A scanning electron microscopic study of the seed coat structure of *Curtia* Chamisso & Schlechtendahl and *Hockinia* Gardner (Gentianaceae)

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ABSTRACT

The six species of *Curtia*, including a hitherto undescribed species published here, as well as the monotypic genus *Hockinia* can be distinguished from each other by the seed coat structure. The anticlinal walls and the cuticle provide the most useful information. *Curtia tenuifolia* appears to be a complex species, but subsp. *tenella* can be readily separated from this complex by the seed coat structure. Heterostyly has been found in *C. tenuifolia* subsp. *tenuifolia*, *C. obtusifolia*, and *Hockinia montana*, but differences in seed coat structure can not be correlated with long-, short-, and equal-styled flowers.

The differences in seed coat structure, the length of the seeds, and the number of cells per seed plead for maintaining *Hockinia* (closely related to *Curtia*) as a distinct genus.

One new species of Curtia is described and a new combination is made.

INTRODUCTION

Curtia, a neotropical genus of Gentianaceae, consists of six species occurring in South and Central America on wet sandy savannas from 0-1600 m altitude. The monotypic genus *Hockinia*, which resembles *Curtia* and is possibly a close relative of it, was also studied. The present study of the micromorphology of the seed coat formed a part of a study of the taxonomy of the genus *Curtia* (Grothe & Maas, Revision of the genus *Curtia*. In prep.).

MATERIALS AND METHODS

Seeds were collected from dehisced capsules of herbarium specimens. Seeds taken from mature closed capsules were compared with those from open capsules and were found to be the same. The seeds were sputter-coated with gold-palladium for about 4 minutes and observed and photographed with a Cambridge Stereoscan mark 2a. Material from the following herbaria was used in this study: BM, BR, C, CGE, E, F, GH, GOET, HAL, HB, K, L, LE, M, MG, MO, NY, OXF, P, RB, S, U, US, VEN, W. For the descriptions the terminology as proposed by Barthlott & Ehler (1977) was followed, although it was necessary to introduce two new terms. They are:

MOLARIFORM: – Resembling a molar; anticlinal wall boundary (the junction of two cells) sunken; anticlinal wall with irregular transverse insertions (Plate 3F). ERUCIFORM (from the latin 'eruca' = caterpillar): – suggesting the shape of a caterpillar; anticlinal wall is marked by regular transverse insertions; the anticlinal wall boundary is marked by a slight ridge between small depressions (not shown clearly in the photograph) running the full length of the wall (Plate 2B, C).

The number of cells mentioned in the descriptions is obtained by counting the cells on the photograph, *without* using any correction factor.

DESCRIPTIONS

I. Curtia Chamisso & Schlechtendal

Capsule 6-to ca. 100-seeded. Seeds 90-510 μ m long (longest axis), mainly pyramidal to subglobose, triangular-cylindrical (prism-shaped), cubiccylindrical (shaped like an elongated tube), truncate, bowl-shaped, or irregularly shaped, one or more sides depressed. Outer epidermal cells (testa cells) 10-70 per seed, show a regular brickwall-like arrangement, sometimes a net-like arrangement; cells mainly elongated (rectangular), sometimes isodiametric in outline, 4-5(-7)-gonal; curvature of outer epidermal cells flat to concave, cells rarely deep. Anticlinal walls straight (sometimes with perforations) to sinusoidal (S-type); anticlinal wall boundary raised to form a ridge, slightly raised between small depressions or sunken. Cuticle smooth, sometimes with stretch-marks or wrinkled, in some cases showing the reticulate or pitted pattern of the underlying periclinal wall, sometimes the cuticle is covered with irregularly shaped dots or a salt deposit.

1. Curtia diffusa (Martius) Chamisso Fig. 1A-C.

Capsule 6–10-seeded. Seeds 390–490 μ m long, mainly pyramidal to triangularcylindrical or cubic-cylindrical. Outer epidermal cells show a regular brickwalllike arrangement; cells elongated, sometimes isodiametric in outline; curvature of outer epidermal cells flat to concave. Anticlinal walls sinusoidal, with about 5 waves per cell-length; anticlinal wall boundary only marked by a small line. Cuticle covered with irregularly shaped dots (Fig. 1C).

2. Curtia confusa Grothe & Maas spec. nov. (see p. 42) Fig. 2 D-F.

Capsule ca. 90-seeded. Seeds $300-510 \ \mu m$ long, variously shaped from cylindrical to subglobose or pyramidal, often one or more sides depressed. Outer epidermal cells show a regular brickwall-like arrangement; cells elongated,



Plate 1. C. diffusa (A–C. Mexía 5750). A. seed (120 \times); B. sinusoidal anticlinal wall and dotted cuticle (530 \times); C. detail of B (1060 \times). Hockinia montana (D–F. Martinelli 227). D. seed (70 \times); E. anticlinal wall showing lateral swellings with pronounced perforations; testa cells deep (530 \times); F. as E. (800 \times).



Plate 2. C. obtusifolia (A, B. Maguire 34597; C. Huber 1578). A. seed (120 \times); B. detail of eruciform anticlinal wall, facing deeply depressed side; cuticle smooth with stretch-marks (265 \times); C. detail of eruciform anticlinal wall; cuticle wrinkled (2500 \times). C. confusa (D, E. Dusén 4392; F. Brade s.n.); D. seed (120 \times); E. anticlinal wall boundary raised, anticlinal wall showing perforations (535 \times); F. raised anticlinal wall boundary and deeply depressed cell (1000 \times).



Plate 3. C. verticillaris (A, D. Duarte 9210; B. Mexía 5812; C. Duarte 2148). A. seed (100 \times); B. salt (?) deposit on the cuticle (500 \times); C. salt (?) deposit on anticlinal wall and cuticle (1000 \times); D. anticlinal wall boundary deeply sunken (2500 \times). C. conferta (E, F. Bunting et al. 4111); E. seed (135 \times); F. detail of molariform anticlinal wall; cuticle pitted (790 \times).



Plate 4. C. tenuifolia subsp. tenuifolia (A. Allen 994; C. Koyama & Agostini 7334; E. Ratter 3027) and subsp. tenella (B. Standley 14548; D, F. Koyama & Agostini 7390). A. seed, with straight partly sinusoidal anticlinal walls ($250 \times$); B. seed ($105 \times$); C. beaded anticlinal walls ($250 \times$); D. pitted cuticle, ruptures along the anticlinal walls ($1000 \times$); E. beaded anticlinal walls; cuticle smooth with stretch-marks ($500 \times$); F. pitted cuticle ($2500 \times$).

sometimes isodiametric in outline; curvature of outer epidermal cells flat to deeply concave. Anticlinal walls straight, with perforations; anticlinal wall boundary with prominent ridge. Cuticle smooth, showing reticulate pattern of underlying periclinal wall.

3. Curtia verticillaris (Sprengel) Knoblauch Fig. 3A-D.

Capsule ca. 100-seeded. Seeds $(245-)300-470 \ \mu m \log r, cylindrical to globose, subpyramidal or truncate, often one or more sides depressed. Outer epidermal cells show a regular brickwall-like arrangement; cells elongated, sometimes isodiametric in outline; curvature of outer epidermal cells mainly flat. Anticlinal walls straight, sometimes reticulate thickenings of inner periclinal wall are visible; anticlinal wall boundary deeply sunken. Cuticle smooth, covered with what is probably a salt deposit.$

COMMENTS:

In order to obtain some information concerning the nature of the deposit on the seeds, seeds were soaked for two hours in 70% alcohol or chloroform, or were soaked for 15 minutes with nitric acid. Treatment with alcohol or chloroform did not solve the salt deposit. Seeds treated with nitric acid, however, did not show any deposit on the S.E.M. images, indicating the deposit may have been a salt deposit indeed. This deposit might have formed by evaporation of the liquid originally surrounding the seeds in the capsule.

4. Curtia obtusifolia (Bentham) Knoblauch Fig. 2A-C.

Capsule ca. 15-seeded. Seeds $280-470 \ \mu m$ long, ellipsoidal or irregularly shaped, often one or more sides depressed. Outer epidermal cells show a netlike arrangement; cells isodiametric in outline; curvature of outer epidermal cells flat to concave. Anticlinal wall straight with regular transverse insertions, eruciform; anticlinal wall boundary marked by a slight ridge between small depressions running the full length of the wall. Cuticle wrinkled or smooth with stretch-marks in the anticlinal field.

COMMENTS:

This species, restricted to the Amazon region of Venuzuela and Brazil, is heterostylous. Seeds with smooth or wrinkled cuticles could not be correlated with long- or short-styled forms.

5. Curtia conferta (Martius) Knoblauch Fig. 3E-F.

Capsule up to 15-seeded. Seeds $250-440 \ \mu m$ long, bowl-shaped, ellipsoidal to subglobose. Outer epidermal cells show a regular brickwall- to net-like arrangement; cells elongated to isodiametric in outline; curvature of outer epidermal cells flat. Anticlinal walls with sunken central part and irregular transverse insertions, molariform.Cuticle wrinkled with pits of underlying periclinal wall.

6. Curtia tenuifolia (Aublet) Knoblauch

Capsule ca. 100-seeded. Seeds $90-350 \mu m \log$, cylindrical to subglobose or irregular. Outer epidermal cells show a regular brickwall-like arrangement; cells elongated, sometimes isodiametric; curvature of outer epidermal cells flat to concave. Anticlinal walls straight, sinusoidal (with up to 10 small waves per cell length) or beaded. Cuticle smooth, or wrinkled with pits of underlying periclinal wall, or wrinkled to smooth with stretch-marks (occasionally a net-like structure is visible).

6.a. Curtia tenuifolia subsp. tenuifolia Fig. 4A, C, E.

Seeds with straight, beaded, or partly sinusoidal anticlinal walls. Cuticle wrinkled or smooth with stretch-marks (occasionally a net-like structure is visible). Curvature of outer epidermal cells flat to concave.

6.b. Curtia tenuifolia (Aublet) Knoblauch subsp. tenella (Martius) Grothe & Maas comb. nov. Fig. 4B, D, F.

Schuebleria tenella Martius, Nov. Gen. & Sp. Pl. 2: 117. 1827. Type. Pohl 5211 (holotype, W), Ponte Feita, Goiás, Brazil.

Curtia tenella (Martius) Knoblauch, Bot. Centralbl. 60(12): 357. 1894.

Seeds with sinusoidal, rarely beaded anticlinal walls. Cuticle smooth to wrinkled with pits of underlying periclinal wall. Curvature of outer epidermal cells mainly flat.

II. 1. Hockinia montana Gardner Fig. 1D-F.

Capsule ca. 40-seeded. Seeds $665-860 \mu m \log n$, ellipsoidal, almost rhombic (with obtuse ends), pear-shaped or irregularly shaped, seeds often flattened. Outer epidermal cells 80-100 per seed, showing a regular brickwall-like arangement; cells mainly elongated in outline; curvature of outer epidermal cells deep-ly concave. Anticlinal walls straight, showing alternating lateral swellings, with pronounced perforations between them.

DISCUSSION

The species of *Curtia, Hockinia*, and the two subspecies of *C. tenuifolia* appear all clearly distinct by their seed-coat structure. Characteristic for *C. tenuifolia* subsp. *tenella* are the distinct sinusoidal anticlinal walls (Plate 4B). Conversely, the seeds of subsp. *tenuifolia* have straight (rarely beaded) anticlinal walls. There are transitional forms within *C. tenuifolia*, however, in which the anticlinal walls are beaded (Plate 4C) or partly sinusoidal (Plate 4A).

The cuticle of *C. tenuifolia* subsp. *tenella* can be smooth or can show the pits of the underlying periclinal wall (Plate 4F). There are, however, indications that what appears as the smooth cuticle is in fact the periclinal (or inner periclinal) wall, which has become visible after detachment of the cuticle (or the cuticle and outer periclinal wall). Both types of surfaces have been found in the same seed. A great many seeds of subsp. *tenella* show ruptures along the anticlinal wall and the cuticle (Plate 4D), which indicate that the cuticle becomes detached.

C. tenuifolia has long-, short-, and equal-styled flowers, C. obtusifolia has long- and short-styled flowers, but no correlations with seed coat structures could be found.

Sinusoidal cell walls are found in C. diffusa and C. tenuifolia. The photograph 2C of C. obtusifolia, which has an eruciform anticlinal wall, also shows sections in between that somewhat tend towards a sinusoidal shape.

Hockinia seeds are longer than those of Curtia (Hockinia 665-860 μ m, Curtia 90-150 μ m long), and the surface of Hockinia seeds also has more cells than in Curtia. Furthermore, Hockinia has anticlinal walls with pronounced lateral swellings with distinct perforations between these swellings (Plate 1E, F). C. confusa (Plate 2E) also has anticlinal walls with perforations, but the perforations are less pronounced. Hockinia differs from Curtia in the seed coat structure, and also in the seed length and the number of cells per seed. This strongly supports maintaining Hockinia as a distinct genus.

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Grothe, E.H.M. & P.J.M. Maas - Revision of the genus Curtia. In prep.

SPECIMENS SEEN

I. Curtia

- 1. C. diffusa
- L. Damazio 2048 (RB); Y. Mexía 5750 (NY).
- 2. C. confusa

A.C. Brade s.n. (SP), 5891 (S, SP); P. Dusén 4392 (S); F. Sellow s.n. (K).

3. C. verticillaris

J.S. Blanchet s.n. (G); A.P. Duarte 2148 (RB), 9210 (U); Y. Mexía 5817 (F)

4. C. obtusifolia

O. Huber 1578 (U); B. Maguire 34597 (NY).

5. C. conferta

G.S. Bunting et al. 4111 (MY); O. Huber 5688 (U).

6.a. C. tenuifolia subsp. tenuifolia

P.H. Allen 994 (U); A.C. Brade 13618, 17684 (RB); L. Damazio 2057 (RB); P. Dusén 8003a (S); K. Fiebrig 5250 = 4991 (L); E.P. Heringer 8536/730 (NY); T. Koyama & G. Agostini 7334 (VEN); P.J.M. Maas & L.Y.Th. Westra 4340 (U); T.M. Pedersen 8740 (C); E. Pereira 1570 (RB); G.T. Prance 4486 (U); A. Progel 1618 (LE); R. Schomburgk 167 (BM): M. Silva 697 (MG).

6.b. C. tenuifolia subsp. tenella

A.M. Brenes 220 = 5065 (F); G.B. Hinton 5040 (NY); I.V. Johnston 877 (GH); T. Koyama & G. Agostini 7390 (NY); H. Pittier 12788 (VEN); J.B.E. Pohl 5211 (W); P.C. Standley 14548 (F); J.A. Steyermark 13218 (F).

II. Hockinia

1. Hockinia montana

G. Martinelli 227 (U).

2. Curtia confusa Grothe & Maas spec. nov.

Schuebleria conferta G. Don, Gen Syst. Dichl. Pl. 4: 201. 1838, not of Martius 1827.

Curtia conferta E. Gilg in Engler & Prantl, Nat. Pflanzenfam. 4(2): 70. t. 40, f. J-L. 1895 (≡ Schuebleria conferta G. Don).

Herba 10-65 cm alta, saepe ramosa. Caulis 6-10-angulatus, 0.5-3.5 mm in diametro. Petiolus 0-1(-2) mm longus. Folia 3-6(-7)-verticillata, inferiora interdum opposita, saepe anguste ad late ovata, 1-8.5(-12) mm longa, 0.2-4.3(-5) mm lata, apice acuta ad acuminata, e basi 1-3(-5)-nervia. Inflorescentia dichasium 3-multiflorum, pedicellis florum centralium 0-1.5(-3) mm longa, 0.8-1.4 mm lata. Corolla alba (alabastro lilacino), 3.8-4.8 mm longa, tubo cylindrico, 2-2.8 mm longo, prope faucem leviter constricto, lobis ovatis, 1.5-2 mm longis, 0.7-1.4 mm latis, apice acuminatis. Stamina 1.7-1.9(-2.4) mm longa e basi exeuntia, filamentis 0.1-0.2(-0.6) mm longis, antheris parallelis, 0.7-0.8 mm longis et 0.3-0.5 mm latis, connectivo latissimo. Ovarium ovoideum ad anguste ovoideum, $1.5-2.4 \times 0.5-1.6$ mm. Stylus 0.1-0.3(-0.6) mm longus. Stigma 0.4-0.5 mm longum. Capsula anguste ovoidea, $2.5-4.5 \times 0.8-1.8$ mm, ca. 90-seminalis.

Distribution. In marshy places, on savannas; common in SE Brazil; mostly at elevations from 750-1300 m.

Type. P. Dusén 4392 (holotype and isotypes, S), Fernandez Pinheiro (50°30'W-25°30'S), marshy place near railway station, 26 March 1904, Paraná, Brazil.

Curtia confusa is very closely related to C. verticillaris, both having verticillate leaves. It diverges, however, by its differently shaped corolla (salversharped vs. funnel-shaped), a much smaller corolla tube (2-2.8 mm vs. 5-7 mm), much smaller filaments (0.1-0.2(-0.6) mm vs. 1.5-4.5 mm long), and differently shaped pollen as well as seeds.

Up till now this species has been misidentified as *Curtia conferta* (or *Schuebleria conferta*). That species, however, is restricted to Amazonian regions of Colombia, Venezuela, and Brazil.