Research Paper

A Critical Review of the Female Gametophyte in the Podostemaceae - Past, Present and Future Studies

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ABSTRACT: *Anoverview of the habit and habitat of Podostemaceae is briefly discussed. Then,the paper attempts to review the past and present female gametophyte studies in the Podostemaceae. It trace the course and major achievements during the megasporogenesis, megagametogenesis and embryogenesis studies in the Podostemaceae. The evolutionary and functional significance of polarity during two-nucleate stage division of the megagametogenesis is discussed. Antipodal cell in the organized female gametophyte is discussed. Possible courses and causes of single fertilization and nucellar plasmodium organization are suggested. Then, provides and points out areas of special attention and limitations in the study of female gametophyte in future. Attempts are made to relate the ontogenies found in the family to plants with similar single fertilization phenomenon, for example Spinacia and Oenothera. Their embryological significances in the development of the female gametophyte in the angiosperms and gymnosperms are discussed.*
Key words: *Chalazal Megaspore Nucleus,Female Gametophyte, Filiform apparatus, Micropylar Dyad Cell, Nucellar Plasmodium,No Antipodal cells, Two Nucleate Stage, Single Fertilization.*

I. INTRODUCTION

Plant embryology occupies today an important position among disciplines of research in botany. Ever since the publications of Coulter and Chamberlain's (1903)[1], 'Morphology of Angiosperms' this subject has attracted much attention. Later, Schnarf of Vienna published two very valuable books on this subject (Schnarf, 1929[2], 1931[3]). These publications stimulated further research in many laboratories all over the world. Two important publications which appeared at the middle of this century, viz., P. Maheshwari's (1950) [4], 'An Introduction to The Embryology of Angiosperms' and Johansen's (1950)[5], 'Plant Embryology' covered the extensive literature that had accumulated by then. It came to be realized through these publications that angiosperms embryology could be of considerable value and prominence in comprehending the interrelationships among families, genera and species and this could be of great value in taxonomy. P. Maheshwari (1963)[6], Rutishauser (1969)[7], Bhojwani and Bhatnagar (1974)[8], and Johri (1984)[9]have thrown considerable light on the current development in the field. Davies (1966)[10] compiled the available references in her book, "Systematic Embryology of Angiosperms". Recent reference on the subject have been compiled by Nagendran and Dinesh (1989)[11].

The word embryology comprises, in its broader sense, the development of male and female gametophytes, the endosperm, the embryo as well as sporophytic tissues directly engage in fertilization. Some families are specially marked out by embryological characters. As examples, the nucellar plasmodium (=pseudo-embryo sac) in the Podostemaceae, Composite endosperm in the Loranthaceae, and the Oenothera type of embryo sac in the Onagraceae, may be cited. Cocucci (1983)[12] has shown the importance of embryological characters in the Loranthaceae and Hydnoraceae. He [12] also discussed the importance of chemical composition of the pollen as a taxonomic tool.

Podostemaceae commonly called River Weed family constitute one of the most curious dicotyledonous taxon. The prevalence of a high degree of polymorphism in the exomorphic characters of each taxon size, shape and colour varying from habitat and from season to season of the same year by the same individual has baffled taxonomists for a long time. Willis (1902)[13] in this regard correctly remarks, 'It is in the highest degree essential to investigate the entire life history and morphology of these plants before any safe deductions as to their grouping can be drawn." (p.189).

The family is represented by about 275 taxa spread over in 50 genera (Mabberley, 1987)[14]. Members of Podostemaceae show remarkable modified appearance and lack truly recognizable stems and roots which keep them aloof from all other angiosperms. They form a group of herbaceous plants on the rocks of stones in swift-following waters. As they complete their life history in water of gushing mountain streams, their physiognomy is completely different from other flowering plants. The vegetative plant body is a creeping of floating thallus resembling an alga or a bryophyte for superficial observation. The state of the vegetative phase in submerged condition, entirely contrasts the fertile phase. Most of the taxa appear different in different streams. The thallus is relatively small bearing a number of short, determinate secondary shoots. On the abaxial side of the thallus, haptera and/or rhizoids are produced for anchorage. Mostly, the vegetative part of the plant is entirely photosynthetic in nature, exhibiting chlorophyll pigment in abundance. The leaves arising from secondary shoots are quite simple, small and filamentous. As their basal portion transforms into bracts, the upper portion are usually deciduous.

The flowers are regular, bisexual, either solitary or in a cymose inflorescence. When young, the flowers are enclosed in a spathe and enlarged, partially united bracts. The perianth comprises two to many tepals. There are one to four (or sometimes numerous) hypogenous stamens. The filaments are either free or partially united. The anthers have two locules, dehiscing lengthwise. Pollen grains are either shed as dyads or monads. The gynoecium is of two or three united carpels. The superior ovary has two or three locules each with a large axile placenta bearing numerous ovules. The styles are free slender. Two or three stigmas are present, mostly subulate or cylindrically shaped. The fruit is a capsule containing numerous minute seeds which *ab initio* lack endosperm.

The seeds are dispersed during dry season and do not germinate until the next rainy season. As thallus develops from the seed, haptera and/or rhizoides arise from it. Flowers are not produced until the end of the rainy season. After fruiting, the thalloid plant body usually dies. Podostemaceae stands aloof from all other angiosperms in its exclusive embryological features [8].

The following combination of embryological characters make them a unique family among the angiosperms:
(i)   Pollen in Podostemoideae and Tristichoideae is shed as dyads and monads, respectively;
(ii)   The female gametophyte development is highly telescoped and are of special types not observed in any other angiosperm taxa;
(iii)   Single fertilization in contrast to double fertilization;
(iv)   Total absence of endorsperm which is compensated by the organization of pseudo-embryo sac which has no parallel among angiosperms.

Inspite of the work done regarding embryo sac ontogeny (Went, 1908[15], 1910[16], 1912[17], 1926[18]; Magnus 1913[19]; Chiarugi, 1933[20]; Razi, 1949[21], 1955[22], 1966[23]; Mukkada, 1962a[24], 1962b[25], 1964[26], 1969[27]; Walia, 1965[28]; Jäger-Zürn, 1967[29]) in various taxa of the family, their observations and interpretations are not free from criticisms. Also, some work on the female gametophyte ontogeny [28] [29] is considered as insufficient embryological data (Battaglia, 1971)[30].
A total of 73 species of Podostemaceae have been recorded in six areas of Africa. The six areas are, Tropical West Africa, Equatorial West Africa, South Africa, Madagascar, Tropical Central Africa and East Africa. These 73 species belong to the genera, *Butumia G.* Taylor, *Dicraeanthus* Engler*, Endocaulos*C. Cusset,*Inversodicraea*Engler, *Ledermaniella*Engler, *Leiothylax* Warm.*, Letestuella*G. Taylor,*Macropodiella*Engler, *Monandriella* Engler*, Pohliella* Engler*, Polypleurum*(Tayl. *ex* Tul.) Warm., *Saxicolella* Engler,*Sphaerothylax* Bischoff, *Stonesia* G. Taylor*, Tristicha* DU Petit-Thouars and *Winklerella*Engler. Their total number of species, in the family, constitute approximately 26.5% of the total taxa in the world. Of these 16 genera, only *Farmeria, Dicraea (=Dalzellia),Hydrobryum, Hydrobryopsis, Griffithella*, *Inversodicraea, Podostemum, Polypleurum, Tristicha*, *Vanroyenella, Willisia* and *Zeylanidium*are investigated in some of their taxa, for embryo sac ontogeny. In addition, out of the 30 species investigated (approx. 10.9% of the total species in the world), 15 species needs re-investigation [30]. To clarify the controversies raised by[30], detailed investigations and/or re-investigations were done for the Indiana taxa (Arekal and Nagendran, 1975a [31], 1975b [32], 1976[33], 1977a [34], 1977b [35]; Nagendran and Arekal, 1976[36]; Nagendran, Anand and Arekal, 1980[37]; Nagendran, Arekal and Subramanyam, 1977[38]; Nagendran, Subramanyam and Arekal, 1976[39]). Battaglia (1987)[40], raised questions with regard to the embryo sac ontogeny of the Podostemaceae, recorded by earlier investigators. As no such work had been done regarding the embryo sac ontogeny in the Kenyan taxa, thestudies were undertaken (Sikolia and Ochora, 2008[41]; Sikolia and Onyango, 2009[42]). Similar studies were carried in other parts of the world where various species were investigated including*Weddellina squamulosa* (Jäger-Zürn, 1997)[43], *Vanroyenella plumosa* (Murguía-Sánchez, Novelo, Philbrick and Márquez-Guzmán, 2002)[44], *Zeylanidium lichenoides* (Chaudhary, Khanduri, Tandon, Uniyal and Mohan Ram, 2014)[45], *Griffithella hookeriana, Polypleurum stylosum and Zeylanidium lichenoides* (Sehgal, Mann and Mohan Ram, 2014)[46]. These works provided the

picture of the embryo sac ontogeny of genera growing in Kenya, Mexico, India and Germany. This in itself helped in the assessment of the various controversies raised in the embryo sac development and organization in the family of Podostemaceae.

II. PAST STUDIES

The firststudy of the embryo sac ontogeny was carried out in two taxa of the family Podostemaceae, *Oenone imthurnii* Goebel and *Mourera fluviatilis* Aublet by [15]. Later, the occurrence of a similar type of development in several other species of the family, viz., *Apinagiadivertens* Went, *A. goejei* Went, *Cladopus nymani* H. Möller, *Lophogyne capillaeum* Pulle, *Oenone guyanensis* Pulle, *O. marowynensis* Went, *O. treslingiana* Went, *O. versteegiana* Went, *Rhyncholacis macrocarpa* Tul. and *Tristicha hypnoides* (St. Hil.) Sprengel [30]was recorded by him [16][17][18]. According to [15][16][17][18], in all the investigated taxa of the family, there are many anatropous ovules. The megaspore mother cell, which differentites early in the ovule undergoes a heterotypic division resulting in two cells. The upper cell degenerates and only the lower one develops into an embryo sac. The nucleus of the lower cell undergoes division. Given that spindles are in the longitudinal plane of the embryo sac the two resulting nuclei are observed situated at opposite poles of the embryo sac. Of the two, the chalazal one degenerates, while the micropylar nucleus divides twice and participates in the organization of embryo sac consisting of two synergids, an egg and an upper polar cell. At the chalazal end, the egg is situated above the polar cell, while the synergids lie side by side at the micropylar end of the embryo sac. Therefore, the organized embryo sac has nuclear derivatives from the micropylar megaspore nucleus only. Chiarugi (1927) [47] designated the above type of embryo sac development described by [15][16][17][18], for many species of *Oenone* and other genera of Podostemaceae as Oenone type ("Tipo Oenone"). Since Royen (1951)[48] merged the genus *Oenone* in*Apinagia,*[30] renamed the Oenone type of embryo sac as "Apinagia type".

[19]collected *Dicraea elongata*Tul.,*Farmeria metzgeriodes* (Trimen) Willis, *Hydrobryum olivaceum* (Gardn.) Tul.*Lawia zeylanica* (Gardn.) Tul. and *Podostemum subulatus* Gardn., from Ceylon (=Sri Lanka) and investigated the development of female gametophyte. His [30] descriptions for the development of embryo sac in *Lawia zeylanica* (Gardn.) Tul. Tallies with those given by [15][16][17][18], for many species of the family. However, [30] recorded two other embryo sac types of development which markedly differed from the observations of [15][16][17][18].

In *Dicraea elongata*, according to [30] the megaspore mother cell undergoes first meiotic division to produce two unequal dyad cells of which the micropylar one promptly degenerates. The chalazal dyad cell completes second meiotic division and a transverse wall is laid down to give rise to two monocleate cells.

He [30] reported the second type of embryo sac development in *Farmeria metzgeriodes, Hydrobryum olivaceum*and *Podostemum subulatus.* Here, the nucleus of the functional dyad cell undergoes two successive free nuclear divisions to produce four nuclei which contribute to the organization of the embryo sac; this type was designated by [47] as the, "Podostemum type". It develops like Dicraea type but the organized embryo sac consists of two synergids, an egg and a chalazal cell.

According to [3] in the vast majority of cases investigated in Podostemaceae, the chalazal group of cells does not organize at all but is represented by degenerating remains of a nucleus. Further, [3] doubted the type of embryo sac development reported by [19] for *Dicraea elongata* and remarked the explanation is beset with difficulties in many respects. He [3] pointed out that the embryo sac is quite small in the family and occupies essentially the same space as that originally occupied by the primary archesporial cell. He [3] also doubted the reported "Podostemum type" in the taxa investigated by [19].

[20] investigated the embryo sac development in *Weddellina squamulosa*Tul. and reported the Podostemum type. The very existence of the Podostemum type in the family of the Podostemaceae was considered doubtful (Maheshwari, P. 1937a [49]; 1937b [50]; Maheshwari, S.C., 1955 [51]) [30]. *Podostemum ceratophyllum*Michaux was investigated by Hammond (1937) [52] and reported the Apinagia type embryo sac development. P. Maheshwari (1941[53],1950 [54]) [49][50] accepted the observations by [15][16][17][18] but consistently doubted the reported Podostemum and Dicraea types in the family and pleaded for re-investigations of the taxa studied by [19]. While commenting on Hammond's (1937) paper, [50] predicted that embryo sac ontogeny in *Podostemum subulatus* on re-investigation may be found similar to that of *P. ceratophyllum*and not the Podostemum type reported by [19].

After investigating and re-investigating the embryo sac ontogeny in *Griffithella hookeriana* warming and *Lawia zeylanica*, respectively, [21] recorded the Apinagia type in both taxa. Subsequently, [22] re-investigated *Zeylanidium olivaceum* (Gardn.) Engler and observed the Apinagia type contrary to the Podostemum type reported by [19]. In his critical review on bisporic embryo sacs, [51] opined that a re-investigation of *Dicraea elongata, Farmeria metzgeriodes, Zeylanidium olivaceum, Podostemum subulatus* and *Weddellina squamulosa* was necessary. [24]investigated *Dicraea stylosa* Wight and reported an organized embryo sac consisting of a single synergid, an egg both considered sister cells, and two antipodal cells but no

polar nuclei. He [24] differed from the observations recorded by [19] and [21] [22] for the embryo sac ontogeny. After studying *Dicraea elongataand Dicraea stylosa,* [26] emphasized on the positive existenceof Dicraea type of embryo sac in the genus, its complements differing fromother embryo sac types in number and organization. A similar type of embryo sac was reported by [23] in *Dicraea agharkarii* Nandi.

Chopra and Mukkada (1966) [55] investigated *Indotristicha ramosissima* (Wight) Van Royen for embryo sac ontogeny and reported the occurrence of the Apinagia type.

The embryology of eight species of *Hydrostachys* were investigated by Jäger-Zürn (1965) [56], listing various characters by which Podostemaceae differ from Hydrostachaceae. Later, [29] recorded the occurrence of an Apinagia type of embryo sac in all the four taxa of Podostemaceae investigated, viz., *Anastrophea abyssinica* Wedd., *Inversodicraea bemarivensis (*H. Perr.) H. Perr., *Inversodicraea minutiflora* (H. Perr.,) H. Perr and *Tristicha trifaria* (Bory *ex* Willd.) Sprengel.

[27] re-investigated the embryo sac ontogeny in *Terniola zeylanica* (Gardn.) Tul. and confirmed the presence of Apinagia type. He [27] differed in many respects with the observations regarding the embryo sac ontogeny previously reported by [19] [21] [22].

[30] extensively reviewed the literature on the embryo sac ontogeny of the Podostemaceae and pointed out several incongruities in the earlier works [19] [20] [21] [22] [26] [28] warranting thorough re-investigations of the taxa, to clarify several points raised by him. He [30] erected different forms within the Apinagia type and are as follows:
(i)   Apinagia type form A (5-nucleate, 4 celled);
(ii)   Apinagia type form B (4-nucleate, 4 celled);
(iii)   Apinagia type form C (5-nucleate, 5 celled); and
(iv)   Apinagia type form D (6-nucleate, 4-5-6- celled).

Also, [30] doubted the Podostemum type of embryo sac ontogeny in the family and sought a re-investigation of *Weddellina, Farmeria* and *Podostemum subulatus.*

A critical analysis of the works of earlier investigators by [30] exposed pertinent controversies not completely resolved regarding the embryo sac ontogeny of Podostemaceae. Careful investigations and/or re-investigations of embryo sac development became necessary to clarify the disputed points raised, in diverse taxa pf Podostemaceae. In this respect, Nagendran and his co-workers [40] studied many Indian taxa to resolve the controversial points.

Nagendran (1974) [57] reinterpreted the Apinagia type as monosporic and four nucleate since only one megaspore nucleus contributed to all the four nuclei present in the mature embryo sac. He (Nagendran, 1975) [58] renamed the Dicraea type of embryo sac as Polypleurum type because taxonomically the genus *Dicraea* Du Petit-Thouars according to Hall (1971) [59] is *Polypleurum* (Tayl. *ex* Tul.) warm. [31] recorded Apinagia type of embryo sac ontogeny in *Farmeria indica*Willis emend. Arekal *et* Nagendran. For the first time, [32]reported the occurrence of both the Podostemum and Dicraea types of embryo sac ontogeny in *Hydrobryopsis sessilis* (Willis) Engler. After investigating *Hydrobryum griffithii* (Wall. ex. Griff.) Tul.for embryo sac ontogeny, [39] reported the existence of the Apinagia type. Further, [33] reported a peculiar type of embryo sac organization in *Willisia selaginoides* (Bedd.) Warming *ex* Willis, a monotypic and endemic taxon from southern part of India. The organized embryo sac has nuclei derived from both the micropylar and chalazal nuclei of the two-nucleate embryo sac. Therefore, it is similar to the Podostemum type and Polypleurum type, in being truly bisporic. The complements of the embryo sac are disposed in unusual planes; it could be decussate or isobilateral arrangement [33]. A re-investigation of*Griffithella hookeriana* by[36]confirmed the presence of the Apinagia type form B recorded by [21] [22]. However, [36] differed from [21] [22] on various aspects regarding embryo sac ontogeny in this taxon. The embryo sac development in *Polypleurum dichotomum* (Gardn.) Hall, *P. filifolium* (Ram. *et* Joseph) Nagendran, Arekal *et* Subramanyam, and *P. munnarensis,* Arekal*et*Nagendran was investigated by [38]. For the first time, the Apinagia type of embryo sac ontogeny existence was reported in the taxon *P. filifolium,* where the chalazal megaspore nucleus of the two-nucleate embryo sac degenerates as in the majority embryologically investigated taxa in the family [29] [30] [31] [38]. Previously, only the Polypleurum (=Dicraea) type of embryo sac development had been recorded in two taxa of this genus. Investigators [38] confirmed the observations reported by [24] [25] [26] in *P. elongatum* (Gardn.)Hall, and *P. stylosum* (Wight)Hall and [23] in *P. agharkarii* (Nandi) Nagendran, Arekal *et* Subramanyam, for the presence of four-celled embryo sac reported by [19] but refuted the latter's observation of wall formation after the first nuclear division of the functional dyad cell. Further, [38] reinterpreted the Dicraea type of embryo sac as consisting of two synergids, one egg and one polar cell. After investigating the female gametophyte ontogeny in three taxa of *Zeylanidium (*Engler) Tul. (=*Hydrobryum* Engler), [34] refuted the reported existence of two types of embryo sacs in this taxon [28]. In addition, [33] studied the embryo sac ontogeny in *Indotristicha*and *Dalzellia.* [36] re-investigated the female gametophyte development in *Podostemum subulatus* and reported the existence of the Apinagia type contrary to Podostemum type recorded by [19] which was questioned time and again. Also, a re-

investigation was sought for *Weddellina squamulosa* to finally assess the independent existence of the so-called Podostemum type of embryo sac ontogeny in the family.

Critical review on the embryo sac ontogeny in Podostemaceae raised several questions [40]. He [40] accepted that Apinagia type forms C and D of embryo sac [30] were no longer tenable, a point earlier discussed by [32]. He categorically stated that all embryological cases not assigned to the Apinagia type are doubtful cases to be re-investigated. However, Battaglia's views [30] [40] were wholly based on theoretical and analytical approach arising from earlier investigators, and therefore required to be treated cautiously. Despite the limitations, his work stimulated more research studies that providedremarkable contribution to our understanding of the embryo sac development in Podostemaceae.

As early as 1908, Went noted peculiar behavior of nucellar cells located below the developing embryo sac. These cells stretch and lose their walls resulting in the formation of a cavity; [15] designated it "pseudo-embryo sac". Its occurrence consistently has been recorded in all the investigated taxa, in the family. After the investigations of *Indotristicha ramosissima, Tristicha trifaria* and *Dalzellia zeylanica* carried out by [55], [27]and [27] respectively, it was clearly observed in these taxa that the walls of the nucellar cells breakdown only after fertilization, an observation also reported by [35], thus contradicting Razi's report [21] of its origin before fertilization. Etymologically,[32] renamed pseudo-embryo sac as 'Nucellar plasmodium' as it is organized by the fusion of uninucleate protoplasts after the breakdown of the separating cell walls in the nucellus. Further, [32] observed healthy nuclei in the pseudo-embryo sac ('Nucellar plasmodium') earlier reported by [29], contrary to the observations made earlier [15] [16] [17] [18] in the family of Podostemaceae. It has been reported that nucellar plasmodium organizes before fertilization in all the investigated genera of Podostemaceae, while it is a post-fertilization phenomenon in all the genera of Tristichoideae [32] 35].

Some investigators [19] [22] reported double fertilization whilethe others (Mukkada, 1963 [60])[15] [27] [28] [33] [35] [55] [58] reported single fertilization. The observed embryo sac stages showing double fertilization in *Zeylanidium olivaceum* [19] [22] were highly criticized [28] after re-investigating this taxon. She [28] did not observe any evidence to support the contention held by [19] [22] that double fertilization in Podostemaceae takes place normally as in other angiosperms. Similarly, the reported fusion of the second male gamete with polar nucleus in *Terniola zeylanica* [19] [22]was not observed [27]; nor discharged, but it is retained in the tip of pollen tube, as observed in *Indotristicha ramosissima*[55]. However, the very presence of antipodal cells in the family [23] [24] [25] [26] has been strongly refuted by mostinvestigators [31] [32] [33] [34] [35] [36], especially [37]. [30] [40] set his own criteria for considering the chalazal cell of the embryo sac as antipodal without clear embryological evidence.

Inspite of several studies and interpretations, the nature of the embryo sac in Podostemaceae remains controversial [40]. Even after Nagendran and his co-workers [36] [37] [38] [39] exhaustively investigated and/or re-investigated all Indian genera and provided embryological data on embryo sac ontogeny, [40] raised questions with regard to it. On account of taxa endemism, inaccessibility for collection and availability of flowers and fruits only during a limited span of time, several taxa have remained uninvestigated [42]. As, noted by [37], Apinagia type of embryo sac ontogeny has been found in both the investigated species of *Podostemum*, it would be desirable to investigate the embryo sac ontogeny in other species of the genus and re-investigated *Weddellina squamulosa* to finally assess the independent existence of the so-called Podostemum type of ontogeny which to-date has been re-investigated [43]. Therefore there is scope for further research work on the embryo sac ontogeny in the family, Podostemaceae. Similarly, a critical perusal of the previous literature indicates the Apinagia type of embryo sac ontogeny has been recorded only in two taxa of the genus *Inversodicraea, viz., I. bemarivensis* and *I. minutiflora*, while other species of the same genusremains uninvestigated. Further, embryological data for the investigated species of *Inversodicraea* was regardedinsufficient [30] [40]. Furthermore, in Kenyan taxa, the mode of embryo sac ontogeny had not been investigated.

After being aware of these gaps in our knowledge of the embryo sac ontogeny in the family of Podostemaceae, an attempt was made [41] [42] to provide the status of embryological studies in the Kenyan taxa to understand and resolve some of the critical issues. Thus, past and recent studies [41] [42] [43] [44] [45] led to the next parts of discussion. First, to discuss the course of the development and organization in the family Podostemaceae. Secondly, to urge the status of the antipodal cells in the course of female gametophyte ontogeny. Thirdly, to present the mode of fertilization because there are reports of the presence of both double and single fertilization in the family. Fourthly, to discuss the organization of the pseudo-embryo sac in the family of Podostemaceae and rationalize the need to rename it the nucellar plasmodium. Fifth, attempt to discuss the causal factors determining the course of the female gametophyte ontogeny. Lastly, to provide the conclusions and areas for possible research focus in future studies.

III.   PRESENT STATUS

Most of the investigations have been studied using improved rotary microtome serial sized sections (i.e. 2-4µm) of the female gametophyte stages observed under fluorescence microscopy[44] [45] [46].These efforts are acknowledged but transmission electron scanning microscopy of the female gametophyte stages are regrettablymissing in literature.

3.1 Morphology of the Female Gametophyte of Podostemaceae

The potential value of embryological characters is now being realized in scientific studies because they are yielding useful data. Embryological characters have proved to be of great significance, especially when correlated with data from other botanical disciplines of accepted value in solving disputed taxonomic problems. A single embryological character can mark out a family, for example, the composite endosperm in the Loranthaceae. Evidences, from many botanical disciplines includingembryology have to be considered to make the system of classification of flowering plants a 'natural' one, reflecting the required phylogenetic history of the taxa concerned. Amongst the biological disciplines, the significance of embryological characters as indicators of taxonomic affinities have been pointed out by a great number of workers(Johansen, 1945 [61]; Cave, Arnott and Cook, 1961[62]; Kapil, 1962[63]; Subramanyam, 1967[64]; Venkateswarlu and Prakasarao, 1972[65]; Arekal and Ramaswamy, 1973[66]) [6][8] [9] [10], who often illustrated their statements with specific example. Of the many embryological characters listed by Schnarf (1933 [67], Cave (1962)[68], Herr (1984) [69][3][4][8][10], the development and structure of female gametophyte, the embryo and the endorsperm receive much attention from embryologists. More contribution of embryological data are reported useful in biochemical, genetical as well as physiological studies [8] in understanding how embryological systems work, in plants.

From literature, it is evident that the Podostemaceae is specially marked out by embryological characters not observedin any other family [8].Among them, the type of female gametophyte and organization of the nucellar plasmodium (=pseudo-embryo sac) are significant. Digital imaging of the female gametophyte stages are available but more data is required, especially during fertilization period.

3.2 Ovary and Ovules of Podostemaceae

The gynoecium in all the investigated taxa is syncarpous. While it is bicarpellary in *Inversodicraea bifurcata*and *I. keniensis* [42],*Zeylanidium lichenoides*[45], *Griffithella hookeriana*, *Polypleurum stylosum* and *Zeylanidium lichenoides*[46] but tricarpellary in *Tristicha trifaria*[41]. The ovary in *Tristicha trifaria* is trilocular [41] while in the latter two taxa*Inversodicarea*, it is bilocular [42]. [30] reported varied structure of gynoeciumin the Podostemaceae showing "…the main part, above an ascidiate region as syncarpous zone with an axile placenta, superimposed on this is a paracarpous region that lacks a septum. The uppermost section develops an additional 'apical septum', a mode structurally connected with anacrostyly". This morphogenetic variant or form could be the effects of the dynamic environmental factors the taxa of Podostemaceae thrive into, the aquatic habitat. In all theinvestigated taxa, the ovary bears numerous minute, tenuinucellate, anatropous and bitegmic ovules on a massive axile placenta [55] [33] [41] [42] [45].False impression of parietal placentation is not true. Previous reports of the ovary being unilocular with free central placentation (Rendle, 1925[70]; Ramamurthy and Joseph, 1964[71]) are erroneous records for the family [42][58]. Flowering commences under but near the water surface with the preceding anthesis and pollination above water; and often than not spathella rupture is an indicator of the anthesis time. Further, early morning hours (7.30am to 8.30am in Kenya or East Africa) associated with flowers inside the green spathella can be reliable source mitotic and meiotic stages (Personal observation-Sikolia, 2017).

The ovular primordial arise on the placental hump as protuberances. In a longitudinal section of an ovular primordium, four cells in an axial row are observed early in the ovule which bends in the form of a hook causing its distal region to move close to the placenta. Differentiation of the two integuments is simultaneous.The outer and inner integuments arise from the nucellus; the former arise first forming the micropyle whereas the latter is restricted to the basal region of the nucellar plasmodium (= Pseudo-embryo sac).

3.3 The Female Gametophyte of Podostemaceae

The differentiation of anatropous, bitegmic and tenuinucellate ovules from ovular primordial is almost uniform in the family[33] [41].Thereafter, the development and organization of the female gametophyte followwell-defined and related patterns in different taxa of the family. Provisionally, they are classified as the Apinagia type, the Dicraea type, the Podostemum type (cf. Battaglia, 1971) and the Willisia type[31].Irrespective of the type of the female gametophyte, a densely cytoplasmic, large nucleate hypodermal archesporial cell differentiates early in the nucellus. It enlarges and directly transforms into a megaspore mother cell and remains at this stage until the level of water subsides in the streams in which these plants grow [38]. Then, the megaspore mother cell undergoes the first meiotic division to form two unequal dyad cells (Sehgal,

Khurana, Sethi and Ara, 2011) [72] [41][42][45] [46]. Of these, the smaller micropylar one degenerates regularly without further division, and appears as an inverted cap of dark mass at the top of the developing female gametophyte. Similar observations have been reported in other investigated taxa in the family [49]. Occasional atypical division (Meiosis-II) of the micropylar dyad cell [19][21][22][23][28][32]or its persistence without further division [29], were refuted [41] [42]. This corroborate the degeneration phenomenon of the micropylar cell in *Vanroyenella plumosa* in the family[44]. However, the meiotic products of this dyad cell don't participate in cellular organization of the female gametophyte. Probably, theyrepresent abnormal divergence from the normal course of the female gametophyte development in the family. This is reflected in *Zeylanidium olivaceum*where the nucleus of the non- functional dyad cell may divide which may or may not be followed by wall formation [34]. This inconsistency fails to conform to the normal ontogeny of monosporic type because, …… in the bisporic types, the upper dyad cell when dividing (meiosis-II) gives rise to a binucleate stage instead of two mononucleatecells as in monosporic types" [30] (Pp.. 406). Possibly, the bicelled dyad [28], uninucleate or binucleate dyad [34] are variations of the of the degeneration phase occurring in the micropylar dyad cell which subsequently may persist as the fully degenerated dark mass in the female gametophyte. From a functional point of view, the degenerated dyad cell never take part in the cellularization of the female gametophyte [42] [44] [45][46] [72].

Haig (1986) [73] points out that subsequent position of the spore generally related to the pattern of callose deposition in the cell walls surrounding it and later in the cross walls separating the megaspore nucleus in the developing female gametophyte. Rodkiewicz and Bednara (1976) [74] have shown, by the end of the first meiotic prophase the callose wall is usually thin or absent at the chalazal pole of the megaspore mother cell, in those species in which the germinal spore occurs at the chalazal position whereas the micropylar pole of the megaspore mother cell has less callose in those species in which the micropylar spore forms the female gametophyte. The former case may be applicable in the present study, because it is only the chalazal dyad cell which is functional and undergoes the second nuclear meiotic division. Callose deposition generally reduce the permeability of the cell wall to nutrients and other substances. This may explain the inconsistency in the micropylar dyad cell degeneration which may undergo occasional anticlinal division with or without wall formation [28][34]. Alternatively, if callose depositions does not form around the megaspore mother cell, as in *Oenothera biennis* and *O. suaveolens*, boththe micropylar and chalazal spore of a tetrad of different complexes, compete within the ovule until one degenerates (Noher de Halac and Harte, 1975[75]; Noher de Halac, 1980[76]); a case where none of the spores is isolated from maternal tissues through selective close deposition, as part of the whole process to suppress somatic spores by maternal genome. In species of *Oenothera,*showing a similar type of ontogeny was observed in the *Inversodicraea bifurcata*, *I. keniensis*[42] and*Tristicha trifaria*[41], indicating that a correlation exists between callose deposition and determination of the germinal spore [73]. The situation in *Oenothera* complex heterozygotes is instructive because callose is not deposited in the megaspore mother cell wall but in cross walls. In *O. biennis*, close deposits are thicker in the walls between dyads than in the walls between megaspores within dyads because spores between the dyads share the chromosomal complex unlike between the dyads [73]. This may be explained by the reduction phenomenon, where the micropylar dyad cell is further isolated from the chalazal region through callose deposition in the cross wall between the two dyads thus resulting in its degeneration, as in the majority of the investigated taxa of Podostemaceae showing the Apinagia type. Similarly, the ubiquity of monosporic development may be the result of the somatic spores or their derivatives being eliminated as non-functional during the female gametophyte ontogeny. In this respect, the theory of megaspore conflict of [73] suggests that the somatic spores or their derivatives having a role in successful gametophyte function should have a tendency to become non-functional. Further, [41] have discussed the effects of physical stress in the developing nucellar plasmodium producing pressure on the cell wall precursor (pre-prophase microtubules cell plate) which is sensitive to mechanical stress or its effects.For instance, they may be shear-sensitive elements capable of processing into constant and predictable orientations with respect to externally applied stress by the unequal integumentary rates of growth and morphology, where only the outer integument forms the micropyle after meiosis-I. Then the micropylar region is impaired with respect to the corrective and regular organization of the stress and strain of the unequal integumentary rates of growth. Thus, the normal cell plate formation followed by cell division of the upper dyad cell cannot take place properly.Due to the callose deposition, lack of sustainable nutrient supply and consistency mechanical stress at the forming two nucleate stage, ensues micropylar dyad cell degeneration. May be these causal factors explains the unexpected binucleate states which are not complete in terms of cell wall formation. This may ascertain the 'Strike' phenomenon of [10] reported in Podostemaceae [38] and observed during the post-meiotic mitoses in the preceding studies [44] [42] [72] [45] [46]).Thus, the theory of megaspore conflict of [73] opines that the somatic spores of their (here as the 'micropylar dyad') derivatives having a role in successful gametophyte functions should have a tendency to become non-functional. The nucleus of the chalazal dyad cell completes the second meiotic division to produce two megaspore nuclei without cell wall formation between them. The report of wall formation at this two nucleate embryo sac (here referred as the 'Nucellar Plasmodium')in *Polypleurum elongatum*[19]and *Griffithella hookeriana*[21]were not confirmed

inlater studies [45] [42] [73] [45] [46]), thus corroborating the observation recorded in *P. elongatum, P. stylosum*[26], *P. dichotomum, P. filifolium* and *P. munnarensis*[38]. Then the 'Strike' phenomenon sets-in, where the primary chalazal nucleus of the developing female gametophyte begins to degenerate without further division. Until this primary two-nucleate stage, the female gametophyte development is fairly uniform in the family. Later, great variation in the cytological development and organization of the female gametophyte follows, showing different well-defined and related patterns.

Of the two nuclei separated by a distinct vacuole at the primary two-nucleate stage, only the micropylar one undergoes the first and second nuclear mitotic divisions in a successive manner, producing four nuclei. This micropylar quartet of nuclei alone participates in the cellularization of the female gametophyte [34][38] [44] [42] [72] [45] [46]). The primary chalazal nucleus fully degenerates and disappears in the female gametophyte. The organized female gametophyte consists of two synergids, an egg cell and a polar cell. Filiform apparatus has been observed in the synergids, for the first time in the family [41] [42]. Variation in the morphological orientation of a mature egg in the organized female gametophyte exists, among the investigated species. It is in the median region placed below the synergids in *Inversodicraea bifurcata* and *Inversodicraea kenie*nsis[42],*Vanroyenella plumosa*[44], *Zeylanidium lichenoides*[45] but descends from the micropylar end positioned behind the synergids in *Tristicha trifaria*[41],*Dalzellia zeylanica*[46]thus tallying with earlier observations [31][32][33][34][35][36][39].

According to embryological data, Apinagia type of embryo sac ontogeny is recorded in the genera, *Apinagia, Dalzellia, Farmeria, Hydrobryum, Griffithella, Indotristicha, Mourera, Oserya, Podostemum, Polypleurum, Weddellina* and *Zeylanidium*[30][40].[57] reinterpreted Apinagia type as monosporic and not reduced bisporic embryo sac development as earlier embryologists who investigated this type of embryo sac type considered. This classification of embryo sac [57] corroborate the present observation that only the micropylar megaspore nucleus contributes to all the four nuclei which alone participate in the cellular organization of the mature embryo sac. Concomitantly, the criterion is explicitly expressed in the original definition of classifying embryo sac types in the angiosperms [49], p. 360) that, "….. There is a general consensus of opinion about the first four nuclei formed after the reduction division as equivalent to megaspore nuclei; laying down of a wall separating them is a matter of secondary importance. Consequently, an embryo sac formed from the divisions of a single megaspore nucleus should be called monosporic; when two take part in its development, it is bisporic; and when all four contributes to it, it is tetrasporic". [46]observes at this stage,"….the smaller chalazal megaspore nucleus degenerates immediately after its formation, making the developing FG functionally unipolar in the sense that chalazal development is highly underdeveloped" and therefore nonfunctional in subsequent female gametophyte development. Further, it was observed that this primary chalazal nucleus fully degenerateswithout division and disappears in the embryo sac[41][42][45], as reported in earlier investigations [27][36][34][38][31][32][33][39]. The view that the mode of ontogeny is imperfect representation of the reduced bisporic, Allium type because of chalazal strike….or pseudo-monosporic as eventually only one megaspore becomes functional and contributes to the formation of a mature female gametophyte [46]…requires an embryological caution and careful approach in the understanding of the ontogeny. Thus, three embryological parameters, the number of megaspore nuclei at the prime stage of stage of the megagametophyte formation, its nuclear mitotic products and their subsequent participation in cellular organization of the mature female gametophyte needs to be considered while classifying female gametophyte types in angiosperms. Then, the Apinagia type is justified to be re-interpreted as monosporic because onlyone megaspore nucleus undergoes the first and second nuclear mitotic divisions resulting in all the four nuclei that alone take part in the cellularization and are present in an organized female gametophyte. This criterion should be explicitly outlined as above, to avoid confusion created by some authors, as pointed out by [57], while classifying female gametophyte development in angiosperms. Therefore, the Apinagia type should be considered as monosporic. A view upheld in the ontogeny of *Weddellina squamulosa* after reinvestigation of the species [29]. Further, Apinagia type B is four-nucleate, four-celled [30][40](?) and not five nucleate, four celled as reported by [29].

The value(s) of typological differences in categorizing various female gametophyte types recorded in angiosperms have been variously discussed by embryologists (Johansen, 1945[77]; Johri, 1963[78], 1967[79])[4][7][9]who often cite causal factors for the differences in the typology. Swamy and Krishnamurthy (1975)[80] criticized the classification of the female gametophyte types and proposed a new one based on the functional aspect of the establishment of polarity at the two-nucleate stage as a major feature. [33] subsequently pointed out that in their classification[80] there is no discussion about the Podostemaceae embryo sac. According to Willemse and Van Went (1984) [81]the classification provided [80] does not add any new characteristics compared to the other more elaborate typologies. It only provides fixed and variable points in the development of the female gametophyte. In addition to these, observations in different plants have shown other factors like nutrient supply, seasonal influences and variable parameters in the internal and external environment can influence the sequence of the female gametophyte ontogeny although it is genetically predetermined as exemplified by polarity. A shift in the nutrient supply in *Spinacia*has been observed to influence female

gametophyte ontogeny (Wilms, 1980)[82]. In the ovules of *Capsicum*, bisporic type is preferred during winter but monosporic type of female gametophyte in summer (Dharamadhaj and Prakash, 1978)[83]. Furthermore, it has been pointed out that aberrations visible in the nucellar tissue and the effect of environmental factors indicate that the female gametophyte ontogeny is a summative effect of genetic as well as environmental influences[81]. While one cannot but admit the rationale of polarity is an important factor operating from the time of differentiation of the megaspore mother cellthus determining the existence of the micropylar-chalazal axis, other determinant factors occurring in nature are equally significant during the ontogeny and cannot be ruled out.

A critical review and reinterpretation of the female gametophyte development and organization of the female gametophyte development and organization in the family of Podostemaceae [30] raised pertinent questions largely considered in subsequent investigations and /or re-investigations [35][38][37]. The investigation revealed that the chalazal megaspore nucleus degenerates soon after the first mitotic division in Podostemoideae for example*Vanroyenella plumosa*[44],*Inversodicraea*[42] and*Zeylanidium lichenoides*[45]but it disappears only after the second mitotic division, which is soon followed by cellularization of the female gametophyte in Tristichoideae for example the *Tristicha*[41] and*Dalzellia zeylanica*[27][72]. These observations confirmed earlier findings in all the investigated taxa belonging to Podostemoideae [35] and in *Dalzellia*[27][35], *Indotristicha*[55][35], and *Tristicha*[29]ofTristichoideae of Podostemaceae.

The two successive nuclear mitotic divisions of the micropylar megaspore nucleus results in a four nucleate stage. Due to precocious degeneration and disappearance of the chalazal megaspore nucleus, the female gametophyte never attains a five nucleate stage. Thus, after organization, the female gametophyte consists of four-nucleate, four-celled unit which conforms to the Apinagia type, form B of Battaglia's [30].He [40] withdrew form C and D of the Apinagia type based on the points raised by [35] and confirmed in the later studies [41].

The contention of Battaglia's [40] (Pp. 43) that all the embryological cases not assigned to the Apinagia type are……doubtful cases…" appeared to be too drastic and may not be trueas raised [42], because many embryologists after investigations and/or reinvestigations have confirmed their existence. The Podostemum type has been observed in *Farmeria metzgeriodes, Podostemum subulatus*[19], *Hydrobryopsis sessilis*[32], the Polypleurum type in in *Polypleurum agharkarii*[23], *Polypleurum dichotomum*[38],*Polypleurum elongatum*[19][24][26][38],*Polypleurum munnarensis*[38], *Polypleurum stylosum*[24][25][38], Willisia type in *Willisia selaginoides*[33].[29]refuted the observed and existence of Podostemum type [20]for Apinagia type B on re-investigation of *Weddellina squamulosa* (Tristichoideae) of Podostemaceae. Further, [30][40]never studiedand reported any type of female gametophyte development in the family and his work was only analytical, a commendable scientific insight.

Different types of female gametophyte recorded in the family of Podostemaceae are as follows: Apinagia type observed in *Podostemum subulatus* [37], *Farmeria indica*[31], *Podostemum ceratophyllum* [52], *Anastrophea, Apinagia, Cladopus, Dalzellia, Griffithella, Indotristicha, Inversodicarea, Mourera, Oserya, Podostemum, Tristicha Zeylanidium* genera [30] and *Hydrobryum*[39], the Podostemum type observed in *Farmeria metzgeriodes* (Magnus, 1913)[19] (refuted by [31]), *Hydrobryopsis sessilis* (Arekal and Nagendran, 1975b)[32], the Polypleurum type in in *Polypleurum agharkarii*[23], *Polypleurum dichotomum*[38], *Polypleurum elongatum*[19][24][25][38], *Polypleurum munnarensis*[38], *Polypleurum stylosum*[24][25][38], Willisia type in *Willisia selaginoides*[33]; and in *Willisia arekaliana*, the four nucleate megagametophyte, a product of two megaspore nuclear division and therefore bisporic, forms a three-nucleate, three-celled female gametophyte with one chalazal nucleus degenerating. Thethree ontogenies, the Podostemum type, Willisia type and Polypleurum type (female gametophyte with inverted polarity) of the bisporic female gametophyte development, both the micropylar dyad and chalazal dyad participates in the formation of the female gametophyte but differ in the final arrangement of the synergids, egg cell and polar cell in the mature female gametophyte. Further studies[32]reported the occurrence of both the Podostemum type and Dicraea type (= now as Polypleurum type) of bisporic ontogenies in *Hydrobryopsis sessilis* for the first time. This occurrence of two types of embryo sac within the same species is unique in the family of Podostemaceae. This situation of two different arrangement of cells in an ovule is strikingly unique given the whole gamete specification in the embryo sacs is strictly under the regulation of asymmetric distribution of auxins in plants [45].The monosporic Apinagia type in *Vanroyenella plumosa* of Podostemoideae [44]was reported in the*Tristicha trifaria*[29][41], *Dalzellia zeylanica*[27][35], *Indotristicha*[35][55],*Podostemum* [37], *Weddellina squamulosa*[43] of the Tristichoideae of the Podostemaceae. New subfamily, Weddellinoideae for *Weddellina squamulosa*species was proposed based on the embryological data recorded [43]. Thus, all members of subfamily Tristichoideae studied to date possesses a monosporic Apinagia type of female gametophyte and include: *Tristicha trifaria*[29][42],*Indotristicha ramosissima, Dalzellia zeylanica* [35]and*Weddellina squamulosa*[43]. Additionally, new subfamily, Weddellinoideae for *Weddellina squamulosa*species was proposed based on the embryological data recorded [43].

3.4 Antipodal Cell in the organized Female Gametophyte

Investigations of the female gametophyte clearly revealed that the primary chalazal nucleus fully degenerates and disappears in the organized female gametophyte in Podostemaceae. This 'Strike' phenomenon has been observed in other species of Podostemaceae: *Farmeria metzgeriodes, Podostemum subulatus*[19],*Polypleurum elongatum,*[19][24][26][38],*Dicraea stylosa (=Polypleurum stylosum)*[24], *Polypleurum stylosum*[24][26][38], *Polypleurum agharkarii*[23], *Farmeria indica*[31], *Hydrobryopsis sessilis*[32], *Tristicha trifaria*[29],*Griffithella hookeriana*[33],*Hydrobryum griffithii*[39], *Willisia selaginoides*[33],*Zeylanidium olivaceum, Z. johnsonii*[34], *Polypleurum dichotomum*, *Polypleurum filifolium, Polypleurum munnarensis*[38], *Willisia selaginoides*)[33], species of Tristichoideae[35], *Podostemum subulatus*[37],*Vanroyenella plumosa*[44], *Zeylanidium lichenoides*[45], *Griffithella hookeriana, Polypleurum stylosum and Zeylanidium lichenoides* [46]. [15](Pp.7) who first investigated the female gametophyte ontogeny in *Oenone imthurnii* and *Mourera fluviatilis* of Podostemaceae observed a four-celled mature embryo sac stage; "………….. of these four two, the synergids, lie at the top, next to each other; then follow the other two, one under the other, the upper one of the pair being the egg and the lower one of the pair being the egg and the lower one all that remains of the embryo sac with the upper polar". The interpretation [15] is accepted in this review, because only the micropylar megaspore nucleus of the primary2-nucleate stage contributes to all four nuclei present in the organized female gametophyte. Consequently, the micropylar quartet of nuclei, can only organize into two synergids, an egg cell and the upper polar cell. The latter's nucleus, undergoes degeneration. Unlike in the present case, if the opposite mode of ontogeny happened, then the chalazal nucleus, would form three antipodal cells and the lower polar cell. But the disintegration of the primary chalazal nucleushas been shown to commence after the disorganization of the nucellar cells below the embryo sac and completely disappears when a distinct central vacuole separating it from the micropylar megaspore nucleus becomes invisible in *Inversodicarea bifurcata* and *I. keniensis*[42], *Zeylanidium lichenoides*[45], *Griffithella hookeriana*, *Polypleurum stylosum* and *Zeylanidium lichenoides*[43][46]. However, this occurs immediately after cellularization in *Tristicha trifaria* (Sikolia and Ochora, 2008)[43], and *Dalzellia zeylanica*[72].This disintegration phenomenon has been reported in all the investigated taxa of Podostemoideae and Tristichoideae showing the Apinagia type of ontogeny [38].

The 'Strike' phenomenon which completely eliminates the antipodal complement in the Podostemaceae has also been observed in *Epipogium roseum* (Arekal and Karanth, 1981)[84]. One researcher[10] defines the 'Strike' phenomenon as the failure of certain nuclei of the developing gametophyte to undergo some or all of the post-meiotic mitoses. This is extreme in Podostemaceae because the functional nuclei in the female gametophyte are derivatives of the primary micropylar nucleus. To qualify this view, the chalazal megaspore undergo further divisions either partly or wholly, resulting in gradual elimination of the antipodal nuclei in a similar specialized family likeOrchidaceae. Concomitantly, this gradual elimination embryo sac known as the 'Strike' phenomenon is observed embryo sac throughout the family, Orchidaceae (Abe, 1972)[85]. Rodkiewicz (1970)[86][43][74]have reported, in which the micropylar spore forms the female gametophyte, as is the case of Podostemaceae, it is the micropylar pole which has less deposition of callose. Callose is known to reduce the permeability of cell wall to nutrients and other substances; and its deposition is part of the process by which the maternal genome suppresses somatic spores[73]. This may shift nutrient supply to the advantageously placed megaspore nucleus as observed in *Spinacia*[80], during embryo sac ontogeny. Callose deposition in the stigmatic papilla below the incompatible pollen tube, inhibits the latter's growth [9] (Pp. 250-252, 285-291). It is also deposited in sieve tubes in the abscission zone of a senescing leaf (Scott, Miller, Webster and Leopold, 1967)[87]. Given that, there is a high degree of self-pollination possibility in the taxa of Podostemaceae because the spate usually encloses flowers, then, homozygosity is favored. Consequently, deposition favored as in *Oenothera hookeri*which possess complex homozygote[73], which disappears from the micropylar tip before the end of the first meiotic prophase resulting in the micropylar spore forming the female gametophyte. Then, the chalazal region of the developing embryo sac may be physiologically depressed or hypofunctional as suggested by [30][40].This may explain the disintegration of the primary chalazal nucleus in the embryo sac of Podostemaceae because it is disadvantageously placed towards the direction of nutrient supply unlike the micropylar component. Degeneration of the chalazal megaspore nucleus is evident in terms of its decrease in size which is likely indication of reduction in physiological activities [30], and its division must be a quite an uncommon event. Additional causal factors for the 'Strike' occurrence might include physical stress and strain[42], and competition (or conflict) amongst the megaspores. Then, how can antipodal cell(s) form, if its predecessor the primary chalazal nucleus completely disappear without further division in the female gametophyte ontogeny? Accordingly, only the primary nucleus through two free nuclear divisions, contribute to all the four nuclei in the organized female gametophyte[31][34][35][37][41][42][43][44][45]. Consequently, the micropylar quartet can only organize into two synergids, an egg cell and polar cell [88].Concomitantly, many embryologists who investigated the female gametophyte of Podostemaceae accept that the cell in the chalazal region of the embryo sac as a proendospermatic cell with one polar cell contrary to Battaglia's (1971, 1987) [30][40]view as the antipodal

cell. The former interpretation is more acceptable. While, Kapil and Bhanagar (1978)[89] emphasized the need to re-evaluate antipodal cell in the family, [37] reported its complete absence in the organized female gametophyte consisting of two synergids, an egg cell and a polar cell, after investigating *Podostemum subulatus.*

The concept that, "Since the days of Hofmeister and Strasburger …. The antipodal cell (Strasburger's 'Antipoden Oder Gegenfusslerinnen is a cell situated at the chalazal end of the mature ES, usually 1-nucleate, regularly degenerating at fertilization or, rarely, showing a mitotic activity (Phenomenon of Polyantipody", [40], p.46); could be accepted only if it is derivative of the primary chalazal nucleusduring the female gametophyte ontogeny. Thus, the status of any component present in the organized female gametophyte must reflect its ontogenetic origin at megagametogenesis and its functional role before its morphological location is taken into consideration. This is valid even if reverse polarity or other factors plays role in determining the course and causes of the ontogeny in other species of the angiosperm [42]. This point has been repeatedly stressed in all reviews on the angiosperm female gametophyte (Maheshwari, P., 1947[90]; 1948[91]; Kapil and Tiwari, 1978[92]; Kapil and Bhatnagar, 1981[93])[51][88]. In the Polygonum type of female gametophyte reported by Strasburger (1877)[94], the chalazal quartet of nuclei form three antipodal cells and lower nucleus whereas the micropylar quarter of nuclei results in two synergids an egg cell and upper polar nucleus [4], Pp. 84). A view held by [30]while justifying the presence of antipodals opined that the free nuclei of the Polypleurum type attain their cellular morphology based on their axial position along the female gametophyte, consisting one synergid, an egg cell, both considered as sister cells, and two antipodals [20][26]. However, [38]reinterpreted the Polypleurum type as an inverted female gametophyte consisting of two synergids, an egg cell and a polar cell. Thus, there are no antipodals in the Polypleurum type. Similar reports of reverse polarity in angiosperms have been reported (Pace, 1913[95]; Tackholm, 1915[96]; Dutt and Subba Rao, 1933[97]; Narayanaswami, 1940[98]; Joshi and Venkateswarlu, 1941[99]; Thirumalachar and Khan, 1941[100]; Gopinath, 1943[101]; Swamy, 1946[102]). The 'Strike' phenomenon is completed so that then nucleus which is to degenerate is not produced at all, in both the Polypleurum type and the Podostemum type of female gametophyte ontogeny [38]. Polarity manifestation should integrate the functional value of the female gametophyte product for the survival of the megaspores. This could arise as a result of positional and nutritional form of gradients and mechanical effects in the developing megagametophyte. Furthermore, [32] have reported the occurrence of both these female gametophyte types in a single taxon, *Hydrobryopsis sessilis.* Thus, [38] observed that the Polypleurum type of the female gametophyte need not to be rationalized as unique in possessing, "…… the peculiar egg apparatus with a single synergid and egg both considered assister cells, the absence of polar nuclei and the presence of two (occasionally only one) antipodal cells are rare combination of characters not found in any other angiosperm – not even in any other member of Podostemaceae" [26] (Pp. 291).

It follows, then that the presence of two synergids, an egg cell is undisputed observation in an organized female gametophyte in the family of Podostemaceae. But the reasons [30][40] has adduced in designating he fourth cell as 'antipodal' and not polar cell, are not cell, are not convincing established because of the following objections:-

(i) Mere chalazal position of this cell in the specialized and most reduced female gametophyte found in the family cannot be the basis for considering it as an antipodal cell; and further, location is bound by the function of the cell;

(ii) The smaller size of this cell should be evaluated and analyzed from the functional and spatial points of view;

(iii) Since triple fusion is absent in the family, the question of male nucleus penetrating this cell does not exist.

Transmission electron microscope investigations on this cell in question needs to be pursued to arrive at valid conclusions. There are observations that the antipodal cells persist beyond fertilization even when the other cell types are no longer present in the female gametophyte of *Arabidopsi*s and thus organizes into seven cells and four cell types rather than four cells and three cell types (Song, Li and Venkatesan, 2014)[103]. Studies in *Weddellina squamulosa*[43]does show degenerating chalazal megaspore nucleus, evidence for the antipodal cell absence in the developing organized female gametophyte. Thefluorescence and confocal laser scanning microscopic studies[46]has also confirmed the degeneration of the chalazal megaspore nucleus in the *Griffithella hookeriana, Polypleurum stylosum* and *Zeylanidium lichenoides* species. Thus, there is no antipodal cell in the organized female gametophyte in the Podostemaceae family.

3.5Fertilizationin the Family of Podostemaceae
Pollination show chronological events in the aquatic environment leading to self-pollination. The best time to observe these unfolding process is between 7.25a.m. – 8.25a.m in the morning, especially when the level of water subside. The floral shoots begin to reach the water surface and open to expose the spathe. Studies [46] described the pollination in more concise method as follows… "The spathe bends away from the substratum to

emerge above water level and ultimately ruptures, exposing the young flower. The stigma and anthers exhibit unique spatial relationship where the two stigmas bend over the ovary and the anthers of the two, young stamens remain pressed against the ovary wall lying well below the stigmas. With the passage of time, the pedicel further elongates and pushes the young flower considerably above the spathe. During subsequent growth, the two stigmas begins to straighten up, the andropodium elongates, and the two filaments carrying the bright red reach the same level as that of reddish-green stigmas. At this stage, the two anthers of a flower not only touch their stigmas but also eventually get clasped between them. Subsequently, the anthers…dehisce introrsely depositing copious pollen dyads on the stigmas of the same flower which results in self-pollination under aerial conditions. The plants therefore show autogamy though allogamy cannot be excluded"… [46]. Further, the present author (Sikolia, 2017) observed aquatic caterpillars scavenging on the Podostemaceae species in the aquatic habitats in Kenya that point to cross pollination but didn't confirm this event. Thus, self-pollination and fertilization occurs in Podostemaceae but cross pollination cannot be ruled out. Further, self- incompatibility can inhibit the germination of pollen tube on the stigma surface way through the styleas the pollen tube grow up to ovular region limiting cross-pollination and therein fertilization of the egg cell in the female gametophyte.

Fertilization is porogamous. The vegetative nucleus leads followed by the germinal cell but soon the germinal cell moves ahead with the former trailing behind. The pollen tube growth ceases at the synergid site and releases two sperm cells. The sperm cell that fuses with the egg cell to bring about single fertilization is health unlike the smaller one which later degenerates[46]. Further, reports of the pollen tube penetrating the embryo sac destroying one of the synergids on its pathway, contrary to Razi's[22]observation between the two synergidsexists [42][46]. Also, there are observations [46] showing the pollen tube passing through the micropyle and growing beyond the filiform apparatus to enter the female gametophyte from the side or the chalazal end in a few instances. The pollen tube cease once they reach the synergid.The receptive synergid degenerates as a response to pollen tube entry [46]. The other synergid, only degenerates during embryogenic stages. This mode of pollen tube pathway has been recorded in *Mourerafluviatilis*[15], *Dalzelliazeylanica*[53]and*Indotristicha ramosissima*[27] [46].The contents of the pollen tube overlaps with the protoplasmic mass of the destroyed synergid and area often a visible as a dark mass with one of the two nuclei in a degenerated state. Also, one of these nuclei may be the undischarged second male gamete. These observations confirm the reports of earlier investigators[15][27][28], that the second male gamete is not discharged, in the family. However, there indications of the release of the second sperm cell that degenerates later [46]. According to [27], often the tip of the pollen tube and one of the synergids persist as late as quadrant and octant stages of the proembryo. At this time, the polar cell is degenerated or absent, a process that starts before the pollen tube enters the female gametophyte, and observed in other taxa of Podostemaceae[32][34][35][39]. The chances of the polar cell being fertilized by second male gamete does not arise because none of the preparations depicted the fusion of the second male gamete with the polar nucleus. Further, there is no endosperm in the family and the primary endosperm nucleus never forms in the investigated taxa of Podostemaceae [27][32]. The observation that fusion of the second male gamete with a polar nucleus occurs [22]were not confirmed in *Inversodicarea bifurcata, I. keniensis*[42]nor in *Tristicha trifaria*[41]. The function of the endosperm, a product of double-fertilization found in other families of angiosperms, is taken over by the nucellar plasmodium in Podostemaceae. The egg cell fertilized (often 24-26 hours after pollination[46]) by a male gamete produces the zygote which increases in size considerable, extending towards the chalazal region of type of fertilization has been reported in other investigated taxa of Podostemaceae [15][28][53][27][31][34][35][36][46]. Thus, only single fertilization takes place in the family.

3.6Nucellar Plasmodium (often referred as "Pseudo-Embryo Sac")of the Podostemaceae

Studies clearly show that the so-called pseudo embryo sac ontogenetically corresponds to the nucellar cells situated at the base of the female gametophyte. This exceptional structure in the family was discovered for the first time by [15]. It functions until it is destroyed by the enclosed growing embryo. It is a feature of Podostemaceae, not documented in any other family. The nucellar plasmodium begins to develop before fertilization in the Podostemoideae sub family but commences development only after fertilization in the Tristichoideae sub family of Podostemaceae)[38][41].

The origin and development of pseudo-embryo sac is of late being understood. In the initial stages, the smaller unclear cells located below the megaspore mother cell, elongate. They become densely cytoplasmic containing a conspicuous enlarged nuclei the primary two-nucleate female gametophyte stage. These cells are enclosed by inner integument. Usually, rapid cell divisions take place in the integument, as compared in the nucellar layer, acropetally [41]. This results in the differential growth rates between the two layers. Although, the growth rate of the outer integument is faster than the two layers, it has little effect on the nucellar layer because of its separation from the inner integument. It only provides an enclosed system.

The differential growth rates between the nucellar layer and inner integument continues to intensify as the cell walls of the former becomes thin and takes lighter stain[31][32][42][44]. This possibly creates opposing tensions between the layers. The net tension of the thicker inner integumentary walls may cause the thin walls

of the nucellar layer to disorganize by stretching, shearing and subsequent disruption. The 'naked' or uninucleate protoplasts forms in the long cavity coalescing in group of less than eight protoplasts [41]. [15] suggested that the development of the pseudo-embryo sac by the stretching and dissolution of the cell walls of the nucellar layer, indicates in many cases that the developing female gametophyte exercise a solvent action on the surrounding tissues of the nucellus, which can take place on the surrounding nucellar cells towards the chalazal region. This hints to the view raised by Davis (1961)[104]in*Podolepisjaceoides* that the tip of the functional megaspore liberates enzymes which dissolve the middle lamella of the adjoining nucellar epidermis. This possibility of lytic enzymes from the developing female gametophyte, is further highlighted through histochemical studies carried in diverse taxa of the angiosperms (Poddubnaya-Arnoldi and Zinger, 1961)[105], which aid in the complete breakdown of the hyaline walls. As an assumption, the disappearance of hyaline layer after wall disruption in the nucellar cells, provides ample evidence to the effect of the lytic action. It can be construed that the physical factor as a result of tension set up by differential growth rates of the nucellar layer and inner integument, also suggested by [52], and the chemical factor (=lysis of hyaline walls of nucellar cells by enzymatic action) are part of the whole process responsible for the disintegration of wall of the nucellar cells; which begins at the chalazal part of the ovule and proceeds micropylar-chalazal axis.

According to [16], who called the pseudo-embryo sac as 'Hahlraumes' gave the impression that it is a hollow space, possessing nuclei in a degenerating phase and undergoing fragmentation phenomenon; whereas, Engler (1930)[106] opined that the central and peripheral layer of cells gradually grow very much so that it becomes indistinct and finally disappears; the nucleus probably becomes a fragmented and ultimately a big space containing protoplasm results. On the contrary, the disintegration of walls of the nucellar cells result in vacuolated cytoplasmic mass consisting of large and healthy nuclei [38]. The 'naked' protoplasts begin to fuse one by one, often clumping at a point and in longisection of the ovule, their number often is less than eight. Later, the long cavity consisting of multinucleate protoplasts is formed. The resultant structure organized never appears like an embryo sac, either in its ontogeny or organization and appearance. By the time it is fully organized, the multinucleate protoplast do not have their walls [41]. Precision lacks in referring this structure 'pseudo-embryo sac'. Because it is organized by the fusion of individual nucleate protoplasts of as a consequence of the disintegration of cells walls separating them in the nucleus, the term 'Nucellar Plasmodium' [32] is etymologically more acceptable. This has received quite wide acceptance in embryologist who have studied female gametophyte in Podostemaceae [43][44][45][46].Subsequently, the embryo grows into it and completes its development.

It is now an established fact that the organization of the nucellar plasmodium in the investigated genera of Podostemoideae, as shown in *Inversodicraea* takes place before fertilization[42] and other investigated species of Podostemoideae including *Vanroyenella plumosa* (Murguía-Sánchez, Novelo, Philbrick and Márquez-Guzmán, 2002)[44], *Zeylanidium lichenoides* (Chaudhary, Khanduri, Tandon, Uniyal and Mohan Ram, 2014)[45], *Griffithella hookeriana, Polypleurum stylosum and Zeylanidium lichenoides* [46]; while it is a post-fertilization phenomenon on Tristichoideae[41][43]. However, the precise stage for the nucellar plasmodium formation varies among species in the Podostemoideae. The more common report is for the nucellar plasmodium to begin development at the two-nucleate embryo sac as seen in *Griffithella hookeriana*[20], *Farmeria indica* and *Hydrobryopsis sessilis*[32],*Hydrobryum griffithii*[39],*Willisia selaginoides*[33],Polypleurum *dichotomum*, *Polypleurum filifolium, Polypleurum munnarensis*[38], Polypleurum *elongatum*[38], *Zeylanidium olivaceum, Z. johnsonii*[34]as observed by[44]. But, this phenomenon commences at the time the megaspore mother cell undergoes meiosis-I in *Podostemum subulatus* [37], at early meiosis-I in *Dicraea stylosa*[25], after meiosis-I in *Vanroyenella plumosa*[44]. In the sub family of Tristichoideaenucellar plasmodium formation commences or occurs at different times, but after fertilization. The nucellar plasmodium commences when the female gametophyte is mature in *Weddellina squamulosa* [43] but at the stage when the nucellar plasmodium is mature in *Lawia zeylanica* (=*Dalzellia*)[20] or at the time of fertilization or early zygote development in *Indotristicha ramosissima*[55] and immediately after fertilization in *Dalzellia zeylanica* ([35] and *Tristicha trifaria*[29] [41].Thus, the commencement of nucellar plasmodium in Podostemaceae is variable and worth taxonomic implications.

All the embryologists who investigated the female gametophyte of Podostemaceae have pointed out the value of nucellar plasmodium as a source of nourishment for the growth of the embryo sac. This is acceptable because the inner integumentary cells in proximity to the developing proembryo, stain negative for starch in provision to receive the growing embryo which subsequently occupies the entire space of the nucellar plasmodium [27]. Because of the limited size of the female gametophyte to provide enough space to the developing embryo, its area with that of nucellar plasmodium is suffice. During embryogenic stages, the liquid medium of the nucellar plasmodium becomes useful, because the plants are suddenly exposed as water level in the streams subsides. This adaptation has enabled a successful mode of life in all the numbers of Podostemaceae in aquatic ecosystem. In this connection, Arber (1920) [107] calls the nucellar plasmodium ('pseudo-embryo sac,) as an 'ideal water reservoir'. It follows that, in the absence of the endosperm i.e. lack of double fertilization, nucellar plasmodium serves to conserve food materials, drawn by the developing embryo,

suspended in its fluid of the cytoplasmic mass. This unit, also maintains the internal maternal environment from collapsing through the tension effect of its fluid mass to counteract the inward pressure from the surrounding sporophytic tissues and other external sources where the plants grow. Probably, it furnishes certain morphogenetic substance necessary for differentiation of the developing embryo [27]. These may include enzymes, growth hormones and osmo-components and their subsequent roles as they effect the developing embryo needs detailed investigations. Further, the nucellar plasmodium might be protecting the developing female gametophyte from desiccation [44].

Investigators who have studied the female gametophyte ontogeny of Podostemaceae now prefer pseudo embryo sac to be referred as the nucellar plasmodium in *Willisia arekaliana*(Khanduri, Chaudhary, Uniyal and Tandon, 2014 [108]) [31][34][35][36][37][43][44][41][42][45]. Thisis based on the nature of the coalescing uninucleate protoplasts or 'naked' nucellar cells within the forming nucellar plasmodium in the Podostemaceae with time.

IV. CONCLUSIONS AND RECOMMENDATIONS
4.1Conclusions
The gynoecium is superior, syncarpous and possess ovary with numerous tenuinucellate, anatropous ovules on a swollen axile placenta.The micropyle is organized by the outer integuments only as the inner integument lags behind the former. The micropylar dyad cell undergoes degenerates and antipodal cell does not exist in the organized female gametophyte.The micropylar nucleus of the chalazal dyad cell undergoes two free nuclear division and the meiotic products only organize the four nucleate and four celled female gametophyte in the Podostemaceae. The mature female gametophyte includes the egg cell, two synergids and a polar cell. Both, the monosporic and bisporic female gametophyte occurs in the Podostemaceae

The nucellar plasmodium forms before fertilization in the Podostemoideae and Weddellinoideae but after fertilization in the Tristichoideae of the Podostemaceae. The timing in the formation of the nucellar plasmodium in either subfamilies varies and is species-specific. Also, the development of the female gametophyte is species-specific with limited life span of the central cell. The second male cell degenerates such that there is no endosperm rather nucellar plasmodium is thought to offer the nourishment to the developing zygote.

Uniquely, two different type of embryo sacs within the same species has been recorded in *Hydrobryopsis sessilis* of the Podostemaceae. The two embryo sacs are the Polypleurum type and the Podostemum types that co-exist in *H. sessilis* of the Podostemoideae.Apinagia type of female gametophyte ontogeny appears common in the Podostemaceae family, often described as 4-nucleate, 4-celled mature female gametophyte organized into an egg cell, two synergids and polar cell but few cases of 3-nucleate, 3-celled into egg cell and two synergids. Further, Podostemum type, Polypleurum type (=formerly as Dicraea type) and Willisia type of embryo sacs have been reported in the different species of Podostemaceae.Single fertilization occurs in the family of Podostemaceae.

The genetics of the Podostemaceae appears very promising with further examples of species-specific characters emerging from recent studies.

4.2Recommendations for Future Studies
Transmission Electron scanning studies of the male gametophyte and female gametophyte are highly missed, especially where controversies exist in investigated species.Determine more embryological species-specific characters to help in the genetical studies (as evidence of gene action over time like the case of *Arabidopsis thaliana* and *Oenothera* species) and taxonomical studies. Also, to be applied to determine the suggested elevation of Weddellinoideae group to the subfamily level

Provide further cytoplasmic transmission electron scanning microscopy images during nuclear fusion between generative male nucleus and egg cell nucleus of the female gametophyte up to the globular embryogenic stages, for the species of thePodostemoideae, Weddellinoideae and Tristichoideae subfamilies of the Podostemaceae.

Study the callose deposition around the walls of the megaspore mother cell, in the cross-walls between the dyad cells, nutrient supply shift, in relation to the significances of the female gametophyte ontogeny and 'Strike' phenomenon in the family.Further embryological data is required to establish whether or not the central cell degeneration occurs before the release of sperm cells or not.Attempt should be made to understand the functional significance of the nucellar plasmodium in the family.

valid citation. It is my hope the paper provides the status of the female gametophyte research work and some of the salient missing gaps, an impetus for future studies.

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