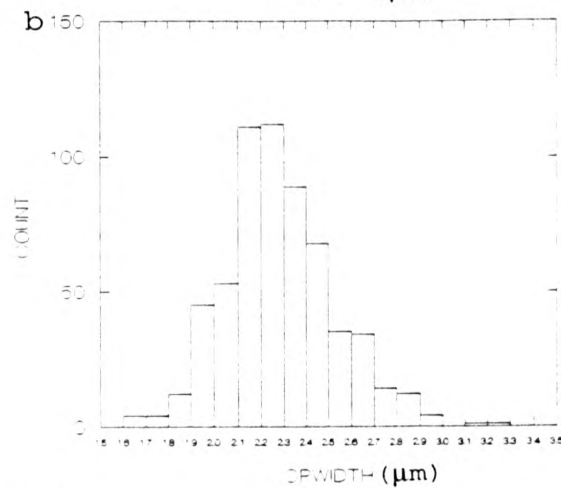
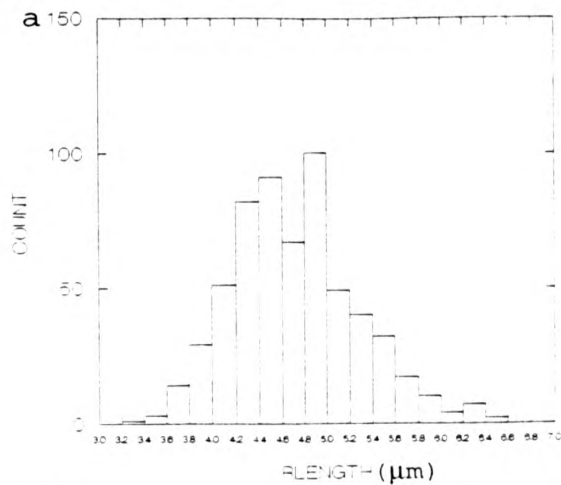
*Trichodina domerguei* exhibits variation in size of skeletal structures similar to the data given by Lom & Stein (1966). The histograms (Figures 6.5, 6.6 and 6.7) show the range and normality of each variable which can be described by skewness and kurtosis. Skewness describes the asymmetry of distribution of a histogram about its mean. If the longer "tail" is to the left this is described as negative skewness, to the right positive skewness. Most of the histograms exhibit significant positive skewness (significance =  $2 \cdot \sqrt{6/n}$  where  $n$  = sample size), indicating that wider variation between specimens occurs in the upper end of the size range where measurements are greater than the mean. The exceptions being border membrane width and denticle length which are negatively skewed, indicating that variation of these variables is greatest for measurements smaller than the mean. Kurtosis (significance =  $24/n$  where  $n$  = sample size) describes a histogram relative to "normal distribution", a positive value indicating it to be longer tailed (more widely distributed about the mean) than "normal", a negative value being flatter (more closely distributed about the mean) than "normal". Again, most of the variables exhibit significant positive kurtosis apart from border membrane width, central part width and ray length which exhibit negative kurtosis. The Pearson's correlation matrix (Table 6.5) summarises the relationships between variables. To ascertain the significance of the relationships summarized in the matrix, regression analysis was undertaken between key variables and adhesive disc diameter. Denticle ring diameter ( $r^2=0.883$ ,  $p<0.0001$ ), denticle span ( $r^2=0.666$ ,  $p<0.0001$ ) (of which blade length, central part width and ray length are components), denticle length ( $r^2=0.488$ ,  $p<0.0001$ ) and denticle number ( $r^2=0.176$ ,  $p<0.0001$ ) were positively correlated with



**Figure 6.5.** Histograms of: a, adoral disc diameter; b, border membrane and c, denticle ring diameter in *Trichodina domerguei*.



**Figure 6.6.** Histograms of: a, denticle number; b, denticle length and c, blade length in *Trichodina domergeui*.



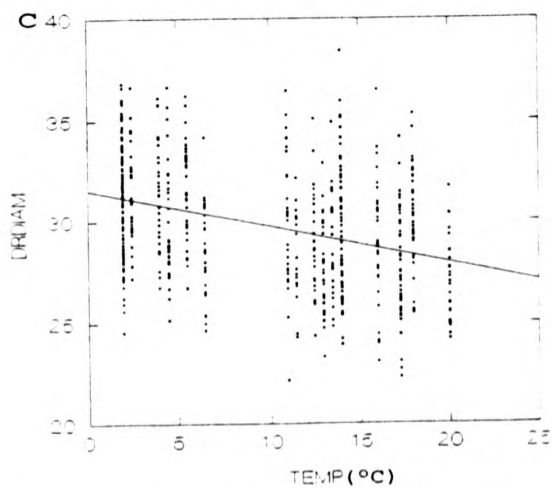
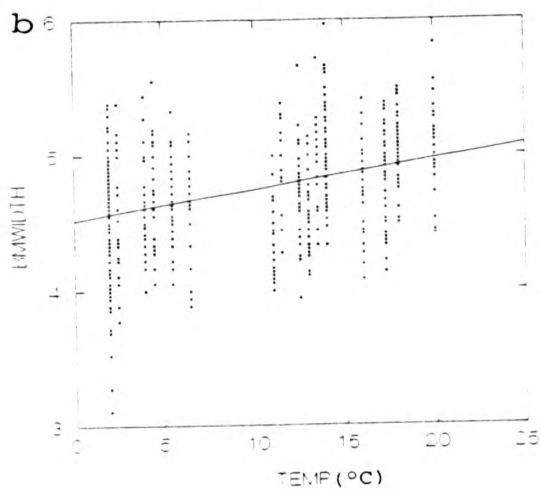
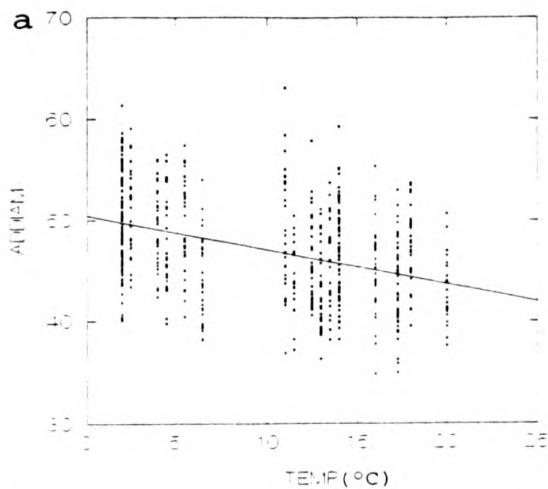
**Figure 6.7.** Histograms of: a, ray length; b, central part width and c, denticle span in *Trichodina domerguei*.

	A. d. diam	B. length	B. m. width	C. p. width	Date	Dent. no	Dent. span	D. length	D. r. diam	R. length	Temp
A. d. diam	1.000										
B. length	0.850	1.000									
B. m. width	0.158	-0.056	1.000								
C. p. width	0.328	0.241	0.104	1.000							
Date	-0.050	-0.124	0.104	0.297	1.000						
Dent. no	0.400	0.310	-0.104	-0.014	-0.125	1.000					
Dent. span	0.820	0.853	0.092	0.399	0.042	0.155	1.000				
D. length	0.685	0.685	-0.036	0.232	0.018	-0.019	0.714	1.000			
D. r. diam	0.932	0.786	0.025	0.300	-0.034	0.470	0.773	0.685	1.000		
R. length	0.363	0.297	0.196	0.102	0.132	-0.118	0.676	0.407	0.372	1.000	
Temp.	-0.348	-0.450	0.345	0.186	0.526	-0.505	-0.137	-0.193	-0.360	0.304	1.000

**Table 6.5.** Correlation matrix of the variables used in the analysis of seasonal variation in *Trichodina domerguei*.

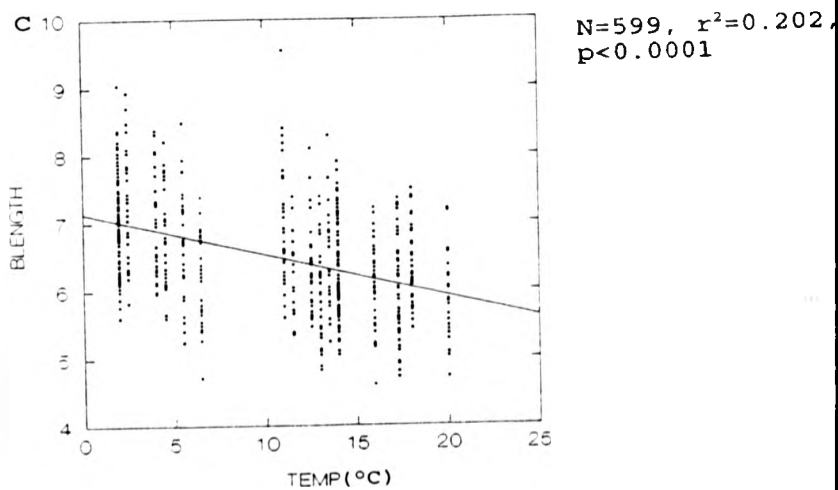
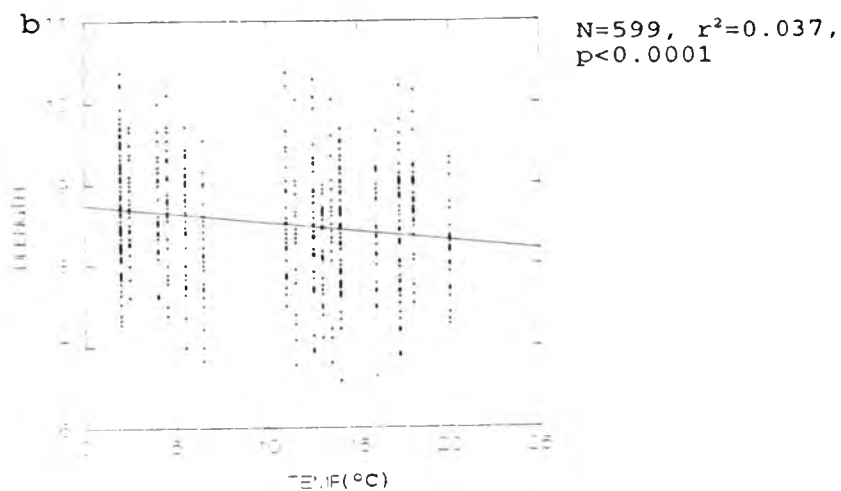
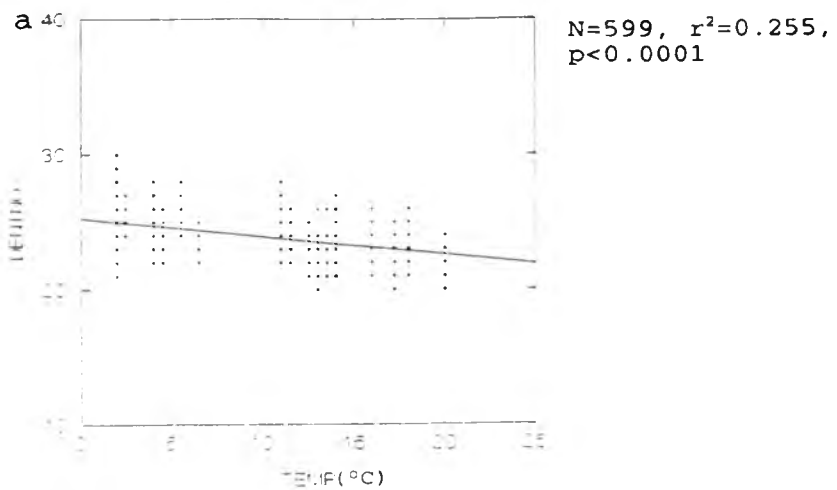
adhesive disc diameter, border membrane width was the exception displaying no significant correlation. Thus, the size and number of denticles increases with diameter of adhesive disc.

Temperature dependent morphological variation is evident in many trichodinid populations, as discussed in the introductory section of this chapter. The relationships between the morphological variables and temperature in *Trichodina domerguei* are summarised in the regressions illustrated in Figures 6.8, 6.9 and 6.10. Figure 6.11 displays the temperature profile in Airthrey Loch, rising from 2°C in the winter to 20°C during the summer. Adhesive disc diameter decreases significantly with increase in temperature, decreasing from 50.4µm at 2-2.5°C (N=150) to 44.6µm at 17.25-20°C (N=103). Denticle ring diameter shows a similar significant decrease from 31.4µm in winter to 28.5µm during the summer months, while denticle number decreases significantly from 25 to 22.7. Increase in temperature also illustrates a significant negative correlation with denticle length. Denticle span decreases significantly with increasing temperature from 13.9µm (low temperatures) to 13.4µm (high temperatures), but to a lesser degree than the variables previously mentioned. The trend explained by the regression of denticle span against temperature is an approximate sum of the variables: blade length, central part width and ray length. Ray length and central part width can be seen to increase significantly with increasing temperature, and therefore mask the significant decrease in blade length when considered jointly as denticle span. Border membrane width in addition to central part width and ray length increase with increasing temperature. These last three variables are opposed to the general trend, interestingly they are the three smallest morphometric variables considered in the analysis ranging from approximately 1.5-6.5µm.

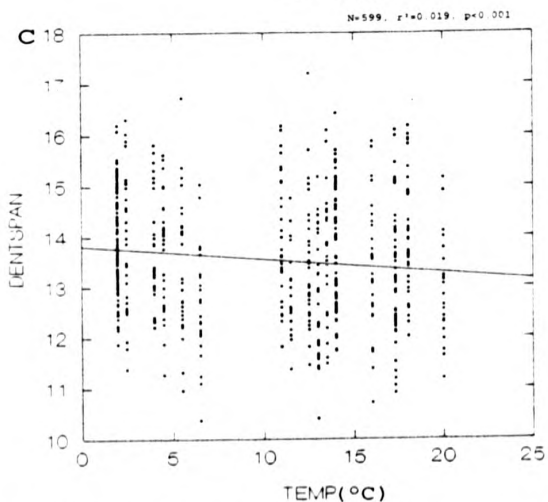
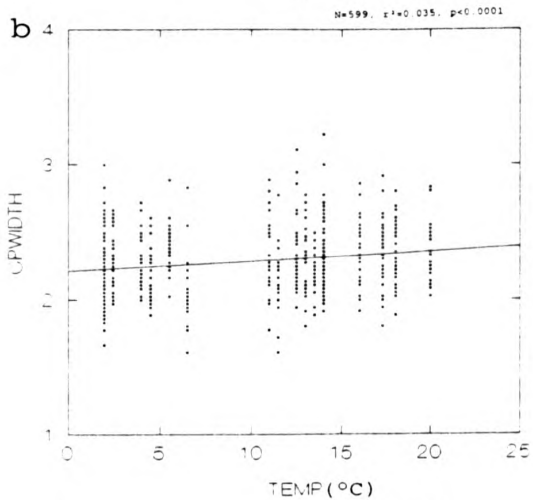
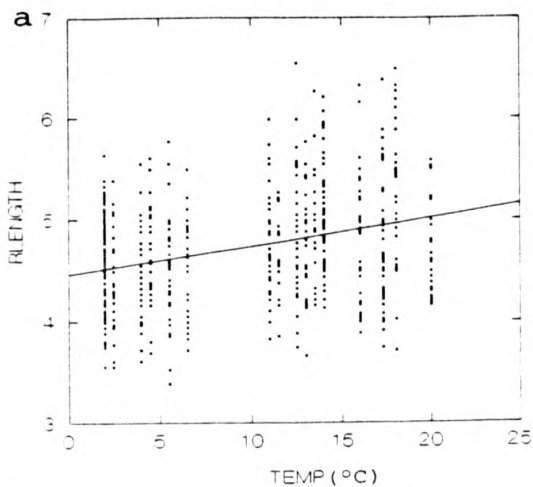


**Figure 6.8.** Regressions of a, adoral disc diameter; b, border membrane width and c, denticle ring diameter against temperature in *Trichodina domerguei*.

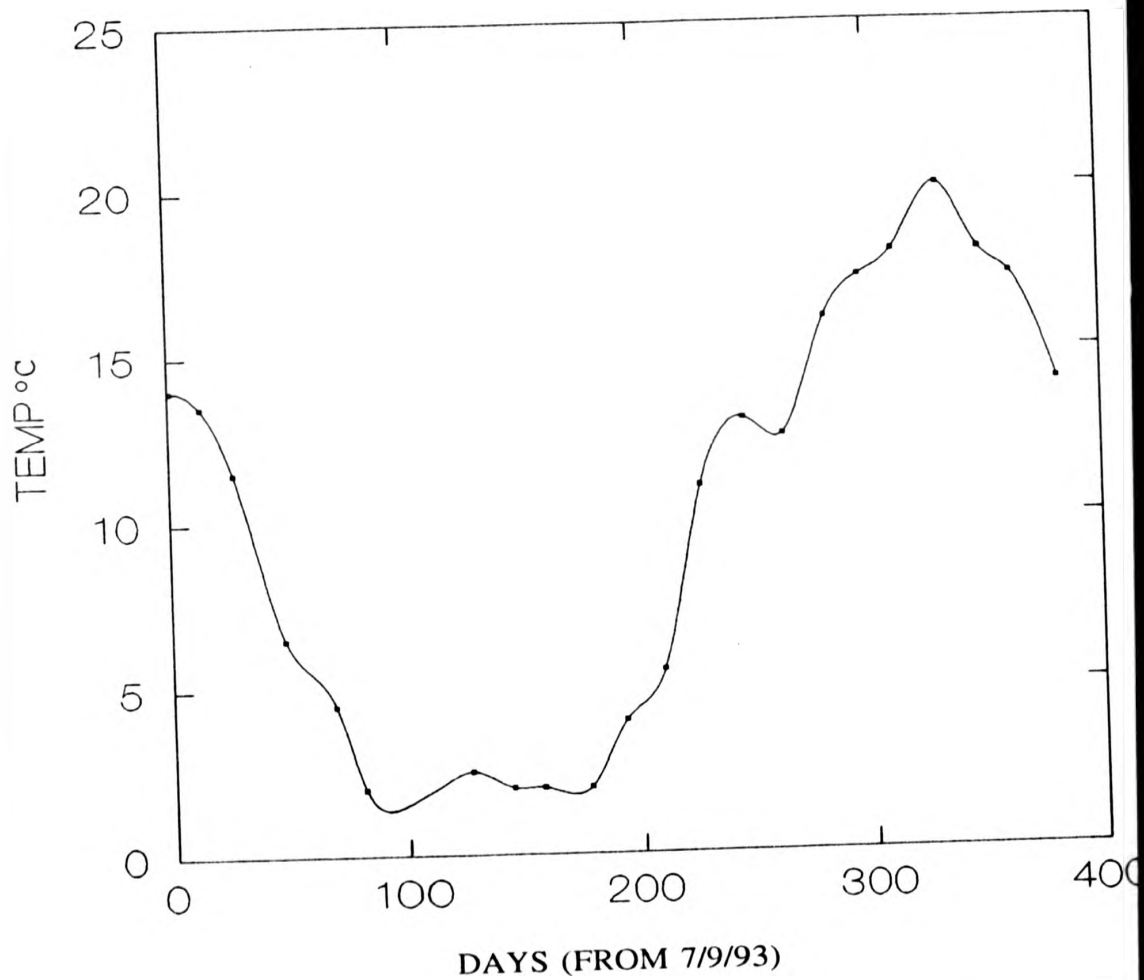




**Figure 6.9.** Regression of a, denticle number; b, denticle length and c, blade length against temperature in *Trichodina domerguei*.



**Figure 6.10.** Regression of a, ray length; b, central part width and c, denticle span against temperature in *Trichodina domerguei*.



**Figure 6.11.** Temperature profile of Airthrey Loch from September 1993 to September 1994.

The gill population comprising of *Trichodina tenuidens* specimens were variable in character with many specimens conforming to the data and photomicrographs given by Lom & Stein (1966) for *Trichodina tenuidens*.

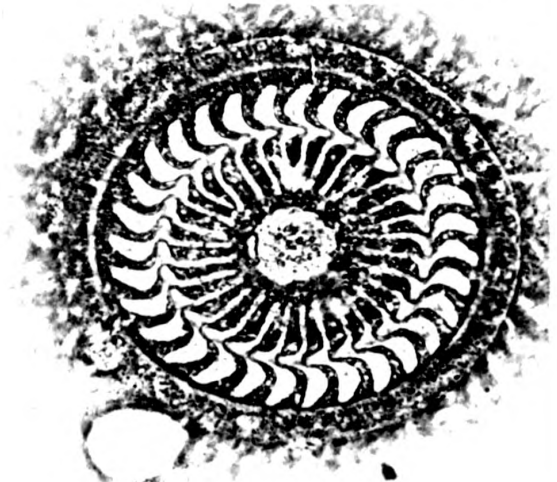
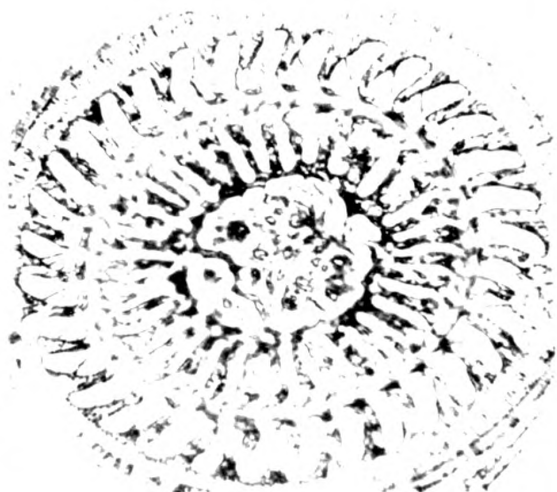
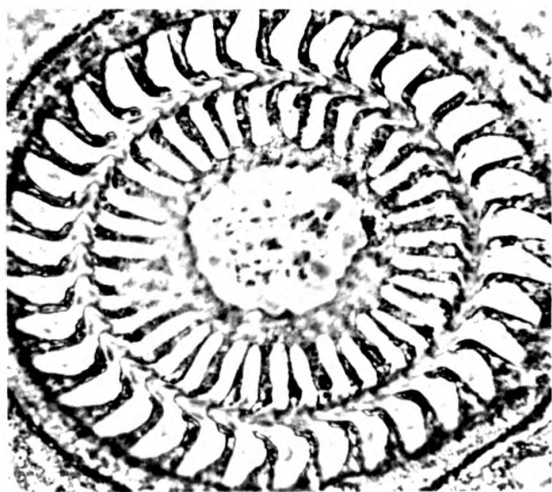
**Table 6.6.** Morphometric and meristic data for *Trichodina tenuidens*.

Author of description	Lom & Stein, 1966	our findings
Host	<i>G. aculeatus</i>	<i>G. aculeatus</i>
Localisation	gills	Gills, rarely skin
Locality	Lake Mamry, Poland	Airthrey Loch
A. d. diam	40.0-62.0 (50.0)	39.0-75.6 (55.9±7.3, 590)
B. m. width	4.5-5.0	3.3-5.5 (4.4±0.3, 590)
D. r. diam	25.0-41.0 (31.0)	25.2-48.6 (36.0±4.7, 590)
Dent. no	25-33 (28)	26-40 (31.4±2.3, 590)
D. length	7.0-9.0	5.3-9.6 (7.2±0.7, 590)
B. length	4.5-7.0	4.8-9.1 (6.7±0.9, 590)
R. length	5.0-7.0	4.8-9.4 (6.9±0.9, 590)
C. p. width	2.0-2.5	1.2-3.1 (2.0±0.3, 590)
Dent. span	-	9.6-20.7 (15.6±1.6, 590)

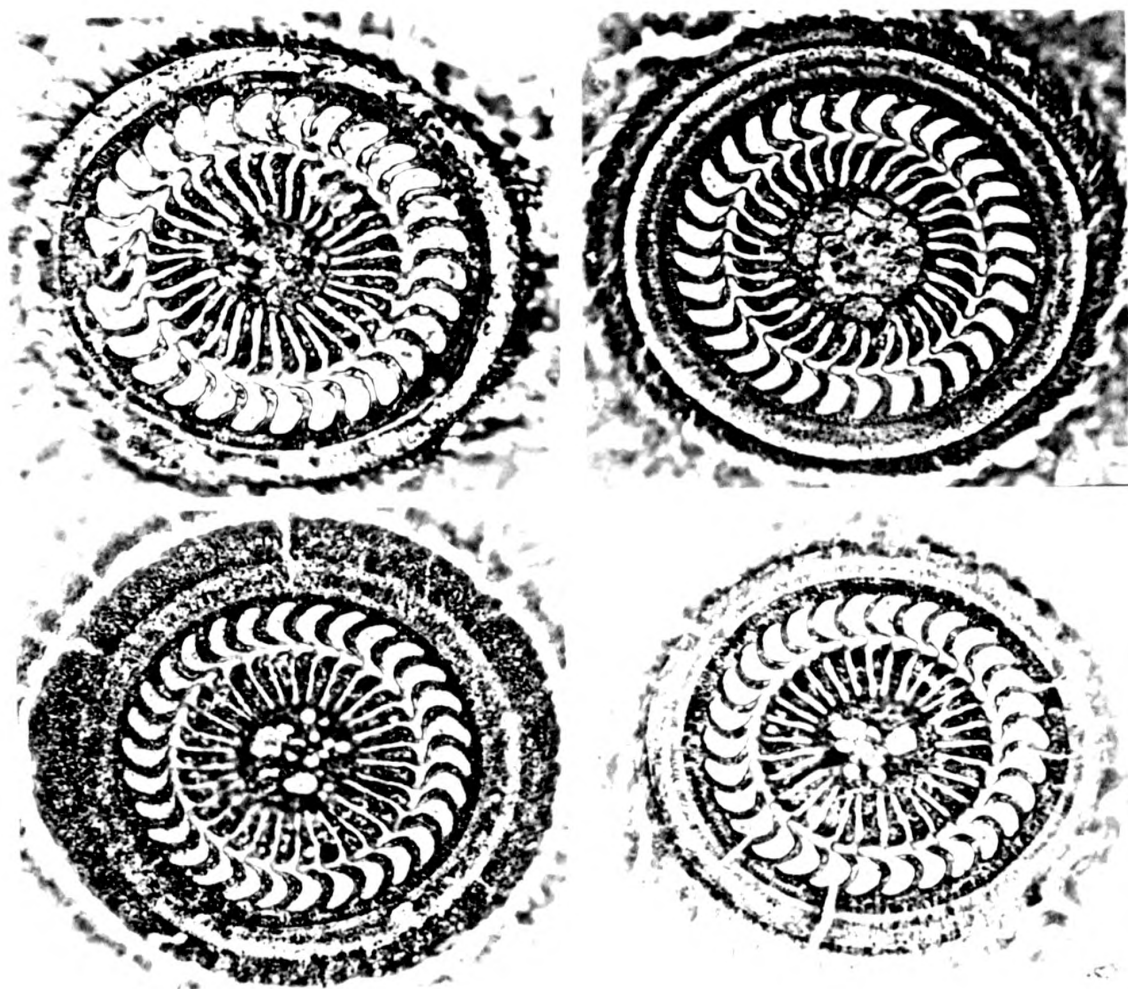
However, there were many specimens which looked significantly distinct to suggest that a second form might be present. Cluster analysis was carried out on the factor scores of a second PCA, run using data from the gill specimens. Two sub-sets of the gill data were looked for by determining two clusters from the factor scores. These were cross referenced with the photomicrographs, but the separation appeared random. Cluster analysis was therefore unable to differentiate two forms which suggests that a single highly variable form was present. Details of the morphometric and meristic data for *Trichodina tenuidens* are given in Table 6.6. The range of denticle form in

*Trichodina tenuidens* is illustrated in Figures 6.12, 6.13 and 6.14. Blade shape varies considerably, from an almost rectangular shape with slight anterior and posterior margin curvature and a pronounced flattened distal surface, to a crescent or semi-lunar form displaying considerable curvature and no distal surface. The central part of the ray is less distinct than in *Trichodina domerguei*, with no visible apophysis or "step" at the junction with the denticle ray. This last structure is also extremely variable, being straight and perpendicular to the central part in some specimens, angled slightly to the posterior in others and in a few cases angled significantly to the anterior of the denticle. Most rays have a rounded tip, and vary in width from fine to robust in character. The argentophilic central circle although present in some form, varies considerably in *Trichodina tenuidens* from a large continuous disc to a much smaller or fragmented arrangement. Individuals with shorter more curved blades seem to correlate with a small or fragmented disc, individuals with straighter blades and more robust rays appear to possess a more *Trichodina domerguei* "like" central circle. Denticle form is extremely variable, with specimens from opposite ends of the range appearing to be significantly different.

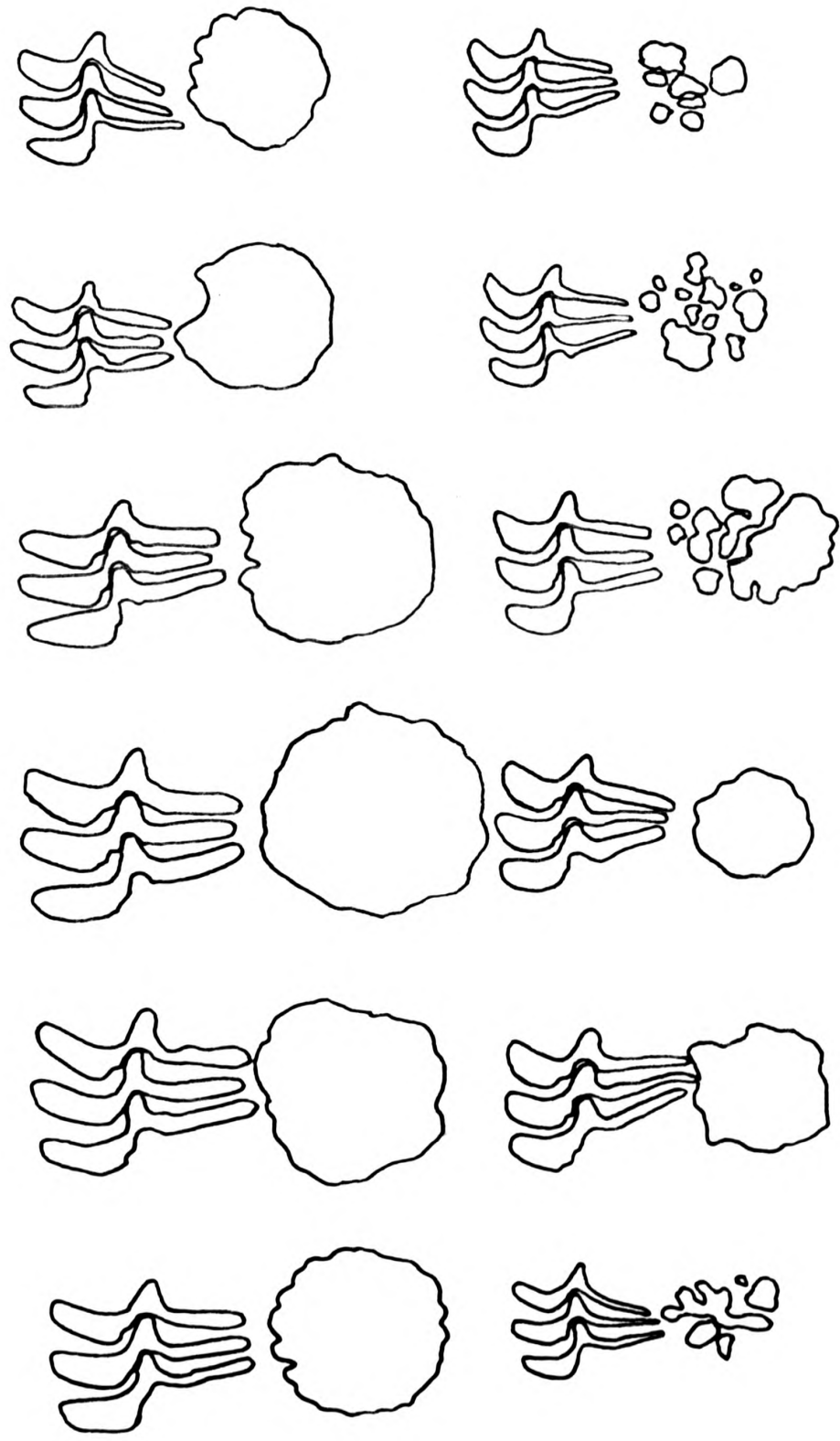
*Trichodina tenuidens* displays greater variation (also noted by Lom & Stein, 1966) when the data is compared to that of *Trichodina domerguei*, the size of the adhesive disc, number of denticles and denticle span stand out as being significantly more variable. Figures 6.15, 6.16 and 6.17 illustrate the range in size of the measured variables in *Trichodina tenuidens*, as well as the normality of the data. Blade length, denticle number and denticle length exhibit a greater degree of positive skewness than in *Trichodina domerguei*, indicating greater variation between specimens for these variables where values are greater than the mean. The remaining variables also show



**Figure 6.12.** Silver stained adhesive disc morphology in specimens of *Trichodina tenuidens*. (1200 $\times$ ).



**Figure 6.13.** Silver stained adhesive disc morphology in specimens of *Trichodina tenuidens*. (1200 $\times$ ).



**Figure 6.14.** Range of morphological variation in denticle form of *Trichodina tenuidens*.

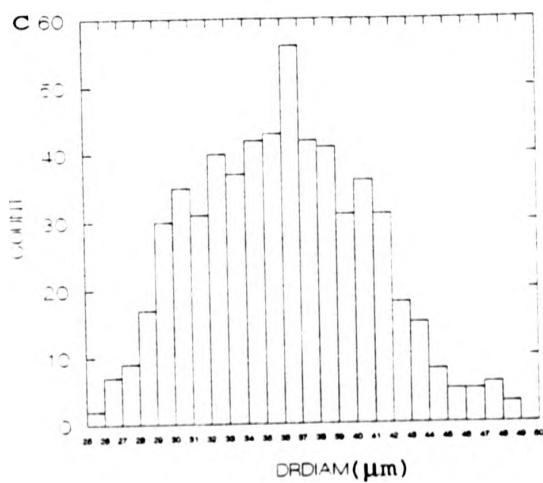
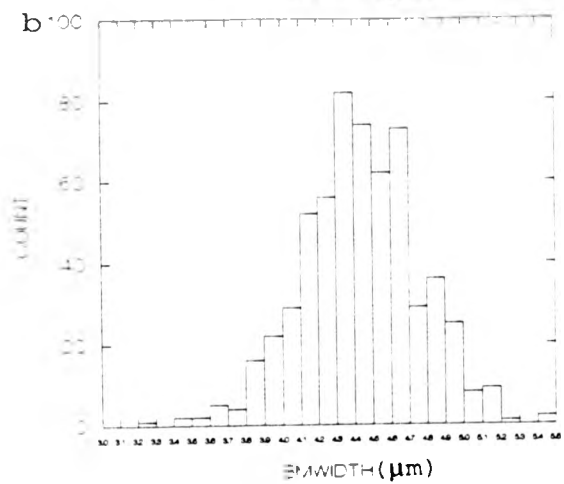
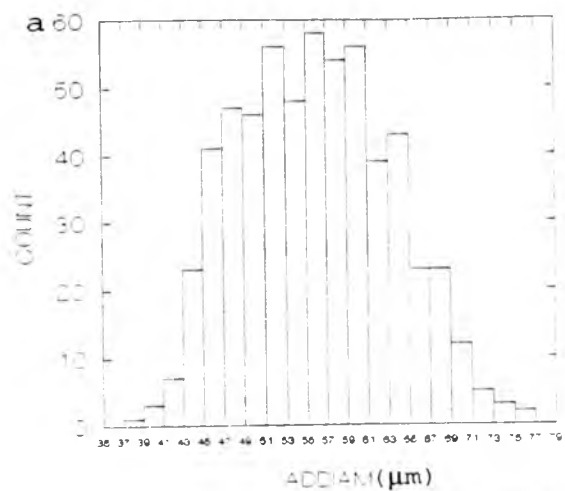


significant positive skewness, apart from border membrane width which, as in *Trichodina domerguei*, is negatively skewed. The distribution of values about their mean as described by kurtosis is greater for denticle number, denticle span and denticle length than in *Trichodina domerguei*. The values for ray length in *Trichodina tenuidens* are more closely distributed about the mean illustrating negative rather than positive kurtosis as in *Trichodina domerguei*. A Pearson's correlation matrix (Table 6.7) describes the relationships between variables for *Trichodina tenuidens*, regressions were carried out between variables to ascertain the significance of correlations. The correlations between adhesive disc diameter and the other variables were similar to those described for *Trichodina domerguei*. Denticle number illustrated a stronger positive correlation with adhesive disc diameter in *Trichodina tenuidens*. Border membrane width was negatively correlated against adhesive disc diameter whereas no significant relationship was observed in *Trichodina domerguei*. A stronger positive correlation between denticle span and denticle number was also apparent in *Trichodina tenuidens*.

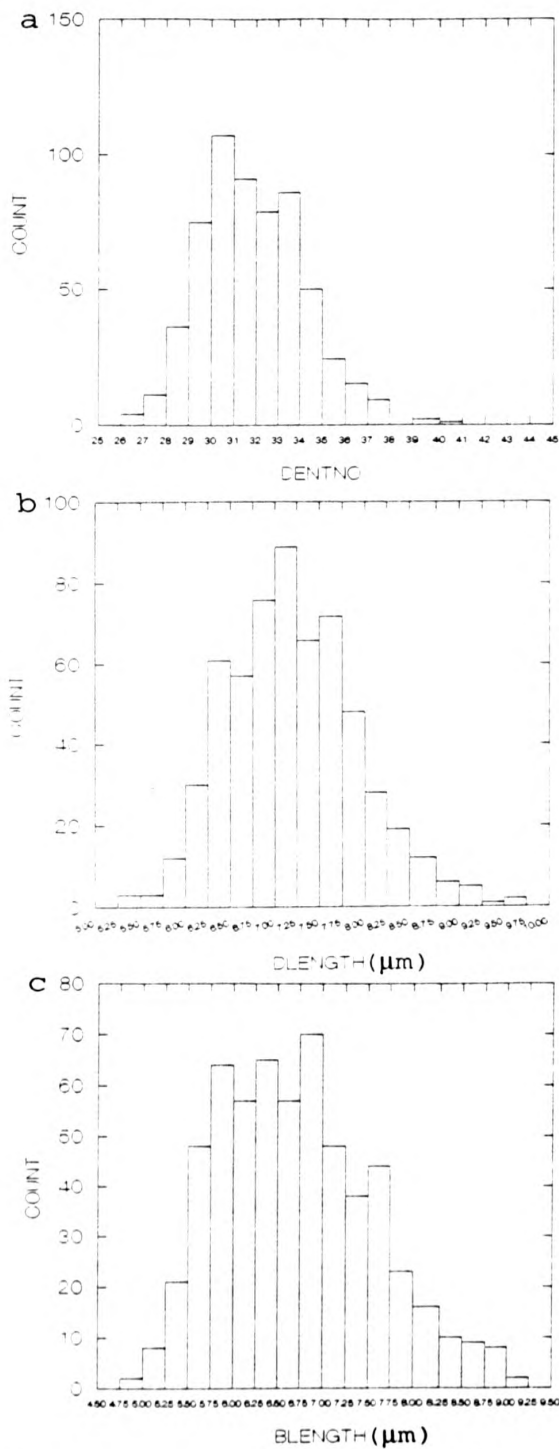
The temperature related trends in morphological variation of *Trichodina tenuidens* are illustrated in Figures 6.18, 6.19 and 6.20. Regressions of each variable against temperature illustrate a remarkable similarity to those of *Trichodina domerguei*. Adhesive disc diameter decreases from 60.8 $\mu\text{m}$  (2-2.5°C, N=115) to 52.6 $\mu\text{m}$  (17.25-20°C, N=153), denticle ring diameter decreases from 38.6 $\mu\text{m}$  to 34.0, denticle number from 33.0 to 30.4, and denticle span from 16.3 $\mu\text{m}$  to 15.1 $\mu\text{m}$  with increasing temperature. Denticle span decreases more noticeably with increase in temperature in *Trichodina tenuidens*, because ray length shows no significant trend and central part width decreases very slightly; as opposed to the increase in the size of both variables in *Trichodina domerguei*. Blade length decreases from 7.2 $\mu\text{m}$  (low temperature) to

	A. d. diam	B. length	B. m. width	C. p. width	Date	Dent. no	Dent. span	D. length	D. r. diam	R. length	Temp
A. d. diam	1.000										
B. length	0.878	1.000									
B. m. width	-0.119	-0.094	1.000								
C. p. width	0.531	0.503	-0.030	1.000							
Date	-0.140	-0.116	0.042	0.194	1.000						
Dent. no	0.651	0.569	-0.154	0.341	-0.054	1.000					
Dent. span	0.775	0.809	-0.058	0.507	-0.067	0.443	1.000				
D. length	0.736	0.711	-0.037	0.427	-0.076	0.288	0.674	1.000			
D. r. diam	0.946	0.848	-0.099	0.554	-0.115	0.677	0.755	0.718	1.000		
R. length	0.396	0.371	0.017	0.130	-0.077	0.158	0.752	0.418	0.400	1.000	
Temp	-0.409	-0.341	0.274	-0.139	0.547	-0.393	-0.256	-0.247	-0.372	-0.065	1.000

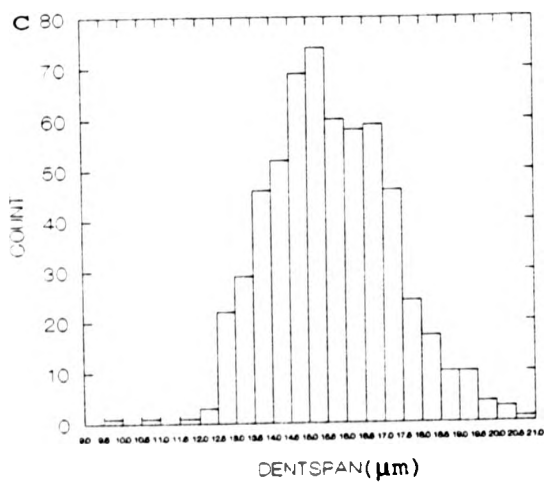
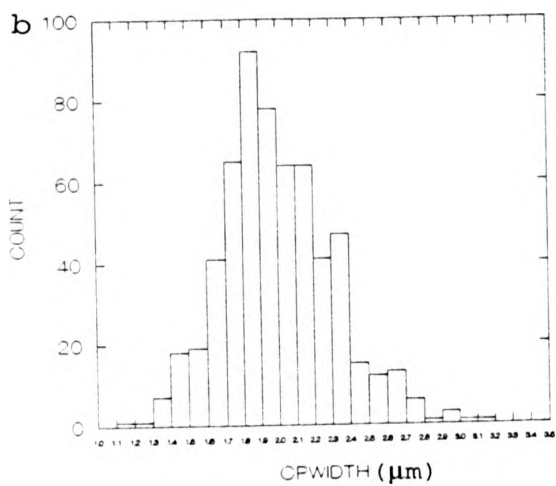
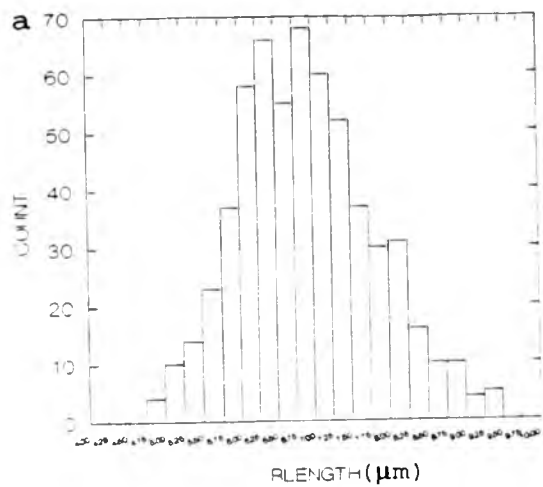
Table 6.7. Correlation matrix of the variables used in the analysis of seasonal variation in *Trichodina tenuidens*.



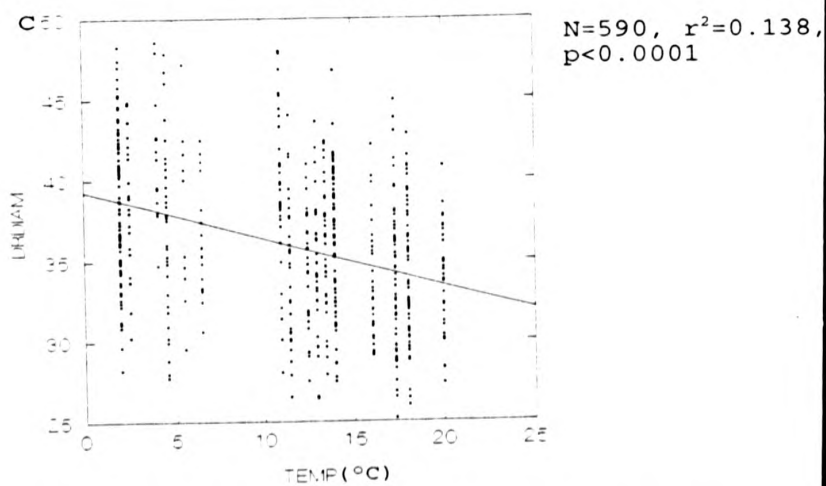
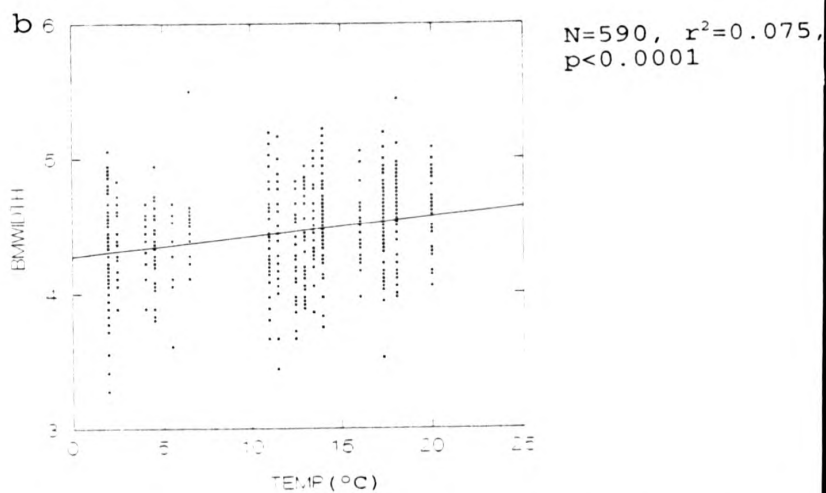
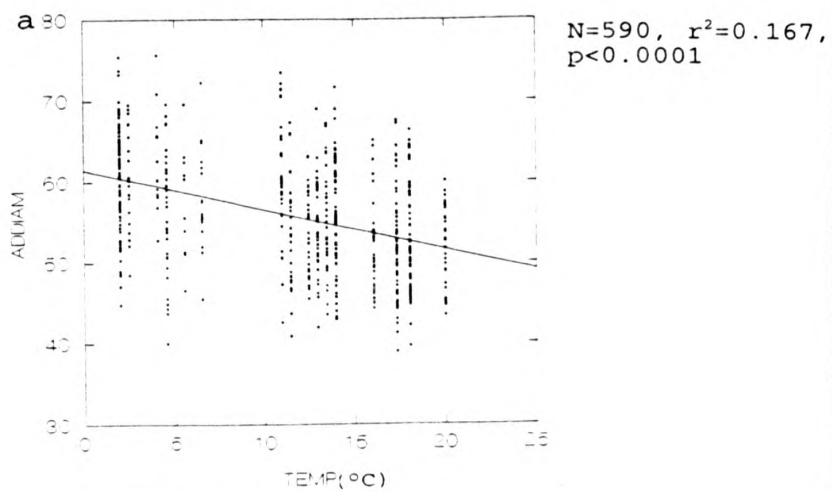
**Figure 6.15.** Histograms of: a, adoral disc diameter; b, border membrane and c, denticle ring diameter in *Trichodina tenuidens*.



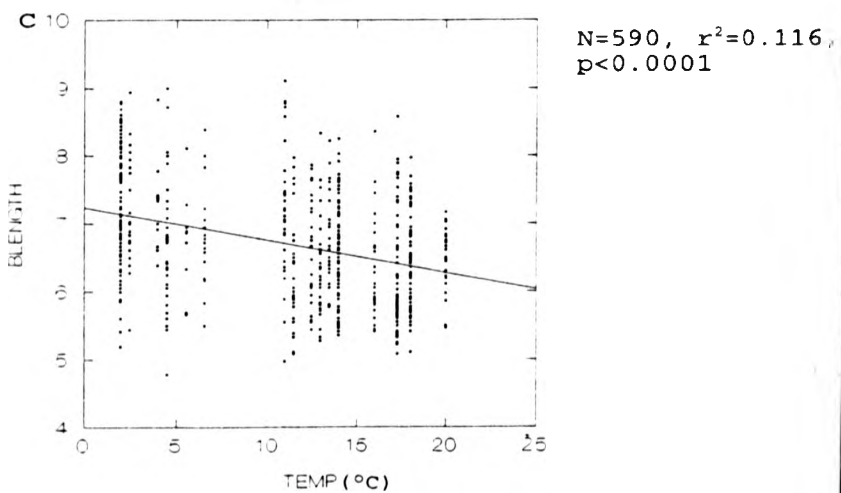
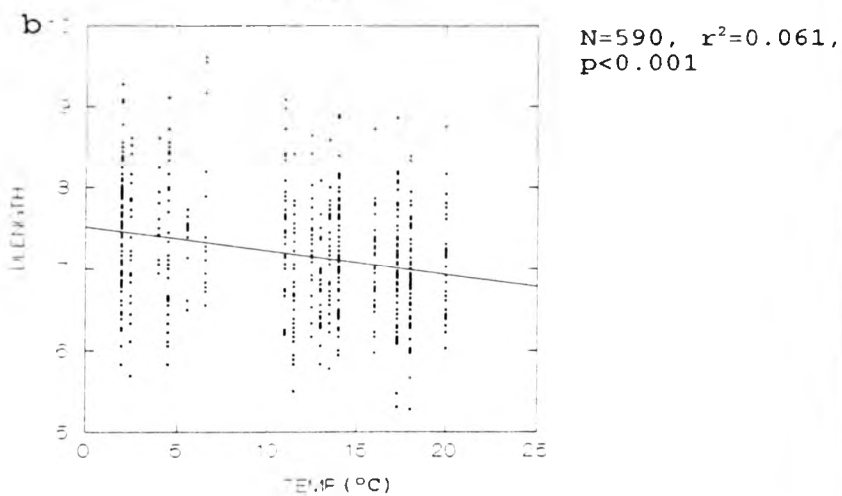
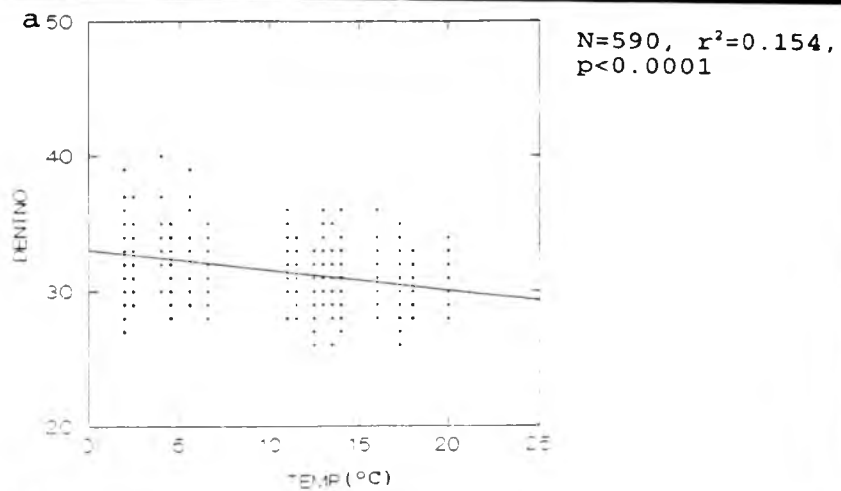
**Figure 6.16.** Histograms of: a, denticle number; b, denticle length and c, blade length in *Trichodina tenuidens*.



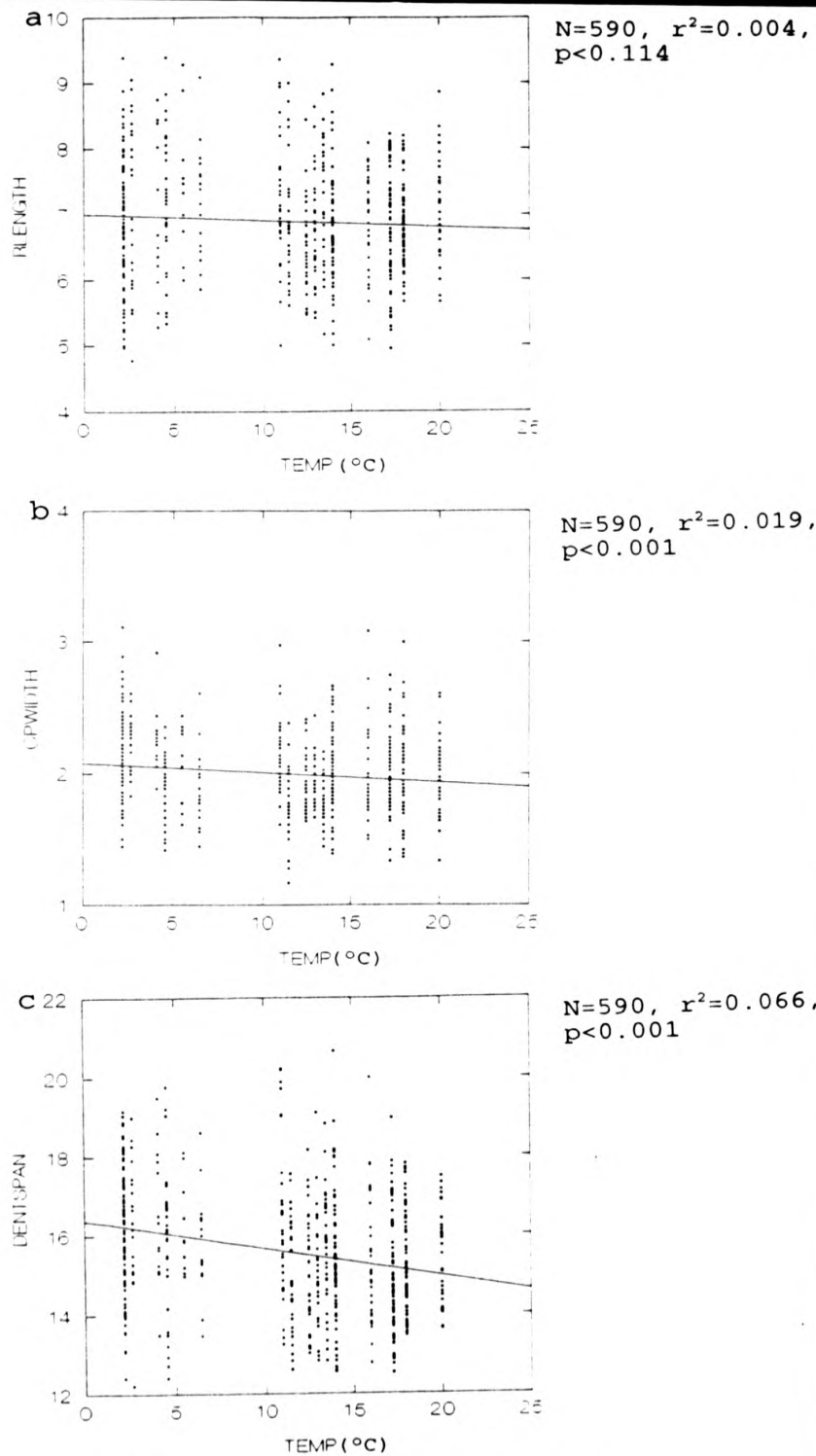
**Figure 6.17.** Histograms of: a, ray length; b, central part width and c, denticle span.



**Figure 6.18.** Regression of a, adoral disc diameter; b, border membrane width and c, denticle ring diameter against temperature in *Trichodina tenuidens*.



**Figure 6.19.** Regression of a, denticle number; b, denticle length and c, blade length against temperature in *Trichodina tenuidens*.



**Figure 6.20.** Regression of a, ray length; b, central part width and c, denticle span against temperature in *Trichodina tenuidens*.



6.4 $\mu$ m (high temperature) in *Trichodina tenuidens*, which is also very similar to that displayed in *Trichodina domerguei*. Variability of border membrane width is consistent with the findings for *Trichodina domerguei*, with a significant increase in size with increasing temperature.

## DISCUSSION

In the PCA plot (Figure 6.1) it can be seen that *Trichodina domerguei* and *Trichodina tenuidens* are well separated, reinforcing the hypothesis that two distinct species are present. Between the two ellipses are a group of points where the two species overlap slightly, indicating that the extreme variants of each species share the same morphometric characters. Lom & Stein (1966) stated that the species of these intermediate forms could not be determined; the examination and comparison of many thousands of silver stained specimens during this study would suggest that the species can be discriminated visually but only with an "experienced" eye. An hypothesis for the presence of intermediate forms is that as *Trichodina domerguei* and *Trichodina tenuidens* have been recorded from the same host over a wide geographic range, an ancient relationship between parasite and host exists. The two species probably have common ancestry, with the gill-skin interface allowing subsets of an original species to occupy different niches and therefore diverge genetically to form two species. This divergence may be sufficiently recent that extreme variants of each species may not be very genetically distinct, or sexual reproduction may be possible between species to give intermediate forms but is usually inhibited by the niche preferences exhibited.

Lom & Stein (1966) describe atypical smaller specimens which exhibit clear central circles, or have denticles similar in form to typical *Trichodina tenuidens*. In these

small individuals the adhesive disc has a mean diameter of 26 (21-33) $\mu\text{m}$ , denticle ring diameter 17 (13-21) $\mu\text{m}$ , and denticle number 21-22 (15-26). These specimens were suggested to be extreme variants of *Trichodina tenuidens* rather than a separate species. However, in the population analysed from Airthrey Loch (N=590) no atypical smaller specimens were found. The measurements given by Lom & Stein are well below the lower limits of the size ranges presented in this chapter, and this would suggest that the smaller specimens referred to may well have been another species.

The range of denticle form in *Trichodina domerguei* is illustrated in Figure 6.4, this species is reported as exhibiting variation similar to other trichodinids (Lom & Stein, 1966). However, the specimens observed during this study illustrated a greater variation in denticle form than previously described, although the range in adhesive disc diameter was less than that reported by Lom & Stein.

The population of *Trichodina tenuidens* examined during this study illustrated considerable variation in denticle form, which was greater than that documented by Lom & Stein (1966) (discounting the small atypical specimens described by the previous authors). The variation of morphometric characters such as adoral disc diameter and denticle number was also much greater than reported by these authors. The variation in appearance of the central circle in *Trichodina tenuidens* was suggested as being the result of changes in the microbiotope by Lom & Stein, although it is more likely to be the product of differing maturity or inconsistent reabsorption of the old denticles (see Chapter 5 on developmental morphology).

In this study the morphometric and meristic characteristics of *Trichodina domerguei* fall within the range of *Trichodina tenuidens*, with the exception of blade length which is considerably greater in the former, and the upper limit of denticle

number and denticle ring diameter values which is higher in the latter species. Thus, the main discriminating features of *Trichodina tenuidens* are a significantly higher denticle number, with a smaller denticle ring - adhesive disc ratio produced by shorter denticle blades. The relatively long denticle rays produce a mean denticle span similar in both species. For this reason it is unwise to replace the component denticle measurements with the single character denticle span, as advocated by Kazubski & Migala (1968).

The size of the adhesive disc components increase significantly in both *Trichodina domerguei* and *Trichodina tenuidens* with an increase in the diameter of the adhesive disc. In some instances exceptions to this general rule occurs for the very small morphometric variables. Measurements of small structures, even when made from enlarged photomicrographs will always be more open to error than those of larger structures. Kazubski (1967) found no significant correlation between denticle ring diameter and denticle number in *Trichodina pediculus*, however a positive correlation was found between ray length and denticle ring diameter, agreeing with the findings of this study. Laird (1953, in: Kazubski & Migala, 1969) observed a high correlation between the diameter of the skeletal ring and the number of denticles in *Trichodina parabranchiola* Laird, 1953 and *Trichodina multidentis* Laird, 1953.

Causes of variation in mature trichodinid adhesive disc morphology are probably due to genetic differences, seasonal changes in water temperature, water chemistry parameters, host effects and inconsistent staining.

Genetic variation in trichodinids will be manifested by morphological differences as in any species of organism, which may be produced by sexual reproduction and selective pressures exerted by environmental factors on geographically distinct populations as demonstrated by Darwin's finches. As trichodinids are thought to mainly

reproduce asexually, genetic changes may be slower than in an equivalent sexually reproducing organism.

Seasonal fluctuations in water temperature have been shown to effect trichodinid morphology Kazubski (1971, 1976, 1979, 1982a and 1991a, b and c), Kazubski & Migala (1968) and Kazubski & Pileckarapacz (1981). Kazubski & Migala (1968) reported qualitative changes in the denticle form of *Trichodina mutabilis* (N=167, 10 sample dates in a 12 month period), and increasing dimensions of the adhesive disc with decreasing water temperature in *Trichodina mutabilis*, *Trichodina nigra*, *Trichodina acuta*, *Trichodinella subtilis* from *Cyprinus carpio*; *Trichodina reticulata* from *Carassius carassius* and *Trichodina pediculus* from hydra. *Trichodina mutabilis* was described as having a mean size in winter specimens over 30% greater than summer specimens. During this study, populations of *Trichodina domerguei* (N=599, 22 sample dates in a 12 month period) and *Trichodina tenuidens* (N=590, 22 sample dates) from *Gasterosteus aculeatus* displayed significant increases in adhesive disc and denticle ring diameter, denticle number and denticle span. Winter specimens were 20% and 25% greater than those of summer specimens in *Trichodina domerguei* and *Trichodina tenuidens* populations described during this study. Qualitative changes in denticle form were not observed in *Trichodina domerguei* and *Trichodina tenuidens*, in contrast to the findings of Kazubski & Migala (1968). It is possible that the previous authors were only describing normal intrapopulational rather than seasonal variation, but given the much larger number of specimens examined during this study the complete range of morphological variation at any point in time may have been observed. Thus, obscuring apparent trends due to sampling error. This study constitutes the most comprehensive and detailed analysis of intrapopulational and seasonal variation in trichodinids to date.

Kazubski & Migala (1968) suggest that assuming continual growth in trichodinids the stage at which fission occurs will affect adhesive disc diameter and this will in turn effect the number of denticles. There is no evidence that as trichodinids grow the number of denticles increases, although the number is directly correlated with adhesive disc diameter. During this study (Chapter 5) it was found that immature specimens undergoing reorganisation of the adhesive disc sometimes show an increase or decrease in new denticle number compared to the parental cell. For example, a daughter cell with 12 parental denticles may have a developing ring with 25 denticles. If this process was temperature dependent it would account for the seasonal variation in denticle number and thus denticle ring and adhesive disc diameter.

Water chemistry may effect trichodinid morphology. Marine specimens of the euryhaline species *Trichodina domerguei* were described as being significantly smaller than those from freshwater by Lom & Stein (1966). Although marine specimens found during this study were very small (N=6), only adhesive disc diameter extended below the range given for any variable from the seasonal freshwater sample (N=599). Given that the marine sample was taken in August, seawater temperature would be relatively high, thus, the small size of the marine specimens might not be due entirely to low water temperature.

Host induced morphological variation was reported by Chen-Chih-leu (1963) who observed rapid changes in the size of morphometric variables of *Trichodina acuta* transferred between fish, tadpole and crustaceans, although no details of statistical significance are given. Adhesive disc morphology for individuals parasitising all the hosts did not seem to vary significantly in form. Kazubski (1991c) studied host induced variation in *Trichodina pediculus* parasitising carp, tadpoles and hydra and could find

no significant difference between samples taken at the same time of year. In this study the morphometrics of different populations of *Trichodina acuta*, *Trichodina nigra*, *Trichodina intermedia*, *Trichodinella epizootica* and *Paratrachodina incisa* were compared using Principal components analysis. In no instance was it possible to associate observed morphological variation with host species.

Lastly, inconsistent silver impregnation is a significant factor in observed morphological variation of small species with delicate denticle structure. During this study (Chapter 4), scanning electron microscopical observations of isolated adhesive disc structure in *Trichodina intermedia*, *Paratrachodina incisa* and *Trichodinella epizootica* indicated that actual blade form may be considerably different from that observed in silver stained specimens.

## CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS

Comparisons of morphometric data, aided by multivariate statistical techniques were utilised during this study to discriminate trichodinid species. This approach can be described as phenetic (Ridley, 1986) or numerical classification (Sokal & Sneath, 1963). Phenetic classification aims to represent a hierarchy of form among living things (Ridley, 1986) in an objective and repeatable manner. However, the method used during this study cannot be described as totally objective, because of subjective selection of the characters of trichodinid adhesive disc morphology. This selection is made partly for reasons of practicality. The adhesive disc constitutes a significant structure resistant to deformation and illustrates apparent species specific morphological variation, in an animal with few easily visualised morphological features. Sokal & Sneath (1963) maintain that not less than forty characters should be used in numerical classification, whether they are morphological, physiological, ethological or distributional. However, this number does not consider the total number of features which may be available to the taxonomist. In the case of simple protozoa with few readily identifiable characteristics, a relatively small number of features may constitute a large proportion of those available. The hypothesis of nonspecificity (Sokal & Sneath, 1963) assumes that there are no single large classes of genes exclusively affecting one class of characters. If this assumption is correct, obtaining a disproportionately large number of characters from one body region would not restrict information to one special class of genes (Sokal & Sneath, 1963). In vertebrate systematics this hypothesis holds true, as classification is often based on skeletons or even bone fragments.

Developments in the understanding of genetics, cytology and geographic

variation during the 1930s and 40s led to a "New Systematics", originating with the work of Huxley (1940 in: Sokal & Sneath, 1963) which was predominantly concerned with classification at the species and infraspecies levels, developing a biological species concept. This explained the existence of (and defined) species by interbreeding, not by similarity of form (Ridley, 1986). If species were recognised by their form, it was because this indicated interbreeding. Other taxonomic levels could not be recognised using this criterion, and were defined by similarity of form. A species is therefore a relatively "real" category and higher levels relatively "artificial" (Ridley, 1986). This highlights the difficulty of classifying predominantly asexually reproducing microorganisms such as trichodinids, in which evidence of interbreeding is scarce or nonexistent. It could be assumed that interbreeding takes place in a trichodinid population of uniform adhesive disc morphology, which would constitute a population belonging to a single "species". However, many such "species" occur in mixed populations on a single host population (Table 7.1), which precludes any assumption of a single interbreeding population. These "species" must therefore be discriminated phenetically on morphology alone.

The concept of a "phenetic" species based on form has limitations, especially in parasites or commensals. For example, the appearance of a sterility barrier (Sokal & Sneath, 1963) will instantly divide a genetic species into two sibling species. Sterility barriers may occur with isolation of host individuals from their original population, or small differences in habitat selection by individuals leading to relative reproductive isolation. The sibling species may remain a single phenetic group, because any differences which may have caused the sterility barrier (plus any accumulated differences after separation) will be insignificant compared to the many attributes shared



**Table 7.1.** Trichodinids species identified from mixed populations during this study.

Host population	Trichodinid species in given population
<i>Cyprinus carpio</i> (Norfolk)	<i>Trichodina acuta</i>
<i>Phoxinus phoxinus</i> (Allt Loin)	<i>Trichodina intermedia</i> , <i>Paratrichodina incisa</i> <i>Tripartiella lata</i>
<i>Phoxinus phoxinus</i> (Castle Semple water)	<i>Trichodina acuta</i> , <i>Trichodina intermedia</i> , <i>Paratrichodina incisa</i> , <i>Tripartiella lata</i>
<i>Phoxinus phoxinus</i> (College Mill)	<i>Trichodina mutabilis</i> , <i>Trichodina rostrata</i> , <i>Trichodina intermedia</i> , <i>Trichodina sp.</i> , <i>Paratrichodina incisa</i> , <i>Tripartiella lata</i>
<i>Phoxinus phoxinus</i> (Lake Bala)	<i>Trichodina intermedia</i> , <i>Paratrichodina incisa</i> , <i>Tripartiella lata</i>
<i>Gasterosteus aculeatus</i> (Airthrey Loch)	<i>Trichodina domerguei</i> , <i>Trichodina tenuidens</i> , <i>Trichodina pediculus</i>
<i>Gasterosteus aculeatus</i> (Ardtoe)	<i>Trichodina domerguei</i> , <i>Trichodina tenuidens</i>
<i>Abramis brama</i> (Yorkshire)	<i>Trichodina modesta</i> , <i>Paratrichodina incisa</i>
<i>Oncorhynchus mykiss</i> (Moffat, Loch Fad)	<i>Trichodina nigra</i> , <i>Trichodina acuta</i>
<i>Oncorhynchus mykiss</i> (Dollar)	<i>Trichodina nigra</i> , <i>Trichodina acuta</i> , <i>Trichodinella epizootica</i>

by them (Sokal & Sneath, 1963).

Definition of the species category is one of the most important problems in taxonomy (Simpson, 1967) who gives the following important species definitions.

**Biospecies:** species as defined by the biological species concept described previously.

**Taxonomic species:** a general expression for any taxon that has been called a species and given a specific name available under the International Rules of Nomenclature.

**Morphospecies:** established by morphological similarity regardless of other considerations.

Trichodinid species described in the literature and during this study fall into the

category defined as the taxonomic species. More importantly, some definitions can equally be described as morphospecies. Cain (1954 in: Simpson, 1967) describes these as species which "have been established solely on morphological evidence". Simpson (1967) objects to this definition, pointing out that many species definitions including genetical and evolutionary are also established in this way. It was suggested that the concept of a morphospecies applies to a purely typological species concept, derived by selecting arbitrary types among specimens suspected or known to be part of a single biological population or biospecies. This definition also encompasses some trichodinid identifications in the literature, where species are proposed from "type" specimens or a very small number of specimens. The problems associated with this practice are eloquently summarised by Simpson (1967) when he suggests that "there are legitimate and violent objections to the construction of non-biological groups and subsequent classification as if they were biological, providing Linnaean names with which taxonomists are forced to deal, if only as synonyms". These "type" specimens are therefore defined as species, indistinguishable from species conforming to the biological species concept.

The problems raised by the use of the morphospecies concept is the main reason for the need to investigate morphological variation in trichodinids. For this reason species definitions were made during this study, based on mean characteristics from as many specimens from as many populations as practically possible. The application of a method to describe trichodinid morphology using fixed reference points on a drawing or photomicrograph (Chapter 2) proposed by Van As & Basson (1989), inevitably encourages species discrimination on the basis of a small number of specimens. While this is inevitable to a certain extent because of the scant nature of some species

descriptions in the literature, emphasis should be placed on differentiation of homologous populations rather than individuals. For this reason the technique proposed by Van As & Basson (1989) was not utilised during this study.

Intraspecific morphological variation and the identification of discrete populations or demes has led to the creation of subspecies or morphotypes (Simpson, 1961). This practice was common in naming trichodinids until it was totally dispensed with by Arthur & Lom (1984). Instead of naming separate demes assumed to belong to a single species, authors such as Kazubski began to document morphological variation. As previously discussed variation can be attributed to three main factors, geographical location, host factors and environmental factors such as water temperature.

Morphological variation was investigated in *Trichodina domerguei* and *Trichodina tenuidens*, from a single population of *Gasterosteus aculeatus* over a twelve month period. In large samples of approximately 600 specimens of each species, considerable variation was observed in size and form of adhesive disc morphology. A significant increase in adhesive disc diameter was noted at low water temperatures, as described in *Trichodina nigra*, *Trichodina acuta*, *Trichodina mutabilis* and *Trichodinella subtilis* by Kazubski & Migala (1968). Seasonal trends in *Trichodina domerguei* and *Trichodina tenuidens* denticle form were not observed during this study, although this is not statistically quantifiable and therefore difficult to detect. The range in adhesive disc diameter observed in populations of the two previous species analysed in this study were remarkably similar to those given for them by Lom & Stein (1966). Lom & Stein's sample size of approximately 50 specimens appears to illustrate the range in adhesive disc diameter quite well. The lower values for adhesive disc diameter from both studies are almost identical, whilst the higher values were greater in the larger

samples examined during this study. This latter disparity may be due to the large sample size or the inclusion of "winter" data from samples taken at times of particularly low water temperatures.

Immature trichodinid specimens were examined during this study, and developmental morphology of the adhesive disc associated with binary fission and subsequent reorganisation in *Trichodina acuta*, *Trichodina domerguei*, *Trichodina tenuidens* and *Trichodina intermedia* closely resembled that described for *Trichodina pediculus* by Kazubski (1967). With only one specimen observed displaying adhesive disc morphology indicative of conjugation, it is likely that sexual reproduction is a rare occurrence in trichodinids.

Variation between different populations of *Trichodina acuta*, *Trichodina nigra*, *Trichodina intermedia*, *Trichodinella epizootica* and *Paratrachodina incisa* was investigated using Principal Components Analysis. In all cases variation between populations appeared to be genetic (caused by geographic separation) or seasonal. No host induced variation was apparent in populations of trichodinid species sampled from different host species.

Thirteen trichodinid species were identified from Great Britain during this study, summarised in Table 7.2. Four of the species identified have already been recorded from Great Britain: *Trichodina acuta* from *Cyprinus carpio* (Salas, 1991); *Trichodina domerguei* and *Trichodina tenuidens* from *Gasterosteus aculeatus* (Chubb, 1970) and *Gasterosteus aculeatus* and *Pungitius pungitius* (Dartnall *et al.*, 1972, 1973); and *Trichodinella epizootica* from *Perca fluviatilis* (Abolarin, 1966 in: Chubb, 1977). These records are the only previous trichodinid species descriptions from Great Britain.

All the species described from Great Britain for the first time including

*Trichodina pediculus*, *Trichodina modesta*, *Trichodina nigra*, *Trichodina mutabilis*, *Trichodina rostrata*, *Trichodina intermedia*, *Tripartiella lata*, *Tripartiella copiosa* and *Paratrichodina incisa* have all been previously reported from Europe. From this study it would appear that the British freshwater trichodinid fauna is very similar to that of continental Europe. Given the similarity of the fish fauna between Great Britain and continental Europe this is perhaps to be expected.

**Table 7.2.** Summary of trichodinid species found during this study.

Trichodinid species	Host species
<i>Trichodina acuta</i>	<i>Cyprinus carpio</i> , <i>Carassius auratus</i> , <i>Oncorhynchus mykiss</i> , <i>Phoxinus phoxinus</i>
<i>Trichodina domerguei</i>	<i>Gasterosteus aculeatus</i>
<i>Trichodina tenuidens</i>	<i>Gasterosteus aculeatus</i>
<i>Trichodina pediculus</i>	<i>Gasterosteus aculeatus</i>
<i>Trichodina modesta</i>	<i>Abramis brama</i>
<i>Trichodina nigra</i>	<i>Cyprinus carpio</i> , <i>Salmo trutta</i> , <i>Oncorhynchus mykiss</i>
<i>Trichodina mutabilis</i>	<i>Phoxinus phoxinus</i>
<i>Trichodina rostrata</i>	<i>Phoxinus phoxinus</i>
<i>Trichodina intermedia</i>	<i>Phoxinus phoxinus</i>
<i>Trichodinella epizootica</i>	<i>Perca fluviatilis</i> , <i>Oncorhynchus mykiss</i>
<i>Tripartiella lata</i>	<i>Phoxinus phoxinus</i>
<i>Tripartiella copiosa</i>	<i>Rutilus rutilus</i>
<i>Paratrichodina incisa</i>	<i>Phoxinus phoxinus</i> , <i>Abramis brama</i> , <i>Rutilus rutilus</i>

The host specificity of trichodinid species found during this study generally agrees with the comments of previous authors. *Trichodina acuta*, *Trichodina nigra*, *Trichodinella epizootica* and *Paratrichodina incisa* were all found on more than one host species. The first three species were described by Lom (1970a) as displaying little

host specificity. *Paratrichodina incisa* was specific to cyprinids in this study, which appear to be its main hosts (Lom, 1963a; Lom & Haldar, 1977; Calenius, 1980) despite being initially described from *Barbatula (Nemacheilus) barbatulus* (Lom, 1959). Of the species found in significant numbers from more than one geographical location, *Trichodina intermedia* from *Phoxinus phoxinus*, *Trichodina domerguei* and *Trichodina tenuidens* from *Gasterosteus aculeatus* displayed a high degree of host specificity. These findings are in accordance with previous publications where these species were only reported from one or two host species, although *Trichodina domerguei* has been reported from several other marine hosts (Lom & Stein, 1966). No correlation between degree of host specificity and trichodinid genus was evident. Gunkovsky (1995) cites the confused taxonomy within the trichodinids and the translocation of farmed and ornamental fish transferring trichodinids to "unnatural hosts" as problems in the study of host specificity.

A sonication technique modified from Shinn *et al.* (1993) was used to liberate the skeletal structures of the adhesive disc in *Trichodina domerguei*, *Trichodina intermedia*, *Paratrichodina incisa* and *Trichodinella epizootica*. The subsequent electron microscopical observations elucidated some previously undescribed features, and allowed an accurate comparison of the four genera commonly found on fish hosts. This study also highlighted some potential taxonomic problems due to the limitations of silver staining.

Although *Trichodina domerguei* possesses substantial denticles which can be accurately visualised using Klein's silver staining technique, an anterior blade and ray apophysis, previously undescribed in trichodinids, were observed either side of the central conical part of the denticle, acting as articulation surfaces. A posterior blade

apophysis was observed, similar to that described for *Trichodina dampanula* (Van As & Basson, 1990). Small peg like structures also previously undescribed in trichodinids were visible on the anterior centrifugal surface of some denticles.

The denticle form of *Trichodina intermedia* appeared to be slightly different from that observed in silver stained material, with the very delicate anterior blade margins being more rounded than the "*Paratrichodina*-like" blades observed using light microscopy. A ridge-like junction of radial pins and finer pins designated denticular pins was observed on the adoral surface of the denticle ring, which has not been previously described. An anterior and posterior blade apophysis and a posterior ray apophysis were visible.

Blade form in *Paratrichodina incisa* was significantly different from that seen in silver stained material, with the anterior blade margins appearing continuous rather than notched. Only the posterior blade apophysis was apparent, with the substantial anterior projection of the central part of the denticle wedging against it.

Denticle form in *Trichodinella epizootica* deviated from that apparent in stained specimens, the anterior blade margins being incompletely visualised. The small crook-like inner rays could be seen in their entirety, which is uncommon in stained material.

These findings highlight the care needed in classifying the smaller *Paratrichodina*, *Tripartiella* and *Trichodinella* species, to ensure populations are not differentiated on the basis of staining inconsistencies.

In conclusion, this study has shown criteria which must be fulfilled in species identifications and especially the description of new taxa include: a significant sample size, exclusion of immature specimens, consideration of morphological variation, water temperature and consideration of staining artifacts in some genera which can be ideally

elucidated using scanning electron microscopy. Genetic studies are required to further our knowledge of trichodinid taxonomy, which would eliminate the complications of environmentally induced morphological variation. Increased insight into the genetic make up of trichodinids may enable clarification of evolution and phylogeny within the group.

The problems faced by taxonomists reflect the complexity of life itself. Singer (1959 in: Sokal & Sneath, 1963) in discussing classificatory schemes suggests that;

"They can be little better than mnemonics mere skeletons or frames on which we hang somewhat disconnected fragments of knowledge".



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