Morphology and Chromosome Numbers of Selected *Argostemma* spp. (Rubiaceae) in Thailand

Saithip Aphinyanan

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Botany

Prince of Songkla University

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ชื่อวิทยานิพนธ์ สัณฐานวิทยาและจำนวนโครโมโซมของพืชบางชนิดในสกุล *Argostemma* Wall. (Rubiaceae) ในประเทศไทย

ผู้เขียน นางสาวสายทิพย์ อภิญญานันท์

สาขาวิชา พฤกษศาสตร์

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บทคัดย่อ

การศึกษาโครโมโซมของพืชบางชนิดในสกุล *Argostemma* Wall. (Rubiaceae) ในประเทศไทยในครั้งนี้ สืบเนื่องจากลักษณะทางสัณฐานวิทยาของพืชในสกุล *Argostemma* ที่พบว่าแบ่งออกได้เป็น 2 กลุ่ม คือ กลุ่มดอกรูประฆัง (bell-shaped) และกลุ่มดอกรูปดาว (star-shaped) พบว่า พืชทั้ง 21 ชนิดและ 1 ชนิดย่อย จากทั้ง 2 กลุ่ม มีจำนวนโครโมโซมเท่ากัน คือ 2n = 22 จากการศึกษาครั้งนี้ได้รายงานจำนวนโครโมโซมของพืช 18 หน่วยอนุกรมวิธาน (taxa) เป็นครั้งแรก และได้พบโครโมโซม 1 คู่ที่มี satellites หรือ secondary constrictions ในพืช 4 หน่วยอนุกรมวิธาน คือ *A. condensum* Craib; *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith; *A. diversifolium* Ridl. และ *A. lobulatum* Craib var. *variabile* K. Sridith จากข้อมูลโครโมโซมแสดงให้เห็นความสัมพันธ์ของพืชในสกุลนี้ว่าควรคงสกุล *Argostemma* Wall. เอาไว้ ไม่ควรแยกเป็นสกุลย่อย อย่างไรก็ตาม ควรมีการศึกษาจำนวนโครโมโซมและคาริโอไทป์ของพืชสกุลนี้เพิ่มขึ้น เพื่อใช้สนับสนุนความสัมพันธ์ระหว่างพืชชนิดต่างๆในสกุล ทั้งนี้ได้พบเทคนิคใหม่ในการศึกษาโซมาติคโครโมโซมของพืชในสกุล*Argostemma* โดยใช้ส่วนของกลีบดอกอ่อน

Thesis Title	Morphology and Chromosome Numbers of Selected *Argostemma* spp. (Rubiaceae) in Thailand
Author	Miss Saithip Aphinyanan
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ABSTRACT

The new information on the chromosomes of selected species in the genus *Argostemma* Wall. (Rubiaceae) in Thailand have been achieved. Concerning the fact that there are two major groups of *Argostemma* due to the morphological characters i.e. the group with bell-shaped flowers and the other with star-shaped flowers. The selected 21 species and one subspecies from both groups of *Argostemma* from Thailand have the same somatic chromosome numbers 2n = 22. Eighteen taxa were counted for the first time. One chromosome pair of satellites or secondary constrictions were found in four taxa : *A. condensum* Craib; *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith; *A. diversifolium* Ridl. and *A. lobulatum* Craib var. *variabile* K. Sridith. The relationships between species in the genus due to the chromosome numbers have been discussed. It is suggested here that *Argostemma* Wall. might remain a "good genus". However, more information on chromosome numbers together with the karyotype patterns of some selected species would be needed in order to support the relationships between various taxa in the genus. Moreover, a new technique for somatic chromosome investigation of *Argostemma* was found by using corolla parts of young flowering buds.

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Saithip Aphinyanan

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CHAPTER 1

INTRODUCTION

The genus *Argostemma* Wall. is a rather large genus in family Rubiaceae, subfamily Rubioideae, tribe *Argostemmatae* (Robbrecht, 1988). They are all perennial herbs. The genus comprises about 100 species (Mabberley, 1987), confined to the South-East Asia but two species occur in tropical West Africa (Sridith & Puff, 2001).

The taxonomic status of the genus *Argostemma* has been questioned, whether it should be divided into the infrageneric taxa or not, due to the morphological differences in growth habits; corolla shape; fusion of the stamens etc. Ridley (1927); Bakhuizen van den Brink, Jr. (1953) and Schumann (1981) have further subdivided the genus based on vegetative characters and floral features in the past. However, none of these infrageneric classifications seem satisfactory, and all of them appear to be highly artificial later (Sridith, 1999[b]). According to the floral morphology, two major types of corolla shapes are recognized due to the different proportions between corolla tube and corolla lobe length, e.g. bell-shaped and star-shaped corolla (Sridith & Puff, 2001).

Besides morphological evidences, the chromosome characters are also useful sources of comparative data in taxonomy, due to the fact that these structures contain the genetic material which is responsible for maintaining reproductive barriers and the integrity of species and other taxa (Stuessy, 1989). The cytotaxonomy can be usefully considered under three headings: chromosome numbers, chromosome

structure and chromosome behaviour. The number of chromosomes is as important as the number of carpels (Stace, 1989). The structure or shape of chromosomes is to be considered in the same way as the shape of leaves or petals. The pairing behaviour of chromosomes at meiosis is determined in which governs the level of fertility of hybrids and hence the breeding behaviour and pattern of variation of populations (Stace, 1989).

There are few cytological works on the genus *Argostemma*. The only six species showed different numbers of chromosomes in the genus (Mangenot & Mangenot, 1962; Khoshoo & Bhatia, 1963; Hellmayr *et al.*, 1994; Kiehn, 1996; Puangsomlee & Puff, 2001). Nevertheless, more karyological works are still needed in order to get the information on numbers and shapes of chromosomes of all representatives of each morphological group. Since the karyological information together with other morphological data may lead to the understanding of how "good genus" *Argostemma* Wall. is.

Objective

To study chromosome numbers from somatic and germ-line cells of selected *Argostemma* spp. in Thailand.

CHAPTER 2

LITERATURE REVIEW

Taxonomic study of *Argostemma* Wall. in Thailand and the adjacent South-East Asian regions

The genus *Argostemma* (Rubiaceae) was described by Wallich in Flora Indica. There were four species in the original publication of the genus, i.e. *A. sarmentosum* Wall., *A. verticillatum* Wall., *A. rostratum* Wall. and *A. pictum* Wall. The genus was described as a small succulent and elegant herbaceous plant, with unequilateral leaves, snow-white flowered, four- or five-parted, largish with respect to the size of the plant (Wallich in Roxburgh, 1824).

Indo-chinese Region

In the Indo-chinese region, seven species of *Argostemma* were recorded and the members of this genus were separated based on the number of merous (four- or five- merous) (Pitard, 1922). Recently, a new species, *A. fasiculata* K. Sridith & K. Larsen, was described from Cambodia (Sridith & Larsen, 2005).

Malesian Region

King and Gamble (1903) reported that there were twenty-three taxa of this genus occurring in the Malay Peninsula, and these taxa were distinguished from each other in terms of size of leaves and number of leaf-pairs. Later, Ridley (1923)

reported forty-one with some additional new taxa from the region. Ridley (1927) recorded 28 species of Malay Peninsular *Argostemma* spp. and divided them into two sections, i.e. *Eu-argostemma* K. Schum. and *Pomangium* (Reinw.) Ridl. Later, Bakhuizen van den Brink, Jr. (1953) recorded 16 species of this genus for the whole Malesian region and proposed five sections, i.e. *Eu-argostemma* K. Schum., *Pomangium* (Reinw.) Ridl., *Argostemmella* (Ridl.) Bakh.f., *Elatosteemoides* K. Schum and *Borragineum* Bakh.f. Recently, Schumann (1981) proposed three sections, i.e. *Euargostemma* K. Schum., *Monophyllum* K. Schum. and *Elatosteemoides* K. Schum.

In 1989, Bremer studied the genus in Borneo. Twenty-eight species were reported, which included six new and endemic species. Members of this genus are distinguished from each other by leaf arrangement, i.e. pseudo-verticillate and scattering along the stem.

Thailand

In Thailand, 33 taxa of *Argostemma* were recorded in Florae Siamensis Enumeratio (Craib, 1932). Later, Sridith (1999[a]) reported four additional species to the flora of Thailand, i.e. *A. monophyllum* K. Sridith, *A. rotundicalyx* K. Sridith, *A. thaithongae* K. Sridith and *A. puffii* K. Sridith, one new variety: *A. lobulatum* Craib var. *variabile* K. Sridith and changed the status of *A. setosum* Geddes to *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith. He also revised the genus for the Flora of Thailand, in which thirty-one taxa were reported for the flora together with illustrations, distributional records and ecological information (Sridith, 1999[b]). In

2000, Sridith and Puff reported the distribution of this genus with special reference to Thailand and surrounding areas. They noted that seventeen species were endemic to Thailand (Sridith & Puff, 2000).

In 2001, Sridith and Puff have discussed the floral diversity in the genus *Argostemma* Wall. according to the morphological characters. They have proposed four major groups, i.e. star-shaped and 5-merous flower group (assumed as the "basic" *Argostemma*), star-shaped and 4-merous flower group (only one species: *A. khasianum* C.B. Clarke), bell-shaped and 5-merous flower group and bell-shaped and 4-merous flower group (believed to be more derived). However, there are intermediate species among groups which still be taxonomic problem (Sridith & Puff 2001).

Chromosomal study of *Argostemma* Wall.

Chromosome numbers

There were few chromosomal data of *Argostemma*. Just only nine of 100 species chromosome numbers were reported. In 1962, Mangenot and Mangenot reported chromosome number of *A. pumilum* Benn., which was one of the two African species of the genus as 2n = 22. Later, Khoshoo and Bhatia (1963) studied cytology of some Rubiaceae of the North-Western Himalayas and reported chromosome number n = 14 for *A. verticillatum* Wall. A few decades later, Hellmayr *et al.* (1994) presented an unidentified *Argostemma* species from the Malay Peninsular 2n = 22. After that, Kiehn (1996) reported chromosome numbers of three *Argostemma* species: *Argostemma* sp. 2n = 22 from Papua New Guinea, *Argostemma*

sp. 2n = 22 and *A. hookeri* King 2n = 32 from the Malay Peninsula. He also proposed the basic chromosome number of this genus is x = 11. Recently, Puangsomlee and Puff (2001) have reported chromosome numbers of Thai Rubiaceae, i.e. *A. diversifolium* Ridl., *A. pictum* Wall. and *A. neurocalyx* Miq., n =11, n = 11, 2n = c.22 respectively.

Chromosome morphology

Kiehn (1995) surveyed the chromosome of Rubiaceae. He reported that normally Rubiaceae have small chromosomes and clump together, the length and shape of all chromosomes within a diploid set are more or less uniform. In generally, one or two pairs of satellite chromosomes are found in Rubiaceae and tannins in the tissues of plants often interfering with fixation and/or staining procedures.

The chromosome studies of various *Argostemma* spp. in Thailand has been studied in the present work in order to seek the taxonomic relationship among different taxa.

CHAPTER 3

MATERAILS AND METHODS

I. Plant collecting

Materials

1. plant collecting materials

- spade
- plastic bags
- label tags
- field notebook
- hand lens
- altimeter
- wooden presses and rope
- pressing paper (newspaper)
- corrugated cardboard
- digital camera

2. plant investigation materials

- stereo microscope
- laboratory needle
- Petri dishes
- razor blade
- forceps
- related taxonomic literatures

3. herbarium specimens preparing materials

- hot air oven
- mounting paper
- latex glue
- white paper cover
- needle and thread
- label pad
- deep freezer
- 70 % ethanol

Methods

1. The herbarium specimens of the genus *Argostemma* available in the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University (PSU) and the Forest Herbarium (BKF), Department of National Park Wildlife and Plant Conservation has been studied.

2. Exploration and collection

- Field collections were made in the localities, following Sridith (1999b).
- Plant collections were made with field notes. The morphological characters of each specimen, such as color, habit etc. were noted and photographed.
- Specimen processing following the directions specified in "The Herbarium Handbook" (Foreman and Bridson, 1992).
- The collected specimens were identified and described by using both keys and descriptions from taxonomic literatures.
- The duplicates of plant specimens were deposited at the herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University (PSU); The

Forest herbarium, Department of National Park Wildlife and Plant Conservation (BKF), Royal Botanic Gardens, Kew, Surrey, UK (K), Singapore Botanic Gardens (SING), Royal Botanic Gardens Edinburgh (E), Leiden Botanic Garden (L) and The herbarium of the Forest Research Institute of Malaysia (KEP).

II. Chromosomal study

Materials

- the 22 specimens of *Argostemma* spp. from all over Thailand
- 0.1 % colchicine
- glacial acetic acid
- 95% ethanol
- 70% ethanol
- carbol fuchsin
- immersion oil
- absolute ethanol
- acetone
- nail enamel
- vials
- beaker 50 ml.
- hot plate
- needle
- scalpel
- forceps
- microscopic slides and cover-glasses

- label stickers
- light microscope: Nikon Optiphot-2, Olympus BX51
- Exposure control unit: Nikon UFX-DX II, Olympus PM-30, Olympus DP11
- black-white negative films

Methods

1. Preparation of meiotic study : Smear technique (modified from Chaiyasut, 1989; Sharma & Sharma, 1980)

1. Fixation : Fix the young flower buds in 95% ethanol-glacial acetic acid (3:1) (Carnoy's solution) for 24 hours at 10 ^{0}C.

2. Washing : Wash in 95% ethanol 3 times.

3. Storage : Store in 70 % ethanol at 10 ^{0}C.

4. Washing : Wash in water 3 times.

5. Staining : Transfer the young flower buds to carbol fuchsin for 5 minutes at room temperature.

6. Smearing : Dissect out anthers from the flower bud. Put the anthers in a drop of carbol fuchsin on a clean dry slide, cut off the edges of the anthers with a scalpel, squeeze out the inner fluid and reject the empty anther lobes, then smear the fluid with a clean scalpel and cover with a cover-glass on the fluid.

7. Observation : Observe under the microscope. Count 10-30 of first metaphase cells and take photographs 10 well spread first metaphase cells at 100x, using an oil immersion objective.

2. Preparation of mitotic study : Squash technique (modified from Chaiyasut, 1989; Sharma & Sharma,1980)

1. Pretreatment: Pretreat fresh young flower buds in 0.1% colchicine for 5 hours at 10 ^{0}C.

2. Fixation : Transfer to 95% ethanol-glacial acetic acid (3:1) (Carnoy's solution) for 24 hours at 10 ^{0}C.

3. Washing : Wash in 95% ethanol 3 times.

4. Storage : Store in 70 % ethanol at 10 ^{0}C.

5. Washing : Wash in water 3 times.

6. Staining : Transfer the young flower buds to carbol fuchsin for 5 minutes to 5 hours at room temperature.

7. Squashing : Cut off the corolla part of the young flower buds and put it in a drop of carbol fuchsin on a slide. Place a cover-glass on the material and squash by applying uniform pressure on the cover-glass with the tip of pencil through a piece of blotting paper.

8. Observation : Observe under the microscope. Count 10-30 metaphase cells and take photographs 10 well spread metaphase cells.

CHAPTER 4

RESULTS

Twenty-two taxa of *Argostemma* spp. from all over Thailand were collected from July 2004 to September 2005 as follows[1]:

Genus description

Perennial herb with rhizomes or tubers. **Stem** erect, prostrate, creeping; unbranch or slightly branched. **Leaves** opposite, isophyllous, to strongly anisophyllous, either only one well developed leaf pair per plant (large leaf) or few leaf pairs in pseudo-verticillate arrangement (internode very short), or many leaf pairs scattered along stem (leafy stem), distichous arrangement. **Stipules** interpetiolar, acute or bifid. **Inflorescences** terminal, many- to few- and 1-flowered, cymose or umbel-like. Bracts free or fused. **Flowers** hermaphrodite, 4- or 5-merous, actinomorphic or slightly zygomorphic. **Calyx** persistent, chartaceous or succulent, calyx tube and lobe mostly short. **Corolla** white, either bell-shaped with a relatively long tube and short lobes or star-shaped with a short tube and long lobes, valvate in bud. **Stamens** inserted near or at base of corolla tube; filament usually free, sometimes fused at least part of their length; anther either in cone-like arrangement (anther either fused as a true anther cone, or covinent into a pseudo anther cone) or entirely free, yellow to whitish, seldom bluish; opening by longitudinal slit or apical pores; connective often prolonged in apical and sometimes enlarged. **Ovary** 2 locules,

[1] Including one new record taxon for Thailand: *A. kurzii* C. B. Clarke and one new described species: *A. argostemon* K. Sridith.

each locule with numerous ovules on axile placenta; style filiform, with club- or globular- or disc-shaped; stigma exserted from anther cone. **Fruit** a capsule, crowned by persistent calyx, opening by an apical operculum. **Seeds** small, numerous.

The morphological characters of the studied taxa.

Star-shaped and 5-merous flower

1. Argostemma argostemon K. Sridith, Nord. J. Bot. (Sridith, inpress[b]) (Fig.1, a)

Perennial herb with tubers. **Stem** erect, unbranched, 2.5-4.6 cm, internode very short, glabrous. **Leaves** 2-3 pairs, pseudo-verticillate, strongly anisophyllous, one large leaf ovate, apex acuminate, base cordate, 6.1-9.3 by 3.7-6.3 cm, upper surface dark green with white streaks on midrib and veins, sparsely pubescent, lower surface lighter green, glabrous; petioles subobsolete. **Stipules** elliptic, tip acute, 4-6 mm long, pubescent. **Inflorescences** 4-9 flowers in compound cyme; peduncles 11-40 mm, green, glabrous; bracts 5 lobes, fuse, ovate, 3-5 mm long, glabrous; pedicels 6-11 mm, white, pubescent. **Flowers** 5-merous, actinomorphic. **Calyx** chartaceous, whitish green; calyx lobes ovate, 3.8-4.0 mm long, suberect, glabrous. **Corolla** star shaped, glabrous; corolla lobes ovate, c. 6.3 mm long, spreading; corolla tube c. 2 mm long. **Stamens** 5, free, inserted at the base of the corolla tube; filaments c. 1.3 mm long; anther connivent into a pseudo anther cone, white without apical appendage, c. 6 mm long, sagittate, semibasifixed, opening by means of longitudinal slits. **Ovary** glabrous; style filiform, c. 8.2 mm, long exserted from anther cone, glabrous; stigma globular shape. **Fruit** not seen.

Ecology : on moist rock in decidous forest.

Flowering : June

Specimens studied : Saithip & K. Sridith & K. Maneenoon 047

2. Argostemma condensum Craib, Kew Bull. 212. 1931. (Fig.2, a)

Perennial herb with rhizomes. **Stem** prostrate, unbranched, 4-15 cm, internode c. 0.5 cm, glabrous. **Leaves** many pairs along the stem, strongly anisophyllous, distichous, oblong or elliptic, apex acute, base acute, 0.8-1.8 by 0.3-0.5 cm (big leaf), upper surface yellowish green, sparsely hairy along midrib and margin, lower surface pale green, glabrous; petioles subobsolete. **Stipules** ovate, tip acute, 4-5 mm long, glabrous. **Inflorescences** 1 (rarely 2-3) flowers; pedicels 10-22 mm, white, glabrous. **Flowers** 5-merous, slightly zygomorphic. **Calyx** chartaceous, green; calyx lobes narrowly triangular, c. 1.6 mm long, suberect, glabrous. **Corolla** star shaped, glabrous; corolla lobes ovate, c. 4 mm long, suberect; corolla tube c. 1 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments c. 1 mm long, free; anther fused, curved in s-shape anther cone like, yellow with white apical appendage, c. 4.2 mm long, sagittate, basifixed, opening by longitudinal slits. **Ovary** glabrous; style filiform, c. 4.4 mm, long exserted from anther cone, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist rock by stream in evergreen forest.

Flowering : March-June

Specimens studied : K. Sridith 732

3. Argostemma dispar Craib, Kew Bull.: 213. 1931. (Fig.3, a)

Perennial herb with rhizomes. **Stem** decumbent or prostate, unbranched 9.5-19 cm long, internode 0.5-1.8 cm, densely covered with long hair. **Leaves** many pairs scattered along stem, distichous, strongly anisophyllous, big leaf oblique-lanceolate, apex acute, base oblique, 1.9-7.5 by 1.1-2.2 cm, small leaf cordate , upper surface grayish green, lower surface whitish, densely pubescent on both surfaces; petioles c. 3-8 mm long, pubescent. **Stipules** ovate, tip acute, 4-7 mm long, densely covered with long hairs. **Inflorescences** terminal or occasionally axillary, 1-11 flowers in simple cyme/compound cyme; peduncles 3-17 mm long, white, densely covered with long hairs; bracts 4 at any node, free, elliptic, c. 3 mm long, densely pubescent; pedicels 2-7 mm long, white, densely covered with long hairs. **Flowers** 5-merous, zygomorphic. **Calyx** chartaceous, light green; calyx lobes narrowly triangular, c. 1 mm long, suberect, pubescent. **Corolla** star-shaped, pubescent outside; corolla lobes narrowly triangular, 5-6 mm long, spreading and tips recurved; corolla tube c. $\leq$ 2 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments c. 1 mm long, free; anther fused, curved in s-shape anther cone like, yellow with white apical appendage, c. 9 mm long, oblong, semi-basifixed, opening by longitudinal slits. **Ovary** pubescent; style filiform, c. 10 mm, shortly exserted from anther cone, glabrous; stigma club shape. **Fruit** globose, pubescent. **Seeds** numerous.

Ecology : ground cover on moist soil in evergreen forest.

Flowering : March-May

Specimens studied : J. Wai 48

4. Argostemma diversifolium Ridl., J. Straits Branch Roy. Asiat. Soc. 57: 52. 1911. (Fig.4, a)

Perennial herb with tubers. **Stem** erect, unbranched, 4.8-22.5 cm, internode very short; glabrous. **Leaves** 1-2 pairs, pseudo-verticillate, slightly anisophyllous, ovate, apex acuminate or acute, base round, 8.8-23.0 by 5.2-10.0 cm upper surface shinny green, pubescent, lower surface lighter green; petioles 3-6 mm, glabrous. **Stipules** ovate, tip acute or bifid, 7-12 mm long, glabrous. **Inflorescences** 4-14 flowers, a dense scorpioid cyme; peduncles 32-61 mm, white, pubescent; bracts 3-4, free, elliptic, 3-5 mm long, pale purple; pedicels 7-15 mm, color purple, pubescent. **Flowers** 5-merous, slightly zygomorphic. **Calyx** chartaceous, green or pale purple; calyx lobes ovate, 4-5 mm long, suberect, glabrous. **Corolla** star shaped, glabrous; corolla lobes ovate, 6.2 mm long, spreading, tip recurved; corolla tube c. 0.5 mm long. **Stamens** 5, free, inserted at the base of the corolla tube; filaments c. 0.5 mm long; anther curved in s-shape, bluewish without apical appendage, 4-5 mm long, sagittate, semibasifixed, opening by apical pore. **Ovary** glabrous; style filiform, c. 7 mm, shortly exserted from anther cone, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist limestone

Flowering : June-August

Specimens studied : Saithip & K. Sridith & K. Maneenoon 046

5. Argostemma elatostemma Hook.f. in Hook.f., Fl. Brit. Ind. 3: 45. 1880. (Fig.5, a)

Perennial herb with rhizomes. **Stem** creeping, unbranched 3-18 cm long, internode 8-17 cm with adventitious roots at any nodes, densely pubescent.

Leaves many pairs scattered along stem, strongly anisophyllous, distichous, big leaf ovate, apex obtuse, base auriculate-oblique, 3.2-4.9 by 1.5-2.4 cm, small leaf cordate, upper surface dark green, sparsely pubescent, lower surface whitish, densely pubescent; petioles 2-14 mm long, densely pubescent. **Stipules** ovate, tip acute, 3-4 mm long, margin ciliate. **Inflorescences** 2-8 flowers in simple or compound cyme; peduncles 22-45 mm long, white, densely covered with short fine hairs; bracts 2-3 at any node, free, linear, c. 2 mm long, green, pubescent; pedicels 3-8 mm long, white, densely pubescent. **Flowers** 5-merous, actinomorphic. **Calyx** chartaceous, green; calyx lobes triangular, 1-5 mm long, suberect, densely pubescent. **Corolla** star-shaped, sparsely pubescent outside; corolla lobes lanceolate, 4-7 mm long, spreading and tip recurved; corolla tube c. 0.5 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments < 1 mm long, free; anther fused forming an anther cone like, yellow with white apical appendage, 5-6 mm long, oblong, semi-medifixed, opening by longitudinal slits. **Ovary** pubescent; style filiform, shortly exserted from anther cone ≤ 1 mm, glabrous; stigma globular shape. **Fruit** not seen.

Ecology : on moist soil by stream in evergreen forest.

Flowering : March-May

Specimens studied : Saithip 039

6. Argostemma kurzii C.B. Clarke in Hook.f., Fl. Brit. Ind. 3: 43. 1880. (Fig. 7, a)

Perennial herb, attached to substrate with dense, matted roots. **Stem** erect, unbranched, 8.3-28.2 cm, internode very short, glabrous; **Leaves** 2 pairs, pseudo-verticillate, strongly anisophyllous, one large ovate, apex acuminate, base cordate , 4.5-19.2 by 3.1-12.9 cm, upper surface shinny green, lower surface lighter

green, glabrous on both surfaces, petioles subobsolete. **Stipules** elliptic, tip acute, c. 8 mm long, glabrous. **Inflorescences** 5-18 flowers, umbel-like; peduncles 1.3-6.5 mm, green, glabrous; bracts 4, free, ovate, 10-13 mm long, glabrous; pedicels 8-12 mm, white, glabrous. **Flowers** 5-merous, actinomorphic. **Calyx** chartaceous, white; calyx lobes triangular, c. 1 mm long, erect, glabrous. **Corolla** semi star-shaped as corolla tube shorter than corolla lobes, glabrous; corolla lobes narrowly triangular, c. 4 mm long, reflexed; corolla tube c. 2 mm long. **Stamens** 5, free, inserted at the base of the corolla tube; filaments c.1 mm long; anther conivent into a pseudo anther cone, yellow without apical appendage, c. 2 mm long, slightly sagittate, semibasifixed, opening by an apical pore. **Ovary** glabrous; style filiform, c. 3.2 mm, long much exserted from anther cone, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist limestone in evergreen forest

Flowering : June

Specimens studied : Saithip & K. Sridith & K. Maneenoon 045

7. Argostemma laeve Benn. ssp. **setosum** (Geddes) K. Sridith, Nord. J. Bot. 19(2): 178. 1999. (Fig. 8, a)

Perennial herb with rhizomes. **Stem** prostrate and suberect, unbranched or sometimes slightly branched 9-23 cm long, internode 0.5-1.1 cm, pubescent. **Leaves** many pairs scattered along stem, strongly anisophyllous, distichous, elliptic, apex acute, base acute, 2.4-5.4 by 1.3-1.9 cm (big leaf), upper surface shiny and setose-ciliate pubescent along midrib, lower surface green, margin setose; petioles 7-14 mm long, glabrous. **Stipules** elliptic, tip acute, 5-6 by 2-3 mm, glabrous.

Inflorescences 1-4 flowers in simple or compound cyme; bract 4-5 at any node, free, linear, c. 3 mm long, glabrous; peduncles 9-20 mm long, white or pale green, glabrous; pedicels 7-10 mm long, glabrous. **Flowers** 5-merous, zygomorphic. **Calyx** chartaceous, green; calyx lobes narrowly triangular, erect, glabrous. **Corolla** star-shaped, entirely glabrous; corolla lobes lanceolate, 11 by 5 mm, spreading; corolla tube c. 2 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments c. 3 mm long, free; anther fused, curved in s-shape anther cone, yellow with white apical appendage, sagittate, c. 7 mm long, semi-medifixed, opening by longitudinal slits. **Ovary** glabrous; style filiform, shortly exserted from anther cone, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist soil in evergreen forest.

Vernacular : Pra-dub-hin-khoa-lung

Flowering : May-July

Specimens studied : Saithip 001, Saithip 031

8. Argostemma laxum Geddes, Kew Bull.:166. 1927. (Fig. 6, a)

Perennial herb, attached to substrate with dense, matted roots. **Stem** erect, branched, 4.5-13.0 cm, internode very short, glabrous. **Leaves** 3 pairs, pseudo-verticillate, slightly anisophyllous, apex lanceolate-acuminate, base acute, 2.4-7.5 by 0.7-1.5 cm, upper surface dark green, sparsely pubescent, lower surface lighter green, glabrous; petioles 2-13 mm, glabrous. **Stipules** ovate, tip acute or bifid, 2.6-3.0 mm long, glabrous. **Inflorescences** 2-10 flowers, compound cyme; peduncles length 7-15 mm, white, glabrous; bracts 5, free, elliptic, 2-7 mm long, glabrous; pedicels 3-5 mm, white, glabrous. **Flowers** 5-merous, slightly zygomorphic. **Calyx** chartaceous, green;

calyx lobes triangular, c. 0.8 mm long, suberect, glabrous. **Corolla** star shaped, glabrous; corolla lobes ovate, c. 5 mm long, spreading; corolla tube c. 1 mm long. **Stamens** 5, free, inserted at the base of the corolla tube; filaments c. 0.3 mm long; anther yellow without apical appendage, c. 4 mm long, slightly sagittate, semibasifixed, opening by apical pore. **Ovary** glabrous; style filiform, c. 3.8 mm, as long as anther, glabrous; stigma globular shape. **Fruit** not seen.

Ecology : on mossy trunks in evergreen forest

Flowering : June-July

Specimens studied : Saithip & K. Sridith & K. Maneenoon 051

9. Argostemma ophirense Maingay ex Hook.f. in Hook.f., Fl. Brit. Ind. 3: 45. 1880. (Fig. 9, a)

Perennial herb with rhizomes. **Stem** erect, unbranched 12-30 cm long, internode 3-8 cm, pubescent. **Leaves** many pairs scattered along stem, strongly anisophyllous, entire, elliptic, apex acute or cuspidate, base oblique, 4.8-13.2 by 1.2-5.1 cm (big leaf), upper surface green, sparsely pubescent, lower surface pale green, pubescent on the vein and midrib; petioles 3-12 mm long, pubescent. **Stipules** ovate, tip acute, 2-4 by 1.2-1.5 mm, pubescent. **Inflorescences** 4-33 flowers, a lax compound cyme; peduncles 15-37 mm long, redish, glabrous; bracts 2-3 at any node, free, lanceolate, c.1.5 mm long, glabrous; pedicels 6-12 mm long, white, glabrous. **Flowers** 5-merous, actinomorphic. **Calyx** chartaceous, green; calyx lobes triangular, c. 2 mm long, spreading, glabrous. **Corolla** star-shaped, glabrous; corolla lobes ovate c. 4 by 2-3 mm, spreading, tip recurved; corolla tube 0.5-1 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments c. 1 mm long, free; anther fused,

forming an anther cone like, yellow with white apical appendage, 5-6 mm long, oblong, semi-medifixed, opening by longitudinal slits. **Ovary** glabrous; style filiform, shortly exserted from anther cone >1 mm, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist soil by stream in evergreen forest.

Flowering : April-June

Specimens studied : *Saithip 038*

10. Argostemma pictum Wall. in Roxb., Fl. Indica (ed. Carey & Wall.) 2: 327. 1824. (Fig. 10, a)

Perennial herb, attached to substrate with dense, matted roots. **Stem** erect, unbranched, 6-30 mm long, pubescent. **Leaves** one pairs, slightly or strongly anisophyllous, ovate or slightly triangular, apex acute, base truncate, 2.9-8.4 by 3.1-6.6 mm (big leaf), upper surface dark green with or without white streaks along midrib and veins, lower surface light green, sparsely pubescent on both surface; petiole subobsolete. **Stipules** ovate, tip acute, 1.3-4 mm long, pubescent. **Inflorescences** 6-20 flowers, a dense scorpioid cyme; peduncles 28-75 mm long, glabrous; bracts 3-6 lobes, ovate, 2-4 mm long, pubescent; pedicels 1-9 mm long, glabrous. **Flowers** 5-merous, actinomorphic. **Calyx** chartaceous, green, calyx lobes triangular, c. 1 mm long, spreading, glabrous. **Corolla** star-shaped, covered with very short fine hairs outside; corolla lobes narrowly triangular, c. 3.5 mm long, spreading, tips recurved; corolla tube c. 0.5 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments $\leq$ 1 mm long, merging into large connectives, free; anther connivent, forming a cone-like structure, yellow, oblong, c. 3 mm long, basifixed,

opening by longitudinal slits. **Ovary** glabrous; style filiform, c. 3.5 mm long, long exserted from anther cone > 1 mm, glabrous; stigma disc shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist soil or rock in evergreen forest.

Flowering : June-August

Specimens studied : Saithip 004, Saithip 017

11. Argostemma propinquum Ridl., J. Straits Branch Roy. Asiat. Soc. 57: 53. 1911. (Fig. 11, a)

Perennial herb with rhizomes. **Stem** suberect, unbranched 15-40 cm long, internode 1.4-3.5 cm, densely covered with short fine hair. **Leaves** many pairs, scattered along stem, strongly anisophyllous, oblanceolate, apex acute, base oblique, 14.3-8.0 by 5.7-2.8 cm (big leaf), upper surface and margin sparsely scabrous, lower surface densely pubescent along midrib and veins; petioles 8-62 mm long, densely pubescent. **Stipules** ovate, tip acute or bifid, 9-20 mm long, sparsely pubescent on both surfaces. **Inflorescences** 4-16 flowers in compound cyme; peduncles 40-66 mm long, purplish/pinkish, densely pubescent; bracts 2-4 at any node, free, linear, c. 3 mm long, densely pubescent; pedicels 8-16 mm long, white, pubescent. **Flowers** 5-merous, actinomorphic. **Calyx** persistent, purplish /pinkish; calyx lobes ovate, c.3 mm long, suberect, densely pubescent outside. **Corolla** star-shaped, pubescent outside along veins; corolla lobes lanceolate, 5-8 mm long, reflexed with inrolled tips; corolla tube 1-1.5 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments c. 1 mm long, free; anther fused, forming an anther cone like, yellow with white apical appendage, c. 6 mm long, oblong, semi-medifixed, opening by longitudinal slits.

Ovary pubescent; style filiform, shortly exserted from anther cone, glabrous; stigma globular shape. **Fruit** globose, sparsely pubescent. **Seeds** numerous.

Ecology : on moist soil in evergreen forest.

Vernacular : Lin-Ku-Rum

Flowering : April-June

Specimens studied : Saithip 028, Saithip 040, Saithip & K. Maneenoon & J. Wai 041

12. Argostemma rotundicalyx K. Sridith, Nord. J. Bot. 19(2): 173. 1999. (Fig. 12, a)

Perennial herb, attached to substrate with dense, matted roots. **Stem** suberect, unbranched, 5.3-11.4 cm long, glabrous, internode very short. **Leaves** 3-4 pairs, pseudo-verticillate, slightly anisophyllous, lanceolate or elliptic, apex acuminate, base acute, 5.3-12.3 by 3.8-4.5 cm, upper surface dark green, lower surface pale green, covered with long hair on both sides; petioles subobsolete, glabrous. **Stipules** *triangular, tip acute or bifid, c. 2 mm long, pubescent.* **Inflorescences** 3-12 flowers, a lax scorpioid cyme; peduncles 30-50 mm long, green, glabrous; bracts 2 at any node, free, oblong, c. 2 mm long, pubescent; pedicels 7-19 mm long, white, glabrous. **Flowers** 5-merous, slightly zygomorphic. **Calyx** chartaceous, green; calyx lobes very short, round, c. 0.2 mm long, erect, glabrous. **Corolla** star-shaped, glabrous; corolla lobes ovate, 4-14 mm long, spreading and tip slightly recurved; corolla tube c. 3 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments c. 1 mm long, free; anther fused, forming a distinctly stout anther cone, yellow with long white apical appendages 6-8 mm long, slightly basifixed, opening by longitudinal slits. **Ovary** glabrous; style filiform, 8-10 mm

long, long exserted from corolla tube, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist limestone in evergreen forest.

Flowering : June-July

Specimens studied : Saithip 053

13. Argostemma subcrassum King, J. Asiat. Soc. Bengal 72: 152. 1903. (Fig.13, a)

Perennial herb with rhizomes. **Stem** erect, unbranched, 48.5 cm long, internode 0.4-1.2 cm, glabrous. **Leaves** many pairs scattered along stem, strongly anisophyllous, oblanceolate, apex acute or acuminate, base acute, 9.5-16.0 by 2.9-4.0 cm (big leaf), upper surface green, lower surface lighter green, glabrous; petioles 3-10 mm long, glabrous. **Stipules** ovate, tip acute or bifid, c. 4 mm long, glabrous. **Inflorescences** 5-41 flowers, a lax compound cyme; peduncles 15-26 mm long, light green, glabrous; bracts 4 at any node, free, triangular, c. 3 mm long, glabrous; pedicels 7-13 mm, white, glabrous. **Flowers** 5-merous, zygomorphic. **Calyx** succulent, green; calyx lobes triangular, 1.5-1.7 mm long, spreading, glabrous. **Corolla** star-shaped, glabrous; corolla lobes lanceolate, 6-7 mm long, spreading and tip enrolled; corolla tube c. 1 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments very short (c. < 1 mm long), free; anther fused, forming an anther cone like, yellow with white apical appendage, c.8 mm, oblong, semi-basifixed, opening by longitudinal slits. **Ovary** glabrous; style filiform, 8-9 mm, shortly exserted from anther cone, glabrous; stigma club shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist floor in shade by stream in evergreen forest.

Flowering : April-August

Specimens studied : Saithip & J. Wai 037, J. Wai 31

14. Argostemma unifolioides var. **glabra** King, J. Asiatic Soc. Bengal 72(2): 148. 1903. (Fig.14, a)

Perennial herb with tubers. **Stem** erect, unbranched, c. 15 cm, glabrous. **Leaves** in 1 pairs, pseudo-verticillate, strongly anisophyllous, one large leaf ovate, apex acuminate, base acute, 14-20.5 by 8.6-13 cm, upper surface dark green, glabrous, lower surface pale green, sparsely pubescent on margin and midrib; petioles subobsolete. **Stipules** ovate, tip acute, 5-6 mm long, glabrrous. **Inflorescences** 9-14 flowers in compound cyme (rarely scorpioid cyme); peduncles 50 mm long, green, glabrous; bracts 4 lobes, fuse at base, ovate, c. 3 mm long, glabrous; pedicels 4-13 mm, white, glabrous. **Flowers** 5-merous, actinomorphic. **Calyx** chartaceous, green; calyx lobes triangular, c. 0.8 mm long, spreading, glabrous. **Corolla** star-shaped, glabrous; corolla lobes narrow ovate to lanceolate, c. 5.2 mm long, spreading; corolla tube c. 1.3 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments 0.5 mm long, free; anther fused, forming an anther cone, yellow with white apical appendage, c. 4 mm long, oblong, semibasifixed, opening by longitudinal slits. **Ovary** glabrous; style filiform, c. 5.2 mm, long exserted from anther cone, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist rock in evergreen forest.

Flowering : October-December

Specimens studied : Saithip 054, K. Sridith 746

15. Argostemma verticillatum Wall. in Roxb., Fl. Indica (ed.Carey & Wall.) 2: 324. 1824. (Fig.15, a)

Perennial herb with rhizomes or tubers. **Stem** erect, unbranched, 2.9-3.9 cm, internode very short, glabrous. **Leaves** 2 pairs, pseudo-verticillate, strongly anisophyllous, elliptic, apex acute, base acute, 2.3-5.0 by 0.7-1.2 cm (big leaf), upper surface dark green, pubescent on margin and midrib, lower surface lighter green, glabrous; petioles subobsolete. **Stipules** elliptic, tip acute, 2-3 mm long, glabrous. **Inflorescences** 2-7 flowers, a lax compound cyme; peduncles 7-17 mm, green, glabrous; bracts 4 ,free, elliptic, c. 2.8 mm long, glabrous; pedicels 3.5-6 mm, green, glabrous. **Flowers** 5-merous, slightly zygomorphic. **Calyx** chartaceous, green; calyx lobes triangular, c. 0.2 mm long, spreading, glabrous. **Corolla** star-shaped, glabrous; corolla lobes narrowly triangular, c. 4.3 mm long, suberect; corolla tube c. 0.2 mm long. **Stamens** 5, inserted at the base of the corolla tube; filaments 1.8 mm long, fused; anther free, conivent into a anther cone, yellow with white apical appendage, c. 2 mm long, oblong, basifixed, opening by oblique elongated pore. **Ovary** glabrous; style filiform, c. 3.2 mm, shortly exserted from corolla tube, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist rock in evergreen forest

Flowering : June-July

Specimens studied : Saithip & K. Sridith & K. Maneenoon 052

Star-shaped and 4-merous flower

16. Argostemma khasianum C.B. Clarke in Hook.f., Fl. Brit. Ind. 3: 43. 1880. (Fig.16, a)

Perennial herb, attached to substrate with dense, matted roots. **Stem** erect, unbranched, 3.5 cm long, internode very short, pubescent. **Leaves** in 2 pairs, pseudo-verticillate, slightly anisophyllous, elliptic, apex acute, base attenuate, 16 by 6 cm, sparsely pubescent on both leaf surface; petioles 1 mm long, glabrous. **Stipules** very small, much reduced. **Inflorescences** 3 flowered, a laxed scorpioid cyme; peduncles 18 mm long, white, densely pubescent; bracts 4, free, elliptic, c. 1.3 mm long, pubescent; pedicels c. 2 mm long, white, pubescent. **Flowers** 4-merous, actinomorphic. **Calyx** chartaceous, green; calyx lobes ovate, c. 1 mm long, spreading, densely pubescent. **Corolla** star-shaped, pubescent outside; corolla lobes narrowly triangular, c. 2 mm long; corolla tube ≤ c. 0.3 mm long. **Stamens** 4, inserted at the base of the corolla tube; filaments c. 0.5 mm long, free; anther fused, forming an anther cone like, yellow with white apical appendage, c. 2.2 mm long, oblong, basifixed, opening by longitudinal slits. **Ovary** pubescent; style filiform, c. 2 mm long, include in anther cone, glabrous; stigma bilobate. **Fruit** globose, pubescent. **Seeds** numerous.

Ecology : on moist limestone by stream in evergreen forest.

Flowering : June

Specimens studied : Saithip & K. Sridith & K. Maneenoon 049

Bell-shaped and 5-merous flower

17. Argostemma ebracteolatum Geddes, Kew Bull.: 165. 1927. (Fig.17, a)

Perennial herb, attached to substrate with dense, matted roots. **Stem** erect, unbranched, c. 12 cm, internode very short, glabrous. **Leaves** 2-3 pairs, pseudo-verticillate, slightly anisophyllous, elliptic or ovate, apex acute or acuminate, base acute or attenuate, 5.4-6.5 by 1.6-2.0 cm, upper surface dark green, pubescent, lower surface lighter green, pubescent; petioles 7 mm, pubescent. **Stipules** very small and caduceous. **Inflorescences** c. 20 flowers in compound cyme; peduncles c. 5 mm, whitish, pubescent; bracts 5, free, narrowly triangular, 2.1 -6.2 mm long, pubescent outside; pedicle c. 5 mm, white, pubescent. **Flowers** 5-merous, actinomorphic. **Calyx** chartaceous, green; calyx lobes ovate, c. 2.7 mm, erect, densely pubescent outside. **Corolla** bell shaped, densely pubescent outside; corolla lobes triangular, c. 1.3 mm long, spreading; corolla tube c. 0.9 mm long. **Stamens** 5, free, inserted at the base of the corolla tube; filaments c. 0.7 mm long; anther yellow without apical appandage, c. 1.9 mm long, sagittate, semibasifixed, opening by apical pore. **Ovary** glabrous; style filiform, c. 1 mm, shortly exserted from corolla tube, glabrous; stigma globular shape. **Fruit** not seen.

Ecology : on moist rock by the nature trail.

Flowering : June-July

Specimens studied : Saithip & K. Sridith & K. Maneenoon 048

18. Argostemma lobulatum Craib var. **variabile** K. Sridith, Nord. J. Bot. 19(2): 177. 1999. (Fig.18, a)

Perennial herb, attached to substrate with dense, matted roots. **Stem** suberect, unbranched, 8.2-12.5 cm, internode very short, glabrous. **Leaves** 3 pairs, pseudo-verticillate, strongly anisophyllous, leaves lanceolate or slightly falcate, apex acuminate, base attenuate or oblique, 9.9 -16 by 3.3 - 4.1 cm (big leaf), upper surface shinny green with sparsely short hairs, lower surface lighter green with sparsely short hairs on margin; petioles 3-25 mm, texture glabrous. **Stipules** ovate, tip acute, 5-10 mm long, glabrous. **Inflorescences** 2-9 flowers, a lax compound cyme; peduncles c. 38 mm, white, glabrous; bracts 5, fused at base, ovate, c. 3 mm long, glabrous; pedicels c. 8 mm, white, glabrous. **Flowers** 5-merous, actinomorphic. **Calyx** succulent, white; calyx lobes broad triangular, 1-2 mm long, spreading, glabrous. **Corolla** bell-shaped, glabrous; corolla lobes triangular, 2-3 mm long, spreading; corolla tube c. 2 mm long. **Stamens** 5, free, inserted at the base of the corolla tube; filaments c. 0.7 mm long; anther, pale yellow without apical appandage, c. 2 mm long, oblong, semibasifixed, opening by longitudinal slits. **Ovary** glabrous; style filiform,c. 5.2 mm, long exserted from anther cone, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist rock in evegreen forest

Flowering : July-August

Specimens studied : Saithip 006, Saithip 019

19. Argostemma puffii K. Sridith, Nord. J. Bot. 19(2): 175. 1999. (Fig. 19, a)

Perennial herb with tubers. **Stem** erect, unbranched, c. 13.3 cm, internode very short, glabrous. **Leaves** 3-4 pairs, pseudo-verticillate, slightly anisophyllous, elliptic, apex acute, base acute, 7.4-9.0 by 2.7–3.9 cm (big leaf), upper surface dark green, pubescent, viens white, lower surface whitish, glabrous; petioles 15-30 mm. **Stipules** ovate, tip bifid, c. 2 mm long, glabrous. **Inflorescences** 8-12 flowers, a lax compound cyme; peduncles c. 34 mm, white, glabrous; bracts 5 at any node, fused at base, triangular, c. 0.2 mm long, glabrous; pedicels 3-4 mm, white, glabrous. **Flowers** 5-merous, actinomorphic. **Calyx** persistent, green; calyx lobes triangular, 0.8 -1.0 mm long, spreading, glabrous. **Corolla** bell shaped, glabrous; corolla lobes ovate, c. 1.2 mm long, spreading, tip recurved; corolla tube c. 1.2 mm long. **Stamens** 5, free, inserted at the base of the corolla tube; filaments 0.5-1.0 mm long; anther connivent into an anther cone, yellow without apical appendage, c. 1.7 mm long, oblong, connective with 3-lobe outgrowth on abaxial side, opening by longitudinal slits. **Ovary** glabrous; style filiform, 3-4 mm, long exserted from anther corolla lobe, glabrous; stigma globular shape. **Fruit** not seen.

Ecology : on moist limestone in evergreen forest.

Flowering : August

Specimens studied : Saithip 025

Bell-shaped and 4-merous flower

20. Argostemma neurocalyx Miq., Ann. Mus. Ludg.-Bat. 4(8): 229.1869. (Fig. 20, a)

Perennial herb with tubers. **Stem** erect, unbranched, 2-50 mm long, internode very short, pubescent. **Leaves** one or two pairs, slightly (or rarely strongly anisophyllous), ovate or triangular, apex acute, base obtuse, 3.1-8.5 by 2-6.5 mm, upper surface dark green and sparsely hairy with or without white dot or streaks along midrib and veins, lower surface light green and covered with short fine hair; petiole subobsolete. **Stipules** triangular, acute at tip 1-9 mm long, pubescent. **Inflorescences** 3-21 flowers, an umbel-like; peduncles 28-57 mm long, glabrous; bracts 4 lobes, ovate, 3-4 mm long, pubescent; pedicels 3-9 mm long, pubescent. **Flowers** 4-merous, slightly zygomorphic. **Calyx** chartaceous, pale green; calyx lobes triangular c. 1 mm long, spreading, sparsely pubescent outside. **Corolla** bell-shaped, glabrous; corolla lobes triangular, 0.4-1 mm long, slightly reflexed; corolla tube 0.4-1 mm long. **Stamens** 4, free, inserted near the base of the corolla tube; filaments c. 0.5 mm long, adnate to corolla tube; anther yellow without apical appandage, oblong, c. 2 mm long, basifixed, opening by subapical pores. **Ovary** sparsely pubescent; style filiform, c. 2-3 mm long, shortly exserted from corolla tube, glabrous; stigma globular shape. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist rock by the stream in evergreen forest.

Flowering : June-August

Specimens studied : Saithip 014, Saithip 018, Saithip & K. Sridith & K. Maneenoon 042

21. Argostemma neurosepalum Bakh.f., Blumea. 7: 331. 1953. (Fig. 21, a)

Perennial herb, attached to substrate with dense, matted roots. **Stem** erect, unbranched, 2.0-4.0 cm long, internode very short, pubescent. **Leaves** 2-3 pairs, pseudo-verticillate, slightly or strongly anisophyllous, leaf lanceolate or elliptic, apex acute, base acute, 4.6-15.2 by 1.9-3.7 cm (big leaf), upper surface dark green, sparsely pubescent, lower surface lighter green, sparsely pubescent; petioles 2-15 mm long, pubescent. **Stipules** triangular, tip bifid, c. 1 mm long, pubescent. **Inflorescences** 1-15 flowers, an umbel-like; peduncles 1.6-3.6 cm long, green, pubescent; bracts 4 lobes at any node, ovate, 2-4 mm long, pubescent; pedicels 6-14 mm long, pubescent. **Flowers** 4-merous, slightly zygomorphic. **Calyx** chartaceous, pale green; calyx lobes triangular, c. 1 mm long, spreading, sparsely pubescent outside. **Corolla** bell-shaped, glabrous; corolla lobes triangular, 2-4 mm long, slightly reflexed; corolla tube 2-3 mm long. **Stamens** 4, free, inserted near the base of the corolla tube; filaments c. 0.5 mm long; anther yellow without apical appandage, oblong, c. 3 mm long, semi-medifixed, opening by subapical pores. **Ovary** sparsely pubescent; style filiform, c. 4 mm long, shortly exserted from corolla tube, glabrous; stigma globular shape, yellow. **Fruit** globose, glabrous. **Seeds** numerous.

Ecology : on moist limestone in evergreen forest.

Flowering : June

Specimens studied : Saithip & K. Sridith & K. Maneenoon 044

22. Argostemma plumbuem Craib, Kew Bull.: 263. 1916. (Fig. 22, a)

Perennial herb with tubers. **Stem** erect, unbranched, c. 0.5 cm, internode very short, pubescent. **Leaves** 2-3 pairs, pseudo-verticillate, slightly

anisophyllous, oblong to slightly rounded, apex round, base rounded, 4.5-5.2 by 4.0-4.5 cm, upper surface dark green with white dot on mid rib and veins, pubescent, lower surface lighter green, pubescent; petioles subobsolete. **Stipules** very small. **Inflorescences** 6-9 flowers, a lax compound cyme; peduncles 40 mm, green, pubescent; bracts 4, free, narrowly triangular, c. 2 mm long, pubescent; pedicels 3-7 mm, white, pubescent. **Flowers** 4-merous, slightly zygomorphic. **Calyx** chartaceous, pale green; calyx lobes triangular, 2 mm long, spreading, pubescent outside. **Corolla** bell shaped, pubescent outside; corolla lobes triangular, c. 2 mm long, slightly recurved; corolla tube c. 2 mm long. **Stamens** 4, free, inserted at the base of the corolla tube; filaments 1 mm long; anther yellow without apical appandage, oblong, c. 3 mm long, yellow, semibasifixed, opening by apical pore. **Ovary** glabrous; style filiform, c. 3.5 mm, shortly exserted from corolla tube, glabrous; stigma globular shape. **Fruit** not seen.

Ecology : on moist rock in stream

Flowering : June-December

Specimens studied : Saithip & K. Sridith & K. Maneenoon 050

Results of the chromosomal study

The somatic chromosome numbers of 21 species and one subspecies of the genus *Argostemma* from four morphological groups were examined from corolla part in the young flowering buds. All taxa have the same chromosome numbers 2n = 22. (Table1, Figures 1b - 22b).

The microsporocytes of two taxa : *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith and *A. kurzii* C.B. Clark (star-shaped and 5-merous flower group) showed 11 bivalent with normal segregation (Figures 7c, 8c).

The chromosomes of *Argostemma* are relatively very small size. The satellites were found in one chromosome pair of four taxa : *A. condensum* Craib, *A. diversifolium* Ridl., *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith (star-shaped and 5-merous flower group) (Figures 2b, 4b, 7b) and *A. lobulatum* var. *variabile* (Geddes) K. Sridith (bell-shaped and 5-merous flower group) (Figure 18b).

Table 1 Chromosome numbers of *Argostemma* spp. in Thailand.

Morphological characteristics		**Scientific name**	**2n**	**n**
Corolla shape	**Number of corolla lobe**			
Star-shaped	5-merous	*A. argostemon*	22	
		A. condemsum	22	
		A. dispar	22	
		A. diversifolium	22	
		A. elatostemma	22	
		A. kurzii	22	11II
		A. laeve ssp. *setosum*	22	11II
		A. laxum	22	
		A. ophirense	22	
		A. pictum	22	
		A. propinquum	22	
		A. rotundicalyx	22	
		A. subcrassum	22	
		A. unifolioides var. *glabra*	22	
		A. verticillatum	22	
	4-merous	*A. khasianum*	22	
Bell-shaped	5-merous	*A. ebracteolatum* ,	22	
		A. lobulatum var. *variabile*	22	
		A. puffii	22	
	4-merous	*A. neurocalyx*	22	
		A. neurosepalum	22	
		A. plumbeum	22	

Star-shaped and 5-merous flower

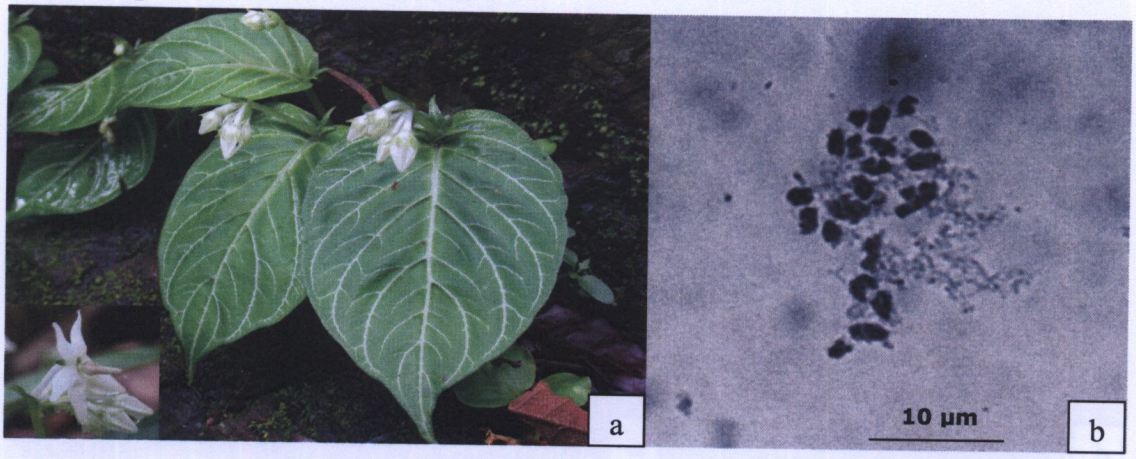

Figure 1 *Argostemma argostemon* K. Sridith

a. Habit b. Chromosome numbers 2n = 22

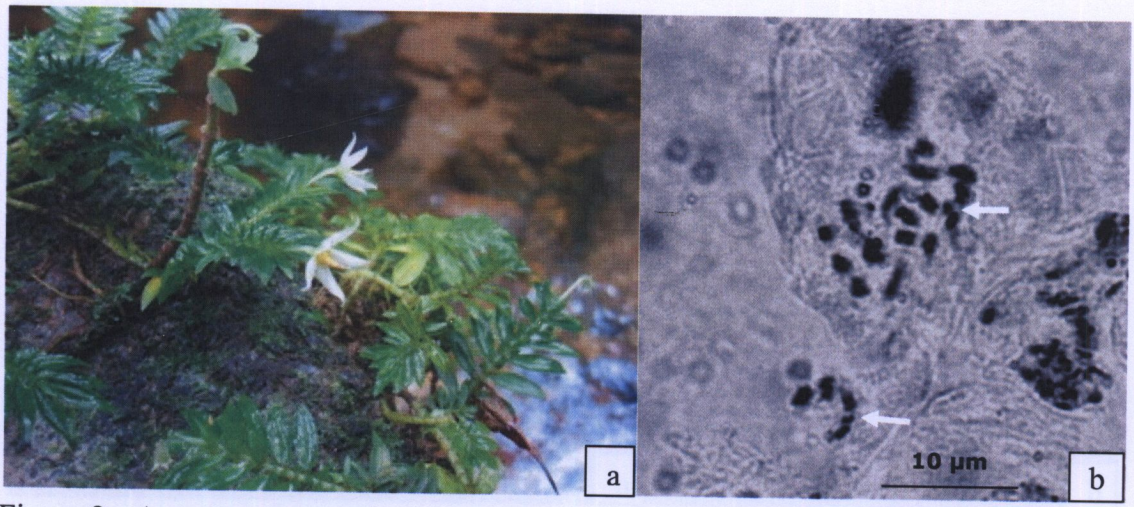

Figure 2 *Argostemma condensum* Craib

a. Habit b. Chromosome numbers 2n = 22

Arrows point out the satellites

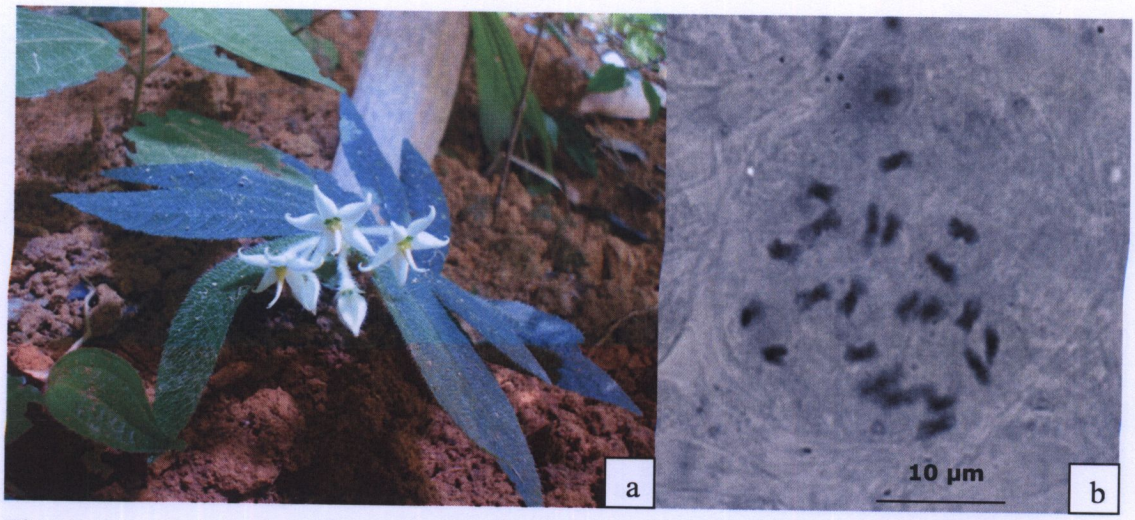

Figure 3 *Argostemma dispar* Craib

a. Habit b. Chromosome numbers 2n = 22

Figure 4 *Argostemma diversifolium* Ridl.

a. Habit b. Chromosome numbers 2n = 22

Arrows point out the satellites

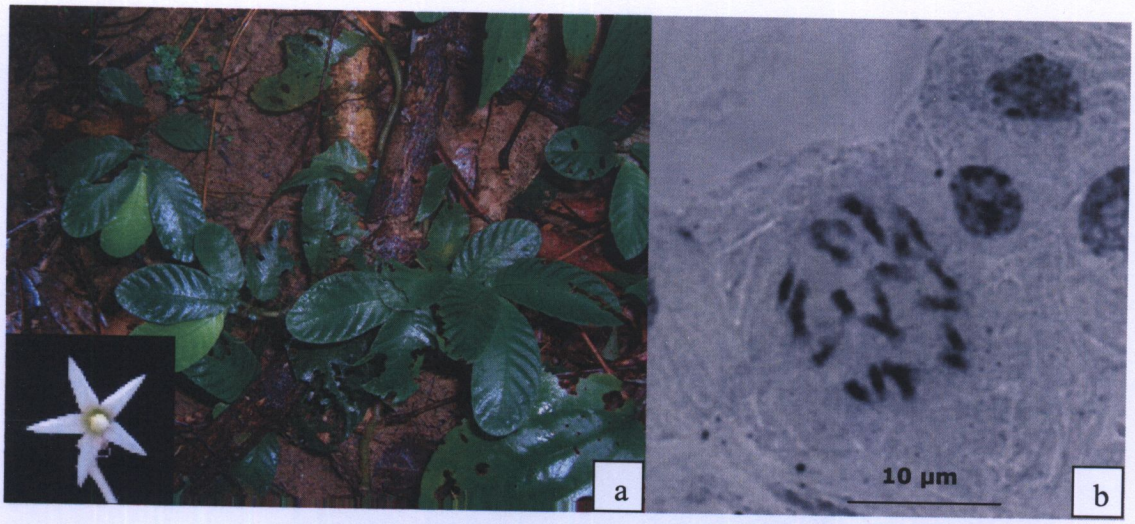

Figure 5 *Argostemma elatostemma* Hook.f.

a. Habit b. Chromosome numbers 2n = 22

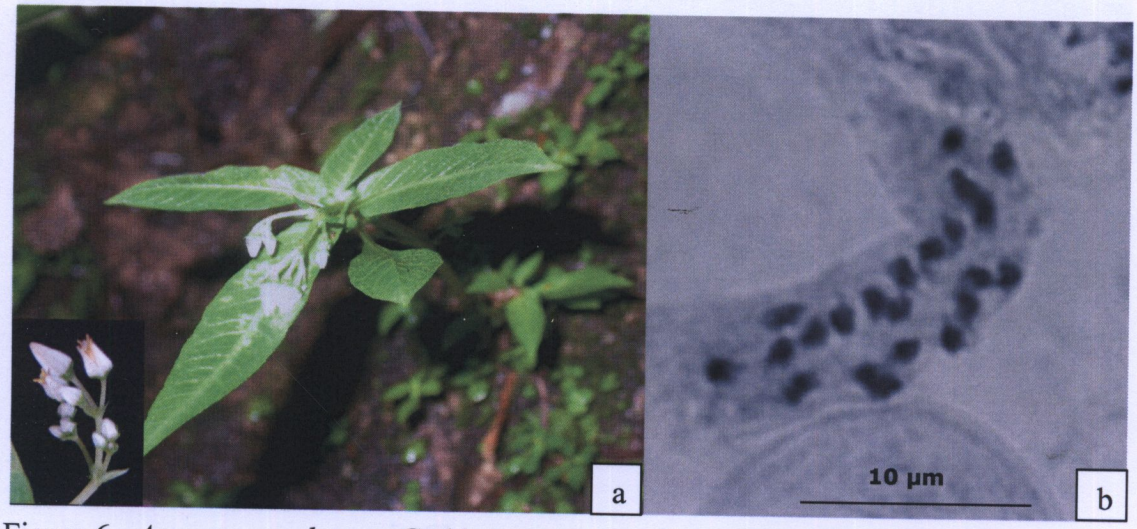

Figure 6 *Argostemma laxum* Geddes

a. Habit b. Chromosome numbers 2n = 22

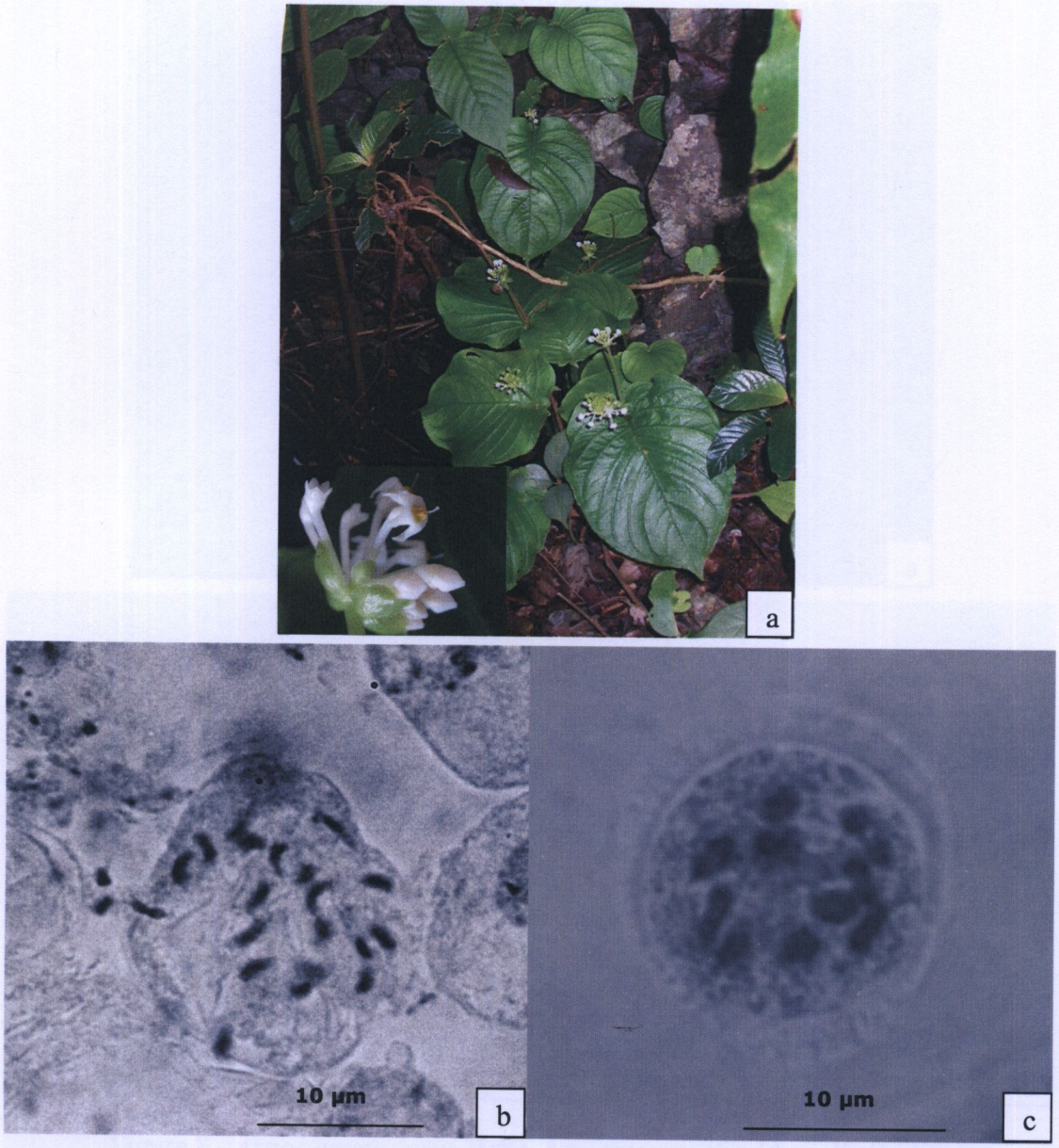

Figure 7 *Argostemma kurzii* C.B. Clark

a. Habit

b. Chromosome numbers 2n = 22

c. Microsporocyte shows 11II

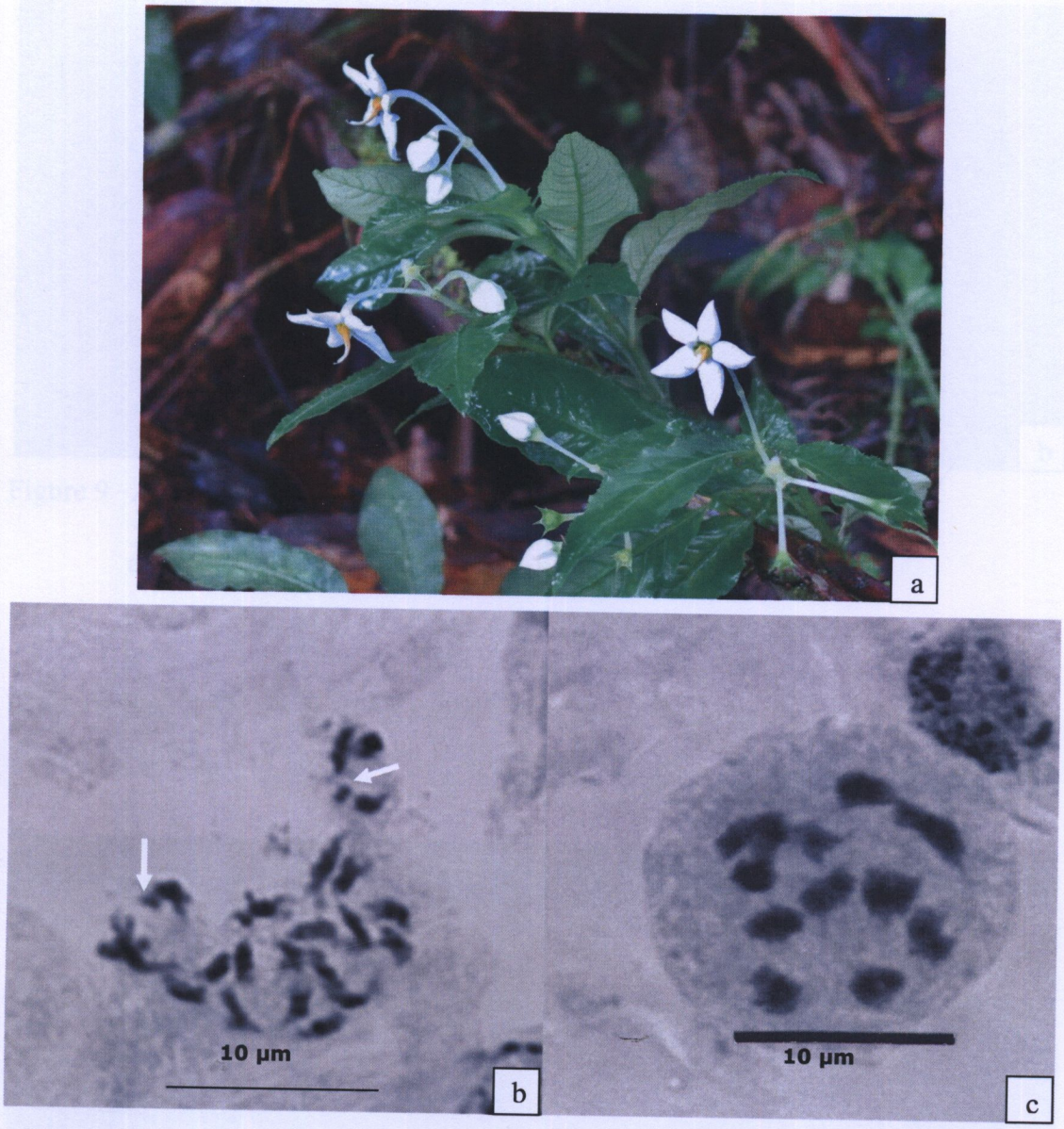

Figure 8 *Argostemma laeve* Benn. ssp. *setosum* (Geddes) K. Sridith

a. Habit

b. Chromosome numbers 2n = 22

c. Microsporocyte shows 11II

Arrows point out the satellites

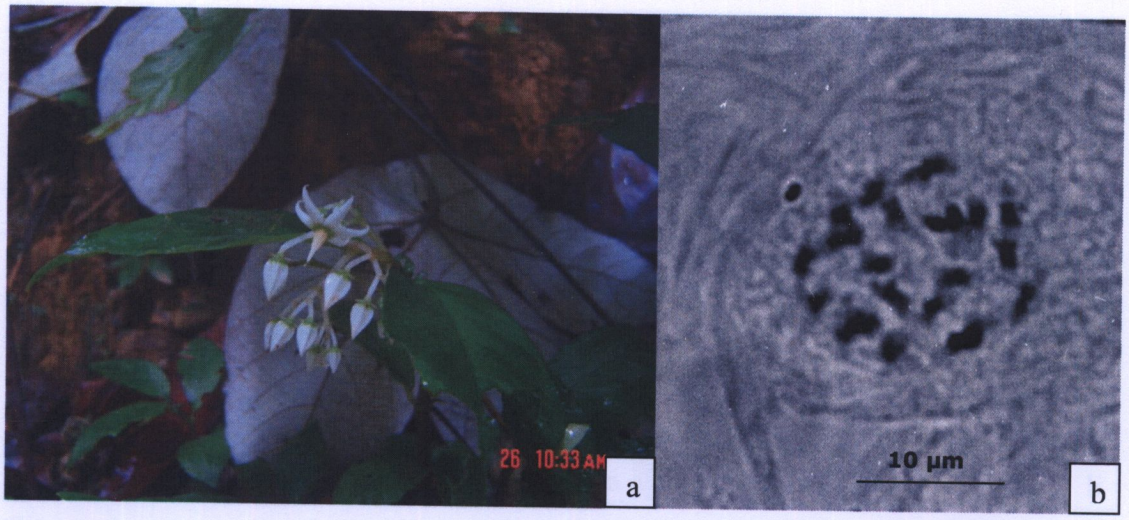

Figure 9 *Argostemma ophirense* Maingay ex Hook.f.

a. Habit b. Chromosome numbers 2n = 22

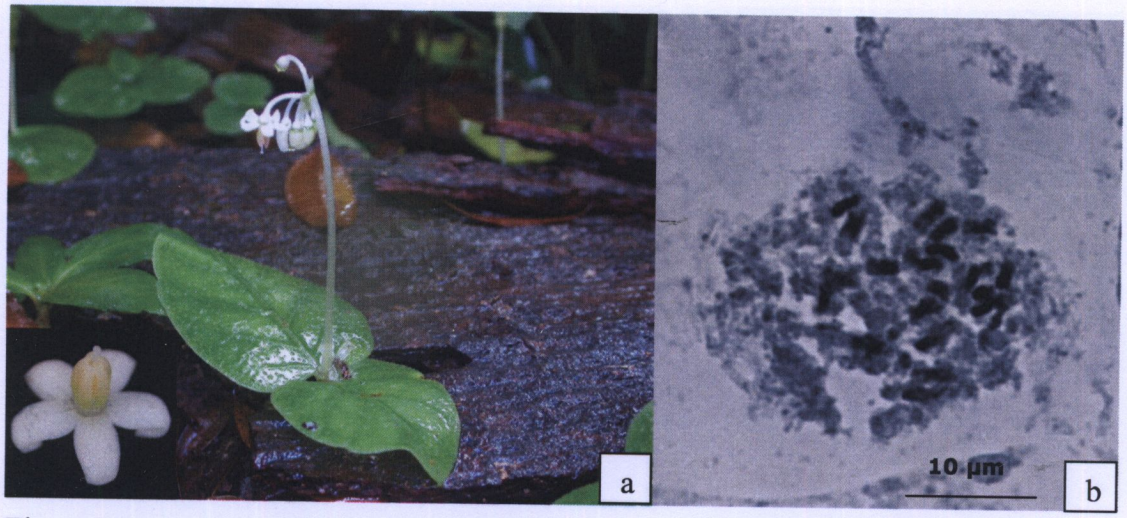

Figure 10 *Argostemma pictum* Wall.

a. Habit b. Chromosome numbers 2n = 22

Figure 11 *Argostemma propinquum* Ridl.

a. Habit b. Chromosome numbers 2n = 22

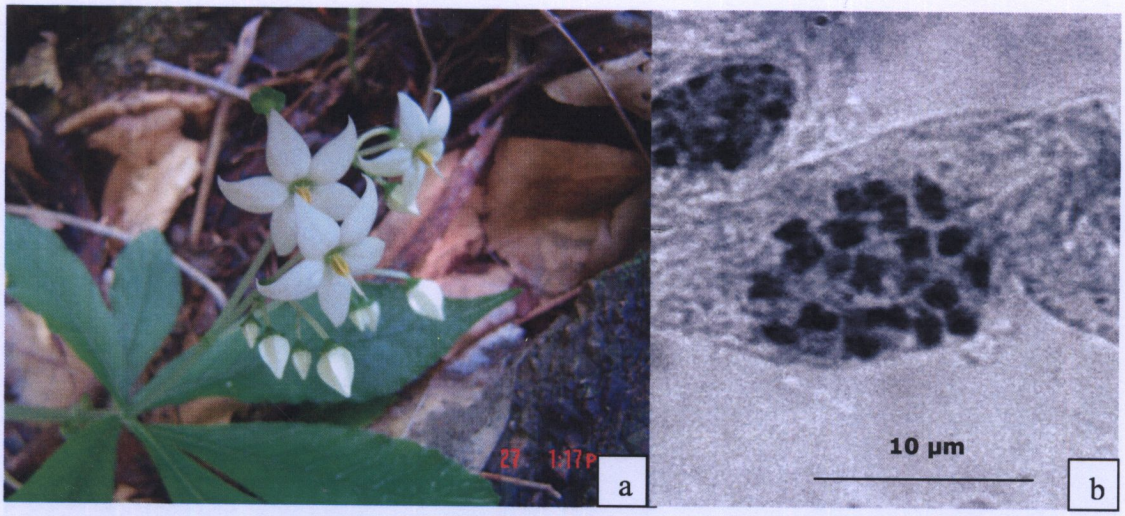

Figure 12 *Argostemma rotundicalyx* K. Sridith

a. Habit b. Chromosome numbers 2n = 22

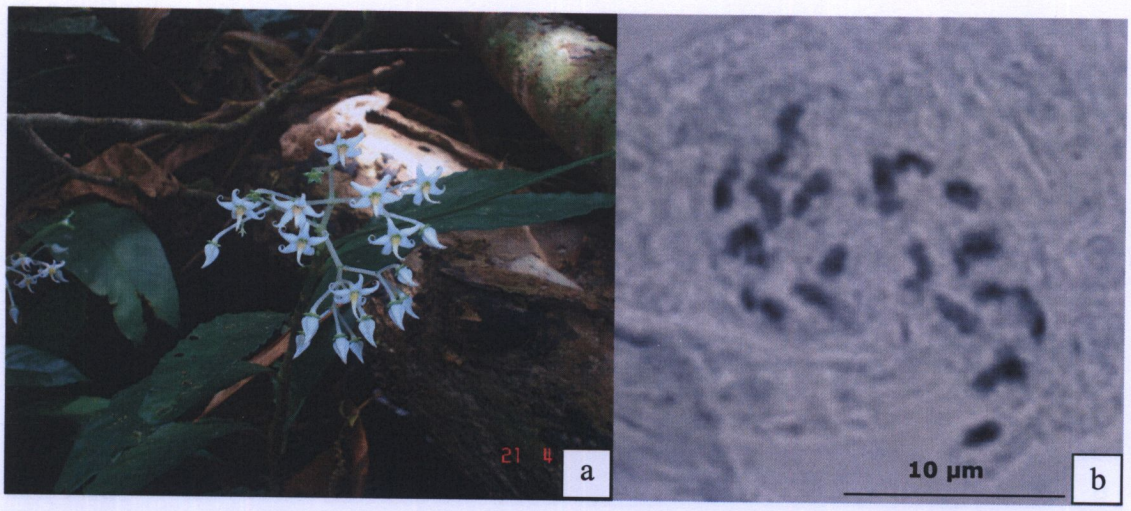

Figure 13 *Argostemma subcrassum* King

a. Habit b. Chromosome numbers 2n = 22

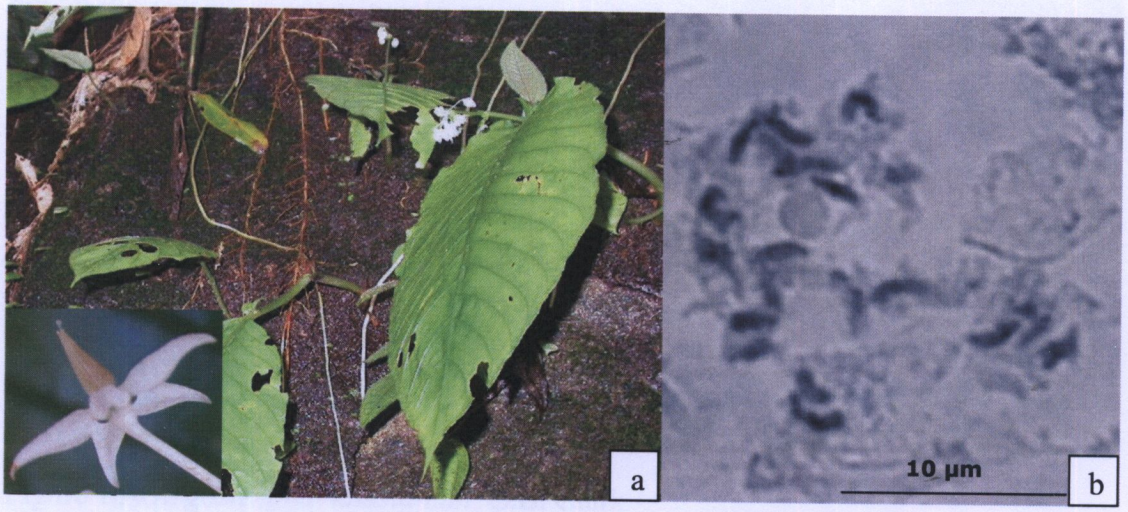

Figure 14 *Argostemma unifolioides* var. *glabra* King

a. Habit b. Chromosome numbers 2n = 22

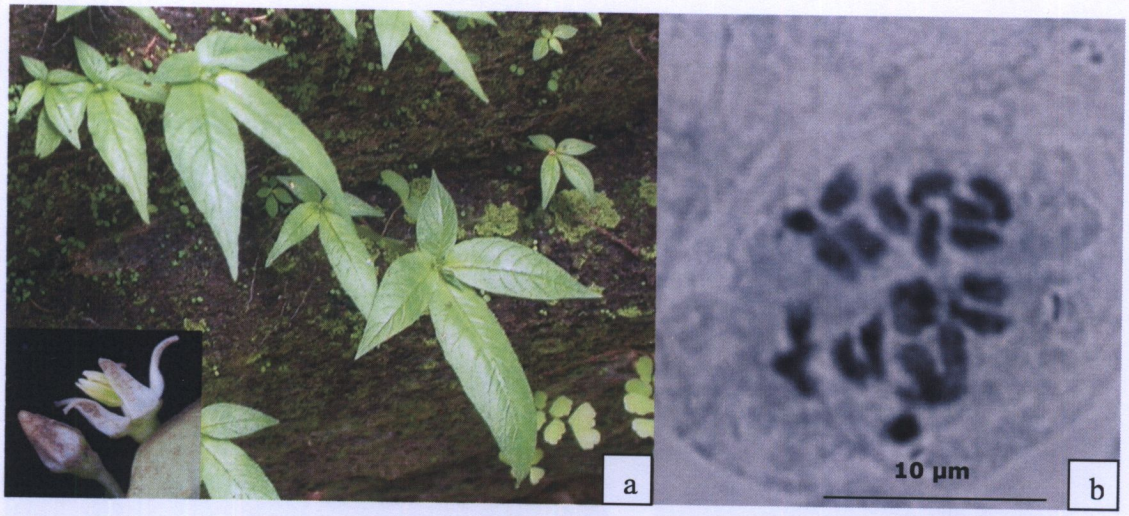

Figure 15 *Argostemma verticillatum* Wall.

a. Habit b. Chromosome numbers 2n = 22

Star-shaped and 4-merous flower

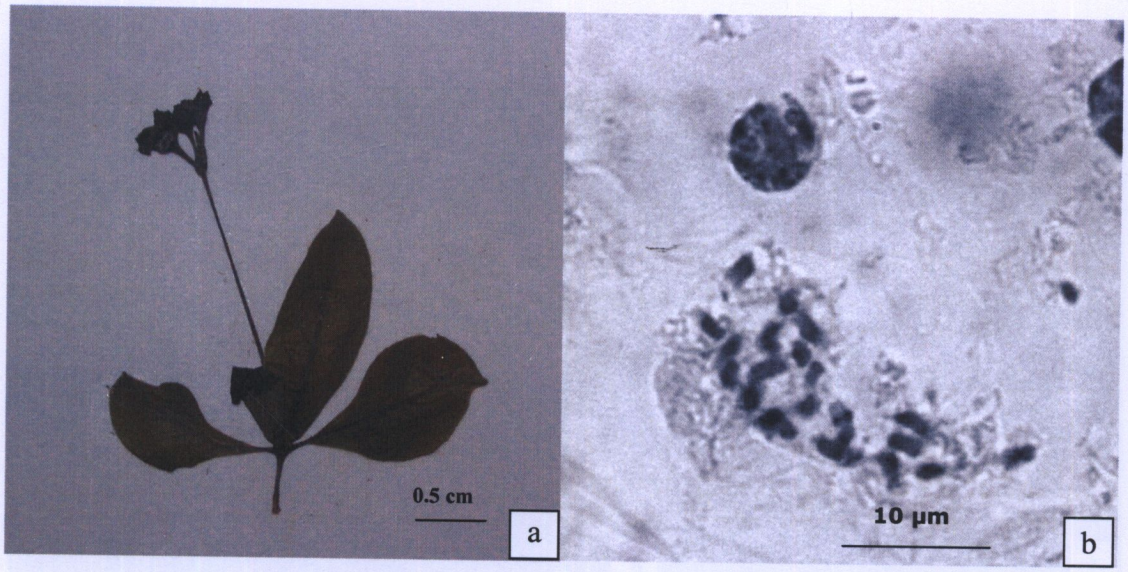

Figure 16 *Argostemma khasianum* C.B. Clark

a. Habit b. Chromosome numbers 2n = 22

Bell-shaped and 5-merous flower

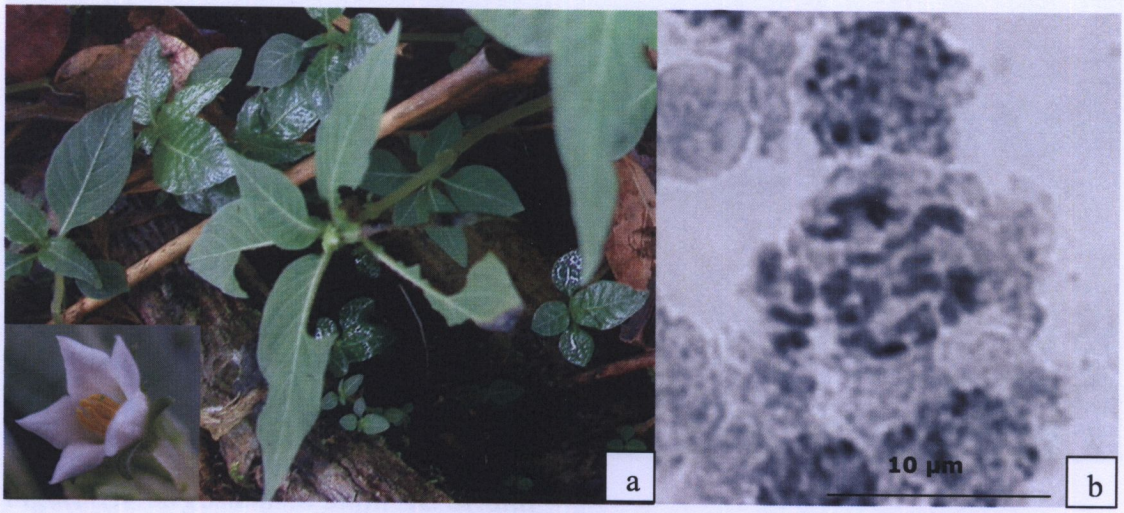

Figure 17 *Argostemma ebracteolatum* Geddes

a. Habit b. Chromosome numbers 2n = 22

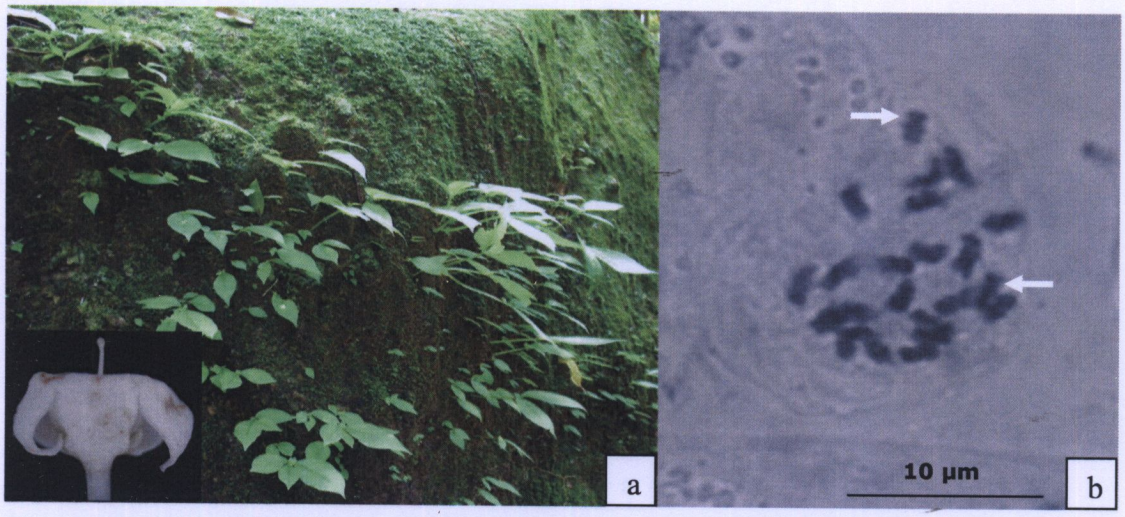

Figure 18 *Argostemma lobulatum* var. *variabile* (Geddes) K. Sridith

a. Habit b. Chromosome numbers 2n = 22

Arrows point out the secondary constrictions

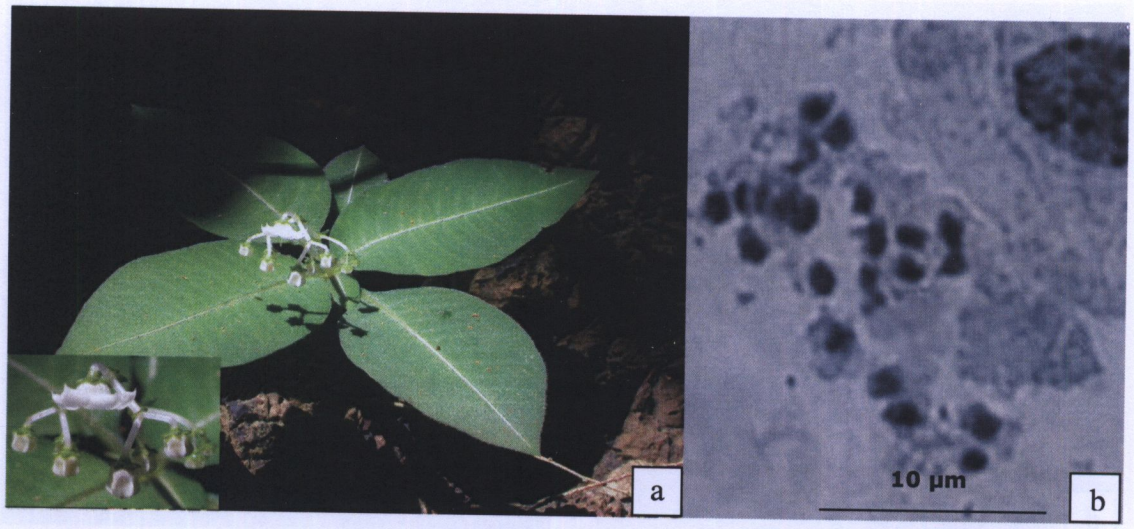

Figure 19 *Argostemma puffii* K. Sridith

a. Habit b. Chromosome numbers 2n = 22

Bell-shaped and 4-merous flower

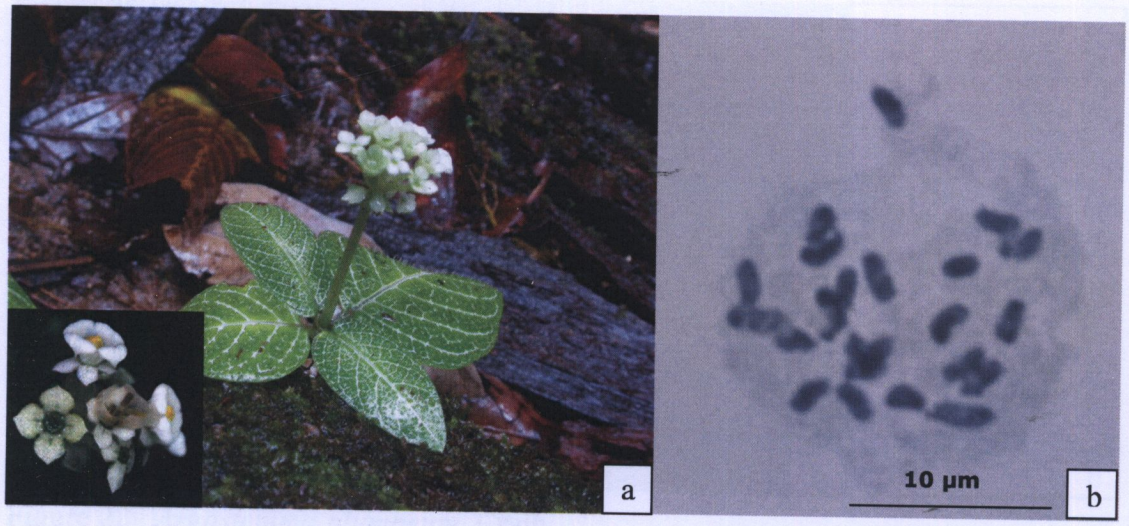

Figure 20 *Argostemma neurocalyx* Miq.

a. Habit b. Chromosome numbers 2n = 22

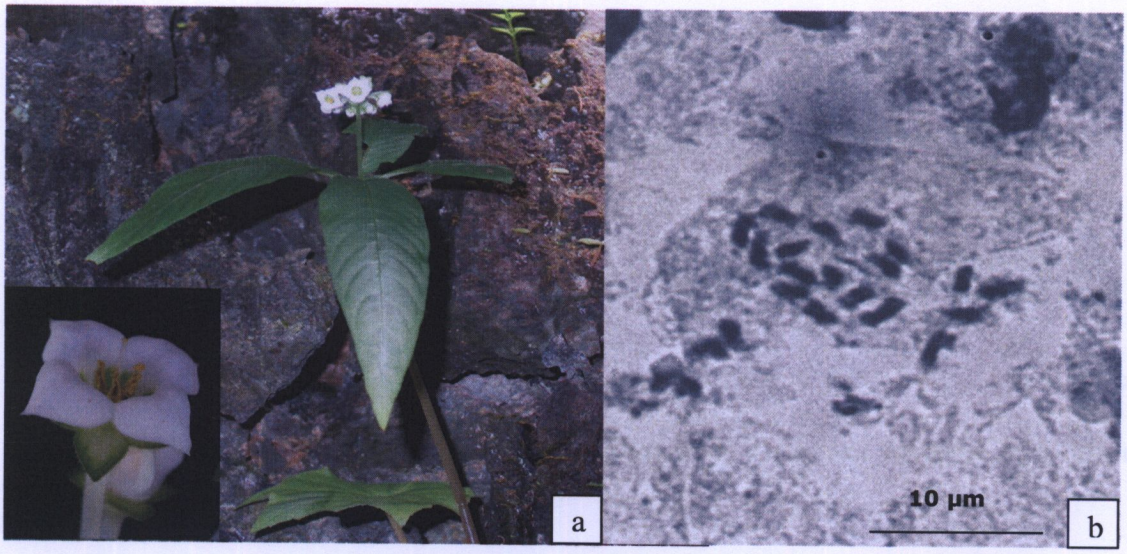

Figure 21 *Argostemma neurosepalum* Bahk.f.

a. Habit b. Chromosome numbers 2n = 22

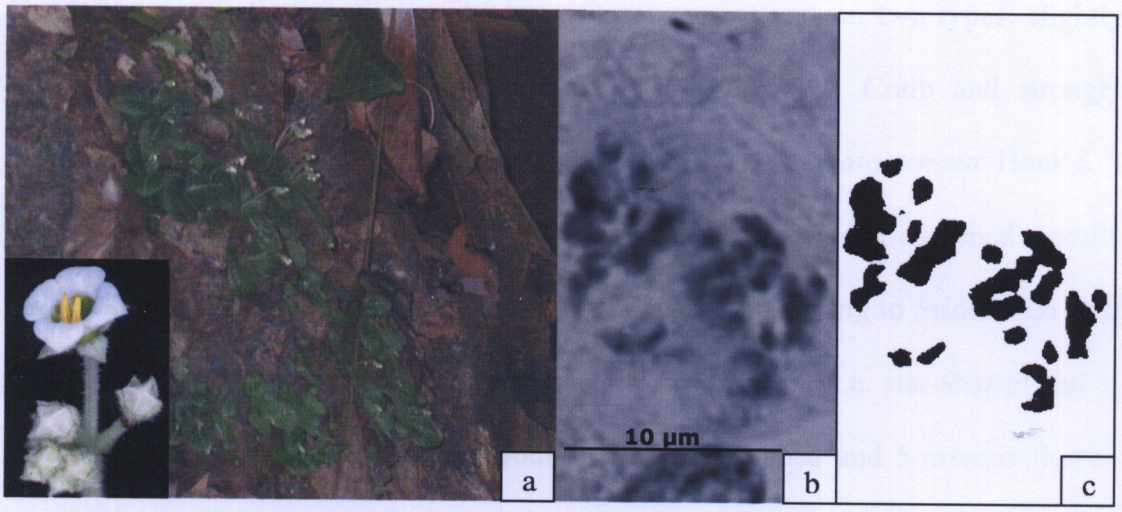

Figure 22 *Argostemma plumbeum* Craib

a. Habit b. Chromosome numbers 2n = 22 c. Drawing

CHAPTER 5

DISCUSSIONS

Taxa in the present study

Twenty-two taxa of *Argostemma* in Thailand have been collected to achieve the karyological studies, which are 66.66 % of Thai taxa reported by Sridith (1999[b]).

The morphological characters of *Argostemma* exhibit high variation. In the present study, both vegetative and reproductive organs play the greatly different appearances.

The growth forms vary in leafy stems (Sridith, inpress[a]) e.g. *A. condensum* Craib, *A. dispar* Craib and pseudo-verticilate e.g. *A. ebracteolatum* Geddes, *A. verticillatum* Wall.. The leaves arrangement vary in two types: slightly anisophyllous leaves e.g. *A. neurocalyx* Miq., *A. plumbeum* Craib and strongly anisophyllous leaves e.g. *A. unifolioides* var. *glabra* King, *A. elatostemma* Hook.f.

The reproductive organs show two types of distinguished corolla shapes: the star-shaped corolla and bell-shaped corolla. According to Sridith and Puff (2001), who have proposed four major groups in *Argostemma*, i.e. star-shaped and 5-merous flower, star-shaped and 4-merous flower, bell-shaped and 5-merous flower, bell-shaped and 4-merous flower. In this study, almost of star-shaped corolla are found anthers fused and forming an anther cone like structure with the anthers opening by longitudinal slits (Sridith & Puff, 2001). These characters are found in ten taxa: *A. condensum* Craib, *A. dispar* Craib, *A. elatostemma* Hook.f., *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith, *A. ophirense* Maingay ex Hook.f., *A. propinquum*

Ridl., *A. rotundicalyx* K. Sridith, *A. subcrassum* King, *A. unifolioides* var. *glabra* King and *A. khasianum* C.B. Clark (the only one star-shaped species which have 4-merous). But some star-shaped species the stamens are free with anthers opening by apical pores (Sridith, inpress[a]). These characters are found in six taxa: *A. argostemon* K. Sridith, *A. diversifolium* Ridl., *A. kurzii* C.B. Clark, *A. laxum* Geddes, *A. pictum* Wall. and *A. verticilatum* Wall., except *A. argostemon* K. Sridith and *A. pictum* Wall. which the anthers opening by longitudinal slits and *A. verticilatum* Wall. the anthers opening by oblique elongate pores. All of the bell-shaped flower are found with free stamens and anthers opening by apical pores (*sensu* Sridith, inpress). These characters are found in six taxa from both 4-and 5-merous flower: *A. ebracteolatum* Geddes, *A. lobulatum* var. *variabile* Geddes, *A. puffii* K. Sridith, *A. neurocalyx* Miq., *A. neurosepalum* Bahk.f. and *A. plumbeum* Craib.

Moreover, there are two addition records to the previous list by Sridith (1999b). The newly described species are star-shaped corolla. *A. argostemon* K. Sridith, free stamens and opening by longitudinal slits, distinguish from the others by white anthers. The other one is a new record to Thailand, *A. kurzii* C.B. Clarke. The corolla shape of this species is different from the others by the semi star-shaped as corolla tube and shorter than corolla lobes, free stamens with opening by apical pores.

According to the tremendous diversity, the infrageneric division was proposed by many taxonomists, in the last five decades, based on vegetative characters and floral features. The taxonomic status of this genus is discussed in cytological aspect in order to support the relationships between various taxa in the genus.

Karyological study in *Argostemma* Wall.

Chromosome numbers

All twenty-two taxa from four morphological groups (star-shaped and 5-merous flower, star-shaped and 4-merous flower, bell-shaped and 5-merous flower, bell-shaped and 4-merous flower) have the same somatic chromosome numbers as 2n = 22. These chromosome numbers agree with six taxa which were reported (Mangenot & Mangenot, 1962; Hellmayr *et al.*, 1994; Kiehn, 1995; Puangsomlee & Puff, 2001). The chromosome numbers of three species revealed that *A. diversifolium* Ridl., *A. pictum* Wall. and *A. neurocalyx* Miq., are coincident with Puangsomlee and Puff (2001). The microsporocytes of *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith and *A. kurzii* C.B. Clark showed 11 bivalents at first metaphase cells. This number agrees with previous reports of *Argostemma*'s basic chromosome numbers x = 11 (Kiehn, 1996; Puangsomlee & Puff, 2001). From the data suggested that *Argostemma* in Thailand are diploid with basic chromosome numbers x = 11 (2n = 2x = 11).

A. verticillatum Wall. from Thailand showed chromosome numbers of 2n = 22, which differed from the report of Khoshoo & Bhatia (1963). This chromosome number n = 14 of *A. verticillatum* Wall. was achieved from Himalayan materials. The contradiction with the previous study is probably due to the misidentifying or the difficulties in obtaining proper methods to determine chromosomal identities remained the imprecise counting in Rubiaceae. So commented by Kiehn (1995). In any case, there were reports of different chromosome numbers in a given species from different geographic ranges of variations (Soejima & Peng, 1998). Moreover, the nondisjunction in asexual reproduction could be one cause of the different chromosome numbers in plants. Concerning the herbaceous plants with

rhizomes like *Argostemma*, Sharma & Bhattacharya (1969) suggested that the origin of different basic chromosome numbers in various genera of Zingiberaceae, a herbaceous taxon which propagate with rhizomes are connected with asexual reproduction.

The meiotic chromosomes of all twenty-two taxa were investigated, however, only two taxa were achieved. This might due to the time of plant collecting is not coincident with the proper flower-development period (too old developed flowers). In addition, most *Argostemma* have flowers once a year (annual herbs) and the downfall in cultivation together with the limiting time of this research, the numbers of taxa with success in meiotic chromosome study in this work are also limited.

New chromosome counts of *Argostemma* are reported for eighteen taxa, i.e. star-shaped and 5-merous flower group: *A. argostemon* K. Sridith, *A. condensum* Craib, *A. dispar* Craib, *A. elatostemma* Hook.f., *A. kurzii* C.B. Clark, *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith, *A. laxum* Geddes, *A. ophirense* Maingay ex Hook.f., *A. propinquum* Ridl., *A. rotundicalyx* K. Sridith, *A. subcrassum* King and *A. unifolioides* var *glabra* King; star-shaped and 4-merous flower group: *A. khasianum* C.B. Clark; bell-shaped and 5-merous flower group: *A. ebracteolatum* Geddes, *A. lobulatum* Craib var. *variabile* K. Sridith and *A. puffii* K. Sridith; bell-shaped and 4-merous flower group: *A. neurosepalum* Bahk.f. and *A. plumbeum* Craib.

Chromosome size and shape

Most of four morphological groups seem to have chromosomal similarity in size and shape. The chromosomes are relatively small and clump together in metaphase as in many taxa of Rubiaceae (Kiehn, 1995). The difficulty in observing the centromeres is an obstruction to study the karyotype of *Argostemma*.

The satellites were usually found in one or two pairs of chromosomes in Rubiaceae (Kiehn, 1995). Naiki and Nagamasu (2004) reported that one pairs of satellite chromosomes were found in six taxa and two pairs of satellite chromosomes were found in one taxa of *Damnacanthus* C. F. Gaertn.. Four taxa of *Argostemma*, i.e. *A. condensum* Craib, *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith and *A. diversifolium* Ridl. and *A. lobulatum* Craib var. *variabile* K. also possess one pair of satellite chromosomes.

The satellites, however, are difficult to be observed in metaphase, whereas in prophase or pro-metaphase are obvious to be distinguished in those taxa as in *Sipanea hispida* Benth. Ex Wernham (Rubiaceae) (Kiehn, 1995). Additionally, more details information on satellites of other *Argostemma* 's chromosomes may be needed for supporting the classification in the genus level as it was achieved in *Citrus* L. (Guerra *et al.*, 1997) and *Hordeum* L. (Linde-Laursen *et al.*, 1995).

Recently, the banding techniques are used to distinguish the differences between chromosomes and allow more details comparisons between complements of different taxa (Stuessy, 1989). Although, Kiehn (1995) reported the failure in Giemsa technique to various taxa of Rubiaceae with small chromosomes size and/or the interfering of the presence of tannin, the chromosomal differentiate of

small size and uniform chromosomes in *Coffea* L. (Rubiaceae) could be distinguished by the C and NOR banding with the DAPI and CMA_3 (Pinto-Maglio, 2006). Thereafter, the chromosomes study of *Argostemma*, which have small chromosomes by banding techniques might be achieved.

Infrageneric relationship of *Argostemma* Wall. according to the karylogical point of view

The same chromosome numbers in a genus is an evidence of the natural groups in most cases, i.e. *Zingiber*: the 27 species are 2n = 22 (Beltran and Kiew, 1984; Chen, 1989; Saensouk, 2000; Eksomtramage *et al.*, 2001; Eksomtramage *et al.*, 2002; Augsonkitt *et al.*, 2004) and *Lathylus* 2n=14 (Seijo & Fernández, 2003). In this study, chromosome data has supported that *Argostemma* could be a natural group. The regular cell division in meiosis could be assumed that the genus is normal fertile in their natural habitat. The variation within the genus is rather high which could be easily seen from the morphological variations, in spite of the chromosomal similarities according to the karyological view points. The morphological variations are possibly due to the physical environments (Fosket, 1994) or changing in the gene or molecular level as in *Jatropha* L. (Soontornchainaksaeng & Chaiyasut, 1999). Currently, the molecular techniques have been considered useful to support the relationship among taxa. Vanijajiva *et al.* (2003) have studied the molecular phylogenetic in Zingiberaceae by using isozyme analysis and RAPD technique. The isozyme patterns and RAPD fingerprintings have indicated that *Bosenbergia* is closer to *Scaphochlamys* than to *Kaempferia*. Therefore, the morphological differences

among the populations or between the taxa in the genus might be investigated more with isozyme analysis technique.

The chromosome data in the present work suggested that the genus *Argostemma* might remain a good taxon. And the infrageneric division of the genus might not be necessary at the moment.

Note on chromosomal studying technique

The sources of mitotic data commonly are from meristem of shoots, young leaves and roots (Stuessy, 1989). But in this study, the somatic chromosomes could not be achieved from root-tips due to their fibrilliform roots that always attached to substrate, especially in the species with tubers which are often found specificly to limestone areas. The cultivation of the collected plants to get root-tips were impossible due to the specific environments needed, such as high humidity; low temperature etc. Nevertheless, the new finding technique of chromosomal study for this genus was proposed here. All of the somatic chromosomes were investigated from *corolla part of the young flowering buds*. The suitable corolla length was 1-3 mm. And it is to be affirmed that the metaphase cells would be detected in the flowering buds at the age of the length 1-3 mm only. From the study, the division of the nucleus were rarely found in more than 3 mm corolla length. Additionally, the region of cell division is at the base of the corolla whereas the other corolla regions are rarely found especially at corolla tips.

The tannins are found in some species of *Argostemma*, i.e. *A. argostemon* K. Sridith, *A. ebracteolatum* Geddes, *A. kurzii* C. B. Clarke, *A. laxum* Geddes, *A. ophirense* Maingay ex Hook.f., *A. pictum* Wall., *A. plumbeum* Craib, *A.*

propinquum Ridl., *A. puffii* K. Sridith, *A. rotundicalyx* K. Sridith, *A. subcrassum* King and *A. unifolioides* var. *glabra* King as in many Rubiaceae (Kiehn, 1995). The self-tanning effects are often encountered in chromosome fixations by using Carnoy's solution, resulting in changing the tissues to tan color (Figures 23-26 in appendix) and reducing the stainability. These problems could be avoided by using formaldehyde as a fixative (Greilhuber, 1988). Anyhow, the hydrolysis process for Feulgen staining may be necessary in somatic chromosomes study from root-tips or other tissues (Sharma & Sharma, 1980). But in this study, the staining without hydrolysis process could be obtained even though it was interfered by self-tanning effects. These outcomes were due to the thin and soft tissues of corolla parts. The tissues could be stained at least 5 minutes to 5 hours, depending on the tissues of a given species. The over staining resulted that the dye could be imbued thoroughly to the cytoplasm.

CHAPTER 6

CONCLUSION

1. Twenty two taxa of *Argostemma* in Thailand have been collected. Sixteen taxa are in star-shaped corolla group and six species are in bell-shaped corolla group. Among those, two species i.e. *A. argostemon* K. Sridith and *A. kurzii* C.B. Clarke are two new records to the previous list.

2. Chromosomal study in *Argostemma* Wall.

2.1 All 22 taxa of Thai *Argostemma* were diploid with the same somatic chromosome numbers 2n = 22. Eighteen taxa were reported for the first time: *A. argostemon* K. Sridith, *A. condensum* Craib, *A. dispar* Craib, *A. elatostemma* Hook.f., *A. khasianum* C.B. Clark, *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith, *A. ophirense* Maingay ex Hook.f., *A. propinquum* Ridl., *A. rotundicalyx* K. Sridith, *A. subcrassum* King, *A. unifolioides* var. *glabra* King. *A. kurzii* C.B. Clark, *A. laxum* Geddes, *A. ebracteolatum* Geddes, *A. lobulatum* Craib var. *variabile* K. Sridith, *A. neurosepalum* Bahk.f., *A. plumbeum* Craib and *A. puffii* K. Sridith

2.2 *A. verticilatum* Wall.: its chromosome numbers are different from the previous report.

2.3 Satellites on one pair of chromosomes were found in four taxa : *A. condensum* Craib; *A. laeve* Benn. ssp. *setosum* (Geddes) K. Sridith; *A. diversifolium* Ridl. and *A. lobulatum* Craib var. *variabile* K. Sridith.

3. The chromosome data in the present work suggested that the genus *Argostemma* might remain a good taxon. And the infrageneric division of the genus might not be necessary at the moment.

4. A new technique for studying somatic chromosomes of *Argostemma* is to use corolla part of the young flowering buds of the length 1-3 mm.

Problems and Suggestions

1. In this study, 0.1% colchicine, PDB and α-Bromonaphthalene were used for pretreatment but the two latter were not succeeded. Although, somatic chromosome numbers study in *Argostemma* have been achieved by using 0.1% colchicine, but the proper pretreatment methods for chromosome number studies in *Argostemma* might be needed in order to achieve better results of metaphase chromosome complements.

2. The flowering period of *Argostemma* did not coincide with some of the collecting times therefore many more *Argostemma* species in Thailand have not been collected and studied in the present work. Moreover, the disturbances of the natural habitats of *Argostemma* spp. were the big problems in finding them according to the previous records.

3. *Argostemma* spp. are very fragile herbs, confine only to the primary forest in moist/wet habitats. It is nearly impossible to grow them in the greenhouse to get flowers or roots for chromosome study. All of the investigations in the present work had been undertaken from the fresh material collected from the natural habitats.

4. The molecular level of DNA in different populations of various *Argostemma* spp. should be proved that it is genetically, or according to this physical environment. Then further discussion on relationship of the taxa in the genus, based on those morphological differences, could be provided better understanding of the classification in the genus.

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APPENDIX

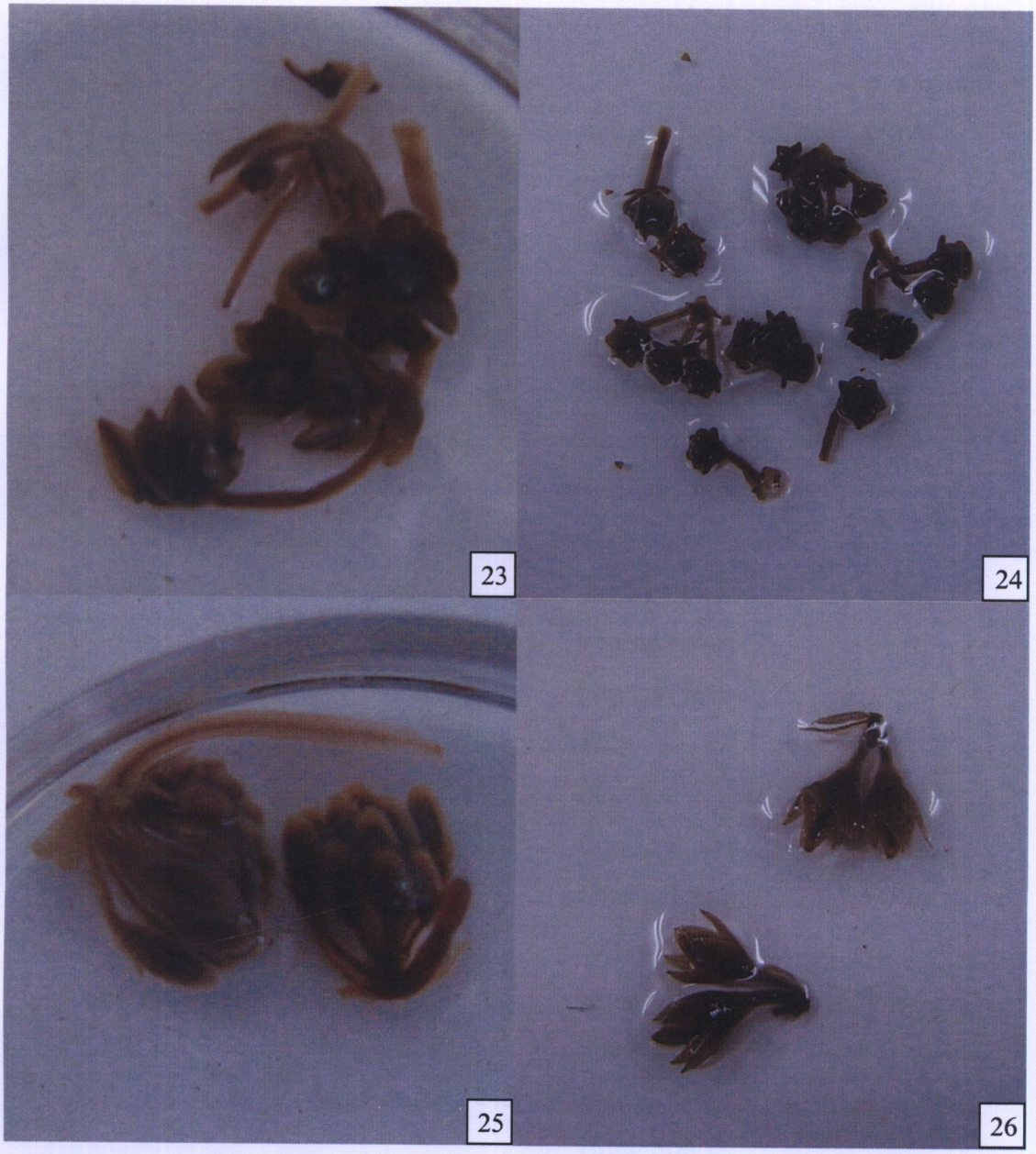

Figure 23-26 The *Argostemma* tissues were changed to tan color.

Fig.23 *A. unifolioides* var. *glabra* King

Fig.24 *A. puffii* K. Sridith

Fig.25 *A. pictum* Wall.

Fig.26 *A. diversifolium* Ridl.

Appendix Table1 List of scientific name, specimen number, specimen locality

scientific name	specimen number	locality	provenance
1. *A. argostemon* K. Sridith	Saithip & K. Sridith & K. Maneenoon 047	Pha-Sua Fall National Park, Muang, Mae Hong Son. N 19° 28' 51.3", E 97° 57' 51.0" Altitude 1,217 m	PSU
2. *A. condensum* Craib	K. Sridith 732	Ton-Nga-Chang Fall, Had Yai, Songkla	PSU
3. *A. dispar* Craib	J. Wai 48	Ban Piyamitr 2, Betong forest Reserve, Betong, Yala	PSU
4. *A. elatostemma* Hook.f.	Saithip 039	Ban-Tha-Khoa, Tum Bon Li Phang, Pra Lhean, Trang Altitude 200 m.	PSU
5. *A. khasianum* C.B. Clark	Saithip & K. Sridith & K. Maneenoon 045	stream opposite Pha Sua Fall National Park, Muang, Mae Hong Son	PSU
6. *A. leave* Benn. subsp. *setosum* (Geddes) K. Sridith	Saithip 001, Saithip 031	Khoa-Ram-Rom, Ronpiboon,Nakornsri thammarat Altitude 928 m.	PSU, K, BKF
7. *A. ophirense* Maingay ex Hook.f.	Saithip 038	Ban Tha Khoa, Tum Bon Li Phang, Pra Lhean, Trang Altitude 200 m.	PSU

Appendix Table 1 (Continued)

scientific name	specimen number	locality	provenance
8. *A. propinquum* Ridl.	Saithip 028, Saithp 040, Saithip& K. Maneenoon & J.Wai 041	Khoa-Ram-Rom, Ronpiboon,Nakornsri thammarat Altitude 928 m.	PSU, BKF, L, E, KE, SING
9. *A. rotundicalyx* K. Sridith	Saithip 053	Trail behide Klong Panom National Park office, Ban Ta Khun, Surathanee	PSU, BKF, L, E, KE, SING
10. *A. subcrassum* King	Saithip & J. Wai 037, J. Wai 31	Ban Chantharat-Ban Hua Muang, Sirikit Forest (Hala) edge, Betong, Yala Altitude 500 m.	PSU, BKF, SING, L
11. *A. unifolioides* var.*glabra* King	Saithip 054, K. Sridith 746	Sirinthorn Fall, Bala-Hala National Park, Wang, Narathiwad	PSU
12. *A. diversifolium* Ridl.	Saithip & K. Sridith & K. Maneenoon 046	Trail behind Pachumyothee Temple, Muang, Pang-Nga	PSU, K, BKF, L, E
13. *A. kurzii* C.B. Clark	Saithip & K. Sridith & K. Maneenoon 045	Trail behind Pachumyothee Temple, Muang, Pang-Nga	PSU, BKF, SING, L
14. *A. laxum* Geddes	Saithip & K. Sridith & K. Maneenoon 051	road side, on the way to Mae Jam, Doi Inthanon,Chaing Mai. N 18° 32' 7.9" E098° 31' 26.7" Alt. 1,243m	PSU, BKF, L

Appendix Table1 (Continued)

scientific name	specimen number	locality	provenance
15. *A. pictum* Wall.	Saithip 004, Saithip 017	Prai-Sa-Wan Fall, Pra Lhean, Trang; Ton-Nga-Chang Fall, Had Yai, Songkla Altitude 200 m.	PSU, BKF
16. *A. verticillatum* Wall.	Saithip & K. Sridith & K. Maneenoon 052	Monthatharn Fall, Doi Inthanon, Chiang Mai	PSU, BKF
17. *A. ebracteolatum* Geddes	Saithip & K. Sridith & K. Maneenoon 048	Pha-Sua Fall National Park, Muang, Mae Hong Son. N 19° 28' 51.3", E 97° 57' 51.0" Altitude 1,217 m	PSU
18. *A. lobulatum* var. *variabile* Geddes	Saithip 006, Saithip 019	Prai-Sa-Wan Fall, Pra-Lhean, Trang	PSU, BKF
19. *A. neurocalyx* Miq.	Saithip 014, Saithip 018, Saithip & K. Sridith & K. Maneenoon 042	Ton-Tok Fall and Prai-Sa-Wan Fall, Pra Lhean, Trang; Ramun Fall, Muang,Pang-Nga Altitude 135 m.	PSU, BKF
20. *A. neurosepalum* Bahk.f.	Saithip & K. Sridith & K. Maneenoon 044	Trail behind Prachumyothee Temple, Muang, Pang-Nga	PSU, BKF, L, E, KE
21. *A. plumbeum* Craib	Saithip & K. Sridith & K.	Thum-Pla National Park, Muang, Mae-	PSU

Appendix Table1 (Continued)

scientific name	specimen number	locality	provenance
	Maneenoon 050	Hong-Son. N 19° 25' 39.0", E 079° 59' 24.6" Altitude 296 m	
22. *A. puffii* K. Sridith	Saithip 025	Khoa-Toh-Ngay, Petra National Park, La-Ngu,Satun	PSU, K, BKF

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List of Publication and Proceeding

1. Saithip Aphinyanan, Ladda Eksomtramage, Kitichate Sridith. 2004. **Chromosome study of selected *Argostemma* spp. (Rubiaceae) in Thailand.** The 9th Biological Science Graduate Congress. Department of Biology Faculty of Science Chulalongkorn Uiversity. at Chulalongkorn University. 16-18 October 2004.
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