Cystoliths in the leaves of the genus *Pseuderanthemum* (Acanthaceae) in Thailand

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ABSTRACT

The morphology and distribution of calcium carbonate crystals or cystoliths in mature leaves of fifteen taxa, included 2 exotic species of the genus *Pseuderanthemum* (Acanthaceae) in Thailand were studied. All studied taxa contained calcium crystals. The size of cystoliths varied even in the same species. The crystals ranged from 65 µm to 300 µm in length and 17.5 µm to 100 µm in breadth. Length and breadth ratio of cystoliths varied for the genus, but the ratio of the most common type of cystoliths, i.e., elongated, tapering at one end remains constant. The cystoliths in the leaves were mainly found as eyebrow-shaped or rod-shaped crystals. Their shape was classified to seven categories. All calcium carbonate crystals or cystoliths were located in the epidermal lithocysts. In the leaves of *Pseuderanthemum*, cystoliths mostly occurred in the adaxial epidermis; however, the cystoliths in *Pseuderanthemum axillare* were located equally in both surfaces. The orientation of cystoliths can occur in two forms: (a) cystoliths arranged randomly in central area, and (b) cystoliths arranged parallel to midrib and leaf margin.

Keywords: Cystoliths, Leaves, *Pseuderanthemum*, Acanthaceae, Thailand

INTRODUCTION

In many plant species, calcium crystals (calcium oxalate and calcium carbonate) are commonly formed under ordinary conditions (Arnott and Pautard, 1970). These crystals are components in the leaves of many higher plant families. Their type and location are often used in plant taxonomic classification (Solereder, 1908; Hsieh and Huang, 1974; Genua and Hillson, 1985). The occurrence of cystoliths (consisting of calcium carbonate) in various parts of the plants, including even xylem and phloem rays but especially in the leaves, is restricted to a few families, particularly Cannabaceae, Moraceae, Urticaceae, and Acanthaceae (Mauseth, 1988; Metcalfe and Chalk, 1950; Pireyre, 1961). They are most frequently found in the epidermis, in trichomes or in special large cells, which are termed lithocysts (Fahn, 1967). In general, the pedicel and the cystolith body are composed of callose, cellulose and pectin (Fahn, 1967; Pireyre, 1961). They are irregular in shape and occasionally completely fill up the cell. The cystoliths are surrounded by a sheath and some cytoplasmic strands were found to be connected with the cystolith sheath. The presence of cystoliths in the vegetative organs is one of the useful characters in identification of the Acanthaceae. Cystoliths are usually clearly visible in dried herbarium material as short white streaks (Figure 1). According to Metcalfe and Chalk (1950), their nature and distribution are valuable for the recognition of genera and species. The presence of cystoliths in the leaves is one of the characters that has been used to delimit plant taxa, i.e., for dicotyledons (Solereder, 1908), for Hernandiaceae (Kubitzki, 1969; Ben, 1980), and for dividing the subfamily Acanthoideae into two tribes (Scotland and Vollesen, 2000). Cystoliths are restricted to the taxa with retinacula but are absent in Acantheae (Scotland and Vollesen 2000). Linsbauer (1921), Scott (1946), and Rabiger (1951) also studied particulars concerning their development. However, our knowledge on the occurrence of cystoliths in Acanthaceae is fragmentary. The present study deals with the occurrence, shape, size, distribution and orientation of cystoliths in the leaves of fifteen taxa belonging to *Pseuderanthemum*, Acanthaceae in Thailand.

MATERIALS AND METHODS

Fifteen taxa belonging to the genus *Pseuderanthemum* (Acanthaceae) in Thailand were selected for study (Table 1). They were collected during 2010-2011 from different localities, including cultivated species, throughout the country. The voucher specimens are kept at the Center for Technological and Scientific Equipment, Suranaree University of Technology. Cystoliths were investigated using a digital camera and scanning electron microscope (Figure 1). A few representative mature leaves of each taxa were used to prepare epidermis following the procedure of Dilcher (1974). In this process, a piece was cut from the median area of the leaf along the margin. This piece was placed in 5% sodium hypochlorite until bleached white. The preparation was then placed in distilled water and the upper and lower epidermises were separated using flattened needles. The preparation was then dehydrated in ethanol series, stained with safranin O in 100% ethanol, transferred to xylene and mounted in Permount. Photographs of the epidermis were taken with a compound light microscope (Olympus CX40). Thirty cystoliths per species (10 cystoliths per leaf) were observed as to shape, size, distribution and orientation. The systematic value of cystoliths in the genus *Pseuderanthemum* was considered.

Table 1. The genus *Pseuderanthemum* (Acanthaceae) in Thailand.

Taxa	Collection number	Locality
Pseuderanthemum axillare J.B. Imlay	T.Choopan 2011-326	Pha Taem, Ubon Ratchathani
P. bracteatum J.B. Imlay	T.Choopan 2010-079	Yod Dom, Ubon Ratchathani
P. carruthersii (Seem.) Guill. (Form A)	T.Choopan 2011-322	Cultivated, Maha Sarakham
P. carruthersii (Seem.) Guill. (Form B)	T.Choopan 2011-323	Cultivated, Nakhon Ratchasima
P. crenulatum (Wall. ex Lindl.) Radlk.	T.Choopan 2011-306	Khao Nan, Nakhon Si Thammarat
P. graciliflorum Ridl.	T.Choopan 2010-016	Khao Soi Dao, Chanthaburi
P. latifolium (Vahl) B. Hansen	T.Choopan 2011-210	Umphang, Tak
P. laxiflorum (A. Gray) F.T. Hubb. ex L.H. Bailey	T.Choopan 2011-324	Cultivated, Nakhon Ratchasima
P. longistylum J.B. Imlay	T.Choopan 2011-048	Tat Ton, Chaiyaphum
P. palatiferum (Nees) Radlk. ex Lindau	T.Choopan 2011-328	cultivated, Maha Sarakham
P. parishii (T. Anders.) Lindau	T.Choopan 2010-111	Phu Kradueng, Loei
P. reticulatum Radlk. (Form A)	T.Choopan 2011-320	cultivated, Saraburi
P. reticulatum Radlk. (Form B)	T.Choopan 2011-321	cultivated, Maha Sarakham
P. sp.1	T.Choopan 2010-036	Khao Chamao,Rayong
P. sp.2	T.Choopan 2011-316	Thung Raya-Nasak, Ranong

RESULTS

Cystoliths were found in all taxa of *Pseuderanthemum*. The cystoliths in the leaves were mainly found as eyebrow-shaped or rod-shaped crystals. All calcium carbonate crystals or cystoliths were located in the epidermal lithocysts. They never completely fill up the cell lumen. The surface is always more or less granular, but without the sharp point characteristic for crystals. Pedicels were rarely observed in these materials, which were considered pedicels absent. Their shapes varied from round to long cylindrical and could be divided into seven types of shape: (a) elongated, tapering at one end (Figure 3A, I), (b) elongated, tapering at both ends, (c) elongated blunt at both ends (Figure 3A-I), (d) short, blunt at both ends (Figure 3F), (e) rounded (Figure 3D, F, G), (f) irregular (Figure 3A), and (g) double (Figure 3H). The most common shape for all of the materials was elongated cystoliths tapering at one end and then elongated cystoliths blunt at both ends. Both types occurred in all the investigated species. Cystoliths are occasionally elongated, tapering at both ends, in *Pseuderanthemum axillare*, *P. graciliflorum*, *P. longistylum*, *P.* sp.1, and *P. reticulatum* (Form B: ovate leaves). Rounded cystoliths were found in all taxa except *P. latifolium*. Irregular cystoliths were rarely found in *P. axillare*, *P. carruthersii* (Form A: elliptic leaves), *P. graciliflorum*, *P. laxiflorum*, *P. palatiferum*, and *P. reticulatum* (Form B). Cystoliths are mostly simple or solitary crystals, but double cystoliths rarely occurred in *P. axillare*, *P. bracteatum*, *P. carruthersii* (Form A), *P. graciliflorum*, *P. palatiferum*, *P. parishii*, and *P. reticulatum* (Form B). As a result, *P. reticulatum* (Form B) was characterized by the greatest number of types of cystoliths, while *P. latifolium* showed the lowest number of cystoliths types. Moreover, the outlines of cystoliths were round, oval, or oblong in cross sections of selected fresh materials from additional investigation.

The size of the cystoliths varied in the genus *Pseuderanthemum* and even within the same species. The length and breadth ranged from 65 µm to 300 µm and 17.5 µm to 100 µm, respectively. The minimum length was noticed in *Pseuderanthemum bracteatum* and maximum in *P. latifolium*, while *P. bracteatum* and *P. palatiferum* exhibited minimum and maximum breadth, respectively.

Length and breadth ratio varied for this particular genus, with the types of cystoliths for the genus demonstrating the difference. This ratio varied from 1.9 to 9.1. The length and breadth ratio of cystoliths was highest in *Pseuderanthemum* sp.2, *P. parishii*, and *P.* sp.1, respectively. As a result, the highest ratio of *P.* sp.2 showed that their cystoliths were very long cylindrical or linear, while the lowest ratio of *P. palatiferum* indicated that their cystoliths were oblong or oval (Table 2 and Figure 3).

Table 2. Size, length and breadth ratio, and type of cystoliths in the leaves of *Pseuderanthemum* in Thailand.

Taxa	Size (μm)		Length/ Breadth	Type
	Length	Breadth		
Pseuderanthemum axillare J.B. Imlay	112.5–212.5	25.0–37.5	4.5–5.9	a-b-c-d-f-g
P. bracteatum J.B. Imlay	65.0–147.5	17.5–32.5	3.1–5.2	a-c-d-g
P. carruthersii (Seem.) Guill. (Form A)	82.5–182.5	50.0–67.5	1.7–2.8	a-c-d-e-f-g
P. carruthersii (Seem.) Guill. (Form B)	75.0–125.0	30.0–50.0	1.5–3.9	a-c-d
P. crenulatum (Wall. ex Lindl.) Radlk.	137.5–175.0	25.0–30.0	5.3–6.4	a-c-d
P. graciliflorum Ridl.	100.0–200.0	37.5–40.0	2.7–5.4	a-b-c-d-f-g
P. latifolium (Vahl) B. Hansen	225.0–300.0	40.0–62.5	3.6–7.1	a-c
P. laxiflorum (A. Gray) F.T. Hubb. ex L.H. Bailey	100.0–175.0	27.5–37.5	3.5–5.4	a-c-d-e-f
P. longistylum J.B. Imlay	107.5–175.0	27.5–37.5	3.4–6.1	a-b-c-d
P. palatiferum (Nees) Radlk. ex Lindau	95.0–180.0	50.0–100.0	1.8–2.3	a-c-d-e-f-g
P. parishii (T. Anders.) Lindau	150.0–220.0	25.0–37.5	4.7–8.7	a-c-d-g
P. reticulatum Radlk. (Form A)	127.5–175.0	30.0–57.5	2.7–4.7	a-c-d
P. reticulatum Radlk. (Form B)	100.0–132.5	25.0–40.0	2.9–4.2	a-b-c-d-e-f-g
P. sp.1	175.0–275.0	30.0–42.5	4.4–8.8	a-b-c-d
P. sp.2	167.5–250.0	22.5–25.0	7.5–11.2	a-c-d

a: elongated, tapering at one end, b: elongated, tapering at both ends, c: elongated blunt at both ends, d: short, blunt at both ends, e: rounded, f: irregular, g: double.

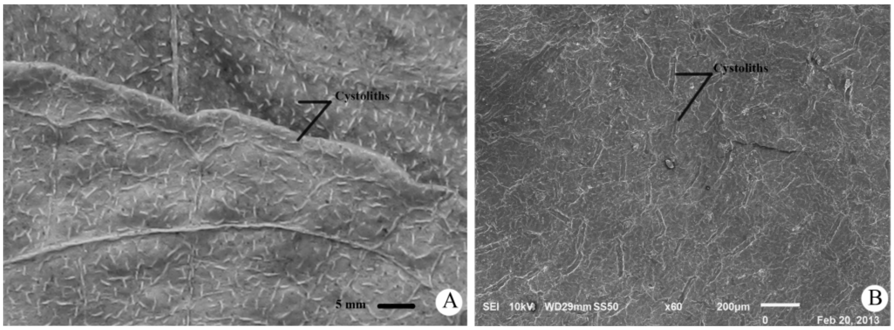

Figure 1. The nature of cystoliths in the leaves of Thai *Pseuderanthemum*.
A: with digital camera, B: with SEM.

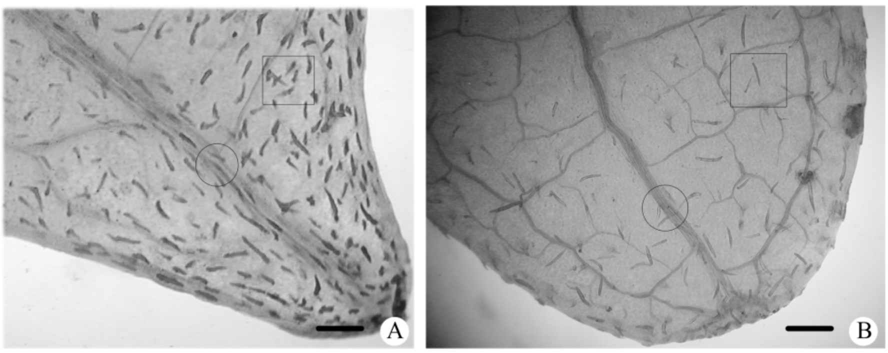

Figure 2. The orientation of cystoliths in the leaves of Thai *Pseuderanthemum*. (scale bars = 300 μm)
A: parallel to midrib and leaf margin with random orientation in central area
B: parallel to leaf margin with random orientation in central area and midrib

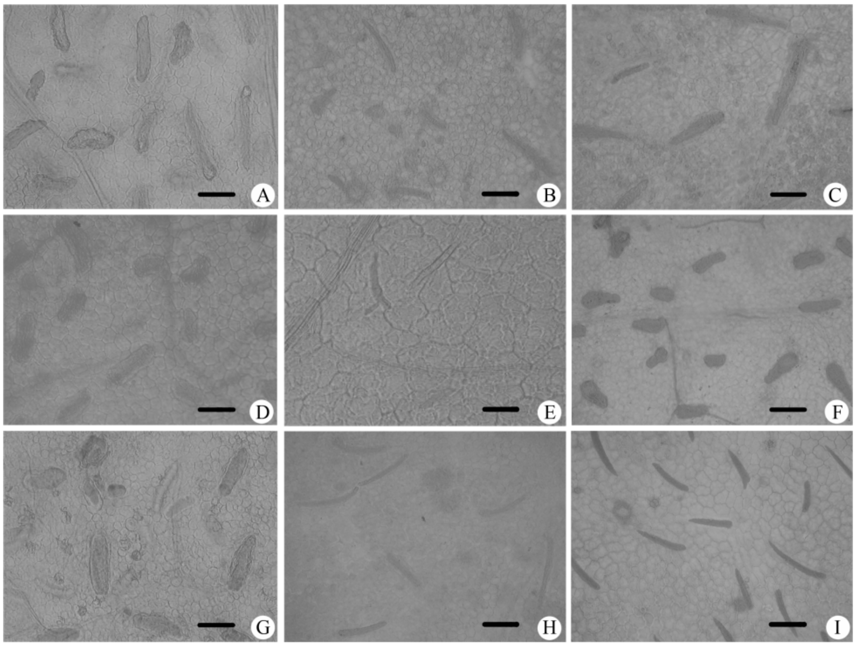

Figure 3. The type of cystoliths in the leaves of Thai *Pseuderanthemum*. (scale bars = 100 µm)
A: *Pseuderanthemum carruthersii* (Form A), B: *P. crenulatum*, C: *P. latifolium*,
D: *P. laxiflorum*, E: *P. longistylum*, F: *P. palatiferum*, G: *P. reticulatum* (Form B),
H: *P.* sp.1, I: *P.* sp.2.

The distribution of cystoliths in the leaves of *Pseuderanthemum* mostly occurred in the adaxial epidermis with variation in the density for this genus; however, the cystoliths in *Pseuderanthemum axillare* were located equally in both surfaces.

The orientation of cystoliths in *Pseuderanthemum* occurred in two patterns: (a) cystoliths arranged randomly in the central area of leaves, and (b) cystoliths arranged parallel to midrib and leaf margin in 2–3 marginal cell lines along the leaf margin. Almost all *Pseuderanthemum* spp. showed their cystoliths arranged parallel to midrib and leaf margin and randomly in the central area of leaves, except *Pseuderanthemum axillare*, with cystoliths arranged randomly even to the midrib (Figure 2).

DISCUSSION AND CONCLUSIONS

Cystoliths were found in all examined taxa of the genus *Pseuderanthemum* in Thailand. Their shape and size were variable, both within a sample and between different samples. The nature and distribution of cystoliths is a valuable character in the Acanthaceae (Metcalfe and Chalk, 1950). During the present investigation the following types of cystoliths were observed: (a) elongated, tapering at one end, (b) elongated, tapering at both ends, (c) elongated, blunt at both ends, (d) short, blunt at both ends, (e) rounded, (f) irregular, and (g) double. The most common shape for all of the material was elongated cystolith tapering at one end but Karlstrom (1980) controversially reported the elongated cystoliths blunt at both ends dominated in *Pseuderanthemum*. The short and broad cystoliths blunt at both ends were used to distinguish *Pseuderanthemum bicolor* from *Eranthemum roseum* with elongated narrow cystoliths, tapering at one end. However, more categories of cystoliths have been reported in *Pseuderanthemum* (Ahmad, 1974; Karlstrom, 1980; Inamdar *et al.*, 1990) which were also seen in this study of *Pseuderanthemum* spp. Moreover, *P. reticulatum* (Form B) revealed the presence of various types of cystolith in the same sample, while *P. latifolium* showed various sizes, although variation in shapes of cystolith were rarely observed. So, the various types may be used as a taxonomic tool.

All calcium carbonate cystoliths of *Pseuderanthemum* were located in the epidermal lithocysts. Lin, Yen, and Kuo-Huang (2004) explained that the calcium carbonate in the cystolith was concentrically accumulated. There were cytoplasmic strands connected with the cystolith sheath. In this study, they never completely fill the cell lumen, although they nearly fill it in some cases. The surface is always more or less granular, but without the sharp point characteristic for crystals like druses. Only occasionally the cystoliths show faint radiating lines. The presence of a pedicel is of essential importance for the development of cystoliths (Fahn, 1967). However, pedicels were rarely observed in these materials, which were considered as pedicels absent.

The size of the cystoliths varied even in the same species. The minimum length and breadth was noticed in *Pseuderanthemum bracteatum*. Short cystoliths were also found in *P. carruthersii* (Form B) and *P. reticulatum* (Form B), but cystolith shapes were different. *P. latifolium* exhibited long cystoliths distinct from those of other species in the genus and showed the lowest number of cystolith types. The maximum breadth was observed in *P. palatiferum*, which was distinguished from the rest. *P. bracteatum*, *P. crenulatum*, and *P.* sp.2 showed narrow cystoliths but differed in length. *Pseuderanthemum* sp.1 is quite similar morphologically to *P. longistylum*, which was also collected from the same area of south-eastern Thailand. It is separate from *P. longistylum* by having longer cystoliths and is different in the length and breadth ratio. This difference along with pubescence of the leaves and difference in flower color permitted the 2 species to be distinguished from each other. Furthermore, the lower length and breadth ratio of cystoliths conformed with the very crispy leathery leaves and the habit of shrubs, i.e., *P. carruthersii* and *P. reticulatum*.

The length and breadth ratio of cystoliths has been employed to determine the nature of cystoliths (Inamdar *et al.*, 1990; Karlstrom, 1980). As a result, the highest ratio of *P.* sp.2 showed that their cystoliths were very long cylindrical or linear. The lowest ratio of *P. palatiferum* indicated oval or oblong cystoliths. These features can be used to distinguish both species from the rest. Moreover, the size of cystoliths in *Pseuderanthemum* spp. in Thailand showed a higher range (65–300 µm in length and 17.5–100 µm in breadth) than in *P. bicolor*, *P. alatum*, *P. cinnabaricum*, *P. indicum*, and *P. tunicatum*, which range from 68–95 µm in length and 18–36 µm in breadth. These species did not differ significantly in the ratio from the Thai species, except *P. tuberculatum*, which is 197 µm, 18 µm, and 11.4 in length, breadth, and ratio, respectively (Karlstrom, 1980).

The distribution of cystoliths in the leaves of *Pseuderanthemum* mostly occurred in the adaxial epidermis where the light intensity is higher (Kuo-Huang and

Yen, 1996). It has been reported that the origin and maturation of the lithocysts are genetically controlled, but the accumulation of the calcium carbonate in the cells is mostly dependent on the calcium ion supply (Zindler-Frank, 1980; Wu, 1995). The physiological role of cystoliths is unclear. However, it has been suggested that cystoliths may serve as calcium and carbon dioxide reservoirs for photosynthesis (Lin *et al.*, 2004; Okazaki *et al.*, 1986). Cystoliths in *P. axillare* were mostly located in both surfaces. It may be caused by their leaves being narrow and arranged with less angle to the stem or branches, so that both surfaces can be exposed to the light at the same level. The different ratios of light intensities between the adaxial and abaxial surfaces of the leaves may play a role in the formation of lithocysts and the cystolith inside.

Most *Pseuderanthemum* spp. showed their cystoliths arranged parallel to the midrib and leaf margin and random in the central area of leaves, except in *P. axillare*, with cystoliths arranged randomly even to midrib. So, the distribution and orientation of cystoliths are also important for identifying this species. Furthermore, cystoliths can be found in leaf trichomes, or they are formed in hair-like lithocysts (Yu and Li, 1991; Wu and Kuo-Huang, 1997). There were trichomes on both adaxial and abaxial epidermises of leaves in some taxa of *Pseuderanthemum*, but in these trichomes no cystoliths could be found.

As already mentioned above, cystoliths occurred in the leaves of all examined samples of *Pseuderanthemum*. Cystolith size, shape and distribution are variable between species in the genus, their occurrence is constant, and therefore they constitute a character of systematic value. Moreover, the cystolith margin in *Pseuderanthemum* is nearly smooth, while that of *Ruellia* and *Hemigraphis* represent more undulate surfaces. In the future work, cystolith density and other cystolith locations should be investigated. Several characters of cystoliths can be used to support the delimitation of species together with morphological, anatomical, palynological, and molecular characters.

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