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SEM Studies on the Compound OOSPORE Ornamentation of Some Members of Charales from South India

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Abstract: ***The fossa ornamentation of the mature oospore wall of three species of the genus Chara, viz., C. zeylanica Willd., C. braunii Gmelin. and C. corallina Klein ex. Willd. and two species of the genus Nitella viz., Nitella furcata Agardh. and N. pseudoflabellata Braun., of South India have been observed. There are no published reports on SEM studies on both Chara and Nitella species from South India. Various patterns ranging from spongy to rough surface, granular to pusticular, and reticulate ornamentation are observed for the various specimens. Oospore features are presented in detail which includes the length and width ratio of the oospores, the fossa width, the number of striae, and, clawed base, etc. There are very few old reports on the presence and absence of clawed base oospore, which is discussed in the present work. The fossa ornamentations of above specimens are compared with the earlier reports of Chara zeylanica Klein. Ex. Willd. var. sejuncta f. sejuncta, C. braunii f. schweinitzii (A. Br.) R. D. W., Nitella furcata subsp. Megacarpa f. tumulosa and N. pseudoflabellata subsp. pseudoflabellata var. imperialils f. wattii. The present SEM work thus helps to identify the above species at the infraspecific level.***

Keywords: *Chara*, *Nitella*, SEM, Fossa ornamentation

1. Introduction

Taxonomy of Charophytes have received due attention in the past by a number of workers and a voluminous literature exists on the taxonomy of Charophytes. However the Characeae has been continued to be considered as a group in which the taxonomy needs revision. This is due to the reason that concept of species varied widely from worker to worker (Wood, 1965). The chief differences in Charophytes were based on various morphological characters which could be viewed under light microscope. Much work has been done only on the morphological characters of Charophytes in India (Pal *et al.*, 1962; Dixit, 1935, 1940, 1942; Allen, 1942; Groves, 1924; Iyengar, 1958). The morphology of the various Charophytes found in South India has been extensively studied and reported by Sundaralingam (1959); Barathan (1983, 1987). In the recent years, application of molecular tools and techniques has led to a revival of interest in structural as well as functional aspects of plants (Kapil and Bhatnagar, 1977). Scanning electron microscope (SEM) has added knowledge about the finer details of plant structure. This has led taxonomists to synthesize plant characters from different minute characters and thus modify the classification of Charophytes (Anand and Langer, 2002).

In the members of the order Charales, the female sex organ consist of a large egg cell (oosphere) surrounded by five sinistrally wound spiral cells. After fertilization rapid changes occur in the walls of the newly formed zygote and the ensheathing sterile cells, which result in the formation of a resistant multilayered thick wall which is pigmented and often overlaid by calcite. According to Horn af Rantzien (1956) the outermost layer is the ectosporostine layer and is variously sculptured due to differential cell wall deposition and has a vital role in species identification. This ornamented layer is resistant to ecological factors and thus has a genetic link to its variation in ornamentation patterns (Casanova, 1997).

Oospore characters that are visible using Light microscopy are thought to be taxonomically useful for identification of Charophytes (Williams 1959; Wood 1972; John and Moore, 1987), and have been used to identify species of *Nitella* and *Chara* (Hagg 1983; Casanova and Brock 1990). John *et al*. (1990) studied 28 species of *Chara* and showed that oospore wall characters can be used to justify some of the taxonomic realignments made by Wood (1965), to reduce *C. Schweinitzii* to a form of *C. braunii*. Conversely, *C. baltica* and *C. pedunculata* are two species included under *C. hispida* by Wood (1965), but the outermost oosporangial wall of these taxa is of a fundamentally different structure. These observations are significant with regard to the oospore wall ornamentation pattern, and they profoundly affect the classification and identification of different Charophyte taxa proposed by Wood (1965) in his monograph. An identification key for the species of *Chara* has been created only on the basis of oospore wall ornamentation pattern and several species have been grouped under certain broad categories of ornamentation (John *et al*., 1990; Hass, 1994). However for accurate classification both the morphological and oospore characters have to be considered.

According to Anand and Langer (2002), the wall ornamentation pattern of oospores in *Chara* is not only helpful in categorizing species but also applicable in delimiting the different infraspecific taxa. Taking this into consideration, it was thought worthwhile to study Charophytes under SEM in order to trace out chief structural details of oospores to establish and confirm their status. Oospores examined under SEM is becoming increasingly evident that ultrastructural features are proving to be more important taxonomic characters. Extensive SEM studies on oospore ornamentation has been carried out in *Chara* and *Nitella* species of West Bengal (Mandal and Ray 2004) and on *Chara zeylanica* and *Chara vulgaris* of Jammu and Kashmir (Chalotra *et al*. 2013). However there is not much published data available on SEM studies on *Chara* in South India.

Hence, three species of the genus *Chara* and two from *Nitella*, are collected from South India and examined for the various ornamentation of the oospore wall using SEM.

2. Materials and Methods

The oospores were collected from natural population in various parts of Tamil Nadu and Kerala. Table 1 provides the details of the collection spots with their GPS locations. The vegetative and reproductive characters including the morphometric analysis of the various parts were first analyzed under a Binocular Olympus CH20i microscope. A portion of the collected samples bearing the female gametangia were placed in a beaker containing water until the mature oospores were collected at the bottom of the beaker. These mature oospores for SEM analysis were prepared following the procedures of John and Moore, (1987) and analyzed using a SEM (S-3400, Hitachi, Japan) at an accelerated voltage of 15kV.

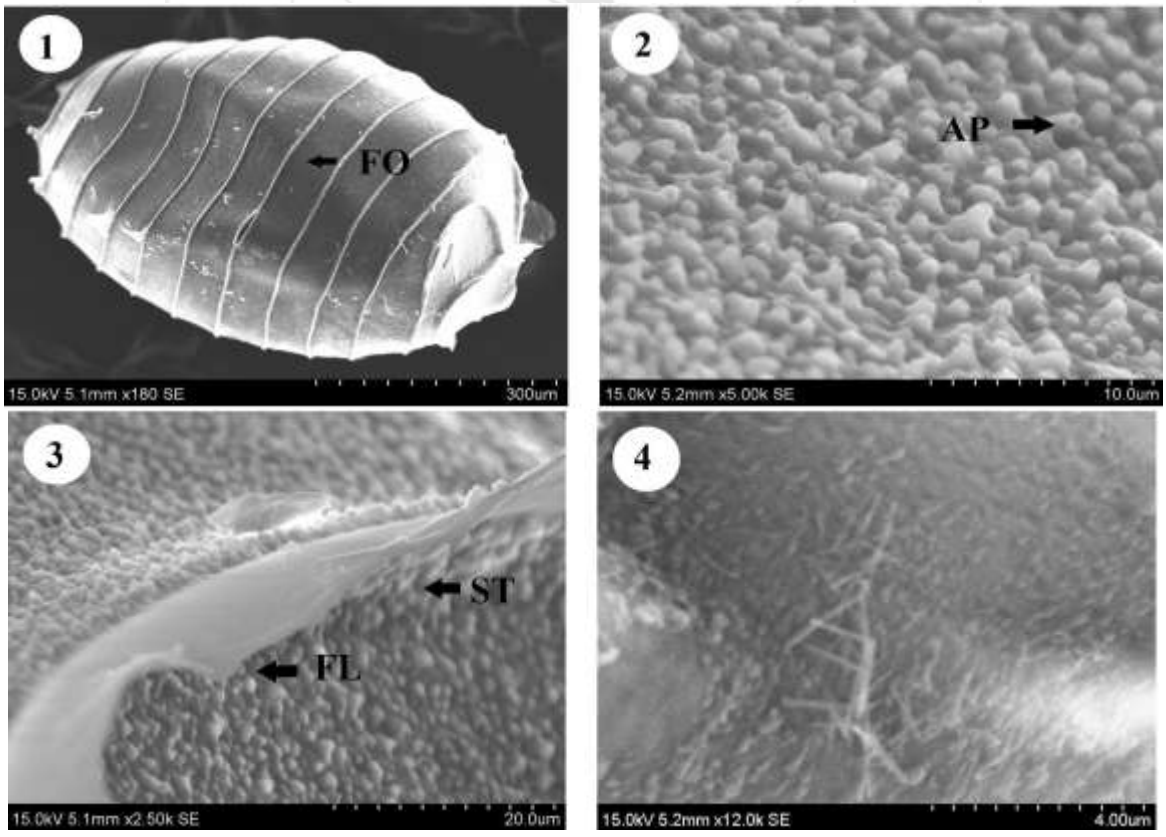
3. Results

***Chara zeylanica* Willd**

The mature oospores are black ovoid structures measuring 631X431µm (±2). There are 11-12 striae which possess the flange, which is well developed. The width of the fossa is 43.3 µm and

Table 1: Collection spots with GPS data

Location	*Latitude, Longitude*	*Specimen collected*
Vandalur lake, Chennai	12°53'22.5"N 80°04'26.9"E	*Chara zeylanica*
Malabar botanical garden, Kerala	11°14'16.7"N 75°49'40.3"E	*Chara braunii, Chara coralline*
In a canal in Karunagapulli, Kerala	9°03'56.0"N 76°31'59.0"E	*Nitella furcata, Nitella pseudoflbellata*



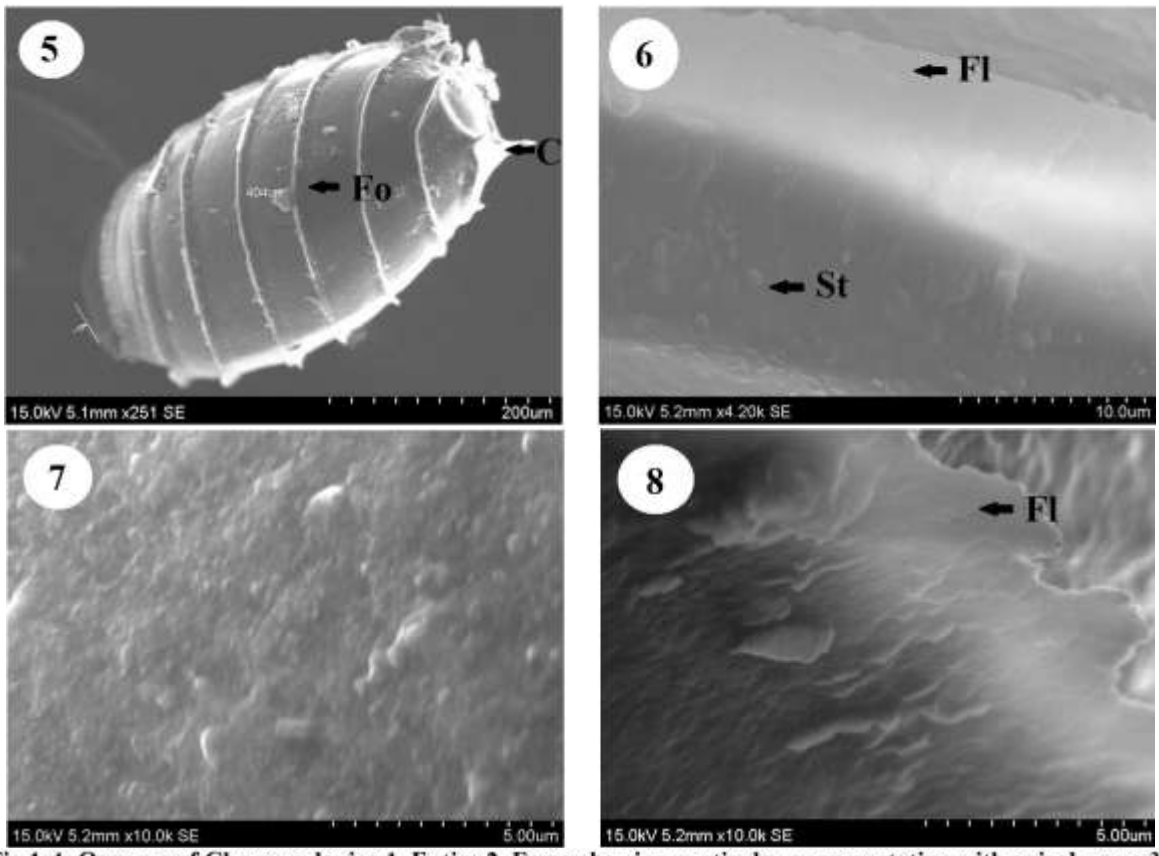


Fig 1-4: Oospore of Chara zeylanica 1. Entire 2. Fossa showing pusticular ornamentation with apical pores 3. Striae with flange 4. Flange ornamentation Fig 5-8: Oospore of *Chara braunii* 5. Entire 6. Striae with flange 7. Fossa showing roughened surface ornamentation 8. Flange ornamentation
(Fo – Fossa, Fl – Flange, St – Striae, Ap – Apical pores, C – Clawed base)

has irregular pusticular ornamentation with apical pores in each pusticule. Flange also shows pusticular ornamentation (Fig 1-4).

Previous reports:
C. zeylanica Klein Ex Willd var. *zeylanica* f. *zeylanica* from India is reported to have ruggulate and granular fossa ornamentation with the presence of flange (Mandal and Ray, 2004). *C. zeylanica* Klein. Ex. Willd. var. *sejuncta* f. *sejuncta* (*C. foliolosa*) is reported to be nodulated with few pits, pores and flange (John *et al*. 1990).

***Chara braunii* Gmelin.:**
The black mature oospores measure 404X238 µm (±1) and are elliptical in shape. They consist of 9-10 striae which are prominent and bear the flange which is not ornamented. The width of the fossa is 48.4 µm and has a roughened surface (Fig 5-8). Clawed oospore is observed clearly in the present specimen.

Previous reports:
According to Mandal and Ray (2004) *C. braunii* Gmelin f. *braunii* and *C. braunii* f. *schweinitzii* (A. Br.) R. D. W. reported in India has a rough fossa ornamentation due to the presence of pits, pores and minute granular elevations and flange absent.

In *C. braunii* f. *schweinitzii* (A. Br.) R. D. W. reported by John *et al*. (1990) fossa is nodular with fused strands. In another form reported by Mandal and Ray (2004) viz. *C. braunii* f. *oahuensis* (Meyen) R. D. W. the fossa is rough due to the presence of pits and pores.

***Chara corallina* Klein. Ex. Willd.:**
The ovate oospore is black when mature and measures 969X486 µm (±2). The 9 striae are very prominent showing nodular ornamentation. Flange is well developed and ornamented with nodules. The fossa is 110µm wide with irregular nodular ornamentation. (Fig 9-12).

Previous reports:
C. corallina Klein. Ex. Willdenow reported in India by Mandal and Ray (2004), and *C. corallina* reported from India by John *et al*. (1990) has fossa wall covered with pits and pores or irregularly scattered pustular elevations with apical pores. In the specimen reported by Mandal and Ray (2004) the flange is absent while it is present in *C. corallina* Klein. Ex. Willdenow reported by John *et al*. (1990). In *C. corallina* var. *wallichii* from India, the fossa ornamentation is a dense network of fused nodulated strands in-between which are small and irregular gaps. Flange is absent in this specimen (John *et al*. 1990).

***Nitella furcata* Agardh.:**
The mature oospore is brown in color measuring 240X233.3µm (±1). 6-8 striae present, flange absent. The ornamentation of the fossa is trabeculae, each trabecule basally fused with each other and arranged in parallel rows perpendicular to the striae (Fig 13-16).

Previous reports:
As reported by Mandal and Ray (1999), *N. furcata* subsp. *Mucronata* var. *mucronata* f. *dictyosperma* has a reticulate ornamentation of the fossa, with each reticulae elevated at their point of insertion, and the ornamentation extending to the striae. According to them in *N. furcata* subsp. *Megacarpa* f. *tumulosa* the fossa wall is papillate with sporadic fusion of papillae. The fossa ornamentation of *N. furcata* subsp. *Furcata* var. *sieberi* is reticulate and beaded (Cartajena and Carmona, 2009).

***Nitella pseudoflabellata* Braun:**
The mature oospore is brown in color measuring 254X173µm (±1). 6 striae with no ornamentation on flange. The fossa width is 35.4µm and the ornamentation is strioreticulate (Fig 17-20)

Previous report:
N. pseudoflabellata oospore shows tuberculate ornamentation according to Casanova, 1991. The *N. pseudoflabellata* var. *imperialis* f. *watti* (J. GR) R. D. W. exhibits strioreticulate ornamentation as described by John *et al.*,, 1990. *N. pseudoflabellata* subsp. *Pseudoflabellata* var. *leptodactyla* (J. G. R) R. D. W. f. *leptodactyla* exhibits granulate ornamentation of the oospore (Mandal and Ray, 1999).

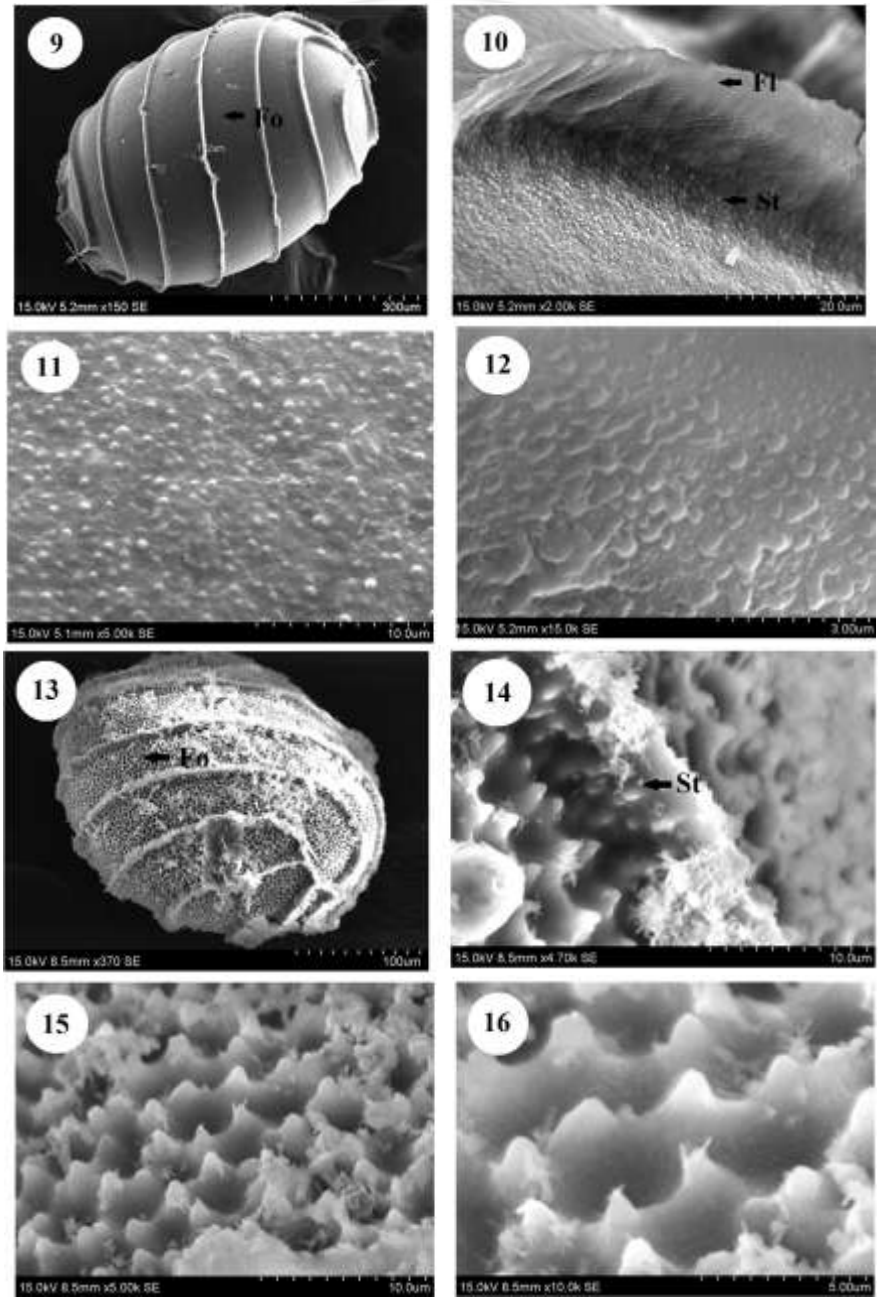


Fig 9-12: Oospore of *Chara Corollina* 9. Entire 10. Striae with flange 11. Fossa showing nodular ornamentation 12. Flange ornamentation

Fig 13-14: Oospore of *Nitella furcata* 13. Entire 14. Striae, Flange-absent, 15. Fossa showing trabeculae ornamentation, 16. Traeculae are basally fused

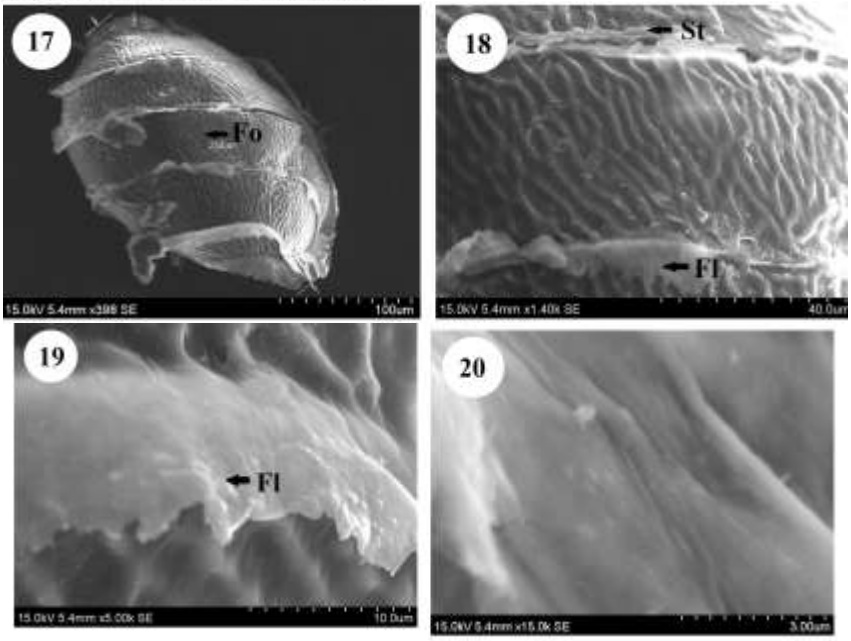


Fig 17-20: Oospore of *Nitella pseudoflabellata* 17. Entire 18. Fossa showing strioreticulate ornamentation 19. Striae with flange 20. Flange – no ornamentation seen

4. Discussion

In the earlier taxonomic studies (Wood,1965; Zeneveld, 1940; Groves, 1924; Sundaralingam, 1959; Pal et al., 1962) morphological characters have played a major role in classifying Charophytes upto species level and the chromosomal studies (Sarma and Khan, 1965; Chakrabarty and Ray, 2016) have helped to classify chara species at intraspecific level. Recent studies on Chara includes scanned micro-structural features which are employed for the delimitation of supra-specific categories. There is need to coin the suitable terminology for most of the scanned micro structures which might be of immense help for better understanding of morphology, anatomy, ecology, taxonomy and evolution of Charophytes (Anand and Langer, 2002).

Scanning electron microscopic studies have highlighted the variation in different taxonomic features of Charophytes which otherwise are not visible under light microscope. SEM studies on the oospore wall ornamentation have brought many interesting points of taxonomic discussion. In the present work three species *Chara* and two species of *Nitella* revealed significant difference in oospore characteristics.

In SEM work on *Chara zeylanica* Klein Ex Willd, fossa shows the pusticular ornamentation with pits, pores and flange as reported in *Chara zeylanica* Klein Ex Willd. var. *sejuncta* f. *sejuncta* by John *et al*., (1990). Hence this specimen from South India is identified as *Chara zeylanica* Klein. Ex. Willd. var. *sejuncta* f. *sejuncta*.

The oospore of *C. corallina* is pustular with flange as seen in John *et al*.,(1990) specimen. However it differs from Mandal and Ray (2004) where the flange is absent and in the present specimen flange is present.

In *C. braunii*, the flange is prominent, not ornamented and fossa is roughened. John *et al.,* (1990) have treated *C. braunii* as a different species, *C. schweinitzii Braun (syn: C. braunii* Gmelin f. *schweinitzii* (Braun) Wood). However Sundaralingam (1959) has reported two varieties of *C. braunii* Gmelin, based on morphological characters which were similar to those described by Zeneveld, namely *C. braunii* var. *coromandelina* (A. Br.) and *C. braunii* var. *Kurzii* . John *et al.,* (1990) and Mandal and Ray (2002, 2004) have identified formas in *C. braunii* species. Mandal and Ray (2004) have published the SEM studies on oospore for the first time in India on *C. braunii* and identified it as *C. braunii* f. *oahunensis* based on the absence of flange and rough pits and pores. Since the present material agrees in vegetative characters with that of *C. braunii* var. *coromandelina*, reported by Sundaralingam (1959) from South India, it is accepted as *C. braunii var. coromandelina* (A. Br.), though it shows similar fossa ornamentation as noted by Mandal and Ray (2004) in the three above mentioned forms. However, in the present work the presence of clawed base of the oospore of *C. braunii* is reported for the first time though earlier works of *C.*

braunii by Mandal and Ray (2004) and John *et al*. (1990) have not reported the clawed base in their SEM studies.

In the present study SEM work is done on two species of *Nitella*, namely *N. furcata* and *N. pseudoflabellata*. The specimen *N. furcata* is identified as *N. furcata* subsp. *Megacarpa* f. *tumulosa* described by Mandal and Ray (1999) which also shows papillae with sporadic fusion of papillae.

N. pseudoflabellata shows prominent flange in the present species which is neither mentioned by Mandal and Ray (1999) nor by Casanova (1991). However, Strioreticulate fossa of the present species is also reported by Mandal and Ray (1999) in *N. pseudoflabellata* subsp. *Pseudoflabellata* var. *imperialils* f. *wattii*. Hence the present material which also shows only strioreticulate fossa is identified as *N. pseudoflabellata* subsp. *Pseudoflabellata* var. *imperialils* f. *wattii*.

From these observations, it becomes evident that some of the details thus scanned are quiet considerable and have relevance for the taxonomy of different species of Charophytes. Since all of these characters are qualitative, it would be utilized for the delimitation of supra specific categories of Charophytes.

According to Wood (1965) such plasticity in ecological expressions demands care in making decisions as it is difficult to know which of the "phenotype" being environmentally induced and which ones are genetically controlled. It, therefore, becomes very important to make the comparative SEM studies of the representative taxa of different population of same species under natural and laboratory conditions as well. This would confirm whether the effect of environmental fluctuation could lead to structural changes in microfeatures or not. This would help in splitting some taxa and synonymizing the others.

Thus the present SEM work on South Indian species has revealed the occurance of two new forms of Chara viz. *Chara zeylanica* Klein. Ex. Willd. var. *sejuncta* f. *sejuncta* and *C. braunii* f. *schweinitzii* (A. Br.) R. D. W. and two new form of Nitella viz. *N. furcata* subsp. *Megacarpa* f. *tumulosa* and *N. pseudoflabellata* subsp. *Pseudoflabellata* var. *imperialils* f. *wattii*.

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