VEGETATIVE ANATOMY AND THE TAXONOMIC STATUS OF ILEX COLLINA AND NEMOPANTHUS (AQUIFOLIACEAE)

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IN 1974 Clark clarified part of the confusion in the literature with respect to three deciduous North American species of *Ilex* L.: *I. longipes* Chapman, *I. decidua* Walter, and *I. collina* Alexander. Because *I. collina* has apopetalous corollas, calyx lobes that are semipersistent on the fruits and inconspicuous in staminate flowers, and free stamens, he transferred it to *Nemopanthus* Raf., noting that *I. longipes* as understood by most authors should be treated as a synonym of *Nemopanthus collinus*. In my accounts of the vegetative anatomy of the Aquifoliaceae (Baas, 1973, 1975, 1978), I noted the close anatomical similarities of *Nemopanthus* with several species of *Ilex* from subg. PRINUS (L.) Maxim. and concluded (1975, p. 355) that merging *Nemopanthus* with *Ilex* "would not meet with any opposition from vegetative anatomists," the peculiar cuticular markings of *Nemopanthus* leaves being the only distinguishing anatomical character.

Dudley and Eisenbeiss (U. S. National Arboretum) drew my attention to *Ilex collina* and expressed their view that the transfer to *Nemopanthus* by Clark might after all have been unjustified. In view of the desirability of a proper understanding of the affinities and taxonomic status of this species as well as of *Nemopanthus mucronatus* (L.) Trel., and also because of the potential or actual importance of these species in horticulture, leaf and xylem anatomy of *Ilex collina* was studied in considerable detail, and additional specimens of *Nemopanthus mucronatus* were examined in order to check the constancy (and hence the diagnostic and taxonomic value) of its anatomical characters.

MATERIALS AND METHODS

The techniques employed have been described earlier (Baas, 1973, 1975). Sections and macerations were studied by light microscopy, and gold-coated surfaces of critical-point dried, previously FAA-fixed material were examined with a scanning electron microscope. All material used for this study was from the herbarium or the living collections of the U. S. National Arboretum, Washington, D. C. The comparative part of the work is largely based on material from the Rijksherbarium collections, detailed previously (Baas, *op. cit.*).

Journal of the Arnold Arboretum **65**: 243–250. April, 1984.

RESULTS

ILEX COLLINA

LEAF ANATOMY

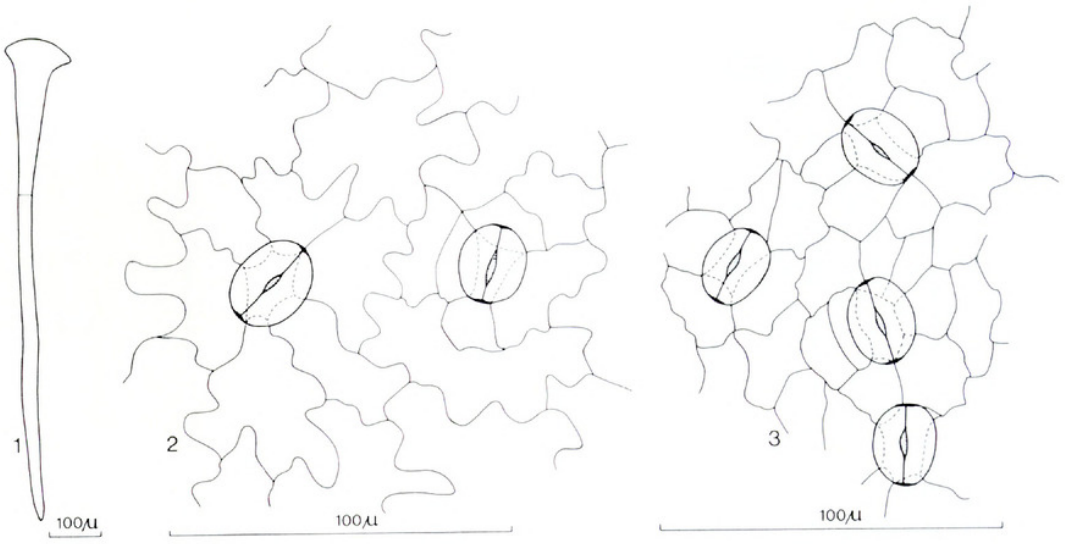
Material studied. U.S.A. Virginia: Mountain Lake, *Shanks s.n.* (NA). West Virginia: Cheat R., *Sharp 1070* (NA); Cheat Mt., *Hutton s.n.*, 13 Aug. 1940 (NA). Pennsylvania: cult. Arbor. Barnes Foundation, Merion, *Fogg s.n.* (NA). Washington, D. C.: cult. from seed collected in 1975 at type locality, *U. S. National Arboretum 38173* (NA); from seed collected in 1974, *Dudley & Eisenbeiss 400 1978* (NA); from seed collected in 1975, *U. S. National Arboretum s.n.* (alcohol-preserved material; L).

Surface view. Long, unicellular, partially septate hairs usually present, especially on veins and abaxial surface (FIGURE 1). Cuticle of adaxial and abaxial surfaces smooth to rather faintly striate (FIGURES 4, 5). Epidermal cells of adaxial surface with straight to (especially in leaves of juvenile specimens) undulating anticlinal walls, those of abaxial surface with strongly undulating (zigzag pattern) walls. Epidermal cell pattern modified over major veins and midrib only. Stomata confined to abaxial surface, irregularly anomocytic to cyclocytic with (3 or) 4 to 7 (to 10) neighboring/subsidiary cells (FIGURE 2), occasionally resembling anisocytic or laterocytic stomata (cf. Den Hartog–Van Ter Tholen & Baas, 1978), guard cell pairs (21–)26–32(–35) by (18–)20–26(–29) μm. Peristomal rims and cuticular T-pieces absent.

Transverse section. Lamina dorsiventral, 80–140 μm thick. Cuticle ca. 1 μm thick. Unspecialized epidermal cells usually flattened, the adaxial cells much larger than abaxial, often bulging, rarely with periclinal wall divisions and/or mucilaginous. Hypodermis absent. Mesophyll composed of 1 (rarely 2) layers of short to tall palisade cells and fairly loose spongy tissue. Midrib grooved adaxially, prominently raised abaxially, supplied with single shallowly arc-shaped collateral vascular bundle; vascular bundle with abaxial cap of thin-walled sclerenchyma fibers, very rarely (only in *Hutton s.n.*) with incurved margins. Ground tissue of midrib parenchymatous to collenchymatous. Veins mostly embedded, with parenchymatous bundle sheath poorly differentiated and extending to upper and lower epidermis only in major veins. Leaf margin with chlorenchymatous ground tissue. Petiole vascularization similar to that of midrib, proximal part with single bundle (type 1; cf. Baas, 1975), distal portion with additional small latero-dorsal wing bundles (types 3 and 4). Crystals present as druses especially in vicinity of vascular bundles.

WOOD ANATOMY

This description is based on a stem 6 mm in diameter from an alcohol-preserved specimen cultivated in the U. S. National Arboretum. Quantitative values should be considered with caution because the specimen was from an immature plant (3 years old). The presence of spiral thickenings was checked

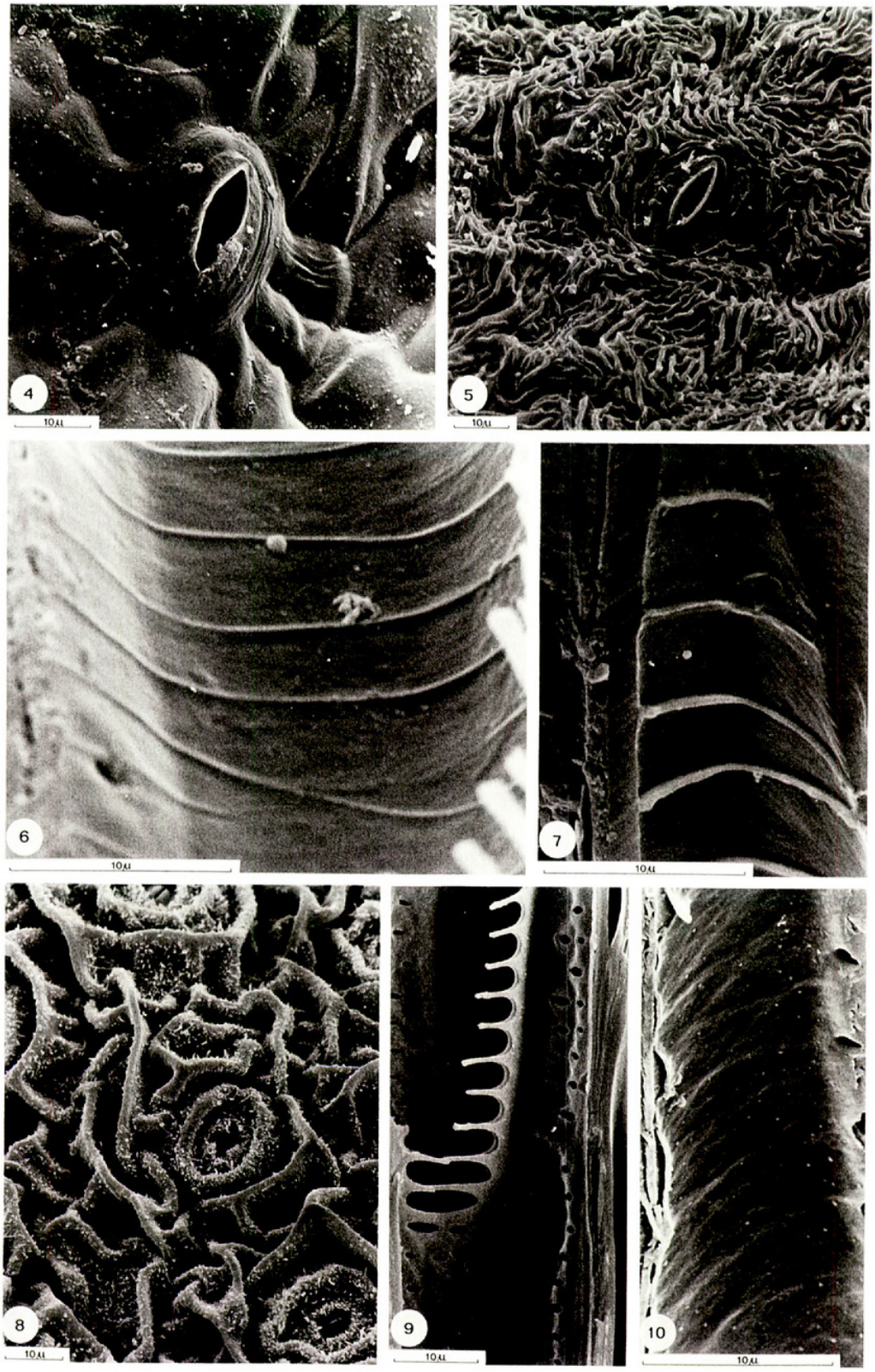


FIGURES 1–3. 1, 2, *Ilex collina:* 1, unicellular, septate hair; 2, anomocytic (left) and irregularly cyclocytic (right) stomatal complex. 3, *Nemopanthus mucronatus,* cyclocytic, anomocytic, and laterocytic stomata.

and confirmed in thin twigs of all herbarium specimens used in studying the leaf anatomy (see above).

Wood semi–ring porous. Growth rings distinct. Vessels ca. 160 per mm^2, solitary (ca. 30%) and in radial multiples of 1 to 6, angular to oval in transverse section, tangential diameter (17–)27(–40) μm, vessel member length (270–)540(–690) μm. Intervessel pits mainly opposite (some transitional or tending to alternate), oval or elongate, 5–8 μm in horizontal diameter, with slitlike apertures enclosed within pit borders. Vessel–ray and vessel–parenchyma pits similar but half bordered. Perforations scalariform in oblique end walls with (11–)14(–20) bars. Spiral thickenings well developed (FIGURE 6). Vessel contents and tyloses absent. Ground tissue composed of thin-walled fiber-tracheids (570–)710(–870) μm long with bordered pits most numerous on radial walls, the pits 5–6 μm in diameter, with slitlike apertures extending only slightly beyond pit borders. Spiral and annular thickenings prominent (FIGURE 7). Parenchyma diffuse, diffuse in small aggregates, and rarely scanty paratracheal, in strands of (3 or) 4 to 6 (or 7) cells. Rays heterogeneous II, uniseriate rays ca. 8 per mm, multiseriate rays 3 (or 4) cells wide and ca. 4 per mm, tallest rays up to 0.6 mm high. Sheath cells and crystals absent.

Judging from comparisons of juvenile and mature secondary xylem in other *Ilex* species, one would expect mature wood of *Ilex collina* to have considerably broader rays, somewhat fewer vessels, and somewhat wider and longer axial elements. Qualitative or even major quantitative differences with the above description are unlikely, however.



FIGURES 4–10. 4–7, *Ilex collina:* 4, smooth abaxial cuticle; 5, finely striate cuticle; 6, secondary xylem vessel with helical thickenings; 7, fiber-tracheid with spiral to helical thickenings. 8–10, *Nemopanthus mucronatus:* 8, strongly ridged cuticle with fine wax

NEMOPANTHUS MUCRONATUS

VEGETATIVE ANATOMY[1]

Material studied.[2] CANADA. Nova Scotia: *Gorham 45.1351* (L). U.S.A. Maine: Hancock Co., *Dudley 8031* (L). New Hampshire: *Schweinfurth & Correll 11235* (L). Pennsylvania: Pike Co., *Brumbach 7982* (L); cult. Arbor. Barnes Foundation, Merion, *Fogg s.n.* (L).

Surface view. Glabrous (except in *Schweinfurth & Correll 11235,* which has few short, unicellular hairs on adaxial side of midrib). Cuticle of adaxial surface smooth or faintly to conspicuously striate, that of abaxial surface with conspicuous, rather widely spaced ridges (FIGURE 8). Epidermal cells of adaxial surface with straight to curved (to undulating) walls, those of abaxial epidermis with curved to strongly undulating walls. Epidermal cell pattern modified over major and most minor veins (cells elongate parallel to venation). Stomata confined to abaxial epidermis, irregularly cyclocytic (FIGURE 3), sometimes tending to anomocytic or laterocytic with (3 or) 4 to 6 (or 7) subsidiary cells, guard cell pairs (18–)21–25(–30) by (17–)18–21(–26) μm. Peristomal rims present. Cuticular T-pieces absent.

Transverse section. Lamina dorsiventral, 90–190 μm thick. Cuticle usually ca. 1 (rarely up to 2) μm thick. Unspecialized epidermal cells usually flattened, the adaxial cells larger than abaxial, often with convex inner periclinal walls and periclinal wall divisions, only rarely resembling mucilage cells. Hypodermis absent. Mesophyll composed of 1 layer of palisade cells and fairly loose spongy tissue. Midrib very shallowly grooved to raised adaxially, prominently raised abaxially, supplied with single shallowly arc-shaped collateral bundle (sometimes with additional small dorsal bundle) having poorly differentiated abaxial sclerenchyma cap. Ground tissue of midrib collenchymatous. Veins mostly vertically transcurrent through parenchymatous to collenchymatous bundle-sheath extensions, only smallest veins embedded in mesophyll. Petiole with single collateral bundle having incurved margins at basal end (type 2; cf. Baas, 1975) and additional latero-dorsal wing bundles at distal end (type 4). Crystals present as druses, especially in vicinity of vascular bundles.

WOOD ANATOMY

The wood specimens described in Baas (1975), as well as twigs of the herbarium specimens cited above, were examined for presence or absence of spiral thickenings. Such tertiary thickenings were absent from the fiber-tracheids in

[1]An amplified, nearly identical version of the previously published account (Baas, 1975).
[2]In addition to that studied previously, listed in Baas (1973, 1975).

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platelets; 9, secondary xylem vessel without spiral thickenings; 10, detail of vessel tip with very faint wall thickenings.

TABLE 1. **Differences between Ilex collina and Nemopanthus mucronatus.**

CHARACTER	SPECIES	
	Ilex collina	*Nemopanthus mucronatus*
Leaf		
Abaxial cuticle	Smooth or striate	Characteristically ridged
Indumentum	Long, partly septate hairs	Usually absent; if present, hairs short, nonseptate
Stomata	Strongest tendency toward anomocytic	Strongest tendency toward cyclocytic
Average length of guard cell pairs (μm)	26–32	21–25
Midrib	Typically grooved, with poorly developed collenchyma in ground tissue	Usually raised, with well-developed collenchyma in ground tissue
Veins	Mostly embedded and not prominent in epidermal cell pattern	Mostly vertically transcurrent, prominent in epidermal cell pattern
Spiral thickenings in secondary xylem	Prominent in fiber-tracheids and vessels	Typically absent, or very faint and in vessels only

all material studied (see FIGURE 9), although *Dudley 8031* and *Schweinfurth & Correll 11235* showed extremely fine thickenings in some vessel members, especially near the tails (FIGURE 10). No other material studied had any spiral thickening in the vessels (see FIGURE 9).

COMPARISONS AND DISCUSSION

ILEX COLLINA AND NEMOPANTHUS MUCRONATUS

From the above descriptions many similarities in general characters are apparent between the two species. The differences worthy of listing are shown in TABLE 1. Most of them (indumentum, stomatal type and size, midrib and vein characters, spiral thickenings in the wood) are only of interest as diagnostic aids at the species level. However, when these differences are considered together with the more significant one of cuticular sculpturing, they constitute enough evidence to render questionable Clark's (1974) transfer of *Ilex collina* to *Nemopanthus*. Before any decision can be made, it must first be determined whether *Ilex collina* has closer anatomical affinities with other *Ilex* species than with *Nemopanthus mucronatus*.

ILEX COLLINA AND OTHER ILEX SPECIES

The leaf and xylem anatomy of *Ilex collina*, as well as its macromorphological characters and its deciduous habit, are quite similar to those of several species grouped together in subg. PRINUS by Loesener (1942). In fact, in leaf and wood

anatomy a species like *I. decidua* is much more similar than *Nemopanthus mucronatus* to *I. collina*; the only differences are in some minute details of the indumentum and in the outline of the abaxial epidermal cells. I also studied two specimens labeled *I. longipes* Chapman in the Rijksherbarium (U.S.A.: Massachusetts, cult. *Arnold Arboretum 22659* (*Boom 39981*); Tennessee, *Biltmore Herb. 4063*). Apparently these specimens are not *I. collina* because their indumentum is different (short, unicellular hairs instead of long ones; see also description in Baas, 1975). They also differ from the *I. decidua* specimen that I studied (1975); this might have been expected considering Clark's remark that *I. longipes* sensu Trelease in fact belongs to the *I. decidua* complex. Apparently identifications on herbarium labels in this group of *Ilex* species are far from reliable, and I therefore refrain from earmarking a particular *Ilex* species as being anatomically closest to *I. collina*. For the present problem, it is significant only that several species of subg. PRINUS "ser. B" PRINOIDES anatomically resemble *I. collina* more closely than *I. collina* resembles *Nemopanthus mucronatus*. *Ilex serrata* Thunb. and *I. verticillata* (L.) A. Gray of "ser. A" EUPRINUS (*sensu* Loesener) (= PRINUS) also resemble *I. collina* in leaf anatomy but differ markedly in their wood anatomy because they lack spiral thickenings in the fibers and vessels.

NEMOPANTHUS MUCRONATUS AND ILEX

As stated before, the only constant anatomical difference between *Ilex* and *Nemopanthus mucronatus* is the sculpturing of the abaxial cuticle. Within *Ilex* (especially in some tropical species) sculpturing ranges from smooth to conspicuously striate, but it is nowhere near as marked as in *Nemopanthus*. Another distinguishing character of *N. mucronatus* is its lack of conspicuous spiral thickenings, which are typical for all *Ilex* species studied from temperate regions except *I. serrata* and *I. verticillata*. It is clear that although cuticular sculpturing is of considerable diagnostic value, it alone cannot justify the separation of two obviously closely related genera. All characters must be examined to determine whether other differences are sufficient to maintain *Nemopanthus* as a separate genus.

SUGGESTIONS FOR TAXONOMIC TREATMENT

Ilex collina is anatomically much closer to several *Ilex* species belonging to "ser. B" PRINOIDES of subg. PRINUS than it is to *Nemopanthus mucronatus*. Translated into taxonomic practice, this would favor the reinstatement of *Nemopanthus collinus* in *Ilex*, in contrast to Clark's (1974) treatment of the species.

Nemopanthus mucronatus shows anatomical affinities to several *Ilex* species of "ser. A" EUPRINUS rather than to those of "ser. B" PRINOIDES *sensu* Loesener. If it is true that the floral characters of *I. collina* break down the boundaries between *Ilex* and *Nemopanthus*, this implies that *N. mucronatus* should be transferred to *Ilex* and should in the future be treated as *Ilex mucronata*. However, if floral and other macromorphological distinctions remain, the additional differences in cuticular structure can be used in support of maintaining *Nemopanthus* as a monotypic genus.

ACKNOWLEDGMENTS

I am greatly indebted to Theodore Dudley and Gene Eisenbeiss (U. S. National Arboretum, Washington, D. C.) for the generous supply of research material and for drawing my attention to this interesting problem. I also thank Paul van Veen for preparing numerous anatomical slides of troublesome herbarium material.

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