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Four plant species from the Neogene of southwestern China, Northern Vietnam and their paleoenvironmental implications

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Three new fossil records of *Equisetum* (Equisetaceae) from the Neogene of south-western China and northern Vietnam

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Abstract

Paleobotany and paleoenvironmental studies are important to trace plant species distribution in the past and understand species extinction, ecological linkages, between species and their habitats. In this study, three species of fossil Equisetum (Equisetaceae) were reported from the Neogene of southwestern of China and northern Vietnam; fossils were collected from areas that are well known for the occurrence of plant megafossils. Two new fossil species of Equisetum (Equisetaceae) and Equisetum cf. pretense Ehrhart are recorded and described in detail. All species are reported based on well-preserved rhizomes with tubers. Equisetum cf. pretense Ehrhart from the middle Miocene in Yunnan Province, China is characterized by a bunch of three ovate tubers with longitudinal ridges on the surface. Another Equisetum fossil species, Equisetum yenbaiense A.T. Aung, T. Su, T.V. Do and Z.K. Zhou, sp.nov. from the late Miocene of Yenbai Province, Vietnam, is characterized by four bunches of elongate tubers arranged in a whorl on a node. Equisetum yongpingense A.T. Aung, T. Su and Z.K. Zhou, sp. nov. from the late Pliocene in Yunnan is characterized by fibrous bunches of large cylindrical tubers arranged in a whorl on a node. According to the climatic conditions of the habitats of their living relatives, these three species of Equisetum species might have survived in wet and humid environments. Additionally, Trapa pollen grains were investigated from the Upper Pliocene Sanying formation, Yongping, western Yunnan, were investigated. Fossil Trapa grains are recorded and compared with Trapa natans pollen grains from the Xishuangbanna Tropical Botanical Garden. Both of these species have similar morphological characteristics e.g., triangular obtuse in equatorial view and concave in polar view, surface slightly granulated. Trapa usually grows in shallow water under wet and humid climates. This suggests the existence of Trapa in western Yunnan in the late Pliocene and indicates that the climate conditions were similar to those of the present time. Generally, the two genera, including four species, were reported from the Neogene of southwestern China and northern Vietnam, based on detailed morphological comparisons. We provide new fossil evidence for the evolution of plant diversity in the regions. Besides, these new fossil records deepen our understanding of the paleoclimatic background floristic correlation between southwestern China and Southeast Asia. Further paleobotanical investigations are needed to explore the evolution of plant diversity in response to climate changes in the geological past.

Keywords: Diversity, Equisetum, Trapa, Miocene, Pliocene, Rhizome tubers, Pollen

Neogene (ကျောက်ဖြစ်ကာလ)မှပေါက်ရောက်တည်ရှိခဲ့ပြီး မြေဆီလွှာမှတွေ့ရှိရသော အပင်မျိုးစိတ် (၄) မျိုးအားလေ့လာခြင်းနှင့် ၎င်းတို့၏ အတိတ်ကာလပတ်ဝန်းကျင်ဆိုင်ရာ ဂေဟဗေဒအခြေအနေများ နှင့် ပတ်သက်ဆက်နွယ်မှုအကျိုးဆက်များအားလေ့လာခြင်း

အေးသီတာအောင်

တောအုပ်ကြီး

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စာတမ်းအကျဉ်း

Paleobotany နှင့် Paleoenvironmental Studies ဘာသာရပ်များအား လေ့လာမှုနှင့် စပ်လျဉ်း၍ အတိတ်ကာလမှ အပင်မျိုးစိတ်များ ပေါက်ရောက်မှုများအား ခြေရာခံခြင်းမှ အပင်မျိုးစိတ်များမျိုးသုဉ်းခြင်း၊ ဂေဟဗေဒဆိုင်ရာ ဆက်နွယ်မှုများ ပတ်ဝန်းကျင်ဆိုင်ရာ အခြေအနေ ရာသီဥတုပြောင်းလဲမှုများကို ၎င်းတို့ပေါက်ရောက် ပျံ့နှံ့ရာဒေသနှင့် ဆက်စပ်၍ လေ့လာသိရှိနိုင်သည်။ အတိတ်ကာလမှ အပင်များ၏ fossil ကိုအခြေခံ၍ လေ့လာခြင်းဖြစ်သည်။ ဤစာတမ်း၏ သုတေသနအပိုင်း(၁)တွင် *Equisetum* မျိုးစိတ် (၃)မျိုးကို ဖော်ထုတ်လေ့လာခဲ့သည်။ *Equisetum* မျိုးစိတ်(၂)ခုကို ယူနန်ပြည်ယ်ရှိ မြန်မာနိုင်ငံနှင့် တရုတ်နိုင်ငံကြား နယ်နိမိတ် အနီးမှ တွေ့ရှိစုဆောင်းခဲ့သည်။ *Equisetum* မျိုးစိတ် (၁)မျိုးမှာ ဗီယက် နမ်နိုင်ငံမှ ဖြစ်သည်။ ထိုမျိုးစိပ်(၃)ခုအနက် *Equisetum* မျိုးစိတ်(၂)မျိုးမှာ မျိုးစိတ် သစ်များဖြစ်ကြောင်း ဖော်ထုတ်နိုင်ခဲ့သည်။ ထို့အပြင်း ၎င်းတို့ပေါက်ရောက်ရာ ဒေသနှင့် ဆက်စပ်ဂေဟစနစ်ကိုပါ လေ့လာခြင်းဖြင့် အတိတ်မှ ရာသီဥတု ဂေဟစနစ်နှင့် ယခုလက်ရှိကာလ ဂေဟစနစ်တို့၏ ကွာခြားမှုကို သိရှိနိုင်သည်။ ထိုလေ့လာမှုနှင့် စပ်လျဉ်း၍ **Three new fossil records of *Equisetum* (Equisetaceae) from the Neogene of south–western China and northern Vietnam** ဟူသောခေါင်းစဉ်ဖြင့် **Impact Factor 1.8** အဆင့်ရှိ Phytokeys ဂျာနယ်တွင် နိုင်ငံတကာ သိပ္ပံစာတမ်းအဖြစ် ထုတ်ဝေခဲ့ပြီးဖြစ်သည်။ သုတေသနအပိုင်း (၂) တွင် *Trapa* မျိုးစိတ်၏ အတိတ်ကာလက ပေါက်ရောက်ခဲ့သော အပင်၏ fossil မှ သုတေသနလုပ်၍ စုဆောင်းရရှိသော ပန်းဝတ်မှုန်များနှင့် ယခုလက်ရှိကာလ *Trapa* မျိုးစိတ်တူမှ သုတေသနလုပ်၍

စုဆောင်းရရှိသော ပန်းဝတ်မှုန်များအား နှိုင်းယှဉ်လေ့လာခြင်းအား ဓာတ်ခွဲခန်းဆိုင်ရာ သုတေသနပြုလုပ်မှုများကို အခြေခံ၍လေ့လာထားပါသည်။ *Trapa* မျိုးစိတ်များကို ယူနန် ပြည်နယ်ရှိ မြန်မာနိုင်ငံနှင့် တရုတ်နိုင်ငံကြား နယ်နိမိတ်အနီးမှ တွေ့ရှိစုဆောင်းခဲ့သည်။ ၎င်း လေ့လာမှုမှတဆင့် အတိတ်မှ ရာသီဥတု ဂေဟစနစ်နှင့် ယခုလက်ရှိကာလ ဂေဟစနစ်တို့၏ ကွာခြားမှုတို့ကို ဆက်စပ် သိရှိနိုင်သည်။ ထိုလေ့လာမှုနှင့် စပ်လျဉ်း၍ **Impact Factor–4.7** အဆင့်ရှိ The Ecology Society of America ဂျာနယ်တွင် **Trapa (Lythraceae) from the late Pliocene of western Yunnan and its paleoenvironmental implications** ဟူသော ခေါင်းစဉ်ဖြင့် နိုင်ငံတကာသိပ္ပံ စာတမ်းအဖြစ် တင်သွင်းခဲ့ပြီး လက်ရှိတွင် Reviewerများ၏ feedbackကို ဖြေကြားထားပြီးဖြစ်ပါသည်။ ဤသုတေသနစာတမ်းတွင် စုစုပေါင်း plant fossil (၄) မျိုးအား လေ့လာမှုကို အခြေခံ ထားပါသည်။

Three new fossil records of *Equisetum* (Equisetaceae) from the Neogene of south-western China and northern Vietnam

1. Introduction

Fossil records in the past are important for our understanding of the evolution of plant diversity, and paleobotanical records are often used as evidences for the evolutionary histories of plant taxa (Taylor et al., 2008). The assemblage of species components in the fossil flora presents, to some extent, the local vegetation conditions in the past, and the evolution of vegetation and species richness can be studied by investigating floras from the same region at different geological times are studied (Jacques et al., 2011; Popva et al., 2013). Besides, fossils could help in the determination of the climatic conditions of ancient times in different regions (Mosbrugger and Utescher, 1997; Traiser et al., 2005). There is a close relationship between the distribution and morphological traits of ancient groups of plants and the paleoenvironment (Pam, 1989). The related field of science, paleobotany has a long history, but is developing rapidly, mainly in combination with ther disciplines such as geology, geochemistry, and modelling (Spicer et al., 2003; Su et al., 2019; Roy et al., 2020). In addition, the development of new technologies, such as micro-CT provides more detailed morphological structures for the identification (Nishida, 2007). In this sense, paleobotany plays an increasingly important role in understanding the evolutionary history of plants as well as the terrestrial ecosystem as such.

1.1 Importance of Paleobotany

Paleobotany is a discipline to study the morphology of plants in the geological past and to explore the evolution of plants (Gould, 1968). It is vital when investigating the life cycles and distribution patterns of species as well as the reasons for their extinction/diversification. Records of fossil plants could provide solid evidence for the early origin of plant groups, such as the origin of angiosperms in the early Cretaceous, with macrofossils (Sun et al., 1998). One of the most important goals of paleobotany is to describe the morphology of plants in deep time, and to understand the evolutionary relationships amongst these taxa (Taylor et al., 2008). Fossil evidence from Late Cretaceous, Paleocene, and Eocene floras indicates their close biogeographic lineages with modern plants (Johnson and Ellis, 2002; Burnham and Johnson, 2004; Jaramillo et al., 2010). Another goal of paleobotany is to use information about fossil assemblages to deduce the likely characteristics of vegetation (Popova et al., 2013) and their surrounding environments (Spicer et al., 2010). In addition, paleobotany focuses on the evolution of ecosystem such as plant-insect interactions on fossil leaves, which is a direct way to understand the evolution of feeding webs in forests under paleoclimatic changes (Wilf and Labandeira, 1999).

Indicators of climate in the geological past are derived from proxies in nature, not by direct measurement (excluding models). Natural proxies such as ice cores, plants, woods, tree rings, and sediment cores usually use diatoms, foraminifera, micro-biota, pollen, and charcoal within the sediment. The paleoclimate can then be estimated by using a combination of different types of the proxies mentioned above (Geay, 2017). Among those proxies, plant fossils are the most suitable evidence for vegetation dynamics and climate change in long

geological time spans, since they were deposited near local vegetation. Some plant groups are highly sensitive to climatic changes do not adapt easily to a changing climate, requiring specific living conditions. Some of them are abundant in fossil records from numerous fossil sites with different geological ages, making them useful indicators of how vegetation and ecosystems have changed under paleoenvironmental changes in the geological past (Mosbrugger and Utescher, 1997).

1.2 Paleobotanical studies in Yunnan

Yunnan Province is probably one of regions with the richest Cenozoic plant fossil sites around the world, with around100 years of paleobotanical studies (Colani, 1920). The dramatic tectonic activities during the Cenozoic formed numerous sedimentary basins in this region (Ge and Li, 1999), and a series of paleobotanical investigations have been carried out.Many plant fossils in the preservation forms of leaves, fruits, seeds, wood, and pollen/spores have been discovered from the Cenozoic in Yunnan (Ge and Li, 1999); in total 386 fossil species (ferns, gymnosperms, angiosperms) in 170 genera and; 66 families have been reported from the Cenozoic (Huang et al., 2016). Among them, angiosperms from 353 plant species of 155 genera within 60 families have been reported, with Fabaceae, Lauraceae, and Juglandaceae being the most abundant families in Cenozoic fossil records of Yunnan (Huang et al., 2016).

The late Eocene Jianchuan flora is currently the oldest known flora in Yunnan represented by *Acer*, *Quercus*, and *Zelkova* (Writing Group of Cenozoic Plants of China (WGCPC), 1978). This flora is considered to originate in the Miocene and has been recently revised by radiometric dating (Gourbet et al., 2017). The early Oligocene Lühe flora is another well-known flora in Yunnan and, dated back to the early Oligocene (~33 Ma) (Linnemann et al., 2018). Based on macrofossil evidence, 23 genera have been reported, with Betulaceae and Fagaceae being the most important families. Only the three genera, *Sequoia*, *Metasequoia*, and *Palaeocarya* have disappeared naturally in Yunnanwhich suggests that the modernization of plant diversity in southwestern China occurred from the late Eocene to theearly Oligocene, not as late as in the Miocene, as proposed based on molecular data (Linnemann et al., 2018).

During the Miocene, the vegetation in Yunnan was dominated by evergreen broad-leaved forests. The early Miocene Jinggu flora in southern Yunnan was dominated by Lauraceae and Fagaceae, and other evergreen elements such as *Annona* and *Machilus* arehave been collected from the same flora (WGCPC, 1978). The middle Miocene floras aremainly occurred in the southern and western parts of Yunnan (Fig. 2); the Sanzhangtian flora in southern Yunnan is well known by the earliest bamboo fossil record in China (Wang et al., 2013). Additionally, this flora bears the southern most fossil record of *Metasequoia* worldwide (Wang et al., 2019). The late Miocene floras are represented by plants from the Xiaolongtan Formation,w hich are dominated by Fagaceae, Fabaceae and Lauraceae (WGCPC, 1978), most of which are evergreen elements in modern forests.

The Pliocene floras are mainly derived from the Sangying Formation, which is a widely distributed in western and northwestern Yunnan sediments (Ge and Li, 199). In the Sanying Formation, evergreen sclerophyllous oak (*Quercus* section *Heterobalanus*) leaves

are the most abundant ones, suggesting that evergreen sclerophyllous broadleaved forests were dominant in these areas at that time (Tao, 2000). Many taxa including ferns, conifers, and angiosperms have been reported from different sites, indicating a high plant diversity at that time (Huang et al., 2016). Parts of fossil materials used in this study were from the Sanying Formation in Yongping County, western Yunnan Province.

1.3 Paleobotanical studies in Southeast Asia

Southeast Asia is a global biodiversity hotspot with a high plant diversity, containing of around 50,000 flowering plant species (Myers et al., 2000; Corlett and Primack, 2011). Even though some new species are discovered every year in this region, our understanding of the high biodiversity is still far from complete, and species in many plant groups and their distributions are still unresolved (Bebber et al., 2010). In Southeast Asia, the living floras are composed of mixed sources, including plants from Australia, India, and Eurasia (South China, Indochina, Malay Archipelago) (Kooyman et al., 2019). This makes it important to trace the evolution and biogeography of plants in Southeast Asia based on fossil evidence.

In Southeast Asia, paleobotanical investigations have been carried out in areas such as the Hindustan Peninsula (Lakhanpal et al., 1984; Bande and Prakash, 1986). However, in biodiversity hotspots such as Indo-Burma, Sundaland, Wallacea, and the Philippines, fossil localities are still not sufficient (Ian Metcalfe et al., 1984; Buffetaut et al., 2009; Wei et al., 2019). On the one hand, there are only few local paleobotanists studying fossil floras in Southeast Asia, and international cooperations for paleobotanical studies in this region are still rare; on the other hand, most parts of Southeast Asia are covered by very forests with few deposit exposures, which make the exploration of fossil floras in this region much more difficult than in many other regions around the world. However, recent progress on the investigation of ambers in Myanmar sheds light on paleobotanical studies in this area and provides pivotal solid evidence for the evolution of plants. Many fossil plant species have been reported from these Cretaceous ambers in Myanmar (Heinrichs et al., 2014; Schneider et al., 2016; Poinar, 2017), suggesting that more fossil sites with different geological ages are expected to be discovered in the future.

1.4 Modern plant vegetation in study areas

Yunnan is famous for its high plant diversity, with more than 16,000 species of vascular plants, grouped into around 3,000 genera and; 433 families. Among these species, half are endemic (Wu, 1988). Western Yunnan is surrounded by the Tibet-Himalaya region, which has long been recognized for its high plant diversity. Most of the research studies in this region are based on these areas (such as botany, paleobotany, molecular phylogenetics and environmental biology). Previous paleoclimatic studies have pointed out that the climate during the Neogene in; Western Yunnan was warmer and wetter than today. From the late Miocene to the late Pliocene to the present, the floristic composition and plant forms have changed significantly, probably in response to changes in elevation and global coeval cooling. Due to the temperature declined, rainfall is low while the monsoon intensified plant diversity: from this aspect, some plant taxa disappeared while others plant flourished. Plant diversity changes may be investigated via topographic deformations. Yunnan is area with highly complex topographic-conditions, showing large elevations and climate gradients (Wu, 1988).

It extends to the Tibetan-Himalayan region and is located in the conjunctional region between the Eurasian and Indian plates, with cold and alpine climates in the north-west and warm and humid seasonal climates in the south (Wang, 2006). The regions contains all of ecosystem types found in China, such as tropical seasonal rainforests, evergreen broadleaved forests, and deciduous broadleaved forests, as well as warm and cold temperate coniferous forests and, dry and warm valley scrubs (Wu and Zhu, 1987). The province's southern and tropical southeastern areas have similar monsoonal environment and lowland tropical rainforest vegetation. The flora of both regions is dominated by tropical flora (78% in southern Yunnan and 68.83% in southeastern Yunnan), belonging to the Indo Malaysian flora on the northern margins of tropical Asia. Typical Asian families are well represented in the southeastern tropical Yunnan flora, while families typical of tropical Asia are well represented in the southern Yunnan flora. Additionally, in the southeastern region, here are 14 primarily East Asia families that are absent in southern Yunnan. Yunnan is known for its high plant diversity in south-western China, and to understand how this new botanical abundance has evolved, researching the past biodiversity in the geological era is important. A Yunnan had two different floristic areas in the Pliocene: a subalpine floral area in the northwest and two subtropical floristic regions in the southwest and eastern center. Gymnosperms and angiosperms were sparse, and the region was mostly recovered by tropical seasonal rain forest, evergreen broad-leaved forest, seasonal rain forest, and deciduous broad-leaved forest; the climate was warm and cold temperate condition. Its high plant diversity makes Yunnan as an excellent location for botanical studies. The region is also rich in fossil records mainly macrofossils, including plant leaves, wood, fruits, seeds, and flowers.

Yunnan fossil ferns consist of only nine species five families; Davalliaceae, Equisetaceae, Osumundaceae, and Polypodiaceae. Many fossil floras are known from Yunnan for the late Pliocene, including the Longmen formation. In the late Miocene, Yunnan had three floristic regions, a northern subtropical zone in the northeast, a subtropical area in the east, and a tropical area in the southwest. Two distinct floristic regions were in northwest; and another two subtropical floristic regions, respectively, in the southeast and in the east center. In the Neogene, Yunnan was cooler and more humid than today.

Northern Vietnam is currently divided into three parts: (1) the Tonkin Lowland around the lower Red River; (2) the coastal lowlands, and (3) the northwestern highlands. Most of the inhabitants live in the Tonkin lowland. Southern Vietnam is divided into two roughly equal parts: (1) the former province of Cochin China, occupied mostly by the Mekong Delta, and (2) southern Annam, a region with a volcanic plateau sand small coastal mountain. Vietnam's climate is tropical in the south and monsoonal in the north, with a hot, rainy season (May to September) and a warm, dry season (October to March). Humidity averages 84% percent throughout the year. Annual rainfall ranges from 1,200 to 3,000 millimeters, and annual temperatures vary between 5 and 37·C. Seasonal divisions are more clearly marked in the northern half than in the southern half of the country, where, except in some of the highlands, seasonal temperatures vary only by a few degrees and generally range between 21 and 28·C

Vietnam ranks 16th out of 25 countries with the richest biodiversity in the world. On the mainland, there are 15,986 plant species of which. More than 13,200 terrestrial plant species and around 10,000 animal species have been recorded in Vietnam; while over 3,000

aquatic species have been identified within its wetland areas. For southern Vietnam, 1,650 vascular species have been recorded (Hoang, 1960), while in Northern Vietnam, 5.035 plant species have been found. According to the Checklist of Plant Species of Vietnam, the country contains 11,083 vascular plant, including one species of Psilotophytes, two species of Equisetophytes, 691 species of polypodiophytes, 53 species of lycopodiophytes, 69 species of gymnosperms and 10,267 species of angiosperms (Nguyen et al., 2003). Northern Vietnam has a long history of paleoecological records based on its high biodiversity and its position at the boundary between the Indochina and Southern China microplates. The most common vegetation in Vietnam is tropical monsoon forest. The plant fossils area is situated in Yen Bai province; this region is formed by a combination of exposed ancient metamorphic basement rock and eroded marine sediments deposited in the late Paleozoic and the early Triassic. The northern area of Vietnam is characterized by tropical monsoon climate, with high temperatures and abundant precipitation, but rainfall peaks are later in the year than in the other parts of Southeast Asia, and the dry season is less extreme.

1.5 Importance of paleoclimatic implication

The study of past climates is important to understand the climate in the future. Nowadays, climate change is linked with low precipitation, melting glaciers, deforestation, declining biodiversity, increasing frequency of extreme weather event increased frequency of human diseases and a warming climate When our planet suffers an energy imbalance and gains or loses heat, global temperatures changes. Any external effect on the climate are referred to as external forces because by changing the planet's energy balance, they force the climate to change. The paleoclimate is important regarding past, contemporary, and future issues, and understanding past climates can help us to explain how current ecosystems have formed, as the climate typically controls the vegetation type in a particular area. Furthermore, paleoclimatology provides data that can be used to predict current and future climate change situations. Past climate reconstruction can facilitate projections about future climate changes and, consequently, predictions of future distribution of plants and animals. The relationship between vegetation cover and past climate changes indicates how the climate influences the vegetation cover.

2. Objectives of the project

This study focuses on plant fossils in southwestern China and northern Vietnam. Plant macrofossils of *Equisetum* (Equisetaceae) from the fossil areas have been classified as two new species and one species as *Equisetum pretense*, which are within the present species distribution. One pollen record of *Trapa* is reported from the late Pliocene in western Yunnan.

This study has the following objectives;

- To explore new fossil records during the Cenozoic based on plant macrofossils from these study sites;
- To describe the detailed morphology of these new fossil specimens;
- To discuss the diversity history of two selected genera within their modern distribution;
- To understand the ecological and biogeographic implications based on these new fossil records.

3. Three new fossil records of *Equisetum* (Equisetaceae) and *Trapa* pollen grains from the Neogene of southwestern China and northern Vietnam

3.1 Introduction

Equisetum (horsetail) has survived as the only genus in the Family Equisetaceae. In total 15 species of *Equisetum* have been reported worldwide (Crane, 1997). The genus is most closely related to ferns; it does not produce seeds but can reproduce sexually through the formation of spores. *Equisetum* produces an extensive underground rhizome system that can reach a depth of more than four feet. Its leaves are reduced to small scales at the joints, while the stems are perennial, he plant body consists of a long, horizontal, underground rhizome, from which numerous roots arise toward the lower side and many erect shoots toward these upper side. The rhizome is long, creeping and well-branched; it can be divided into nodes and internodes. The horsetail descends from a prehistoric plant which covered entire forests during the Palaeozoic era 400 million years ago. This plant is a reminder of prehistoric times, when plants did not yet possess the ability to flower and had a more primeval relationship to the mineral world, to water and to light. *Equisetum* species were recorded as living fossils since they are relics of the Carboniferous geological period (325 million years ago). The remains of their ancient ancestors have formed the vast deposits of coal throughout the world.

Equisetales (horsetails) have a long stratigraphical occurrence, extending from the Devonian (Taylor et al., 2009), which took place on Gondwanan landmasses from at least the Carboniferous onward (Gutiérrez, 1995; Morris, 1985). The group turned into various species via the overdue Palaeozoic, ncorporating some arborescent species. Rössler and Noll, 2002, 2006). However, progressively declined in diversity and abundance through the Mesozoic, when it was mainly represented by herbaceous taxa. These days, the group is represented by *Equisetum*, an almost cosmopolitan genus of approximately 15 extant species and numerous extinct species (Hauke, 1963, 1978). Indigenous *Equisetum* is significantly absent from New Zealand, Australia, Antarctica, and a few Pacific islands, despite the fact that species thrive in these regions as weeds (Clifford and Constantine, 1980; Brownsie and Perrie, 2015).

Pole and McLoughlin (2017) proposed that aggregates of sizeable environmental alternates (specifically drying and abrupt warming/cooling) and opposition from diversifying angiosperm that probably contributed to the extinction of *Equisetales* across New Zealand–Australia–Antarctic in the late Mesozoic and Paleogene. The youngest *Equisetaleas* record from Australia was from the Cenomanian to the Maastrichtian strata (see McLoughlin et al., 2010). Pole and McLoughlin (2017) recorded *Equisetum* fossils from two Miocene deposits in New Zealand indicating that the group survived the cease-Cretaceous mass extinction and was persevered in wetter components of this location until it become extinct due to Cenozoic warming and drying (in Australia) and cooling (in New Zealand).

Equisetum (Equisetaceae) belongs to the order Equisetales and; has a long evolutionary history. Calamitaceae, Tchernoviaceae, Gonduanostachyaceae and Equisetaceae, which can be dated back to the Devonian (Gu and Shi 1974; Wang et al., 2005), belong to the same order as Equisetaceae. Among those families, Equisetaceae is extant and could be traced back to the Carboniferous, with *Equisetites hemingwayii* from the early Pennsylvanian of Yorkshire, UK (Kidston, 1883). *Equisetum* is the only living genus among the family Equisetaceae, with 15 species distributed all around the world, with the exception of

Antarctica (Kenrick and Crane, 1997). The genus is characterized by its hollow aerial stems with distinct nodes and reduced leaves similar to its arborescent ancestors. Among the different species, *Equisetum laterale* is the oldest fossil species in *Equisetum* has and has been from the Middle Triassic of Australia (Gould, 1968). Even some *Equisetum*-like fossils from the Mesozoic have been identified as *Equisetum*. According to molecular data, divergence of *Equisetum* species occurred during the late Eocene (~40 Ma), with the main occurrence during the Neogene (Des Marais et al., 2003; Elgorriaga et al., 2018). In *Equisetum* fossils, tuberous rhizomes are the most commonly preserved organs from the Cenozoic strata, found in North America (Lesquereux, 1878; Bell, 1949; Becker, 1969; Skog and Dilcher, 1994), Europe (Watson and Batten, 1990; Denk et al., 2005), and Asia (Kon'no, 1962; Wu, 1999; Sun et al., 2001). In China, the fossil records are rich, but most fossils are limited to northern China, for example from the Lower Cretaceous Yixian Formation and the Fuxin Formation in Liaoning (Chen et al., 1988; Sun et al., 2001), the Lower Cretaceous Muling Formation of the Jixi Basin, Heilongjiang (Yang, 2003) and the Middle Eocene Hunchun Formation in Jilin (Guo, 2000). In southwestern China, only four fossil records of *Equisetum* have been found, i.e. *Equisetum* cf. *oppositum* from the Paleocene-Eocene of the Niubao Formation in Nima Basin, northern Tibet (Yang et al., 2016), *Equisetum oppositum* from the Lower Oligocene Lawula Formation in eastern Tibet (Ma et al., 2012), *Equisetum* cf. *pratense* from the Lower Oligocene in the Lühe coal-mine in south-central Yunnan (Zhang et al., 2007) and *Equisetum* sp. from the Middle Miocene Wulong Formation in southern Tibet (Geng and Tao, 1982).

In Asia, some *Equisetum* fossils have survived and can still be found today, such as *Equisetum diffusum*, *Equisetum hyemale* and *Equisetum pratens.*

*Trap*a fossil sediments are collected from the Sanying Formation, Sanying Formation is the location of *Equisetum yongpingense* A.T.Aung, T.Su & Z.K. Zhou, sp.nov. collected area. *Trapa* (the family Lythraceae) is a genus of floating aquatic plants with a wide distribution around the world, containing about 10 living species. In this genus, the intraspecies classification is still unresolved. Even Lythraceae has a long fossil history since the late Cretaceous (Taylor et al., 2008), plenty of *Trapa* fossil records have been reported from the Cenozoic, many of them from Europe, North America and Asia (Wo´jcicki et al., 1999).

Fossil *Trapa* pollen grains are morphologically similar to modern *Trapa* pollen grainsThey have the same paleoenvironmental implications than the studied *Equisetum* fossils. Fossil pollen grains from sediments are relatively abundant compare to macrofossils, making them important pieces of evidence when studying the ; evolution of plant groups. Palynological studies along a section could trace the changes in both vegetation and climate if the chronology of the sediment is well constrained (Mosbrugger and Utescher, 1997). In this study, well-preserved pollen grains of *Trapa* are reported and described, adding new data of fossil records for *Trapa* and imporving our understanding of the diversity history of *Trapa* in southwestern China.

3.2 Materials and Methods

For the first study, all *Equisetum* species (subtropical and temperate species) were collected from sediment formations. According to these studies, three fossil species of

Equisetum (Equisetaceae) were reported from the Neogene of Southwestern China and Northern Vietnam based on well-preserved rhizomes with tubers.

3.2.1 Morphological techniques

Equisetum fossils were imaged to view the gross morphology, using a virtual digital camera (Nikon D 700) with a Kaiser 5510 stand and oblique light.

To observe the morphological characteristics in detail, fossils were photographed via stereoscope microscopes (Leica A8AP0) and ZEISS clever zoom 5);-adjustments were done using.

Adobe Photoshop (model CC 2018)

Morphological characteristics measured by Image J (model 1.52)

For comparison with formerly reported fossil taxa, we checked fossil statistics from on-line databases such as Web of Science and Google Scholar.

All fossil specimens used in this study were deposited in the paleoecology collections, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, and Vietnam National Museum of Nature, Vietnam Academy of Science and Technology.

Three fossil species of Equisetum are from the Neogene of southwestern China and northern Vietnam.

3.3 Fossil areas of three *Equisetum* fossil species (Equisetaceae)

Equisetum fossils were collected from the following three different areas.

- Dajie Formation, Sanzhangtian Village, Zhenyuan County (middle Miocene);
- Yen Bai, northern Vietnam (late Miocene);
- Yongping, southwestern China (late Pliocene).

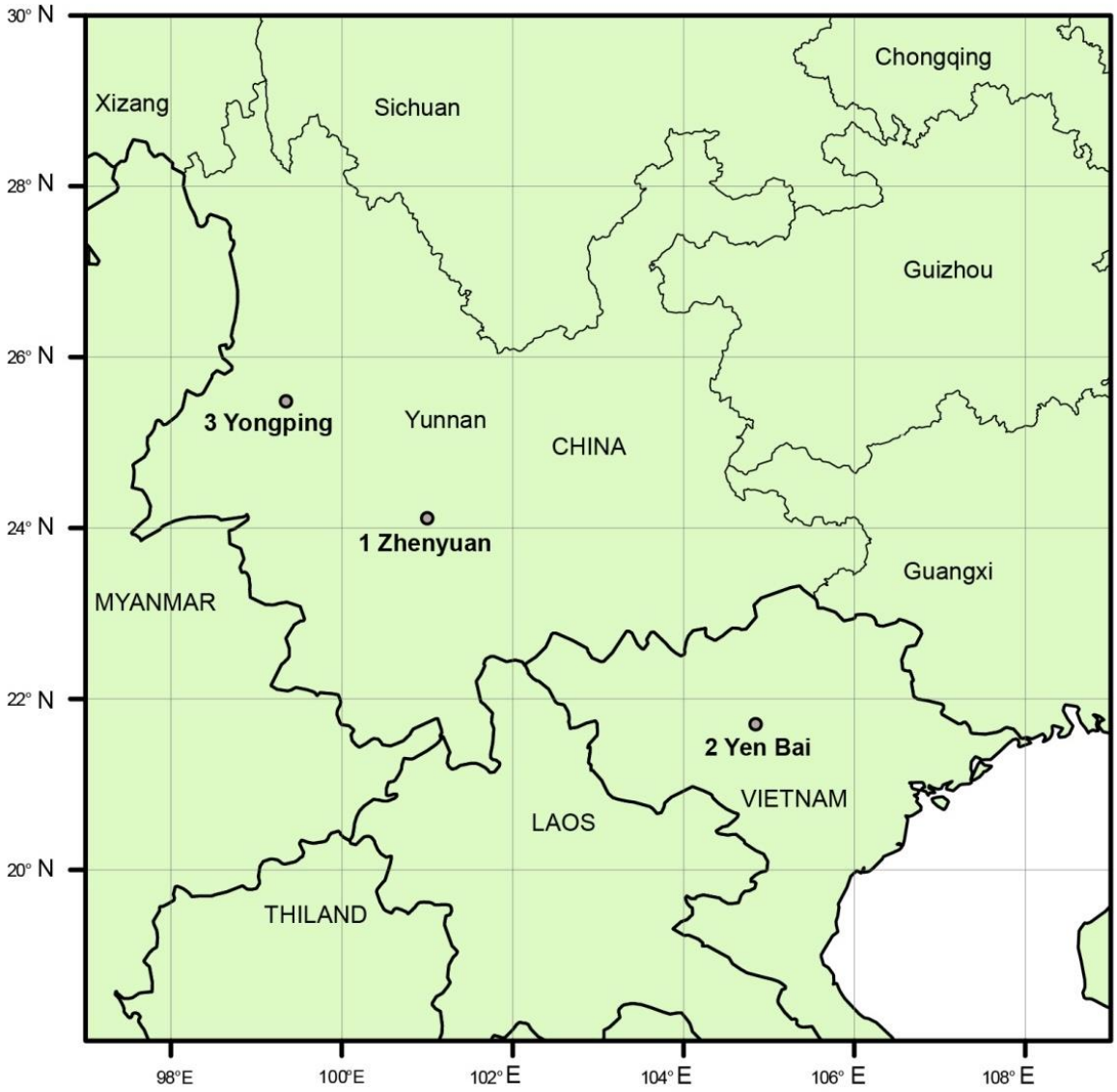

Figure 3.1 Map showing the locations of the fossils collected in this study. 1. Sanzhangtian, Zhenyuan County, Yunnan, southwestern China (middle Miocene); 2. Hop Thanh Village, Tuy Loc Commune, Yen Bai Province, northern Vietnam (late Miocene); 3. Longmen, Yongping County, Yunnan Province, southwestern China (late Pliocene).

3.3.1 Dajie Formation, Sanzhangtian Village, Zhenyuan County

This formation is situated in central Yunnan Province, southwestern China. It contains numerous well-distributed fossils from the middle Miocene in age, according to the lithological and palynological comparisons (Bureau of Geology and Mineral Resources of Yunnan Province (BGMRYP), 1990). Most of these fossils were collected from light-yellow mudstone of the upper layer in the stratum. In this area, many plant fossils remained including*Palaeosorum ellipticum* (Jacques et al., 2013), *Bambusium angustifolia*, *B. latifolia*, *Bambusiculmus angustus*, *B. latus* (Wang et al., 2013), *Celastrus caducidentatus* (Liang et al., 2016a), *Populus zhenyuanensis* (Liang et al., 2016b), *Cladium zhenyuanensis* (Liang et al.,

2017), *Zygogynum poratus* (Liang et al., 2018), and *Metasequoia cf. glyptostroboides* (Wang et al., 2019).

3.3.2 Yen Bai, northern Vietnam (the late Miocene)

Equisetum fossils were found in the Co Phuc Formation, Hop Thanh Village, Tuy Loc Commune, Yen Bai Province, northern Vietnam. The fossil site is located in the Yen Bai Basin along the Red River Fault Zone, which is a main Cenozoic strike-slip zone in Southeast Asia. The Co Phuc Formation dates back to the late Miocene and are composed of siltstone and fine sandstone (Wysocka and Świerczewska, 2010). The fossils were collected from the layers that are included and from the yellow siltstone layers. Plant fossils such as Polypodiaceae, Palmae, and Lauraceae have already been reported from this site (Zeiller, 1903; Colani, 1920).

3.3.3 Yongping, southwestern China (late Pliocene)

Fossils were collected from the Sanying Formation, Longmen Village, Yongping County, Yunnan Province, southwestern China. According to a recent paleomagnetic study, the Sanying Formation was dates back to the late Miocene/ early Pleistocene (Li et al., 2013). This formation ranges from the western to the north-western Yunnan Province (BGMRYP, 1990). Fossils were collected from the middle part of the stratum, which is covered by siltstone layers and is dominated by *Quercus Heterobalanus* (evergreen sclerophyllous oak) (Su, 2010). *Drynaria callispora* (Su et al., 2011) and *Cedrus angusta* (Su et al., 2013b) have also been reported for this area. Based on the reconstruction of leaf assemblages, during the late Pliocene, the climate in western Yunnan was warmer and more humid (Su et al., 2013a).

3.4 Results

Order Equisetales Dumortier
Family Equisetaceae A. Michaux ex Alph. De Candolle
Genus *Equisetum Linnaeus*

Equisetum fossils from Zhenyuan, southwestern China
Equisetum cf. *pratense* Ehrhart
Species checked: XTBGSZTF0001
Locality: Dajie Formation, Sanzhangtian Village, Zheyuan County, central_ Yunnan Province, southwestern China (24.100N, 101,216E).
Age: Middle Miocene.
Repository: Paleoecology Collections, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

3.4.1 Description

Rhizomes have both internodes and nodes (Fig. 2D). The internode is ~0.2–0.3 cm in width, the length could not be observed; three longitudinal ridges are on the surface of the internode (Fig. 2D). The node is round and ~0.2 cm in diameter (Fig. 2D). Four bunches of tubers are attached to one node and arranged in a whorl (Fig. 2D). Only one tuber is preserved on each bunch, but it is likely that there are more than one tuber on each bunch

(Fig. 2E–F). Most tubers are elongate (Fig. 2D); few are elliptical (Fig. 2F), being~ 0.8 to 3.0 cm long and 0.4 to 1.0 cm wide. Two to four ridges on the surface of each tuber (Fig. 2D). The tip of the tuber is mucronate (Fig. 2F).

Equisetum fossils from Yen Bai, northern Vietnam

Equisetum yenbaiense A.T. Aung, T. Su, T.V. Do & Z.K. Zhou, sp. nov.

Holotype: XTBGVNMN4001 (Fig. 2D).

Paratypes: XTBGVNMN4002-4004 (Fig. 2C, E–F).

Locality. Hop Thanh Village, Tuy Loc Commune, Yen Bai Province, northern Vietnam (21.725N, 104.849E).

Age: Late Miocene.

Repository: Paleoecology Collections, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences and Vietnam National Museum of Nature, Vietnam Academy of Science and Technology.

Etymology: The species name '*yenbaiense*' means that fossils are from Yen Bai Province, northern Vietnam.

Diagnosis: Rhizomes with internodes and nodes, node round; four bunches of tubers arranged in a whorl on a node (Fig. 2D); most tubers elongate in shape, with one to two tubers in each bunch (Fig. 2C–D); longitudinal ridges on the surface of the tuber; the tip of the tuber is mucronate (Fig. 2F).

Description: Rhizomes have both internodes and nodes (Fig. 2D). The internode is ~0.2–0.3 cm in width, the length could not be observed; three longitudinal ridges are on the surface of the internode (Fig. 2D). The node is round and ~0.2 cm in diameter (Fig. 2D). Four bunches of tubers are attached to one node and arranged in a whorl (Fig. 2D). Only one tuber is preserved on each bunch, but it is likely that there are more than one tuber on each bunch (Fig. 2E–F). Most tubers are elongate (Fig. 2D); few are elliptical (Fig. 2F), being~ 0.8 to 3.0 cm long and 0.4 to 1.0 cm wide. Two to four ridges are on the surface of each tuber (Fig. 2D). The tip of the tuber is mucronate (Fig. 2F).

Equisetum fossils from Yongping, southwestern China

Equisetum yongpingense A.T.Aung, T.Su & Z.K. Zhou, sp.nov.

Holotype. XTBGYP 0748

Paratypes: _XTBGYP 0747, XTBGYP 1014, XTBGYP 0750, XTBGYP 049

Locality: _Sanying Formation, Longmen Village, Yongping County, Western Yunnan Province, southwestern China.

Age: _Late Pliocene

Repository:_Paleoecology Collections, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

Etymology: _The species name "*yongpingense*" means that fossils are from Yongping County, southwestern China.

Diagnosis: _ Rhizomes with internodes and round nodes; fibrous roots on most nodes; two to four bunches of tubers arranged in a whorl on a node (Fig. 3A); tubers cylindrical in shape, one to two tubers on each bunch (Fig. 3A–B); longitudinal ridges not observed on the surface of the tuber; the tip of the tuber is mucronate (Fig. 3A)

Description; Rhizomes have both internodes and nodes (Fig. 3A). The internode is ~0.5 to 0.8 cm wide; the length is up to 5.1 cm (Fig. 3A). The node is round and ~0.7 to 1.0 cm in

diameter (Fig. 3A–B). Most nodes have fibrous roots ~ 0.1 to 0.2cm wide. Two to six bunches of tubers are attached on one node and arranged in a whorl (Fig. 3A–B). One to two tubers are preserved on each bunch (Fig. 3A–B). Tubers are cylindrical, ~ 1.5 to 3.4 cm long and 0.6 to 1.2 cm wide (Fig. 3A–F). No ridges on the tuber surface (Fig. 3A–B, E). The tip of the tuber is mucronate (Fig. 3A).

3.5 Discussion

Here, we reported three new fossil records of *Equisetum* from southwestern China and northern Vietnam, based on well-preserved rhizomes with tubers. In *Equisetum*, tubers are the most commonly preserved organs in the fossil records. They are mainly characterized by either a single tuber or one bunch of tubers with longitudinal ridges on the surface, which were present on the fossils we collected (Figs. 2–4). These three new fossil records vary in morphological characteristics, such as tuber shape and size (Table 1). They are also different from living species of *Equisetum*, for example; *Equisetum pratense*, as they have lager tubers (Zhang et al., 2007; Sun et al., 2013). *Equisetum* cf. *pratense* from the middle Miocene of Zhenyuan, southwestern China, has an ovate tuber shape, similar to most fossil records of *Equisetum*, but different from some fossil species; for example, the tubers of *Equisetites longevaginatus* are elliptical or round in shape (Table 1). The tubers in *E. cf. pratense* are smaller than those of other fossil species, such as *Equisetum oppositum*, *Equisetum* cf. *oppositum*, and *Equisetum hunchunense* (Table 1). Generally, all observed morphological characteristicss of the fossil, such as tuber size and shape, as well as the mucronate tuber tip, were in accordance with *E.* cf. *pratense*, a fossil species previously reported from the early Oligocene inthe Lühe coal-mine, south-central Yunnan (Zhang et al., 2007). However, we only found one bunch of tubers at the fossil site whereas the fossils from the Lühe coal-mine have one to four bunches of tubers with an acervate arrangement of rhizome nodes. More fossils are needed to further determine the arrangement of tubers to better understand the systematic relationship between these two fossil records. Judging by the high morphological similarity, we assign the fossil from Zhenyuan as to *E.* cf. *pratense*.

In *Equisetum yenbaiense* from the late Miocene of northern Vietnam, the tubers are arranged in a whorl on a rhizome node, which also occurs in *E. hunchunense* and *E.* cf. *arcticum*. However, both *E. hunchunense* and *E.* cf. *arcticum* have two to three tubers per bunch, whereas *E. yenbaiense* only has one or two tubers per bunch, which might be partly due to the preservation of the fossil. In addition, there are more than three tubers per bunch in *E. oppositum* and *E.* cf. *oppositum* (Table 1). Tuber shape in *E. yenbaiense* is elongate, and the ratio between length and width is higher than in most other fossil species, except for *E.* cf. *arcticum*. We noted that the tuber size in *E. yenbaiense* is larger than that in *E.* cf. *arcticum* (Table 1). Therefore, we designate the new fossil specimens from northern Vietnam as a new species, namely *E. yenbaiense* A.T. Aung, T. Su, T.V. Do & Z.K. Zhou, sp. nov. In *Equisetum yongpingense*, there are two to four bunches of tubers arranged in a whorl on a node, which has the same pattern as in *E. hunchunense* and *E.* cf. *arcticum*. The tip of the tubers is mucronate in *E. yongpingense*, which is also the case for other previously reported fossil species (Table 1). However, the tuber shape of *E. yongpingense* is cylindrical, which is different from that of other fossil records. Ridges on the surface of tubers are not prominent in *E. yongpingense*, whereas ridges are present in other fossil species.

Table 3.1 Morphological comparisons between the new fossil records in this study and previously reported *Equisetum* and *Equisetites* fossil species

Species	Tuber arrangement	Number of tubers per bunch	Shape of tuber	Tuber length	Tuber Width	Age	Locality	References
Equisetum sp.	?	3	Elliptical	1.0-1.2	0.6-0.8	Miocene	Iceland	Denk et al., 2005
Equisetum cf. *arcticum*	Whorl	2-3	Elongate, ovate, Round	1.5-2.5	0.5-0.7	Oligocene to Miocene	America	Backer, 1969
Equisetum hunchunense	Whorl	2-3	Elliptical, ovate	1.5	0.4-0.9	Eocene	China	Guo, 2000
Equisetum jiuquanense	Single to acervate	2-3	Elliptical, ovate, round	0.3-0.8	0.4-0.7	Early Cretaceous	China	Sun et al., 2013
Equisetites longevaginatus	Single or opposite	2-3	Elliptical, round	0.5-0.8	0.3-0.5	Cretaceous	China	Sun et al., 2001
Equisetum oppositum	Opposite	6	Round, elliptical, Ovate	1.0-1.6	0.8-1.2	Early Oligocene	China	Ma et al., 2012
Equisetum cf. *oppositum*	Single	5-6	Elliptical, ovate and nearly round	1.0-1.6	0.8-1.2	Paleocene-Eocene	China	Yang et al., 2016
Equisetum cf. *pretense*	?	3	Ovate	0.9-1.2	0.6-0.8	Middle Miocene	China	This study
Equisetites vittatus	Whorl	1-2	Elliptical, elongate	0.8-3.0	0.4-1.0	Late Miocene	Vietnam	This study
Equisetum yongpingense	Whorl	1-2	Cylindrical	1.5-3.4	0.6-1.2	Late Pliocene	China	This study

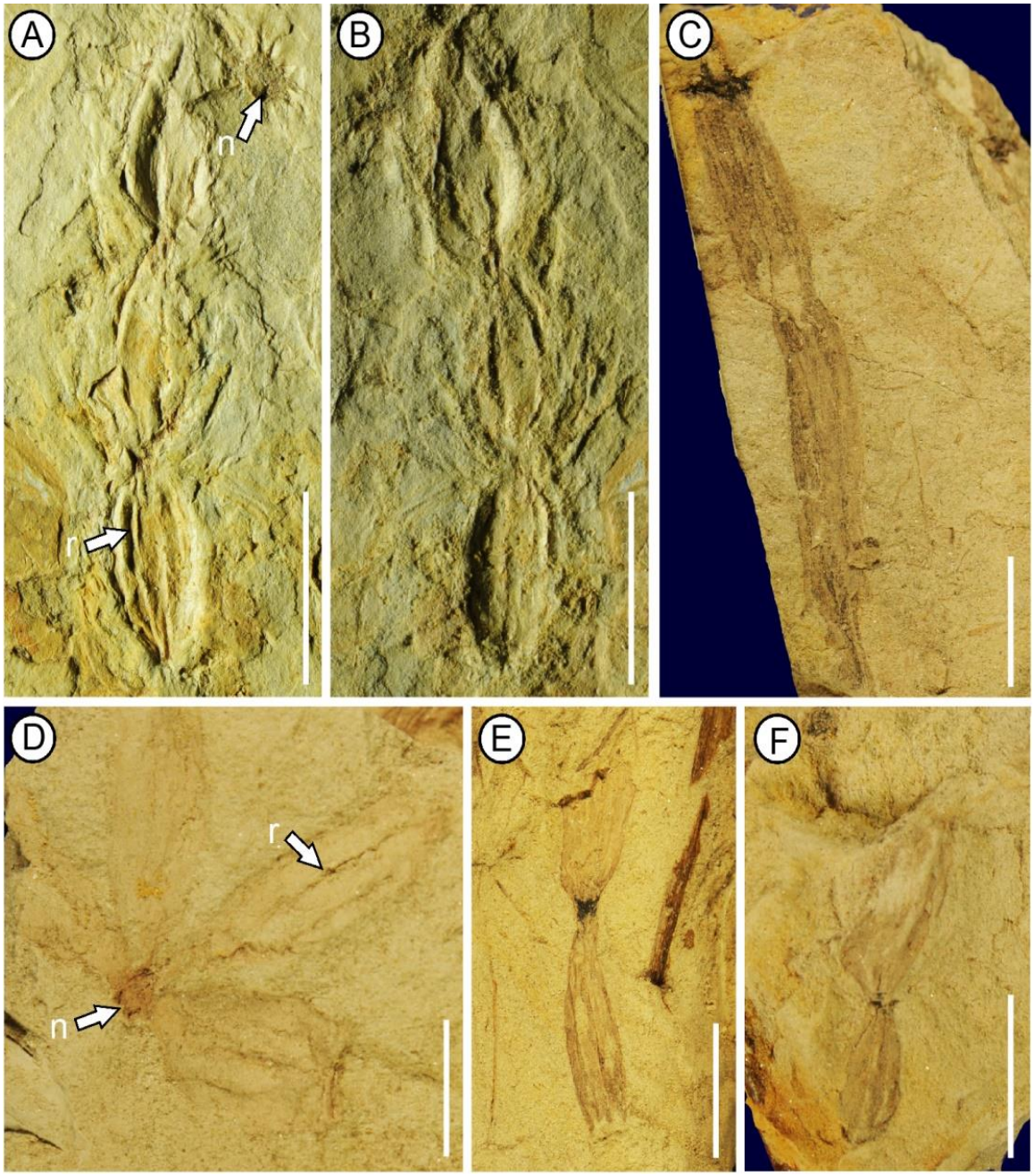

Figure 3.2 A–B *Equisetum* cf. *pratense* Ehrhart, C–F *Equisetum yenbaiense* A.T. Aung, T. Su, T.V. Do & Z.K. Zhou, sp. nov. Specimen numbers: A–B XTBGSZTF0001 (counterparts); C XTBGVNMN4002; D XTBGVNMN4001; E XTBGVNMN4003; F XTBGVNMN4004. n = node; r = ridge. Scale bars: 1cm.

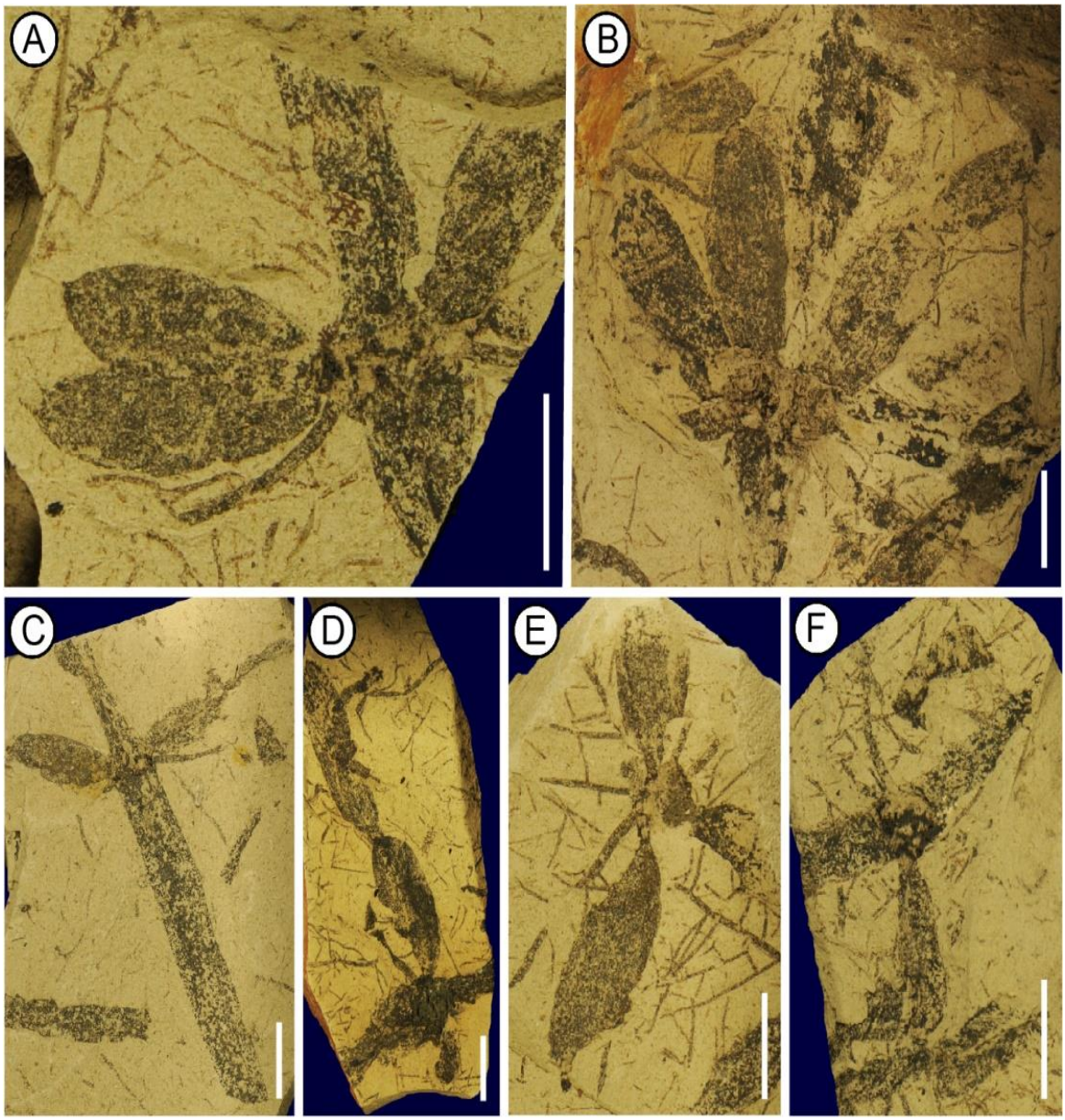

Figure 3.3 *Equisetum yongpingense* A.T. Aung, T. Su & Z.K. Zhou, sp. nov. Specimen numbers: A. XTB- GYP0748; B. XTBGYP0747; C. XTBGYP1014; D. XTBGYP1015; E XTBGYP0750; F XTBGYP0749. Scale bars: 1cm.

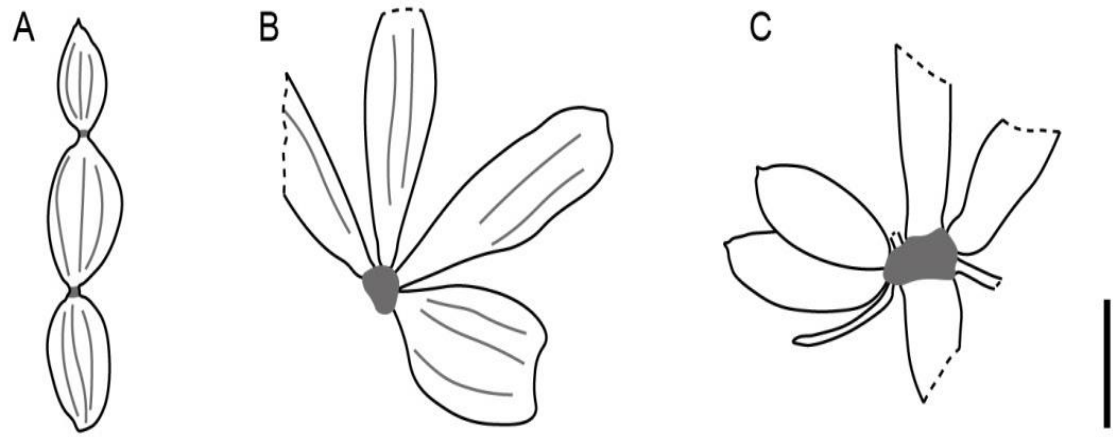

Figure 3.4 Reconstruction of *Equisetum* from: A. Zhenyuan, southwestern China, specimen number XTBGSZTF0001; B. Yanbei, northern Vietnam, specimen number XTBGVNMN4001; and C. Yongping, southwestern China, specimen number XTBGYP0748. Scale bar: 1.5 cm.

In *E. yongpingense*, fibrous roots were also observed on the rhizome node, which was not observed for other fossil species. Therefore, we named these fossil specimens from Yongping *E. yongpingense* A.T. Aung, T. Su & Z.K. Zhou, sp. nov**.**

The three new fossil records described in this study expand the distribution of *Equisetum* during the Neogene in Asia. These findings and previous fossil records indicate that *Equisetum* has been widely distributed in Asia since the Paleocene and has become diverse in Asia since the Oligocene. According to fossil assemblages, *Equisetum* mainly grew under wet conditions. Other taxa that were reported from the same stratum in Zhenyuan, such as *Palaeosorum ellipticum* (Jacques et al., 2013), *Bambusium angustifolia*, *B. latifolia*, *Bambusiculmus angustus*, *B. latus* (Wang et al., 2013), and *Metasequoia* cf. *glyptostroboides* (Wang et al., 2019), tend to grow near rivers or lakes.Therefore, we considered that *E.* cf. *pratense* might favor a similar habitat. In addition, *E. yongpingense* was found from the Upper Pliocene in western Yunnan, where numerous fruits from *Trapa*, an aquatic plant, were also found in the same layer (Su, 2010). In this sense, we assume that this pattern persisted throughout the Cenozoic. Tubers, storing starch indicate a perennial life form and contribute to the adaptation of *Equisetum* under seasonally unfavorable conditions (Taiz and Zeiger, 2010). Eventually, tubers became important elements in the local vegetation, which was evidenced by the abundance of specimens in many fossil sites (Becker, 1969; Guo, 2000; Ma et al., 2012; Yang et al., 2016).

The three species of *Equisetum* described here might have been distributed in wet and humid environments. We assume that they survived near water areas, which have favorable climatic conditions for these species.

Trapa pollen grains were investigated from the Upper Pliocene Sanying formation, Yonping, western Yunnan. *Trapa* usually grows in shallow water under wet and humid conditions. Since one species of *Equisitum* and one of*Trapa* are from the same sediment of the late Pliocene Sangying Formation in Yongping, we suggest that they prefer a similar climate.

3.6 *Trapa* pollen grains from the late Pliocene of Western Yunnan

Trapa fossil sediments were collected from the Sanying formation, Upper Pliocene in western Yunnan. As mentioned before, *E. yongpingense* was found in the Upper Pliocene in western Yunnan, where *Trapa* fossils are abundant (Su, 2010). Observation and description of the pollen grains from the Trapa fossil sediments that intended to contribute as the fourth fossil plant species of the whole study. Fossil *Trapa* pollen grains from the sediment may be used as a measure for past plant diversity, since fossil pollen from sediment might be a more accessible tool for the study of floral diversity. The fossil records of the family (Lythraceae) are represented by leaf impressions, pollen sediments, seeds and fruits.

The pollen grains in this study resemble those of *Trapa natans* commonly known as water chestnut, which is an annual plant introduced from Asia and has become abundant in the northeastern United States where it creates issues in lakes, ponds, canals, and other slow-moving water bodies. *Trapa natans* survives nearby water and is an annual, thermophilus plant; it appears in eutrophic water bodies with neutral to somewhat alkaline water (Zetter and Fergusson, 2001). The species is distributed globally-including Eastern, Middle, and Southern Europe and most parts of Asia (Hulten 1971) (Samuelesson, 1934). In the flowering period, the temperature may be beyond 20·C (Vankina, 1970; Mai, 1985; Tornadore et al., 2000). The fruits have a variety of size and shape (Velichkevich and Zastawniak, 2006).

Figure 3.6 *Trapa natans* L. from Xishuangbanna Tropical Botanical Garden.

3.7 Materials and Methods

3.7.1 Morphological observation

For fossil *Trapa* pollen grains, sediments yielded Trapaceae pollen were also analyzed. The ground sediment was removed to prevent contamination with later pollen grains. Subsequently the sediment was placed upside down in a mortar and lightly boiled in HF in a copper pan to remove silicates. After this the samples were transferred into large polyethylene beakers with water and left until the solids had settled. After decanting the liquid, the residue was boiled in conc. HCl in a glass beaker for 5 mins to avoid the formation of calcium fluoride. Subsequently, the sample was left to settle at the bottom of the beaker and any remaining HCl was decanted. The sample was then washed in distilled water and centrifuged three to four times prior to acetolysis (chlorination plus acetylation). At first, the fabric turned in a test tube, then ca. 1.five cm glacial acetic acid brought, accompanied via ca. 3 cm freshly prepared solution of saturated sodium chlorate, ensuring that those drinks did not blend. Subsequently three to four drops of conc. HCl were added and then the mixture was stirred with a glass rod. The test tube was then placed to a boiling water bath for 3 mins, followed by centrifugation at 2,000 rpm for 20 seconds; subsequently, the liquid fraction was removed. The sample was then washed and centrifuged three times. To be able to eliminate the water, the samples were washed in conc. acetic acid or acetic anhydride. A combination of 9 elements acetic anhydride and one element conc. H2SO4 were now delivered to the check tube (Erdtman, 1943). The take a look at tube changed into located in a heat water bath for 3–4 mins, and then the aggregate turned into centrifuged and the liquid fraction decanted. Finally, the residue was washed in acetic acid and water for three times. In some cases, the natural fraction needed to be separated from the fabric with the use zinc bromide. Glycerin was combined with the organic residue to form a suspension, using a pipette. A pipette a drop of this liquid was transferred to a glass slide the a usage of the specially adapted needle (see above) those grains which have been of specific hobby had been then brushed to the threshold of the glycerine, where in they can be positioned and transferred to another glass slide with a sparkling drop glycerine for photography below a light microscope. The rest of the method is identical to that used for pollen grains. Pollen grains which may be mentioned the Trapaceae are regularly encountered inside the path of palynological evaluation of Cenozoic sediments. Up to now two species had been described based totally on microscopic (LM) examination. Pollen grain slides are restored in the Paleoecology Research Laboratory, Xishuangbanna Tropical Botanical Garden,Academy of Sciences.

The modern pollen grains were collected from the flower garden of the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, in November; 2019. At the first step, *Trapa natans* is collected in order to take their flowering parts in the pond of flower garden, Xishuangbanna Tropical Botanical Garden for chemical processing. Pollen grains were taken from flowers, dried, and treated with acetic anhydride. Subsequently, they were placed on the slides and observed under microscope (Leica DM 750).

3.7.2 Brief morphological description of fossil *Trapa* species

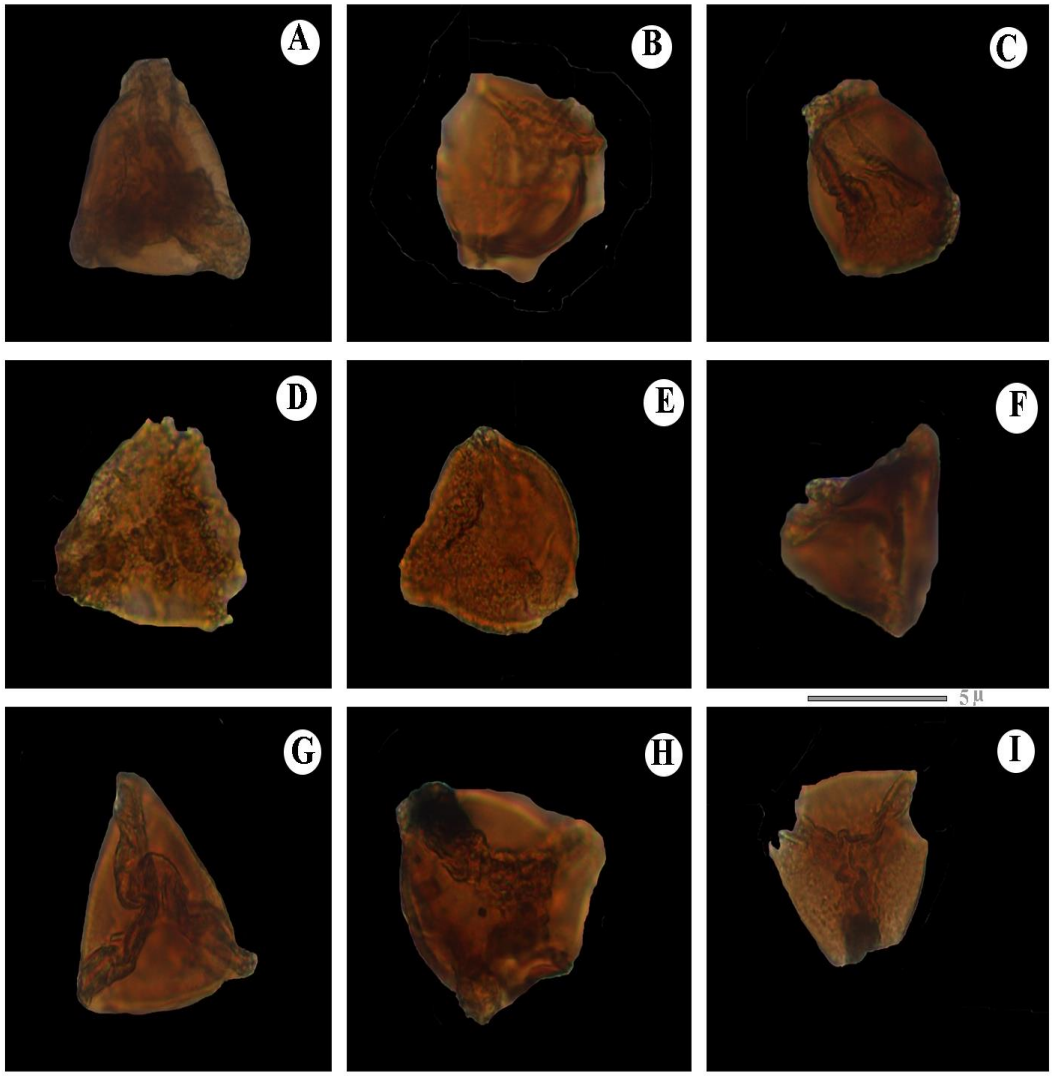

Figure 3.7.2 Different views of pollen grains in *Trapa* sp. under the microscope. Samples were from the late Pliocene of the Sanying Formation, Yongping County, western Yunnan Province, southwestern China.

Fossil pollen grains are quite distinct in shape and size. Pollen grain in Figure(1-A) is symmetric trichotomosulcate while other pollen grains (1-B, E) are more or less oblate asymmetric triangular. Asymmetric trich-otomosulcate grains may be exclusive within a sample. Some pollen grains are more or less spheroidal. The pollen grain is folded (1-C), but most of the pollen grains are exclusively symmetric trichotomosulcate (1-D, G, H, F, I).

3.7.3 Brief morphological description of modern species *Trapa natans*

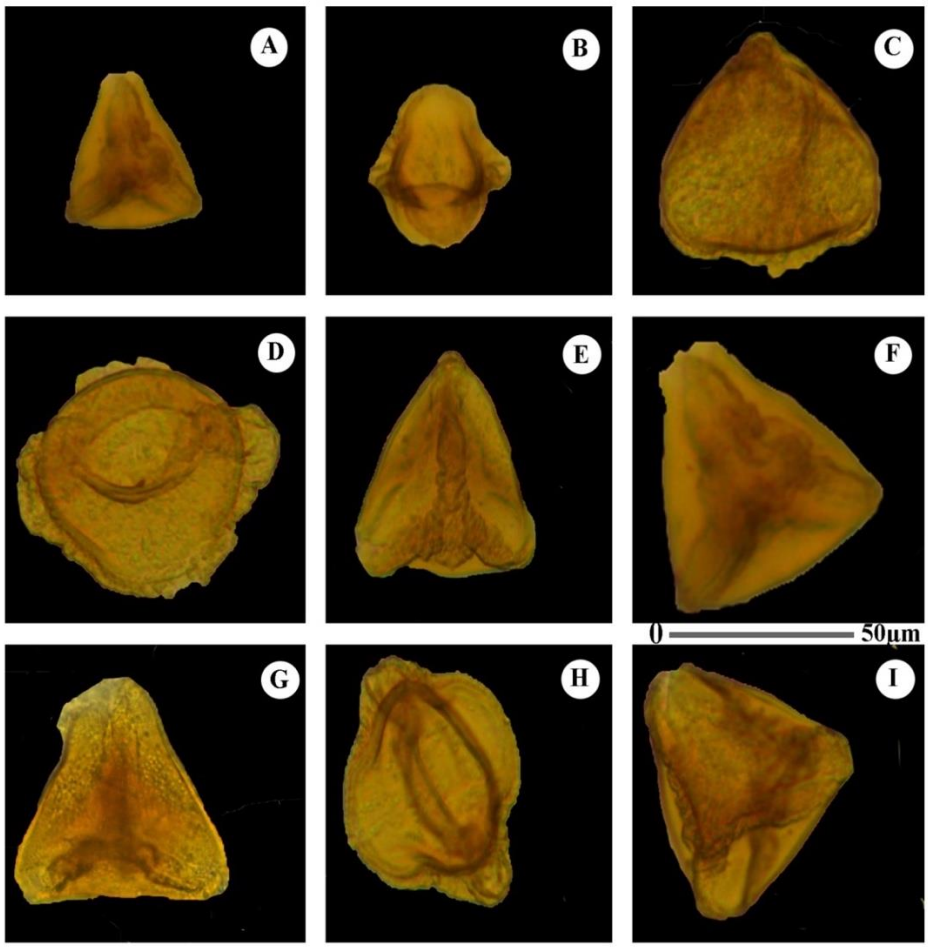

Figure 3.7.3 Different views on pollen grains of the modern species *Trapa natans* under the microscope (collected from Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences).

Modern pollen grains are asymmetric, three-armed sulcus (Fig. 2-A) and different from other pollen grains (Fig. 2-B). The shape is spheroidal. Most of the modern pollen grains have similar shape, exclusively symmetric tricholtomosulcate (Fig-E, F, G, I), while other pollen grains (Fig-B) are circular oblate. Similarly, another pollen grain (Fig-B) three circular pores positioned in the oblate spheroidal shape because pollen grain is folded or split under pressure. Some pollen grains are asymmetric triangular- but lacking three-armed sulcus (Fig-C).

3.8 Ecological implications of *Trapa*

Trapa usually lives in streams and ponds (Ridley, 1930). Mostly *Trapa* species are confined to shallow water, with adventitious roots. Before *Trapa* plants become free-floating, their submerged plant parts die back because of self-shading. For this reason macrofossils of *Trapa* seem to be either allochthonous or autochonous. Pollen grains of *Trapa natans* can be produced very early in the year; production is confined to places where fruits were occurred endorses autochthony. Thus, the occurrences of these pollen grains can favor the adaptation

to different environmental conditions. *Trapa natans* is an annual plant (hydro-therophyte), and winter temperature are not critical for its survival. However, flower and fruit formation require water temperature above 20·C (Zetter, 2001). According to the fossil records, *Trapa* was distributed across a wide latitudinal range by the end of the Pliocene.

4. Conclusions

In this study, three *Equisetum* macrofossil records and one *Trapa* pollen record are reported and described in detail, and their paleoenvironmental implications are discussed. The conclusions are as follows:

The three species *Equisetum yongpingense*, *Equisetum yenbaiense*, and *Equisetum* cf. *pretense* are from the Miocene and the Pliocene flora of southwestern of China and northern Vietnam. These three new fossil records differ in their morphological characteristics, such as tuber shape and size. Two new fossil species of *Equisetum*,namely *Equisetum yenbaiense* and *Equisetum yongpingense*, are proposed; another *Equisetum* species was assigned to *Equisetum* cf. *pretense*.

According to the previous fossil records and to our findings, *Equisetum* has been widely distributed in Asia since the Neogene. Fossil assemblages suggest that *Equisetum* lived under wet conditions. *Equisetum* cf. *pretense* was collected from the same strata as previously recorded species such as *Palaeosorum ellipticum* (Jacques et al., 2013), *Bambusium angustifolia*, *B. latifolia*, *Bambusiculmus angustus*, *B. latus* (Wang et al., 2013), and *Metasequoia* cf. *glyptostroboides* (Wang et al. 2019). These species can grow near lakes or streams in humid areas, which is further evidenced by *Equisetum* fossils in this study.

Numerous *Trapa* pollen grains were extracted from the late Pliocene in western Yunnan via the single pollen technology, showing a high morphological similarity to the living species *Trapa natans*, e.g., three distinctive crests and an obtuse outline. This suggests that *Trapa* existed in shallow water bodies in western Yunnan in the late Pliocene, and this genus still lives in this region. Previously, one fossil record of *Hemitrapa* was reported from the early Oligocene in Mangkang, the southeastern margin of the Qinghai-Tibetan Plateau, which shows close affinity to *Trapa* based on the morphology. The pollen grains differ from those of *Hemitrapa* by thesignificantly larger size of *Trapa* pollen grains from the late Pliocene from Yongping (Su et al., 2018). These pollen records indicate the long evolutionary history of the high plant diversity.

These new fossil records deepen our understanding of the information on the Cenozoic plant diversity in southwestern China and Southeast Asia. However, considering the high plant diversity of these regions, the fossil records are far from still far from being sufficient. More paleobotanical studies are urgently needed to better understand the evolution of plant diversity in response to dramatic environmental changes in these biodiversity hotspots.

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Publication

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