

Nanoflagellates: functional groups and intraspecific variation*

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Abstract: The ecology of the smallest eukaryotes, i.e., nanoflagellates, is a comparatively young but rapidly developing research area. Whereas most of the ecological research focused on functional groups, autecological data, specifically with regard to intraspecific variation, are sparse. I will briefly address two complementary approaches in nanoflagellate ecology: (i) the level of rough functional groups and (ii) the level of specific model strains. The latter approach increasingly confirms the suspicion of FOISSNER (1991) and others that the value of flagellate species lists is limited, which is at least partly due to inappropriate identification methods. As a further consequence of the insufficient flagellate identification, the choice of model strains for laboratory studies was often too indiscriminate.

Key words: Ecology, identification problem, model strains.

Introduction

Flagellated protists are the most numerous eukaryotes on earth and of tremendous importance in all ecosystems (LAYBOURNE-PARRY & PARRY 2000; BOENIGK & ARNDT 2002). Some taxa, such as *Spumella* spp., *Paraphysomonas vestita* complex, *Oikomonas* spp., *Bodo/Neobodo* spp., *Rhynchomonas nasuta*, and *Cercomonas* spp. are specifically of quantitative importance in many different habitats. These ubiquitous taxa (occurring in freshwaters, soil, and marine waters) account for a significant fraction, often exceeding 10–20 % of the total nanoflagellate abundance (e.g. FOISSNER 1991; PREISIG et al. 1991; PATTERSON & LEE 2000; BOENIGK & ARNDT 2002; VON DER HEYDEN & CAVALIER-SMITH 2005). Most of these taxa seem, furthermore, to be globally distributed, i.e. they are reported from Arctic/Antarctic sites as well as from tropical sites (DIETRICH & ARNDT 2004; TONG et al. 1997; BUTLER et al. 2000; VØRS et al. 1995; FINLAY & FENCHEL 2004). However, FOISSNER (1991) pointed out that flagellate species lists are often too indiscriminate, i.e. probably pool different taxa in one binomen.

The historical development of the field provided many valuable insights on the heterotrophic nanoflagellates as a group, yet variation within (or between closely related) species has long been considered but became only recently a research focus (BOENIGK et al.

2004, 2006, BOENIGK 2005; KOCH & EKELUND 2005; LOWE et al. 2005).

Functional groups

The ecology of nanoflagellates was, historically, largely based on studies concerned with the differentiation between animals and plants: From the mid-19th century onwards the mode of nutrition, i.e. heterotrophy and specifically particulate food uptake versus autotrophy, became the dominating criterion in differentiating between animals and plants (e.g. STEIN 1878; KENT 1880/81). PERTY (1852) was one of the first who discussed the problem of animal/plant affiliation thoroughly and his proposal of the order Phytozoa illustrates the historic problem of the animal-plant distinction. Subsequently, during the 1850s and 1860s, the green coloration of several flagellates was attributed to the presence of chlorophyll, further heating the debate on the animal or plant nature of the flagellates (cf. BÜTSCHLI 1883–87). In general, zoologists tended to include many pigmented forms in the Flagellata (Protozoa) based on observed food uptake or their mode of movement (cf. KENT 1881; BÜTSCHLI 1880–89; DOFLEIN 1916). In contrast, botanists increasingly documented a structural similarity between some pigmented taxa and unpigmented taxa and tended to regard an increasing number of unpigmented flagellates as colorless algae (PASCHER & LEMMERMANN 1914; PRINGSHEIM 1963). The affiliation of an increasing number of nanoflagellates to both kingdoms (based on partly di-

* This article is dedicated to Wilhelm FOISSNER in honour of his 60th birthday.

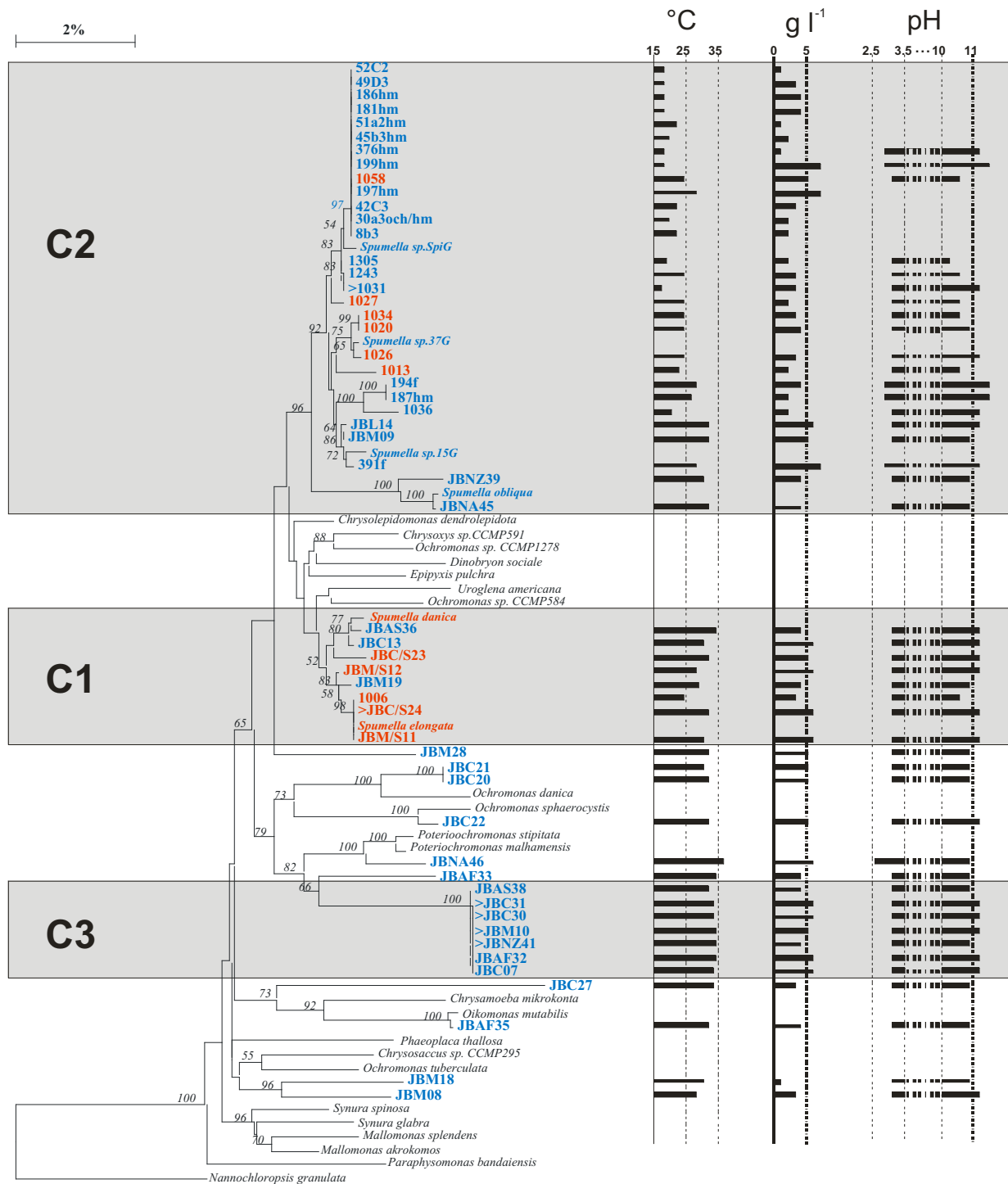


Fig. 1: Neighbor-joining tree showing the affiliation of 18S rRNA gene sequences from *Spumella* isolates with the Chrysophyceae sensu stricto. The numbers at the nodes of the tree indicate the percentage of bootstrap values for each node out of 100 bootstrap resamplings (values above 50 are shown). The scale bar indicates 2% estimated sequence divergence. Bold face indicates *Spumella* strains, blue indicates freshwater origin and red indicates soil origin. The subclusters C1, C2 and C3 are indicated by grey areas. Maximal temperature tolerance (°C), salinity tolerance (g NaCl l⁻¹) and pH range are shown. Several distinct lineages have obviously been pooled under the name of *Spumella* – further each of the monophyletic clusters most probably comprises a number of cryptic species. Even though some trends can be inferred, the ecophysiological adaptations are hardly resolved. This indicates the strong need for comparative studies on different strains affiliated with the same morphotype or binomen. Data partly taken from BOENIGK et al. (2006, 2007).

verging arguments) yielded in different, even though interconnected, nanoflagellate systematics. However, this dispute provided valuable insight into the nutritional ecology of nanoflagellates as functional groups (i.e. heterotrophic nanoflagellates versus microalgae).

As long as the ecology of nanoflagellates was largely extrapolated from the ecology of functional groups, the potential for conflicting evidence remained low: The historical development of protistology with the strong research focus on the animal-plant distinction resulted in a comprehensive background knowledge on the functional differences between 'animal-like' and 'plant-like' flagellates: Life observations on food uptake provided early insights in these functional differences (e.g. EHRENBURG 1838). As food uptake was basic to the functional differentiation between animals and plants, specifically during the second half of the 19th century (e.g. STEIN 1878; KENT 1880/81), a considerable research activity was directed towards the mode of nutrition. The more recent research foci on the plant-animal-fungi-protist distinction (WHITTAKER 1969; MARGULIS 1970b; CORLISS 1986) and the bacterial ingestion as basic in the serial endosymbiosis theory (SET: formulated in the 1970's, e.g. MARGULIS [1970a]; based on former suggestions by MERESCHKOWSKY [1905] and WALLIN [1927]) further extended this research direction. In sum, this long-lasting research focus on feeding and food uptake provided a strong basis for formulating a general ecology of the heterotrophic nanoflagellates as a functional group (AZAM et al. 1983).

Strain-specific differentiation

A high ecophysiological and molecular variation, specifically within the 'ubiquitous' morphotypes, has long been suspected. For instance, inconsistencies between ecophysiological studies on the same species but carried out with different strains have been ascribed to strain-specific variation (e.g. CHOI & PETERS 1992; PÅLSSON & DANIEL 2004). FOISSNER (1991) already pointed out that 'some or even many of the soil flagellates are new species and only superficially resemble those found in freshwater'. In fact, even at the 18S rRNA level almost identical and morphologically indistinguishable protist 'sibling' species (MAYR 1942) may carry unique phenotypic traits that enable specialized exploitation of resources within their natural habitat (NANNEY et al. 1998; DE VARGAS et al. 1999; BOENIGK et al. 2006, 2007). Corresponding to the ecophysiological variation, and therefore not surprisingly, a high intraspecific molecular diversity, i.e., microdiversity, has been demonstrated for several protistan morphospecies, such as for *Oxyrrhis marina* (Alveolata, Dinoflagellata; LOWE et al. 2005), *Neobodo designis* (Euglenozoa, Kine-

toplasta; KOCH & EKELUND 2005; VON DER HEYDEN & CAVALIER-SMITH 2005), *Micromonas pusilla* (Chloroplastida, Chlorophyceae; ŠLAPETA et al. 2005) and *Spumella* sp. (Chromista, Stramenopiles; BOENIGK 2005).

However, the degree of similarity between (clonal) strains or populations affiliated with the same morphospecies has still been insufficiently addressed. In fact, ecophysiological variation below morphospecies level became apparent in recent studies (KOCH & EKELUND 2005; LOWE et al. 2005; BOENIGK et al. 2004, 2006; Fig. 1).

In the light of these findings the choice of model strains for ecological laboratory experiments during the past decades seems often too indiscriminate. A number of typical model organisms not even originated from the type of habitat they were used for. This holds true specifically for mixotrophic strains as these were easily available from culture collections, e.g. *Ochromonas danica* (strain SAG L 933-7) and *Poterioochromonas malhamensis* (strain 933/1a, originally described as *Ochromonas malhamensis*). Heterotrophic strains are, in general, less available from culture collections and, as a consequence, were more often directly isolated before a set of experiments (examples are *Paraphysomonas* spp., *Bodo* spp. and *Rhynchomonas nasuta*; but compare the CCAP strain *Spumella elongata* [CCAP 955/1]). None of the three mentioned strains, which are available from culture collections, originates from the pelagial: *Ochromonas danica* originates from rotten cabbage turnip (*Brassica oleracea* var. *gongyloides*) collected in an acidic moor (PRINGSHEIM 1955); *Poterioochromonas malhamensis* originates from a benthic biofilm, i.e. from a red film on a stone (PRINGSHEIM 1952); and *Spumella elongata* originates from a damp soil site (BELCHER & SWALE 1976). However, all three strains have repeatedly been used as pelagic model organisms.

It is currently uncertain to what extent the choice of model organisms may have biased our view of nanoflagellate ecology. With the increasing awareness of ecophysiological differentiation within and between morphotypes, the generalisability of nanoflagellate ecology across rough functional groups became more and more questioned. Systematic comparative studies on intraspecific variation in relation to molecular differentiation and the place of origin (habitat type and geographic region) may solve the problem but such studies are, so far, rare (BOENIGK et al. 2004, 2006, BOENIGK 2005; KOCH & EKELUND 2005; LOWE et al. 2005).

Conclusion

Even though ecophysiology is certainly strain specific, it is likely that the basic behavioural patterns can be generalised among morphologically similar organisms. For instance, despite the considerable ecophysiological variation (Fig. 1) the basic mechanisms of swimming behaviour, feeding and food selection are generally similar among the morphologically similar *Spumella* spp. (BOENIGK 2005). It remains a future task to identify those characters, which can be generalised, and to separate those, which cannot. The currently open research questions stress the demand for overcoming the black box approach and for specific monitoring of microbial species and ecotypes in the field. Our knowledge will be comparable with that on the “classical food chain” not until single microbial species and ecotypes are differentiated in ecological investigations. Due to the complex behaviour of the “*Spumella*-like” flagellates in combination with their relatively simple organisation and the comparatively good knowledge of their ecophysiology, these organisms are promising as model organisms for investigating key questions of general ecological relevance (BOENIGK 2005).

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