IN VITRO CULTURE OF *PTERYGONEURUM COMPACTUM* (MUSCI, POTTIACEAE): CONTROL OF TAXONOMICAL CHARACTERS

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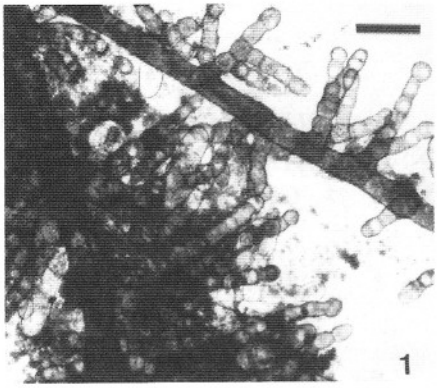
ABSTRACT — The in vitro culture of *Pterygoneurum compactum*, a species recently described by the authors, was realized from adult leaves. From the plant material thus obtained the characters proposed as differential were confirmed.

RÉSUMÉ — La culture, in vitro, de *Pterygoneurum compactum*, une espèce récenment décrite par les auteurs a été réalisée à partir de feuilles adultes. Elle permet de confirmer les caractères spécifiques attribués.

Pterygoneurum compactum Cano, J. Guerra & Ros was described by Cano *et al.* (1994) based on two samples deposited in the MUB (Murcia) and BCB (Barcelona) herbaria. Although our experience led us to believe that differentiating characters proposed within the *Pterygoneurum ovatum* complex were sufficient for it to be considered a new species easily distinguishable from *P. ovatum*, we decided to investigated further since the group possesses numerous characters which may be subject to variation in different enviromental conditions. For this reason, following our research line on the control and validity of the morphological characteristics used in Pottiaceae taxonomy, we carried out an in vitro experiment with *P. compactum* in an attempt to amass more data on its phenotypical plasticity.

The taxa which make up this complex are short-live shuttle species (*sensu* During 1979) which grow in the Mediterranean Region on clayey, loamy or saline soils after rainy periods at the end of autumn and at the beginning of spring. They are xerophytic taxa exposed to harsh environmental conditions and show morphological adaptations (incurved leaves, lamellae, supracostal chlorophilic filaments, leaf papillae, hyaline basal leaf cells, etc.) that retain water for the largest possible time (cf. Guerra *et al.* 1992). It was the variations in these characters which we decided to study in order to ascertain their permanence and use in taxonomy.

Szweykowski (1984) approached the problem of interpreting the results of the in vitro culture of bryophytes intended for taxonomic studies by maintaining, along with Zander (1982), that a previous taxonomic study by traditional methods is necessary. In our case, we studied numerous herbarium samples of the genus *Ptery-*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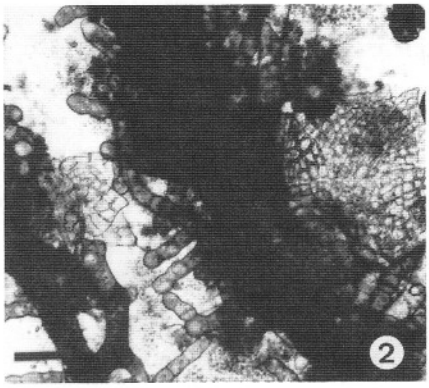
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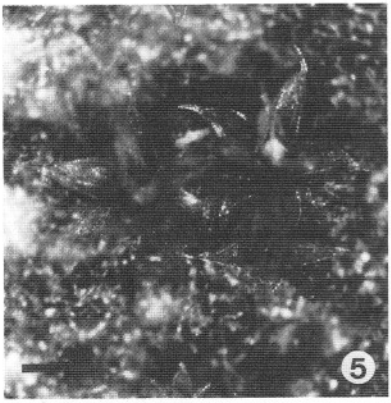
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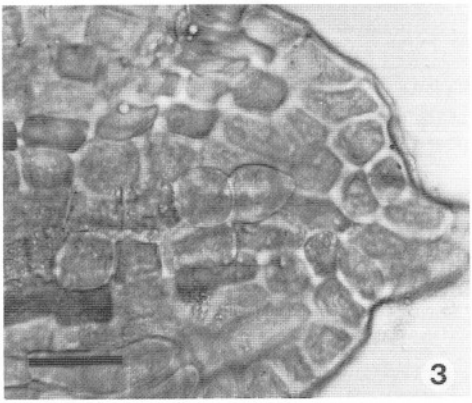
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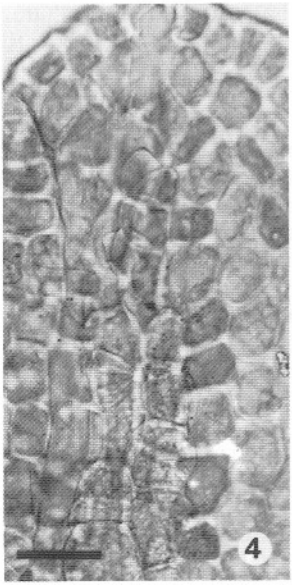
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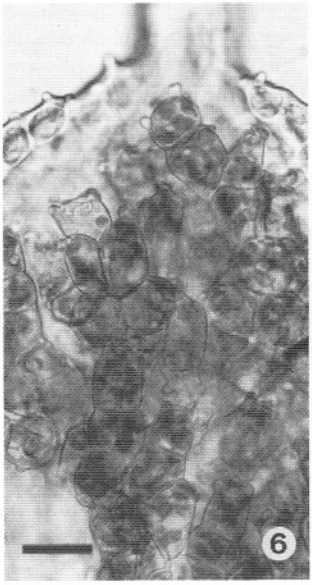
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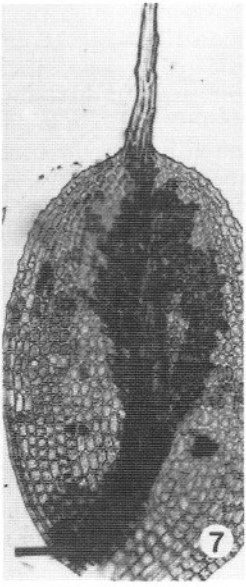
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goneurum which established a base line study. In vitro culture was begun to confirm whether the characters proposed as differential were merely phenotypical within the *P. ovatum* complex or were genotypical variation (Smith 1979).

The main differences between *P. compactum* and *P. ovatum*, a species with which it usually grows, are the papillose leaf cells of the former, particularly on the abaxial side of the leaves, and the presence of ramified filaments with papillose cells which start from the supracostal lamellae and form a cushion of compacted filaments near the leaf apex. All the other gametophytic and sporophytic characters are very similar to those of *P. ovatum* (cf. Cano *et al.* 1994).

MATERIAL AND METHODS

The original material for describing *P. compactum* consisted of two collections deposited in the MUB and BCB herbaria. Although one of the samples had sporophytes and the spores seemed to be sufficiently mature, there were not enough sporophytes to initiate a culture and so mature leaves were used instead. The leaves from several stems were separated and submerged in water contained in a vial and cleared by ultrasound for approximately 5 minutes at 40 Khz. They were then sterilized in 0.1 % commercial sodium hypochlorite solution and a drop of detergent for 5 minutes. The cultures were initiated by placing 5-6 leaves in each of 5 Petri dishes containing 2 % Knop-agar according to the method described by Bopp *et al.* (1964). These were then kept in a culture chamber at 20°C with a photoperiod of 20 h light and 4 h darkness, under a luminous intensity of 1500-3000 lux. The cultures were kept in these conditions for three months, after which the plant material obtained was submitted to microscopic examination every week.

RESULTS

Abundant chloronematic and caulonematic filaments (Fig. 1) and small buds consisting of 4-5 leaf primordia (Fig. 2) were observed. These began to differentiate a costa very early, and form the first supracostal cells which would later make up the corresponding lamellae (Figs. 3 and 4). As small erect caulidia began to form (Fig. 5) so the number of leaves increased and they began to take on an adult appearance. Filaments made up of subspherical, cilindrical, papillose or smooth cells developed over both sides of the supracostal lamellae. The terminal cells of these filaments were always papillose with 2-5(8) simple or bifurcate papillae (Fig. 6).

At the leaves grew in size, the supracostal filaments ramified to form a cushion of filaments near the leaf apex, the basal part of the lamellae remaining without filaments and maintaining their similarity with other species of the genus. (Fig. 7).

Figures 1-7. — *Pterygoneurum compactum* obtained in vitro cultures. 1, 2: chloronematic and caulonematic filaments. 3, 4: young leaves showing first supracostal cells (arrows). 5: young caulidia with leaves. 6, 7: adult leaves showing papillose laminal cells and filaments. (Scales: 1, 2, 7 = 0.1 mm, 3, 4, 6 = 50 μm, 5 = 1 mm).

The juvenile state of the leaves which formed the buds had completely smooth lamina cells although papillae began to differentiate very soon on the abaxial side of the apical cells of the lamina and costa (Fig. 6). Due to their small size, these papillae were difficult to detect in the herbarium sample so that only under SEM could they be observed clearly (cf. Cano *et al.* 1994). However, they could easily be distinguished on the culture specimens since these were not covered with the normal debris which accumulates on samples collected from the field.

CONCLUSION

The material obtained in vitro culture under conditions totally distinct from those in which *P. compactum* usually grows (saline or gypsiferous soils in sunny, dry places) (cf. Cano et al. 1994), showed that the gametophytic characters differentiating it from *P. ovatum* persisted, lending validity to this taxon, which until now had passed unnoticed due to its affinity with *P. ovatum.*

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