

Paraluffisphaera tuba gen. n., sp. n. - a Newly-discovered Eukaryote

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Summary. *Paraluffisphaera tuba* gen. n., sp. n. is a very small (~ 3 µm), enigmatic organism, that was isolated from montane grassland in Scotland, and from inland saline water in Spain. The cell surface of this new protist is covered with scales of two types: base scales and tubular scales. No flagella or pseudopodia were observed. The gross morphology resembles scale-bearing protists of the genus *Luffisphaera*, but the new organism is distinctly different. Like *Luffisphaera*, *Paraluffisphaera* should be considered an *incertae sedis* protist.

Key words: *Luffisphaera*, nanoplankton, picoeukaryotes, picoplankton, scaly protists, soil protozoa.

INTRODUCTION

We present a new genus of eukaryotes. The micro-organisms within the genus are unicellular, probably free-living protists that live in aquatic environments, including re-wetted soils. They are very small, and almost indistinguishable from other particles when using light microscopy alone. Isolation of individual specimens for establishment of cultures has so far proved impossible. As in other cases where micro-organisms were found to be too small to resolve and study optically, we relied on the electron microscope examination of direct preparations of aquatic samples to initially detect and then examine the new genus (e.g. Vørs 1993, Tong 1997). The small size of the new organism and the difficulties associated with detecting and handling it

presented a severe challenge in probing its natural history.

Ironically, the most interesting thing we can report is how little we have been able to discover about this organism. Thus far, it has been detected only in two distinctly different habitat types separated by about 1500 km. As the abundance of protist species populations is inversely proportional to cell size (Finlay 2002), we might predict that this minute enigmatic organism is probably represented worldwide by vast numbers of individuals (although this is not usually obvious because they are so small as to be almost undetectable) and filling an ecological niche about which we still know very little.

MATERIALS AND METHODS

Field sites. Samples were taken from two contrasting habitats: montane grassland in Scotland, and inland saline groundwater in Spain. Grassland samples were obtained from the experimental site at

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the Macaulay Land Use Research Institute's Sourhope Research Station, situated 15 miles south of Kelso. The land rises to 605 m and the annual rainfall is 1015 mm (10 year mean). The experimental site (Grid reference NT854 196) is representative of mid-altitude (304–313 m) temperate upland grasslands on base-poor, damp, mineral soils (see Finlay *et al.* 2000). The source of the new protist was a sample taken in November 1998. It included turf and soil down to grass-root depth, plus a centimetre of soil below this. In the lab, the soil was dampened with 0.2 µm-pore size membrane-filtered Volvic mineral water, covered and placed on a shaded bench at room temperature. At this stage of the investigation, we were recording all the protist species we could find in the sample.

The sampling site in NE Spain is located at Los Monegros (Zaragoza), a semi-arid plateau at 340 m above sea level placed on a Tertiary basin rich in marl and gypsum with limestone in the upper part (Alcorlo *et al.* 2001). The local climate is semi-arid Mediterranean with low annual rainfall (average 300 mm) and high evaporation (Pueyo and Inglés 1987). Winds are strong and persistent, and mainly from the north-west. We sampled the saline spring water of a permanent concrete-built well (41° 25'229"N and 0° 1'669" W) next to Laguna de la Playa, where the salinity was 13ppt. Samples were collected on 22nd and 23rd December 2003 using screw-capped sterile centrifuge tubes, which were kept cold (*ca* 4 °C) until they reached the laboratory. *Artemia salina* and a variety of protists typical of saline environments were observed in the well samples (Esteban and Finlay 2004).

Electron microscopy. Transmission Electron Microscopy (TEM) was carried out on whole cells. 4 µl of a "weak dilution" from the superficial sediment of Sourhope's re-wetted soil material was placed on specimen grids pre-coated with a Formvar support film. The "weak solution" was prepared as a sub-sample from the re-wetted soil that was diluted subjectively to the point where it would give sufficient separation of dried material on the grid to enable clear examination of individual cells and the scales. Each sub-sample for TEM examination was fixed for 30 seconds with osmium tetroxide vapour, dried, and shadowed with chromium metal.

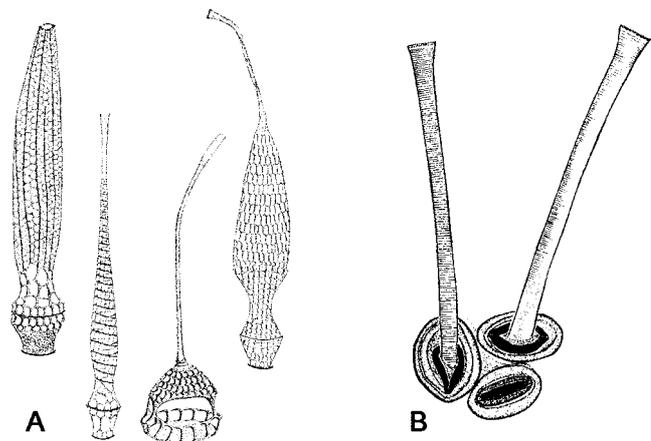
For TEM investigations of the Spanish saline well samples, sub-samples were fixed with osmium tetroxide or glutaraldehyde fixatives; replicates of these fixed samples were washed twice in distilled water to remove salt and fixative, using centrifugation and Eppendorf tubes. Each sample was then resuspended before a 3–4 µl-sized drop was placed on Formvar-coated specimen grids. The dried samples were shadowed with chromium. Cell dimensions were measured from EM negatives.

Deposited material. Following established practise in descriptions of new species of scale-bearing protists (see, e.g. Vørs 1993, Tong 1997, Backe-Hansen and Thronsdén 2002, Schroeckh *et al.* 2003), we use uninterpreted illustrations as type material.

RESULTS

Paraluffisphaera gen. n.

Diagnosis: Probably free-living protists, oval in shape and with the cell surface entirely covered with two types of scales: (1) base scales that are elliptical, flat, and with



Figs 1A, B. Line drawings of the scales that cover the cell surface of the protists *Luffisphaera* and *Paraluffisphaera*, respectively, based on electron microscope micrographs. **A** - examples of the elongate scales of *Luffisphaera*, illustrating their reticulated architecture (after Belcher and Swale 1975); **B** - *Paraluffisphaera tuba* gen. n., sp. n.: schematic representation of the base scales and the tubular scales.

a "closed" slit in the centre, and (2) structures resembling the base scale whose slit is open to house a tubular structure. With the present stage of knowledge, it is not clear whether the tubular structure is attached at its proximal end to the open base scale, or if it emerges from the slit opening. No flagella or similar swimming structures were observed. This diagnosis may change if further species of the genus are found.

Type species: *Paraluffisphaera tuba* sp. n.

Etymology: *para* from Greek, "alongside, near", meaning "resembling *Luffisphaera*", and *Sphaera* from Greek *Sphairā*, a sphere.

The original description of the genus *Luffisphaera* Belcher *et* Swale, 1975 does not provide the etymology of the generic name. However, the type species *Luffisphaera cucumiformis*, and the shape of some of its scales, suggest "*cucumiformis*" must refer to the cucumber-shaped cylindrical scales that project from the cell surface (Fig. 1A). The cucumber-shaped fruit of the tropical plant *Luffa* are similar in form to *L. cucumiformis* scales - hence we credit the generic name *Luffisphaera* to a sphere bearing *Luffa*-fruit-shaped scales.

Differential diagnosis: There is no other described eukaryote such as *Paraluffisphaera* - and this alone justifies its erection as a new genus. The presence of two types of scales wrapping the cell surface resembles organisms of the genus *Luffisphaera* - hence the generic name *Paraluffisphaera*. However, the organisational level of *Paraluffisphaera* is far simpler

than that of *Luffisphaera*. The base and the tubular scales of *Paraluffisphaera* lack the lattice network configuration of *Luffisphaera*. In the latter, the lattice structure is present in the basal and tubular scales. The structure of this lattice network is one of the characters used for the discrimination of species. As this character does not appear in *Paraluffisphaera*, the segregation of species is based on the shape of the base and/or tubular scales, e.g. base scale flat and elliptical, or cup-shaped, etc. Furthermore, in *Paraluffisphaera* the tubular scales do not sit on prominent basal scales, as in *Luffisphaera*. Finally, both basal and tubular scales of *Paraluffisphaera* lack the conspicuous rigidity of *Luffisphaera* scales (for assessment see Figs 1A, B; 3A, B).

***Paraluffisphaera tuba* sp. n.**

Diagnosis: Scale-bearing protists with the characteristics of the genus. Cells are oval, with a size range of 3 to 3.8 μm in length and 2.4 μm to 3 μm in width. The base scales are $0.4 \times 0.3 \mu\text{m}$; the elongate scales have a base scale and a trumpet-shaped tubular structure that is 1.5 μm long and projects upright from a basal elliptical scale. The cell surface is associated with one or several bacteria but the nature of this association is unknown.

Etymology: *tuba* (feminine) from Latin “the straight war-trumpet of the Romans”, in reference to the shape of the tubular scales.

Type location: Montane grassland soil from Sourhope Research Station, Macaulay Land Use Research Institute, Natural Environment Research Council, near Kelso, Southern Scotland (UK).

Type specimen: The uninterpreted illustration of the specimen in Fig. 2A.

Holotype: The uninterpreted illustration of the specimen in Fig. 2D.

Description: This description of *Paraluffisphaera tuba* gen. n., sp. n. is based on observations of shadow-cast cells using transmission electron microscopy. Cells are ovoid (Figs 2A, D) and, in the specimens we recorded, range in size from $3 \times 2.4 \mu\text{m}$ to $3.8 \times 3 \mu\text{m}$. We could not observe flagella, pseudopodia or any other structures that might be noticeably involved in locomotion or feeding. However, the specimens appeared associated with bacteria of various sizes and with detrital particles (see below and Figs 2A, D).

The cell surface of *P. tuba* is entirely covered with scales of two types (Figs 2B, C): (1) base scales, these are flat and elliptical, $0.4 \times 0.3 \mu\text{m}$ in size, they interweave in all directions, and have a slit across the scale surface, with a thickened rim that gives the appearance

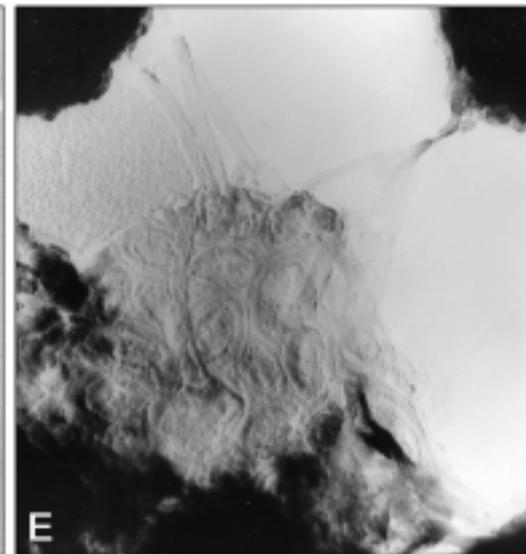
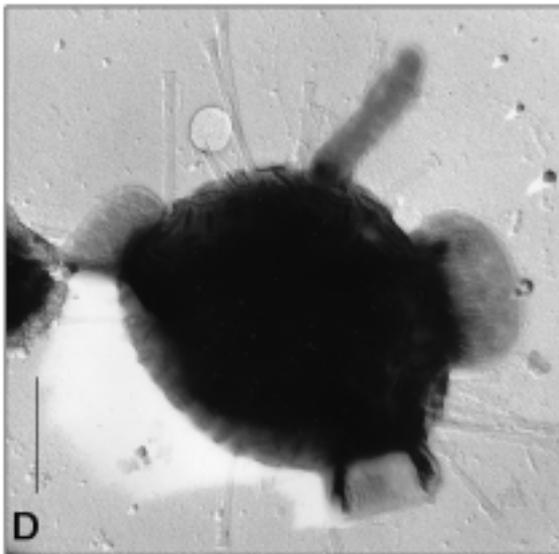
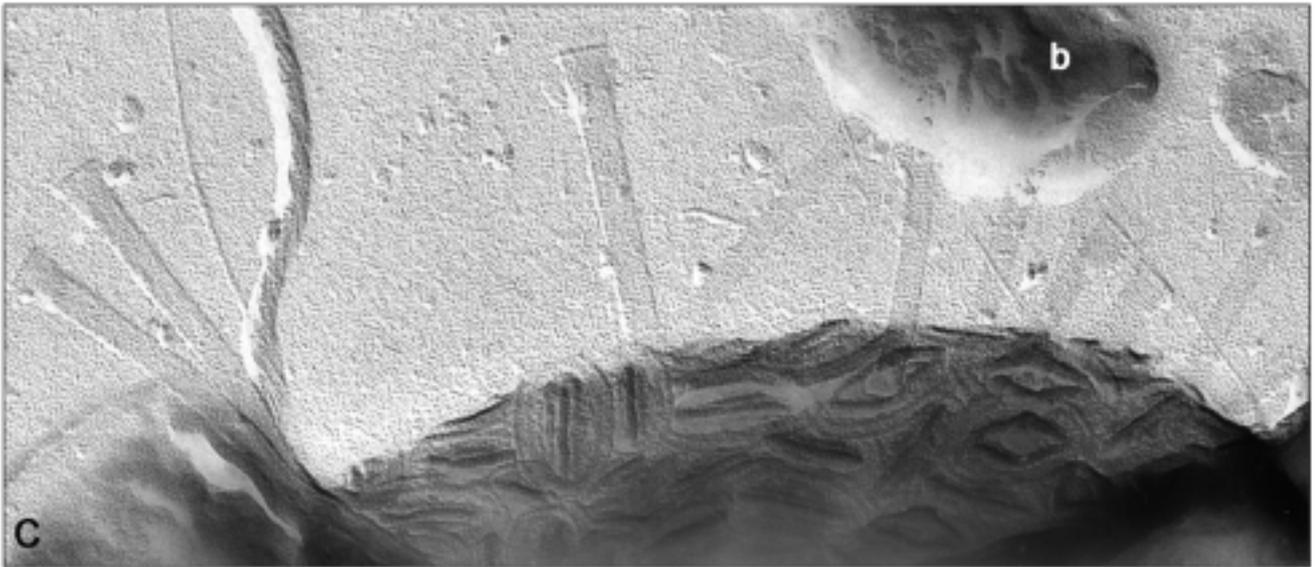
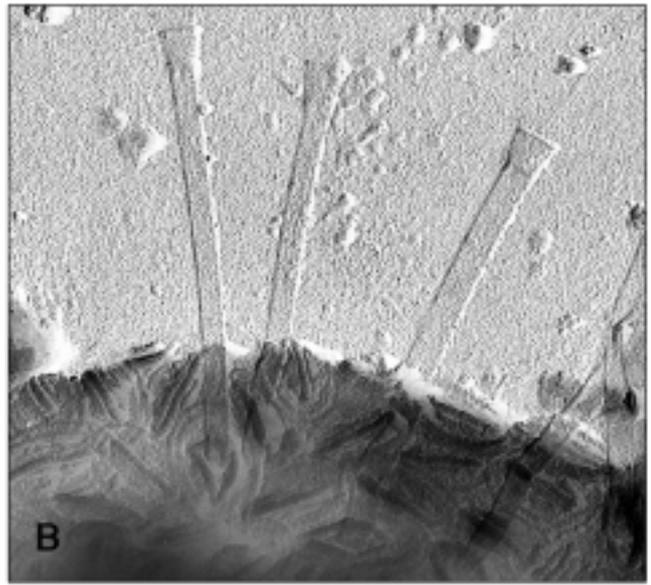
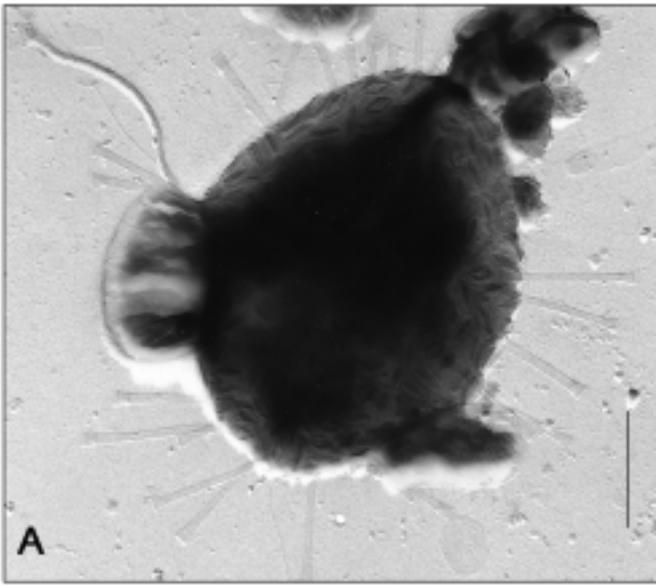
of two lips in the centre of the scale (Figs 1B; 2B, C); (2) elongate scales, which seem to arise from the “open” slit of some of the base scales described above. The elongate scales are composed of two parts: (a) a trumpet-shaped structure consisting of a tube (maximum 1.5 μm long and 0.06 μm wide in our specimens) of cylindrical bore ending in a flared bell, with thickened rim. The tubular structures do not show any pattern or network on their surface apart from an inconspicuous transversal striation (Fig. 2C). The tubes seem to emerge through (b) the labiated ‘open’ slit of base scales, and in most specimens they look as though they are in contact with or embedded in detritus particles and bacteria (Figs 2C-E), but this might be a preparation-generated artefact. Most scales are of the base-type described above (Fig. 2A). The cell surface of *P. tuba* also appears associated with one or several bacteria whose size relative to the protist becomes immediately obvious (see, e.g. Fig. 2A). The nature of this association is unknown.

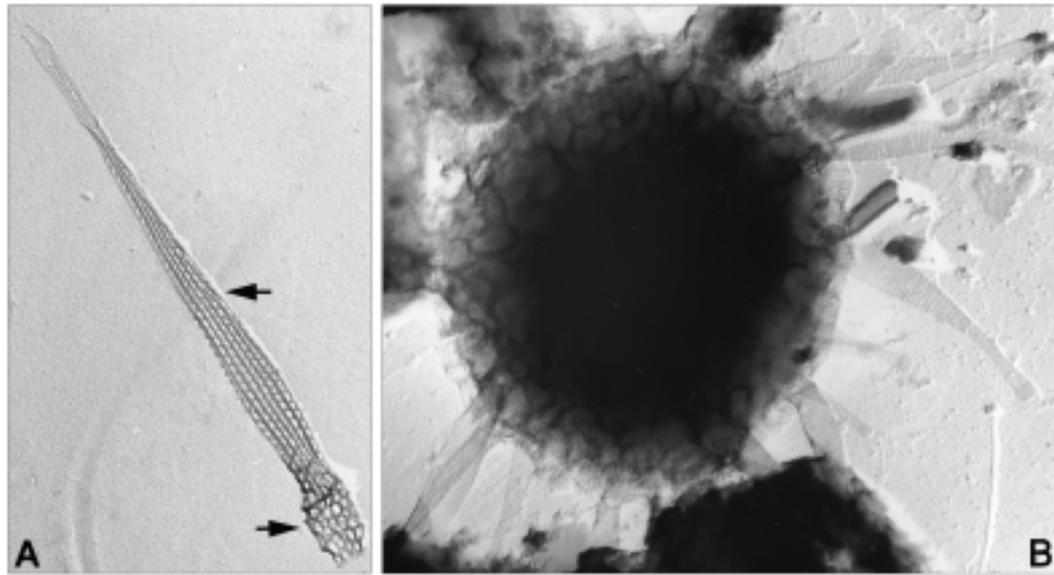
DISCUSSION

The EM micrographs of *Paraluffisphaera tuba* gen. n., sp. n. show no flagella, pseudopodia or any other structure that might enable the organism to glide or swim. We may speculate that if *P. tuba* is a planktonic organism, the tubular scales might endow some buoyancy.

The EM preparations of *P. tuba* show that the tubular scales seem to be in contact with bacteria and detrital particles (Figs 2A-E). This could be the result of the flattening of cells that occurs when they are dried onto EM grids. Alternatively, the tubular scales may actually be ingesting food. Their function may bear some resemblance to the tentacles of suctorian ciliated protozoa (which ‘suck out’ the internal content of the prey). However, suctorian tentacles are contractile, which is probably not the case in the tubular scales of *Paraluffisphaera*. We cannot begin to answer the question until thin sections are cut, and electron micrographs are obtained.

Species of *Luffisphaera*, the protistan genus that morphologically most resembles *Paraluffisphaera*, also appear to have attached bacteria to the cell surface (Belcher and Swale 1975, Vørs 1993, Backe-Hansen and Thronsen 2002), although none of these authors comment on them in the context of a potential food source. We have also considered the possibility of





Figs 3A, B. Specimen of *Luffisphaera lanceolata* from a productive freshwater pond ('Priest Pot') in the UK. Original TEM direct preparations. **A** - elongate scale showing the reticulated scale base (arrow, bottom of the image) and the narrowly lanceolate, reticulated scale (arrow, centre of the image). The split end of this scale is atypical for this species. **B** - specimen (less than 3 μm in diameter) showing the cell scales.

P. tuba being a dormant resting form or spore of some kind rather than an active protist. However, although spores and other dormant forms can be quite ornamented, they also have conspicuous protective layers that can be resolved by electron microscopy; and these are scale-free and reticulated. In contrast, all scale-bearing protists, whether photo- or heterotrophic, have their cell surfaces covered by scales and other kinds of extraordinary structures - as also in *Paraluffisphaera*. We can safely say that *P. tuba* is not a spore, resting cyst or dormant form.

Similar species

Apart from size and shape, *Paraluffisphaera* shares with *Luffisphaera* the characteristics of being without flagella and having a long-form and an elliptical-form of scale. But the ultrastructural morphology of the scales is entirely different (see "Differential diagnosis" above), e.g. the tubular scales of *Paraluffisphaera* lack the elaborated networked pattern typical of *Luffisphaera* scales (Figs 1A, B; 3A, B).

Luffisphaera was first reported from a ditch in Cambridge UK, (Belcher and Swale 1975), and all species described since have been found in marine environments (Thomsen 1982; Vørs 1992a, b, 1993; Tong 1997; Backe-Hansen and Thronsen 2002). However, a recent intensive investigation of "scaly" protists in a productive freshwater pond ("Priest Pot") in England recorded 220 scale-bearing protist species. These included a small number of ciliates and a larger number of amoebae and flagellates, sixteen of which were *Luffisphaera* (e.g. Fig. 3) - the five originally described from the ditch in Cambridge, and ten that have not been reported before and that are probably new to science (Clarke - unpublished results), but none of the marine ones. *Paraluffisphaera tuba* was not found. Similar intensive investigations took place at the grassland site of Sourhope, but *P. tuba* was reported only once throughout the three-year project. In contrast, the finding of *P. tuba* in the Spanish brackish water occurred on a single isolated sampling occasion. It may be that *P. tuba* is more common in saline or marine environments.

Figs 2A-E. Transmission electron microscopy of *Paraluffisphaera tuba*. Direct preparations. **A** - specimen from re-wetted soil (Scotland, UK) with the scales easily visible. Note the flagellated bacterium (left of the image) in relation to the small size of *P. tuba*. **B** - close-up of the scale-like structures of *P. tuba*: base scales and tubular scales; **C** - attachment (but see text) of tubular scales to bacteria (b); **D** - specimen from re-wetted grassland soil in Scotland (UK) is also attached to bacteria of different shapes, and to detrital particles. **E** - specimen from a saline-water spring in NE Spain, partially hidden by detritus. Scale bars 1 μm ;

Another scale-bearing protist observed in the well was *Paraphysomonas imperforata*, a common freshwater and marine flagellate, and in the adjoining Laguna de la Playa, the marine choanoflagellates *Platypleura* sp. and *Cosmoeca* sp. were also found.

Paraluffisphaera should be considered an *incertae sedis* Taxon (International Code of Zoological Nomenclature), i.e. a protist of uncertain taxonomic position, possibly heterotrophic, and possibly related to *Luffisphaera*, an *incertae sedis* genus of protists itself.

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