CALLITRICHE NANA (CALLITRICHACEAE), A NEW SPECIES FROM JAVA, INDONESIA

W.J.J.O. De Wilde, B.E.E. Duyfjes, B. Gravendeel, R.W.J.M. Van der Ham

Nationaal Herbarium Nederland, Universiteit Leiden Branch, P.O. Box 9514, 2300 RA Leiden, The Netherlands

B.C. Ho

National University of Singapore, 10 Kent Ridge Crescent, Singapore **Rugavah**

Herbarium Bogoriense, Pusat Penelitian Biologi–LIPI, Bogor, Indonesia G.T.P. Vo

University of Natural Sciences-Hochiminh City, 227 Nguyen Van Cu St., Dist.5, Hochiminh City, Vietnam

W.J.J.O. De Wilde, B.E.E. Duyfjes, B. Gravendeel, R.W.J.M. Van der Ham, B.C. Ho, Rugayah, G.T.P. Vo. 2003. *Callitriche nana* (*Callitrichaceae*), jenis baru dari Jawa, Indonesia. *Floribunda* 2(3): 57–66. — *Callitriche nana* (*Callitrichaceae*) B.C. Ho & G.T.P Vo merupakan jenis *Callitriche* pertama yang ditemukan di Jawa. Jenis ini adalah tumbuhan terestrial. Di antara 50 jenis yang ada di dunia, *Callitriche* yang ditemukan di Jawa mempunyai ukuran paling kecil dan mempunyai karakter morfologi yang berbeda sekali dengan jenis lainnya. Analisis filogeni dengan menggunakan urutan *rbcl* menunjukkan bahwa *Callitriche nana* menduduki posisi yang terpisah dalam genus *Callitriche*.

Kata kunci: Callitriche nana, Callitrichaceae, Indonesia, Jawa, filogeni.

W.J.J.O. De Wilde, B.E.E. Duyfjes, B. Gravendeel, R.W.J.M. Van der Ham, B.C. Ho, Rugayah, G.T.P. Vo. 2003. *Callitriche nana* (*Callitrichaceae*), a new species from Java, Indonesia. *Floribunda* 2(3): 57–66. — *Callitriche nana* B.C. Ho & G.T.P. Vo, the first *Callitriche* species from Java, is described. It is a terrestrial species, not a terrestrial form of an aquatic species. It has the smallest stature among the some 50 species known world-wide. The species is distinct in several other morphological characters as well. Phylogenetic analyses of its *rbcL* sequences also indicate an isolated position in the genus *Callitriche*.

Keywords: Callitriche nana, Callitrichaceae, Indonesia, Java, phylogeny.

Callitriche L., the sole genus in the family Callitrichaceae, is distributed world-wide, and comprises some 50 species. It is most abundant in temperate areas, whereas in the tropics it is confined to mountainous areas. The genus is relatively poorly represented in Asia, particularly in Malesia, from where only a few collections are known: Sumatra (aquatic), Java (terrestrial; this paper), Borneo (aquatic), New Guinea (terrestrial and aquatic), and Philippines (aquatic). The material from New Guinea was referred to C. papuana Merrill & Perry (1941), and later to C. verna L. (Merrill & Perry 1948; Backer 1951) and C. palustris L. (Hoogland 1958; van Royen 1983). More recently collected terrestrial plants, also from New Guinea, were described as C. cycloptera Schotsman (1985). The Java material comes from a single locality in the Cibodas Botanic Mountain Garden, W Java, at 1400 m altitude, namely from a rather shaded stone-paved place beside the old guesthouse. Material from this locality, collected in 1950 by Van Ooststroom & Kern, was treated by Backer (1951, with figure; 1963) as a terrestrial

form of *C. verna*, but its status was doubted later on; Schotsman (1985) regarded it as an undescribed terrestrial species, because of various (not stated) morphological and anatomical characters.

The genus Callitriche is difficult because of its minute flowers, and its plasticity in habit according to its environment: either completely submersed or with floating leaves in a spurious rosette, or amphibious, or partly or completely terrestrial. In recent times most authors agree on the occurrence of strictly terrestrial species, a feature being one of the main characters for species distinction. Most important, however, are the characteristics of the ripe fruits, the disposition of the male and female flowers in the leaf axils, the presence or absence of bracteoles and characters of the flowers (stamen, ovary) and pollen. In addition, leaf morphology, anatomical characters and chromosome numbers are helpful. The pollination biology of *Callitriche* is extremely varied (Philbrick 1984; Philbrick & Les 2000; Miller 2001).

The material used for the description of *C. nana* was collected in 1987 by Veldkamp, and in 2002 by De Wilde et al. (Fig. 1 & 2). The latter material was extensively studied during the international training course on taxonomy, Bogor

2002, resulting in the present formal description by Ho & Vo, two of the course participants. Although Schotsman (1985) stated that the Cibodas material concerned an undescribed species, the sole locality within the Mountain



Fig. 1. Collecting of *Callitriche nana* B.C. Ho & G.T.P. Vo, at the type-locality, Aug. 2003, beside the guesthouse in the Cibodas Botanic Mountain Garden. (Photo P.H. Hovenkamp).

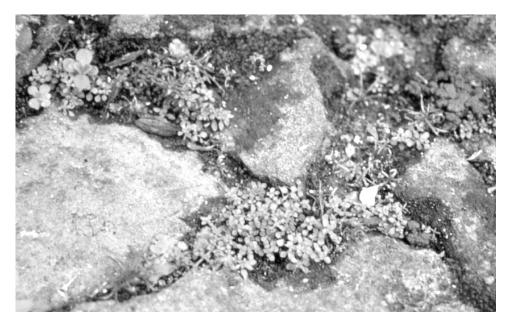


Fig. 2. *Callitriche nana* B.C. Ho & G.T.P. Vo, at the type-locality, Aug. 2003, beside the guesthouse in the Cibodas Botanic Mountain Garden. (Photo P.H. Hovenkamp).

Garden could mean that the material was introduced, and before deciding to describe C. nana the following regional taxonomic treatments and floras were checked: Fassett (1951) for the New World; Mason (1959) for Australia & New Zealand; Ohwi (1965) for Japan; Aston (1973) for Australia; Cheng & Ming (1980) for China; Schotsman (1981) for Europe; Kak & Javeid (1982) for the western Himalayas; Schotsman (1988) for Tropical Africa; Ho (1993) for Vietnam; Ming (1997) for Yunnan; Yang & Hsu (1998) for Taiwan; Tzvelev (2000) for the European part of Russia and bordering regions; Hedberg & Hedberg (2001) for Tropical Africa; Miller (2001) for SE United States. With these works we failed to match the Java material, which appeared to be by far the smallest-sized Callitriche known. Perhaps C. japonica Engelm. comes closest, but it is different e.g. by longer prostrate stems and a stouter habit.

In Callitriche 2 or 3 sections can be recognized regionally. The Java plants fit more or less into section Microcallitriche Fassett (to be emended), comprising the terrestrial species (Fassett 1951; Miller 2001). As concerns the position of Callitrichaceae within the system of Angiosperms, Miller (2001) gives a survey, and concludes that Callitrichaceae are a monotypic family, possibly related to Plantaginaceae and Schrophulariaceae. However, the Callitrichaceae are sunk into the Plantaginaceae by the APG II (2003).

Callitriche nana B.C. Ho & G.T.P. Vo., spec. nov. – Fig. 3 & 4.

Callitriche verna auct. non L.: Backer 251. 1951 f. 1 (terrestrial form); (1963) 267.

Plantae minutae terrestres sine rosula apicali, folia spathulata trinervia, caules foliaque pilis glandulis gaudentes, flores solitarii in folii axillis aut masculi aut feminei haud uterque, flores masculi e stamine solitario constantes ebracteolati anthera biloculari lutei, flores feminei sebsessiles stylo stigmateque valde papilloso, fructus plus minusve cordiformes lateraliter visi, latior quam longi, semina fusca margine anguste allata. – Typus: W.J. de Wilde et al. 22275 (holo BO; iso K, L, P), West Java.

Plant annual, minute, 1–2.4 cm tall, delicate, terrestrial, erect, sometimes the primary stem creeping only at very base, with erect secondary stems (branches), rooted at the base and basal nodes. Roots to 18 mm long, infrequently branched. Stems slender, 0.3–0.6 mm in diameter; internodes 1–3 mm long.

Leaves dispersed, not forming a spurious rosette at apex of stem; lamina spathulate, 3-4.5 mm by 1-2.2 mm; nerves 3, distinct, the main nerve ending at the apex with secondary nerves on both sides, anastomosing with the main nerve near the apex of the lamina often at different distances below the apex, each secondary nerve infrequently with few free branching nerves rarely anastomosing; apex \pm rounded, sometimes slightly mucronate. Glandular hairs on stem more or less evenly distributed on the surface of the stem, protruding, peltate, consisting of a sunken stalk cell, with 6 or 8 radiate, wedge-shaped disc cells, arranged in a fanshaped pattern when viewed from the side, elliptic when viewed from above, diameter 35–65 μm; glandular hairs on lamina present on both sides, scarce, similar to those on the stems, except that the head consists of usually 4, rarely 6 or 8 wedgeshaped cells, 25-40 µm in diameter. Flowers present in most leaf axils, solitary, male and female always single in separate axils, rarely flowers present in one axil of a pair only, mostly in both axils, either both female or one female the other one male, rarely both male. Male flowers (sometimes also found in leaf axils on the shoots below leaf axils with already mature fruit): stamen erect, ebracteolate, filament thin, 175-415 µm long, anther minute, becoming yellowish brown, yellowish when young, reniform, 107–195 μm high, 155-250 µm wide, 2-locular, each locule dehiscing along lateral sutures becoming more or less confluent at the apex; columella indistinct, pollen spherical light yellow. Female flowers: subsessile; ovary (270–)390–490 µm high, (340–) 400–470 µm wide, light green, compressed transversely, styles 300–400(–500) µm long, brown, divergent, densely and strongly papillose, (stigma) somewhat pendulous, ascending towards the apex. Fruits (schizocarps): broader than long, compressed, more or less heart-shaped in lateral view, double winged, 800-880 µm high, 980-1045 µm wide, dark brown, apex distinctly emarginate, commissural groove V-shaped and rather wide, remnants of the style persistent, short; mericarps rounded at the apex, wings thin and translucent, broadest at apex, on lateral sides narrow; exocarp with undulate walls, becoming less undulate towards the margin, mesocarp composed of two fibrous layers, in oblique position to each other, endocarp composed of collenchymatous cells. Seeds obliquely ovoidoblong, rather thick, dark brown, embryo slightly bent.



Fig. 3. Habit of *Callitriche nana* B.C. Ho & G.T.P. Vo: 1. part of a flowering shoot; 2. habit of vegetative young plant; 3. adaxial leaf surface showing stomata (×10) (1–3: De Wilde et al. 22275, drawn by G.T.P. Vo).

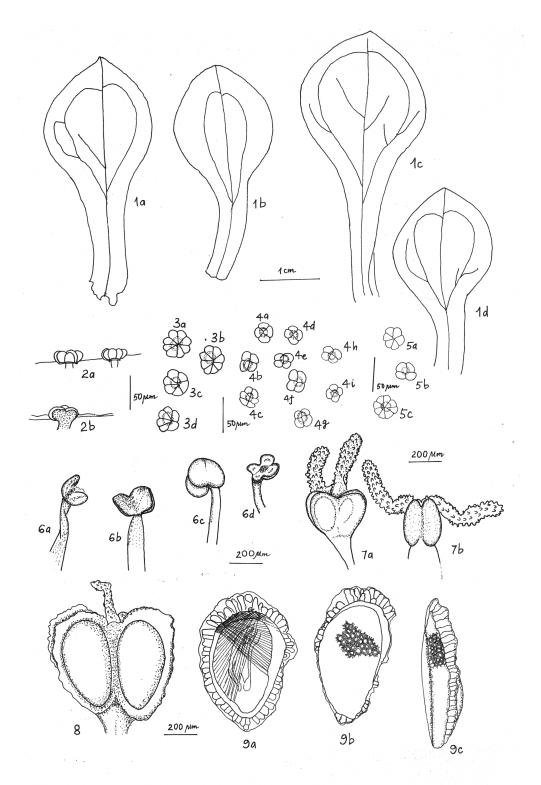


Fig. 4. Various plant parts of *Callitriche nana* B.C. Ho & G.T.P. Vo: 1a–d. leaf showing different venation patterns (×4); 2a–b. lateral views of glandular hairs on stem surface (×40); 3a–d. top view of glandular hairs on stem surface (×40); 5a–c. top view of glandular hairs on abaxial surface of leaf (×40); 6. male flowers / stamens at different stages of maturation, a. back view of half-opened anther, b. front view of half-opened anther, c. anther before anthesis, d. top view of fully-opened anther (×10); 7. young female flowers, a. front view, b. side view (×10); 8. a developing fruit from lateral view (×10); 9. seeds, a. showing mesocarp layer (fibrous) with visible embryo, b. showing endocarp layer (collenchymatous) at lateral view, c. side view (×10) (all: De Wilde et al. 22275, drawn by G.T.P.Vo).

Distribution. Only known from the type locality: Indonesia, West Java, Cibodas, Botanic Garden, beside the guesthouse.

Habitat & Ecology. On moist soils between the pavement stones, open or partly shaded, at 1400 m altitude. Sparsely distributed in small patches, thriving well and producing viable seeds copiously. Fl. & fr. in August. About the pollination nothing is known.

Cytological work. Tissue of shoots and roots was investigated, but the chromosome number remained unclear, due to the small sizes of the cells and chromosomes. About 20 chromosomes were counted in some cells. Chromosome numbers are variable in *Callitriche*, with somatic numbers between 10 and 40 (Philbrick & Les 2000; Hedberg & Hedberg 2001).

Pollen morphology (Fig. 5). Unacetolysed pollen sampled from W.J. de Wilde et al. 22275 was studied with LM and SEM. The pollen grains are small (P x E = $13.2 \times 15.4 \mu m$), suboblate (P/E=0.86) using LM, more prolate with SEM, and 3-aperturate. The apertures are ill-defined colpi (probably without endoapertures), though recog-

nisable using LM by the clearly thinner exine and thicker intine (oncus). The mesocolpial intine is distinct, as thick as or slightly thicker than the overlying exine. The exine is c. 1 μ m thick, and consists of a thin nexine, a columellate infratectum (distinct in the mesocolpa using LM) and a reticulate tectum with c. 0.5 μ m long microechinae (SEM).

Phylogenetic position. DNA sequences of the *rbcL* gene of *C. nana* were collected using the methods described in Les et al. (1993) and submitted to the GenBank database under accession number AY289597. Molecular and morphological data of *C. nana* were added to the matrix compiled by Philbrick and Les (2000; Tables 1 & 2; TreeBASE SN1429) and analysed using PAUP* 4.0b10 under the options described by Philbrick and Les (2000). Phylogenetic analyses of the molecular and combined data indicate that *C. nana* occupies an isolated and basal position in the genus *Callitriche* (Fig. 6).

Material studied. W.J. de Wilde et al. 22275 (BO, K, L, P); Van Ooststroom (with Kern) 13073 (L); Veldkamp 8616 (L).

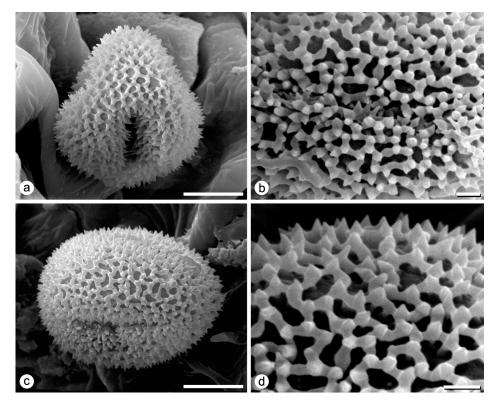


Fig. 5. Pollen micrographs (SEM), all from W.J. de Wilde et al. 22275 (L). a. nearly polar view, showing a trilobate grain with invaginated colpi; b. detail of an invaginated colpus; c. equatorial view, showing a prolate grain (colpi invaginated); d. detail of the pollen wall surface, showing the columellate infratectum and the reticulate tectum with microechinate ornamentation. Scale bar = $5 \mu m$ (a and c) or $1 \mu m$ (b and d).

Table 1. List of characters and character states used for the morphological analyses (slightly modified from Philbrick and Les 2000).

- 1. Endothecial thickenings: 0 = absent; 1 = present.
- 2. Inflated floral bracts: 0 = absent; 1 = present.
- 3. Internal geitonogamy: 0 = absent; 1 = present.
- 4. Fruit wing: 0 = absent; 1 = present.
- 5. Fruit pedicel: 0 = absent; 1 = present.
- 6. Fruit orientation: 0 = upright; 1 = reflexed.
- 7. Style orientation: 0 = upright; 1 = reflexed.
- 8. Peltate scales on leaves: 0 = absent; 1 = present.
- 9. Pollen size: $0 = \text{small} (<18 \, \mu\text{m}); 1 = \text{large} (>19 \, \mu\text{m})$
- 10. Aperture number: 0 = 4-6 apertures; 1 = 3 apertures; 2 = no apertures.
- 11. Exine thickness: $0 = > 0.7 \mu m$; $1 = < 0.5 \mu m$ or exine absent.
- 12. Sexine structure: 0 = tectate; 1 = pointed scabrae; 2 = rounded scabrae; 3 = microechinate scabrae; 4 = finely granular; 5 = sexine absent.
- 13. Fruit position: 0 = aerial; 1 = geocarpic.
- 14. Flower type: 0 = bisexual; 1 = unisexual.
- 15. Calyx: 0 = present; 1 = absent.
- 16. Carpel number: 0 = unicarpellate; 1 = bicarpellate.
- 17. Leaf arrangement: 0 = whorled; 1 = opposite.

Table 2. Data matrix of morphological characters used. Polymorphic characters are indicated by all states possible and inapplicable or unknown characters by a question mark. Data were collected from own observations, Cooper et al. (2000), Engel (1978) and Philbrick and Les (2000).

									chara	cter							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
C. albomarginata	?	1	0	0	0	0	0	1	?	?	0	?	0	1	1	1	1
C. brutia	0	0	0	1	1	1	1	1	1	2	1	5	0	1	1	1	1
C. cophocarpa	1	1	0	0	0	0	0	1	1	2	0	2	0	1	1	1	1
C. cribrosa	1	1	0	1	0	0	0	1	0	2	0	3	0	1	1	1	1
C. deflexa	1	0	0	1	0	0	0	1	0	1	0	1	0	1	1	1	1
C. hamulata	0	1	0	1	0	0	1	1	0/1	2	1	4	0	1	1	1	1
C. hermaphroditica	0	0	0	1	0	0	1	0	1	2	1	5	0	1	1	1	1
C. heterophylla var. heterophylla	1	1	1	0	0	0	0	1	0	1	0	2	0	1	1	1	1
C. heterophylla var. bolanderi	1	1	1	0	0	0	0	1	1	1	0	2	0	1	1	1	1
C. heteropoda	1	1	1	0	0	0	0	1	?	?	0	2	0	1	1	1	1
C. lusitanica	1	0	0	1	0	0	1	1	0	2	1	5	0	1	1	1	1
C. marginata	1	1	0	1	1	1	0	1	0	1	0	1	1	1	1	1	1
C. nana	1	0	?	1	1	0	0	1	0	1	0	1	0	1	1	1	1
C. nuttallii	1	0	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1
C. peploides	1	0	0	0	0	1	0	1	0	1	0	1	0	1	1	1	1
C. platycarpa	1	1	0	1	0	0	0	1	1	1	0	2	0	1	1	1	1
C. stagnalis	1	1	0	1	0	0	0	1	1	2	0	1	0	1	1	1	1
C. terrestris	1	0	0	0	0	1	0	1	?	1	0	2	0	1	1	1	1
C. truncata	0	0	0	1	0	0	1	0	0	2	1	5	0	1	1	1	1
C. trochlearis	1	1	1	1	0	0	0	1	1	1	0	2	0	1	1	1	1
C. verna	1	1	1	1	0	0	0	1	1	1	1	2	0	1	1	1	1
H. vulgaris	1	?	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
H. lanceolata	1	?	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0

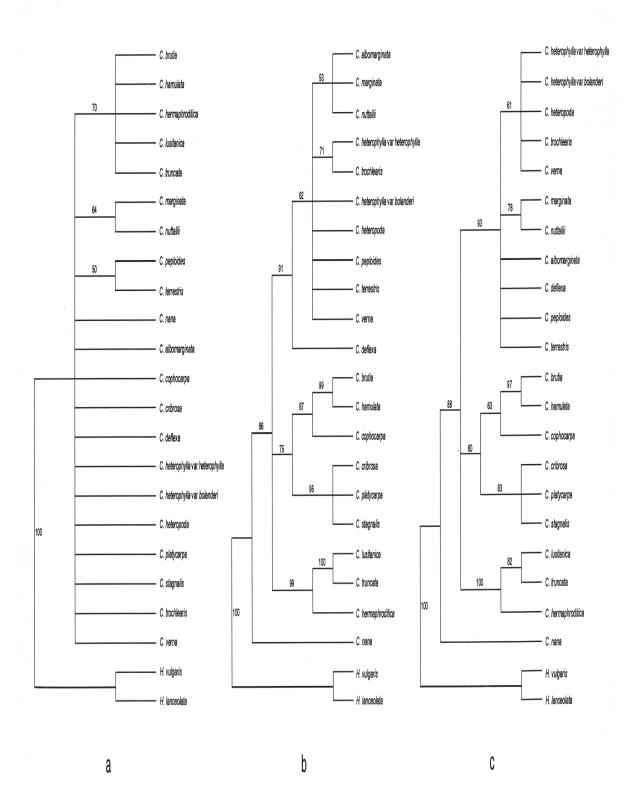


Fig. 6. Resulting trees from parsimony analyses of separate and combined data with bootstrap support values above the corresponding nodes. Only values higher than 50% are indicated. a. strict consensus of 135 MPTs of analysis of morphological data (l=36 steps; CI=0.61; RI=0.80); b. single MPT of analysis of *rbcL* sequences (l=103 steps; CI=0.84; RI=0.91); c. strict consensus of 24 MPTs of analysis of combined morphological and molecular data (l=155 steps; CI=0.61; RI=0.80).

DISCUSSION

Based on macromorphological characters, Callitriche nana fits more or less into Fassett's section Microcallitriche (Fassett 1951): plants terrestrial, fruits wider than high; stamens short, not elongating as the fruit develops; anthers 0.1-0.2 mm wide; flowers ebracteolate; peltate hairs absent; leaves narrowly oblong, spathulate, or oblanceolate. The pollen characters also place C. nana among the terrestrial species. As compared to the data presented by Cooper et al. (2000; 17 species included), C. nana pollen matches that of the terrestrial C. deflexa, C. nutalii, C. peploides, C. terrestris and the amphibious C. marginata most: small grains with relatively well-developed apertures and a thick, clearly stratified (welldefined infratectum) exine. The microechinate ornamentation might be another argument for including C. nana in the terrestrial group. Outside this group, only C. stagnalis has a microechinate tectum. Sequences of the rbcL gene, however, do not place C. nana with the other terrestrial species analysed, but suggest an isolated and basal position in the genus Callitriche. This position is further supported by the up to 0.25 mm wide anthers and peltate hairs, characters not occurring in any of the other terrestrial species. Callitriche nana does not belong to the aquatic sections either, because it never bears dimorphic leaves, and never forms a spurious leaf rosette at the plant apex. According to Schotsman (1954), terrestrial forms of Callitriche are usually rosette forming and/or more or less exhibiting leaf dimorphism. The present species is terrestrial and not a terrestrial form of an aquatic species.

Comparison of C. nana with the other known terrestrial species in Malesia (New Guinea), C. cycloptera, shows that both are clearly distinct especially in general habit and floral characters. In C. cycloptera, male flowers are situated together with a female flower in a single leaf axil (Schotsman 1985), in contrast, there is always a single flower of either male or female on each leaf axil in C. nana. The habits of these two species are rather different too. C. cycloptera is a much larger plant (shoots 20 cm long) and matforming, where C. nana is minute (less than 2.5 cm long), occurring as dispersed individual, erect plants. Leaf size of C. cycloptera is larger (6 by 2.8 mm) and the shape of the widened part of the lamina is rather elliptic-ovate, rhomboid or subtriangular, whereas leaves of C. nana are smaller (3-4 by 1-2 mm) and the shape of the

widened part of the lamina rather orbicular-ovate. Glandular hairs in *C. cycloptera* are of two types, peltate and eventail, the peltate hairs on the lamina consist of 8(-10) disc cells; *C. nana* has only peltate hairs, found on leaves and stems, consisting of 4–8 disc cells.

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