

# AN EMBRYOLOGICAL APPROACH TO THE TAXONOMIC STATUS OF VACCINIACEAE

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Received November 21, 1972

## ABSTRACT

The structure and development of male and female gametophytes, endosperm and embryo are described for *Vaccinium nummularia* Hook., *V. retusum* Hook. and *V. serratum* Weight. A comparison of embryological information presented here with that of other species given by previous workers shows general agreement in all the vacciniums. In view of the similarities between the vacciniums and the subfamilies of Ericaceae, the grouping of Vaccinioideae as a subfamily with the rest of Ericaceae, as against the erection of the family Vacciniaceae, is supported.

## INTRODUCTION

BENTHAM AND HOOKER (1862-83) treated the vacciniums and allied genera as belonging to a separate family Vacciniaceae under Ericales. Following them Hutchinson (1959) also recognised the family Vacciniaceae as distinct from the Ericaceae. However, according to Drude's (1889) classification (in Engler and Prantl, 1887-99) the Vacciniaceae are given a lower taxonomic status as a subfamily under Ericaceae along with Rhododendroideae, Arbutoideae and Ericoideae. Rendle (1938) and Gundersen (1950) agreed with Engler and Diels (1936).

Embryological information of this group is fragmentary if not incomplete (Schnarf, 1931; Davis, 1966) with the exception of *Vaccinium*. Stushnoff and Palser (1969) who studied five taxa of this genus—three species and two cultivars of hybrid origin at different ploidy levels—observed embryological conformity among them. Since their materials are from North America and Europe, embryological studies were extended to three more species (*viz.*, *Vaccinium nummularia* Hook., *V. retusum* Hook., and *V. serratum* Weight) growing in India (of uncertain provenance) and the results reported briefly earlier (Venkateswarlu and Maheswari Devi, 1960) are now presented in detail.

#### MATERIAL AND METHODS

Material of *V. nummularia*, *V. serratum* and also some material of *V. retusum* were kindly collected from Darjeeling area and fixed in formalin-acetic-alcohol by Dr. R. Seshagiri Rao, Botanical Survey of India, to whom we are thankful. Customary methods of dehydration, embedding and microtoming were followed. The sections were stained in Delafield's haematoxylin.

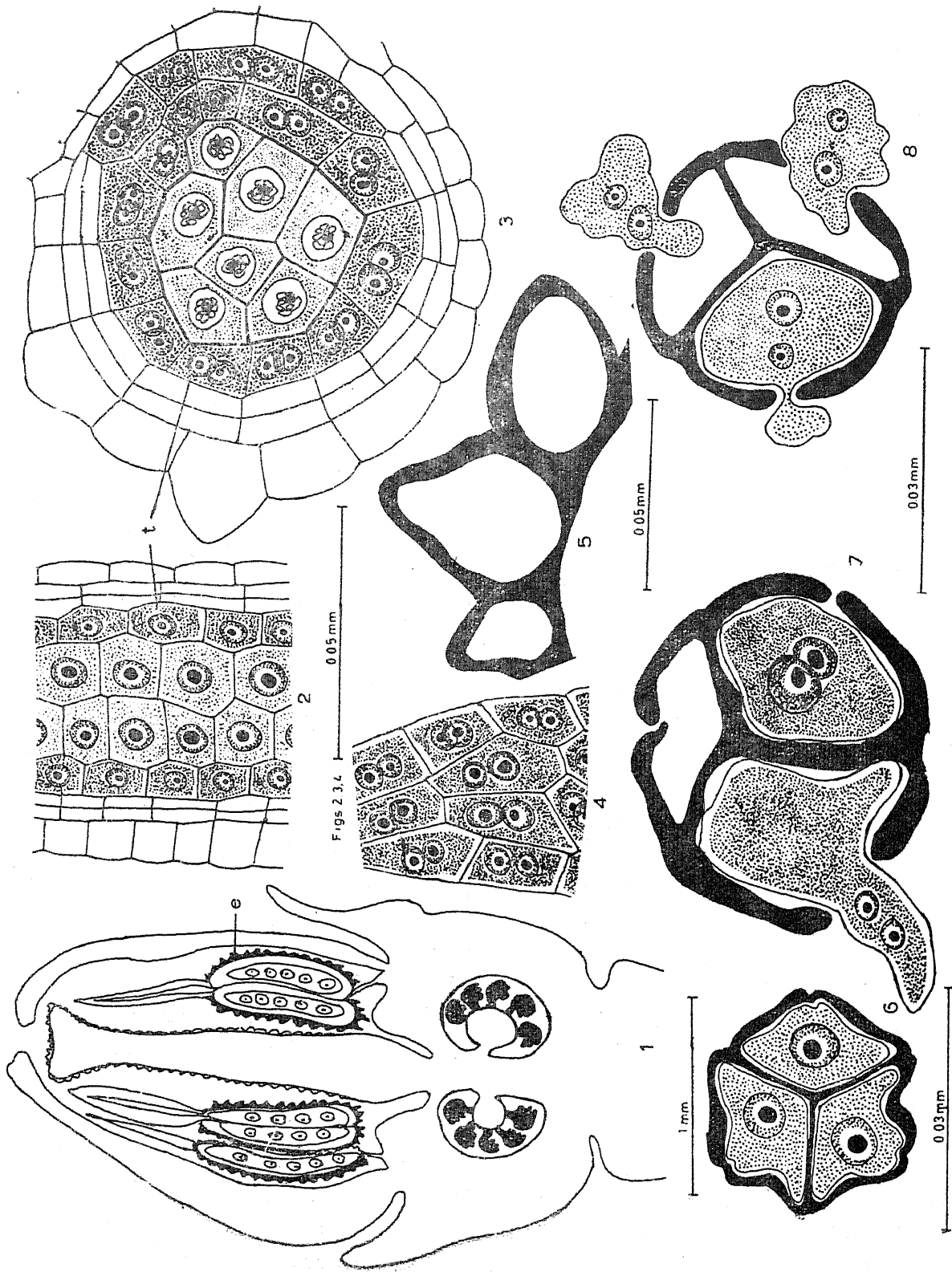
#### OBSERVATIONS

*Floral morphology.*—The flowers are bisexual, pentacyclic, sympetalous, actinomorphic and epigynous. The stamens are introrse, spurless and dehisce by pores at the tips of the awns. The gynoecium is pentacarpellary, syncarpous and the ovary is inferior, five locular with two rows of ovules in each on axile placentae. The floral whorls develop in an acropetal succession.

*Microsporangium, microsporogenesis and male gametophyte.*—The anther is tetrasporangiate. Its wall consists of the epidermis, two middle layers and tapetum (Figs. 2, 3). An endothecium is not differentiated. The tapetum is parietal in origin and is of the secretory type. Its cells become binucleate by the time the pollen mother cells are in meiotic prophase. It remains uniseriate except on the connective side where it becomes multi-seriate (Figs. 3, 4). The middle layers become crushed by the time the pollen tetrads are formed. However, the walls of the epidermal cells become thickened and project out giving a wavy appearance (Figs. 1, 5).

The primary sporogenous cells undergo repeated divisions and produce a large number of pollen mother cells which undergo meiosis with simultaneous cytokinesis and produce both bilateral and tetrahedral tetrads. Divisions in all the four locules within an anther are not synchronous. The pollen grains remain united in tetrads at the time of liberation (Fig. 6). The pollen grains are 2-nucleate, with three germinal furrows at the time of shedding. The four pollen grains of a tetrad do not germinate at the same time (Figs. 7, 8).

*Megasporangium, megasporogenesis and female gametophyte.*—The ovule is anatropous, unitegmic and tenuinucellate with a narrow micropyle. The innermost layer of the integumentary primordium is differentiated as the endothelium by the time meiosis takes place in the megaspore mother cell. It remains uniseriate in *V. nummularia* but becomes biseriate in



Figs. 1-8. *Vaccinium serratum*. Fig. 1. L.s. flower, Fig. 2. L.s. part of anther lobe showing middle layers, secretory tapetum and pollen mother cells. Fig. 3. T.s. anther lobe showing middle layers, binucleate tapetal cells and pollen mother cells in meiotic prophase-I. Fig. 4. Part of multiserial anther tapetum. Fig. 5. Thick-walled epidermal cells of anther. Fig. 6. Pollen tetrad. Figs. 7, 8. Germinating pollen tetrads. e., epidermis; t., tapetum.

*V. serratum* and *V. retusum* (Fig. 10). The nucellar epidermis becomes completely disorganised at about the two or four-nucleate stage of embryo sac.

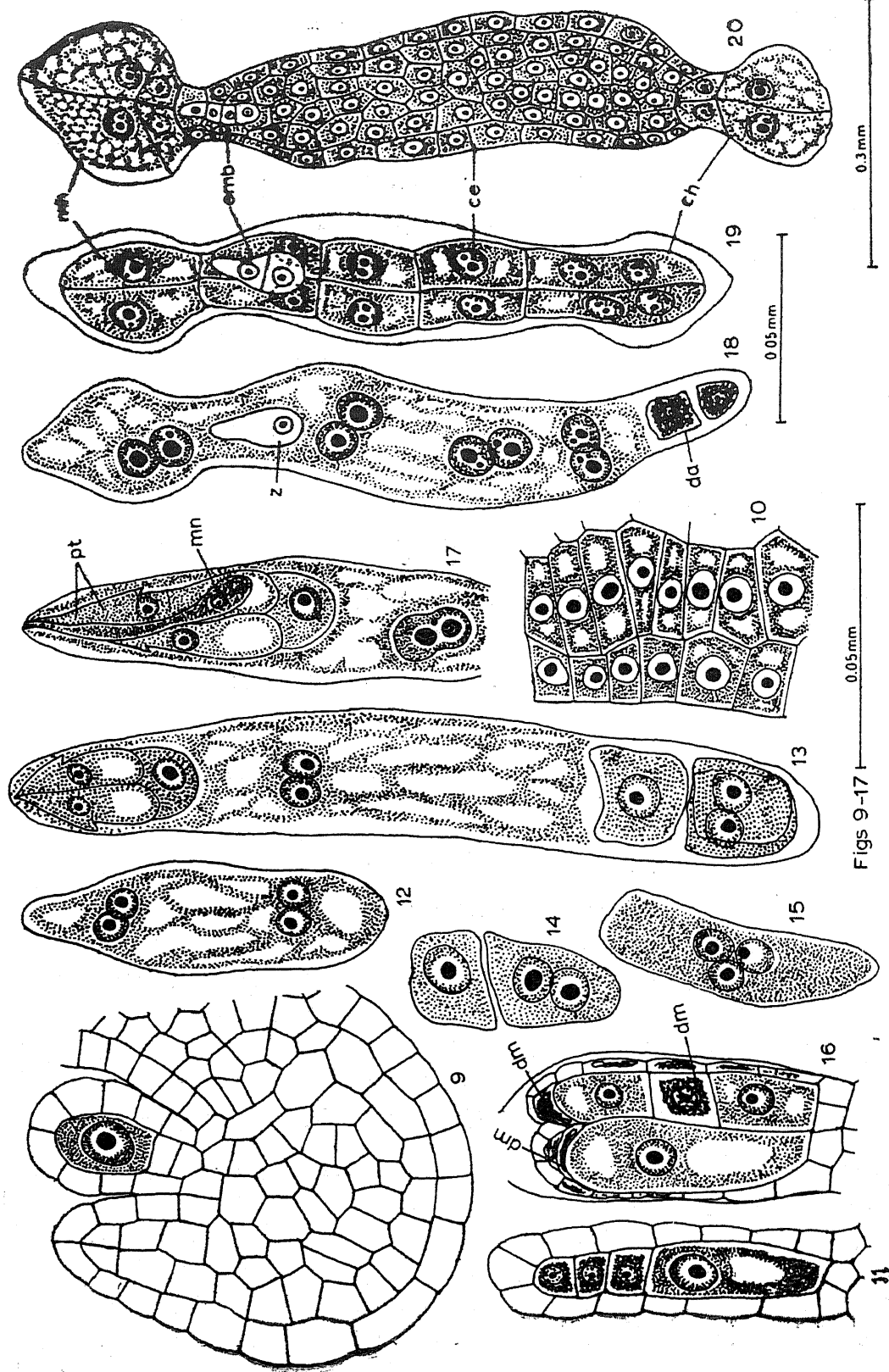
The archesporium is hypodermal (Fig. 9). It functions directly as the megaspore mother cell without formation of any parietal layers. Meiosis occurs normally and a linear tetrad of megaspores is formed, of which the chalazal one develops into an eight-nucleate embryo sac of the Polygonum type (Figs. 11–13). The synergids are hooked and are organised earlier than the egg cell. The two polar nuclei fuse in the vicinity of the egg apparatus. In *V. retusum* three uninucleate antipodal cells are formed (Fig. 13). However, in the other two species their number seems to vary. A single three-nucleate condition is noted in *V. serratum* (Fig. 15). In *V. nummularia* there are 2 antipodal cells, of which one is binucleate (Fig. 14). The antipodal cells persist until the endosperm haustorium develops in the chalazal region of the embryo sac.

Occasionally ovules with two archesporial cells and two megaspore tetrads are met with (Fig. 16). Apparently only one of them is functional as occurrence of two embryo sacs in an ovule has not been observed.

*Fertilisation and endosperm.*—Fertilisation is porogamous (Fig. 17) with syngamy and triple fusion taking place normally.

The endosperm is of the Nuclear type. The primary endosperm nucleus divides earlier than the zygote. After the first division the two nuclei move apart and divide twice successively. The eight free nuclei so formed are arranged in four groups of two each (Fig. 18). At this stage wall formation commences and divides the embryo sac into three cells—two 2-nucleate cells one at each pole and a large central cell which is 4-nucleate. The cells at either end undergo one more vertical division (Fig. 19) and eventually develop into chalazal and micropylar endosperm haustoria which enlarge, crush and absorb all but one or two layers of cells of the integument. In the meanwhile, the four nuclei of the middle cell undergo further divisions followed by cell wall formation and give rise to the endosperm proper (Figs. 18–20).

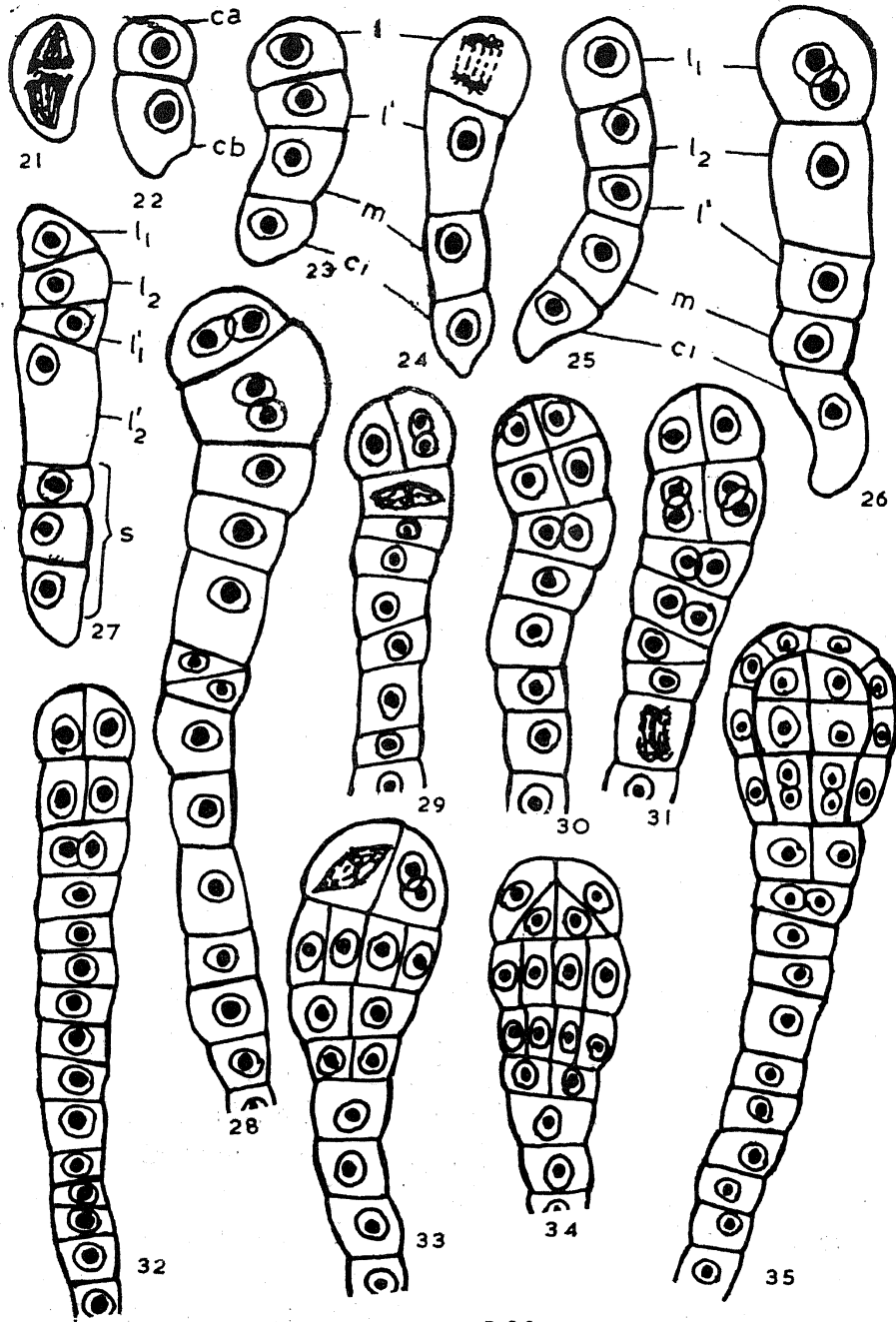
*Embryo.*—The first division of the zygote is transverse and each of the two cells *ca* and *cb* undergo one more transverse division; the cells of the linear proembryo are termed *l*, *l'*, *m* and *ci* (Figs. 21–23). Two superposed cells *l*<sub>1</sub> and *l*<sub>2</sub> are then derived from *l* (Figs. 24–27). In each of these cells, quadrants are formed by two vertical divisions (Figs. 28–32). The derivatives of the tier *l*<sub>1</sub> contribute to the formation of cotyledons and stem tip



Figs. 9-20. *Vaccinium retusum*. Fig. 14. *V. nummularia*. Figs. 15-20. *V. serratum*. Fig. 9. L.s. ovule showing megaspore mother cell. Fig. 10. Part of multiseriate endothelium. Fig. 11. Megaspore tetrad. Figs. 12 and 13. Four- and organized eight-nucleate embryo sacs respectively. Figs. 14, 15. Antipodal cells. Fig. 16. Twin megaspore tetrads. Fig. 17. Upper part of embryo sac showing egg apparatus, pollen tube with two male nuclei and secondary nuclei. Fig. 18. Embryo sac showing nuclear endosperm, zygote and degenerating antipodal cells. Figs. 19, 20. Embryo sac showing embryo, cellular endosperm micropylar and chalazal endosperm haustoria.

ce., Cellular endosperm; ch., chalazal endosperm haustorium; da., degenerating antipodal cells; dm., degenerating megaspores; emb., embryo; mt., micropylar endosperm haustorium; pt., pollen tube; mm., male nucleus; z., zygote.

and that of  $l_2$  to the hypocotyledonary region (Figs. 31-35). The cell  $l'$  divides transversely resulting in two cells  $l'_1$  and  $l'_2$ . The cell  $l'_1$  undergoes further divisions in all directions and contributes to the formation of all



Figs. 21-35 | 0.03 mm

Figs. 21-35. *Vaccinium serratum*. Embryogeny.

parts of root. The cells of  $l_2'$ ,  $m$  and  $ci$  undergo several transverse divisions and produce an elongated uniseriate suspensor (Figs. 27-35).

The four-celled proembryo is linear and the embryo proper is derived from a large part of the cell  $ca$  of the two-celled proembryo, whereas the suspensor is formed from the basal cell  $cb$  and a few cells contributed by its sister cell  $ca$ . Thus the embryo development in the three *Vaccinium* species follows the Solanad type.

#### DISCUSSION AND CONCLUSIONS

The salient embryological features of the three *Vaccinium* species studied are: The microsporangium has two middle layers, endothecium is not differentiated, the tapetum is secretory with binucleate cells; the pollen are liberated through apical pores and they remain united as tetrads and pollen grains are two-celled. The ovule is anatropous, unitegmic and tenuinucellate; an integumentary tapetum is present; the embryo sac is of the Polygonum type; antipodals are one, two or three nucleate depending upon their number varying from one to three in each embryo sac. The endosperm is free nuclear and develops both micropylar and chalazal haustoria. The embryo development is of the Solanad type.

*Vaccinium* species of different ploidy levels, including some cultivars of hybrid origin, have been embryologically studied by Gilbert (1952), Bell and Burchill (1955), Bell (1957), Batygina *et al.* (1963), Rousi (1967) and Stushnoff and Palser (1969). Although the chromosome numbers or the ploidy levels of the material investigated here are not known, it was interesting to observe greater embryological agreement between the North American-European taxa and the Indian species. However, some species show differences in certain embryological details. Thus the tapetum is believed to be sporogenous in origin in the three *Vaccinium* taxa studied by Batygina *et al.* (1963) whereas it is parietal in origin in the rest of the species. In *V. serratum* and *V. retusum* the endothelium is biseriata while it is uniseriate in *V. nummularia* (present work) as has also been reported in the five taxa of the genus investigated by Stushnoff and Palser (1969). A greater variability seems to exist in the endosperm development. In our study it conforms to the Nuclear type as in *Rhododendron japonicum* and *R. mucronatum* (Creech, 1955), *Vaccinium augustifolium* (Bell, 1957), *V. corymbosum* (Merrill, 1936) and *V. leschenaultii* (Reddy and Narayana, 1965). In contrast Stevens (1919) observed both Nuclear and Cellular types

TABLE I

*A comparison of morphological and embryological features in different subfamilies of the Ericaceae*

	Rhododendroideae	Arbutoideae	Vaccinioideae	Ericoideae
Anther	Appendages absent	Appendaged	Awned anthers; appendages absent	Appendaged
Fibrous endothecium	Present in some members and absent in others	Present in some members and absent in others	Absent	Absent
Tapetum	Secretory with binucleate cells	Secretory	Secretory with binucleate cells	Secretory
Pollen at liberation	Both tetrads and free pollen grains present	Both tetrads and free pollen grains present	Remain united as tetrads	Both tetrads and free pollen grains present
Pollen grains	Two-celled	Two-celled	Two-celled	Two-celled
Ovary	Superior	Superior	Inferior	Superior
Ovule	Anatropous, uni-tegmic and tenuinucellate	Anatropous, uni-tegmic and tenuinucellate	Anatropous, uni-tegmic and tenuinucellate	Anatropous, uni-tegmic and tenuinucellate
Embryo sac	Polygonum type	Both Polygonum and Bisporic types	Polygonum type	Polygonum type
Antipodals	Variable	Variable	Variable	Variable
Endosperm	Nuclear	Cellular	Both nuclear and cellular	..
Embryo	Solanad type	Solanad type	Solanad type (only <i>V. corymbosum</i> shows Caryophyllad type)	Solanad type
Fruit	Septicidal capsule	Loculicidal capsule or berry	Berry or drupe	Loculicidal capsule
Seed	Winged	Not winged	Not winged	Not winged



of development in *V. corymbosum* and more recently Gilbert (1952) and Stushnoff and Palser (1969) have described cellular types in the materials studied by them. In embryo development Solanad type seems to be the most common (Veillet Bartoszewaska, 1958; Stushnoff and Palser, 1969 and present report) with the exception of *V. corymbosum* which shows the Caryophyllad type (Gilbert, 1952).

The embryological features of *Vaccinium* and those of the other sub-families of Ericaceae, together with their morphological features, are tabulated in Table I. An analysis of the data shows that there is greater uniformity in the embryological features of these diverse groups which support the retention of Vaccinioideae as a subfamily rather than its elevation to the rank of a distinct family, Vacciniaceae. In fact, Watson (1965) while supporting a similar status for this group on the basis of anatomical features is severely critical of Hutchinson's (1959) treatment who promoted the Vaccinioideae into the taxonomic hierarchy of a full family.

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\* Originals not seen.

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4403/73. Published by B. S. Venkatachar, Editor, "Proceedings of the Indian Academy of Sciences", Bangalore and Printed by V. J. F. Jesudason at the Bangalore Press, Bangalore-560018.

Supplement to the

"Proceedings of the Indian Academy of Sciences"

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