

New species of algae *Actinastrum lagerheim* and *Closterium nitzsch* ex Ralfs from Middle Miocene Sediments of Chiang Muan basin, Phayao, Thailand, with tropical pollen composition

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Received 23 May 2003

Accepted 24 Dec 2003

ABSTRACT: Two forms of algae were described including *Actinastrum bansaense* n. sp. and *Closterium thailandicum* n. sp. They occurred abundantly in some sedimentary horizons of Middle Miocene age from Chiang Muan basin. Abundant occurrence of the two algal species with some rare forms of algae *Botryococcus* and *Striatriletes susannae* aquatic fern spores reveals that the Chiang Muan basin formation occurred in a freshwater depositional environment. Abundant spores of *Crassoretiriletes vanraadshoovenii* (*Lygodium microphyllum*-type) were also recovered with common Dipterocarpaceae, *Lagerstroemia*, *Ilexpollenites*, *Myrtaceidites*, and Combretaceae with rare forms of *Florschuetzia*, *Homonoia*, *Calophyllum*, *Striatriletes susannae*, *Botryococcus* and Mimosaceae. There are abundant *Laevigatosporites haardtii* fern spores in some horizons with various forms of as yet unidentified tricolporate and tricolpate pollen. Three acme palynological zones were established and used for describing variation in paleoenvironment through time of sedimentation. The sporomorphs representing tropical palynofloras derived from tropical monsoon forests, accumulated mainly in lacustrine depositional environments. Origins and distributions of the families Dipterocarpaceae and Myrtaceae are discussed and criticised and new criteria elucidating their paleophytogeographic histories in relation to northern Thailand are proposed.

KEYWORDS: new species, freshwater algae, palynology, Middle Miocene, northern Thailand.

INTRODUCTION

The history of vegetation change in northern Thailand has been mainly based on palynological and paleobotanical studies.¹⁻¹⁰ Major climatic fluctuations are recorded by major vegetation changes from Oligocene through Miocene time.^{6,9,10} Northern Thailand is considered in a tropical region. Tropical monsoon forests mainly occupy lowland areas with some warm temperate forests on mountainous areas recognisable as successive overlapping altitudinal ranges of various plant species. However, both latitudinal and altitudinal geographic settings control the occurrence and distribution of tropical forests and warm temperate plant species. The first indication that northern Thailand used to have a warm temperate climate was determined on the basis of macrofloral remains – leaves, catkins, cones, and scales – found in assumed Paleogene sediments from the Li Basin.¹⁻³

Thereafter, several palynological studies confirmed this.⁴⁻¹⁰ On the basis of both palynology and vertebrate palaeontology it was determined that tropical forests also occurred in northern Thailand at least as early as late Early Miocene. These types of fossil material, pollen, spore and vertebrate remain, are reliable and provide acceptable clues to the understanding of plant and animal communities within particular depositional environments.

Chiang Muan basin is a Cenozoic basin (Fig 1) in Chiang Muan District of Phayao Province. It has a north-south elongated shape and is located between 100° 10' and 100° 25' east longitude and 18° 45' and 19° 05' north latitude (Fig 2). The basin contains Middle Miocene sediments overlain by Quaternary deposits.¹² The basin is bounded by Pre-Tertiary rocks including Jurassic sandstone, siltstone, shale, and redbed formations with some Triassic clastic rocks exposed in the south. There are some Jurassic rhyolite, tuffaceous

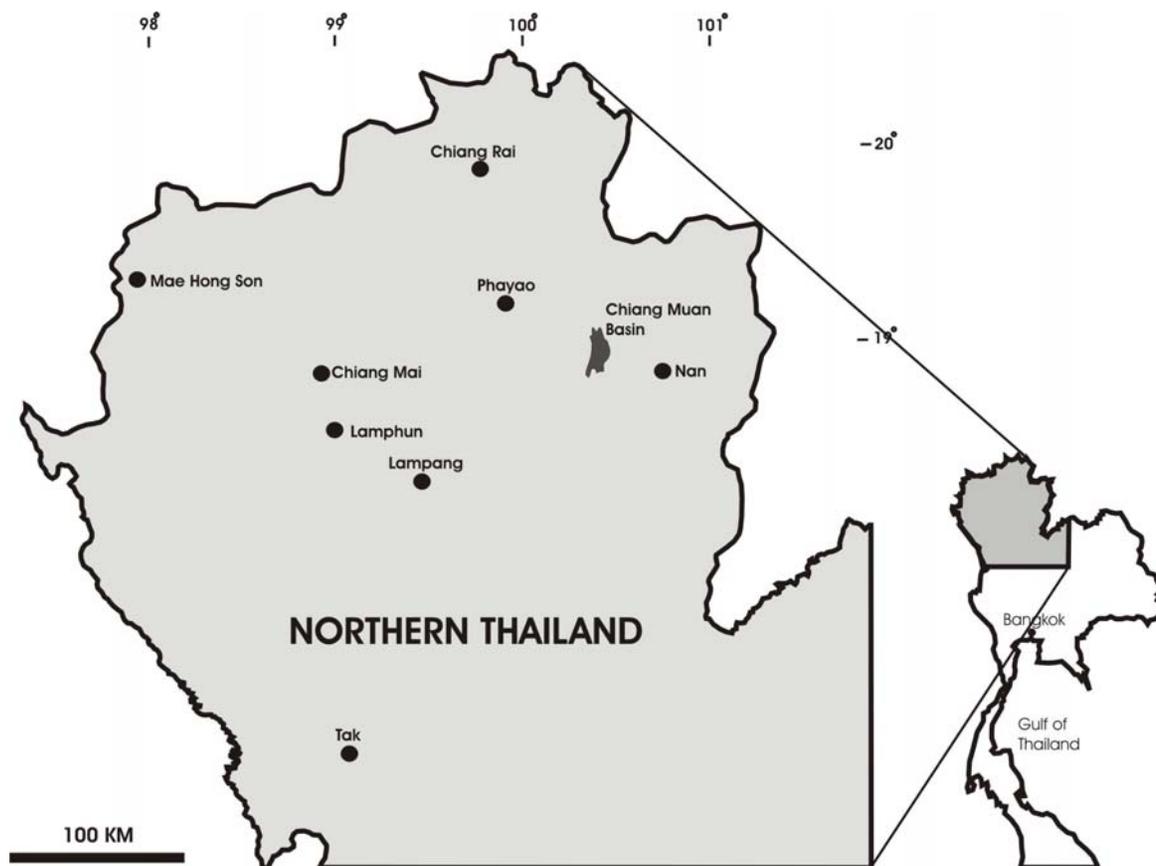


Fig 1. Map of northern Thailand showing location of Chiang Muan basin.

shale, and sandstone outcrops along the western flank of the basin (Fig 2).

Tertiary sediments consist of a series of claystone, sandstone, ligneous claystone, and coal layers with common vertebrate and plant remains. Abundant gastropod molluscs occur in some horizons, as seen in Chiang Muan coal mine in Ban Sa, in the northwest of the basin. The formation includes coal measures exploited for a cement factory in Saraburi Province. Various forms of vertebrate remains have been reported, including primate, proboscidean, rhinoceros, pig, barking deer, deer, bovid, bird, reptile, and many indeterminate forms.¹¹ Presence of a new primate species, *Lufengpithecus chiangmuanensis*, was claimed as an ancestral orangutan ape.¹² Age of the sediments is thought to be late Middle Miocene, between 13.5 and 10 Ma yrs, on the basis of magnetic polarity changes.¹²

We present evidence for tropical late Middle Miocene palynological assemblages in Chiang Muan basin and establish three new palynological acme zones to help elucidate changes in the depositional environment. Two new species of freshwater algae are described. Some ideas on floristic development of the

late Middle Miocene forests are also presented.

MATERIALS AND METHODS

Twenty-four coal and sedimentary samples were collected from outcrops in the Chiang Muan coal mine. About 5 grams of each sample was first soaked with 48% HF to digest minerals under heating on the hotplate for 30 minutes. The residues (also coal samples) were treated with 2 ml concentrated HNO₃ with a few droplets of saturated sodium chlorate solution, NaClO₃, to oxidise for 5 minutes at room temperature. After washing the residues with distilled water, 10% KOH solution was added then washed with distilled water. After that the residues were sieved to obtain 11 to 133 microns fractions. The sporomorphs were then permanently mounted with Eukit mounting media for light microscopic work. For SEM work, the sporomorphs were coated with a 30 nanometres layer of gold then examined under an SEM (Model JSM-5410) at the National Science and Technology Development Agency (NSTDA) in Pathumthani. The laboratory work was carried out at the Palynology

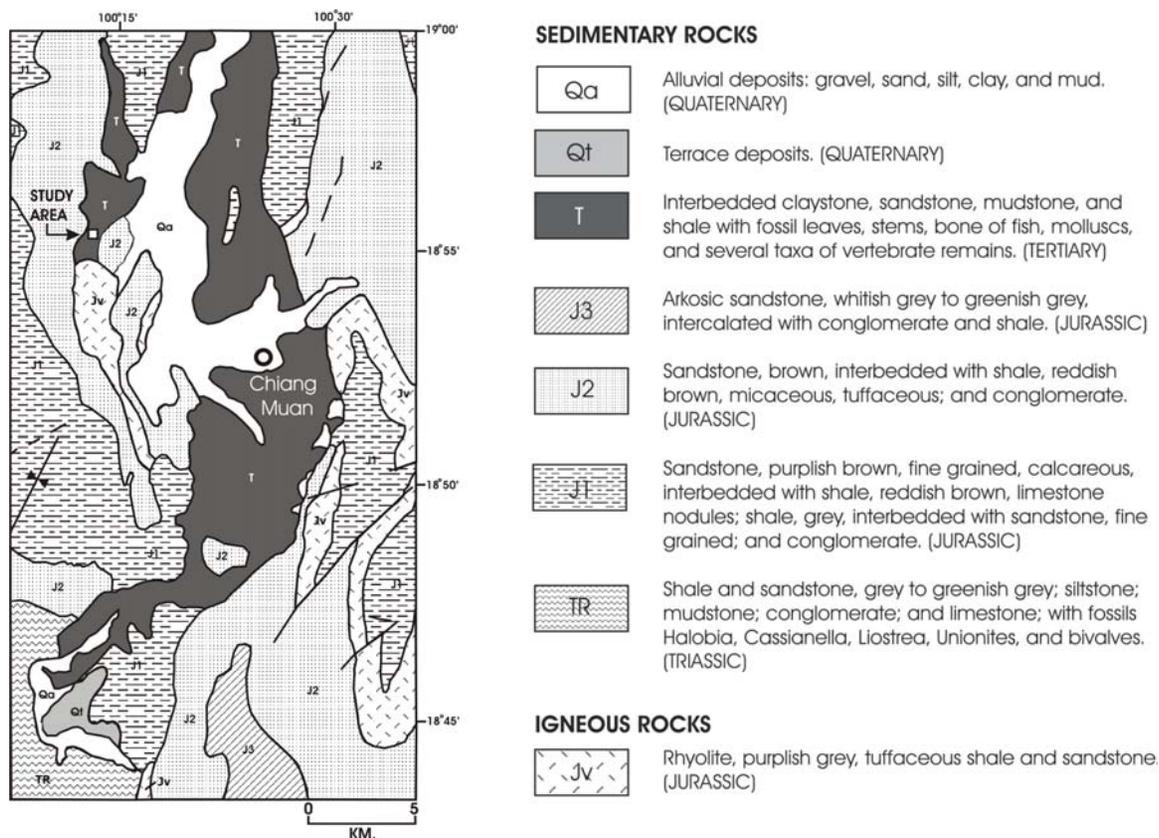


Fig 2. Geological map of Chiang Muan basin.⁴⁴

Laboratory of the Department of Mineral Resources in Bangkok.

SYSTEMATIC PALYNOLOGY

The systematic section of the paper involves description of some important freshwater palynomorphs including *Actinastrum bansaense* n. sp., *Closterium thailandicum* n. sp., which occur abundantly in some samples. It also includes discussion on *Crassoretitriletes vanraadshoovenii* Germeraad, Hopping, and Muller. Other forms of palynomorph will not be described but will be cited in the text and some are illustrated. Please refer to diagramme in Fig 3, locations of the sample collected and pollen counts are shown in Table 1.

Colonial green algae

Order: CHLOROCOCCALES

Family: SCENEDESMACEAE

Some authors place *Actinastrum* into a separate family, Coelastraceae, with close affinities to *Scenedesmus*.¹³⁻¹⁵

Genus *Actinastrum* Lagerheim, 1882

Actinastrum bansaense Songtham, Ratanasthien, and Mildenhall, n. sp.
Plate 1, figs 1-2

Etymology: Name of a village, Ban Sa, where the fossils were first found.

Holotype: slide no. CM-37a; co-ordinates N19/3 (England reference).

Paratype: slide no. CM-37a; co-ordinates N20/3, S21/3 (England reference).

Description: The species is characterised by a shuttle-like or cigar-like cell body. It is swollen in the middle portion and tapers to both sides with blunt ends. The cell length between both ends ranges from 20 to 37 μm ; the cell width in the middle portion of the cell body ranges between 7 to 13 μm . Surface ornamentation is smooth. Twenty specimens were measured.

Remarks: The fossil form is comparable in its general morphology to the extant freshwater green alga *Actinastrum*, family Scenedesmaceae¹⁶. However, when compared with *Actinastrum gracillimum* Smith and

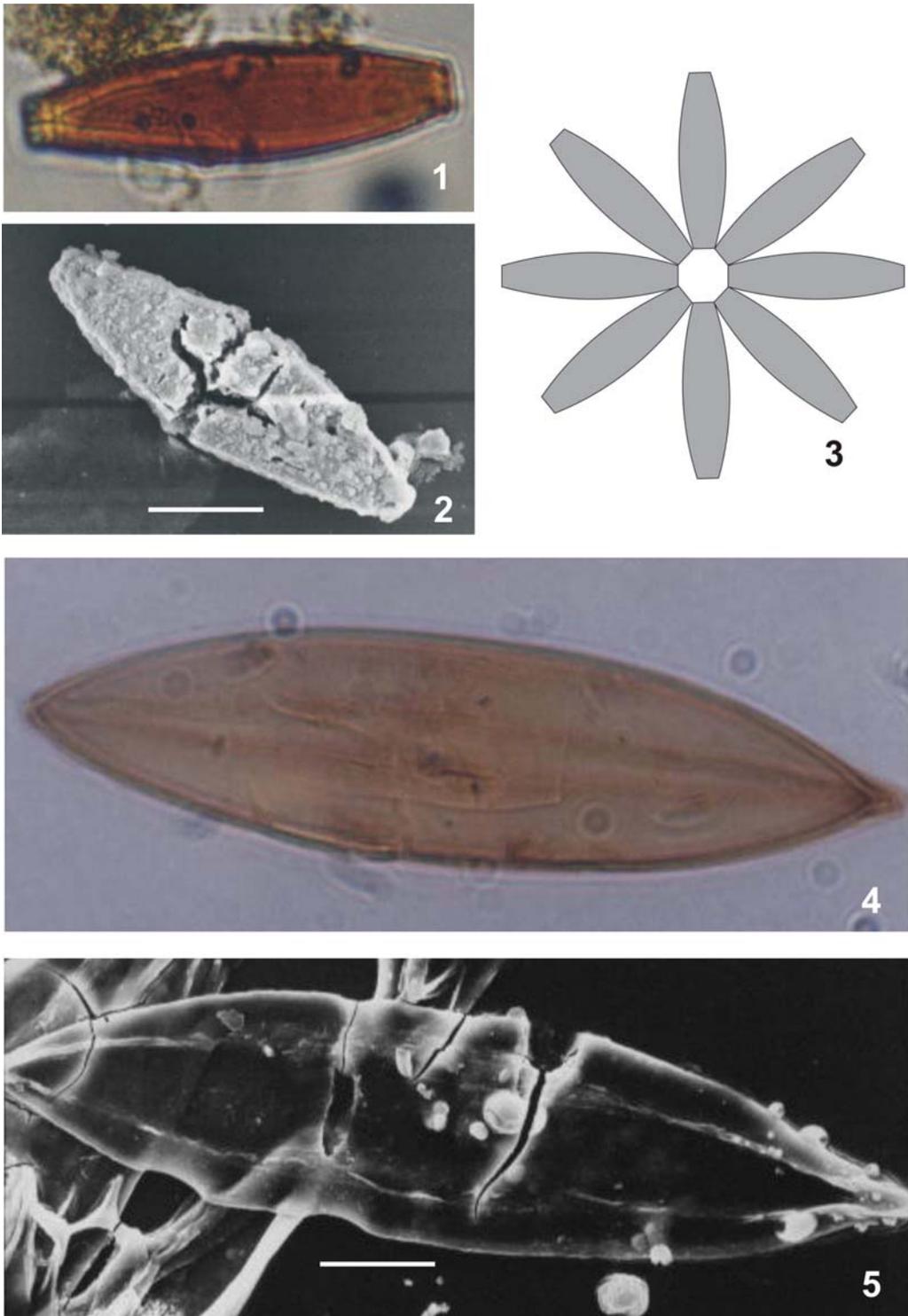


PLATE 1: All at same scale (bar = 10 μ m.).

1. *Actinastrum bansaense* Songtham, Ratanasthien, and Mildenhall, n. sp., LM.
2. *Actinastrum bansaense* Songtham, Ratanasthien, and Mildenhall, n. sp., SEM.
3. Reconstructed *Actinastrum* colony, 8 cells in star-like form, two dimension cell arrangement.
4. *Closterium thailandicum* Songtham, Ratanasthien, and Mildenhall, n. sp., LM.
5. *Closterium thailandicum* Songtham, Ratanasthien, and Mildenhall, n. sp., SEM.

Table 1. Percentages of each sporomorph taxon counted from 24 sedimentary samples.

Sample No.	<i>Striatriletes susannae</i>	<i>Closterium thailandicum</i>	<i>Botryococcus</i>	<i>Laevigatosporites haardtii</i>	<i>Homonoida</i>	<i>Calophyllum</i>	<i>Ilexpollenites</i>	<i>Myrtacidites</i>	<i>Hopea</i>	<i>Florschuetzia</i>	Combretaceae	<i>Crassotritriletes vanraadshoovenii</i>	<i>Actinastrum bansaense</i>	Indeterminant sporomorphs	Total percentage	Number of count
CM-69													92.0	8.0	100	200
CM-66													96.0	4.0	100	200
CM-65													95.5	4.5	100	200
CM-63													95.0	5.0	100	200
CM-60													90.5	9.5	100	200
CM-49													87.0	13.0	100	200
CM-42													89.5	10.5	100	200
CM-39													98.5	1.5	100	200
CM-37													97.0	3.0	100	200
CM-31													98.5	1.5	100	200
CM-25													99.5	0.5	100	200
CM-21													97.5	2.5	100	200
CM-17													96.5	3.5	100	200
U1-6-B1												100			100	200
U1-4-B1					5.4							20		74.6	100	56
U2-9				100											100	200
U2-8						7.3					4.9			87.8	100	41
U2-4				23										77.0	100	125
U2-2							15		10			9		66.0	100	78
LM-A-6				57			8.9			1.8				32.3	100	56
LM-A-4			6.3				17							76.7	100	48
LM-A-3			7.5	16	0.5	2.5		9.5						64.0	100	200
LM-A-1		100													100	200
LS-B-1	8.3													91.7	100	36

Actinastrum hantzschii Lagerheim, the fossil forms have a greater size in both middle portion and the length between both ends than the extant forms. *Actinastrum* is a colonial green alga comprising a fixed number of cells (4, 8, 16, 32 or more). The cells in the colony are arranged into 2 to 3 dimensions by adjoining one end of each cell together at a center point while the another end of each cell points outwards forming a star-like (Plate 1, fig 3), circular or spherical colony (coenobium). However, we have never seen any fossil colonial forms because they were probably disarticulated forming

individual cells during diagenesis and sample treatment.

There are only two published reports on fossil forms of *Actinastrum*, both from Early Triassic rocks, from Germany¹⁷ and Western Australia.¹⁴ The later report was later re-illustrated.¹⁵

Occurrence: Abundant in some horizons from Oligocene to Miocene sediments of northern Thailand. This includes the entire overburden unit (OB unit) of Chiang Muan coalfield in this study, *Pediastrum* Zone of Ban Pa Kha coalfield (Li basin), and Mae Lamao basin.

Green algae

Order: DESMIDIALES

Family: DESMIDIACEAE

Genus: *Closterium* Nitzsch ex Ralfs (1848)

Remarks: Many species of *Closterium* are found in most parts of the world or cosmopolitan. All species in this genus are solitary with elongate-cylindric and curved (at least at the ends). A few species have elongate-fusiform straight cells. Cells gradually narrow toward both apices that may be acutely pointed, rounded, or truncate. Cell wall may be smooth, have fine longitudinal striae, or have coarse costae.

Closterium thailandicum Songtham, Ratanasthien, and Mildenhall, n. sp.

Plate 1, figs 4-5

Etymology: named after Thailand, where the fossils were first found.

Holotype: slide no. LM-A-1a; co-ordinates F23/3 (England reference).

Paratypes: slide no. LM-A-1a; co-ordinates G19 and D15/4 (England reference).

Description: The species is characterised by a single, long, straight cell. The cell wall is thin with a smooth surface and some longitudinal lines. The cell is usually flattened forming a long, frond-like shape tapering at both ends. Both ends have sharply acute to small, rounded points. Width of the middle portion of the cell ranges from 10 to 26 μm and length between both ends ranges between 43 to 91 μm . The ratio between the width of the middle portion and the length between both ends is about 1: 4.5. Twenty specimens were measured.

Comparison: The cell of extant species of *Closterium* are generally of crescentic shape. Only a few species of the subgenus *Closterium* (*Holopenium*) have straight cells,

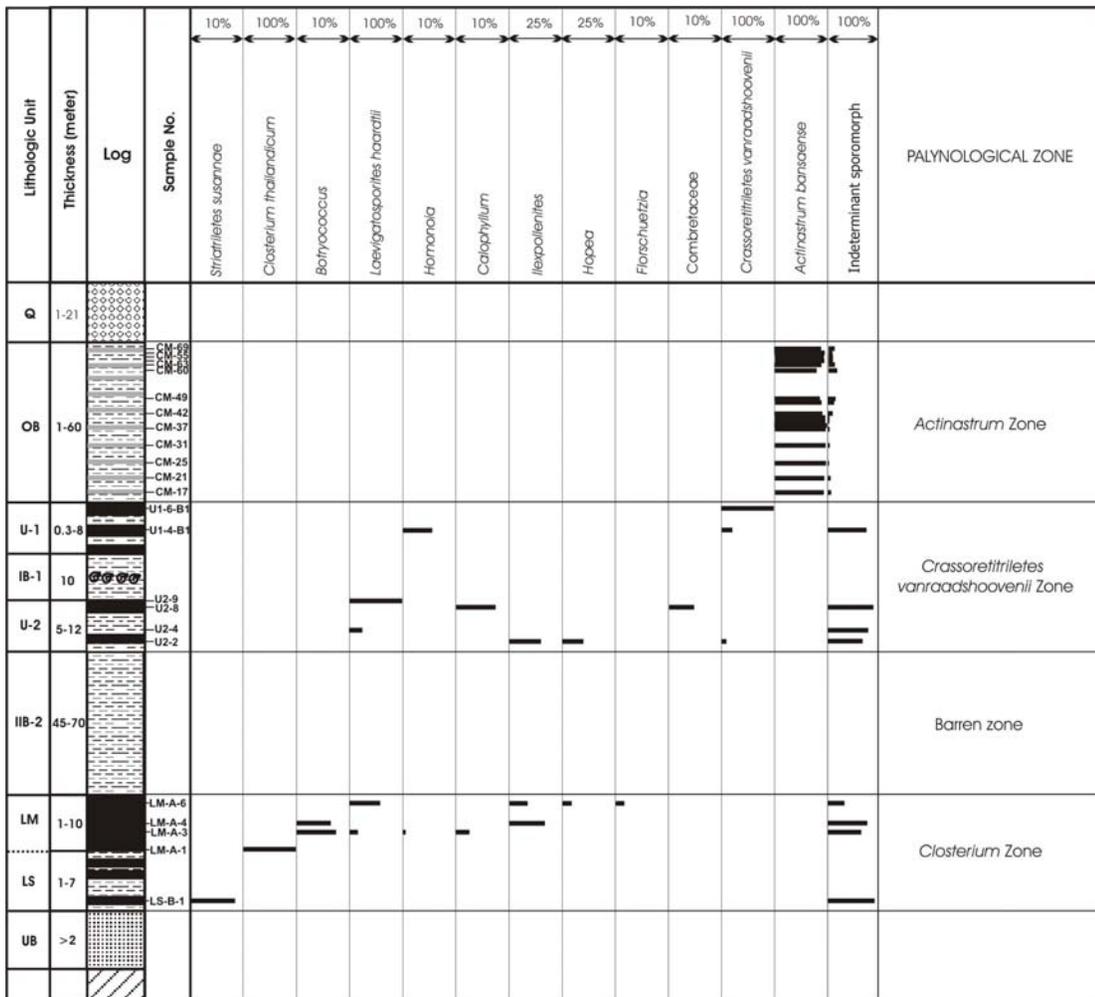


Fig 3. Pollen diagram showing plots of pollen occurrence in percentage against lithostratigraphic units and palynological acme zones of Chiang Muan coalfield.

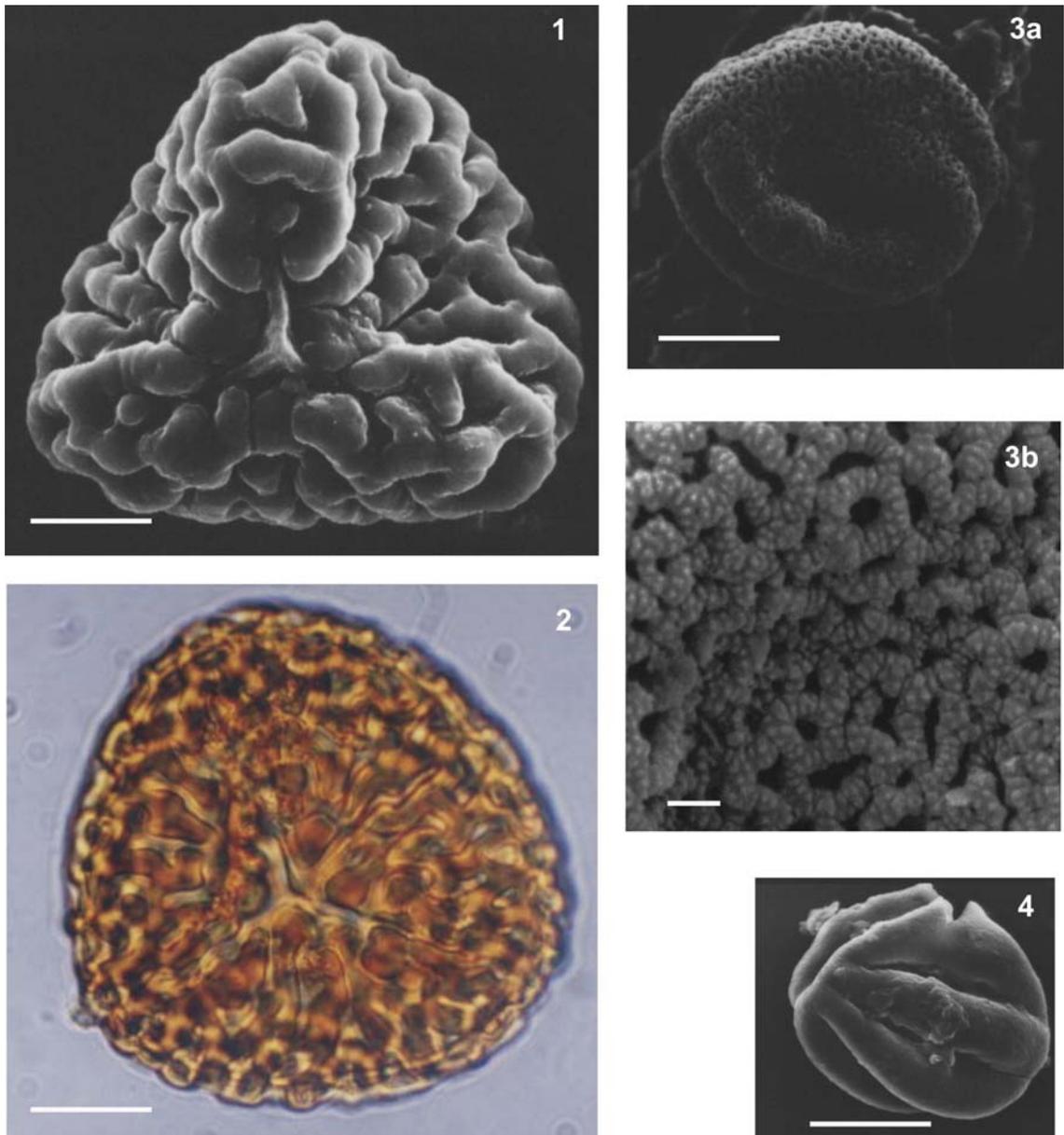


PLATE 2: All at same scale (bar = 10 μm .) except 3b (scale bar = 1 μm .)

1. *Crassoretitriletes vanraadshoovenii* Germeraad, Hopping, and Muller, SEM.
2. *Crassoretitriletes vanraadshoovenii* Germeraad, Hopping, and Muller, LM.
3. *Hopea* sp., tricolpate form with reticulate sculpture.
4. Combretaceae, tricolporate form with pseudocolpi.

including *Closterium libellula* (Focke) and *Penium navicula* (Lütkemüller). These two modern forms are much bigger than fossil *Closterium thailandicum*.

Remarks: So far, there are no reports of fossil *Closterium* from sediments, except for zygospores of *Closterium idiosporum* West and West from Holocene sediments in the Netherlands.¹⁸ *Closterium* is a green alga belonging to the family Desmidiaceae occurring in freshwater environments.

Occurrence: They are abundant in the lower coal unit (LS and LM coal units) of Chiang Muan coalfield. One sample (LM-A-1) contains nearly 100% *Closterium thailandicum*.

Pteridophytic spores

Trilete spores

Genus ***Crassoretitriletes*** Germeraad, Hopping, and Muller (1968)

Crassoretitriletes vanraadshoovenii Germeraad, Hopping, and Muller (1968)

Plate 2, figs 1-2

Description: These spores from Chiang Muan coalfield are spherical to slightly triangular in polar view. They have broadly convex sides, with a trilete mark on the proximal surface clearly observable in polar view. The surface sculpturing is entirely coarsely reticulate with wide undulating muri, about 3 to 5 μm wide, covering the whole grain on both distal and proximal sides. The lumina of the reticulum are 2 to 5 μm wide. Exine thickness is indistinct. The grains range from 52 to 74 μm and average about 62 μm , based on 20 measured grains.

Remarks: The above characteristics well match the descriptions of *Crassoretitriletes vanraadshoovenii* reported from Borneo, Nigeria and South America¹⁹ and from Thailand.⁵ However, first designation¹⁹ describes the size as ranging between 58 and 101 μm , while the forms from Thailand⁵ describes the size as ranging from 62 to 90 μm . *Crassoretitriletes vanraadshoovenii* is comparable to the extant spores of *Lygodium microphyllum* (Cav.) of the family Lygodiaceae.^{19,20} *L. microphyllum* is a climbing fern, common in humid marsh and swamp forests in open to semi-open forests in tropical to subtropical areas of West Africa, Australia, and Indo-Malesia, but absent today from South America.

Occurrence: Abundant fossils were recovered from the U-1 and U-2 units of the late Middle Miocene Chiang Muan coalfield. They were previously reported from Mae Tip basin in Lampang Province.⁵ The species has been widely reported from Late Eocene sediments to the present day from Australia, China, South America, India, and South East Asia. They were reported from

the late Early Miocene of South America and Early Miocene to Recent of Borneo and Nigeria.¹⁹ In Australia the species first appears in the late Eocene and dies out in the late Miocene²¹ although they were previously reported from the Pleistocene of Tasmania, southern Australia considerable as recycled specimens.²⁰ In addition, they were also reported from Eocene to Early Miocene and Oligocene sediments of India.²²⁻²⁵

PALYNOSTRATIGRAPHY

Most palynofloras from Chiang Muan sediments contained identifiable tropical elements and abundant freshwater green algae. Three palynological acme zones are defined - *Closterium thailandicum* Acme Zone, *Crassoretitriletes vanraadshoovenii* Acme Zone, and *Actinastrum bansaense* Acme Zone - from the base to the top (Fig 3).

1. *Closterium thailandicum* Acme Zone, new zone

This zone covers the whole part of the lower coal zone. It is dominated by the freshwater alga *Closterium thailandicum*. Some coenobia of the colonial chlorophycean alga *Botryococcus* and fossil spores *Striatriletes susannae* (van der Hammen), related to the extant aquatic fern *Ceratopteris thalictoides* (L.), family Parkeriaceae, also occur. These freshwater elements occur in association with several forms of pollen from predominantly tropical plants, including *Calophyllum*, Combretaceae, *Florschuetzia*, *Homonoia*, *Ilexpollenites*, *Lagerstroemia*, and Mimosaceae. This zone reflects a freshwater depositional environment surrounded by tropical rainforests. The climate was tropical.

The forms of *Ilexpollenites*, *Homonoia*, and *Florschuetzia* represent taxa occurring near swamps and rivers at the present day. *Ilexpollenites* (*Ilex*-type) is a fossil pollen grain from a plant genus typically common in swampy environments. The fossil *Homonoia* pollen matches well pollen from the extant species *Homonoia riparia* Lour., family Euphorbiaceae, a species of shrub growing along streams or riversides in Thailand. *Florschuetzia* (Sonneratiaceae-type) has a pollen morphology close to sonneratiaceous pollen forms from plants living in coastal mangrove as well as terrestrial environments. There is today only one species of Sonneratiaceae in northern Thailand, namely *Duabanga grandiflora* (DC.), occurring well inland,²⁶ in the moist areas. Even though the fossil *Florschuetzia* pollen does not match well the pollen of *Duabanga grandiflora* or *Sonneratia*, they may be best regarded as representing an early stage in the phylogenetic evolution of *Duabanga grandiflora* pollen. The fossil *Florschuetzia* pollen type cannot be from *Sonneratia*, a mangrove taxon, because it occurred in a distinctly freshwater palynological assemblage.

2. *Crassoretiriletes vanraadshoovenii* Acme Zone, new zone

This zone covers the upper coal zone including the units U1, U2, and IB-1. The zone is dominated by *Crassoretiriletes vanraadshoovenii*, the ancestral spores of the extant fern *Lygodium microphyllum* Link., with several forms of tropical pollen. These include cf. *Bauhinia*, *Calophyllum*, *Combretaceae*, *Homonioia*, *Hopea*, *Ilexpollenites*, *Lagerstroemia*, *Myrtaceidites* (Myrtaceae), cf. *Shorea* with abundant *Laevigatosporites haardtii* R. Pot. et Venitz spores, of uncertain, but probably mixed botanical affinity, in some horizons.

This zone reflects a moist environment probably along marginal areas of the Chiang Muan Miocene lake. The pollen and spore assemblages from this zone come from tropical rainforests. Climate during deposition was tropical.

3. *Actinastrum bansaense* Acme Zone, new zone

This zone covers the total thickness of the overburden unit (OB unit). It contains almost entirely cells from the freshwater alga *Actinastrum bansaense*. Pollen and spores are absent but some indeterminate fungal spores are present. This zone is interpreted as an ancient freshwater lake. The abundant occurrence of freshwater algae probably resulted from algal bloom events during sedimentation of the overburden, but why spores and pollen are not preserved is not known unless they were differentially oxidized out of the sediment or completely overwhelmed by the abundance of algal material.

All three palynological zones of the Chiang Muan Formation provide evidence of tropical climate during sedimentation. The vegetation around the Chiang Muan Miocene lake during basin formation was comprised of tropical rainforests. The age of the sedimentary sequence is between 13.5 and 10 Ma yrs (late Middle Miocene).¹²

DISCUSSION

Chiang Muan sediments yielded some distinctive sporomorph forms representing tropical megathermal floras dominated by members of the family Dipterocarpaceae. The origin and distribution through time of this family has often been debated. Dipterocarps today have their greatest diversity in Southeast Asian rain forests. They also occur in some parts of India and Sri Lanka. Two genera, *Marquesia* and *Monotes*, occur in tropical Africa,^{27,28} and one genus, *Pakaramaea*, occurs in northern South America.²⁹ This was maintained that the family did not originate in Southeast Asia, but in either South America or Africa and that their descendants have not substantially changed their paleoecological preferences.²⁹ However, a new revision was recently made placing African and American

dipterocarps into a new family, Monotoaceae, comprising two subfamilies Monotoideae (*Monotes* and *Marquesia*) and Pakaramaeoideae (*Pakaramaea*). Therefore, dipterocarps (*sensu stricto*) today survive just in the western region of Wallace's line including western Indonesia, the Melasian archipelagoes, Southeast Asian mainland, India and Sri Lanka. *Monotes* is characterised by endoapertures meanwhile Dipterocarpaceae is tricolpate. On the basis of aperture characteristics, it is believed that Dipterocarpaceae is more primitive than the Monotoideae. Dipterocarps were clearly extensively represented in the monsoon forests of the Sunda region during the Late Oligocene and Early Miocene.²⁹ Meanwhile, dipterocarps first appeared in India during Neogene³⁰⁻³⁴ and without any record of dipterocarps from the Paleogene of India.³⁵ Thus it is possible that the family originated in Southeast Asia and later expanded to India via Thailand and Myanmar sometime in the Early Miocene which was previously proposed.³⁵ The occurrence of dipterocarpaceous pollen, *Hopea* and cf. *Shorea*, from the Middle Miocene Chiang Muan sediments reveals that the dipterocarp vegetation developed under a tropical monsoon climate.

Myrtaceidites is also an important fossil sporomorph with a distinctive morphology found primarily in the family Myrtaceae, making it easy to tracing the family's origin and distribution through time. Abundant fossil Myrtaceae pollen were previously reported from Chiang Muan sediments, which they related to *Syzygium*, and claimed that the dominance of this pollen type and the swampy environment indicated a dispersal corridor to Africa.¹² Myrtaceae is a family of trees and shrubs found in the tropics, subtropics, Polynesia, and temperate Australia and New Zealand comprising 140 genera and 3000 species and including the well known gum tree, eucalyptus. *Syzygium* is native to Australia, New Zealand and elsewhere, as well as Southeast Asia including Thailand. There are many native species of *Syzygium* reported from northern Thailand²⁶ including the Chiang Muan area. Accordingly, there is no reason to regard these *Syzygium*-like sporomorphs from Chiang Muan sediments as indicating an African connection. In addition, most myrtaceous pollen from Tertiary sediments have been identified only at the family level and assigned the fossil form generic name *Myrtaceidites*, and it appears impossible to accurately determine botanical affinity to the generic level in many cases.³⁶ Use of the scanning electron microscope to accurately identify the botanical affinity of myrtaceous fossil pollen is possible in some cases and probably impossible in others where the pollen morphology of the family is so conservative and the pollen grains so small. Some species of *Syzygium* are distinctive in that this pollen type often has a more triangular appearance in polar view with more acute angles and an ectexinous polar island

reduced to scabrae and granules^{36,37} [as *Eugenia*] than other myrtaceous grains.

It is not known when Myrtaceae first appeared in Thailand but it is at least Early Miocene.⁶ The first appearance of Myrtaceae pollen types in Australia and New Zealand in the Paleocene³⁸⁻⁴⁰ probably represents its worldwide first appearance. Therefore, the presence of this Gondwanan family in northern Thailand in the Miocene may relate to the tectonic movement of the Indian subcontinent. India used to be a part of Gondwana, together with Australia, and broke up when it moved northward acting as a Noah's ark²⁹ carrying the southern fauna and flora, including the Myrtaceae, northward. India collided and welded onto South Asia during the Eocene forming the massive Himalayan Ranges and Tibetan Plateau. The Gondwanan flora then entered the Eurasian supercontinent and subsequently migrated southeastward to occupy the Southeast Asia region.

A Middle Miocene marine incursion into the Chiang Muan area may have occurred.⁴¹ This conclusion came from sulfur isotope values from the upper coal seam unit, which is remarkably high in sulfur. On the other hand, palynological results from the same unit yielded no closely related sporomorphs representing marginal marine environments. The unit contains abundant *in situ* fern spores of *Crassoretitriletes vanraadshoovenii* and *Laevigatosporites haardtii* with other sporomorphs representing inland environments, without any trace of marginal marine pollen evidence. The lack of palynological evidence for a marine incursion needs corroboration by further geochemical, sedimentological and other studies to see if the high sulfur content could have been caused by conditions other than a marine incursion. From a palynological point of view, absence of mangrove pollen does not mean no marine incursion occurred if the Chiang Muan basin was occupied by a temperate climate. However, the Chiang Muan sediments yielded sporomorphs clearly representing an inland tropical, monsoon vegetation and if there was a marine incursion then the sediments should have contained significant numbers of mangrove pollen.

However, if a marine interval is shown to be present by further sedimentological and geochemical studies then more detailed investigations of the palynology are required to find the coastal taxa associated with the transgression and more detailed oxygen isotope and magnetostratigraphic studies to accurately date the timing of the transgression.

ACKNOWLEDGEMENTS

We appreciate the sponsorship of Royal Golden Jubilee Ph.D. Programme, The Thailand Research Fund

for financial support for this research. We thank the Department of Mineral Resources, Chiang Mai University, and Institute of Geological and Nuclear Sciences, New Zealand in providing financial, logistical, and equipment support. We thank all the staff of Chiang Muan Mine Company who warmly welcomed us and permitted us to collect samples in the mine. Special thanks also to Mr. Nikorn Wongchai and his family for hospitably providing us with many things.

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