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The epidermis in *Passerina* (Thymelaeaceae): structure, function and taxonomic significance

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Keywords: anatomy, cuticle, epicuticular waxes, epidermis, *Passerina*, southern Africa, stomata, taxonomy, Thymelaeaceae

ABSTRACT

Epidermal features were studied in all 17 species of *Passerina*, a genus endemic to southern Africa. Leaves in *Passerina* are inversely ericoid, the adaxial surface concave and the abaxial surface convex. Leaves are inversely dorsiventral and epistomatic. The adaxial epidermis is villous, with unicellular, uniseriate trichomes and relatively small thin-walled cells, promoting flexibility of leaf margins owing to turgor changes. In common with many other Thymelaeaceae, abaxial epidermal cells are large and tanniniferous with mucilaginous cell walls. The cuticle is adaxially thin, but abaxially well developed, probably enabling the leaf to restrict water loss and to tolerate high light intensity and UV-B radiation. Epicuticular waxes, present in all species, comprise both soft and plate waxes. Epidermal structure proves to be taxonomically important at family, genus and species levels. Interspecific differences include arrangement of stomata and presence or absence of abaxial epidermal hair. Other diagnostic characters of the abaxial epidermal cells are arrangement, size and shape, cuticular ornamentation and presence or absence of wax platelets. Two groups of species on the basis of abaxial epidermal cell orientation are recognised. Many leaf epidermal features in *Passerina* are interpreted as structural adaptations to the Mediterranean climate of the Cape.

CONTENTS

INTRODUCTION

The genus *Passerina* L. comprises about 17 species, all endemic to southern Africa (Thoday 1924; Bond & Goldblatt 1984). Despite the now outdated revision by Thoday (1924), taxonomic boundaries in *Passerina* remain a problem, mainly owing to the apparent lack of marked morphological differences between the species. The present paper emanates from a comparative leaf-anatomical survey of the genus, undertaken as part of a monographic study of the group. This survey highlighted the importance of the epidermis as a source of taxonomic evidence.

The combined distribution of all the *Passerina* species is shown in Figure 1. Most species of *Passerina* are

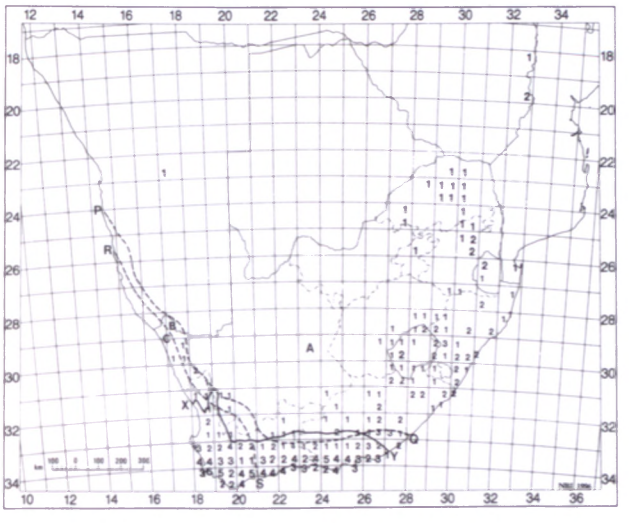

FIGURE 1—Number of species per grid in the distribution of *Passerina*. Lines PQ and RS: boundaries between summer (A), intermediate (B) and winter (C) rainfall areas. Line XY shows northern boundary of Cape Supergroup rock outcrops.

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TABLE 1.—*Passerina* specimens examined and housed at PRE

Species	Collector	Locality
burchellii Thoday	*Bredenkamp 1545*	WESTERN CAPE.—3319 (Worcester): Jonaskop, (–DC).
	*Bolus *687; Stokoe *2542*	WESTERN CAPE.—3419 (Caledon): Baviaanskloof, Genadendal, (–BA).
comosa C.H.Wright	*Andreae *1288; MacDonald *2125*	WESTERN CAPE.—3321 (Ladismith): Seweweekspoort, (–AD).
drakensbergensis Hilliard & B.L.Burtt	*Edwards 974*	KWAZULU-NATAL.—2828 (Bethlehem): Royal Natal National Park, (–DB).
	*Bredenkamp *1018, 1019, *1020*	KWAZULU-NATAL.—2829 (Harrismith): Ndedema Gorge, Cathedral Peak Forest Reserve, (–CD).
ericoides L.	*Bredenkamp *962*	WESTERN CAPE.—3418 (Simonstown): Cape Maclear, (–AD).
	*Bredenkamp *+956*	WESTERN CAPE.—3318 (Cape Town): Milnerton, (–CD).
	Taylor 4042	WESTERN CAPE.—3419 (Caledon): Pearly Beach, (–CB).
falcifolia C.H.Wright	*Bredenkamp *+917*	WESTERN CAPE.—3323 (Willowmore): Gouna, (–CC).
	*Bredenkamp *915*	WESTERN CAPE.—3324 (Steytlerville): opposite Tsitsikamma Lodge, (–CD).
	Tyson 1449	WESTERN CAPE.—3423 (Knysna): Knysna, (–AA).
filiformis L.	*Killick 238*	KWAZULU-NATAL.—2930 (Pietermaritzburg): Table Mountain, (–CB).
	*Bredenkamp *1016, *1017*	KWAZULU-NATAL.—3030 (Port Shepstone): Oribi Gorge, (–CB).
	*Bredenkamp *1012; Van Wyk & Bredenkamp 1*	KWAZULU-NATAL.—3130 (Port Edward): Umtamvuna River Bridge, (–AA).
	Bredenkamp 896	EASTERN CAPE.—3327 (Peddie): Kiwane, (–BA).
	Bredenkamp 1036	WESTERN CAPE.—3418 (Simonstown): Steenbras River Mouth, (–BB).
galpinii C.H.Wright	*Bredenkamp *946*	WESTERN CAPE.—3420 (Bredasdorp): De Hoop Nature Reserve, (–AD).
	Galpin 4491	WESTERN CAPE.—3421 (Riversdale): Melkhoutfontein, (–AD).
	*Bredenkamp *932*	WESTERN CAPE.—3421 (Riversdale): Riethuiskraal, (–AD).
	Bredenkamp 933	WESTERN CAPE.—3421 (Riversdale): Still Bay, (–AD).
	Bredenkamp 923	WESTERN CAPE.—3422 (Mossel Bay): Mossel Bay, (–AA).
glomerata Thunb.	*Bredenkamp *988, 994, 1002*	WESTERN CAPE.—3219 (Wuppertal): Cederberg Mountains, near Algeria, (–AC).
	Bredenkamp 984	WESTERN CAPE.—3219 (Wuppertal): Citrusdal, (–CA).
	Bredenkamp 977	WESTERN CAPE.—3219 (Wuppertal): Ceres, Karoo, Farm Groenfontein, (–DC).
	*Bredenkamp *973*	WESTERN CAPE.—3319 (Worcester): Tulbagh, (–AC).
montana Thoday	*Giess 13136*	NAMIBIA.—2217 (Windhoek): Auas Mountains, (–CA).
	Bredenkamp 1028	NORTHERN PROVINCE.—2427 (Thabazimbi): Marikele Nature Reserve, (–BC).
	*Bredenkamp *1024*	MPUMALANGA.—2430 (Pilgrim's Rest): World's View, (–DD).
	*Bredenkamp *1025*	MPUMALANGA.—2430 (Pilgrim's Rest): God's Window, (–DD).
	*Bredenkamp 889, *890*	FREE STATE.—2828 (Bethlehem): Golden Gate National Park, (–DA).
	*Bredenkamp *893*	FREE STATE.—2927 (Maseru): Ladybrand, (–AB).
obtusifolia Thoday	*Bredenkamp 971*	WESTERN CAPE.—3319 (Worcester): Karoo National Botanical Garden, (–CB).
	Bredenkamp 967	WESTERN CAPE.—3319 (Worcester): Jonaskop, (–CD).
	*Bredenkamp 1033, *1034*	WESTERN CAPE.—3321 (Ladismith): Seweweekspoort, (–AD).
	*Bredenkamp *929*	WESTERN CAPE.—3321 (Ladismith): Rooiberg, (–CB).
	*Bredenkamp *919*	WESTERN CAPE.—3322 (Oudtshoorn): Perdepoort, (–CD).
paleacea Wikstr.	*Bredenkamp 960*	WESTERN CAPE.—3418 (Simonstown): Kommetjie, (–AB).
	*Bredenkamp *+961*	WESTERN CAPE.—3418 (Simonstown): Cape Maclear, (–AD).
	Bredenkamp 952	WESTERN CAPE.—3418 (Simonstown): Harold Porter National Botanical Garden, (–BD).
	Bredenkamp 950	WESTERN CAPE.—3420 (Bredasdorp): De Hoop Nature Reserve, (–AD).
	*Bredenkamp *949*	WESTERN CAPE.—3420 (Bredasdorp): Waenhuiskrans, (–CA).
	Bredenkamp 940	WESTERN CAPE.—3421 (Riversdale): Puntjie, (–AC).
paludosa Thoday	*Bredenkamp *1035; Jangle *156*	WESTERN CAPE.—3418 (Simonstown): Rondevlei Nature Reserve, (–BA).
	Thoday 100	WESTERN CAPE.—3418 (Simonstown): Riet Valley, Cape Flats, (–BA).
pendula Eckl. & Zeyh.	*Fourcade 3040*	EASTERN CAPE.—3324 (Steytlerville): Suuranys, (–CB).
	*Bredenkamp *908, *909*	EASTERN CAPE.—3325 (Port Elizabeth): Groendal Nature Reserve, (–CB).
rigida Wikstr.	*Ward 7211*	KWAZULU-NATAL.—2832 (Mtubatuba): St. Lucia Park, (–AD).
	*Bredenkamp *1013*	KWAZULU-NATAL.—3130 (Port Edward): Umtamvuna River Mouth, (–AA).
	*Bredenkamp *899*	EASTERN CAPE.—3326 (Grahamstown): Kenton-on-Sea, (–DA).
	Bredenkamp 898	EASTERN CAPE.—3326 (Grahamstown): Port Alfred, (–DB).
	Bredenkamp 897	EASTERN CAPE.—3327 (Peddie): Kleinmond West, (–CA).
	Bredenkamp 911	EASTERN CAPE.—3424 (Humansdorp): Jeffreys Bay, (–BB).
rubra C.H.Wright	*Bredenkamp 914*	EASTERN CAPE.—3324 (Humansdorp): Kareedouw, (–CD).
	*Bredenkamp *905*	EASTERN CAPE.—3325 (Port Elizabeth): Colchester, (–DB).
	*Bredenkamp *900*	EASTERN CAPE.—3326 (Grahamstown): Grahamstown, (–AD).
vulgaris Thoday	*Bredenkamp *926*	WESTERN CAPE.—3321 (Ladismith): foot of Gysberg Pass, (–CC).
	Bredenkamp 907	EASTERN CAPE.—3325 (Port Elizabeth): Groendal Nature Reserve, (–CB).
	Bredenkamp 901	EASTERN CAPE.—3326 (Grahamstown): Grahamstown, (–AD).
	Bredenkamp 943	WESTERN CAPE.—3420 (Bredasdorp): Bontebok National Park, (–AB).
	*Bredenkamp *924*	WESTERN CAPE.—3422 (Mosselbaai): Kleinbrak, (–AA).
sp. nov. 1	*Bredenkamp *1044, *1046, *1047*	WESTERN CAPE.—3319 (Worcester): Waboomberg, (–AD).
sp. nov. 2	*Esterhuysen *12189, *26859*	WESTERN CAPE.—3218 (Clanwilliam): northern Cederberg Mountains, (–BB).
sp. nov. 3	*Stokoe *9302*	EASTERN CAPE.—3322 (Oudtshoorn): Swartberg Pass, Prince Albert area, (–AC).
	*Esterhuysen *28006*	EASTERN CAPE.—3324 (Steytlerville): Cockscomb, (–BD).
sp. nov. 4	*Schlechter *9302*	EASTERN CAPE.—3322 (Oudtshoorn): Montagu Pass, (–AC).
	*Esterhuysen *10734*	EASTERN CAPE.—3323 (Willowmore): Kouga Mountains, (–DA).

* Material used for the SEM study of the ad- and abaxial epidermises. +Fresh material collected for the TEM study.

endemic to the Cape Floristic Region. From here the distribution of *P. filiformis* and *P. montana* extends east and north along the eastern mountains and Great Escarpment of southern Africa. In the Cape the climate is for the most part Mediterranean or semi-Mediterranean. In the west, it rains in winter; along the south coast, winter rainfall is complemented by some summer rain which increases eastwards. The western Karoo and Namaqualand (Succulent Karoo Biome) are characterised by winter precipitation and summer drought. KwaZulu-Natal and the eastern mountains of southern Africa are predominantly summer rainfall areas. Distribution of the species of *Passerina* coincides with the geography and climate along the whole distribution area. *P. ericoides*, *P. paleacea*, *P. paludosa*, *P. galpinii* and *P. burchellii* are endemic to Western Cape. The first three species are found along beaches and salt marshes only, *P. galpinii* grows mainly on calcrete in the Agulhas Plain area (Goldblatt & Manning in press) and *P. burchellii* is found on the high mountains at Genadendal and Villiersdorp. *P. comosa* grows on mountain slopes and summits in the Kamiesberg, Great Winterhoek and Klein Swartberg Ranges. *P. glomerata* is found from Worcester to Tulbagh, in the Clanwilliam area and extends to the Witteberg south of Matjiesfontein. *P. obtusifolia* is ubiquitous in the Cape, distributed from Worcester in Western Cape to Alice in Eastern Cape and on some of the mountain ranges in and around the Little Karoo. A new species, of which the plants are often buried under snow during winter, grows at high altitudes in the Ceres Karoo. *P. vulgaris* is a pioneer with a wide distribution from Western Cape to East London in Eastern Cape. *P. falcifolia* is found on mountain ranges between George and Uitenhage and *P. pendula* is endemic to the KwaZunga Catchment Basin and the Zwartkops River area of Eastern Cape. *P. rubra* is common in the Port Elizabeth to Uitenhage area, with outliers in the Swellendam and Bredasdorp Districts. *P. drakensbergensis* is endemic to the high Drakensberg in the Bergville District of KwaZulu-Natal and *P. rigida* is distributed all along the coast, from northern KwaZulu-Natal to the Cape Peninsula. *Passerina* sp. nov. 2 is found on the northern Cederberg Mountains, *P.* sp. nov. 3 at mountain tops in the Uitenhage area and the Swartberg Pass and *P.* sp. nov. 4 on the Kouga Mountains and the Montagu Pass.

The most important studies applying the 'anatomical method' for the delimitation of the Thymelaeaceae were published by Van Tieghem (1893) and Supprian (1894). The presence of mucilaginous epidermal cells in *P. ericoides* (= *Chymococca empetroides* Meisn.) as opposed to the total lack thereof in the other species, was also mentioned by Supprian (1894). Subsequently, Gilg (1894) critically discussed the 'anatomical method' as applied by Van Tieghem (1893) and Supprian (1894) for the delimitation of the Thymelaeaceae and concluded that certain characters would not uphold criticism. He regarded former systems based on floral morphology as more suitable for a taxonomic grouping of the Thymelaeaceae.

The twentieth century yielded very little anatomical work on the Thymelaeaceae. Standard works were those of Solereder (1908) and Metcalfe & Chalk (1950, 1979). Thoday (1921) described the structure and behaviour in drought of the ericoid leaves of *P. filiformis* and *P.* cf. *falcifolia*; he also supplied some notes on their anatomy. In a discussion of inversely dorsiventral leaves, Kugler (1928) included a description of the leaves of *P. filiformis* (= *P. pectinata* Hort.). More recently, leaf anatomy of the genera *Lachnaea* L. and *Cryptadenia* Meisn. was treated by Beyers (1992) and leaf and involucral bract characters of systematic use in *Gnidia* L. were studied by Beaumont *et al.* (1994). The scanty information on leaf anatomy in Thymelaeaceae calls for further research in this field, especially in the genus *Passerina*.

Previous leaf anatomical studies identified mucilagination of the epidermal cells as being of possible taxonomic importance. Recently Bredenkamp & Van Wyk (1999) clarified the structure of the epidermal cells and origin of the mucilage, concluding that mucilagination of epidermal cells is of taxonomic importance mainly at the family level.

The wide distribution of *Passerina* in the Cape Floristic Region, along the southern and eastern coastline and along the Great Escarpment of southern Africa as far north as Zimbabwe, illustrates the adaptation of these plants to a wide range of habitats, including Mediterranean and summer rainfall regimes. Decreasing rainfall from the eastern Escarpment to the northwestern Cape is reflected by adaptive changes in the leaf structure of the group. The present paper provides a description of epidermal characters in *Passerina* as well as an assessment of their taxonomic significance. It also speculates on the possible adaptive value of the observed structural features of the leaf.

MATERIAL AND METHODS

Fresh leaf material of 17 species of *Passerina* (Table 1) was collected, fixed and stored in a 0.1 M phosphate-buffered solution at pH 7.4, containing 2.5% formaldehyde, 0.1% glutaraldehyde and 0.5% caffeine [modified Karnovsky fixative; Karnovsky (1965)]. Whenever possible, material from at least five different localities was collected, fixed and air-dried for each species and herbarium specimens were made.

Light microscope (LM) studies

The LM was used for general leaf anatomy as well as epidermal studies. Unless stated otherwise, the tenth leaf from the growing point of a twig was used in all comparative studies. To prepare transverse sections of the main vein as well as both leaf margins, a 1 mm wide segment of leaf material was cut from the centre of each leaf. Samples were dehydrated, embedded in glycol methacrylate (GMA) and sectioned according to the methods of Feder & O'Brien (1968). Sections were stained with the periodic acid/Schiff's reaction and in toluidine blue 'O', then mounted in Entellan (Art. 7961, E. Merck, Darmstadt).

The following three methods were followed in the study of the cuticles:

1. GMA transverse sections of leaves were stained for 10 minutes in 1% Sudan Black B dissolved in 70% ethanol. Sections were rinsed twice in 70% ethanol for a few seconds and mounted in glycerine jelly.

2. Cuticular mounts were obtained by maceration according to the method of Kiger (1971). Specimens were slightly over-stained in a 1% acqueous safranin solution, dehydrated in methyl cellusolve and mounted in Entellan.

3. Epidermal mounts were obtained by removing small pieces of ad- and abaxial epidermis manually and by paradermal hand sections. Epidermises were stained in 1% safranin dissolved in 50% ethanol, dehydrated in a graded ethanol series and mounted in Entellan.

Scanning electron microscope (SEM) studies

The SEM was used to study the epidermal surface features (including epicuticular waxes), as well as to verify the structure of the cuticle. Leaves from air-dried material were used for all species. Whole leaves were used as they are small and ericoid, but trichomes were manually removed adaxially to reveal the stomata. Leaves were mounted onto aluminium stubs with silver paint, exposing the ad- and abaxial surfaces separately and sputter-coated with gold. For the purpose of studying epicuticular waxes, the sputter-coating process was modified to prevent high temperatures from changing the wax surfaces. Specimens were sputter-coated for 30 seconds and left to retain their normal temperature for one minute. This was repeated five times after which the specimens were viewed with a Jeol 840 SEM.

For the verification of the authenticity of epicuticular wax droplets and small round protrusions observed in certain species of *Passerina*, leaves were washed in chloroform for one minute, before they were pasted onto aluminium stubs. The procedure described above was used for sputter-coating and viewing.

Transmission electron microscope (TEM) studies

The TEM was used for the study of the structure of mucilaginous epidermal cell walls in *Passerina*. The second, fifth and tenth leaf from the growing points of *P. ericoides*, *P. falcifolia* and *P. paleacea* were used to study the structure of the cell wall. Leaf segments of ± 1 mm^2 were fixed in a modified Karnovsky fixative (Karnovsky 1965). Fixed material was rinsed in 0.075 M phosphate buffer, pH 7.4–7.5, post-fixed for one hour in 0.25% aqueous OsO_4, washed in three changes of water, dehydrated in a graded acetone series and embedded in Quetol 651 resin (Van der Merwe & Coetzee 1992). Ultrathin sections were contrasted in 4% aqueous uranyl acetate for 10 minutes and rapidly rinsed in water three times. The sections were then contrasted with lead citrate (Reynolds 1963), rinsed in water and examined with a Phillips 301 TEM.

For the verification of wettability and possible absorption of water by laminar epidermal hairs, we follow Alvin (1987). He proposed a mechanism through which water is absorbed by the specialised abaxial epidermal trichomes of *Androstachys johnsonii* Prain. This process involves the wettability of the hairs which he investigated by spraying the glabrous adaxial surfaces of the leaves with water. Water seeped round the leaf margins to the abaxial surface, wetting approximately 50% of the abaxial surface within 5 minutes. In the present study, the glabrous abaxial surfaces of five cymbiform leaves (from dried herbarium specimens) were pasted onto a sticky surface, exposing the villous concave adaxial surface. A drop of water was placed in the adaxial groove at the base of each leaf (average leaf size 2.5 × 4.0 mm) and left overnight. This experiment was repeated using 0.5% aqueous safranin, followed after 20 minutes by a rinse with water.

Terminology

Trichome structure

We have followed the terminology of Stace (1965) and Theobald *et al.* (1979).

Cuticle

Although the interpretation proposed by Martin & Juniper (1970) for the cuticle of plants has been widely followed by many workers, Holloway (1982) reviewed the historical perspective of the plant cuticle and attempted to adopt the most workable interpretation of the cuticular membrane (CM) in practice. In response, we follow Jeffree (1986), whose uncomplicated and pragmatic interpretation distinguishes three main zones, namely the cuticle proper, the cuticular layer and the cell wall. The cuticular membrane comprises the cuticle proper plus the cuticular layer and is bonded to the outer periclinal walls of the epidermal cells by a pectin-rich layer, which is equivalent to the continuous middle lamella. A layer of epicuticular wax generally coats the cuticle proper.

Cuticular ornamentation (LM and SEM)

We follow Wilkinson (1979) in our choice of terminology to describe cuticular ornamentation.

Epicuticular wax

The recognition of soft waxes in the present study is based on the criteria proposed by Amelunxen *et al.* (1967), Wilkinson (1979) and Barthlott *et al.* (1998).

RESULTS

Macromorphology of the leaf

Leaf arrangement decussate, sometimes imbricate, closely adherent to stem or spreading at angle of 5°–20°(–60°); spreading of leaves often prominent in juvenile plants. *Lamina* inversely ericoid; adaxial surface concave, often forming a groove facing stem and lined with woolly hairs; abaxial surface convex, orientated more or less acroscopically, thus exposing a large surface area to the environment; cuticle often amber-coloured (in herbarium material) and outline of epidermal cells often macroscopically visible. *Leaf shape* cymbiform (boat-shaped), falcate or cigar-shaped; plane shape linear, oblong, lanceolate, ovate or trullate. *Leaf base* sessile or cuneate. *Leaf apex* truncate and hump-backed, obtuse, rounded, acumi-

nate or acute to almost spine-tipped. *Margins* sometimes ciliate. *Size* (1.5–)2.5–4.0(–8.0) × (0.8–)1.2–2.0(–3.0) mm.

Anatomy of the leaf

Transverse section (LM): *leaves* epistomatic. *Adaxial epidermis* concave, villous, with unicellular, uniseriate trichomes; cuticle relatively thin, 2–5 µm; epidermal cells uniserial, relatively small (10–)15–25(–35) × 10–17(–20) µm; vacuoles large with tanniniferous substances, cell walls thin; stomata present, with guard cells at same level, sunken below, or raised above adjacent epidermal cells. *Abaxial epidermis* convex, glabrous or sparsely hairy; cuticle relatively thick (10–)20–40(–70) µm; epidermal cells relatively large, periclinal diam. of cells (20–)30–60(–65) µm, anticlinal diam. (25–)30–75 (–105) µm (Table 2), tanniniferous, often with mucilaginous cell walls. *Mesophyll* inversely dorsiventral (Kugler 1928); spongy parenchyma situated adaxially and palisade parenchyma abaxially. *Main vascular bundle* collateral, surrounded by parenchymatous bundle sheath with ample amounts of tanniniferous substances; bundle sheath adaxially irregularly biseriate, abaxially strengthened by sclerenchyma. *Secondary vascular bundles* ± 6; bundle sheaths irregular, parenchymatous and tanniniferous. Figure 2A, B.

Adaxial (dorsal) epidermis

Cuticle

Transverse section (LM): cuticular membrane 2–5 µm thick, smooth, ridged along boundaries of guard cells (Figure 2G), gradually thickening close to leaf margins, equalling abaxial cuticle in thickness and sculpturing at margins.

Surface view (LM and SEM): smooth (Figure 2C), except in *Passerina* sp. nov. 1, where markings on epicuticular wax are most probably caused by snow (Figure 3D, E).

Epidermal cells

Transverse section (LM): cells uniserial, irregularly shaped, relatively small with periclinal diam. (10–)15–25(–35) µm, anticlinal diam. 10–17(–20) µm; cell walls thin, outer periclinal wall convex; vacuoles large, containing tanniniferous substances (Figure 2A, F–H). Margin formed by a few rows of conically shaped or anticlinally elongated cells.

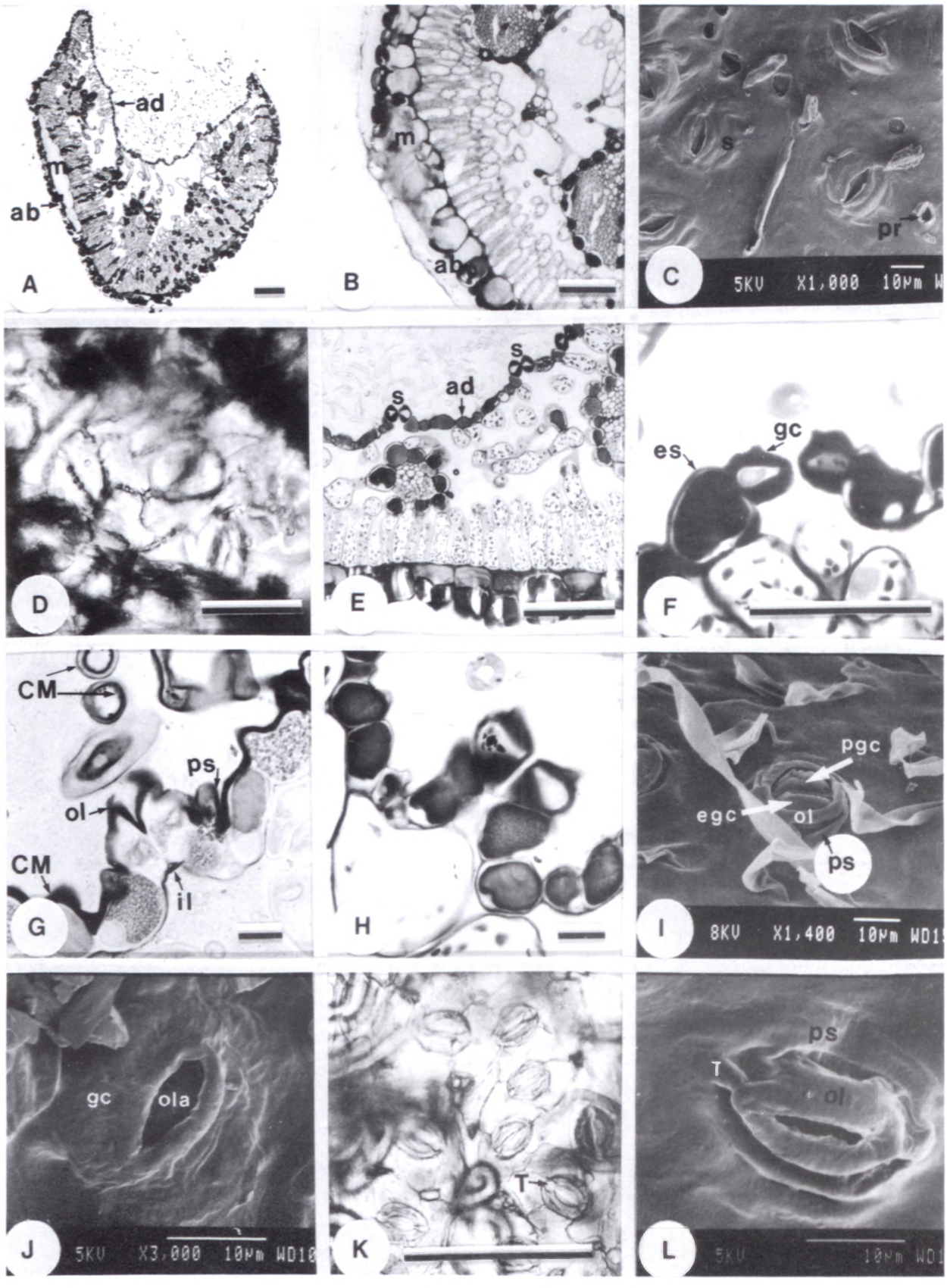
Surface view (LM and SEM): cells polygonal, 4–many-sided, walls usually undulate with loose, wide u-shaped curves of shallow amplitude (Figures 2D, K; 3C), arranged in rows and straight-walled in *Passerina* sp. nov. 1 (Figure 3D, E). Nodular walls observed in *P. falcifolia* (Figure 2D). Vacuoles with ample tanniniferous substances.

Stomatal complex

Transverse section (LM): *lamina* epistomatic; stomata dispersed randomly over adaxial surface, but absent from edges of leaf margin; raised or at same level as other epidermal cells (Figure 2E–H); dispersed in two columns in adaxial epidermal folds, with ± 3–5 rows of epidermal cells in between; raised, sunken or arranged in stomatal crypts in *Passerina* sp. nov. 1 (Figure 3F). *Guard cell* outline in all species varying between widely obtrullate, very widely obtrullate or widely depressed obtrullate, with angles slightly rounded; cell walls thick-

TABLE 2.—Dimensions of abaxial epidermal cells and cuticular membrane (CM) in *Passerina*. Measurements in µm in cross section and surface view

Name	Width of CM	Periclinal diam.	Anticlinal diam.	Length × width	Shape of cell
comosa (Figure 4B, C)	10–40	30–60	70–75	45–55 × 35–40	slightly oblong
glomerata	(20–)30–40(–50)	(20–)30–35(–40)	(25–)30–55(–60)	30–40 × 30–35	isodiametric
ericoides (Figure 4D–F)	20–32	35–60	40–60	35–50 × 40–50	± isodiametric
obtusifolia (Figure 4G–I)	20–30	40–55	55–75(–105)	(30–)40–55(–60) × (45–)50–70(–95)	transversely oblong
burchellii (Figure 4J–L)	60(–70)	45	75	(65–)85(–125) × 45–50	angles rounded, oblong
drakensbergensis (Figure 5A–C)	20	30–35	50–55	50–65 × 30–40	oblong
montana	30–35	30–45	45–65	40–60 × 35–40	isodiametric to slightly oblong
sp. nov. 1 (Figure 5D–F)	20	35	40	45–55 × 35–40	oblong
vulgaris	(10–)20–30	30–45	35–45(–70)	35 × 30–40	transversely oblong
filiformis	20–35	35(–65)	55–75(–90)	35–55 × 25–30	oblong
falcifolia	20	40	40	60–75 × 35–50	oblong
pendula	30	45–55	60–65	50–65 × 30–40	oblong
rigida (Figure 5G–I)	20–30	35–50	35–55	35–45 × 35–40	isodiametric to slightly oblong
galpinii	40–50	30–35	(40–)55–60(–70)	30–40 × 30	isodiametric to slightly oblong
rubra	20–30	30–50	45	(30–)35(–55) × 35–40	isodiametric to slightly oblong
paleacea	20(–40)	35(–65)	50–60	45–50 × 30–35	slightly oblong
paludosa (Figure 5J–L)	20	35–45	60–70	95–100 × 45–50(–95)	oblong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ened (Figure 2F, H); periclinal and anticlinal dimensions for individual guard cells 10.0–12.5(–15.0) × (10.0–) 12.5–15.0(–20.0) µm. *Cuticular membrane* (Figures 2G; 3B) covering outer periclinal walls of epidermal and guard cells, as well as poral epidermal walls of guard cells, smooth or slightly crenate when lining the pore (Figure 3B), contracted into a pair of ± continuous outer stomatal ledges above guard cells, thus forming an entire outer cavity (not divided into compartments); inner stomatal ledges and inner cavity present. *Epidermal cells* surrounding guard cells not different from other epidermal cells in size, shape or staining properties (Figure 2F). *Peristomatal cuticular rims* conspicuous on epidermal cells bordering guard cells (Figure 2G).

Surface view (LM and SEM): *stomata* anomocytic; outline elliptic to circular, dimensions (20–)26–30(–35) × (15–)24–30(–35) µm, circular in *Passerina* sp. nov. 1, dimensions 27.5 × 27.5 µm. *Epidermal cells surrounding guard cells* 3–5(6), irregularly shaped with sinuate walls and long axis parallel to guard cells, corresponding in orientation, size, shape and staining properties to other ± elongated epidermal cells (Figures 2D, K; 3C); pentagonal to heptagonal epidermal cells in *Passerina* sp. nov. 1, with walls slightly sinuate to straight, possibly nodular (Figure 3E). *Stomata* raised above or at same level as other epidermal cells in all species (Figure 2I, J, L); dispersed in two columns in adaxial epidermal folds, with ± 3–5 rows of epidermal cells in between, sunken or arranged in stomatal crypts in *Passerina* sp. nov. 1 (Figure 3D). *Guard cells* often conspicuously raised (Figure 2I, J). *Peristomatal cuticular rims* conspicuous on epidermal cells bordering guard cells (Figures 2I, L; 3A), rims also visible as 1–4 small semilunar protrusions bordering guard cells in cuticular preparations and epidermal peels (Figure 3C) (rims should not be confused with small subsidiary cells, an interpretation which could result in stomata being erroneously classified as paracytic or cyclocytic). *Outer stomatal ledges* ± continuous, present above guard cells (Figures 2I–L; 3A, C). *Stomatal poles* (where guard cells meet) retuse; T-pieces (cuticular thickenings of common walls between guard cells) well developed (Figures 2I, J, L; 3C).

Trichomes

LM and SEM: *adaxial surface* of leaf villous. *Trichomes* nonglandular, unbranched, devoid of surface features or constrictions, mostly strongly spiralled (Figure 3G, H), terete, with central lumen, covered by cuticle (Figures 2G; 3I). *Hair bases* with pore, poral rim somewhat thickened (Figures 2C; 3C, G); hair base cells mostly 4–6 and slightly radially elongated (Figure 3C, G). *Trichomes bordering leaf margin* conspicuous in *P. burchellii*, *P. paludosa* and *P. pendula* (Figure 3I, J). *Trichome foot* scarcely modified, inserted between epidermal cells (Figure 3I), usually straight, but with individual trichomes strongly spiralled (Figure 3J) in *P. pendula* (brown in dried material).

Wettability and the possible absorption of water by the laminar epidermal hairs in *Passerina* were assessed by means of laboratory tests. We found that water had formed a film over the felty layer of hair at the leaf base, whereas the adaxial surface had remained dry. A treatment with 0.5% aqueous safranin revealed that only the exposed parts of the spiralised hairs in the felty indumentum at the leaf bases stained pinkish. Although the longer hairs at the leaf margins were stained, those on the rest of the adaxial surface remained unstained.

Abaxial (ventral) epidermis

Trichomes

Abaxial surfaces of bracts and young leaves in *P. comosa*, *P.* sp. nov. 3 and *P.* sp. nov. 4 tomentose to sparsely hairy (Figure 4B), older leaves often glabrous. Description of trichomes as described under adaxial epidermis.

Epidermal cells

Transverse section (LM and TEM) (Figures 2A, B, E; 3K–L): epidermis uniserial. *Stomata* absent. *Epidermal cells* more or less oblong in outline; outer periclinal walls straight or convex, inner periclinal walls straight, convex or bulging towards mesophyll, often mucilaginous and then superficially resembling a multiple epidermis; periclinal diam. of cells (20–)30–60(–65) µm, anticlinal diam. (25–)30–75(–105) µm (Table 2). *Mucilaginous cell walls* increasing progressively from leaf margin to midrib (Figure 2B), affecting mainly inner periclinal but also anticlinal cell walls (Figure 3K, L); mucilage with a layered appearance (Figures 2E; 3K), eventually occupying about two-thirds of epidermal cell and separated from cytoplasm by innermost cellulose layer of inner periclinal cell wall (Figure 3L). *Cytoplasm* compressed by mucilage, remaining as a thin layer appressed to large, usually tanniniferous vacuole. *Anticlinal layer* of inner periclinal cell wall often plicate but gradually straightening and often disappearing as mucilagination increases, eventually breaking under pressure of accumulating

FIGURE 2.—LM photographs and SEM micrographs of epidermis of inversely ericoid leaf in *Passerina*. A, *P. falcifolia*, *Bredenkamp 917*, ad- and abaxial epidermis with mucilage accumulating abaxially; B, *P. galpinii*, *Bredenkamp 946*, mucilaginous abaxial epidermal cells; C, *P. filiformis*, *Bredenkamp 1016*, smooth adaxial cuticle, stomata and poral rims of hair bases; D, *P. falcifolia*, *Bredenkamp 915*, adaxial epidermal walls undulate, nodular; E, *P. ericoides*, *Taylor 4042*, stomata at different levels in relation to adaxial epidermis; F, *P. comosa*, *Bredenkamp 1034*, PAS staining of guard cell walls and surrounding epidermal cells, showing width; G, *P. pendula*, *Bredenkamp 909*, t/s adaxial epidermis stained with Sudan Black B, showing cuticular membrane; H, *P. pendula*, *Bredenkamp 909*, raised stomata stained with toluidine blue; I, *P. paleacea*, *Bredenkamp 961*, with peristomatal rim, raised epidermal and poral walls of guard cells, conspicuous outer stomatal ledges; J, *P. galpinii*, *Bredenkamp 946*, with distinct outer stomatal ledge aperture; K, *P. filiformis*, *Bredenkamp 1016*, and L, *P. pendula*, *Bredenkamp 909*, with T-pieces at stomatal poles. Abbreviations: ad, adaxial epidermis; ab, abaxial epidermis; CM, cuticular membrane; e, epidermal cell; egc, epidermal wall of guard cell; es, epidermal cell surrounding guard cell; gc, guard cell; ic, inner cavity; il, inner stomatal ledge; l, lumen of trichome; m, mucilage; oc, outer stomatal cavity; ol, outer stomatal ledge; ola, outer stomatal ledge aperture; p, pore; pgc, poral wall of guard cell; pr, trichome poral rim; ps, peristomatal rim; s, stomata; sc, stomatal crypt; t, trichome; T, T-piece at stomatal pole. Scale bars: A, B, D, E, F, K, 100 µm; C, G, H, I, J, L, 10 µm.

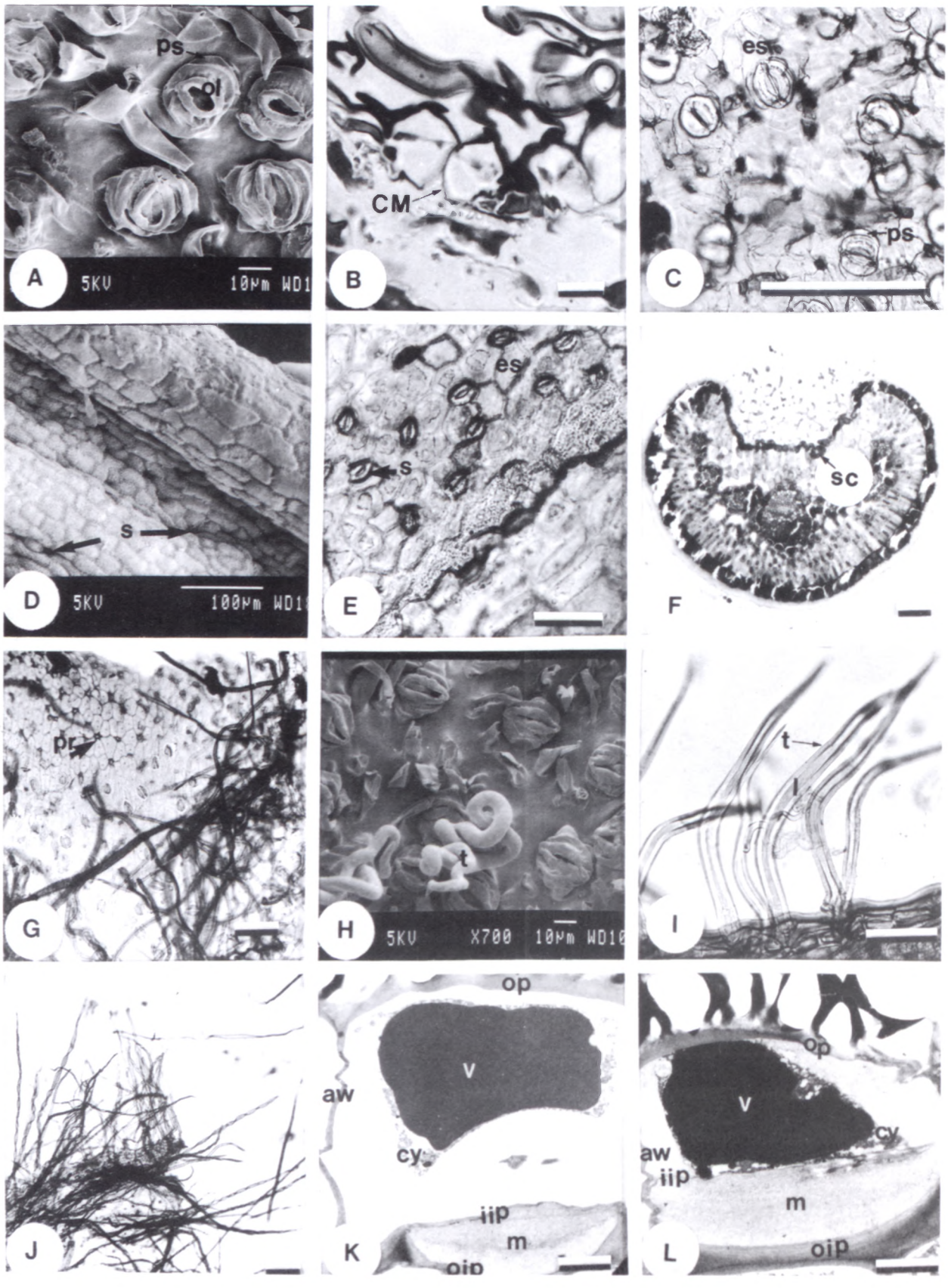
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TABLE 3.—Abaxial epidermal characters in *Passerina*

Name	Epidermal cells		Abaxial hair present	Ornamentation of CM						Epicuticular wax		
					Papillate							
	random	in rows		Smooth	Molar-like crown	One dome per cell	Several domes per cell	Several globular papillae per cell	Striate	Soft wax	Platelets	Plates
Group A												
P. glomerata (Figure 6C)	X			X								X
P. ericoides (Figures 4D–F; 6D)	X			X						X		
P. obtusifolia (Figures 4G–I; 6E)	X					X				X		
P. burchellii (Figures 4J–L; 6F)	X						X			X		X
Intermediate												
P. comosa (Figures 4B, C; 6A,B)		X	X		X						X	X
P. sp. nov. 3		X	X				X					X
P. sp. nov. 4		X	X	X				X			X	
P. drakensbergensis (Figures 5A–C; 6G)		X						X				X
P. montana		X		X				X				X
Group B												
P. sp. nov. 1 (Figure 5D–F)		X					X					X
P. sp. nov. 2		X					X				X	
P. vulgaris		X							X			X
P. filiformis (Figure 6H)		X							X			X
P. falcifolia		X							X			X
P. pendula (Figure 6I)		X							X		X	X
P. rigida (Figures 5G–I; 6J)		X							X		X	X
P. galpinii		X							X	X		
P. rubra		X							X	X		
P. paleacea (Figure 6K, L)		X							X	X		
P. paludosa (Figure 5J–L)		X							X	X		

mucilage, resulting in a mucilage-filled cavity between remains of epidermal cells and adjacent mesophyll (Figure 2A) (Bredenkamp & Van Wyk 1999).

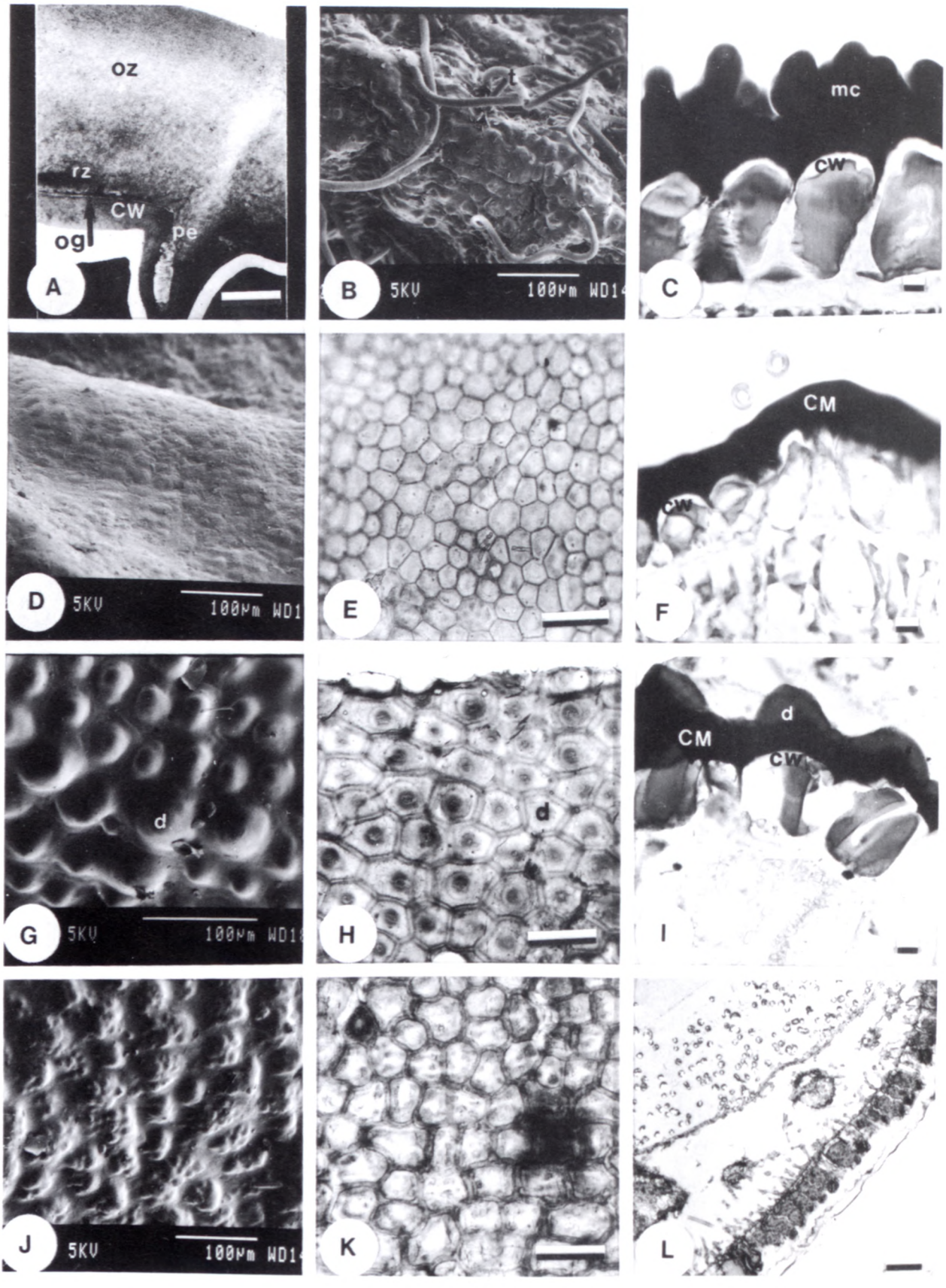
Surface view (SEM micrographs and cuticular preparations): *shape* pentagonal to heptagonal, cells mostly isodiametric or transversely oblong in *P. glomerata*, *P. ericoides* (Figure 4D, E) and *P. obtusifolia* (Figure 4G, H), but oblong in *P. burchellii* (Figure 4J, K); cells mostly slightly oblong or oblong in all other species of *Passerina* (Figure 5; Table 2). *Arrangement* random in *P. glomerata*, *P. ericoides*, *P. obtusifolia* and *P. burchellii* (Figure 4D–K), in rows in all other species of *Passerina* (Figure 5; Table 3).

Cuticle

Transverse section (LM): *epicuticular wax* absent owing to chemical treatment during fixation, embedding and staining. *Cuticular membrane* (CM) well developed, (10–)20–30(–70) µm thick (Table 2); cuticle proper delineated by narrow, lightly stained outer zone and cuticular layer by wider, darker stained zone; cuticular pegs present, formed by layer projecting into grooves between anticlinal walls of adjacent epidermal cells. *Outer periclinal cell walls* not staining with Sudan Black B (Figures 4I; 5C, I, L).

TEM: cuticle structure corresponding to the cuticular structural type 3, described by Holloway (1982). Cuticle proper and CM not distinguishable. *Cuticular membrane* (Figure 4A) comprising a wide, mainly amorphous outer zone and narrow faintly reticulate inner zone; osmiophilic granules aligned on border of clearly defined cell wall; cuticular pegs with unknown (possibly pectinaceous) substance (stained light grey) between cell wall and peg, forming part of middle lamella.

FIGURE 3.—LM photographs and SEM micrographs in *Passerina*. A–F, structure of stomatal complex. A–C, *P. rigida*, *Bredenkamp 1013*, *Ward 7211*: A, surface view of stomata showing peristomatal rims, raised guard cells and pronounced outer stomatal ledges; B, t/s adaxial epidermis stained with Sudan Black B, with crenate surface of cuticular membrane lining poral walls of guard cells; C, epidermal maceration stained with safranin, showing structure of epidermal cells surrounding guard cells, peristomatal rims. D–F, *Passerina* sp. nov. 1, *Bredenkamp1046*: D, sunken stomata in cavity of cymbiform leaf; E, epidermal maceration stained with safranin, with structure of epidermal cells and sunken stomata; F, t/s leaf, with raised stomata as well as stomatal crypts. G–J, structure of trichomes. G, *P. rubra*, *Bredenkamp 905*, with poral rims in relation to adaxial epidermal cells. H, *P. falcifolia*, *Bredenkamp 915*, with unicellular, long, spiralised, pointed trichomes; I, *P. paludosa*, *Bredenkamp 1035*, with trichome foot and conspicuous lumen; J, *P. pendula*, *Bredenkamp 909*, trichomes strongly spiralised. K, L, TEM micrographs of abaxial leaf epidermal cells of *P. falcifolia*, *Bredenkamp 917*, in cross section: K, mucilage accumulated between innermost and outermost cellulose layers of inner periclinal cell wall; L, innermost cellulose layer of inner periclinal cell wall. Abbreviations: aw, anticlinal cell wall; cy, cytoplasm; iip, innermost layer of inner periclinal cell wall; oip, outer layer of inner periclinal cell wall; m, mucilage; op, outer periclinal cell wall; v, vacuole. Scale bars: K, L 5 µm; A, B, H, 10 µm; C–F, G, I, J, 100 µm.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Cuticular ornamentation

In transections and surface view of leaves, LM and SEM studies showed that two groups of species, henceforth called Groups A, Intermediate and B (Table 3), can be distinguished on the basis of the arrangement and shape of epidermal cells as well as cuticular ornamentation.

Group A

Epidermal cells mostly isodiametric or transversely oblong in surface view; arranged randomly; cuticle mostly papillate; *outer periclinal walls* of cells convex in all species. *Cuticular membrane* (CM) smooth in *P. ericoides* and *P. glomerata* (Figures 4D–F; 6C); papillate in *P. obtusifolia*, with one dome per cell, situated ± centrally on outer periclinal wall of pentagonal or heptagonal cells (Figures 4G–I; 6E); with several domes per cell in *P. burchellii* (Figures 4J–L; 6F).

Group B

Epidermal cells mostly oblong in surface view, arranged in rows; concavities (depressions in centre region of cell) and convexities (roundish cells forming a low dome) more or less alternating (Figure 5G, J); cuticle with ridges at junction of epidermal cell walls mostly conspicuously raised, exhibiting a definite striate pattern (Figure 5D, G, J), otherwise ± plane.

Cuticular membrane pronounced at junctions of epidermal cell walls and grooved between anticlinal walls of adjacent cells (Figure 5I), more or less smooth in *P. vulgaris*, *P. filiformis*, *P. falcifolia*, *P. pendula*, *P. rigida*, and *P. galpinii*, except in *Passerina* sp. nov. 1, in which the presence of snow, at the time of collecting, seemed to have caused markings on the cuticular wax (Figure 5D, E). Small globular papillae visible between cuticular ridges in *Passerina* sp. nov. 1 (Figure 5D–E), *P. rubra*, *P. paleacea* and *P. paludosa* (Figure 5J–L).

Intermediate

Epidermal cells arranged in rows but CM less pronounced at junctions of epidermal cell walls and cuticular ridges less conspicuous, were recorded in *P. comosa* (Figures 4B; 6A, B), *P. drakensbergensis* (Figure 5A, B), *P. montana*, *P.* sp. nov. 3 and *P.* sp. nov. 4. CM smooth or with small globular papillae in *P. montana* and *P.* sp. nov. 4; domed with a 'molar'-like crown in *P. comosa* (Figure 4B, C), with several domes per cell in *P.* sp. nov. 3 and with 9 or 10 globular papillae per cell in *P. drakensbergensis* (Figures 5A–C; 6G).

Epicuticular waxes

Soft waxes present, coating entire abaxial surface: wax protruding through amorphous layer of CM in a variety of configurations: droplets conspicuous in *P. comosa*, *P. ericoides* and *P. burchellii* (Figure 6A, D, F); droplets and small round protrusions forming flat, shapeless lumps in *P. paleacea* (Figure 6L). *Crystalloids*: wax platelets and plates present or absent (Table 3); thin wax platelets, with margins entire or non-entire, flaking from wax surface in *P. comosa* and *P. rigida* (Figure 6A, J) and changing to plates as margins become distinctly edged. Upright plates separating from surrounding wax in *P. filiformis* (Figure 6H). Platelets and plates varying from sparse to abundant; platelets ± square to irregularly shaped, plates ± square to oblong and usually arranged perpendicular to cell rows.

The authenticity of epicuticular wax droplets and small round protrusions, observed in *P. ericoides*, *P. obtusifolia* and *P. paleacea* (Figure 7), was verified by washing leaves in chloroform for one minute and comparing them to unwashed specimens under SEM. Epicuticular wax droplets were clearly discernible in unwashed *P. paleacea* (Figure 7A), while small pores appeared in the cleaned, de-waxed cuticle after washing (Figure 7B–E). Similar pores were also present in *P. ericoides* (Figure 7F). No pores were present in the papillate CM of *P. obtusifolia*, but the corroded apices of the papillae clearly showed an accumulation of epicuticular waxes at these points (Figure 7G–I).

DISCUSSION

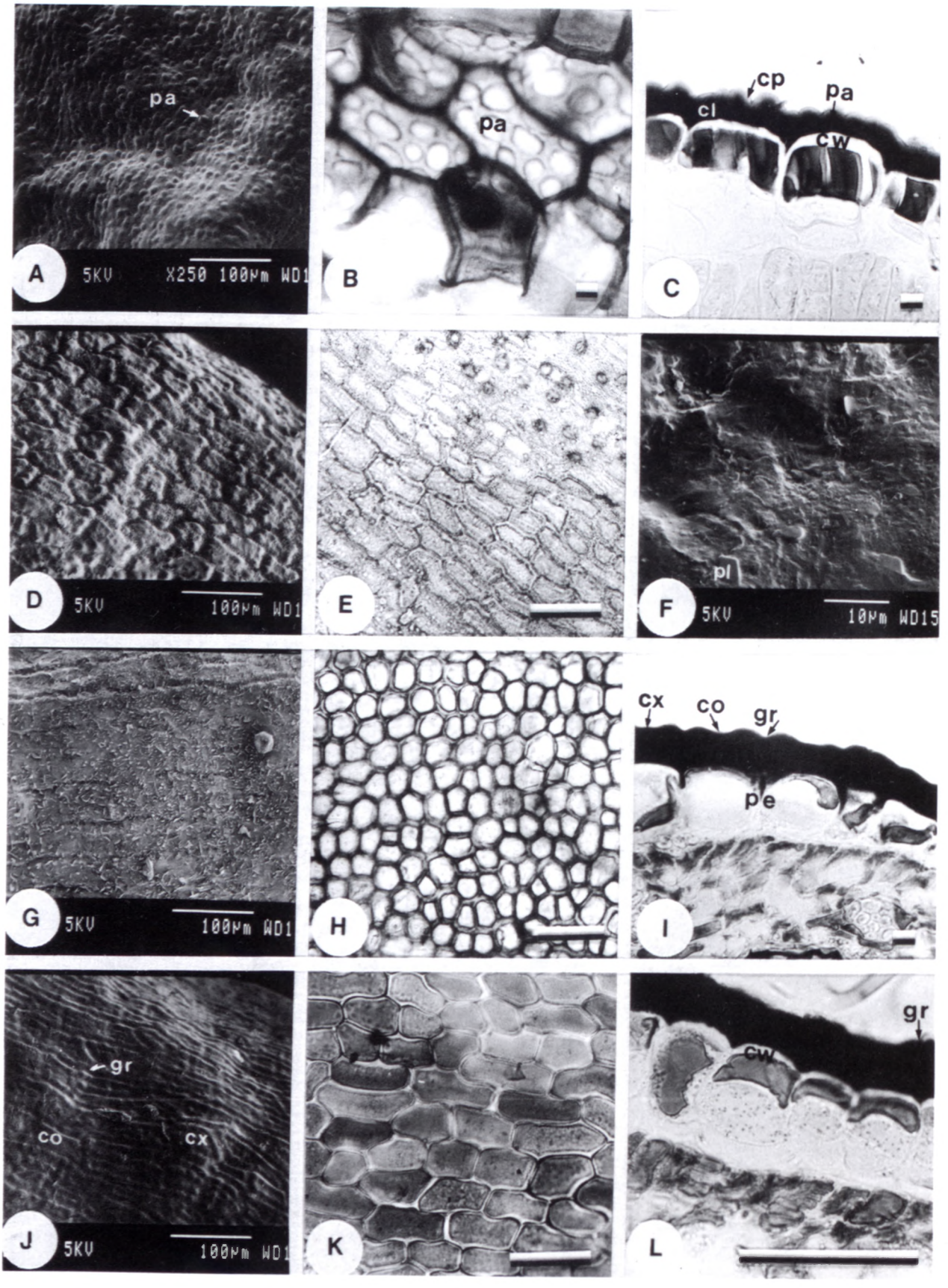
Adaxial epidermis

Plants of high mountains in the tropics usually have straight to curved anticlinal epidermal cell walls, the percentage of species with undulated walls increasing as altitude decreases (Wilkinson 1979). The straight-walled arrangement of the cells in *Passerina* sp. nov. 1 (Figure 3D, E), a high-altitude montane species, seems to comply with this pattern.

Stomatal complex

In all but one species of *Passerina* the stomata are usually raised or at the same level as other epidermal cells (Figure 2E, G, H), indicating that this character is of lim-

FIGURE 4.—A, TEM micrograph of cuticular membrane in *Passerina paleacea*, *Bredenkamp 961*, with wide, amorphous outside zone, narrow faintly reticulate inner zone, osmiophilic granules at border of cell wall and cuticular peg. B–L, LM photographs and SEM micrographs of abaxial leaf epidermis in *Passerina*. Epidermal macerations stained with safranin and t/s of epidermis stained with Sudan Black B. B, C, *P. comosa*, *MacDonald 2125*, *Andraca 1288*: B, trichomes present; C, CM domed, with 'molar-like' crown to each dome. D–F, *P. ericoides*, *Bredenkamp 956*, *962*, *Taylor 4042*: D, CM smooth, epidermal cells randomly arranged, ± isodiametric, outer periclinal cell walls convex; E, cells randomly arranged, ± isodiametric; F, convex outer periclinal walls and smooth CM. G–I, *P. obtusifolia*, *Bredenkamp 1034*: G, CM with one dome per cell; H, epidermal cells randomly arranged, transversely oblong with one dome per cell; I, convex outer periclinal cell wall and CM with one dome per cell. J–L, *P. burchellii*, *Bolus 687*, *Bredenkamp 1545*: J, CM with several domes per cell; K, randomly arranged cells, transversely oblong with rounded angles, several domes per cell; L, t/s epidermis in polarised light showing CM with several domes per cell. Abbreviations: CM, cuticular membrane; cw, outer periclinal cell wall; d, dome; mc, molar-like crown; og, osmiophilic granules; oz, amorphous outside zone; pe, cuticular peg; rz, narrow faintly reticulate inner zone; t, trichome. Scale bars: A, 5 µm; C, F, I, 10 µm; B, D, E, G, H, J–L, 100 µm.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ited taxonomic value at species level, except in *Passerina* sp. nov. 1, which has stomatal crypts or sunken stomata. Classification of the stomatal complex into stomatal types is often a problem owing to the subtle distinction of subsidiary cells (Wilkinson 1979; Van Wyk *et al.* 1982).

Patel (1978) considers subsidiary cells as morphologically and physiologically different from other epidermal cells and proposes a number of criteria to distinguish subsidiary cells in mature epidermis. Of these criteria we used the following in the distinction of subsidiary cells: size, shape, contents and position of cells. We found that the cells adjacent to the guard cells did not differ from other epidermal cells, except that they might be raised or sunken (Figures 2K; 3C). Furthermore, when stained with PAS, periclinal walls of subsidiary cells should be lightly stained compared with other epidermal cells, owing to less carbohydrates in these cell walls according to Patel (1978). In *Passerina* the periclinal walls of the cells adjacent to the guard cells stained homogenously with other cells in the stomatal complex (Figure 2F) and the anticlinal walls are not comparatively thinner than those of other epidermal cells, thus the cells adjacent to the guard cells cannot be considered subsidiary cells (Figure 2F, H). Stained with Sudan Black B, the contents of the cells surrounding the guard cells do not differ from those of other epidermal cells and no lipid bodies are present (Figure 2G).

We therefore conclude that the epidermal cells surrounding the guard cells in *Passerina* are not differentiated as subsidiary cells and we classify the stomatal apparatus in *Passerina* as anomocytic. This corresponds to the prevailing state in the Thymelaeaceae (Solereder 1908; Metcalfe & Chalk 1979). However, although we prefer to regard the epidermal cells surrounding the guard cells as similar to other epidermal cells, the presence of conspicuous peristomatal cuticular rims on the outer periclinal cell walls of epidermal cells around the guard cells may be used in support of a view that these cells are subsidiary cells. The stomatal apparatus could then be classified as staurocytic (Wilkinson 1979) or anomotetracytic (Dilcher 1974). As the number of epidermal cells surrounding the guard cells varies from 3–5(6), it would seem appropriate to classify the stomatal apparatus as anomostaurocytic (Van Wyk *et al.* 1982).

Trichomes

Passerina leaves are often cymbiform with spiralised trichomes densely arranged in the concave ventral space. This indumentum is likely to play an important role in the water relations of the plant. Water droplets precipitating from the atmosphere, or running down from leaves directly above, would accumulate in the concave leaf space. Droplets would be repelled by the hydrophobic cuticle of the trichomes and owing to cohesion forces cause a moisture layer in the upper part of the dense trichomes. One may speculate that water vapour escaping through the stomata would not be drawn outwards by capillary forces because of the water-repelling nature of the cuticle surrounding the trichomes, thus retaining a high concentration of moisture in the vicinty of the stomata. The overall high concentration of water vapour over the adaxial surface of the leaf is likely to decrease the transpiration rate. Laboratory tests to assess the wettability and the possible absorption of water by the laminar epidermal hairs in *Passerina*, suggest that the wettability of the spiralised hairs is quite low and that absorption of water by these trichomes is highly improbable. However, our suggestion of an overall high concentration of water in the adaxial cavity of the leaf, which serves to decrease the transpiration rate, is supported by these tests.

Cuticular ornamentation

Cuticular thickness may be affected by light, temperature, soil, atmospheric moisture and altitude (Wilkinson 1979). In *Passerina*, with many species adapted to the Cape Mediterranean climate, all members have a relatively thick cuticle, but it was the thickest in *P. comosa*, *P. glomerata*, *P. burchellii*, *P. galpinii* and *P. paleacea* (Table 2). The first two species grow in the northwestern parts of the Western Cape and on the mountains in and around the Little Karoo (= Karoo Mountain Centre *sensu* Weimarck 1941), areas with high light intensity, high temperature and low atmospheric moisture. *P. burchellii*, growing on high mountains at Villiersdorp and Genadendal, is exposed to high light intensity as well as high and low critical temperatures. *P. galpinii* grows on calcrete and *P. paleacea* is exposed to salt spray and wind. In *P. drakensbergensis*, *P. falcifolia*, *P. paludosa* and *P.* sp. nov. 1, the thickness of the CM is ± 20 µm. Of these species, *P. falcifolia*, from the mountains between George and Uitenhage, and *P. drakensbergensis*, from high altitudes in the Bergville District of KwaZulu-Natal, are exposed to relatively high atmospheric moisture. However, it is difficult to speculate on the functional significance of the relatively thin cuticles in *P. paludosa*, from salt marshes in the Cape Peninsula, and *P.* sp. nov. 1, a species from Waboomberg, one of the highest points in the Western Cape and often covered by snow in winter.

FIGURE 5.—Abaxial leaf epidermis and structure of CM in *Passerina*. Epidermal macerations stained with safranin and t/s of epidermis stained with Sudan Black B. A–C, *P. drakensbergensis*, *Bredenkamp 1018, 1019*: A, cells arranged in rows with 9 or 10 globular papillae per cell; B, inner surface facing upwards, cells oblong in shape with 9 or 10 papillae per cell; C, CM layered, with cuticular layer and cuticle proper, also globular papillae. D–F, *Passerina* sp. nov. 1, *Bredenkamp 1044, 1046*: D, several domes per cell, CM irregularly marked by ice crystals; E, cells arranged in rows, oblong in shape with CM irregularly marked by ice crystals; F, geometrical plates, flat or slightly raised. G–I, *P. rigida*, *Bredenkamp 1013*, *Ward 7211*: G, cells arranged in rows, plates abundant; H, cells arranged in rows, isodiametric to slightly oblong; I, CM pronounced at junctions of epidermal cell walls, grooved in midline of joining walls, concavities and convexities not conspicuous. J–L, *P. paludosa*, *Bredenkamp 1035*, *Thoday 100*: J, cells arranged in rows, CM pronounced at junctions of epidermal cell walls, grooved in midline of joining walls, concavities and convexities conspicuous; K, cells arranged in rows, cells oblong; L, CM pronounced at junctions of epidermal cell walls, grooved in midline of joining walls. Abbreviations: cl, cuticular layer; co, concavity; cp, cuticle proper; cw, outer periclinal cell wall; cx, convexity; gr, groove in CM; pa, papillae; pe, cuticular peg; pl, plates. Scale bars: A, D, E, G, H, J–L, 100 µm; B, C, F, I, 10 µm.

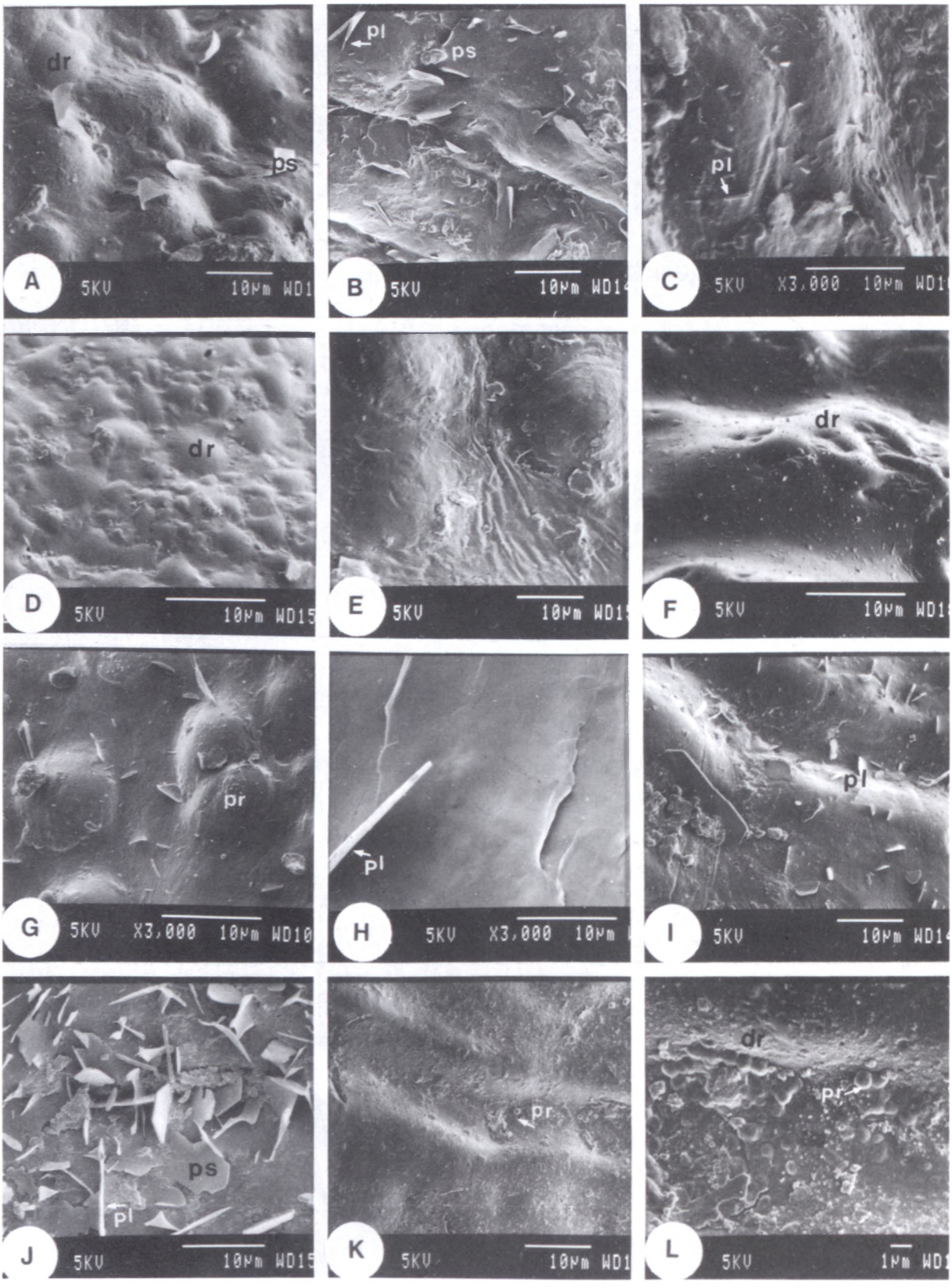

FIGURE 6.—SEM micrographs of abaxial leaf surfaces, the CM and epicuticular waxes in *Passerina*. A, B, *P. comosa*, *MacDonald 2125*: A, droplets present in epicuticular wax, platelets flaking from smooth wax coating; B, wax platelets flaking from smooth wax coating, plates present. C, *P. glomerata*, *Bredenkamp 973*, outer periclinal wall convex, plates scarce, square to oblong, raised 30°–90°; D, *P. ericoides*, *Bredenkamp 956*, droplets present in epicuticular wax; E, *P. obtusifolia*, *Bredenkamp 929*, smooth wax coating also covering domes. F, G, *P. burchellii*, *Stokoe 2542*: F, droplets at apices of domes; G, small round protrusions at apices of papillae. H, *P. filiformis*, *Bredenkamp 1016*, upright plates separate from surrounding wax, orientated at an angle to cell rows; I, *P. pendula*, *Bredenkamp 908*, plates frequent, perpendicular to cell rows, square to oblong, flat or raised; J, *P. rigida*, *Bredenkamp 1013*, platelets and plates ; K, L, *P. paleacea*, *Bredenkamp 961*, wax droplets, protrusions and flat shapeless lumps contributing towards soft wax coating or smooth layer. Abbreviations: dr, droplets in epicuticular wax; pl, plates; pr, small round protrusions of epicuticular wax; ps, platelets. Scale bars: A–K, 10 µm; L, 1 µm.

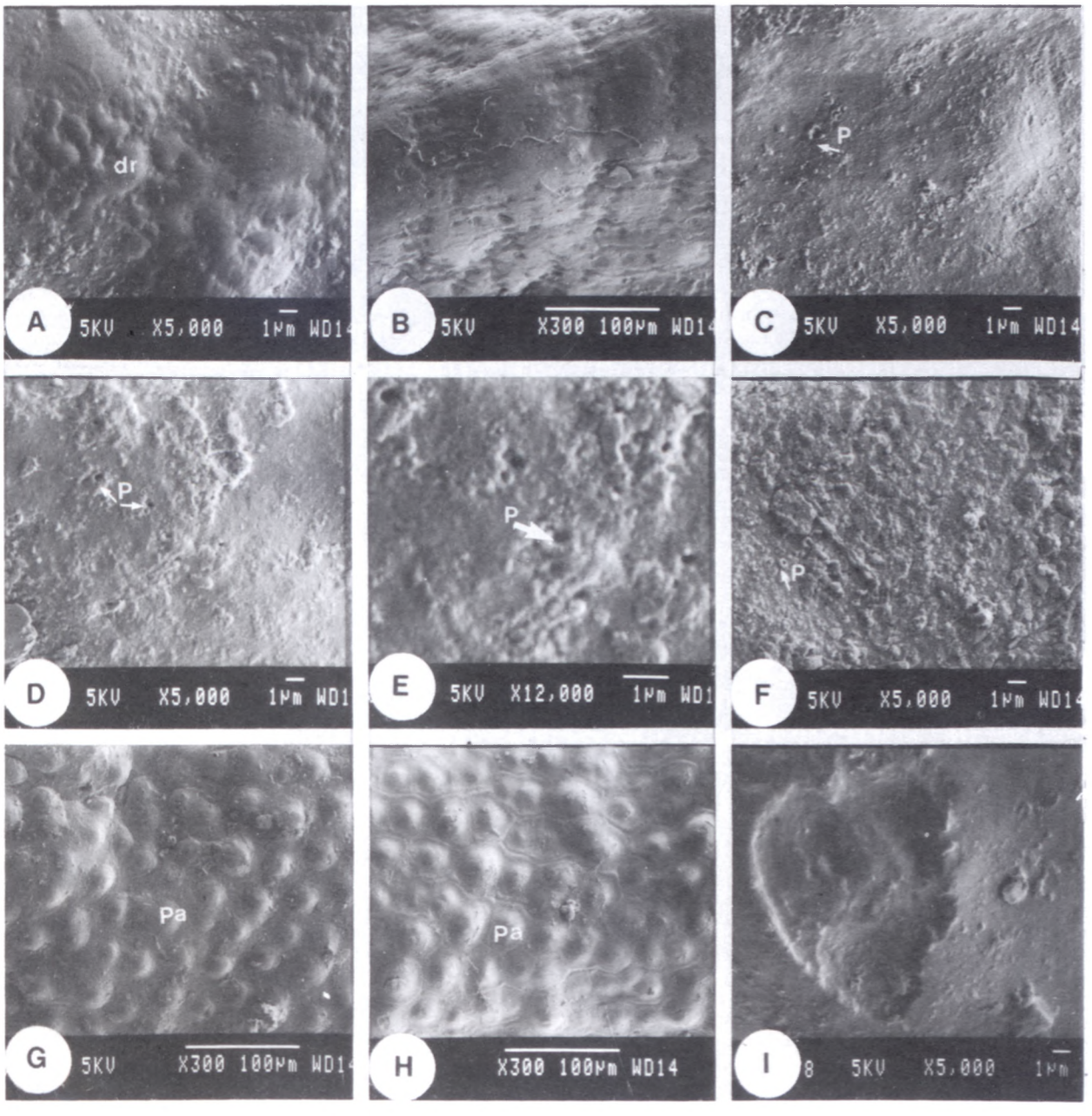

FIGURE 7.—SEM micrographs of abaxial leaf surfaces of *Passerina* washed in chloroform for one minute, compared to unwashed specimens. A–E, *P. paleacea*, *Bredenkamp 961*: A, unwashed leaf showing droplets in smooth wax coating; B, low magnification of washed leaf, showing CM devoid of epicuticular wax; C–E, higher magnifications showing pores in CM. F, *P. ericoides*, *Bredenkamp 956*, washed specimen showing pores in CM. G–I, *P. obtusifolia*, *Bredenkamp 929*: G, unwashed specimen; H, I, washed specimens showing corroded apices of papillae. Abbreviations: dr, droplets in epicuticular wax; p, pore; pa, papillae. Scale bars: A, C–F, I, 1 µm; B, G, H, 100 µm.

Haberlandt (1914), following a study of plants in tropical rain forests, considered the function of papillose epidermal cells as concentrating limited light by acting as lenses. Bredenkamp & Van Wyk (1999) speculate that, in *Passerina*, the convex outer periclinal epidermal cell wall may well focus light rays onto the mesophyll, whereas large vacuoles filled with phenols and the mucilage formed by the cellulose slimes (inner periclinal walls) protect the mesophyll from potentially dangerous UV-B radiation. According to Wilkinson (1979) the presence and prominence of papillae are diagnostically unreliable because they vary with the climate or distribution of the species; only morphologically distinct types can be used for diagnostic purposes. However, distinct epidermal cell papillae characterise *P. comosa*, *P. obtusifolia* *P. burchellii*, *P. drakensbergensis* and *P.* sp. nov. 2 (Figures 4B–C, G–L; 5A–C). The presence of these papillae could have been induced by the high light intensity of the areas in which these plants grow.

Epicuticular waxes

In their study of the epicuticular waxes in the families of the Dilleniidae and Rosidae, Ditsch & Barthlott (1997) documented the numbers of genera, species and hybrids in which different wax types occur, without identifying the various taxa. The epicuticular waxes of 12 genera, 31 species and two hybrids were studied in the Thymelaeaceae. Of these, nine genera and 26 species have wax flakes, one species has angled platelets and four genera and five species have no crystalloids. Our

observations indicate that the simple plate-type waxes found in *Passerina* correspond well to those described by Ditsch & Barthlott (1997) in the Thymelaeaceae. Of the 17 species in *Passerina*, two have wax flakes, eight have platelets or angled plates and seven are devoid of crystalloids (Figure 6, Table 3).

The mechanism of wax extrusion through the cuticle is highly controversial (Baker 1974; Jeffree *et al.* 1975; Hallam 1982). Baker (1982) discusses the extrusion of wax by means of 'pores and channels, the liquid extrusion theory, polymerization theory and the crystallization theory'. Hallam (1982) proposes that wax or wax precursors in their protein or glycoprotein 'shells' move through the cuticle and burst on the surface, liberating the wax from the 'package'; on crystallization, the protein coats stick to the surface as the wax crystals develop.

Our results indicate small pores in the cleaned, dewaxed cuticle of *P. paleacea* and *P. ericoides* (Figure 7B–F), after washing leaves in chloroform. Both Baker (1982) and Hallam (1982) are convinced that detailed investigations by many investigators have failed to confirm the presence of pores or microchannels in certain plant cuticles and that pores have not been shown to connect with the plasmalemma of the epidermal cytoplasm below. Although the presence of pores has been confirmed by our study, further research on the ultrastructure of the CM in *Passerina* could be most informative.

Freeman *et al.* (1979), working on *Citrus*, found amorphous wax layers on immature leaves and fruit, with small protrusions and isolated regions of upright platelets developing, eventually followed by cracks and irregular plates. Similarly in *Passerina*, wax droplets, protrusions and flat, shapeless lumps contribute towards a soft wax coating or a smooth layer. Species of *Passerina* with soft wax coatings, without platelets or plates, are summarised in Table 3. In *P. comosa*, *P. filiformis* and *P. rigida* (Figure 6B, H, J) platelets and plates are formed as a result of cracks developing on the outer wax surface, crystallising into irregularly shaped flakes, which gradually become square or oblong with 'entire' or 'non-entire' margins, often becoming distinctly edged. In *P. filiformis* (Figure 6H) upright plates separate from the surrounding wax, orientating themselves at an angle to the cell rows, eventually resulting in most plates being arranged more or less perpendicularly to the cell rows. Wax type, as well as the presence or absence of plates and platelets, is apparently genetically determined (Baker 1982). For example, *P. ericoides*, *P. rigida* and *P. paleacea* (Figure 6D, J, K) all grow along the sea shore, where they are subjected to wind, salt spray and high light intensity, and yet, *P. ericoides* and *P. paleacea* have coverings of soft waxes only, whereas platelets and plates are abundantly present in *P. rigida*. However, in plate waxes the number of platelets and plates, size, configuration and distribution of the surface wax structures can be considered as environmentally induced (Baker 1974, 1982).

Functions of epicuticular waxes

Possible functions of epicuticular waxes are discussed by Jeffree (1986). In *Passerina*, large areas of the abaxial epidermis are exposed to the atmosphere because the inverse-ericoid leaves are usually closely appressed to the stem. In response to the warm, dry summers of the Mediterranean climate of the Cape, it is proposed that the CM, including the abaxial epicuticular waxes, has a water-proofing function, protecting the leaves against desiccation and limiting transpiration to the adaxial epidermis only. As the leaves are decussately arranged, the water-repelling function of the waxes would cause droplets of water to run off the abaxial epidermis, into the concave, hairy adaxial surface of the lower leaf, resulting in a decreased transpiration rate owing to the higher adaxial water concentration. According to Jeffree (1986) the wettability of the plant surface is determined by its microroughness. The presence of crystalloid platelets and plates, and especially their arrangement perpendicular to cell rows, may facilitate the retention of moisture.

Systematic value

Epicuticular waxes have been proven taxonomically valuable, among others in the study of the Centrospermae (Engel & Barthlott 1988), Dilleniidae and Rosidae, including the Thymelaeaceae (Ditsch & Barthlott 1997), at sectional level in *Eucalyptus* L'Hér. (Hallam & Chambers 1970) and at species level in *Hordeum* L. (Baum *et al.* 1989). In *Passerina* the presence or absence of crystalloid platelets or plates combined with characteristics of the CM and the outer periclinal cell walls of the abaxial epidermis, makes it possible to distinguish between two groups in the genus. This distinction is species-specific for most of the 17 species examined (Table 3).

Ecological aspects of leaf epidermis

The structure and function of the epidermis should be considered in context with gross leaf morphology and arrangement. Leaf arrangement is of vital importance to the physiology of the plant. The epidermis serves as an envelope, physically protecting the mesophyll, the largest part of the abaxial epidermis forming a multifunctional barrier to the environment. The thin adaxial epidermis is concealed in the groove of the cymbiform leaf in most cases; it is almost covered by dense, long, spiralised uniserial trichomes and contains the stomata, which are often raised. This arrangement is likely to reduce the rate of transpiration, especially if moisture can be retained by the indumentum. The abaxial epidermis is probably multifunctional. The whole of the CM has a waterproofing function and the epicuticular waxes also have a water-repelling function. At the same time the CM may play a major part in focusing light rays onto the palisade parenchyma. Large tanniniferous vacuoles may play a role in the possible absorption of UV-B radiation, and mucilage formed by the cellulose slimes (inner periclinal walls) possibly protects the mesophyll from desiccation (Bredenkamp & Van Wyk 1999).

The expansion and inrolling of the leaf margins in *Passerina*, as a result of changing turgor pressure in the epidermal cells, were described by Thoday (1921). He regards the main mechanism involved as the co-ordina-

tion between the turgor pressure and the difference in size and thickness of cell walls of the ad- and abaxial epidermis, whereas the plicate anticlinal cell walls of the abaxial epidermis protect the cells against bending stress. Stomata (or at least the indumentum) are exposed when the leaf margins expand and are protected in a villous groove when the leaf margins are rolled inwards, thus regulating the rate of transpiration.

CONCLUSIONS

Leaf shape and structure in Thymelaeaceae exhibit a transformation series from mainly dorsiventral, the prevailing family feature, to isobilateral or centric in *Diarthron* Turcz., *Pimelea* Banks & Soland. and *Thymelaea* Juss. (Metcalfe & Chalk 1950). All the mentioned states are present in *Lachnaea* and *Cryptadenia* (Beyers 1992) and, as the most advanced state, inversely dorsiventral leaves in *Passerina*. A transformation series can also be illustrated by the presence of amphistomatic, hypostomatic and epistomatic leaves in the Thymelaeaceae (Metcalfe & Chalk 1950), the epistomatic state in *Passerina* considered to be the most advanced (the collateral vascular bundles of the leaves, with xylem arranged adaxially and phloem abaxially, rule out the possibility of resupination of the leaves).

The most pronounced epidermal characters of the Thymelaeaceae are anomocytic stomata (Metcalfe & Chalk 1950), unicellular trichomes and mucilagination of epidermal cells. In the present study the presence or absence, distribution of or changes in the above-mentioned structures, were used as distinguishing characters at both generic and species levels. Mucilagination of epidermal cells is often found both ad- and abaxially in the leaves of Thymelaeaceae. In *Passerina*, mucilagination takes place in the abaxial epidermis only. At species level the sunken stomata and stomatal crypts of *Passerina* sp. nov. 1 are used in the delineation of the new taxon and *P. comosa* is distinguished by the presence of unicellular trichomes on the abaxial surface of the leaves.

On the basis of abaxial cuticular characters, it has been possible to distinguish two groups of species in the genus. Group A comprises *P. burchellii* Thoday, *P. comosa* C.H.Wright, *P. ericoides* L., *P. glomerata* Thunb. and *P. obtusifolia* Thoday. Group B comprises *P. drakensbergensis* Hilliard & B.L.Burtt, *P. falcifolia* C.H.Wright, *P. filiformis* L., *P. galpinii* C.H.Wright, *P. montana* Thoday, *P. paleacea* Wikstr., *P. paludosa* Thoday, *P. pendula* Eckl. & Zeyh., *P. rigida* Wikstr., *P. rubra* C.H.Wright, *P. vulgaris* Thoday, *P.* sp. nov. 1, *P.* sp. nov. 2, *P.* sp. nov. 3 and *P.* sp. nov. 4. Certain species in each of the two groups seem to be naturally allied. Distribution patterns of *P. obtusifolia* and *P. glomerata* coincide at Worcester and transitional types can be clearly distinguished. Transitional types are similarly present in *P. filiformis* and *P. vulgaris* in the Cape Peninsula and in *P. filiformis* and *P. falcifolia* near Knysna.

Hence it can be concluded that the conspicuous differences as well as the concise characters of the ad- and abaxial epidermis, critically described and discussed in this paper, can be used as taxonomic tools at the family, genus and species levels. Furthermore, the leaf epidermis in *Passerina* is probably most valuable to the plant in terms of ecological adaptation, considering the wide distribution of the genus in southern Africa as well as the accompanying geographical and climatic variation. The gross leaf morphology and the ad- and abaxial epidermal characters have been most useful in the interpretation of the possible functioning of the leaves and are of vital importance in the survival strategies of the plant.

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