STUDIES ON THE LEAF-NODULES

Part II (*Rubiaceae*)*

XIII. Leaf-nodular plants and the localization of their leaf-nodules in *Rubiaceae*

By

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Although the root-nodules of leguminous plants have been throughly investigated and reported that almost the plants belonging to the family have the root-nodules, very little is known about the leaf-nodules which occur on the leaves of some higher plants. On *Myrsinaceae*, hitherto, have been published and studied on three species; *Ardisia crispa* (Miehe, 1914, 1919; Jongh, 1938; Yamada, 1960), *A. hortorum* (Hanada, 1952, 1954) and *A. punctata* (Yamada, 1960). Mez (1902), however, disclosed that there are some leaf-nodular plants in the subgenera *Amblyanthus* and *Amblyanthopsis* of *Ardisia* in Assam and Bhotan. On *Dioscoreaceae*, Orr (1923) and Schaede (1939) reported that *Dioscorea macroura* is a leaf-nodular species which can be found in tropical West Africa.

To the above, several hundred species of *Rubiaceae* are known to support bacterial nodules in their leaves or stipules. Although such leaf-nodules had been described earlier by Trimen (1894), Zimmermann (1902) was the first to recognize them as bacterial in the botanical gardens at Buitenzorg, Java. His observations were confined to four species of *Pavetta*; *P. angustifolia*, *P. Zimmermanniana*, *P. lanceolata* and *P. indica* (tentatively termed *Grumilea* but later identified as *Pavetta*).

Besides genus *Pavetta* similar nodules are known to occur in genera *Psychotria*, *Chomelia*, *Kraussia*, *Heterophyllaea*, *Lecanosperma*, and *Coprosma* in *Rubiaceae*. These have been discussed by various authors including Valeton (1908), Boas (1911), von Faber (1912, 1914), Georgeovitch (1916), Rao (1923, b), Korinek (1928), Bremekamp (1933), Humm (1944), and Stevenson (1953), but very little is known about the whole phenomenon.

The author stayed in Australia** in order to isolate the endophytic bacteria from rubiaceous plants and collected some bacteriophilous leaf-nodular species in the Country and New Zealand in 1967.

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* Part I, *Myrsinaceae* (I–XII)

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A. Leaf-nodular plants in *Rubiaceae*.

a. Bremekamp (1933) has made a special study of the occurrence of such nodules in genera *Pavetta* and *Psychotria* and reported that the majority of the 30 or so species which he distinguishes of *Pavetta* have leaf-nodules, and that some 50 species of the several hundred usually recognized in the genus *Psychotria* bear nodules, these particular species occurring in Africa and Madagascar. He classified the bacteriophilous species into three groups. In the first the bacterial nodules follow the midrib, in the second and third they are irregularly scattered; the species of the second group have a ring of hairs at the insertion of the stamens, whereas in those of the third group the corolla-tube is glabrous inside. The first group is confined to a district of tropical West Africa, the third to East Africa; of the species of the second and largest group a few tetramerous corollas are found in the same region as the species of the first group, whereas the far more numerous species with pentamerous corollas occur in East Africa, Rhodesia, the Congo, and Angola. His classification of the bacteriophilous species of the rubiaceous genus *Psychotria* is as follows:

Group I. Nodules along the midrib.

Ps. calva, Ps. kisantuensis, Ps. umbellata, Ps. recurva

Group II. Nodules scattered; a ring of hairs at the insertion of the stamens.

Ps. Verschuereni, Ps. setacea, Ps. kimuenzae, Ps. variopunctulata, Ps. Fleuryi, Ps. faucicola, Ps. griseola, Ps. refractiflora, Ps. stigmatophylla, Ps. Swynnertonii, Ps. Welwitschii, Ps. Kaessneri, Ps. reducta, Ps. beniensis, Ps. lubutuensis, Ps. rutshurensis, Ps. maculata, Ps. sabukuensis, Ps. brevipaniculata, Ps. monticola, Ps. gracilescens, Ps. cinerea, Ps. nairobiensis, Ps. marginatum, Ps. Guerkeana, Ps. punctata, Ps. petroxenos, Ps. pachyclados, Ps. mucronata, Ps. perbrevis, Ps. collicola, Ps. pubifolia. Ps. Kirkii, Ps. Volkensii, Ps. tarambassica, Ps. nigropunctata

Group III. Nodules scattered; corolla-tube glabrous inside.

Ps. alsophila, Ps. Eickii

b. Bremekamp (1960) also reported on seven bacteriophilous species belonging to the *Psychotria* in Madagascar. They are

Ps. expansissima, Ps. tsiandrensis, Ps. polygrammata, Ps. lokohensis, Ps. microgrammata, Ps. pachygrammata, and *Ps. himanthoplylla.*

c. The plants belonging to the genus *Coprosma* are erect, usually dioecious shrubs. Flowers solitary or clustered, axillary or terminal. Anthers exserted, on long filaments. Style divided nearly to the base into two long, filiform branches, more or less hairy, exserted. Fruit drupaceous.

Stevenson (1953) made public in New Zealand that the *Rubiaceae* is re-

presented by a number of herbaceous forms and by the woody genus *Coprosma* with neary thirty, ranging from small trees with large leaves to small-leaved spreading or creeping shrubs. On all the large-leaved species bacterial nodules are conspicuous on the stipules: *C. arborea, C. australis, C. lucida, C. tenuifolia,* and *C. cathartica* with a single apical nodule, up to 5 mm long (*C. lucida* occasionally with 2 or 3); *C. macrocarpa, C. repens,* and *C. serrulata* with several smaller nodules on each stipules. The following smaller-leaved species have one or more slender less conspicuous, stipular nodules: *C. acerosa, C. areolata, C. banksii, C. ciliata, C. colensoi, C. crassifolia, C. cuneata, C. foetidissima, C. linariifolia, C. microcarpa, C. polymorpha, C. propinqua, C. pseudocuneata, C. restusa, C. rhamnoides, C. rotundifolia, C. rugosa,* and *C. virescens.*

d. Taylor (1961) published the genus *Coprosma* has its greatest concentration of species in New Zealand, where there are about forty of the ninety known species. The remainder are scattered through the Pacific, and fall within a line including Tasmania, Eastern Australia, Borneo, Hawaii, Juan Fernandez Islands and New Zealand's subantarctic islands. The genus was first collected by Banks and Solander in New Zealand on Cook's first voyage. He classified the *Coprosma* species into two sections, i.e. large-leaved and small-leaved *Coprosma,* and mentioned 11 and 39 species including two varieties in each section. We can clearly identify 19 leaf-nodulated species and two varieties in his figures. They are

> *C. foetidissima, C. rigida, C. banksii. C. crassifolia, C. spathulata, C. propinqua var. propinqua, C. propinqua var. latiuscula, C. cunninghamii, C. rhamnoides, C. tenuicaulis, C. areolata, C. virescens, C. rubra, C. parviflora, C. rotundifolia, C. ciliata, C. microcarpa, C. antipoda, C. rugosa, C. linariifolia* and *C. colensoi.*

e. The author (1967) found out several leaf-nodular species (Figs. 1~10) in *Rubiaceae* belonging to the genera *Pavetta, Hodgkisonia, Psychotria, Rothmannia,* and *Coprosma* in Australia and New Zealand, and could identify their species names by the courtesy of the staff members of the Botanic Gardens*. Some of them were used as isolation materials of the endophytic bacteria and successfully obtained an isolant** which would probably be classified to *Bacillus.*

B. Localization of leaf-nodules on the rubiaceous plants.

The root-nodules of the leguminous plants are formed by the infection of roof-nodule bacteria (*Rhizobium*) through root hairs from soil. Consequently, the nodules are made limited on the roots. On the other hand, leaf-nodules

* The National Herbarium, N. S. W., in the Royal Botanic Gardens, Sydney

** The details will be published in the following papers.

Fig. 1. *Pavetta australiensis* Brem.

Tree
Nodule along the midrib, oblong, medium size, 2–3 mm; number, 2–4; less conspicuous
Leaf size, 5–15×1–3 cm; color, green
Collected Royal Botanic Gardens, Sydney

Fig. 2. *Pavetta lanceolata* Eckl. et Zeyh.

Shrub
Nodule scattered, round or oblong, small size, 0.5–1.0 mm; number, 20–40 or more; conspicuous on the lower surface
Leaf size, 5–10×1.0–1.5 cm; color, green or yellowish green
Collected Royal Botanic Gardens, Sydney

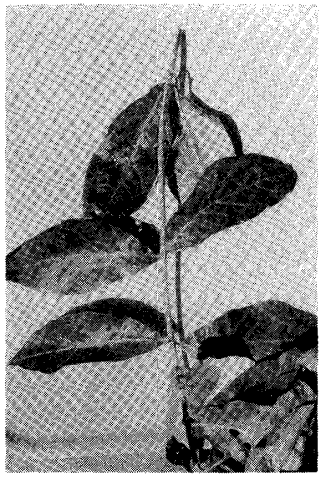
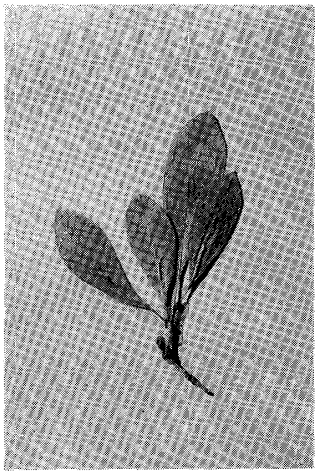


Fig. 3. *Pavetta* sp.

Shrub (P. capensis?)
Nodule along the midrib, round or oblong, medium size, 1.5–2.0 mm; number, 10–15
Leaf size, 5–7×1.5–2.0 cm with a long petiole; color, green or greenish yellow
Collected Brisbane Musium garden

Shrub
Nodule along the midrib, oblong, small size 1.0–2.0 mm; number, 10–20; not conspicuous
Leaf size, 5–8×2–3 cm; color, green
Collected Royal Botanic Gardens, Sydney

Fig. 4. *Hodgkinsonia ovatifolia* F. Muell.



Tree
Nodule along the midrib, oblong, medium size, 2–4 mm; number, 2–10; less conspicuous
Leaf size, 15–20×5–7 cm with a slender petiole; color, yellowish green
Collected Royal Botanic Gardens, Sydney

Fig. 5. *Rothmannia globosa* (Hochst.) Kery (dried leaves)

Tree
Nodule along the midrib, oblong, concaved, medium size, 2–4 mm; number, 5–10
Leaf size, 3–5×2–2.5 cm; color, dark green
Collected Royal Botanic Gardens, Sydney

Fig. 6. *Gardenia thunbergia* L. F.

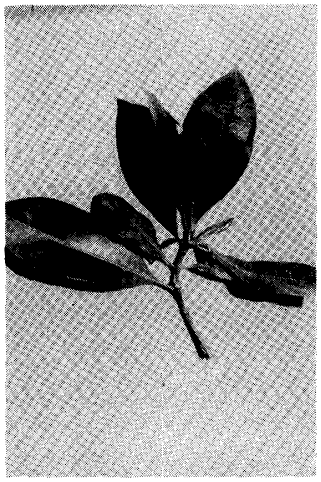


Fig. 7. *Psychotria capensis* (Eckl.) Vätke.

Tree

Nodule along the midrib, oblong, concaved, very conspicuous, big size, 3–5 mm; number, 10–15

Leaf size, 10–15×4–6 cm; color, deep green and glossy on the upper surface

Collected Royal Botanic Gardens, Sydney

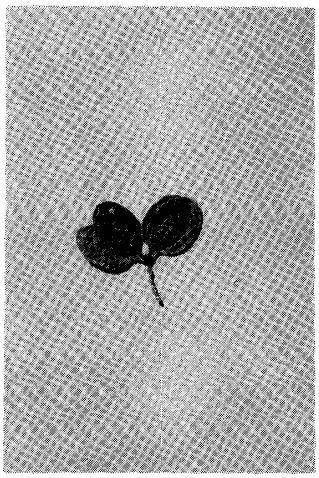


Fig. 8. *Coprosma repens* A. Rich. (dried leaves)

Shrub

Nodule along the midrib, round, small size, 1.0–1.5 mm; number, 3–8

Leaf size, 3–5×2–3 cm; color, bright green

Collected Royal Botanic Gardens, Sydney; New Zealand, Tasmania and Victoria States, Australia

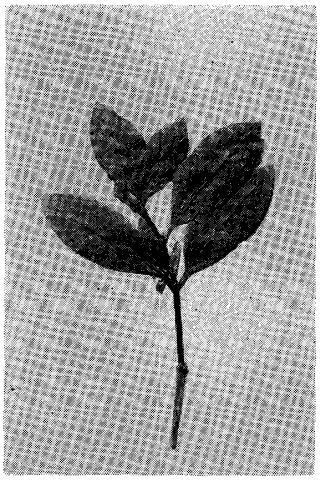


Fig. 9. *Coprosma robsta* Raoul. (dried leaves)

Tree

Nodule along the midrib, round, medium size, 1–2 mm; number, 10–15; not conspicuous

Leaf size, 5–9×2–3 cm; color, bright green, dull green on the lower surface

Collected New Zealand (North Island)

Fig. 10. *Coprosma* sp. (dried leaves)

Shrub	(C. grandfolia ?)
Nodule	along the midrib, oblong, size, 1.5–2.5 mm; number, 5–10; less conspicuous
Leaf	size, 10–15×5–8 cm; color, green
Collected	New Zealand (North Island)

are not formed by the penetration of external microorganisms into the leaves but by the bacteria, inhabit as hereditary symbiont, in the seeds. Although the locality of the leaf-nodules is confined on leaves, it is not identical. As for myrsinaceous leaf-nodular plants, *Ardisia crispa* and *A. punctata*, the bacteriophilous nodules can be found restricted to the position of water pores at the vein ends on each crenated leaf-margin (Fig. 11; A, B), and the number is 20–30 on the former and 40–60 on the latter, respectively. Of the family *Dioscoreaceae*, *Dioscorea macrura*, is known as a nodular and has only one nodule on its leafapex.

On the other hand, the leaves of some species of the tropical and subtropical genera *Pavetta*, *Psychotria*, *Coprosma* and others, members of the

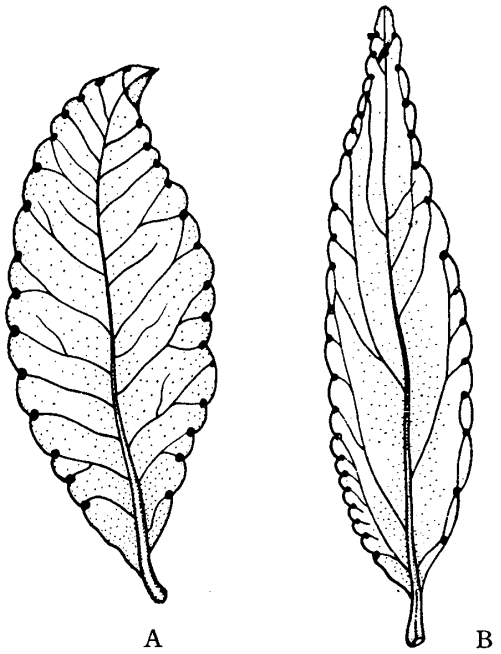


Fig. 11. *Ardisia crispa* (A) and *A. punctata* (B); nodules exist on their leaf-margins.

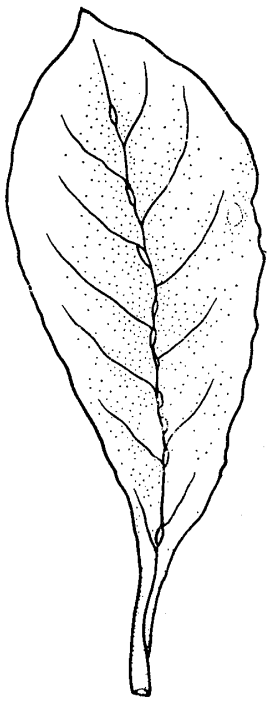


Fig. 12. *Pavetta zimmermanniana* (after Faber)

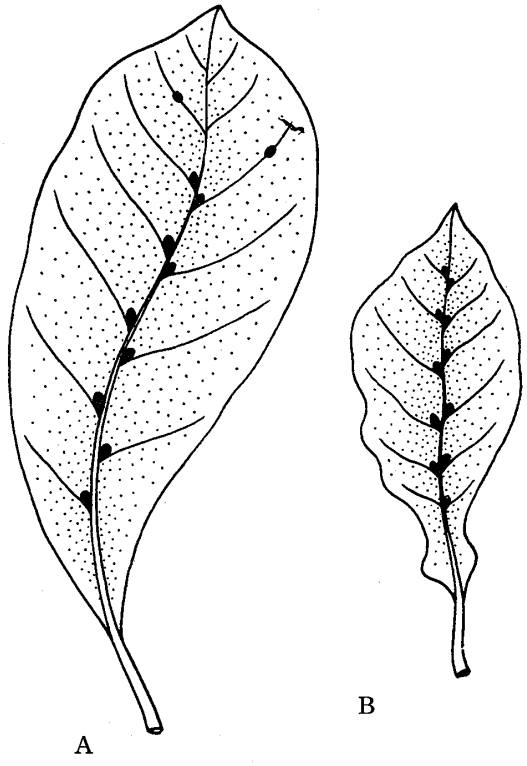


Fig. 13. *Psychotria capensis* (A) and *Gardenia thunbergia* (B)

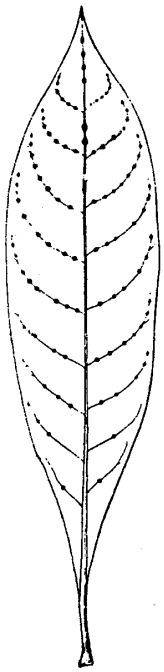


Fig. 14. *Psychotria Revesii*

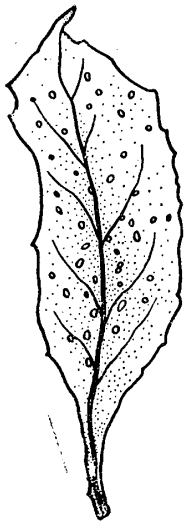


Fig. 15. *Pavetta indica* (after Faber)

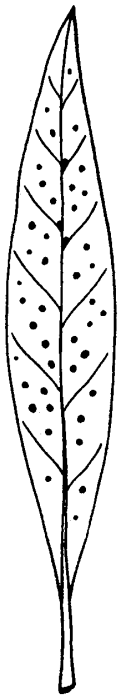


Fig. 16 *Pavetta lanceolata*

Rubiaceae, show some or numerous green, dark or black dots (leaf-nodules) visible to the naked eye, which due to the presence in the leaf tissue of cavities filled by dense growth of bacteria. By the author the locality of the rubiaceous leaf-nodules was reclassified after Bremekamp into three main groups.

Group 1. Nodules along the midrib.

i. Nodules limited along the midrib.
Pavetta Zimmermanianna (after Faber) (Fig. 12)

ii. Nodules limited to the dichotomously branched positions of midrib and primary veins.
Pavetta capensis, Hodgkinsonia ovatifolia, Rothmannia globosa, Gardenia thunbergia (Fig. 13, B), *Psychotria capensis* (Fig. 13, A), *Coprosma australis, C. cunninghamii, C. repens, C. rotundifolia*, etc.

Group 2. Nodules mainly on the primary veins.
Psychotria Reevesii (Fig. 14)

Group 3. Nodules scattered.

i. Nodules scattered all over the leaf surface (on the position of stomata).
Pavetta indica (after Faber) (Fig. 15)

ii. Nodules scattered all over the leaf surface and some are on the dichotomously branched position of midrib and primary veins.
Pavetta lanceolata (Fig. 16)

In any cases, the position of leaf-nodules is closely related to the vascular bundles, especially to the ends of sieve tubes. Namely in *Myrsinaceae* and *Dioscoreaceae* they are formed on the spot of water pore; in *Rubiaceae*, on the stomata, along the midribs or on the primary veins. From these facts it is conceivable that there are some closed metabolic relations between the endophytic bacteria and their host-plants, living together in a symbiotic relation.

SUMMARY

In this paper the author dealt with the leaf-nodular plants and the localization of their leaf-nodules in *Rubiaceae*.

The leaf-nodules have made clear to occur in the genera *Pavetta, Psychotria, Coprosma, Chomelia, Klaussia, Heterophylla, Lecanosperma, Hodgkinsonia, Rothmannia* and *Gardenia* by several investigators, including the author. All these are said bacteriophilous and have been reported that the majority of the 350 or so species of *Pavetta* have nodules and that some 50 species of the several hundred recognized in the genus *Psychotria*, and that these particular species can be found in Africa, Madagascar, India, Indonesia and the other regions.

In the genus *Coprosma* about 40 out of 90 species including large-leaved

and small-leaved are known as nodular, and these are scattered through the Pacific and fall within a line including Tasmania, Eastern Australia, Borneo, Hawaii, Juan Fernandez Islands and New Zealand. The author newly listed the leaf-nodular species in the genera *Pavetta*, *Hodgkinsonia*, *Rothmannia*, *Gardenia*, *Psychotria* and *Coprosma* in Australia and New Zealand, and could successfully isolate a kind of endophytic *Bacillus* from some of them.

Secondly reclassified the locality of the leaf-nodules as shown in the following scheme:

Group 1. Nodules along the midrib.
- i. Nodules limited along the midrib.
- ii. Nodules limited to the dichotomously branched position of midrib and primary veins.

Group 2. Nodules mainly on the primary veins.

Group 3. Nodules scattered.
- i. Nodules scattered all over the leaf-surface.
- ii. Nodules scattered all over the leaf-surface and some are on the dichotomously branched position of the midrib and primary veins.

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葉　粒　の　研　究
第 二 部 (アカネ科)
XIII. アカネ科に於ける葉粒植物と葉粒の所在

山　田　　保

本論文に於て，アカネ科の葉粒植物と葉粒の所在について発表した。

アカネ科の葉粒植物は11属に亘って存在することが明らかにされて来た。内 *Pvvetta* 属には約350種，*Psychotria* 属には約50種，存在し，之等は Africa, Madagascar, India, Indonesia 等の地方に分布していることが知られている。*Coprosma* 属では 90 種の内約 40 種が葉粒植物として認められ，之等は Tasmania, 東部 Australia, Borneo, Hawaii, Juan Fernandez 島（チリー）及び New Zealand を含む線内の島々に分布している。

著者は 1967 年，Australia と New Zealand でアカネ科の *Pavetta*, *Hodgkinsonia*, *Rothmannia*, *Gardenia*, *Psychotria* 及び *Coprosma* 属に新に葉粒植物を発見，記載し其の内の或種のものから葉粒菌の分離に成功した。

次に新たにアカネ科植物の葉粒の所在位置を Bremekamp に従い次の様に再分類した。

I．葉粒は主脈に沿って存在するもの。
　1. 葉粒は主脈に沿って限られて存在するもの。
　2. 葉粒は主脈と枝脈の分岐点に限って存在するもの。
II．葉粒は主として枝脈上に存在するもの。
III．葉粒は散在して存在するもの。
　1. 葉粒は葉面全体に散在するもの。
　2. 葉粒は葉面全体に散在するが一部は主脈と枝脈の分岐点にも存在するもの。