

Comparative Studies of the Gametophytes of Five New World Species of *Tectaria* (Tectariaceae)

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ABSTRACT.—The gametophyte development and morphology of five Mexican species of *Tectaria* were documented and compared with what is known from literature reports of the Old world species of *Tectaria*. Both Old and New World species had the following characteristics in common: spores monoletic, ellipsoidal and with a rugose surface; the perine is folded, brown to dark brown, with wing-like folds, and the laesurae measure 1/2 to 3/4 the length of the spore. The germination pattern is of the *Vittaria*-type and the developmental pattern of the prothallus is of the *Aspidium*-type. Gametangia are of the common type for the advanced leptosporangiate ferns. The presence of multicellular uniseriate hairs on the gametophytes may represent a synapomorphy for the family.

Tectaria Cav. contains about 200 species Worldwide, of which 30 are found in the American tropics and subtropics (Morton, 1966; Smith, 1981; Mickel and Beitel, 1988; Mickel, 1992; Moran, 1995; Mickel and Smith, 2004). Previous research on Old World species has shown that prothallial development is relatively uniform within the Tectariaceae and has found many similarities in developmental patterns among different taxa (Kaur and Devi, 1976). The gametophytes of *Tectaria devexa* (Kunze) Copel., *Tectaria variolosa* (Wall.) C. Chr., *T. fuscipes* (Wall. ex Bedd.) C. Chr., *T. macrodonta* (Fée) C. Chr., *T. polymorpha* (Wall.) Copel., *T. semibipinnata* (Wall.) C. Chr., *T. simonsii* (Baker) Ching, *T. variolosa* (Wall. ex Hook.) C. Chr., *T. amplifolia* (V.A.V.R.) C. Chr., *T. heracleifolia* (Willd.) Underw., and *T. leuzeana* (Gaud.) Copel. have a *Vittaria*-type germination pattern, an *Aspidium*-type prothallial development, and unicellular to multicellular branched hairs (Kachroo, 1956; Mahabale and Venkateswaran, 1959; Nayar and Kaur, 1964; Srivastava, 1968).

As part of a larger study on fern gametophytes, this paper examines the morphology and development of gametophytes in five species of *Tectaria* from Mexico: *T. fimbriata* (Willd.) Proctor et Lourteig, *T. heracleifolia* (Willd.) Underw., *T. incisa* Cav., *T. mexicana* (Fée) C. V. Morton, and *T. transiens* (C. V. Morton) A. R. Sm. In addition to documenting gametophyte development in these New World species we compare them with developmental patterns observed in Old World species of the Tectariaceae.

MATERIALS AND METHODS

Spores were obtained from fertile leaves of the above mentioned species from several Mexican sites. Voucher specimens are: *T. fimbriata*: M.G. Caluff 1101; *T. heracleifolia*: A. Mendoza- Ruiz-308, 311; *T. incisa*: AMR-307; *T. mexicana*: B. Pérez-García 1101, 1106 and *T. transiens*: AMR-415, 554, 574. All

TABLE 1. Collection localities of *Tectaria* used in this study. All voucher materials deposited at UAMIZ.

Taxa	Vouchers	Locality	Habitat/Altitude
<i>T. fimbriata</i> (Willd.) Proctor <i>et</i> Lourtig	MGC 1101	Salto, Soroa, Pinar del Rio, west Cuba	Sobre rocas y paredones humedos, 3000 m asl
<i>T. heracleifolia</i> (Willd.) Underw.	AMR 308	Ca. 5 km before Miguel Hidalgo, Mpio. Catemaco, Veracruz River	Tropical rainforest remanants, 450 m asl
	AMR 311	Malila, highway 105, Pachuca-Molango, Mpio. Molango, Molango, Hidalgo	Cloud forest, 1380 m asl
<i>T. incisa</i> Cav.	AMR 307	Ca. 5 km before Miguel Hidalgo, Mpio. Catemaco, Veracruz	Tropical rainforest remnants, 450 m asl
<i>T. mexicana</i> (Fée) C. V. Morton	BPG 1101	5 km before Valle National, towards Oaxaca, Oaxaca	Cloud forest remnants, 210 m asl
	BPG 1106	10 km after Valle Nacional, towards Oaxaca, Oaxaca	Cloud forest remnants, 510 m asl
<i>T. transiens</i> (C. V. Morton) A. R. Sm.	AMR-415	Between Rincon of Piedra Blanca and Agua Zarca, Mpio Landa of Matamoros, Queretaro	Forest in gully, 1380 m asl
	AMR-554	Chuvejé waterfall, before Escanelilla, Querétaro	River banks, cloud forest, 1290 m asl
	AMR-574	Km 257 highway Jalpan of Serra- Xilitla, Mpio Xilitla, San Luis Potosi	Cloud forest, 810 m asl

vouchers are deposited at the Metropolitan Herbarium "Ramón Riba y Nava Esparza" (UAMIZ) (see Table 1 for detailed information). Pinnae were left to dry at room temperature in paper envelopes to facilitate the opening of the sporangia and the expulsion of the spores. Fragments of leaves and sporangia were separated from spores by means of a sieve with 0.074 mm openings. Spores of each species were sown in 30 petri dishes (three replicates) with agar and Thompson medium (Klekowski, 1969; Pérez-García *et al.*, 1998), with an average density of 100–150 spores/cm². Two dishes of each species were covered with tin foil in order to test for photoblastism. Inoculated petri dishes were placed inside transparent polyethylene bags to reduce contamination and dehydration. The cultures were kept in the lab under artificial light, with 75 W lamps and a photoperiod of 12 hrs light/12 hrs darkness, at a temperature of 20–25°C.

All photomicrographs were taken from live material, with a MicroStar AO optic microscope and a Star Zoom AO 580 stereoscopic microscope, with TMAX-100, black and white film.

RESULTS

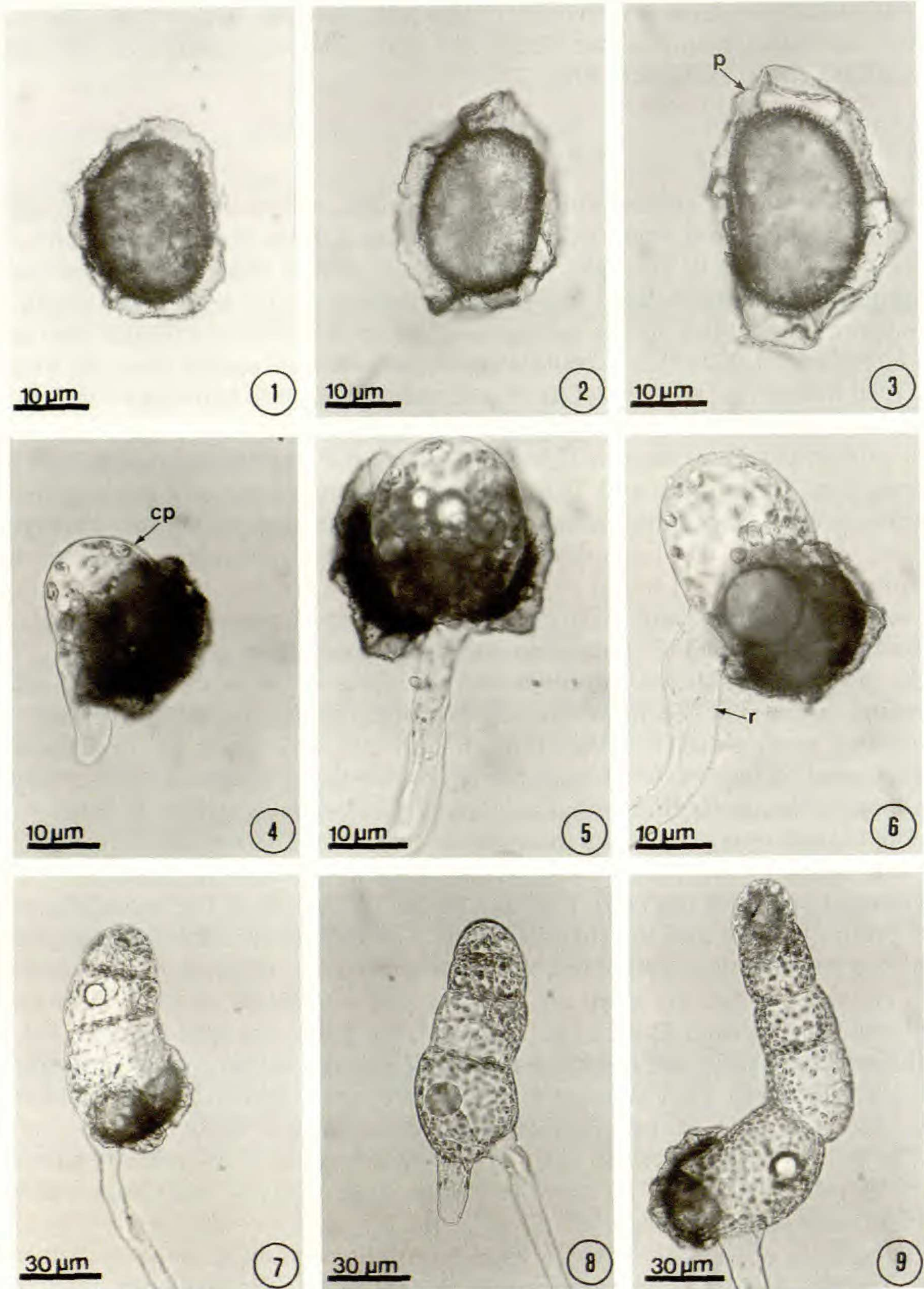
Spores of all five species studied are monolete, ellipsoid, convex-flat when viewed laterally and ovate when viewed from a polar perspective. They are without chlorophyll, are light to dark brown, with a thin to thick perispore arranged in wide undulated folds. The laesurae measure $\frac{1}{2}$ to $\frac{3}{4}$ the length of the spore. The largest spores belong to *T. incisa* (44(48)51 \times 31(34)35 μm) and *T. heracleifolia* (42(47)53 \times 29(33)35 μm), intermediate spores sizes are found in *T. transiens* (32(37)39 \times 24(26)29 μm) and *T. fimbriata* (31(40)44 \times 26(29)31 μm), and the smallest spores belong to *T. mexicana* (31(34)35 \times 24(25)26 μm). The widest perine belongs to *T. heracleifolia* and *T. incisa*, measuring 7 μm on average; in *T. fimbriata* and *T. transiens* it measures 5 μm and the narrowest perine is in *T. mexicana*, measuring 4 μm . According to Tryon and Tryon (1982) and Tryon and Lugardon (1991), *Tectaria* spore ornamentation varies: a spinulose surface is found in *T. heracleifolia*, a slightly perforated pattern is seen in *T. incisa*, and crested, equinated or equinulated ornamentation is found in *T. fimbriata*, *T. mexicana* and *T. transiens* (Figs. 1, 2, 3).

In the species studied, germination took place between 6 to 14 days after sowing. Spores of *Tectaria incisa*, *T. heracleifolia*, *T. mexicana* and *T. transiens* germinated between days 6 and 11, and those of *T. fimbriata* germinated on day 14. Germination begins with the appearance of the rhizoid initial and the prothallial cell. The perispore is persistent (Figs. 4, 5, 6).

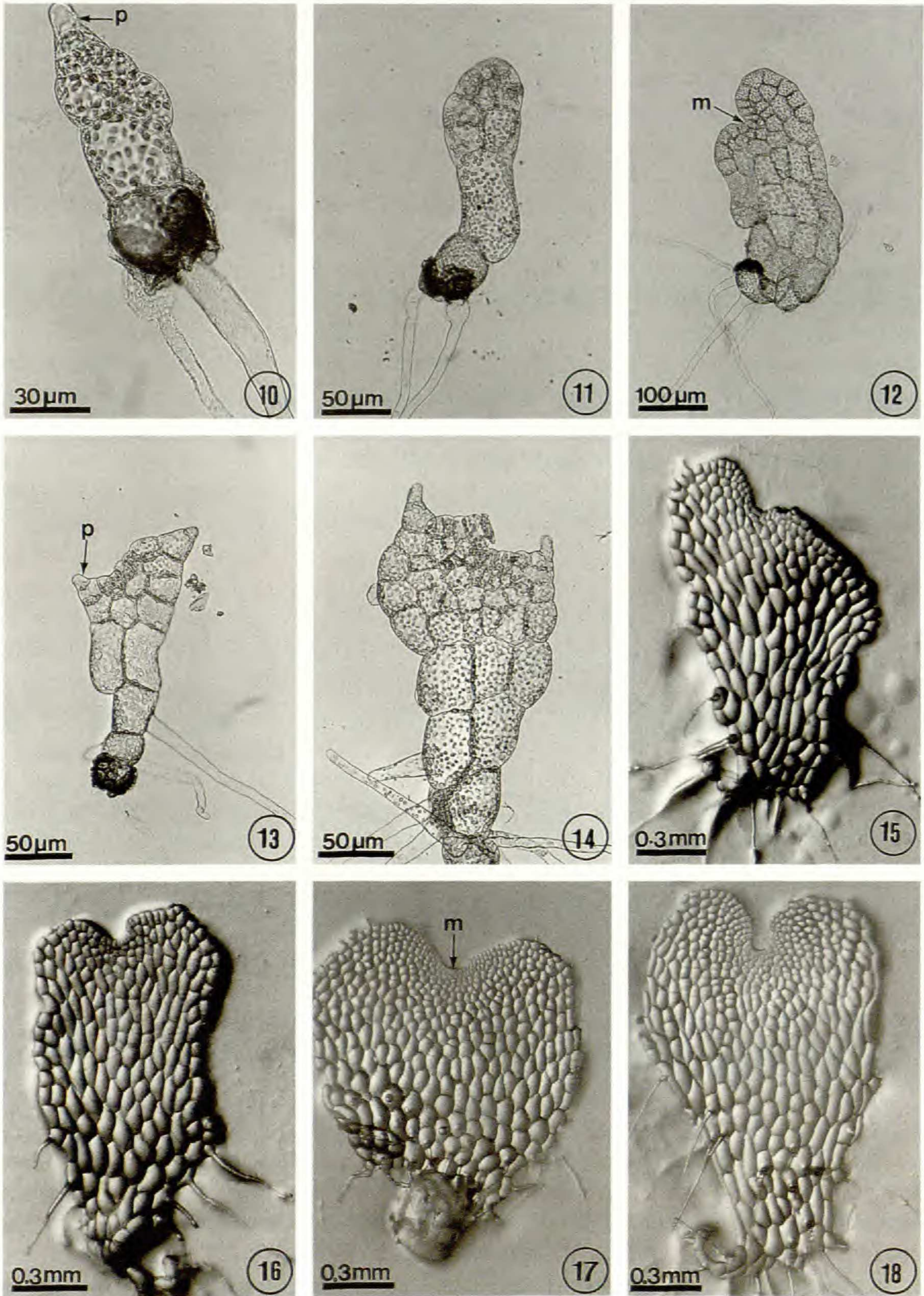
The filamentous phase lasts from day 14 to day 25. The first cellular division of the spore is evidenced by a wall, which is laid down parallel to the equatorial plane (of the cell). It is this division (Figs. 4, 6) that establish the prothallial cell and the rhizoid initial. The rhizoid initial is hyaline, long, and has little evident cytoplasm but some protoplast, whereas the prothallial cell contains numerous small oil globules. The elongation of the first rhizoid cell and the germinal filament is parallel to the polar axis of the spore, and is followed by a series of diagonal divisions giving rise to a short uniseriate germinal filament. This filament is 2 to 6 cells long, and is composed of short, barrel shaped cells with numerous chloroplasts (Figs. 7, 8, 9).

The planes of cell division and the growth direction of the primary rhizoid and germinal filament follows a *Vittaria*-type (Nayar and Kaur, 1971) germination pattern.

All species of *Tectaria* have an *Aspidium*-type prothallial development but the species studied shown variation in cell division sequence and development rate during days 25–50 (Nayar and Kaur, 1969). In *T. fimbriata*, prothallial plate development is initiated on about day 21 when the terminal cell of the filament produces a hair at its apex (Fig. 10) and the intercalary cells of the filament begin to divide. In other species, the last cell of the germinal filament divides longitudinally giving rise to two cells. This latter type is seen



FIGS. 1–9. Spores and filamentous phases of *Tectaria*. Fig. 1. Spore of *Tectaria mexicana*. Fig. 2. Spore of *T. fimbriata*. Fig. 3. Spore of *T. incisa*. Figs. 4–6. Initial stages of germination of *T. mexicana*, 11 days. Figs. 7–8. Filamentous phases of *T. heracleifolia* 10 days. Fig. 9. Filamentous phase of *T. incisa*, 10 days. **cp** = prothallial cell; **pe** = perine; **r** = rhizoid.



FIGS. 10–18. Several stages of prothallial plate development of *Tectaria*. Fig. 10. Initial stages of laminar phase with one hair, *T. fimbriata* (21 days). Figs. 11–13. Young prothallial plate. Figs. 11–12. *T. incisa*, 20 days, notice that one wing is shorter than the other. Fig. 13. *T. fimbriata*, 30 days. Figs. 14–18. Prothallial plate. Fig. 14. *T. heracleifolia*, 21 days. Fig. 15. *T. incisa*, notice one wing is more developed than the other, 38 days. Fig. 16. *T. incisa*, 38 days. Figs. 17–18. *T. mexicana*, 37 days. **m** = meristematic zone; **p** = hair.

in *T. incisa* (12 days) and *T. mexicana* (22–25 days). One of these two cells produces a hair and becomes inactive, whereas the other cell divides to form an asymmetric, spatulate to reniform prothallial plate (Figs. 11, 12). Marginal hairs form in *T. heracleifolia* at 19–21 days, in *T. incisa* at 12–20 days, in *T. mexicana* at 18–28 days, in *T. transiens* at 18–37 days and in *T. fimbriata* at 22–30 days (Figs. 11–18).

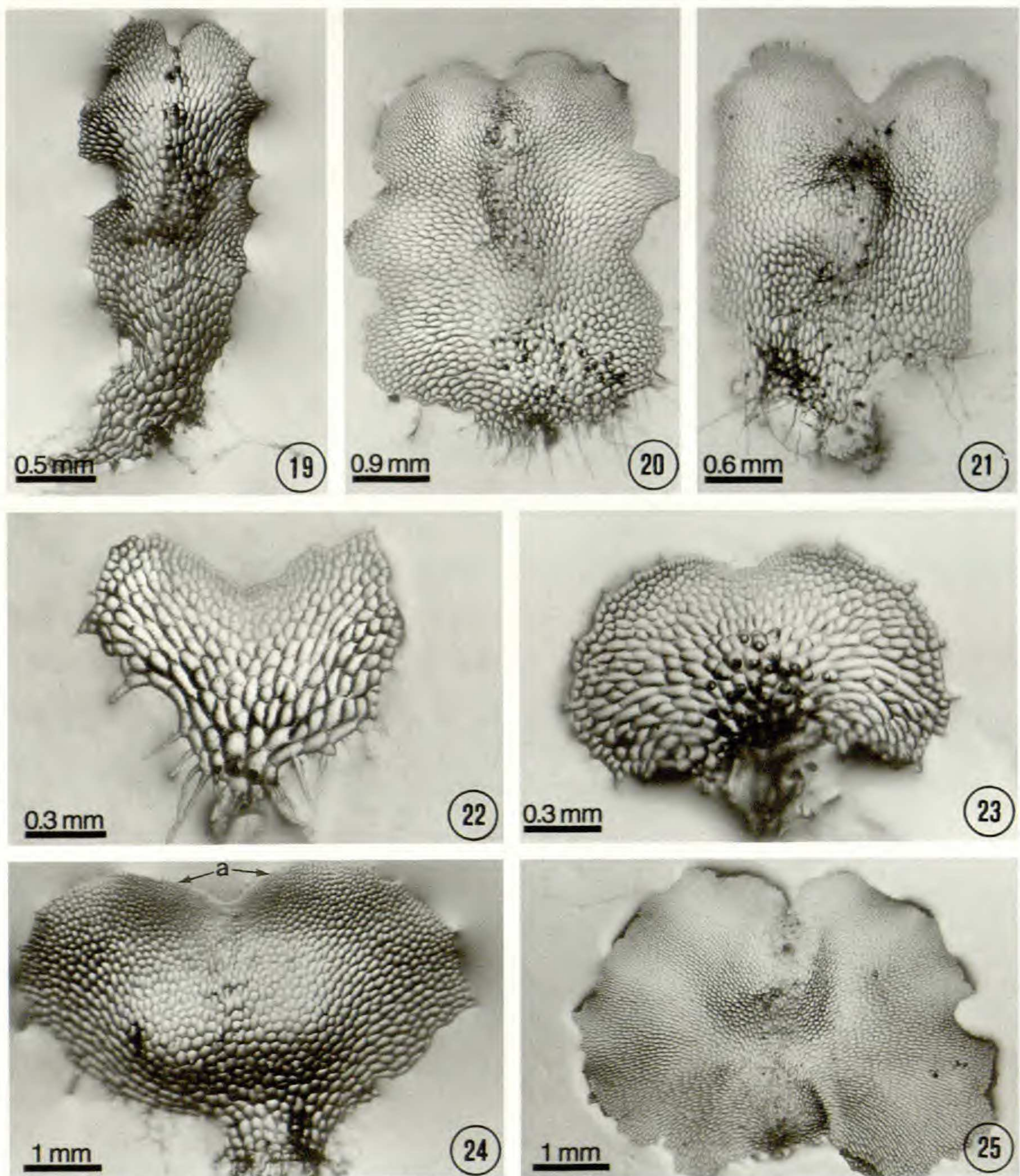
Once the prothallial plate has developed due to the activity of the meristematic cells, reniform-spatulate gametophytes develop. This takes 45 days in *T. heracleifolia* (Fig. 16), 21–38 days in *T. incisa*, 29–37 days in *T. mexicana*, and 43–49 days in *T. transiens* (Figs. 17, 18). The prothalli of all species develop hairs they are on both surfaces and on the margins, a pluricellular apical meristem is differentiated, and eventually a thick midrib appears which is a long spatulate gametophytes, and short in the reniform and cordiform gametophytes.

The adult phase is either spatulate-cordiform with wide wings, a shallow notch, and smooth prothallial plate margins, or is cordiform-reniform with short wings, a shallow notch and a prothallial plate with slightly undulated margins. Cordiform gametophytes with wide wings, marginal hairs on both surfaces, and thin, elongate midribs upon which gametangia develop were found in *T. fimbriata*, *T. incisa*, *T. mexicana* and *T. transiens* (41–120 days) are cordiform. Spatulate gametophytes with a shallow notch, short iso-diametric wings, short marginal and superficial hairs, and a short, thin midrib were found in *T. fimbriata*, *T. heracleifolia*, *T. incisa* and *T. mexicana* (42–134 days). All species have thin, long, hyaline rhizoids, of a soft texture (Figs. 19–27).

Hairs first appear during the filamentous phase and continue to develop until the adult phase. The most commonly observed hair type (Fig. 30) is unicellular, simple, capitate, claviform, hyaline, and secretory; these have an extra-cellular layer similar to a waxy secretion at their apex (Fig. 31). Such hairs occur along the margins and on both surfaces of *T. fimbriata* (69 days), *T. heracleifolia* (20 days), *T. incisa* (41–128 days), and *T. mexicana* (42–63 days). All species also produce multicellular, uniseriate, simples, hyaline hairs between the notch and the midrib. These hairs develop during later stages of prothallus growth: around day 69 in *T. fimbriata*, day 41 in *T. incisa*, and day 42 in *T. mexicana* (Figs. 32, 33).

The adult prothalli are bisexual in *T. fimbriata*, *T. heracleifolia*, *T. transiens*, and *T. mexicana* (Fig. 37) or unisexual as in *T. incisa* (Fig. 34). Gametangia develop along the midrib on the ventral side of the thallus, intermingled with the rhizoids. Antheridia develop between days 65 and 70 in *T. fimbriata*, *T. mexicana* and *T. transiens*, between days 90 and 100 in *T. heracleifolia*, and between days 41 and 294 in *T. incisa*. In *T. incisa* and *T. mexicana* they are small, 58 by 42 μm , and elongate-globose to more or less obovate in general outline. They are characteristically 3-celled with a basal cell, a median cell and an opercular cell (Figs. 37, 38).

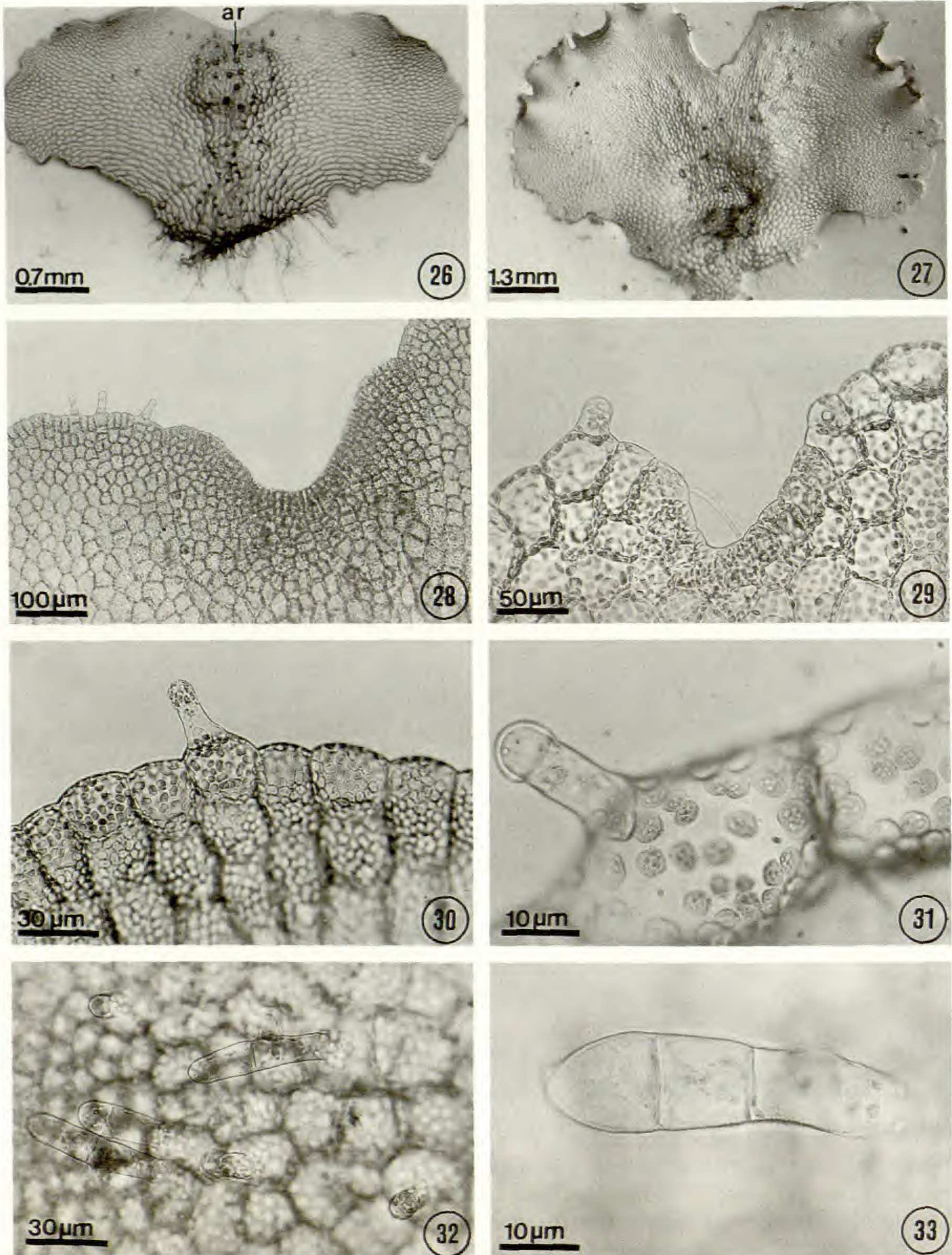
The archegonia develop between days 65 and 70 in *T. mexicana* and *T. transiens*, day 90 to 100 in *T. heracleifolia*, day 41 to day 294 in *T. incisa*, and in *T. fimbriata* on day 68. They are superficial and arranged on the lower



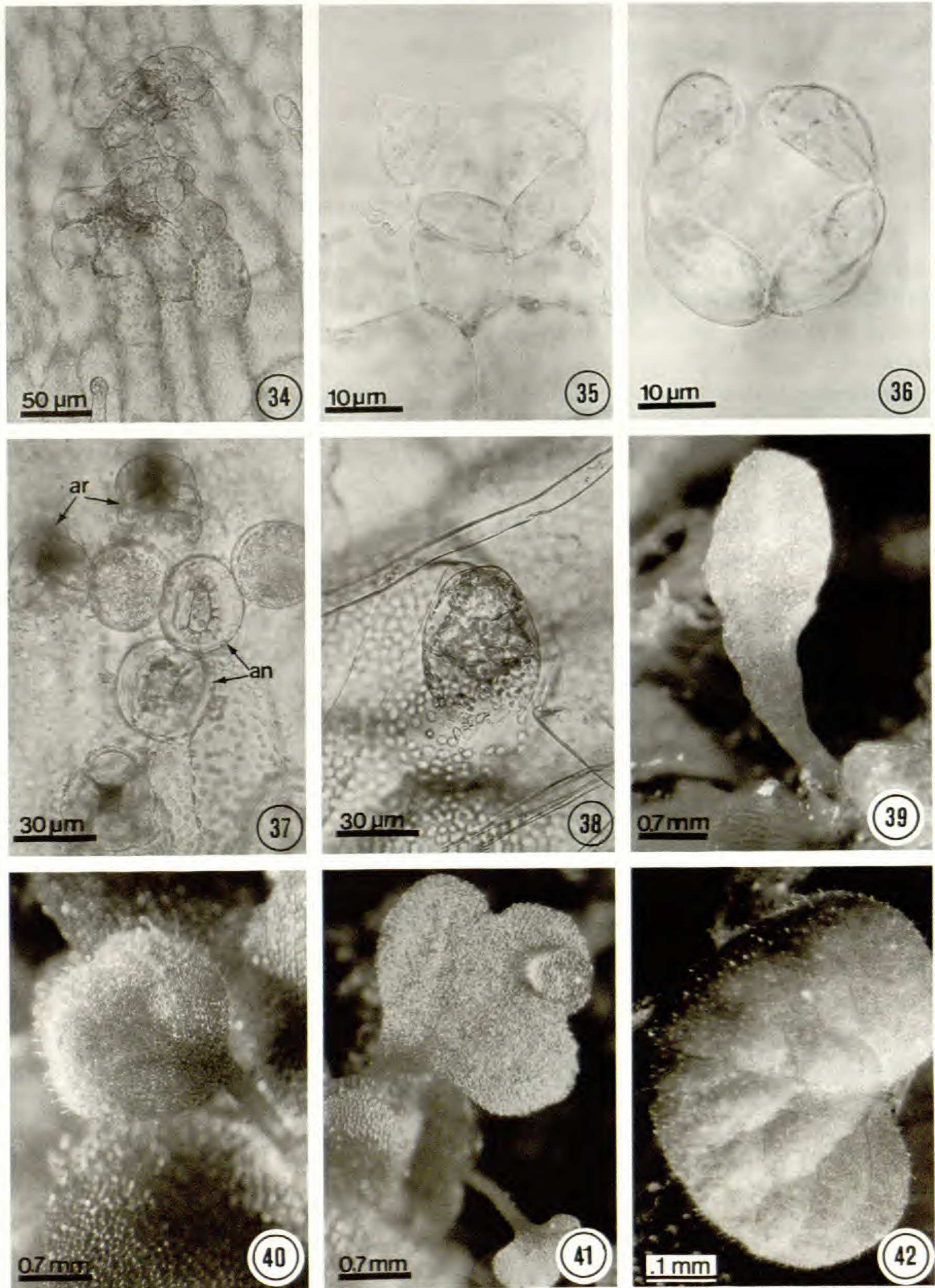
FIGS. 19–25. Adult phase of *Tectaria*. Fig. 19. Spatulate-elongated gametophyte of *T. heracleifolia*, 85 days. Fig. 20. Spatulate-cordiform gametophyte of *T. fimbriata*, 58 days. Fig. 21. Spatulate-cordiform gametophyte of *T. mexicana*, 42 days. Fig. 22. Cordiform gametophyte of *T. transiens*, 40 days. Fig. 23. Cordiform gametophyte of *T. heracleifolia*, 41 days. Figs. 24–25. Cordiform-reniform gametophytes of *T. fimbriata*, 42–64 days. a = wings.

side of the prothallus near the midrib and the meristematic region. The archegonium neck, is short, 3–5 cells long and is directed toward the meristematic zone; the archegonial opening is composed of 4 cells (Figs. 26–27, 34–36).

In *T. mexicana* and *T. transiens* fertilization and subsequent development of the sporophytes takes place after four months (120–125 days, respectively),



FIGS. 26–33. Adult phase of *Tectaria*. Figs. 26–27. Adult gametophytes of *T. mexicana*, 64 days. Fig. 28. Meristematic zone of *T. incisa*, 41 days. Fig. 29. Meristematic zone slightly developed, *T. heracleifolia*, 20 days. Fig. 30. Marginal hair of *T. incisa*, 128 days. Fig. 31. Secretor marginal hair of *T. mexicana*, 42 days. Fig. 32. 2-branched hairs, bi- and tri-cellular, of *T. mexicana*, 42 days. Fig. 33. Multicellular uniseriate hair of *T. fimbriata*, 69 days. **ar** = archegonia; **pm** = multicellular uniseriate hair.



FIGS. 34–42. Young gametangia and sporophytes of *Tectaria*. Fig. 34. Necks of archegonia of *T. incisa*, 41 days. Figs. 35–36. Neck and mouth of archegonia of *T. fimbriata*, 68 days. Fig. 37. Antheridia and archegonia of *T. mexicana*, 63 days. Fig. 38. Antheridia of *T. incisa*, 41 days. Fig. 39. First leaf of sporophyte of *T. heracleifolia*, 116 days. Fig. 40. Young tri-lobate, pubescent leaf of *T. transiens*, 123 days. Fig. 41. Young tri-lobate leaf of *T. mexicana*, 120 days. Fig. 42. Young cordiform leaf of *T. heracleifolia*, 491 days. **an** = antheridia; **ar** = archegonia.

whereas in *T. heracleifolia* and *T. incisa* they develop at five months (166 days). In *T. fimbriata*, sporophyte development occurred after 16 months (491 days).

First leaves are small, entire, and spatulate, with a lobate apex and only one vein (Fig. 39). The lamina of later leaves are reniform and lobed (bi or trilobulate), the margins are entire and the vein divides dichotomously; sparse hairs are present on the adaxial and abaxial leaf surfaces of *T. heracleifolia*, *T. incisa* and *T. mexicana* (Figs. 41, 42). In *Tectaria transiens*, the leaves are more densely pubescent (Fig. 40). All species studied have anomocytic stomata on the abaxial surface. Mature leaves show reticulate venation, with areoles and small, included veins, features characteristic of the group (Nayar and Kaur, 1964).

Sporophytes develop uniseriate, unicellular, and multicellular hairs, similar to those found on adult prothalli. These hairs are found on the petiole and on the lamina of young leaves.

DISCUSSION

The five species of *Tectaria* share monoletic, ellipsoid spores, with perine; *Vittaria*-type germination; *Aspidium*-type prothallial development; gametophytes with unicellular, simple, claviform or capitate, secretory hairs; multicellular uniseriate hairs between the meristematic zone and the midrib; spatulate, cordiform to reniform adult gametophytes; and gametangia of the common type for leptosporangiate ferns.

All *Tectaria* species, including Old World taxa, have *Vittaria*-type germination and *Aspidium*-type prothallial development (Kachroo, 1956). Nayar and Kaur (1964), and Srivastava (1968) do not mention these patterns by name because their studies were conducted prior to those of Nayar and Kaur (1969, 1971) who named the different types of germination and prothallial development in homosporous ferns.

Both New and Old World species develop simple capitate, unicellular, secretory and claviform hairs on the margins and on both surfaces of the prothallial plate. Multicellular branched hairs on the ventral surface of the prothallus near the meristematic zone, as mentioned by some authors for *Tectaria amplifolia*, *T. macrodonta*, *T. polymorpha*, *T. semibipinnata*, *T. simonsii*, *T. fuscipes* and *T. variolosa*, have not been observed in species studied during this research.

With the exception of *T. polymorpha*, antheridia are small and globose, with one basal plate-like cell, a median cell and an opercular cell. In *T. polymorpha*, the basal cell is funnel-shaped (Nayar and Kaur, 1964). In all species the archegonia are typical, with 3–5 tiers of neck cells and with a slight curvature toward the apical meristem.

Sporophytes of all the Old World species are reported to form 4 to 6 months after germination. In the New World species studied taxa develop sporophytes usually after 4 or 5 months. Sporophytes did not appear until much later, 16 months, in *T. fimbriata*.

First leaves have a lobate apex and are more or less spatulate, as in *T. simonsii*. The second and third leaves have defined veins and smooth margins, as in *T. variolosa*, *T. polymorpha* and *T. amplifolia* and the mature leaves show a reticulate venation (Nayar and Kaur, 1964). Mature sporophytes in *T. amplifolia* have been studied in detail by Rao and Khare (1964). Heteroblastic leaf development has been well documented by Wagner (1952), Nayar and Kaur (1964) and Kaur and Devi (1976).

Srivastava (1968) mentioned that old prothalli of *T. amplifolia* undergo vegetative regeneration; we did not observe this process in any New World taxa, nor did we see evidence of apospory or apogamy, as described for *T. trifoliata*. (Steil, 1944).

Our study confirms that spore germination and prothallial development patterns are similar in five taxa of New World *Tectaria* and that these patterns are consistent at the generic level as evidenced by previous studies on Old World species. Also, the presence of multicellular branched hairs on gametophytes appears to be concentrated in *Tectaria* of Old World (Nayar and Kaur, 1964; Stokey, 1960). This paper confirms the strong uniformity in gametophyte development in *Tectaria* and represents a further contribution to our understanding of gametophyte development.

ACKNOWLEDGMENTS

The authors wish to thank Robbin C. Moran, Ana Rosa López-Ferrari and Adolfo Espejo for their careful proof reading, comments, and suggestions to the manuscript. Also, we thank Manuel García Caluff for sending the spores of *T. fimbriata* from Cuba, and to Jorge Lodigiani for his photographic support.

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