KARYOTYPE ANALYSIS OF ZINGIBERACEAE IN THAILAND: SYSTEMATIC IMPLICATION

KANTIWA SAENPROM

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biodiversity at Mahasarakham University

March 2018

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The examining committee has unanimously approved this dissertation, submitted by Mrs. Kantiwa Saenprom, as a partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biodiversity at Mahasarakham University.

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ABSTRACT

Somatic chromosome numbers and karyotypes for 16 species of Thai Zingiberaceae from five different genera *Alpinia, Amomum Elletariopsis, Kaempferia* and *Zingiber* was determined using tissue samples of root tips. The somatic numbers are range from 2*n* = 22 to 2*n* = 48. Eight species in *Alpinia*, *Amomum* and *Elettariopsis* were studied have somatic chromosome number of 2*n* = 48 and found that most of them have 2n = 4x = 48, suggesting that they are tetraploid. The four species of *Kaempferia* were examined with a chromosome number ranging of 2*n* = 22 to 2n = 42. The somatic numbers of *K. angustifolia* and *K. grandifolia* are 2*n* = 22, while the chromosome numbers found in *K. rotunda* and *K. marginata* are 2*n* = 30 and 42, respectively. In addition, according to this study the somatic number of 2*n* = 22 were found in *Z. montanum*, *Z. mekongense*, *Z. officinale* and *Z. ottensii*, confirming that these four *Zingiber* species are diploid with basic chromosome x = 11. All species observed symmetric and asymmetric karyotype. The chromosomes included metacentric (m), submetacentric (sm), acrocentric (a) and telocentric (t). The telocentric chromosomes were observed in the 21^{st} and 22^{nd} medium chromosome pairs in *Elettariopsis biphylla* S. Saensouk & P. Saensouk. All plants in this study is the first time to describe the karyotypes. The difference of karyotypes in these species can be used as potential cytogenetic data and provides an advantage for more accurate cytotaxonomy classification of this family found in Thailand.

Key words : Chromosome number, Karyotype, idiogram, Zingiberaceae

ชื่อเรื่อง	การวิเคราะห์แคริโอไทป์ของพืชวงศ์ขิงในประเทศไทย : นัยทางซิสเทมาติกส์
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บทคัดย่อ

จากการศึกษาจำนวนโครโมโซมและแคริโอไทป์ของพืชวงศ์ขิงที่พบในประเทศไทย จากเซลล์ปลายรากจำนวน 16 ชนิด จัดอยู่ใน 5 สกุลดังนี้ *Alpinia, Amomum Elletariopsis, Kaempferia* และ *Zingiber* พบว่าพืชที่ศึกษามีจำนวนโครโมโซมอยู่ระหว่าง $2n$ = 22 ถึง $2n$ = 48 มีจำนวน 8 ชนิด ในสกุล *Alpinia*, *Amomum* และ *Elettariopsis* ที่มีจำนวนโครโมโซมเท่ากันคือ $2n$ = 48 และจัดเป็นชุดโครโมโซมแบบเตตระพลอยด์ สำหรับการศึกษาพืชสกุล *Kaempferia* จำนวน 4 ชนิด พบจำนวนโครโมโซมอยู่ระหว่าง $2n$ = 22 ถึง $2n$ = 42 โดยพบว่า *K. angustifolia* และ *K. grandifolia* มีจำนวนโครโมโซมเท่ากันคือ $2n$ = 22 ในขณะที่ *K. rotunda* และ *K. marginata* มีจำนวนโครโมโซม $2n$ = 30 และ $2n$ = 42 ตามลำดับ นอกจากนี้เมื่อศึกษาพืชในสกุล *Zingiber* จำนวน 4 ชนิด ได้แก่ *Z. montanum*, *Z. mekongense*, *Z. officinale* และ *Z. ottensii* พบว่ามีจำนวนโครโมโซมเท่ากันคือ $2n$ = 22 ซึ่งจัดเป็นดิพลอยด์ โดยคาริโอไทป์ของพืชตัวอย่างที่พบนั้นมีทั้งเป็นคาริโอไทป์แบบสมมาตร (symmetrical karyotype) และคาริโอไทป์แบบไม่สมมาตร (asymmetrical karyotype) ซึ่งประกอบด้วยโครโมโซมชนิดเมตาเซนตริก (m) ซับเมตาเซนตริก (sm) อะโครเซนตริก (a) และเทโลเซนตริก (t) ในการศึกษานี้พบโครโมโซมแบบเทโลเซนตริก (t) บนตำแหน่งโครโมโซมขนาดกลางคู่ที่ 21 และ 22 ของชนิด *Elettariopsis biphylla* S. Saensouk & P. Saensouk. การศึกษานี้เป็นการรายงานการจัดคาริโอไทป์พืชกลุ่มนี้เป็นครั้งแรก โดยพืชตัวอย่างที่ศึกษาแต่ละชนิดมีสูตรแคริโอไทป์แตกต่างกัน ซึ่งข้อมูลที่ได้จากการศึกษานี้จะเป็นประโยชน์ต่อการศึกษาด้านพันธุศาสตร์และเป็นข้อมูลสนับสนุนในการจัดจำแนกพืชวงศ์ขิงที่พบในประเทศไทยต่อไป

คำสำคัญ : จำนวนโครโมโซม แคริโอไทป์ อิดิโอแกรม พืชวงศ์ขิง

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CHAPTER 1
Introduction

1.1 Background and rationale of the study

Zingiberaceae (ginger family) is the largest of the eight families of the order (Kress, 1990). The members are distributed mainly in tropics and subtropics with the centre of distribution in the Indo-Malayan region, but extending through tropical Africa to central and South America. Zingiberaceae species have been well known as spices, medicinal and condiments such as genera *Alpinia*, *Curcuma* and *Zingiber*. In addition, several species are used as ornamental plants, for example, (*Alpinia purpurata* (Vieill) Schum.), (*Curcuma alismatifolia* Gagnep) and (*Etlingera elatior* (Jack) R. M. Smith.).

Peterson (1889) classification of the family, amended by others over time, recognized four tribes including Globbeae, Alpinieae, Hedychieae and Zingibereae *base* on morphological characters, such as the number of locues and placentation in the ovary, staminoidia development, modifications of the single fertile anther, foliar orientation. According to recent studies as well as the literature, Kress *at al.* (2002) proposed classification of the family Zingiberaceae based on DNA data which is recognized four subfamily and six tribes: Zingibereae, Globbeae, Alpinieae, Riedeae, Tamijioideae and Siphnochiloideae. The later accepted classification of the Zingiberaceae (Larsen and Larsen, 2006) includes 52 genera and four subfamilies, namely Alpinioideae, Tamijioideae, Siphonochiloideae and Zingiberoideae. The current studies, Kress *et al.* (2014) reported the Zingiberaceae approximately 53 genera and over 1,200 specie distributed in all tropical regions of the world, with highest concentration in Southeast Asia. Only one genus of genus of zingiberaceae, *Renealmia*, occurs in Tropical America, and was found in Africa. Three are endemic to Africa (*Aframomum*, *Autotandra* and *Siphonochilus*) and the remainder range from east Asia to the South Pacific.

Thailand is one of the richest countries for Zingiberaceae in the world. Since Larsen (1980) made annotated key to the 29 genera of the family Zingiberaceae for Thailand, the members within the family have been carried on for finish them both in genera and species. In the present at least 300 species from 26 genera in 3 tribes *Alpinieae*, *Zingibereae* and *Globbeae* of Zingiberaceae are found throughout Thailand, however, the number of species will most certainly rise (Larsen and Larsen, 2006). Many workers are studied family Zingiberaceae such as Sirirugsa (1989, 1992a, 1992b), Theilade (1999), Saensouk and Larsen (2001), Saensouk and Saensouk. (2014), Khamtang *et al.* (2014).

Cytological knowledge of the Zingiberaceae is poor in a few genera have been studied for their chromosome numbers. Many workers are studied family Zingiberaceae such as (Eksomtramage and Boontum, 1995); (Theilade, 1999); (Saensouk and Jenjittikul, 2001); (Saensouk and Larsen, 2001); (Eksomtramage *et al.*, 2002); (Sirisawad *et al.*, 2003); (Saensouk and Chantaranothai, 2003); (Saensouk and Saensouk, 2004); (Khamtang *et al.*, 2014); (Saensouk and Saensouk 2014); (Bhadra and Bandyopadhyay, 2016).

The objective of this study were to the chromosome number and analyses the karyotype characteristics of Ginger family from Thailand in order to support species identification and their relationships. Of the data acquired to support the classification type and including notes on chromosome Atlas of plants found in Thailand.

1.2 Research question and hypothesis

The results of this study can be applied to develop knowledge in the research of this plant group.

1.3 Objectives of the study

1.3.1 To study fundamental standardized number of chromosome arm and standardized karyotype of some species Zingiberaceae found in Thailand.

1.3.2 To study fundamental standardized chromosome number, karyotype and idiogram for classification in Zingiberaceae.

1.3.3 To study fundamental standardized idiogram of Zingiberaceae in Thailand.

1.3.4 To study fundamental standardized systematic of Zingiberaceae based on chromosome morphology and fundamental number.

1.4 Conceptual framework

The study between January 2015 and December 2016, living specimens from various locations in Thailand were collecting and preserving. Information on chromosome number, karyomorphological and idiogram of at least 16 species Zingiberaceae in Thailand were provided for support to plant cytotaxonomy.

CHAPTER 2

Literature Review

2.1 Systematic position of Zingiberaceae Family

The pantropical Zingiberaceae is the largest family in the order Zingiberales with approximately 53 genera and over 1,200 species distributed in all tropical regions of the world, with highest concentration in Southeast Asia, where most species are distributed; four genera (*Aframomum, Aulotandra, Renealmia,* and *Siphonochilus*) occur in Africa and the genus *Renealmia* is the sole member that is native to South America. (Kress *et al.*, 2014). Classifications of the family first proposed in 1889 and refined by others since that time recognize four tribes (Globbeae, Hedychieae, Alpinieae, and Zingibereae) (Kress *et al.*, 2002). The systematic position of the family Zingiberaceae is as follows:

Kingdom: Plantae
Division: Spermatophyta
Subdivision: Angiospermae
Class: Monocotyledonae
Order: Zingiberales
Family: Zingiberaceae

Members of the Zingiberaceae family are small to large herbaceous plants with distichous leaves with basal sheaths that overlap to form a pseudostem. The plants are either self-supporting or epiphytic. Flowers are hermaphroditic, usually strongly zygomorphic, in determinate cymose inflorescences and subtended by conspicuous, spirally arranged bracts. The perianth is composed of two whorls, a fused tubular calyx, and a tubular corolla with one lobe larger than the other two. Flowers typically have two of their stamenoids fused to form a petaloid lip, and have only one fertile stamen. The ovary is inferior and topped by two nectaries, the stigma as funnel-shaped. The fruit is a loculicidal capsule or is berrylike.

The members of this family are rhizomatous plants whose leaves form a pseudostem. They produce a terminal inflorescence, either on a leaf-shoot or on a separate shoot growing directly from the rhizome near the base of the leaf-shoot. The inflorescence comprises an axis which bears spirally arranged cincinni supported by primary bracts; in some groups the cincinni are reduced to a single flower (Holttum 1950). The zygomorphic flowers usually possess only one fertile stamen. The main identifying character is the labellum, formed by the fusion of two lateral staminodes of the inner whorl.

Most Zingiberaceae are evergreen (especially members of the Alpinoideae subfamily), as they are distributed in the warm tropical forests. Some, mostly representatives of Zingiberoideae, survive periods of drought in dormant state (Kress *et al.*, 2002). The rhizome of these species (such as *Curcuma*, *Zingiber*) is usually fleshy and adapted as a resting organ; they are therefore able to adapt to a drier climate, although none are truly xerophytic (Holttum, 1950). Epiphytic species are also present, though rarely, for example in the genus *Burbidgea* Hook. f.

In 1999, Sakai *et al.* observed the pollinators of 29 species of Bornean Zingiberaceae and *Costaceae* and discovered three pollination guilds: one was pollinated by spiderhunters (Nectariniidae), one by medium-sized *Amegilla* bees (Anthophoridae), and one by small hallictid bees. These guilds correlated with the flower morphology, especially in the formation of the floral tube and labellum (Sakai *et al.*, 1999).

Zingiberaceae contain aromatic oils and are thus often used as spices or in traditional medicines, such as the commercially grown ginger (*Zingiber officinale* Roscoe) or the green cardamom (*Elettaria cardamomum* (L.) Maton). As their flowers are often large and brightly coloured, they are also grown as ornamental plants (such as species of *Etlingera* Giseke).

The Zingiberaceae are a difficult and still largely unresolved group. The main (and perhaps only) morphological character by which they can be securely distinguished is theflower, and until the development of morphological analyses the taxonomy of Zingiberaceae had been largely based on their flower morphology. The most recent monography of Zingiberaceae remains that written by Schumann in 1904. Zingiberaceae have been intensively researched during the past few decades (Holttum, 1950, Kress *et al.,* 2001, Kress *et al.,* 2002) and the research is continuing with the use of new methods in botany such as next-generation sequencing, while new species are still being discovered in the area of dispersal.

In 2002, Kress *et al.* divided Zingiberaceae into four subfamilies, Siphonochileae, Tamijioideae, Alpinoideae and Zingiberoideae on the basis of the ITS and *mat*K regions and morphological data. The subfamily Alpinioideae, which *Elettariopsis* is a member of, is characterised by the absence or reduction of the two lateral staminodes, a plane of distichy perpendicular to that of the growth of the rhizome, and the lack of a dormant period (Kress *et al.,* 2002). It contains two tribes, Alpinieae and Riedeliae. The largest genus of Alpinioideae, *Alpinia* Roxb., is polyphyletic (Rangsiruji *et al.,* 2000), and so are the remaining two large genera, *Amomum* Roxb. (Kress *et al.,* 2002) and *Etlingera* Giseke (Pedersen, 2004).

The Zingiberaceae form an important group with considerable economic potential, with plants such as *Aframomum, Amomum, Curcuma, Elettaria, Kaempferia* and *Zingiber*. Some genera yield essential oils used in the perfume industry including, *Alpinia* and *Hedychium*. Many gingers are cultivated as ornamental and economically important spice plants, including the *Alpinia zerumbet*, *Curcuma alismatifolia*, *Globba*, *Hedychium Kaempferia*, *Nicolaia*, *Renealmia*, and *Zingiber*. They are important natural resources that provide many useful products for food, spices, medicines, dyes, perfume and aesthetics to man.

2.2 Distribution and habitat specialization

Zingiberaceae are dispered over a wide belt of tropical and subtropical regions, primarily in tropical Asia, where the region's long history of a stable, damp, and hot climate together with its wide array of habitats probably favored the development and differentiation of these plants (Chen *et al.*, 1984). Within tropical Asia, the diversity-rich Malesian region, which includes Malaysia, Indonesia, Brunei, Singapore, Philippines, and Papua New Guinea, contains 24 genera and about 600 species (Larsen, 1996). The members of this family are distributed pantropically, with one genus (Renealmia) in the Neotropics, four (Aframomum, Aulotandra, Siphonochilus, and Renealmia) in Africa, and the rest distributed in East Asia and the Pacific islands (Kress *et al.*, 2002).

The distribution of the Asian genera into different zones in the tropics is distinguished by their various characteristics, which most likely are the result of evolutionary changes and their adaptation to several climatic, geographic, and topographic conditions. For example, the genus Roscoea is found in the Himalayan range and is therefore adapted to the harsh conditions and high elevations of those mountains. Thus, species of Roscoea have closed leaf sheaths, a reduced number of rhizomes, and long roots reaching deeply into the soil since the fleshy rhizomes cannot be accommodated on the surface of the rocky soil (Chen *et al.*, 1989). Similarly, Aframomum is the largest genus of the African Zingiberaceae appoximately 80 species, as well as the largest amonggenera representing the understory herbs of the African rain forest. Its range extends from Senegal to Ethiopia in the north and Angola to Madagascar in the south; it is also found on the Gulf of Guinea islands of São Tomé and Principe (Harris *et al.*, 2000).

2.3 Mode of propagation

The biology of the Zingiberaceae is poorly known. With the exception of a few species, the predominant mode of reproduction in the Zingiberaceae is asexual and propagation is often through underground rhizomes. The plants have prostrate or tubular rhizomes that are sympodial and promote the formation and development of axillary buds. Sexual reproduction is largely diminished or even lost with increasing ploidy levels. Moreover, in some species, such as those of the genus *Globba*, the flowers are often replaced by bulbils (Chen 1989). Due to their vegetative mode of reproduction, crop improvement in zingiberaceous plants is confined to clonal selection, mutation breeding, and the induction of polyploidy (Ravinderan *et al.*, 2005).

2.4 Medicinal gingers

Zingiberaceae forms an important group with economic potential and many members of this family yield spices, dyes, perfumes and medicines and some are ornamental. Many of them are used in ayurvedic and other native systems of medicine

Turmeric is known as ‘golden spice’ as well as ‘spice of life’ and has been used in India as a medicinal plant and has strong association with sociocultural life (Jain and Prakash, 1995; Ravindran, 2007). The word ginger truly refers to the edible ginger of commerce known botanically as *Zingiber officinale* Roscoe. Ginger is also the common term for members of the ginger family (Larsen *et al*., 1999; Sabu and Skinner, 2005).

The presence of essential oils such as limonene, eugenol, pinene, geraniol, etc. in many Zingiberaceae species has made some of them important since the time of ancient Greeks (Larsen *et al*., 1999). The rhizomes of many of them are dietary agents and are also used in traditional medicine (Chirangini *et al*., 2004).

Alpinia calcarata Roscoe is a native of India, the rhizomes of which with sharp odour and pleasant taste are used in the form of infusion for fever, rheumatism and catarrhal affections. The rhizomes also form a major ingredient of several ayurvedic preparations (Kirtikar and Basu, 1975).

Alpinia galanga (L) Sw. is known as greater galangal and is a perennial rhizomatous herb. The rhizome is antibacterial, antirheumatic, antidiabetic, aphrodisiac and carminative (Chatterjee and Pakrashi, 2001; Sabu, 2006). Khare (2007) has reported antispasmodic action for the oils of the species. He has also indicated the antiulcer activity of its roots which has been attributed to the antisecretory and cytoprotective properties of the plant.

Alpinia officinarum Hance. is a native of South China and the plant is a perennial herb with a raceme of showy flowers and ornamental foliage. This is the lesser galangal and the rhizome possesses aromatic spicy odour and pungent taste, like a mixture of pepper and ginger. It is used in cooking, in medicine, for flavouring liquors and to impart a pungent flavour to vinegar (Pandey, 2001). *Alpinia speciosa* (Wendl.) K. Schum is the light galangal. Rhizomes are antiulcerative and spasmolytic (Khare, 2007).

Amomum subulatum Roxb. is known as Nepal cardamom and the dried leathery fruits resemble cardamom in aroma and flavour. In medicine it is used to correct digestive disorders and as an antivomiting drug. Large cardamom is used as preventive as well as curative for throat troubles, congestion of lungs, inflammation of eye lids, digestive disorders (Chatterjee and Pakrashi, 2001; Sabu, 2006).

Curcuma aeruginosa Roxb. is used for the extraction of East Indian arrow root or Travancore starch. It is used as a medicine for stomach disorders. The rhizome is aromatic with a blue colour in the centre which is variable depending on the nature of the soil and age of the rhizome. The rhizome is used for fever, diarrhoea, swellings and skin diseases and its oil is antibacterial, antifungal and antihelmintic (Sabu, 2006; Khare, 2007).

Curcuma amada Roxb. is known as mango ginger. The rhizome is regarded as cooling and carminative and is useful in prurigo. The rhizomes are used externally in the form of paste as an application for bruises and skin diseases and combined with other medicines it is useful in improving quality of blood. The paste is applied externally to bruises, sprains, contusions, rheumatic pains and black eye (Khory and Katrak, 1999).

Curcuma anguistifolia Roxb. is called East Indian arrow root. The rhizome is used as a source of arrow root which is largely manufactured and exported in Malabar. The tubers are used for the extraction of starch. It forms an important food of tribal people in the area. It is cooling, demulcent and nutritious and is used in dysentery, dysuria, typhoid, fevers and ulcerations (Nadkarni, 2005; Skornickova *et al*., 2007).

Curcuma aromatica Salisb. is known as wild turmeric or Cochin turmeric. Rhizomes are bitter, carminative, appetizer and tonic, and are used in combinations with astringents and aromatics for bruises, sprains, bronchitis, cough, leucoderma and skin eruptions. Dried rhizome is used as an aromatic adjunct to other medicines used in skin diseases and impurities of blood. Boiled in oil, it is applied externally as an application to sprains and bruises (Warrier *et al*., 1994; Joshi, 2000).

Curcuma caesia Roxb. is a species with blue rhizome and is commonly called black turmeric. The leaf has a deep violet patch which runs throughout the lamina. Rhizome is aromatic, carminative and stimulant and a paste made from the rhizome is used to cure dysentery and as poultice in rheumatic pain, sprains and bruises (Chatterjee and Pakrashi, 2001; Ravindran, 2007).

Elettaria cardamomum (L) Maton is a perennial herb with thick horizontal root stock. It is known as the queen of spices. The seeds are used as condiment and medicinally. Cardamom has stimulant properties and is used as a spice and masticator. Seeds are useful in asthma, bronchitis, cardiac disorders, anorexia, dyspepsia, gastropathy, debility and vitiated condition of *vata*. Cardamom oil is used in several pharmaceutical preparations (Fischer, 1928; Warrier *et al.*, 1994; Joshi, 2000).

Hedychium coronarium Koenig, is a stout perennial herb commonly grown as ornamental. The rhizome is considered antirheumatic and excitant. The rhizome and leaf paste are applied for headache. The essential oil from rhizome shows anthelmintic and mild tranquilizing property. Among the tribal inhabitants of Orissa, a paste from the essential flowers and black pepper is taken orally, used for dysuria (Parrotta, 2001; Khare, 2007).

Hedychium spicatum Ham ex Smith, the spiked ginger lily and is a rhizomatous perennial tuberous herb. Rhizome is used in dyspepsia and as carminative, stimulant, stomachic and tonic (Joshi, 2000; Chatterjee and Pakrashi, 2001; Sabu, 2006).

Kaempferia galanga L. rhizome is stomachic and anti-inflammatory. In the form of powder or ointment it is applied to wounds and bruises to reduce swellings. They improve complexion and cure burning sensations, mental disorders and insomnia. Decoction of rhizome is used for dyspepsia, head ache and malaria. Roasted rhizomes are applied hot in rheumatism and for hastening the ripening of inflammatory tumors. Leaves are used in lotions and poultices for sore eyes, sore throat, swellings, rheumatism and fevers. The tuber powdered and mixed with honey is prescribed for coughs, asthma and pectoral affections (Joshi, 2000; Nadkarni, 2005).

Kaempferia rotunda L. is an erect herb with tuberous rhizomes. The whole plant is useful in the form of powder or ointment as an application to wounds and bruises to reduce swellings. The powder extracted from *Kaempferia rotunda* is made into ointment and is used for healing fresh wounds. It is taken internally to remove coagulated blood or purulent matter and is used in many ayurvedic preparations. The tubers are useful in vitiated condition of *vata* and *kapha*, gastropathy, dropsy, inflammations, wounds, ulcers, blood clots, tumours and cancerous swellings (Warrier *et al*., 1995; Singh and Panda, 2005; Sabu, 2006).

Zingiber mioga Roscoe, the Myoga ginger or Japanese ginger is a perennial woodland species, endemic to Japan. There it is used as a substitute for true ginger. In Chinese Pharmacopoeia, myoga ginger is suggested to treat fever and also as a vermifuge (Sabu and Skinner, 2005).

Zingiber montanum (K.D.Koenig) Link ex Dietr., the wild ginger or forest ginger is native to India. The rhizome is used in diabetes. The tribals use the rhizome as a substitute for *Curcuma longa* and is an ingredient of many traditional medicines (Sabu and Skinner, 2005; Sabu, 2006).

Zingiber officinale Roscoe is a perennial herb and is one of the reputed drugs employed in indigenous systems of medicine. Both fresh rhizome and dried rhizome are used in medicine (Sivarajan and Balachandran, 1994). In ancient India, ginger was not significant as a spice, but it was called *mahabheshaj* or *mahaoushadhi* meaning the great cure and used in medicine (Ravindran and Nirmal Babu, 2005). The rhizome possesses stimulant, aromatic and carminative properties when taken internally and when chewed it acts as a sialagogue. Externally it is applied as rubefacient. It is anti rheumatic, carminative, diuretic and aphrodisiac. It promotes digestive power, cleans the throat and tongue, dispels cardiac disorders and cures vomiting (Sivarajan and Balachandran, 1994; Joshi, 2000).

Zingiber spectabilis Griff., known as bee hive ginger due to the peculiar shape of the spike, is widely used in Malayan traditional medicine (Burkill and Haniff, 1930).

Zingiber zerumbet (L) Smith, a native of tropical Asia is known as shampoo ginger or pine cone ginger. The inflorescence releases a thick juice when squeezed and is used to make Paul Mitchel and Freeman's shampoo (Sabu and Skinner, 2005). Rhizome is useful in colic, head ache, haemorrhoids, respiratory disorders, cough, asthma, leprosy and skin diseases (Chatterjee and Pakrashi, 2001; Khare, 2007).

2.5 The Zingiberaceae in Thailand

Thailand is one of the countries that contain a highest diversity of Zingiberaceae plants. 26 genera and more than 300 species were reported by Larsen and Larsen (2006). Zingiberaceae plants mostly grow in damp and humid shady places, and also found from the lowlands to the highest elevations in secondary and primary forests. There is a high number of Zingiberaceae species in Peninsular Thailand because the climate and landscape support the plant's growth. Several genera were found and investigated taxonomy such as *Alpinia* (Kittipanangkul and Ngamriabsakul, 2008), *Amomum* (Kaewsri *et al.*, 2009), *Boesenbergia* (Sirirugsa, 1987), *Curcuma* (Maknoi *et al.*, 2005), *Cornukaempferia* (Mood and Larsen, 1997; Mood and Larsen, 1999), *Caulokaempferia* (Ngamriabsaku, 2008), *Etlingera* (Chongkraijak, 2013), *Elettariopsis* (Saensouk and Saensouk., 2014), *Globba* (Larsen, 1972; Picheansoonthon and

Tiyaworanant, 2010), *Geostachys* (Mayoe, 2010), *Hedychium* (Sirirrugsa and Larsen, 1995), *Hemiorchis* (Larsen and Triboun, 2001), *Kaempferia* (Larsen, 1962), *Smithatris* (Kress and Larsen, 2001) and *Zingiber* (Theilade, 1999; Mood and Theilade (2002).

Larsen (1980) first published the annotated key to the genera of Zingiberaceae of Thailand in Natural History Bulletin of Siam Society.

Sirirugsa (1987) described three new species and one combination of *Boesenbergia* in Thailand, i.e. *B. acuminata* Sirirugsa, *B. basispicata* K. Larsen ex Sirirugsa, *B. petiolata* Sirirugsa and *B. siamensis* (Gagnep.) Sirirugsa.

Sirirugsa (1992a) revised genus *Boesenbergia* in Thailand. Thirteen species were recognized as followings, *Boesenbergia basispicata*, *B. prainiana*, *B. curtisii*, *B. petiolata*, *B. xiphostachya*, *B. thorelii*, *B. longipes*, *B. siamensis*, *B. rotunda*, *B. plicata*, *B. acuminata*, *B. parvula* and *B. pulcherrima*.

Sirirugsa (1992b) revised genus *Kaempferia* in Thailand. Fifteen species were recognized, including 3 new species of *Kaempferia*, such as *Kaempferia larsenii* Sirirugsa, *K. siamensis* Sirirugsa and *K. spoliata* Sirirugsa.

Larsen (1996) reported about 200 species in 21 genera in “A Preliminary Checklist of Zingiberaceae of Thailand”. Consist of *Alpinia* (12 species), *Amomum* (15 species), *Boesenbergia* (15 species), *Caulokaempferia* (5 species), *Cautleya* (1 species), *Curcuma* (34 species), *Curcumorpha* (1 species), *Elettaria* (1 species), *Elettariopsis* (3 species), *Etlingera* (5 species), *Gagnepainia* (2 species), *Geostachys* (3 species), *Globba* (34 species), *Haniffia* (3 species), *Hedychium* (25 species), *Hornstedtia* (1 species), *Kaempferia* (15 species), *Pommereschea* (1 species), *Scaphoclamys* (3 species), *Stahlianthus* (3 species) and *Zingiber* (25 species). The numbers in parentheses are the number of species in each genus.

Theilade (1999) published the synopsis of the genus *Zingiber* of Thailand, with 26 species were recognized. Six species are endemic to the Malay Peninsula, including *Zingiber petiolatum*, *Z. puberulum* var. *ovoideum*, *Z. spectabile*, *Z. wrayii*, *Z. peninsulare* and *Z. newmanii*. Four species are cultivated throughout Thailand, for example *Zingiber officinale*, *Z. montanum*, *Z. ottensii* and *Z. zerumbet.*

Eksomtramage *et al.* (2001) reported tribe Alpinieae, genus *Geostachys*, including *Pommereschea* and *Siamanthus*, and tribe Zingibereae, genus *Boesenbergia*, *Caulokaempferia, Cornukaempferia, Kaempferia* and *Scaphochlamys* in Thailand. The result showed 46 species were recognized in both tribes.

Eksomtramage *et al.* (2002) reported 11 species that were new records from Southern Thailand in Natural History Bulletin of Siam Society, i.e. *Boesenbergia flava* (Ridl.) Holtt., *Camptandra parvula* (King ex Bak.) Ridl.

Kharukanant and Tohdam (2003) published a new species, *Boesenbergia regalis*, B. Kharuk. & S. Tohdam from the Halabala Forest Reserve in Southern Thailand.

Larsen (2003) reported about 290 species in 25 genera of Zingiberaceae in Thailand.

Saensouk *et al.* (2003) published notes on the genus *Alpinia* in Thailand. Two new records from Thailand, *Alpinia scabra* (Blume) Baker and *A. blepharocalyx* K. Schum. with two varieties were reported. 21 species were recognized including three introduced species. A new species, *Alpinia peninsularis* was described from southern Thailand.

In 2004, Picheansoonthon and Mokkamul found a new species, *Caulokaempferia khaomaenensis* Picheansoonthon and Mokkamul, from Khao Maen, Khao Luang National Parks.

Picheansoonthon and Mokkamul (2005) reported two new species, *Hedychium thaianum* Mokkamul and Picheansoonthon and *H. khaomaenense* Picheansoonthon and Mokkamul, from Chiang Mai and Nakhon Si Thammarat, respectively.

Picheansoonthon and Koonterm (2008) reported the genus *Kaempferia* L. (Zingiberaceae) is one of the important medicinal plant genera in Thailand. As a result of herbarium investigation and intensive field studies, 16 taxa are enumerated for Thailand in this account. Distinguishable morphological characteristics of these species are given. Photographic illustrations of some key species are included. The ethnobotany of the genus *Kaempferia* in Thailand is also discussed in four main aspects: food, medicine, belief, and horticulture.

Triboun *et al.* (2002) studied biogeography and biodiversity of the genus *Zingiber* in Thailand, 51 species were recognized. Eleven species were new records to Thailand. Eighteen species are endemic.

Larsen and Larsen (2006) reported about 300 species in 26 genera of Thailand Zingiberaceae.

Kittipanangkul and Ngamriabsakul (2008) reported Zingiberaceae diversity in Khao Nan and Khao Luang National Parks was determined from September 2006 - August 2007. 29 species in 11 genera in three tribes of the family were collected from nine stations, less than 30 % of the Zingiberaceae recorded in southern Thailand. Tribe Alpinieae, the highest diversity, comprises five genera and 15 species. Tribe Zingibereae, the second highest diversity, includes five genera and 12 species. The lowest diversity, tribe Globbeae consists of only two species in the genus *Globba*.

Chen *et al.* (2015). Reported *Curcuma woodii*, a new species of *Curcuma* subg. *Ecomata* (Zingiberaceae) from Thailand is described and illustrated here. It differs from *C. rhomba* by the leaf blades abaxially pubescent, the bracts whitish green, the labellum white with orange bands at the center, the lateral staminodes white with orange dots at the apex, and the ovary nearly glabrous.

Saensouk *et al.* (2016). Three tribes, 12 genera and 38 species of the ginger family (Zingiberaceae) along five routes in Nam Nao National Park were surveyed between January 2012 and December 2013.

2.6 Chromosome numbers and idiogram of the Zingiberaceae family

Cytotaxonomy is a branch of cytogenetics, devoted to the comparative study of karyological features for systematic and evolutionary purposes (Siljak-Yakovlev and Peruzzi 2012). Today, a number of data can be obtained by chromosome studies: chromosome number, karyotype structure, karyotype asymmetry, chromosome banding, FISH, GISH and chromosome painting (Stace, 2000; Levin, 2002; Graphodatsky *et al.*, 2011; Guerra, 2012). Among them, one of the most popular, cheap and widely used approaches especially by botanists is that concerning karyotype asymmetry.

Chromosomes within a taxon (e.g. family, genus or even species) may vary concerning their number (including ploidy level and aneuploidy) but may also show variation in absolute and relative chromosome size, in chromosome morphology, and in staining properties (Sharma and Sen, 2002). Morphological characters of chromosomes are mostly due to the position of the centromere (Levan *et al*., 1964). The description of chromosome morphology is a powerful method to characterize genomes in plants and in animals as well. Karyological data are essential information and provideimportant characters for plant systematics and evolutionary anal-ysis (Stace, 2000).

Chromosome numbers of Thai Zingiberaceae were previously reported by several authors, namely: Mahanty (1970), Larsen (1972), (Beltran and Kiew,1984), Sirisawad and Apavatjrut (1995), Eksomtramage and Boontum (1995), Eksomtramage *et al*. (1996; 2001), Weerapukdee and Krasaechai (1997) and Saensouk (2000). (Saensouk and Larsen, 2001); (Eksomtramage *et al*., 2002) (Sirisawad *et al.*, 2002); (Saensouk and Chantaranothai,2003); (Saensouk and Saensouk, 2004); (Khamtang *et al.*, 2014); (Saensouk and Saensouk, 2014). Moreover, several other studies have implicated this below:

Eksomtramage *et al.* (2002) The data shown on chromosome numbers of 22 species belonging to 10 genera of Thailand Zingiberaceae were investigated. The somatic numbers range from 20 to 48 showing diploidy and polyploidy. Ten of these species were firstly reported here, i.e. *Alpinia purpurata* (Vielli) K. Schum. (2*n* = 48), *Boesenbergia* aff. *rotunda* (2*n* = 20), *Cornukaempferia aurantiflora* J. Mood & K. Larsen (2*n* = 46), *Curcuma* aff. *oligantha* Trimen (2*n* = 42), *C. rhabdota* Sirirugsa & M.F. Newman (2*n* = 24), *Etlingera elatior* (Jack.) R.M. Smith (white form) (2*n* = 48), *E. hemisphaerica* (Bl.) R.M. Smith (2*n* = 48), *Hedychium gomezianum* Wall. (2*n* = 34), *H. longicornutum* Bak. (2*n* = 34) and *Zingiber* aff. *wrayi* (2*n* = 22)

Ngamriabsakul (2004). The results shown chromosome counts of *Roscoea alpina* Royle, *R. auriculata* K. Schum., *R. purpurea* Sm. and *Cautleya spicata* (Sm.) Baker are presented. Two species: *R. auriculata*, *R. purpurea* confirm the widely reported number of 2*n* = 24. However, *both R. alpina* and *C. spicata* have chromosome number as 2*n* = 26.

Nair and Sasikumar (2009) reported chromosome numbers in 22 germplasm collections and 28 open-pollinated seedling progenies of turmeric (*Curcuma longa* L.) were determined by counting the chromosomes of somatic metaphase plates. Among the germplasm collections analyzed 20 have 2*n* = 63, the accepted chromosome number of turmeric and 1 collection was 2*n* = 61 and another 1 was 2*n* = 84. The seedling progenies showed various chromosome numbers ranging from 2*n* = 63 to 2*n* = 86, of which 2*n* = 84 was the most frequently. The role of abnormalities during triploid chromosomes segregation in generating chromosome number variation among open pollinated seedling progenies is discussed.

Chen and Xia (2013). studied chromosome numbers of 15 populations representing 11 species of *Curcuma* from China. Results showed that only *Curcuma flaviflora* S. Q. Tong was diploid with 2*n* = 42 and *C. kwangsiensis* S. G. Lee & C. F. Liang was tetraploid with 2*n* = 84. The other species were triploid (2*n* = 63). The study indicated that the basic chromosome number of *Curcuma* from China could be x = 21. which prevented karyotype analysis.

Bhadra and Bandyopadhyay (2015). The karyological investigations of four economically and medicinally important Indian species of Zingiberaceae belonging to the genera *Curcuma* and *Zingiber* were performed. The somatic chromosome number of *Curcuma amada* was 2*n* = 42 and that of *Curcuma longa* was 2*n* = 63, while the chromosome numbers of both *Zingiber officinale*and *Zingiber zerumbet* were 2*n* = 22.

Bhadra and Bandyopadhyay (2016). Describe the new chromosome counts and karyomorphological details for the three important members of the Zingiberaceae family. Root tip cells of plants of *Alpinia zerumbet* (2*n* = 40) and *Globba marantina* showed 2*n* = 52 chromosomes, both being aneuploids, *Hedychium spicatum* were found to be tetraploid with somatic chromosome number of 2*n* = 68.

CHAPTER 3
Methodology

3.1 Plant collection and names

In total, 16 species belonging to five genera namely, *Alpinia*, *Amomum*, *Elettariopsis*, *Kaempferia* and *Zingiber* were studied in Table 1. Living specimens of Zingiberaceous plants were collected from various locations in Thai forests then transplanted into pots at Mahasarakham University Nursery. Actively growing root tips were used for mitotic studies and apical root tips at about 1 to 2 cm in length were used for chromosome counts. The root tips were harvested at different times throughout the day, between May 2015 and August 2016.

Table 3.1 List of Zingiberace species collected in Thailand during May 2015 to August 2016.

No.	Genera	Botanical Name	Locality (Province)
1	*Alpinia*	*Alpinia galanga* (L.) Willd. (No.1)	Sakon Nakhon
2		*Alpinia galanga* (L.) Willd. (No.2)	Chaiyaphum
3		*Alpinia siamensis* K. Schum.	Roi Et
4		*Alpinia zerumbet* (Pers.) B.L.Burtt & R.M.Sm.	Loei
5	*Amomum*	*Amomum biflorum* Jack	Phetchaburi
6		*Amomum schmidtii* (K. Schum.) Gagnep.	Ubon Ratchathani
7	*Elettariopsis*	*Elettariopsis biphylla* S.Saensouk & P.Saensouk, *sp. nov.*	Bueng Kan
8		*Elettariopsis triloba* (Gagnep.) Loes.	Loei
9	*Kaempferia*	*Kaempferia angustifolia* Roscoe	Chaiyaphum
10		*Kaempferia grandifolia* Saensouk & Jenjitt.	Khon Kaen
11		*Kaempferia marginata* Carey ex Roscoe	Maha Sarakham
12		*Kaempferia rotunda* L.	Khon Kaen
13	*Zingiber*	*Zingiber montanum* (J. Koenig) Link ex A.Dietr.	Khon Kaen
14		*Zingiber mekongense* Gagnep.	Phetchabun
15		*Zingiber officinale* Roscoe.	Khon Kaen
16		*Zingiber ottensii* Valeton.	Prachuap Khiri Khan

Note: No.1 = Smooth leaf surface; No.2 = Hairy leaf surfaces.

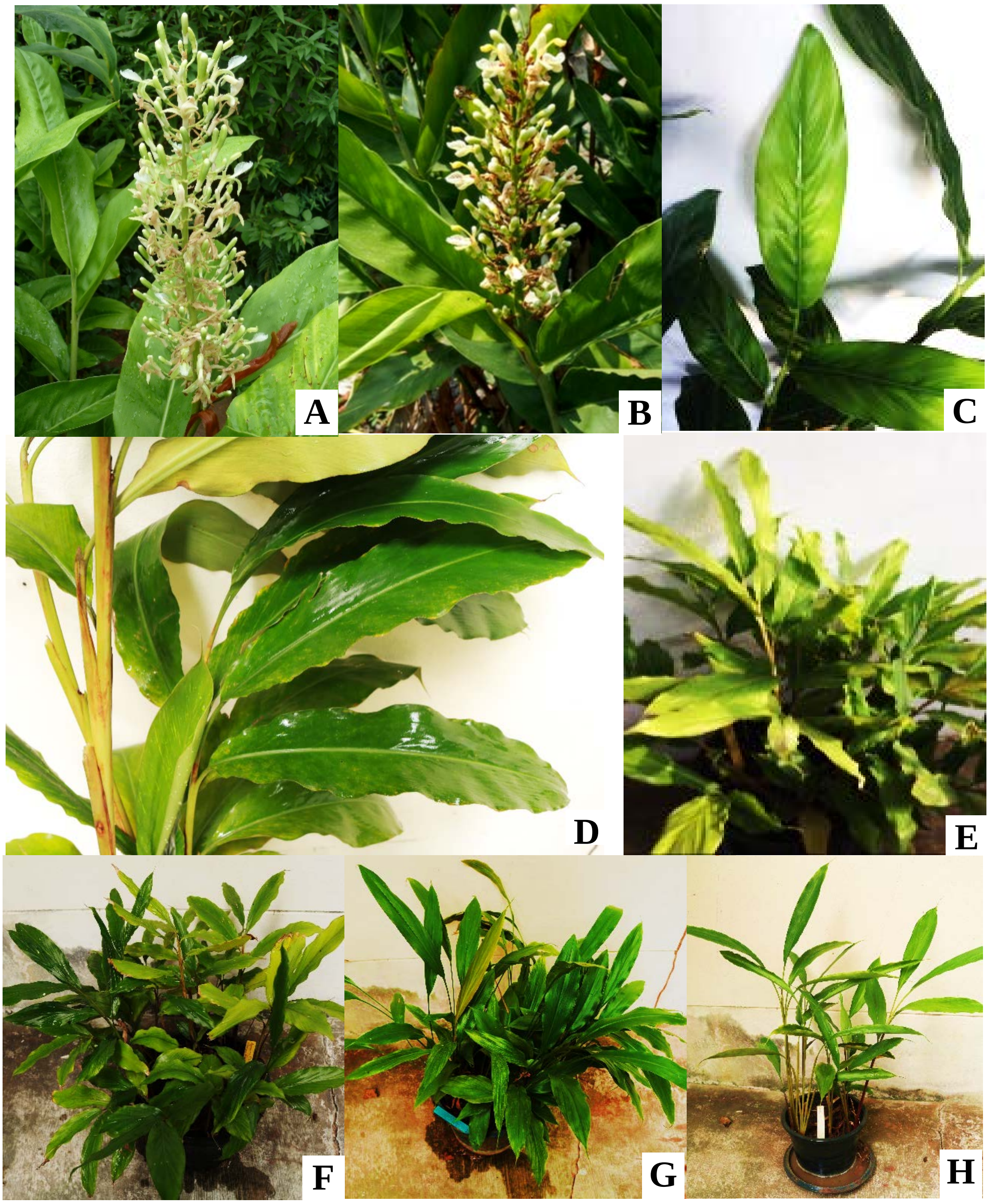


Figure 3.1 Showed all species were collected from Zingiberaceae in Thailand:(A) *A. galanga* (No.1), (B) *A. galanga* (No.2), (C) *A. siamensis*, (D) *A. zerumbet*, (E) *Amomum biflorum*, (F) *Amomum schmidtii*, (G) *E. biphylla*, and (H) *E. triloba* respectively.

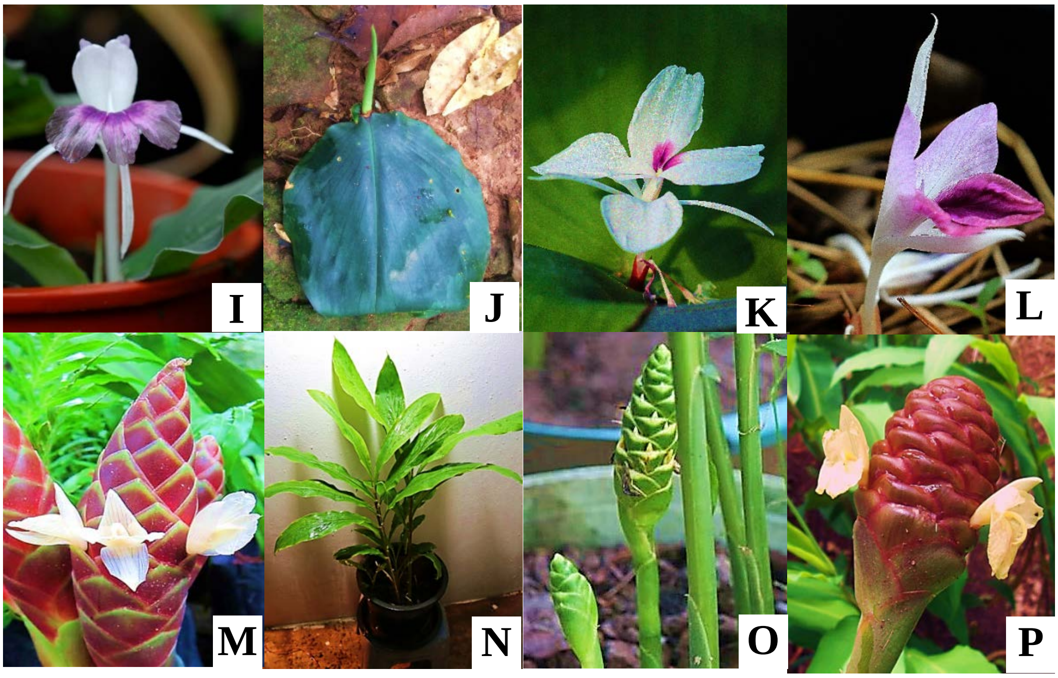


Figure 3.2 Showed all species were collected from Zingiberaceae in Thailand: (I) *K. angustifolia,* (J) *K. grandifolia,* (K) *K. marginata,* (L) *K. rotunda,* (M) *Z. montanum,* (N) *Z. mekongensis,* (O) *Z. officinale*, (P) *Z. ottensii*, respectively.

3.2 Methods

3.2.1 Mitotic analysis

Actively growing root tips were pretreated in a saturated paradichlorobenzens (PDB) solution for 4 hours at 4^{o}C, fixed in Carnoy's solution (3 parts 95% ethanol and 1 part glacial acetic acid) for 30 minutes at 4^{o}C, and stored in 70% ethanol at 4^{o}C. Fixed cells fragments were hydrolyzed with 1 N HCl for 5 minutes at 60°C and stained with 2% aceto-orcein for 5 minutes. Slides were prepared using the squash technique. The well spread metaphase plates of 10 cells in each species were identified using an Olympus model CH30 at 100x magnification and were then photographed using a digital camera (Zeiss Axiostar Plus).

3.2.2 Karyotype analysis

Chromosome counting was performed on mitotic metaphase cells under a light microscope. Twenty cells each of male and female with clearly observable and well-spread chromosomes were selected and photographed. The length of short arm (Ls) and the length of long arm of chromosome (Ll) were measured to calculate the length of total arm chromosome (LT, LT = Ls + Ll). The relative length (RL), the centromeric index (CI), and the standard deviation (SD) of RL and CI were also computed to classify the types and size of chromosomes according to Turpin and Lejeune (1965). All parameters were used in karyotyping and idiograming

3.3 Location of research conducting

Thesis project were worked at:

3.3.1 Walai Rukhavej Botanical Research Institute, Mahasarakham University

3.3.2 Field trip in Thailand.

CHAPTER 4
Results and Discussion

4.1 Chromosome numbers

The chromosome numbers of 16 species belonging to five genera of Zingiberaceae were found ranging from 2*n* = 22 to 2*n* = 48. Comparisons with previous studies by other authors shown in Table 4.1. Chromosome numbers showing difference in chromosome number, shape and size among the genera in Figure 4.1. For all genera are briefly described below.

4.1.1 *Alpinia*

Alpinia was described by Roxburgh in 1810 (Wu and Larsen, 2000). the genus contains more than 300 species which may suggests that it is the biggest genus of the family Zingiberaceae. Most members of the genus have distribution ranges in subtropical and tropical rain forest of Asia, Australia and Pacific Islands (Wu and Larsen, 2000). Some members of the genus are well known for ethnomedicinal importance and food as spicy materials especially *A. nigra* (Gaertner) B.L. Burtt, *A. galanga* Willd., *A. conchigera* Griff., *A. siamensis* K. Schum., etc.

The somatic chromosome number of four *Alpinia* species were determined (Table 4.1). Among them, *A. galanga* (No.1), *A. galanga* (No.2), *A. siamensis* and *A. zerumbet* had the chromosome number 2*n* = 48 (Figures 4.1A-D), which agrees with the *Alpinia* group 2n = 4x = 48 as reported by Chen and Huang (1996). In addition, agrees with the values previous reported for other species in the genus *Alpinia*, (Raghavan and Ven, 1943; Venkatasbban, 1946; Sato, 1948, Ono and Masuda, 1981; Beltran and Kiew, 1984; Eksomtramage *et al.*, 2001; Saensouk and Chantaranothai, 2003). Obviously, these four species of *Alpinia* suggesting that are tetraploid and they were deduced to be x = 12 (Darlington and Wylie, 1945). Whereas, Joseph (1998) also reported a variant somatic chromosome number of *A. zerumbet* 2*n* = 40. Bhadra and Bandyopadhyay (2016) describe the new chromosome counts and karyomorphological details for the important plants of *A. zerumbet* showed 2*n* = 52. Thus, it might be concluded that this is a new cytotype of this species.

4.1.2 *Amomum*

The genus *Amomum* comprises 150–180 species. They are widely distributed in Southeast Asia from the Himalayas to Northern Australia and extend into the central Pacific (Kam, 1982; Smith, 1985). Larsen (1996) listed 14 species of *Amomum* in his preliminary checklist of Zingiberaceae of Thailand. Sirirugsa (2001) estimated that there are around 15–20 species in Thailand.

The somatic chromosome number of two *Amomum* species were determined (Table 4.2). Chromosome number were studied: *A. biflorum* and *A. schmidtii* have the same number, 2*n* = 48. (Figures 4.1E-F). The same chromosome numbers were found of *A. biflorum* by Sharma and Bhattacharyya, (1959); Beltran and Kiew (1989); Chen and Huang (1996) and Eksomtramage *et al*. (2001). While, the first reported the somatic chromosome number of *A. schmidtii* in this study. Obviously, these two species of *Amomum* as 2*n* = 4x = 48, (*n* =24). The most common cytotypes found in this species are tetraploid and they were to be x = 12 (Darlington and Wylie, 1945).

4.1.3 *Elettariopsis*

Elettariopsis is one of the less intensively researched genera in the Zingiberaceae, and the only genus in the subfamily Alpinioideae that is still lacking revision. It is a small genus counting around 36 recognized species (Newman *et al*, 2013), with more species still being discovered and published (Picheansoonthon and Yupparach, 2012; Saensouk and Saensouk, 2014).

The somatic chromosome number of two *Elettariopsis* species were determined (Table 4.3). The somatic number of *E. biphylla* and *E. triloba* is 2*n* = 48 (Figures 4.1G-H). The results correspond to the gametic number (*n* = 24) which is reported for Malayan (Beltran and Kiew, 1989; Chen and Huang, 1996) conclude that is tetraploid and they were to be x = 12 (Darlington and Wylie, 1945).

4.1.4 *Kaempferia*

Kaempferia is a medium-sized genus in the ginger family Zingiberaceae. It comprises approximately 60 species distributed worldwide and about 35 accepted taxa have been recognized. The best-known example is *K. galanga* L. commonly known as kencur or aromatic ginger, or in Thai: proh horm or waan horm. It is one of the most popular, aromatic medicinal plants used in the tropics and subtropics of Asia. The Indochinese Peninsula is regarded as the centre of distribution of this genus, especially in Laos, Vietnam, Myanmar, Thailand and Peninsular Malaysia (Larsen and Larsen, 2006). In Thailand, at least 28 *Kaempferia* species have been recorded (Sirirugsa, 1989, 1992; Saensouk and Jenjittikul, 2001; Picheansoonthon and Koonterm, 2009; Picheansoonthon, 2011;). Several rare and endemic species have been assigned conservation status in Thailand.

The somatic cells of root tips for *Kaempferia* of the four species are examined with a chromosome number ranging of 2*n* = 22 to 2*n* = 42 (Table 4.4). The somatic numbers of *K. angustifolia* and *K. grandifolia* are 2*n* = 22 (Figures 4.1I-J), in this study also indicates that the base chromosome number of *Kaempferia* is x = 11 (Darlington and Wylie, 1945). This result is in full agreement with previous reports (Ramachandran, 1969; Mahanty, 1970; Eksomtramage *et al.*, 2002). The somatic numbers of 2*n* = 30 and 42 found in *K. rotunda* and *K. marginata*, respectively which is disagreement with Nerle and Torne (1984); Chen *et al.* (1988); Omanakumari and Mathew (1991); Eksomtramage and Boontum (1995), who observed somatic numbers of *K. rotunda* 2*n* = 54, 44, 46, 33, respectively. In contrast, a previous study of *K. marginata* from Phu Laenkha National Park, Thailand Khamtang *et al.* (2014), observed a chromosome count of 2*n* = 44, while a different study by Eksomtramage and Boontum (1995) showed a chromosome count of 2*n* = 55. These chromosome numbers may have been the products of aneuploidy. Therefore, chromosome number variations in ploidy level within species in our plant material are detected and they were to be x = 9, 11, 12 (Darlington and Wylie, 1945).

4.1.5 *Zingiber*

The genus *Zingiber* shows constant somatic chromosome number, and very few reports of variations exist. The somatic cells of root tips for *Zingiber* of the four species are examined with a chromosome number ranging of 2*n* = 22 (Table 4.5). Raghavan and Venkatasubban (1943) reported the basic number of this genus to be x = 11, 12 which was confirmed by and overwhelming majority of further studies. Earlier reports on chromosomes of the species *Z. officinale* also shows the diploid number to be 2*n* = 22 (Sugiura 1928, 1931; Morinaga *et al.* 1929; Raghavan and Venkatasubban

1943; Chakraborti 1948; Darlington and Wylie 1955; Sharma and Bhattacharya 1959; Ramachandran 1969; Ratnambal 1979; Omanakumari and Mathew 1985; Goldblatt 1988; Rai et al. 1997; Das *et al.* 1998; Dhamayanthi and Zachariah 1998; Eksomtramage et al. 2002; Saensouk and Saensouk 2004; Sanpote 2004; Nayak *et al.* 2005; Joseph 2010). Other than this, variations in chromosome numbers were reported by some workers. The diploid number of 2*n* = 24 was reported by Kihara *et al*. (1931), Chakraborti (1948), Sharma and Bhattacharya (1959), and Dhamayanthi and Zachariah (1998), while Etikawati and Setyawan (2000) and Daryono *et al.* (2012) reported the diploid number of 2*n* = 32. Further variations were reported by Darlington and Janaki Ammal (1945) (2*n* = 22 + 2B), Chakraborti (1948) (2*n* = 22 + 2f, 55), Sharma and Bhattacharya (1959) (2*n* = 10, 16, 21, 36, 46), Bisson *et al.* (1968) (2*n* = 66), and Daryono *et al*. (2012) (2*n* = 30). In *Z. zerumbet*, a well-established chromosome number of 2*n* = 22 was reported by Raghavan and Venkatasubban (1943), Chakraborti (1948), Ramachandran (1969), Ratnambal (1979), Omanakumari and Mathew (1985), Eksomtramage *et al.* (2001), Li and Chen (2008), and Joseph (2010). In the present study, the chromosome numbers of both the species of the genus *Zingiber* (*Z. officinale* and *Z. zerumbet*) was found to be 2*n* = 22, consistent with the earlier reports. About 25 species occur in Thailand (Larsen, 1996). Counts on most Thai *Zingiber* indicate it is a diploid with 2*n* = 22 (Moringa *et al.*, 1929; Sugiura, 1936; Raghavan and Venkatasubban, 1943; Chakravorti, 1948; Sharma and Bhattacharya, 1959; Eksomtramage *et al.*, 2001 and Saensouk, 2000). According to this study the somatic number of 2*n* = 22 was found in *Z. montanum, Z. mekongense, Z. officinale* and *Z. ottensii* (Figure 4.1M-P), confirming that these four *Zingiber* species are diploid and they were to be x = 9, 11, 12 (Darlington and Wylie, 1945) . The variations in chromosome numbers of these species have been reported from different parts of Thailand as well as that of the world. These chromosome numbers may have been the products of aneuploidy. Therefore, chromosome number variations in ploidy level within species are detected. The evolution processes have relationship with differentiation on geography, climate, soil or ecological environment. Genetic altering in all native population is caused by habitat changing in genetic processing. Genetic altering cause altering of morphological structure and physiological process. Genotype of species could be influenced by environment.

Table 4.1 Chromosome number of *Alpinia* compared with records by other authors.

Species	This study (2*n*)	Previous records			
		2*n*	*n*	Ploidy level (x)	Authors
A. allughas Rose.		48	24	x = 12	Ramachandran (1969)
		48	24		Raghavan (1942)
		48	24		Raghavan and Venkatasubban (1943)
		48	24		Chakravarti (1948)
A. aquatica Rose.		48	24		Chakravarti (1948)
A. bilamellata Makino		50	25		Ono and Masuda. (1981)
A. boninensis Makino		36	18		Ono and Masuda. (1981).
A. bracteata Roxb.		48	24		Chakravarti (1948)
A. calcarata Rose.		48	24		Ramachandran (1969)
		48	24		Raghavan (1943)
		48	24		Raghavan and Venkatasubban (1943)
		48	24		Chakravarti (1948)
A. chinensis		48	24		Satil (1948, 1960)
		48	24		Sato (1948)
A. coerulea Ben th.		48	24		Bisson *et al.* (1968)
A. coriacea T.L. Wu & S.J. Chen		48	24		Chen *et al*. (1982)
A. coriandriodora D. Fang		48	24		Chen and Chen (1984)
A. formosana K.			24		Hsu (1972)
			24		Chuang *et al*. (1963)
		48	24		Mahanty (1970)
A. galanga (L.) Willd.		48	24		Ramachandran (1969)
		48	24		Raghavan (1943)
		48	24		Chen and Chen (1985)
		48	24		Raghavan and Venkatasubban (1943)
A. galanga (L.) Willd. No.1,2	48, 48	48	-		Saensouk and Chantaranothai (2003)
A. intermedia Gagnep.		28	14		Hsu (1972)
A. japonica (Thunb.) Miq.		48	24		Satil (1948, 1960)
		48	24		Sato (1948)
		48	24		Chen *et al.* (1982).
A. katsumadae Hayata		48	24		Chen *et al.* (1982)
A. maclurei Merr.		48	24		Chen *et al.* (1982).
A. malaccensis (Burm. f.) Roscoe		48	24		Chen *et al***.** (1988)
		48	24		Chen *et al.* (1982)
A. malaccensis Rose.		48	24		Chakravarti (1948)
A. nutans Rose.		48	24		Raghavan and Venkatasubban (1943)
		48	24		Chakravarti (1948)
A. officinarum Hance		48	24		Chen and Chen (1986)
A. polyantha D. Fang		48	24		Chen *et al***.** (1988)

Table 4.1 Chromosome number of *Alpinia* compared with records by other authors. (Continued.)

Species	This study (2*n*)	Previous records			
		2*n*	***n***	**Ploidy level (x)**	**Authors**
A. sanderae Sander		48	24	x = 12	Chen *et al* (1982).
			23		Bhattacharyya (1968)
A. stachyodes Hance		48	24		Chen and Chen (1987)
A. siamensis	48	-	-		-
A. tonkinensis Gagnep.		48	24		Chen *et al.* (1982).
A. vittata Bull.		22	11		Chatterji (1964)
		48	24		Raghavan and Venkatasubban (1943)
A. zerumbet	48	-	48		Bhadral and Bandyopadhyay (2016)

Table 4.2 Chromosome number of *Amomum* compared with records by other authors.

Species	This study (2*n*)	Previous records			
		2*n*	***n***	**Ploidy level (x)**	**Authors**
A. aculeatum Roxb.			24	x = 12	Beltran and Kam (1984)
A. austrosinense D. Fang		96	48		Chen *et al.* (1988)
A.chinense W. Y. Chun		48	24		-
A. compactum Sol. ex Maton		48	24		-
A. biflorum Jack	48	48	24		Sharma and Bhattacharyya (1959)
		48	24		Beltran and Kiew (1989)
		48	24		Chen and Huang (1996)
		48			Eksomtramage *et al.* (2001)
A. gagnepainii T. L. Wu & K. Larsen & Turland		48	24		Chen *et al.* (1982)
A. hastilabium Ridl.			24		Beltran and Kam (1984)
A. kravanh Pierre ex Gagnep.		48	24		Chen and Chen (1984)
A. kwangsiense D. Fang & X. X. Chen		48	24		-
A. lappaceum Ridl.			24		Beltran and Kam (1984)
A. longiligulare T. L. Wu		48	24		Chen *et al* (1982)
A. macrodons Scort.			24		Beltran and Kam (1984)
A. maximum Roxb.		24	12		Chen *et al.* (1988)
		48	24		Chen *et al.* (1982)
A. menglunense T. L. Wu & S. J. Chen		48	24		Chen and Chen (1984)
A. muricarpum Elmer		48	24		
A. putrescens D. Fang		48	24		Chen and Chen (1984)
A. rivale Ridl.		48	24		Newman (1986)
A. sericeum Roxb.		48	24		-
A. schmidtii (K. Schum.) Gagnep.	48	-	-		-
A. squarrosum Ridl.			24		Beltran and Kam (1984)
A. subulatum Roxb.		54	27		Das and Das (1999)
		54	27		Das and Das (1999)
		48	24		Sharma and Bhattacharyya (1959)
A. testaceum Ridl.			24		Beltran and Kam (1984)
A. uliginosum J. Koenig			24		Beltran and Kam (1984)
A. villosum Lour.		48	24		Chen *et al.* (1982)
A. villosum var. xanthioides (Wall.) T. L. Wu & S. J. Chen		48	24		-

Table 4.3 Chromosome number of *Elettariopsis* compared with records by other authors.

Species	**This study (2*n*)**	**Previous records**			
		2*n*	***n***	**Ploidy level (x)**	**Authors**
E. burtiana Kam			24	x = 12	Kam (1982)
E. curtisii Baker			24		Kam (1982)
E. smithiae Kam			24		Kam (1982)
E. trilobum (Gagnep.) P.H. H			24		Kam (1982
E. smithiae var. rugosa Kam			24		Beltran and Kam (1984)
E. biphylla	48	-	-		New species
E. triloba	48	-	-		New species

Table 4.4 Chromosome number of *Kaempferia* compared with records by other authors.

Species	This study (2*n*)	Previous records			
		2*n*	*n*	Ploidy level (x)	Authors
K. angustifolia Roscoe		36	18	x = 9, 11, 12	Omanakumari and Mathew (1991)
		54	27		Sharma and Bhattacharyya (1959)
		54	27		Sharma and Bhattacharyya (1959)
	22				-
K.atrovirens N. E. Brown			11		Bhattacharyya (1968)
		22	11		Venkatasubban (1946)
		22	11		Venkatasubban (1946)
		22	11		Chakravorti (1948)
K. candida		22	11		-
K. elegans Wall.		22	11		Mahanty (1970)
K. galanga L.		54	27		Ramachandran (1969)
		54	27		Nerle and Torne (1984)
		54	27		Omanakumari and Mathew (1991)
		22	11		Sharma and Bhattacharyya (1959)
		54	27		Raghavan and Venkatasubban (1943)
		54	27		Raghavan and Arora (1958)
K. grandifolia	22	-	22		Saensouk and Jenjittikul (2001)
K. gilbertii Bull		36	18		Chakravorti (1948)
		36	18		Raghavan and Venkatasubban (1943)
		33			Mahanty (1970)
K. marginata	42	-	55		Eksomtramage and Boontum (1995)
		-	22		Saensouk and Chantaranothai (2003)
K. ovalifolia Roxb.		22	11		Sharma and Bhattacharyya (1959)
K. pulchra Ridl.		22	11		Mahanty (1970)
K. roscoeana Wall.		22	11		Nerle and Torne (1984)
K. rotunda			16		Bhattacharyya (1968)
		44	22		Ramachandran (1969)
		44	22		Chen *et al.* (1988)
		54	27		Nerle and Torne (1984)
		45			Omanakumari and Mathew (1991)
		33			Mahanty (1970)
		33			Chakravorti (1948)
			46		Omanakumari and Mathew (1991)
			33		Eksomtramage and Boontum (1995)
	30				-
K. speciosa Baker *K.*		22	11		Nerle and Torne (1984)

Table 4.5 Chromosome number of *Zingiber* compared with records by other authors.

Species	This study (2*n*)	Previous records			
		2*n*	***n***	**Ploidy level (x)**	**Authors**
Z. cassumunar Roxb.	22	22	11	x = 11, 12	Raghavan and Katasubban (1943)
		22	11		Chakravorti (1948)
Z. chrysostachys Ridl.			11		Beltran and Kam (1984)
Z. corallinum Hance		22	11		-
		22	11		Chen *et al.* (1988)
Z. cylindricum Moon.		22	11		Mahanty (1970)
Z. ellipticum		22	11		-
Z. griffithii Baker			11		Beltran and Kam (1984)
Z. macrostachyum Dalzell		22	11		Ramachandran (1969)
		22	11		Omanakumari and Mathew (1991)
		22	11		Ratnambal (1984)
Z. mioga (Thunb.) Roscoe		22	11		-
		22	11		Chen *et al.* (1988)
		55			Suzuka and Mitsuoka (1968)
		55			Morinaga *et al.* (1929)
		55			Sat (1960)
Z. multibracteatum Holttum			11		Beltran and Kam (1984)
		22	11		Newman (1986)
Z. mekongense Gagnep.	22				Saensouk and Chantaranothai (2003)
Z. nudicarpum		22	11		-
Z. officinale Roscoe	22	22	11		Ramachandran (1969)
		22	11		Li (1989)
		22	11		Ramachandran (1982)
		22	11		Omanakumari and Mathew (1991)
		22	11		Ratnambal (1984)
			11		Adaniya and Shoda. 1998)
		22	11		Das and Das (1998)
		22	11		Dhamayanthi (1998)
		22	11		Rai *et al.* (1997)
		22	11		Das *et al.* (1998)
		24	12		Dhamayanthi, and Zachariah (1998)
		66	33		Bisson *et al.* (1968)
		22	11		Dhamayanthi, and Zachariah (1998)
		22	11		Sugiura (1928)
		22	11		Raghavan and Venkatasubban (1943)

Table 4.5 Chromosome number of *Zingiber* compared with records by other authors. (Continued.)

Species	This study (2*n*)	Previous records			
		2*n*	***n***	**Ploidy level (x)**	**Authors**
Z. officinale Roscoe		22	11		Chakravorti (1948)
		22	11		Sharma and Bhattacharyya (1959)
		24	12		Kihara *et al*. (1931)
		22	11		Sharma and Bhattacharyya (1959)
		22			Saensouk and Chantaranothai (2003)
Z. ottensii Valet.	22		11		Beltran and Kam (1984)
Z. purpureum Roscoe			11		Beltran and Kam (1984)
Z. roseum		22	11		Ramachandran (1969)
		22	11		-
Z. rubens Roxb.		24	12	12	Das *et al*. (1998)
		22	11		Chakravorti (1948)
Z. spectabile Griff.			11		Beltran and Kam (1984)
		22	11		Newman (1986)
		22	11		Mahanty (1970)
Z. striolatum		22	11		-
		22	11		Ramachandran (1969)
Z. wightianum Thw.		22	11		Omanakumari and Mathew (1991)
Z. zerumbet (L.) Roscoe ex Sm.		22	11		Ramachandran (1969)
			11		Bhattacharyya (1968)
		22	11		-
		22	11		Chen *et al*. (1988)
		22	11		Omanakumari and Mathew (1991)
			11		Beltran and Kam (1984)
		22	11		Ratnambal (1984)
		22	11		Raghavan and Venkatasubban (1943)
		22	11		Chakravorti (1948)

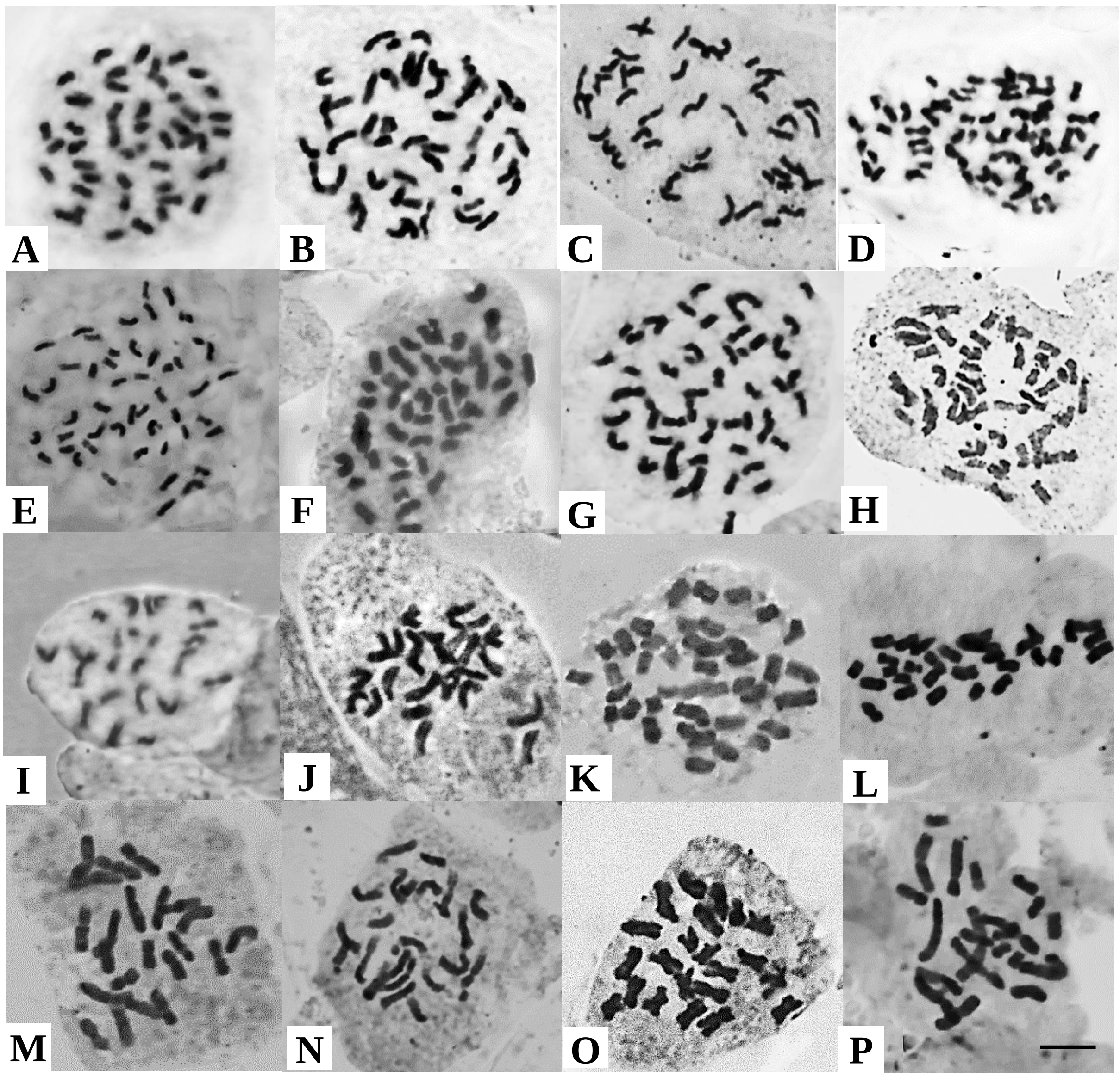


Figure 4.1 Somatic metaphase chromosomes of 16 species from Zingiberaceae. (A) *A. galanga* (No.1), (B) *A. galanga* (No.2), (C) *A. siamensis*, (D) *A. zerumbet*, (E) *Amomum biflorum*, (F) *Amomum schmidtii*, (G) *E. biphylla*, (H) *E. triloba*, (I) *K. angustifolia*, (J) *K. grandifolia*, (K) *K. marginata*, (L) *K. rotunda*, (M) *Z. montanum*, (N) *Z. mekongensis*, (O) *Z. officinale* and (P) *Z. ottensii*, respectively. Scale bars represent 5 μm.

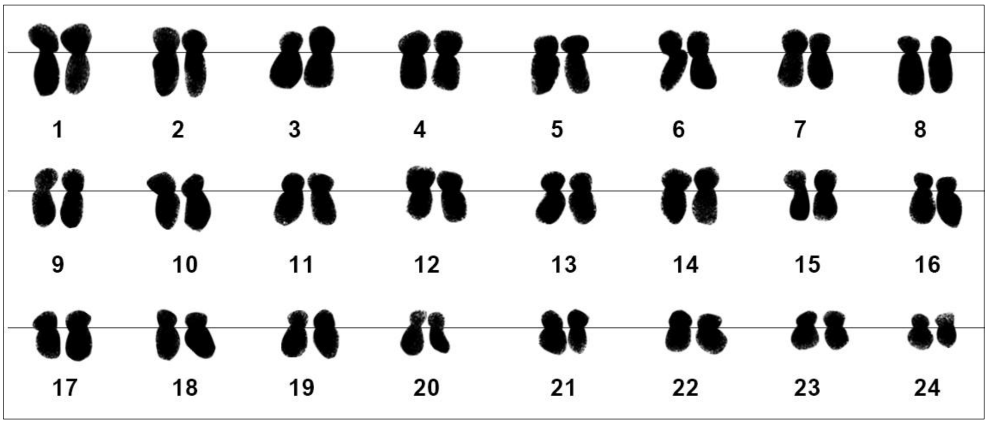
4.2 Karyotype and idiograms

Karyomorphological studies revealed consistent chromosome numbers in the root tips of 16 species from Thailand area was carried out. The karyotypes and idiograms are summarized in each species described below.

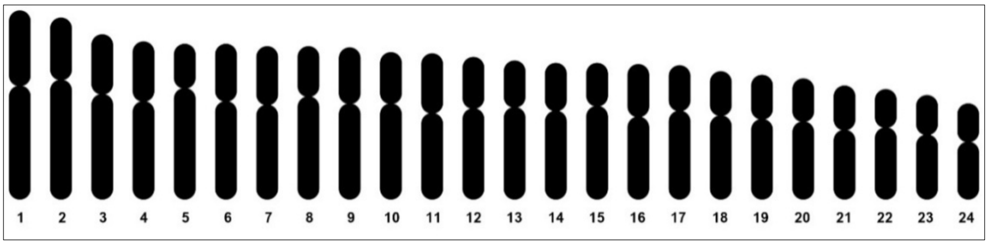
4.2.1 *Alpinia galanga* (L.) Willd. (No.1)

A. galanga (No.1) showed a somatic chromosome number of 2*n* = 48 (Figure 4.1A), the same chromosome number was found by Ravindran *et al.* (2007). The karyotype formula was deduced to be (2*n*, 48) = $L^{sm}_{20} + L^{a}_{2} + M^{sm}_{26}$ (Figure 4.2, Table 4.6). The total chromosome length of individual chromosomes ranged between 0.80 to 1.57 µm. with the length of short arms and the long arms 0.32 to 0.32 µm, and 0.48 to 0.80 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.605 to 0.716. The relative lengths (RL%) in this study ranged from 0.028 to 0.056 (Table 4.6).

This data demonstrate that has 22 large and 20 medium chromosomes, observed asymmetrical karyotypes. An idiogram of *A. galanga* (No.1) of the haploid set (*n* = 24) is shown in Figure 4.2B. This is the first report of karyotype for this species.



A



B

Figure 4.2 Karyotype (A) and idiogram (B) of *Alpinia galanga* (No.1)

Table 4.6 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Alpinia galanga* (No.1), 2*n* (diploid) = 48.

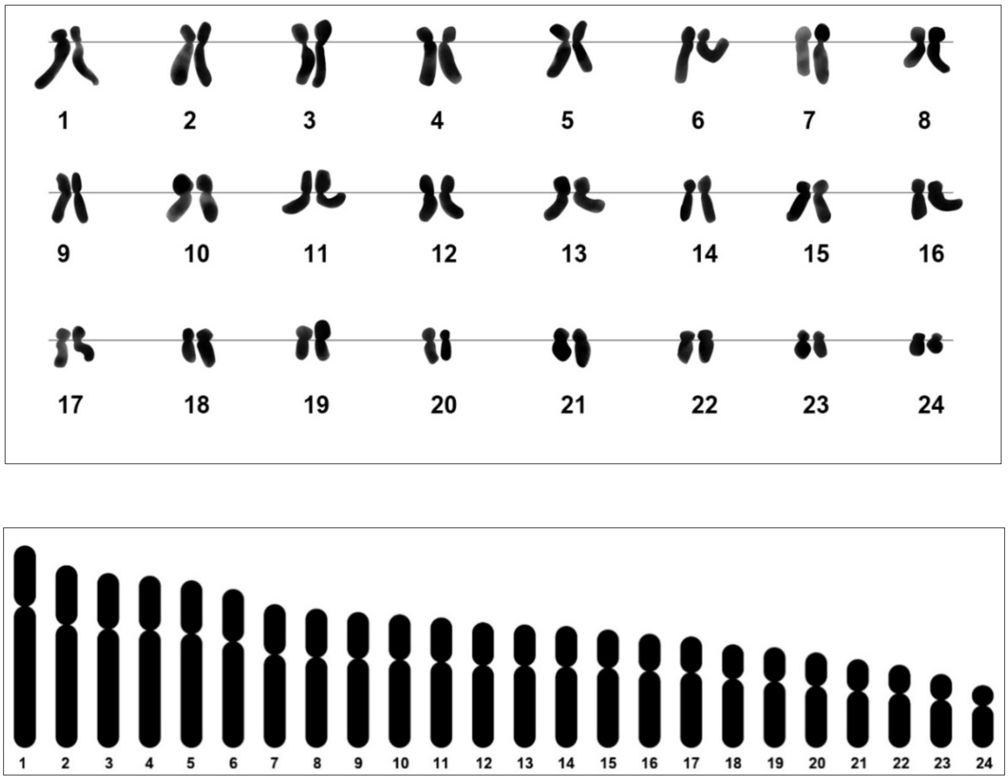
Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	0.63	0.95	1.57	0.601 ± 0.004	0.056 ± 0.001	Large	submetacentric
2	0.52	1.00	1.52	0.657 ± 0.021	0.054 ± 0.001	Large	submetacentric
3	0.50	0.88	1.38	0.636 ± 0.095	0.049 ± 0.001	Large	submetacentric
4	0.50	0.82	1.32	0.620 ± 0.009	0.047 ± 0.000	Large	submetacentric
5	0.37	0.93	1.30	0.716 ± 0.001	0.046 ± 0.000	Large	acrocentric
6	0.48	0.82	1.29	0.632 ± 0.079	0.046 ± 0.000	Large	submetacentric
7	0.49	0.79	1.28	0.620 ± 0.011	0.045 ± 0.000	Large	submetacentric
8	0.42	0.86	1.27	0.674 ± 0.014	0.045 ± 0.000	Large	submetacentric
9	0.47	0.80	1.26	0.631 ± 0.003	0.079 ± 0.048	Large	submetacentric
10	0.43	0.80	1.23	0.649 ± 0.026	0.044 ± 0.001	Large	submetacentric
11	0.50	0.72	1.22	0.600 ± 0.083	0.043 ± 0.000	Large	submetacentric
12	0.43	0.76	1.19	0.637 ± 0.002	0.042 ± 0.001	Medium	submetacentric
13	0.39	0.77	1.16	0.663 ± 0.009	0.041 ± 0.000	Medium	submetacentric
14	0.40	0.74	1.14	0.646 ± 0.017	0.041 ± 0.000	Medium	submetacentric
15	0.36	0.78	1.13	0.685 ± 0.027	0.071 ± 0.043	Medium	submetacentric
16	0.44	0.69	1.12	0.611 ± 0.014	0.071 ± 0.043	Medium	submetacentric
17	0.38	0.74	1.12	0.660 ± 0.047	0.070 ± 0.043	Medium	submetacentric
18	0.37	0.70	1.07	0.651 ± 0.058	0.067 ± 0.039	Medium	submetacentric
19	0.37	0.67	1.04	0.647 ± 0.060	0.037 ± 0.000	Medium	submetacentric
20	0.36	0.65	1.02	0.641 ± 0.060	0.063 ± 0.038	Medium	submetacentric
21	0.37	0.58	0.95	0.612 ± 0.045	0.059 ± 0.035	Medium	submetacentric
22	0.32	0.60	0.92	0.655 ± 0.040	0.033 ± 0.000	Medium	submetacentric
23	0.33	0.54	0.88	0.619 ± 0.015	0.031 ± 0.000	Medium	submetacentric
24	0.32	0.48	0.80	0.605 ± 0.000	0.028 ± 0.001	Medium	submetacentric

Note: Chro. = Chromosome

4.2.2 *Alpinia galanga* (L.) Willd. (No.2)

A. galanga (No.2) showed a somatic chromosome number of 2*n* = 48 (Figure 4.1B), which agrees with the values previous reported for this species (Saensouk and Chantaranothai, 2003). The karyotype formula was deduced to be (2*n*, 48) = L^{sm}_{18} + L^{a}_{2} + M^{sm}_{28} (Figure 4.3, Table 4.7). The total chromosome length of individual chromosomes ranged between 2.34 to 4.26 µm. with the length of short arms and the long arms 0.43 to 1.28 µm, and 0.88 to 2.99 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.641 to 0.701. The relative lengths (RL%) in this study ranged from 0.032 to 0.058 (Table 4.7).

This data demonstrates *A. galanga* (No.2) that has 20 large and 28 medium chromosomes, observed asymmetrical karyotypes. An idiogram of *A. galanga* (No.2) of the haploid set (*n* = 24) is shown in Figure 4.3B. This is the first report of karyotype for this species.



A

B

Figure 4.3 Karyotype (A) and idiogram (B) of *Alpinia galanga* (No.2)

Table 4.7 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI) and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Alpinia galanga* (No.2), 2*n* (diploid) = 48.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	1.28	2.99	4.26	0.701 ± 0.026	0.058 ± 0.002	Large	acrocentric
2	1.25	2.59	3.85	0.674 ± 0.015	0.053 ± 0.001	Large	submetacentric
3	1.18	2.51	3.68	0.681 ± 0.012	0.050 ± 0.001	Large	submetacentric
4	1.15	2.48	3.62	0.684 ± 0.013	0.050 ± 0.001	Large	submetacentric
5	1.12	2.40	3.53	0.682 ± 0.016	0.048 ± 0.001	Large	submetacentric
6	1.10	2.24	3.34	0.670 ± 0.022	0.046 ± 0.001	Large	submetacentric
7	1.06	1.96	3.02	0.650 ± 0.020	0.041 ± 0.001	Large	submetacentric
8	1.02	1.90	2.93	0.650 ± 0.004	0.040 ± 0.000	Large	submetacentric
9	0.98	1.88	2.86	0.657 ± 0.002	0.039 ± 0.000	Large	submetacentric
10	0.95	1.85	2.81	0.660 ± 0.005	0.038 ± 0.000	Large	submetacentric
11	0.94	1.81	2.74	0.659 ± 0.011	0.037 ± 0.001	Medium	submetacentric
12	0.90	1.74	2.64	0.658 ± 0.016	0.036 ± 0.001	Medium	submetacentric
13	0.87	1.73	2.59	0.667 ± 0.030	0.035 ± 0.002	Medium	submetacentric
14	0.86	1.70	2.56	0.666 ± 0.021	0.035 ± 0.001	Medium	submetacentric
15	0.81	1.68	2.49	0.676 ± 0.021	0.034 ± 0.001	Medium	submetacentric
16	0.78	1.61	2.40	0.674 ± 0.029	0.033 ± 0.001	Medium	submetacentric
17	0.76	1.58	2.34	0.677 ± 0.026	0.032 ± 0.001	Medium	submetacentric
18	0.72	1.45	2.17	0.668 ± 0.045	0.030 ± 0.002	Medium	submetacentric
19	0.72	1.39	2.11	0.659 ± 0.035	0.029 ± 0.002	Medium	submetacentric
20	0.71	1.30	2.00	0.647 ± 0.004	0.027 ± 0.000	Medium	submetacentric
21	0.67	1.19	1.86	0.641 ± 0.029	0.025 ± 0.001	Medium	submetacentric
22	0.60	1.14	1.74	0.654 ± 0.012	0.024 ± 0.000	Medium	submetacentric
23	0.54	1.01	1.55	0.651 ± 0.029	0.021 ± 0.001	Medium	submetacentric
24	0.43	0.88	1.31	0.677 ± 0.053	0.018 ± 0.001	Medium	submetacentric

Note: Chro. = Chromosome

4.2.3 *Alpinia siamensis* K. Schum.

The plants of *A. siamensis* showed a somatic chromosome number of $2n$ = 48 (Figure 4.1C), which agrees with the values previous reported for other species in the genus *Alpinia*, (Ono and Masuda, 1981; Eksomtramage *et al.*, 2001; Saensouk and Chantaranothai, 2003). The karyotype formula was deduced to be (2n, 48) = $L^{m}_{4} + L^{sm}_{8} + L^{a}_{16} + M^{m}_{4} + M^{sm}_{10} + M^{a}_{6}$ (Figure 4.4, Table 4.8). The total chromosome length of individual chromosomes ranged between 2.18 to 4.75 µm. with the length of short arms and the long arms 0.75 to 2.07 µm, and 1.36 to 3.68 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.553 to 0.778. The relative lengths (RL%) in this study ranged from 0.037 to 0.056 (Table 4.8).

This data demonstrates that *A. siamensis* has 28 large and 20 medium chromosomes, observed asymmetrical karyotypes. An idiogram of *A. siamensis* of the haploid set (n = 24) is shown in Figure 4.4B. This is the first report of karyotype for this species.

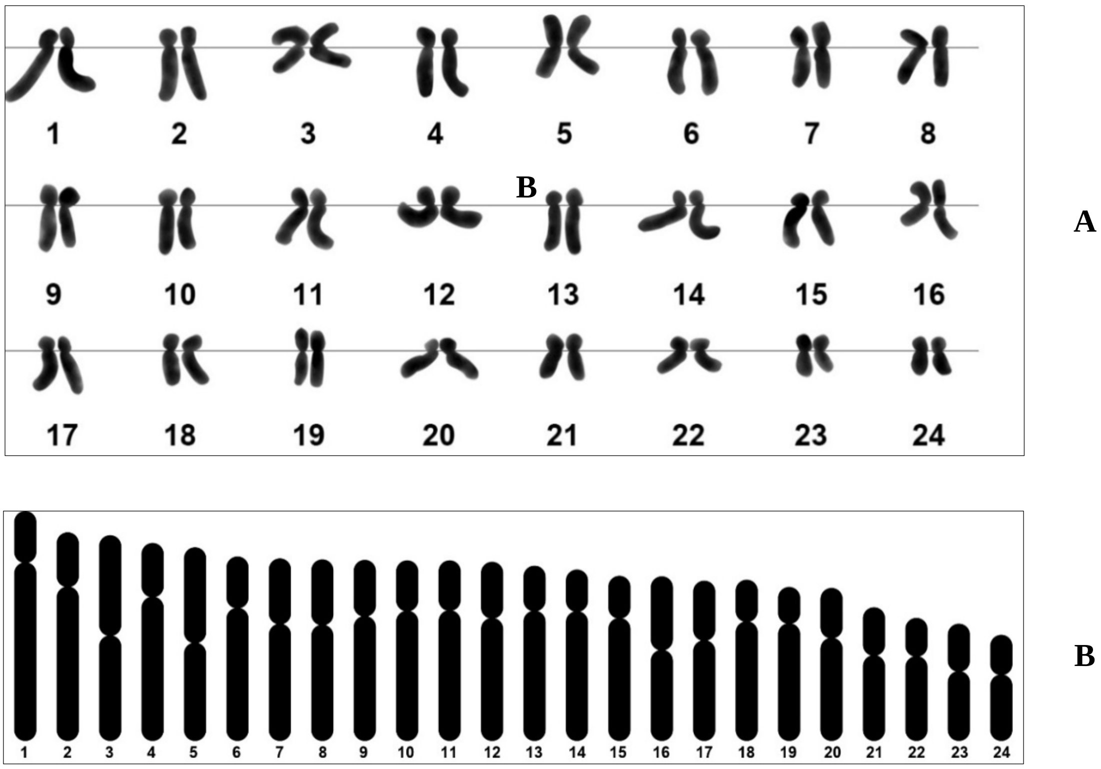


Figure 4.4 Karyotype (A) and idiogram (B) of *Alpinia siamensis*

Table 4.8 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI) and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Alpinia siamensis*, 2*n* (diploid) = 48.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	1.06	3.68	4.75	0.778 ± 0.059	0.056 ± 0.004	Large	acrocentric
2	1.12	3.18	4.30	0.739 ± 0.015	0.051 ± 0.001	Large	acrocentric
3	2.07	2.17	4.23	0.512 ± 0.008	0.050 ± 0.001	Large	metacentric
4	1.11	2.97	4.07	0.728 ± 0.009	0.048 ± 0.001	Large	acrocentric
5	1.97	2.02	3.99	0.506 ± 0.023	0.047 ± 0.002	Large	metacentric
6	1.06	2.74	3.80	0.721 ± 0.002	0.045 ± 0.000	Large	acrocentric
7	1.35	2.41	3.76	0.642 ± 0.038	0.045 ± 0.003	Large	submetacentric
8	1.35	2.39	3.74	0.641 ± 0.037	0.044 ± 0.003	Large	submetacentric
9	1.16	2.57	3.72	0.690 ± 0.021	0.044 ± 0.001	Large	submetacentric
10	1.04	2.68	3.72	0.720 ± 0.029	0.044 ± 0.002	Large	acrocentric
11	1.03	2.69	3.71	0.724 ± 0.039	0.044 ± 0.002	Large	acrocentric
12	1.16	2.53	3.68	0.687 ± 0.048	0.044 ± 0.003	Large	submetacentric
13	0.93	2.68	3.61	0.743 ± 0.035	0.043 ± 0.002	Large	acrocentric
14	0.86	2.67	3.53	0.756 ± 0.019	0.042 ± 0.001	Large	acrocentric
15	0.87	2.53	3.40	0.744 ± 0.002	0.040 ± 0.000	Medium	acrocentric
16	1.52	1.87	3.39	0.553 ± 0.046	0.040 ± 0.003	Medium	metacentric
19	1.23	2.07	3.30	0.626 ± 0.001	0.039 ± 0.001	Medium	acrocentric
17	0.86	2.46	3.33	0.741 ± 0.017	0.037 ± 0.002	Medium	submetacentric
20	0.75	2.42	3.18	0.763 ± 0.012	0.039 ± 0.000	Medium	submetacentric
18	1.03	2.12	3.15	0.674 ± 0.031	0.038 ± 0.001	Medium	acrocentric
21	0.99	1.76	2.75	0.641 ± 0.003	0.033 ± 0.000	Medium	submetacentric
22	0.79	1.74	2.52	0.690 ± 0.049	0.030 ± 0.002	Medium	submetacentric
23	0.98	1.43	2.42	0.594 ± 0.022	0.029 ± 0.001	Medium	metacentric
24	0.82	1.36	2.18	0.623 ± 0.012	0.026 ± 0.000	Medium	submetacentric

Note: Chro. = Chromosome

4.2.4 *Alpinia zerumbet* (Pers.) B.L.Burtt & R.M.Sm.

The plants of *A. zerumbet* showed a somatic chromosome number of 2*n* = 48 (Figure 4.1D). Although not much cytological study has been done previously on *A. zerumbet* by Joseph (1998) reported 2*n* = 48, while a different study by Bhadra and Bandyopadhyay (2016) showed a chromosome count of *A. zerumbet* 2*n* = 52. In this study the karyotype formula was deduced to be (2*n*, 48) = $L^{m}{}_{2} + L^{sm}{}_{14} + M^{m}{}_{20} + M^{sm}{}_{12}$ (Figure 4.5, Table 4.9). The total chromosome length of individual chromosomes ranged between 2.62 to 4.35 µm. with the length of short arms and the long arms 0.65 to 1.76 µm, and 0.87 to 2.59 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.574 to 0.646. The relative lengths (RL%) in this study ranged from 0.025 to 0.070 (Table 4.9).

This data demonstrates that *A. zerumbet* has 16 large and 32 medium chromosomes, observed asymmetrical karyotypes. An idiogram of *A. zerumbet* of the haploid set (*n* = 24) is shown in Figure 4.5. A previous karyotype description has been made for this species by Bhadra and Bandyopadhyay (2016) found (2*n*, 52) = 1 M+ 39 m+ 10Sm + 1 m:Sm +1Sm:Sm.

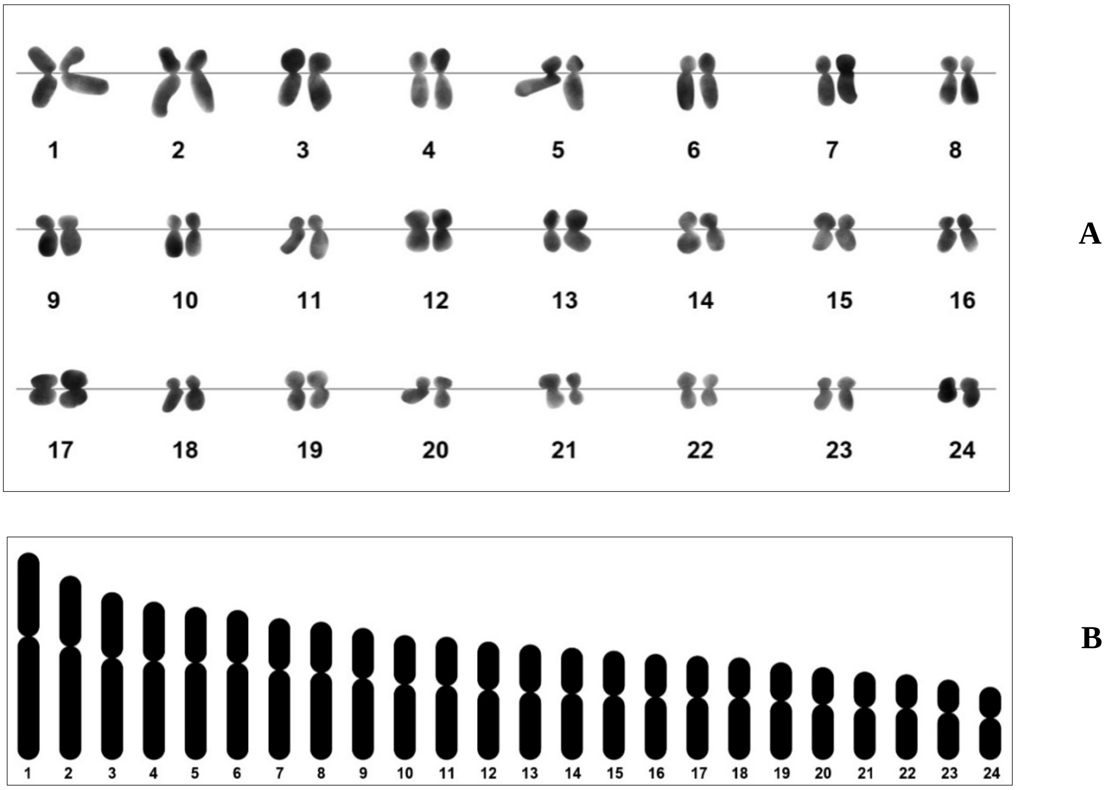


Figure 4.5 Karyotype (A) and idiogram (B) of *Alpinia zerumbet*.

Table 4.9 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI) and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Alpinia zerumbet*, 2*n* (diploid) = 48.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	1.76	2.59	4.35	0.596 ± 0.006	0.070 ± 0.001	Large	metacentric
2	1.48	2.38	3.86	0.617 ± 0.017	0.062 ± 0.002	Large	submetacentric
3	1.38	2.14	3.52	0.608 ± 0.015	0.057 ± 0.001	Large	submetacentric
4	1.25	2.07	3.32	0.625 ± 0.009	0.053 ± 0.001	Large	submetacentric
5	1.17	2.04	3.21	0.637 ± 0.007	0.052 ± 0.001	Large	submetacentric
6	1.11	2.03	3.14	0.646 ± 0.006	0.051 ± 0.001	Large	submetacentric
7	1.09	1.88	2.97	0.634 ± 0.002	0.048 ± 0.000	Large	submetacentric
8	1.06	1.83	2.89	0.633 ± 0.009	0.047 ± 0.001	Large	submetacentric
9	1.06	1.71	2.76	0.617 ± 0.017	0.045 ± 0.001	Medium	submetacentric
10	1.03	1.58	2.61	0.605 ± 0.008	0.042 ± 0.001	Medium	submetacentric
11	1.02	1.56	2.58	0.604 ± 0.004	0.042 ± 0.000	Medium	submetacentric
12	1.01	1.46	2.48	0.591 ± 0.005	0.040 ± 0.000	Medium	metacentric
13	1.00	1.41	2.42	0.585 ± 0.007	0.039 ± 0.000	Medium	metacentric
14	0.96	1.39	2.35	0.590 ± 0.016	0.038 ± 0.001	Medium	metacentric
15	0.94	1.34	2.28	0.589 ± 0.001	0.037 ±0.000	Medium	metacentric
16	0.90	1.31	2.22	0.593 ± 0.002	0.036 ± 0.000	Medium	metacentric
17	0.87	1.30	2.18	0.598 ± 0.010	0.035 ± 0.001	Medium	metacentric
18	0.84	1.30	2.14	0.606 ± 0.012	0.034 ± 0.001	Medium	submetacentric
19	0.81	1.23	2.04	0.605 ± 0.002	0.033 ± 0.000	Medium	submetacentric
20	0.78	1.16	1.94	0.598 ± 0.033	0.031 ± 0.002	Medium	metacentric
21	0.75	1.09	1.84	0.594 ± 0.020	0.030 ± 0.001	Medium	metacentric
22	0.71	1.07	1.79	0.602 ± 0.033	0.029 ±0.002	Medium	submetacentric
23	0.69	0.99	1.68	0.588 ± 0.013	0.027 ± 0.001	Medium	metacentric
24	0.65	0.87	1.52	0.574 ± 0.031	0.025 ± 0.001	Medium	metacentric

Note: Chro. = Chromosome

4.2.5 *Amomum biflorum* Jack

The plants of *A. biflorum* showed a somatic chromosome number of 2*n* = 48 (Figure 4.1E). A previous reported for *A. biflorum* 2*n* = 48, (Eksomtramage *et al.*, 2001). In this study the karyotype formula was deduced to be (2*n*, 48) = L^{sm}_{10} + L^{a}_{2} + M^{sm}_{36} (Figure 4.6, Table 4.10). The total chromosome length of individual chromosomes ranged between 1.26 to 4.39 µm. with the length of short arms and the long arms 0.49 to 1.30 µm, and 0.77 to 3.09 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.617 to 0.704 The relative lengths (RL%) in this study ranged from 0.021 to 0.075 (Table 4.10).

This data demonstrates that *A. biflorum* has 12 large and 36 medium chromosomes, observed asymmetrical karyotypes. An idiogram of *A. biflorum* of the haploid set (*n* = 24) is shown in Figure 4.6. This is the first report of karyotype for this species.

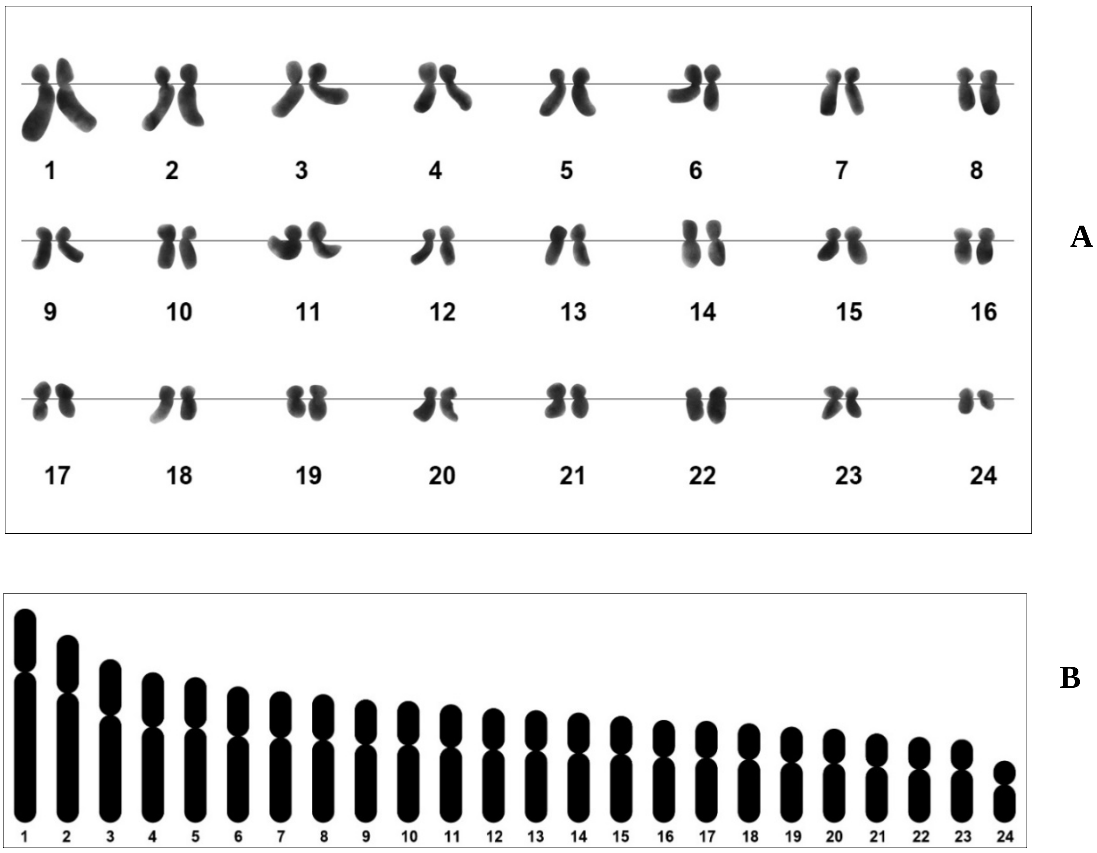


Figure 4.6 Karyotype (A) and idiogram (B) of *Amomum biflorum.*

Table 4.10 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI) and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Amomum biflorum*, 2*n* (diploid) = 48.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	1.30	3.09	4.39	0.704 ± 0.024	0.075 ± 0.003	Large	acrocentric
2	1.19	2.66	3.85	0.693 ± 0.058	0.065 ± 0.005	Large	submetacentric
3	1.15	2.20	3.35	0.658 ± 0.016	0.057 ± 0.001	Large	submetacentric
4	1.12	1.96	3.07	0.638 ± 0.037	0.052 ± 0.003	Large	submetacentric
5	1.04	1.94	2.98	0.652 ± 0.034	0.051 ± 0.003	Large	submetacentric
6	1.02	1.77	2.80	0.634 ± 0.013	0.048 ± 0.001	Large	submetacentric
7	0.95	1.74	2.69	0.646 ± 0.000	0.046 ± 0.000	Medium	submetacentric
8	0.94	1.69	2.63	0.644 ± 0.004	0.045 ± 0.000	Medium	submetacentric
9	0.92	1.60	2.52	0.635 ± 0.009	0.043 ± 0.001	Medium	submetacentric
10	0.90	1.59	2.49	0.638 ± 0.018	0.042 ± 0.001	Medium	submetacentric
11	0.88	1.54	2.42	0.636 ± 0.031	0.041 ± 0.002	Medium	submetacentric
12	0.85	1.49	2.34	0.638 ± 0.015	0.040 ± 0.001	Medium	submetacentric
13	0.83	1.47	2.30	0.640 ± 0.016	0.039 ± 0.001	Medium	submetacentric
14	0.83	1.42	2.25	0.632 ± 0.009	0.038 ± 0.001	Medium	submetacentric
15	0.78	1.40	2.17	0.644 ± 0.003	0.037 ± 0.000	Medium	submetacentric
16	0.76	1.34	2.10	0.638 ± 0.009	0.036 ± 0.001	Medium	submetacentric
17	0.76	1.32	2.08	0.637 ± 0.011	0.035 ± 0.001	Medium	submetacentric
18	0.74	1.29	2.03	0.636 ± 0.001	0.034 ± 0.000	Medium	submetacentric
19	0.73	1.23	1.95	0.628 ± 0.006	0.033 ± 0.000	Medium	submetacentric
20	0.71	1.21	1.92	0.629 ± 0.007	0.033 ± 0.000	Medium	submetacentric
21	0.68	1.14	1.82	0.628 ± 0.037	0.031± 0.002	Medium	submetacentric
22	0.66	1.09	1.75	0.622 ± 0.013	0.030 ± 0.001	Medium	submetacentric
23	0.62	1.08	1.70	0.637 ± 0.024	0.029 ± 0.001	Medium	submetacentric
24	0.49	0.77	1.26	0.617 ± 0.085	0.021 ± 0.003	Medium	submetacentric

Note: Chro. = Chromosome

4.2.6 *Amomum schmidtii* (K. Schum.) Gagnep.

The plants of *A. schmidtii* showed a somatic chromosome number of 2*n* = 48 (Figure 4.1F). According to Beltran and Kiew (1984) and Chen and Huang (1996) chromosome of *Amomum* as 2n = 4x = 48. The total chromosome length of individual chromosomes ranged between 1.65 to 4.39 μm. with the length of short arms and the long arms 1.07 to 2.42 μm, and 0.77 to 3.99 μm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.583 to 0.628 The relative lengths (RL%) in this study ranged from 0.025 to 0.061 (Table 4.11).

This data demonstrates that *A. schmidtii* has 22 large and 26 medium chromosomes and a karyotype formula of (2*n*, 48) = $L^{sm}_{22} + M^{sm}_{16} + M^{m}_{10}$ (Figure 4.7A), observed asymmetrical karyotypes. An idiogram of *A. schmidtii* of the haploid set (*n* = 24) is shown in Figure 4.7B. This is the first report of karyotype for this species.

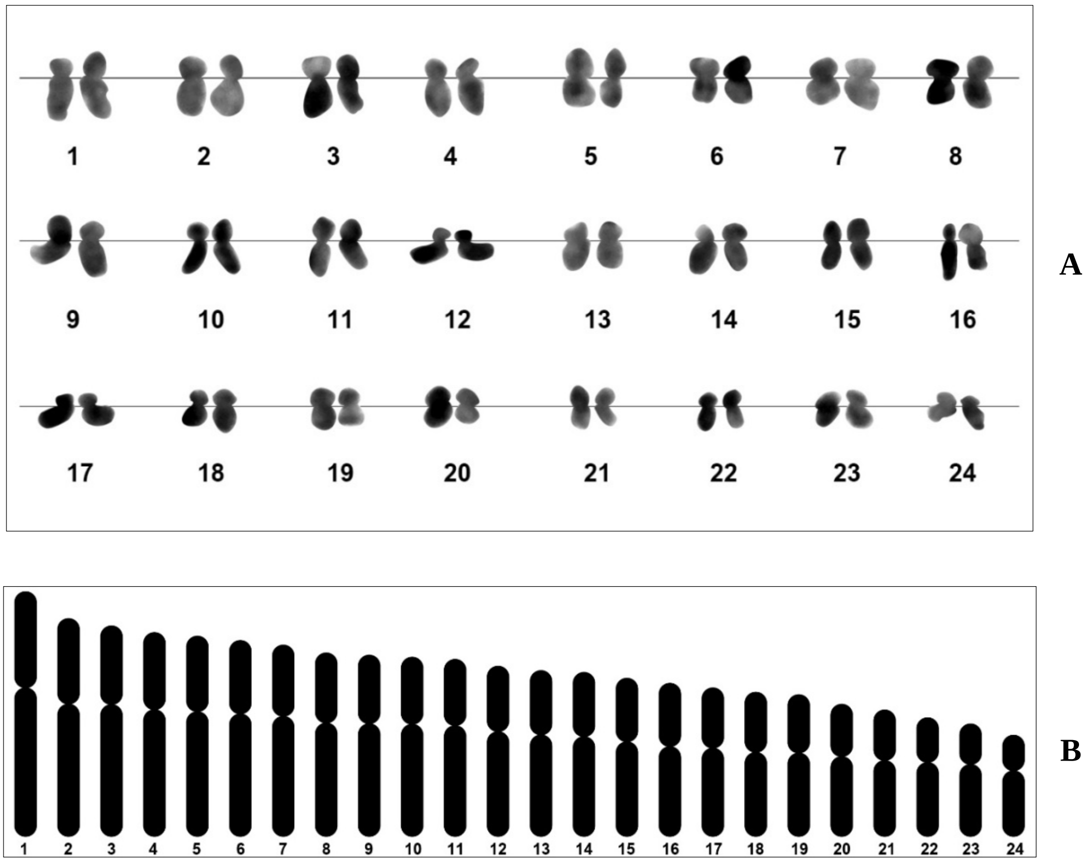


Figure 4.7 Karyotype (A) and idiogram (B) of *Amomum schmidtii.*

Table 4.11 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI) and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Amomum schmidtii*, 2*n* (diploid) = 48.

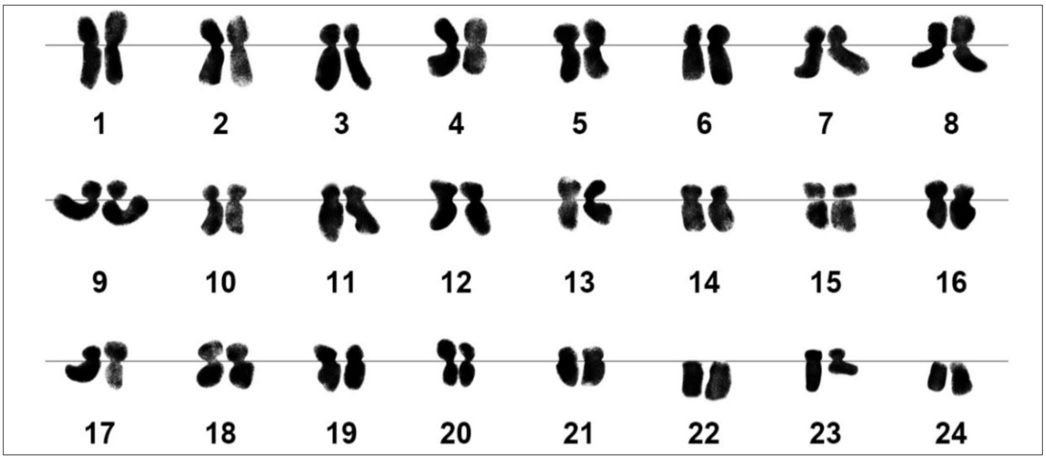
Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	1.57	2.42	3.99	0.607 ± 0.004	0.061 ± 0.000	Large	submetacentric
2	1.39	2.16	3.55	0.607 ± 0.009	0.054 ± 0.001	Large	submetacentric
3	1.28	2.15	3.43	0.626 ± 0.015	0.052 ± 0.001	Large	submetacentric
4	1.26	2.07	3.32	0.622 ± 0.006	0.051 ± 0.001	Large	submetacentric
5	1.23	2.03	3.27	0.623 ±0.005	0.050 ± 0.000	Large	submetacentric
6	1.20	2.00	3.20	0.625 ± 0.000	0.049 ± 0.000	Large	submetacentric
7	1.16	1.96	3.12	0.628 ± 0.010	0.048 ± 0.001	Large	submetacentric
8	1.14	1.85	2.99	0.618 ± 0.003	0.046 ± 0.000	Large	submetacentric
9	1.12	1.84	2.96	0.622 ± 0.011	0.045 ± 0.001	Large	submetacentric
10	1.09	1.83	2.92	0.626 ± 0.017	0.045 ± 0.001	Large	submetacentric
11	1.08	1.81	2.89	0.626 ± 0.022	0.044 ± 0.002	Large	submetacentric
12	1.07	1.71	2.78	0.616 ± 0.014	0.042 ± 0.001	Medium	submetacentric
13	1.05	1.66	2.70	0.612 ± 0.000	0.041 ± 0.000	Medium	submetacentric
14	1.05	1.63	2.68	0.608 ± 0.006	0.041 ± 0.000	Medium	submetacentric
15	1.04	1.54	2.58	0.598 ± 0.010	0.039 ± 0.001	Medium	metacentric
16	1.03	1.46	2.50	0.587 ± 0.021	0.038 ± 0.001	Medium	metacentric
17	0.98	1.44	2.42	0.595 ± 0.019	0.037 ± 0.001	Medium	metacentric
18	0.98	1.37	2.35	0.583 ± 0.017	0.036 ± 0.001	Medium	metacentric
19	0.95	1.36	2.31	0.589 ± 0.019	0.035 ±0.001	Medium	metacentric
20	0.85	1.30	2.16	0.604 ± 0.027	0.033 ± 0.000	Medium	submetacentric
21	0.82	1.24	2.06	0.600 ± 0.004	0.031 ± 0.000	Medium	submetacentric
22	0.73	1.21	1.94	0.624 ± 0.026	0.030 ± 0.001	Medium	submetacentric
23	0.66	1.17	1.84	0.640 ± 0.011	0.028 ± 0.000	Medium	submetacentric
24	0.58	1.07	1.65	0.649 ± 0.001	0.025 ± 0.000	Medium	submetacentric

Note: Chro. = Chromosome

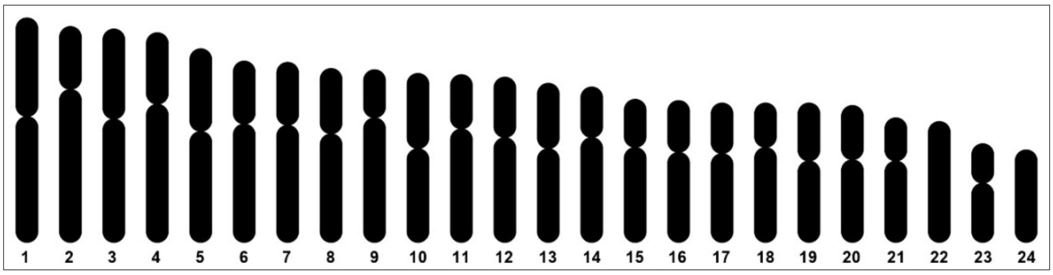
4.2.7 *Elettariopsis biphylla* S.Saensouk & P.Saensouk, *sp. nov.*

E. biphylla, a new species of Zingiberaceae from Thailand, was discovered by Saensouk and Saensouk (2014) and has a somatic chromosome number of $2n = 48$ (Figure 4.1G). This chromosome number agrees with those previously reported for other species in this genus (Beltran and Kiew, 1984 and Kam, 1982). The total chromosome length of individual chromosomes ranged between 0.75 to 1.82 µm. with the length of short arms and the long arms 1.00 to 0.80 µm, and 0.73 to 1.27 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.555 to 1.000 The relative lengths (RL%) in this study ranged from 0.024 to 0.059 (Table 4.12).

The results showed that *E. biphylla* has 26 large and 22 medium chromosomes with the karyotype formula of *E. biphylla* to be $(2n, 48) = L^{m}_{6} + L^{sm}_{16} + L^{a}_{4} + M^{m}_{2} + M^{sm}_{16} + M^{t}_{4}$ (Figure 4.8A), observed asymmetrical karyotypes. In addition, this study found that the 22nd, 24th medium chromosome pairs were of the telocentric chromosomes type. An idiogram of *E. biphylla* showing the haploid set ($n = 24$) is shown in Figure 4.8B. This is the first report of karyotype for this species.



A



B

Figure 4.8 Karyotype (A) and idiogram (B) of *Elettariopsis biphylla.*

Table 4.12 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Elettariopsis biphylla*, 2*n* (diploid) = 48.

Chro. pair	Ls	Ll	LT	CI ± SD	RL ± SD	Chro. size	Chro. type
1	0.80	1.02	1.82	0.559 ± 0.050	0.059 ± 0.004	Large	metacentric
2	0.51	1.24	1.75	0.711 ± 0.017	0.056 ± 0.001	Large	acrocentric
3	0.73	1.00	1.74	0.622 ± 0.004	0.056 ± 0.000	Large	submetacentric
4	0.58	1.12	1.70	0.660 ± 0.046	0.055 ± 0.003	Large	submetacentric
5	0.67	0.90	1.57	0.574 ± 0.003	0.050 ± 0.000	Large	metacentric
6	0.51	0.96	1.47	0.652 ± 0.025	0.047 ± 0.002	Large	submetacentric
7	0.51	0.95	1.47	0.651 ± 0.018	0.047 ± 0.001	Large	submetacentric
8	0.53	0.88	1.41	0.648 ± 0.010	0.045 ± 0.001	Large	submetacentric
9	0.39	1.01	1.40	0.724 ± 0.008	0.045 ± 0.000	Large	acrocentric
10	0.61	0.76	1.37	0.554 ± 0.011	0.044 ± 0.001	Large	metacentric
11	0.44	0.92	1.36	0.678 ± 0.002	0.044 ± 0.000	Large	submetacentric
12	0.49	0.85	1.33	0.651 ± 0.017	0.043 ± 0.001	Large	submetacentric
13	0.53	0.76	1.30	0.647 ± 0.021	0.042 ± 0.001	Large	submetacentric
14	0.41	0.85	1.26	0.675 ± 0.002	0.041 ± 0.000	Medium	submetacentric
15	0.39	0.77	1.16	0.646 ± 0.002	0.037 ± 0.000	Medium	submetacentric
16	0.42	0.73	1.15	0.658 ± 0.002	0.037 ± 0.000	Medium	submetacentric
17	0.41	0.72	1.14	0.651 ± 0.012	0.037 ± 0.001	Medium	submetacentric
18	0.36	0.77	1.13	0.645 ± 0.001	0.036 ± 0.000	Medium	submetacentric
19	0.47	0.66	1.13	0.581 ± 0.003	0.037 ± 0.000	Medium	metacentric
20	0.44	0.67	1.10	0.606 ± 0.008	0.036 ± 0.000	Medium	submetacentric
21	0.35	0.66	1.00	0.656 ± 0.031	0.032 ± 0.001	Medium	submetacentric
22	0.00	0.98	0.98	1.000 ± 0.023	0.032 ± 0.001	Medium	telocentric
23	0.32	0.48	0.80	0.605 ± 0 .277	0.026 ± 0.003	Medium	submetacentric
24	0.00	0.75	0.75	1.000 ± 0.000	0.024 ± 0.000	Medium	telocentric

Note: Chro. = Chromosome

4.2.8 *Elettariopsis triloba* (Gagnep.) Loes.

E. triloba, has a somatic chromosome number of 2*n* = 48 (Figure 4.1H). The results correspond to the gametic number (*n* = 24) which is reported for *Elettariopsis* (Beltran and Kiew, 1984). The total chromosome length of individual chromosomes ranged between 2.30 to 2.72 µm. with the length of short arms and the long arms 1.01 to 2.08 µm, and 1.28to 3.65 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.509 to 0.637 The relative lengths (RL%) in this study ranged from 0.025 to 0.061 (Table 4.13).

This data demonstrates that *E. triloba* has 16 large and 32 medium chromosomes and a karyotype formula of (2*n*, 48) = L^{m}_{4}+ L^{sm}_{10} + L^{a}_{2} + M^{m}_{14} + M^{sm}_{18} (Figure 4.9A), observed symmetrical karyotypes. An idiogram of *E. triloba* of the haploid set (*n* = 24) is shown in Figure 4.9B. The chromosome relative lengths (RL%) in this study ranged from 0.023 to 0.063 (Table 4.13). This is the first report of karyotype for this species.

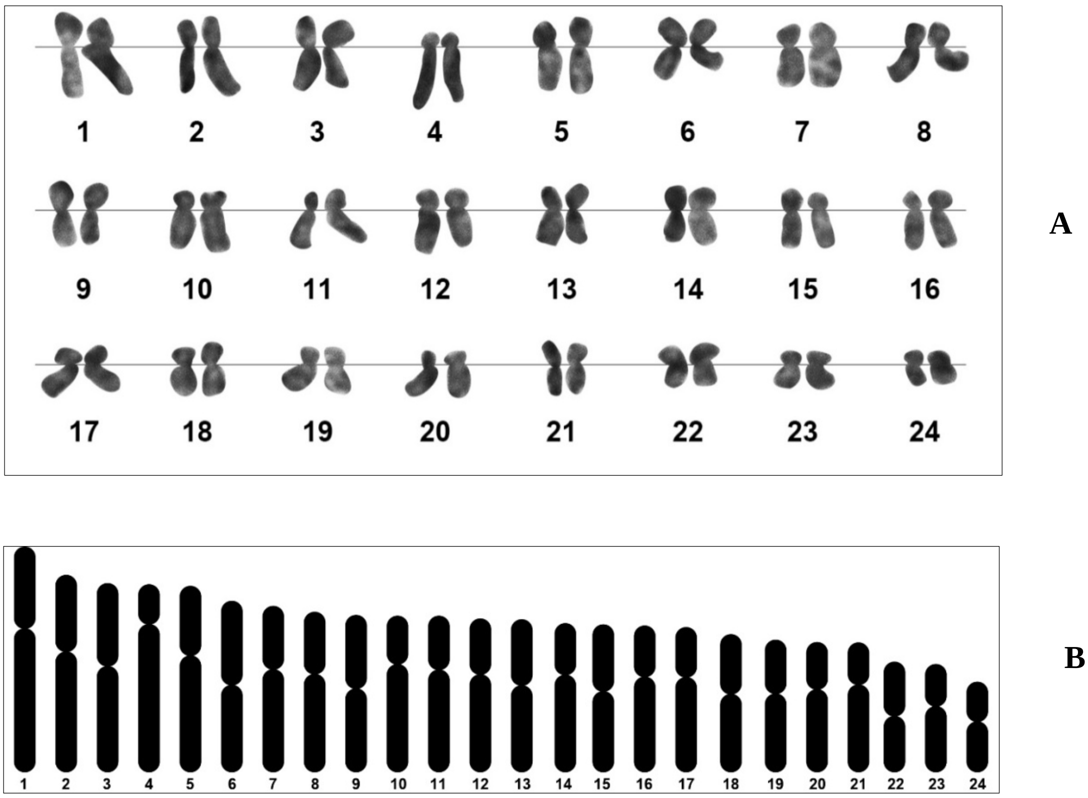


Figure 4.9 Karyotype (A) and idiogram (B) of *Elettariopsis triloba*

Table 4.13 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Elettariopsis triloba*, 2*n* (diploid) = 48.

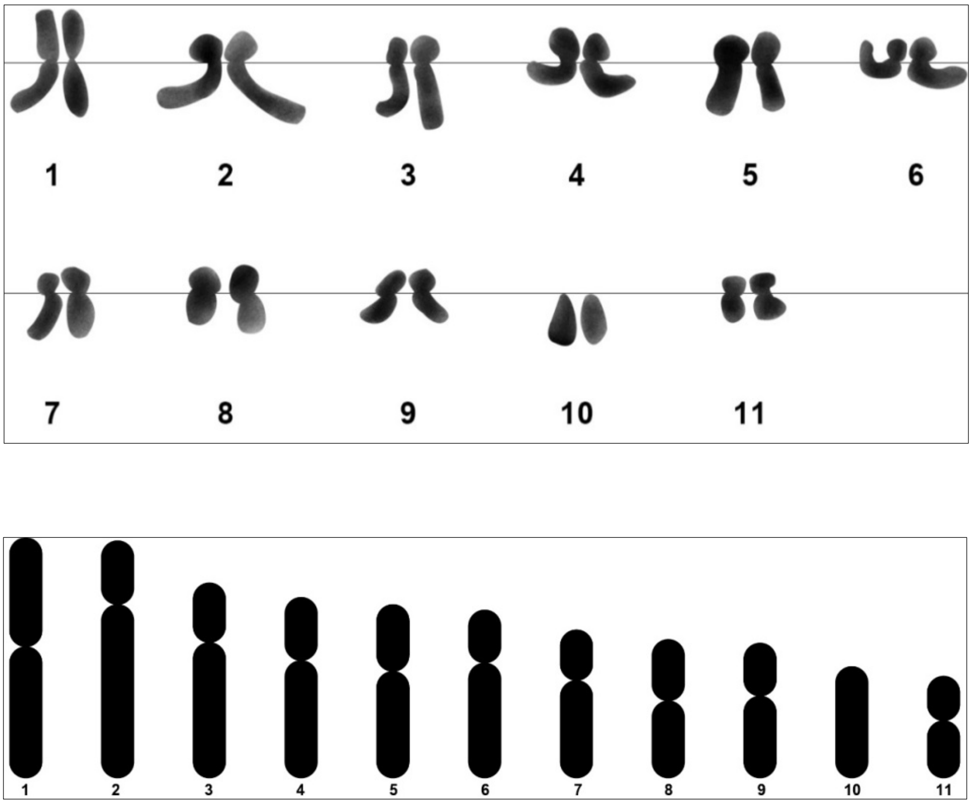
Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	2.08	3.65	5.72	0.637 ± 0.010	0.061 ± 0.001	Large	submetacentric
2	1.95	3.06	5.01	0.611 ± 0.017	0.054 ± 0.001	Large	submetacentric
3	2.11	2.69	4.80	0.560 ± 0.004	0.051 ± 0.000	Large	metacentric
4	1.01	3.76	4.77	0.792 ± 0.070	0.051 ± 0.004	Large	acrocentric
5	1.77	2.96	4.73	0.628 ± 0.049	0.051 ± 0.004	Large	submetacentric
6	2.14	2.21	4.35	0.509 ± 0.021	0.047 ± 0.002	Large	metacentric
7	1.61	2.61	4.22	0.619 ± 0.011	0.045 ± 0.001	Large	submetacentric
8	1.58	2.49	4.07	0.611 ± 0.017	0.044 ± 0.001	Large	submetacentric
9	1.87	2.12	3.99	0.532 ± 0.021	0.043 ± 0.002	Medium	metacentric
10	1.23	2.74	3.97	0.691 ± 0.010	0.042 ± 0.001	Medium	submetacentric
11	1.37	2.60	3.97	0.662 ± 0.095	0.042 ± 0.006	Medium	submetacentric
12	1.42	2.48	3.89	0.637 ± 0.032	0.042 ± 0.002	Medium	submetacentric
13	1.68	2.20	3.88	0.566 ± 0.019	0.041 ± 0.001	Medium	metacentric
14	1.31	2.47	3.78	0.654 ± 0.006	0.040 ± 0.000	Medium	submetacentric
15	1.70	2.05	3.75	0.548 ± 0.020	0.040 ± 0.001	Medium	metacentric
16	1.30	2.42	3.71	0.653 ± 0.049	0.040 ± 0.003	Medium	submetacentric
17	1.27	2.41	3.68	0.655 ± 0.026	0.039 ± 0.002	Medium	submetacentric
18	1.52	1.98	3.50	0.566 ± 0.024	0.037 ± 0.002	Medium	metacentric
19	1.37	1.99	3.36	0.594 ± 0.031	0.036 ± 0.002	Medium	metacentric
20	1.20	2.10	3.29	0.637 ± 0.011	0.035 ± 0.001	Medium	submetacentric
21	1.07	2.22	3.29	0.676 ± 0.024	0.035 ± 0.001	Medium	submetacentric
22	1.38	1.42	2.80	0.507 ± 0.010	0.030 ± 0.001	Medium	metacentric
23	1.07	1.67	2.75	0.610 ± 0.012	0.029 ± 0.001	Medium	submetacentric
24	1.01	1.28	2.30	0.563 ± 0.068	0.025 ± 0.003	Medium	metacentric

Note: Chro. = Chromosome

4.2.9 *Kaempferia angustifolia* Roscoe

The somatic cells of root tips of *K. angustifolia* showed the chromosome number of $2n = 22$, and not different from earlier studies by Mahanty (1970); Saensouk and Saensouk (2004) (Figure 4.1 I). The total chromosome length of individual chromosomes ranged between 1.69 to 3.99 µm. with the length of short arms and the long arms 0.00 to 1.81 µm, and 0.95 to 2.88 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.548 to 1.000. The relative lengths (RL%) in this study ranged from 0.025 to 0.061 (Table 4.14).

This data demonstrates that *E. triloba* has 16 large and 32 medium chromosomes and a karyotype formula. The karyotype formula to be $(2n, 22) = L^{m}_{2} + L^{sm}_{8} + L^{a}_{2} + M^{m}_{4} + M^{sm}_{4} + M^{t}_{2}$ (Figure 4.10A), with observed asymmetrical karyotypes. An idiogram of *K. angustifolia* is shown in Figure 4.10B. This is the first report of karyotype for this species.



A

B

Figure 4.10 Karyotype (A) and idiogram (B) of *Kaempferia angustifolia.*

Table 4.14 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Kaempferia angustifolia*, 2*n* (diploid) = 22

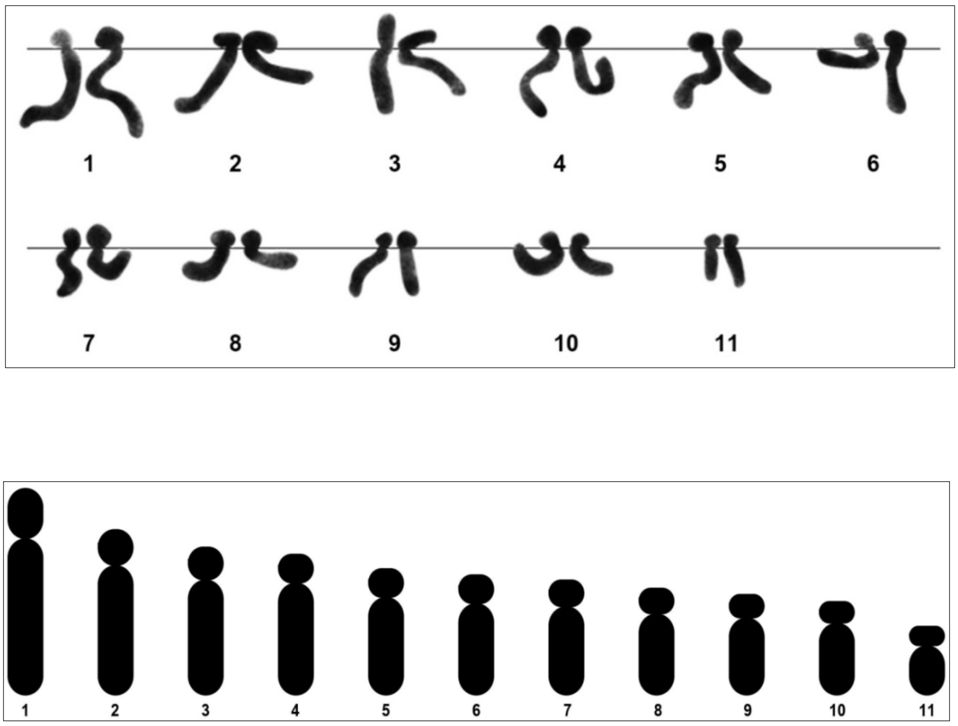
Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	1.81	2.18	3.99	0.548 ± 0.037	0.131 ± 0.003	Large	metacentric
2	1.06	2.88	3.94	0.737 ± 0.100	0.130 ± 0.006	Large	acrocentric
3	0.99	2.25	3.24	0.696 ± 0.056	0.107 ± 0.009	Large	submetacentric
4	1.05	1.95	3.00	0.652 ± 0.023	0.099 ± 0.001	Large	submetacentric
5	1.11	1.77	2.88	0.614 ± 0.020	0.095 ±0.001	Large	submetacentric
6	0.88	1.91	2.79	0.687 ± 0.050	0.076 ± 0.002	Large	submetacentric
7	0.84	1.62	2.46	0.659 ± 0.005	0.092 ± 0.000	Medium	submetacentric
8	1.02	1.28	2.29	0.557 ± 0.031	0.076 ± 0.004	Medium	metacentric
9	0.88	1.36	2.23	0.610 ± 0.047	0.074 ± 0.006	Medium	submetacentric
10	0.00	1.85	1.85	1.000 ± 0.000	0.056 ± 0.000	Medium	telocentric
11	0.74	0.95	1.69	0.563 ± 0.009	0.061 ± 0.000	Medium	metacentric

Note: Chro. = Chromosome

4.2.10 *Kaempferia grandifolia* Saensouk & Jenjitt.

K. grandifolia is a new species from the Phu Phan National Park in NE Thailand, described and illustrated by Saensouk and Jenjittikul (2001). The somatic cells of root tips of *K. grandifolia* showed a chromosome number of 2*n* = 22 (Figure 4.1J). This result is agreement with previous reported by Saensouk and Jenjittikul (2001). The total chromosome length of individual chromosomes ranged between 0.68 to 2.01 µm. with the length of short arms and the long arms 0.19 to 0.49 µm, and 0.48 to 1.52 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.717 to 0.793. The relative lengths (RL%) in this study ranged from 0.050 to 0.145 (Table 4.15).

This data demonstrates that *K. grandifolia* has 8 large and 14 medium chromosomes and a karyotype formula to be (2*n*, 22) = $L^{a}_{8} + M^{a}_{14}$ (Figure 4.11A), with observed asymmetrical karyotypes. An idiogram of *K. grandifolia* is shown in Figure 4.11B and observed asymmetrical karyotypes. This is the first report of karyotype for this species.



A

B

Figure 4.11 Karyotype (A) and idiogram (B) of *Kaempferia grandifolia*

Table 4.15 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Kaempferia grandifolia*, 2*n* (diploid) = 48.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	0.49	1.52	2.01	0.755 ± 0.025	0.148 ± 0.005	Large	acrocentric
2	0.35	1.26	1.62	0.781 ± 0.007	0.119 ± 0.001	Large	acrocentric
3	0.32	1.12	1.44	0.780 ± 0.011	0.106 ± 0.002	Large	acrocentric
4	0.28	1.09	1.37	0.793 ± 0.004	0.101 ± 0.000	Large	acrocentric
5	0.28	0.95	1.23	0.774 ± 0.032	0.091 ± 0.004	Medium	acrocentric
6	0.28	0.89	1.17	0.765 ± 0.045	0.086 ± 0.005	Medium	acrocentric
7	0.26	0.86	1.12	0.764 ± 0.018	0.083 ± 0.002	Medium	acrocentric
8	0.25	0.79	1.05	0.761 ± 0.086	0.077 ± 0.009	Medium	acrocentric
9	0.23	0.75	0.99	0.767 ± 0.049	0.073 ± 0.005	Medium	acrocentric
10	0.21	0.70	0.91	0.766 ± 0.007	0.067 ± 0.001	Medium	acrocentric
11	0.19	0.48	0.68	0.717 ± 0.062	0.050 ± 0.004	Medium	acrocentric

Note: Chro. = Chromosome

4.2.11 *Kaempferia marginata* Carey ex Roscoe

The somatic cells of root tips of *K. marginata* showed a chromosome number of 2*n* = 42, (Figure 4.1K). Whereas, a previous study of *K. marginata* from Phu Laenkha National Park, Thailand by Khamtaeng *et al.* **(**2014**)**, observed a chromosome count of 2*n* = 44. Eksomtramage and Boontum (1995) showed a chromosome count of *K. marginata* 2*n* = 55. The total chromosome length of individual chromosomes ranged between 0.85 to 1.88 µm. with the length of short arms and the long arms 0.37 to 0.57 µm, and 0.48 to 1.32 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.562 to 0.704. The relative lengths (RL%) in this study ranged from 0.033 to 0.073 (Table 4.16).

This data demonstrates that *K. marginata* has 10 large and 32 medium chromosomes and with the karyotype formula being (2*n*, 42) = $L^{m}_{4} + L^{sm}_{2} + L^{a}_{4} + M^{m}_{16} + M^{sm}_{16}$ (Figure 4.12A). An idiogram of *K. marginata* is shown in Figure 4.12B. The observed asymmetrical karyotypes. This is the first report of karyotype for this species.

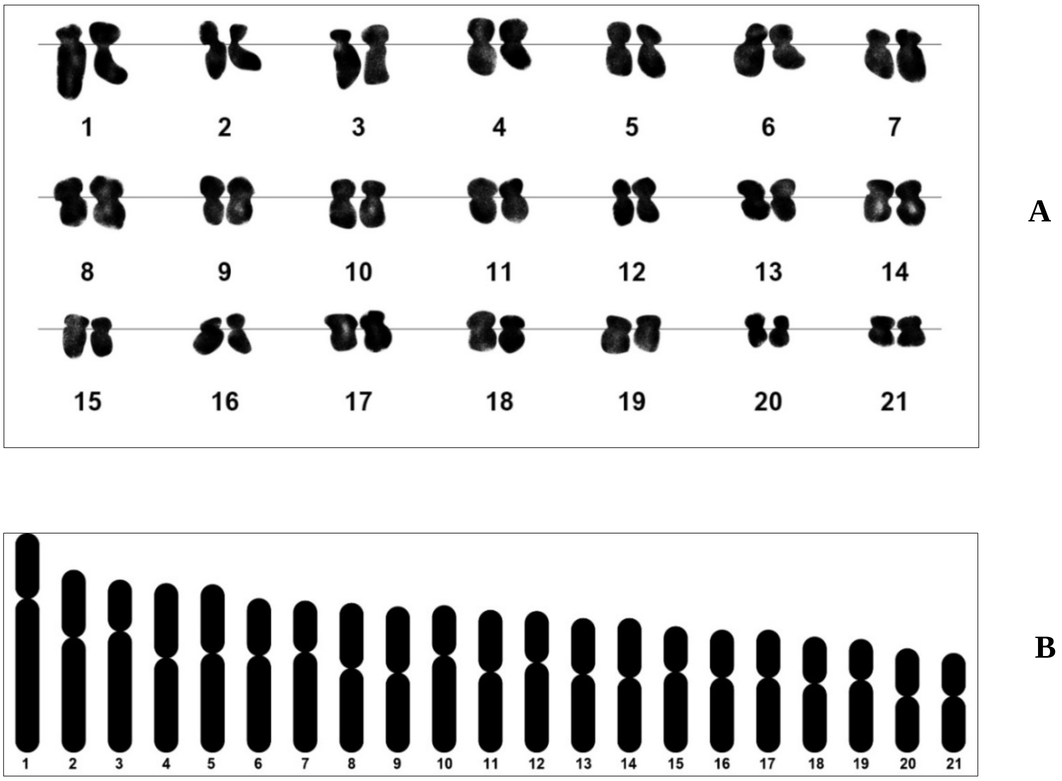


Figure 4.12 Karyotype (A) and idiogram (B) of *Kaempferia marginata.*

Table 4.16 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Kaempferia marginata*, 2*n* (diploid) = 42.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	0.56	1.32	1.88	0.704 ± 0.050	0.073 ± 0.005	Large	acrocentric
2	0.57	0.98	1.56	0.631 ± 0.024	0.061 ± 0.003	Large	submetacentric
3	0.44	1.04	1.48	0.703 ± 0.010	0.057 ± 0.004	Large	acrocentric
4	0.64	0.81	1.45	0.557 ± 0.029	0.056 ± 0.000	Large	metacentric
5	0.59	0.85	1.44	0.593 ± 0.032	0.056 ± 0.001	Large	metacentric
6	0.49	0.83	1.33	0.628 ± 0.023	0.051 ± 0.003	Medium	submetacentric
7	0.44	0.86	1.30	0.661 ± 0.006	0.050 ± 0.003	Medium	submetacentric
8	0.56	0.72	1.28	0.564 ± 0.034	0.050 ± 0.002	Medium	metacentric
9	0.57	0.68	1.26	0.544 ± 0.034	0.049 ± 0.003	Medium	metacentric
10	0.43	0.83	1.25	0.660 ± 0.039	0.049 ± 0.000	Medium	submetacentric
11	0.53	0.69	1.22	0.564 ± 0.044	0.047 ± 0.001	Medium	metacentric
12	0.44	0.77	1.21	0.636 ± 0.006	0.047 ± 0.000	Medium	submetacentric
13	0.48	0.67	1.15	0.583 ± 0.005	0.045 ± 0.002	Medium	metacentric
14	0.51	0.64	1.15	0.559 ± 0.037	0.044 ± 0.000	Medium	metacentric
15	0.39	0.69	1.07	0.640 ± 0.017	0.042 ± 0.003	Medium	submetacentric
16	0.41	0.64	1.06	0.616 ± 0.096	0.041 ± 0.006	Medium	submetacentric
17	0.41	0.64	1.06	0.611 ± 0.052	0.041 ± 0.000	Medium	submetacentric
18	0.40	0.59	0.99	0.594 ± 0.000	0.038 ± 0.003	Medium	metacentric
19	0.35	0.62	0.97	0.641 ± 0.029	0.037 ± 0.002	Medium	submetacentric
20	0.41	0.48	0.89	0.539 ± 0.034	0.035 ± 0.003	Medium	metacentric
21	0.37	0.48	0.85	0.562 ± 0.000	0.033 ± 0.002	Medium	metacentric

Note: Chro. = Chromosome

4.2.12 *Kaempferia rotunda* L.

K. rotunda had a somatic chromosome number of 2*n* = 30 (Figure 4.1L). This chromosome number is inconsistent with that previously reported for *K. rotunda* by Nerle and Torne (1984); Chen *et al.* (1988); Omanakumari and Mathew (1991); Eksomtramage and Boontum (1995); Saensouk and Chantaranothai (2003), who observed a chromosome number of 2*n* = 54, 44, 46, 33 and 22 respectively. The total chromosome length of individual chromosomes ranged between 0.75 to 1.89 µm. with the length of short arms and the long arms 0.30 to 0.70 µm, and 0.46 to 1.89 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.607 to 0.0.690. The relative lengths (RL%) in this study ranged from 0.040 to 0.099 (Table 4.17).

This data demonstrates that *K. rotunda* has 26 large and 4 medium chromosomes. A karyotype formula of *K. rotunda* (2*n*, 30) = $L^{sm}_{14} + M^{m}_{6} + M^{sm}_{10}$ (Figure 4.13A). An idiogram is shown in Figure 4.13B and observed symmetrical karyotypes. This is the first report of karyotype for this species.

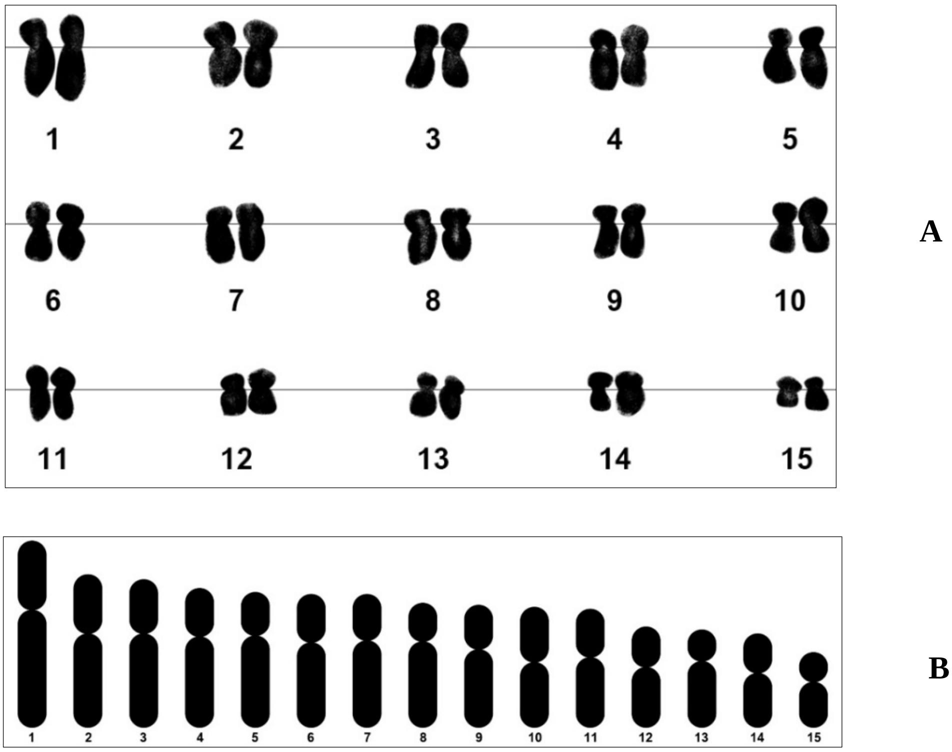


Figure 4.13 Karyotype (A), and idiogram (B) of *Kaempferia rotunda.*

Table 4.17 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Kaempferia rotunda*, 2*n* (diploid) = 30.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	0.70	1.19	1.89	0.631 ± 0.014	0.099 ± 0.002	Large	submetacentric
2	0.60	0.95	1.56	0.612 ± 0.002	0.082 ± 0.000	Large	submetacentric
3	0.55	0.95	1.50	0.632 ± 0.003	0.079 ± 0.000	Large	submetacentric
4	0.49	0.92	1.42	0.652 ± 0.006	0.074 ± 0.001	Large	submetacentric
5	0.44	0.93	1.37	0.681 ± 0.058	0.072 ± 0.006	Large	submetacentric
6	0.49	0.86	1.35	0.635 ± 0.006	0.071 ± 0.001	Large	submetacentric
7	0.47	0.88	1.34	0.653 ± 0.014	0.071 ± 0.002	Large	submetacentric
8	0.39	0.87	1.26	0.690 ± 0.040	0.066 ± 0.004	Medium	submetacentric
9	0.45	0.79	1.24	0.638 ± 0.022	0.065 ± 0.002	Medium	submetacentric
10	0.56	0.66	1.22	0.542 ± 0.041	0.064 ± 0.005	Medium	metacentric
11	0.49	0.71	1.20	0.597 ± 0.056	0.063 ± 0.006	Medium	metacentric
12	0.41	0.61	1.02	0.601 ± 0.029	0.053 ± 0.003	Medium	submetacentric
13	0.32	0.67	1.00	0.677 ± 0.003	0.052 ± 0.000	Medium	submetacentric
14	0.40	0.55	0.95	0.581 ± 0.046	0.050 ± 0.004	Medium	metacentric
15	0.30	0.46	0.75	0.607 ± 0.010	0.040 ± 0.001	Medium	submetacentric

Note: Chro. = Chromosome

4.2.13 *Zingiber montanum* (J. Koenig) Link ex A. Dietr.

The plants of *Z. montanum* showed a somatic chromosome number of 2*n* = 22 (Figure 4.1M) and correspond to a previous count of 2*n* = 22 from this species by Saensouk and Saensouk (2004). The total chromosome length of individual chromosomes ranged between 2.58 to 4.49 µm. with the length of short arms and the long arms 1.02 to 1.88 µm, and 1.56 to 3.06 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.540 to 0.649. The relative lengths (RL%) in this study ranged from 0.062 to 0.119 (Table 4.18).

The data demonstrates that *Z. montanum* has 14 large and 8 medium chromosomes and a karyotype formula of (2*n*, 22) = $L^{m}_{2} + L^{sm}_{12} + M^{m}_{6} + M^{sm}_{2}$ (Figure 4.14A). An idiogram of *Z. montanum* of the haploid set (*n* = 11) is shown in Figure 2B and observed symmetrical karyotypes. This is the first report of karyotype for this species.

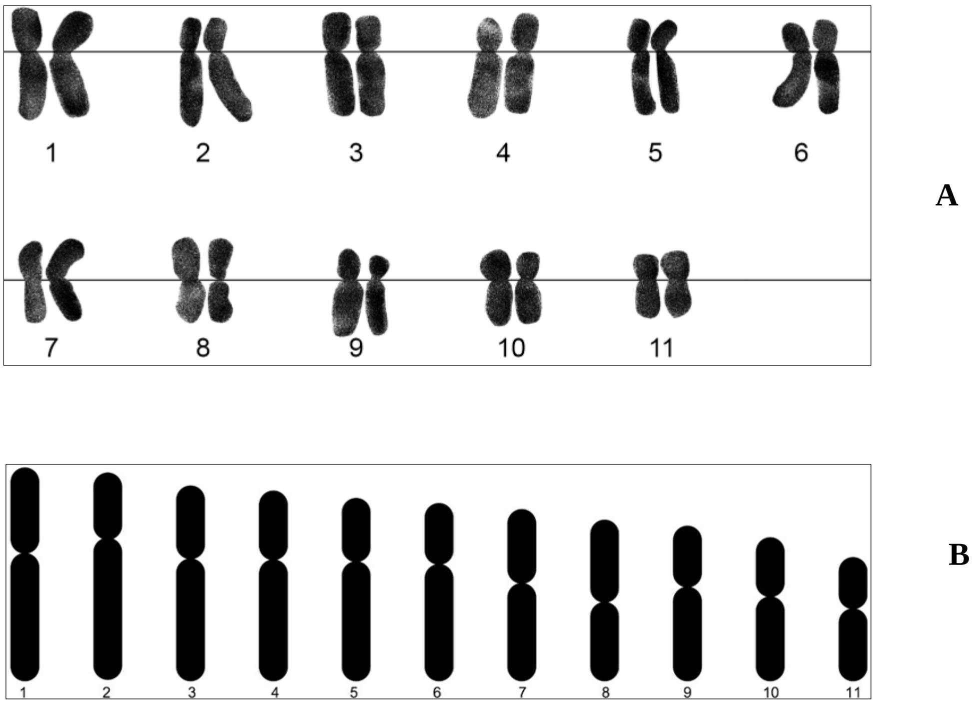


Figure 4.14 Karyotype (A) and idiogram (B) of *Zingiber montanum.*

Table 4.18 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Zingber montanum.* 2*n* (diploid) = 22.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	1.88	3.06	4.94	0.619 ± 0.004	0.119 ± 0.001	Large	submetacentric
2	1.64	2.76	4.40	0.628 ± 0.036	0.106 ± 0.006	Large	submetacentric
3	1.52	2.67	4.19	0.638 ± 0.025	0.101 ± 0.004	Large	submetacentric
4	1.62	2.62	4.24	0.619 ± 0.014	0.102 ± 0.002	Large	submetacentric
5	1.46	2.55	4.01	0.637 ± 0.009	0.096 ± 0.001	Large	submetacentric
6	1.36	2.52	3.88	0.649 ± 0.016	0.093 ± 0.002	Large	submetacentric
7	1.58	2.34	3.92	0.599 ± 0.047	0.094 ± 0.007	Large	metacentric
8	1.31	1.85	3.16	0.584 ± 0.005	0.076 ± 0.001	Medium	metacentric
9	1.28	1.77	3.05	0.581 ± 0.011	0.073 ± 0.001	Medium	metacentric
10	1.47	1.69	3.16	0.540 ± 0.068	0.076 ± 0.010	Medium	metacentric
11	1.02	1.56	2.58	0.606 ± 0.018	0.062 ± 0.002	Medium	submetacentric

Note: Chro. = Chromosome

4.2.14 *Zingiber mekongense* Gagnep.

The plants of *Z. mekongense* showed a somatic chromosome number of 2*n* = 22 (Figure 4.2N), which agrees with the values previous reported for this species, 2*n* = 22, (Saensouk and Chantaranothai, 2003). The total chromosome length of individual chromosomes ranged between 2.31 to 5.40 µm. with the length of short arms and the long arms 0.75 to 1.65 µm, and 1.56 to 3.75 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.570 to 0.750. The relative lengths (RL%) in this study ranged from 0.055 to 0.128 (Table 4.19).

This data demonstrates that *Z. mekongense* has 10 large and 12 medium chromosomes and a karyotype formula of (2*n*, 22) = $L^{m}_{2} + L^{sm}_{4} + L^{a}_{4} + M^{sm}_{6} + M^{a}_{6}$ (Figure 4.15A). In this study, that found the nucleolar organizer regions (NORs), which represents the chromosome marker in located only on the short arms of the 6th medium chromosome pairs were of the acrocentric chromosomes type. An idiogram of *Z. mekongense* of the haploid set (*n* = 11) is shown in Figure 4.15B and observed asymmetrical karyotypes. This is the first report of karyotype for this species.

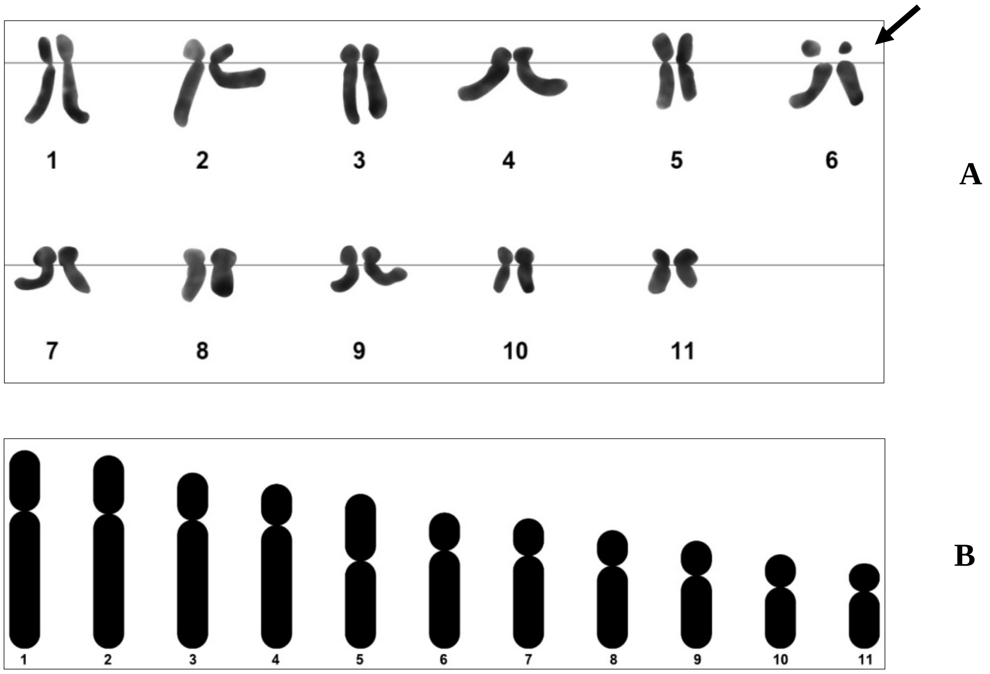


Figure 4.15 Karyotype (A) and idiogram (B) of *Zingiber mekongense.*

Table 4.19 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Zingiber mekongense.* 2*n* (diploid) = 22.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	1.65	3.75	5.40	0.694 ± 0.011	0.128 ± 0.002	Large	submetacentric
2	1.59	3.67	5.26	0.699 ± 0.031	0.124 ± 0.005	Large	submetacentric
3	1.29	3.50	4.79	0.730 ± 0.017	0.113 ± 0.003	Large	acrocentric
4	1.12	3.36	4.48	0.750 ± 0.000	0.106 ± 0.000	Large	acrocentric
5	1.82	2.39	4.21	0.570 ± 0.053	0.099 ± 0.009	Large	metacentric
6	1.03	2.67	3.70	0.722 ± 0.027	0.088 ± 0.003	Medium	acrocentric
7	1.01	2.53	3.54	0.716 ± 0.057	0.084 ± 0.007	Medium	acrocentric
8	0.97	2.25	3.22	0.700 ± 0.002	0.076 ± 0.000	Medium	acrocentric
9	0.94	1.99	2.93	0.679 ± 0.014	0.069 ± 0.001	Medium	submetacentric
10	0.89	1.67	2.56	0.652 ± 0.021	0.060 ± 0.002	Medium	submetacentric
11	0.75	1.56	2.31	0.679 ± 0.078	0.055 ± 0.006	Medium	submetacentric

Note: Chro. = Chromosome

4.2.15 *Zingiber officinale* Roscoe.

The plants of *Z. officinale* showed a somatic chromosome number of 2*n* = 22 (Figure 4.1J), which agrees with the studies previous was reported as 2*n* = 22 (Moringa *et al.,* 1929; Sugiura, 1936; Raghavan and Venkatasubban, 1943; Darlington and Ammal, 1945; Chakravorti, 1948; Sharma and Bhattacharya, 1959; Saensouk, 2005 and Daryono *et al.*, 2012). The total chromosome length of individual chromosomes ranged between 1.76 to 3.83 µm. with the length of short arms and the long arms 0.68 to 1.37 µm, and 1.08 to 2.45 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0595 to 0.643. The relative lengths (RL%) in this study ranged from 0.060 to 0.131 (Table 4.20).

The data demonstrate that *Z. officinale* has 10 large and 12 medium chromosomes and a karyotype formula of (2*n*, 22) = L^{m}_{2}+ L^{sm}_{8}+ M^{sm}_{12} (Figure 4.16A). An idiogram of *Z. officinale* of the haploid set (*n* = 11) is shown in Figure 4.16B and observed symmetrical karyotypes. This is the first report of karyotype for this species.

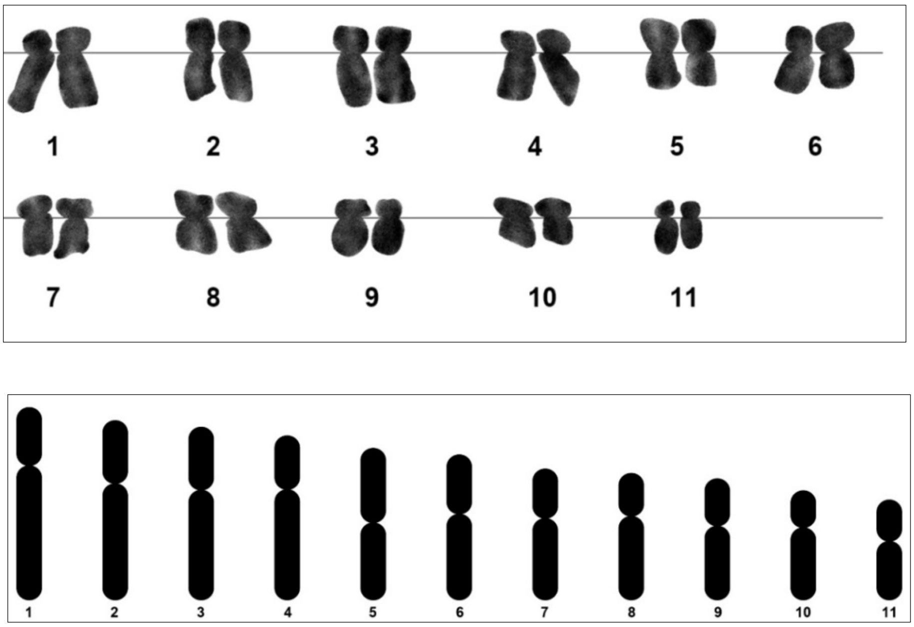


Figure 4.16 Karyotype (A) and idiogram (B) of *Zingiber officinale.*

Table 4.20 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI), and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Zingiber officinale*, *2n* (diploid) = 22.

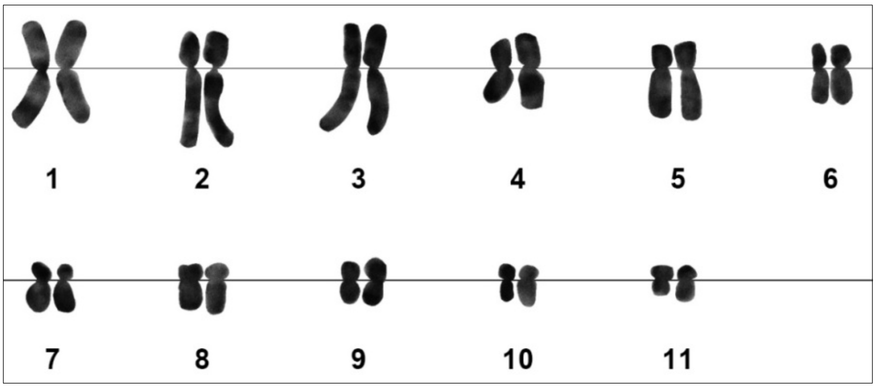
Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	1.37	2.45	3.83	0.643 ± 0.049	0.131 ± 0.010	Large	submetacentric
2	1.29	2.22	3.51	0.632 ± 0.020	0.120 ± 0.004	Large	submetacentric
3	1.12	2.03	3.15	0.644 ± 0.021	0.108 ± 0.004	Large	submetacentric
4	1.07	1.96	3.03	0.648 ± 0.031	0.103 ± 0.005	Large	submetacentric
5	1.04	1.58	2.62	0.595± 0.028	0.089 ± 0.004	Large	metacentric
6	0.98	1.54	2.52	0.610 ± 0.011	0.086 ± 0.002	Medium	submetacentric
7	0.94	1.50	2.44	0.614 ± 0.016	0.083 ± 0.002	Medium	submetacentric
8	0.83	1.44	2.26	0.635 ± 0.008	0.077 ± 0.001	Medium	submetacentric
9	0.77	1.39	2.16	0.642 ± 0.001	0.074 ± 0.000	Medium	submetacentric
10	0.75	1.28	2.04	0.631 ± 0.009	0.069 ± 0.001	Medium	submetacentric
11	0.68	1.08	1.76	0.611 ± 0.011	0.060 ± 0.001	Medium	submetacentric

Note: Chro. = Chromosome

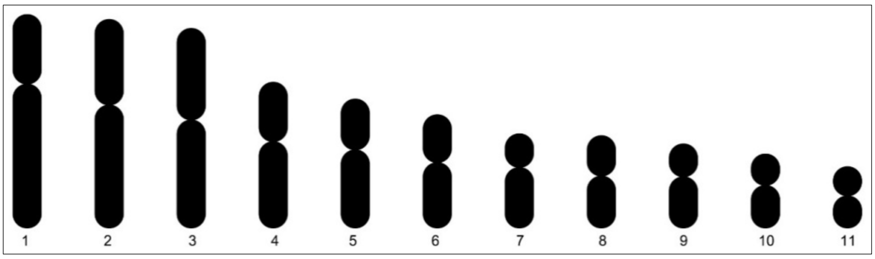
4.2.16 *Zingiber ottensii* Valeton.

The somatic cells of root tips of *Z. ottensii* showed a chromosome number of $2n$ = 22, (Figure 4.2K) and correspond to a previous count for *Z. ottensii* was determined in 1943 to be $2n$ = 22 (Raghavan and Venkatasubban, 1943). The total chromosome length of individual chromosomes ranged between 1.64 to 5.88 µm. with the length of short arms and the long arms 0.78 to 2.45 µm. and 0.86 to 3.43 µm, respectively. The chromosomes according to centromeric index (CI) ranged from 0.533 to 0.624. The relative lengths (RL%) in this study ranged from 0.043 to 0.155 (Table 4.21).

The data demonstrate that *Z. ottensii* has 8 large and 14 medium chromosomes and a karyotype formula of ($2n$, 22) = ($2n$, 22) = $L^{m}_{4} + L^{sm}_{4} + M^{m}_{10} + M^{sm}_{4}$ (Figure 4.17A). An idiogram of *Z. ottensii* of the haploid set (n = 11) is shown in Figure 4.17B and observed symmetrical karyotypes. This is the first report of karyotype for this species.



A



B

Figure 4.17 Karyotype (A) and idiogram (B) of *Zingiber ottensii*.

Table 4.21 Mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), length of total chromosome (LT), Relative length (RL), Centromeric index (CI) and standard deviation (SD) of CI and Rl from 10 metaphase spreads of *Zingiber ottensii*, 2*n* (diploid) = 22.

Chro. pair	Ls	Ll	LT	CI ± SD	RL± SD	Chro. size	Chro. type
1	2.45	3.43	5.88	0.586 ± 0.060	0.155 ± 0.016	Large	metacentric
2	2.29	3.59	5.89	0.611 ± 0.033	0.155 ± 0.008	Large	submetacentric
3	1.86	3.01	4.87	0.621 ± 0.050	0.129 ± 0.010	Large	submetacentric
4	1.64	2.18	3.83	0.582 ± 0.115	0.101 ± 0.020	Large	metacentric
5	1.34	2.22	3.56	0.624 ± 0.027	0.094 ± 0.004	Medium	submetacentric
6	1.25	1.75	3.00	0.583 ± 0.010	0.079 ± 0.001	Medium	metacentric
7	1.10	1.62	2.72	0.597 ± 0.041	0.072 ± 0.005	Medium	metacentric
8	0.92	1.53	2.45	0.624 ± 0.018	0.065 ± 0.002	Medium	submetacentric
9	0.88	1.28	2.16	0.591 ± 0.010	0.057 ± 0.001	Medium	metacentric
10	0.79	1.10	1.89	0.583 ± 0.050	0.050 ± 0.004	Medium	metacentric
11	0.78	0.86	1.64	0.533 ± 0.087	0.043 ± 0.007	Medium	metacentric

Note: Chro. = Chromosome

CHAPTER 5
Conclusion

5.1 Chromosome number and karyotype

Cytogenetics for 16 species of Thai Zingiberaceae from five different genera (*Alpinia*, *Amomum Elletariopsis*, *Kaempferia* and *Zingiber*) was determined using tissue samples of root tips. The somatic numbers range from $2n = 22$ to $2n = 48$ showing diploidy and to explanations below.

5.1.1 *Alpinia*

Four species in *Alpinia* were studied have somatic chromosome number of $2n = 48$ and found that most of them have $2n = 4x = 48$, suggesting that them are tetraploid. The chromosome relative lengths (RL%) in all species ranged from 0.018 to 0.072. The chromosomes consist of large (L) and medium (M) chromosomes size. In addition, three species are observed asymmetrical karyotypes, whereas observed symmetrical karyotypes in *A. zerumbet*. Karyotypes from diploid cell of *Alpinia* were formulated as following:

A. galanga (No.1) $(2n, 48) = L^{sm}_{20} + L^{a}_{2} + M^{sm}_{26}$

A. galanga (No.2) $(2n, 48) = L^{sm}_{18} + L^{a}_{2} + M^{sm}_{28}$

A. siamensis $(2n, 48) = L^{m}_{4} + L^{sm}_{8} + L^{a}_{16} + M^{m}_{4} + M^{sm}_{10} + M^{a}_{6}$

A. zerumbet $(2n, 48) = L^{m}_{2} + L^{sm}_{14} + M^{m}_{20} + M^{sm}_{12}$

5.1.2 *Amomum*

Two species of *Amomum* were studied: *A. biflorum* and *A. schmidtii* have the same number, $2n = 48$ and found that most of them of *Amomum* as $2n = 4x = 48$. The most common cytotypes found in this species are tetraploid. The chromosome relative lengths (RL%) in all species ranged from 0.021 to 0.075. The chromosomes consist of large (L) and medium (M) chromosomes size. In addition, all species are observed asymmetrical karyotypes, with karyotypes from diploid cell of *Amomum* were formulated as following:

A. biflorum $(2n, 48) = L^{sm}_{10}+L^{a}_{2}+M^{sm}_{36}$

A. schmidtii $(2n, 48) = L^{sm}_{10} + L^{a}_{2} + M^{sm}_{16} + M^{m}_{10}$

5.1.3 *Elettariopsis*

Elettariopsis is a small genus counting around 36 recognized species. Two species of *Elettariopsis* were studied: The somatic number of *E. biphylla* and *E. triloba* is 2*n* = 48, conclude that is tetraploid. The chromosome relative lengths (RL%) in two species ranged from 0.023 to 0.158. The chromosomes consist of large (L) and medium (M) chromosomes size. In this study found that the 22nd, 24th medium chromosome pairs were of the telocentric chromosomes type in *E. biphylla*. The karyotypes from diploid cell of *Elettariopsis* were formulated as following:

E. biphylla $(2n, 48) = L^{m}_{6} + L^{sm}_{16} + L^{a}_{4} + M^{m}_{2} + M^{sm}_{16} + M^{t}_{4}$

E. triloba $(2n, 48) = L^{m}_{4} + L^{sm}_{10} + L^{a}_{2} + M^{m}_{14} + M^{sm}_{18}$

5.1.4 *Kaempferia*

Four species of *Kaempferia* were examined with a chromosome number ranging of 2*n* = 22 to 2*n* = 42. The somatic numbers of *K. angustifolia* and *K. grandifolia* are 2*n* = 22, while the chromosome numbers found in *K. rotunda* and *K. marginata* are 2*n* = 30 and 42, respectively. This study also indicates that the base chromosome number of *Kaempferia* is x = 11. The chromosome relative lengths (RL%) in all species ranged from 0.023 to 0.161. The chromosomes consist of large (L) and medium (M) chromosomes size and observed asymmetrical karyotypes. The karyotypes from diploid cell of *Kaempferia* were formulated as following:

K. angustifolia $(2n, 22) = L^{m}_{2} + L^{sm}_{8} + L^{a}_{2} + M^{m}_{4} + M^{sm}_{4} + M^{t}_{2}$

K. grandifolia $(2n, 22) = L^{a}_{8} + M^{a}_{14}$

K. marginata $(2n, 42) = L^{m}_{4} + L^{sm}_{2} + L^{a}_{4} + M^{m}_{16} + M^{sm}_{16}$

K. rotunda $(2n, 30) = L^{sm}_{14} + M^{m}_{6} + M^{sm}_{10}$

5.1.5 *Zingiber*

According to this study the somatic number of 2*n* = 22 were found in *Z. montanum*, *Z. mekongense*, *Z. officinale* and *Z. ottensii*, confirming that these four *Zingiber* species are diploid. The chromosome relative lengths (RL%) in all species ranged from 0.043 to 0.155. The chromosomes consist of large (L) and medium (M) chromosomes size. In this investigation, the nucleolar organizer regions (NORs), which represents the chromosome marker in located only on the short arms of the 6th medium chromosome pairs were of the acrocentric chromosomes type in *Z. mekongense*. The karyotypes from diploid cell of *Zingiber* were formulated as following:

Z. montanum (2*n*, 48) = $L^{m}_{2} + L^{sm}_{12} + M^{m}_{6} + M^{sm}_{2}$

Z. mekongense (2*n*, 22) = $L^{m}_{2} + L^{sm}_{4} + L^{a}_{4} + M^{sm}_{6} + M^{a}_{6}$

Z. officinale (2*n*, 22) = $L^{m}_{2} + L^{sm}_{8} + M^{sm}_{12}$

Z. ottensii (2*n*, 42) = $L^{m}_{4} + L^{sm}_{4} + M^{m}_{10} + M^{sm}_{4}$

Karyomorphological studies of the plants of Zingiberaceae are especially difficult because of the small size and high number of chromosomes.

In the present study, no variable chromosome number was found in the species studied. But it should also be noted that the study was confined to plants from definite geographic locations. The karyotypes of the 16 species studied were documented and analyzed using software-assisted imaging techniques that helped in acquiring accurate data regarding chromosome numbers and types, which is more expedient than manual karyotype analysis. Increasing the range of collection of these plants in further study might help in solving the karyological puzzle that exists in these species. Also, more karyomorphological studies of the plants of Zingiberaceae are needed in order to fill in the gap that exists in our present knowledge of understanding the karyological evolution and speciation in the family Zingiberaceae.

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Appendices

1. Preparation of solution

1.1 Aceto-orcein preparation (2% solution)

Orcein is used in form of a 2% solution in 45% acetic acid. This solution is prepared by pouring 55 mL boiling glacial acetic acid over 2 g orcein powder. The solution is cooled, 45 mL of distilled water added, and filtered. This solution is unstable and should be prepared fresh before use.

1.2 Carnoy's solution I (Farmer's solution)

Carnoy's I is the most commonly used fixative and gives good results for a large number of different species and tissues. In general, fixed material can be stored at 4°C for several months. The following combinations are most commonly used to study plant chromosomes:

1 part glacial acetic acid

3 parts 95% or absolute (100%) ethanol

2. Preparation of squash-sample

2.1 Fixation and maceration (softening of the tissue)

Actively growing root tips were pretreated in a saturated paradichlorobenzens (PDB) solution for 4 hours at 4ºC, fixed in Carnoy’s solution for 30 minutes at 4ºC, and stored in 70% ethanol at 4ºC. Fixed cells fragments were hydrolyzed with 1 N HCl for 5 minutes at 60°C. Do not boil the roots as this will denature the chromosomes making them impossible to stain. Keep heating until the roots are soft (try by pressing the upper part of the root tip with the tweezers).

2.2 Staining and squashing

Add 1-2 drops of 2% aceto-orcein to a microscope slide and transfer one root tip from the fixation solution to the staining solution (move it quickly to make sure it’s not dried) for 5 minutes. In order to achieve a high frequency of mitotic cells in your sample, you need to cut off, using the tweezers, the root tip and keep only the meristem (~1 mm). Pulverize the meristem carefully by grinding it with a plastic rod. In order for the staining solution to reach each individual cell, the tissue fragments need to be very small. The pulverization also makes it easier to flatten the tissue fragments into unicellular layers later on.

The staining solution must not boil as this would destroy the morphology of the chromosomes. By heating the sample, chromosome staining gets more efficient and the cytoplasm gets detained (though this effect becomes more pronounced as the sample is cooled down again). Using the microscope, make sure the chromosomes are significantly stained before you press out the excess staining solution and flatten the cells.

Place your sample between filter papers on the desk end. Take a firm grip on the table and press with your thumb on the sample. Do not let the coverslip slide sidewise. Analyze your sample in the microscope and identify all the phases of mitosis. The samples dry quickly, and in order to increase the stability of your samples you can seal the coverslip with nail polish.

Biography

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Research output

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