CHAPTER 1

Introduction

1.1 Principles of dental microwear

The objective of dental microwear study is to determine the type of food that the animal consumed. When food passes through the mouth, they do not go directly to the stomach. They must be masticated into small size before further digestion process. Food mastication occurs at the molar surfaces. The abrasion between food and molar causes the tiny marks on the dental surface called microwear (Semprebon *et al*., 2004).

Dental microwear is microscopic scar on mammal teeth created by food. The size and shape of those scars can be used to determine types of that food (Todd *et al*., 2007; Rivals *et al*., 2010; Ungar, 2010). On proboscidean (elephants and their ancestors) molars, longitudinal scars called scratch microwear are evidence of grass consumption. On the other hand, rounded scars called pit microwear are evidence of woody plant consumption (Vaughan *et al*., 2011). The percentage of scratch and pit microwear can be used to distinguish types of feeder in proboscideans. For those that prefer eating grass, a high number of scratch are found on their teeth and they are classified into grazers. In contrast, browsers are those that prefer eating woody plant and high pit percentage are found on their teeth. Proboscideans that consume both grass and woody plant, are classified into mixed-feeders based on the equal amounts of scratch and pit microwear (Rivals *et al*., 2012). Moreover, the study of dental microwear cannot only reveal types of food of proboscideans, but also reconstruct paleoecology they lived in the past (Novello *et al*., 2010; DeMiguel *et al*., 2011).

Microwear features recorded the characteristic of the food that the animal consumed a couple meals before they died. The old microwear features were rapidly replaced by the new ones throughout the day. The observed microwear results could only imply the type of food that was available at the time the animal died, not during their whole lifetime. This incidence was called “the last supper phenomenon”. So, the study of dental microwear can reveal types of food in the last few meals that the animal consumed before death (Grine, 1986).

Proboscidean fossils were mostly found in northern and northeastern parts of Thailand from Cenozoic basins. The most abundant fossil site is located at Tha Chang sand pits, Nakhon Ratchasima Province, northeastern Thailand. The age of these fossils range from Early Miocene to Late Pleistocene (23 Ma – 0.01 Ma) (Thasod, 2007). Much research has been done on the classification scheme for these fossils (e.g. Saegusa *et al*., 2005; Chavasseau *et al*., 2009; Thasod, 2007; Thasod *et al*., 2012). However, dental microwear have never been applied to proboscideans in Thailand before. By dental microwear analyses, more specific types of food of proboscidean in Thailand will be discovered and also more detailed dietary ecology in the past, instead of broad interpretation, big forest, proposed by Thasod *et al*. (2012).

Microwear is feature that cannot be directly observed by naked eye, they require microscopic techniques. This study uses low-magnification light stereomicroscopy for larger field of view. High-resolution scanning electron microscopy (SEM) uses for high resolution details.

1.1 Literature review of dental microwear

Teeth play an important role in acquisition and mastication of food in mammal. The four types of mammal teeth are incisors, canines, premolars and molars. Unlike other types of teeth that are usually specialized for various purposes, molars function only the mastication of food. Thus, they are the most useful for paleodiet interpretation. Physical properties of food have tremendous impact on dental microwear pattern, which do not depend on taxonomy but individual preferences (Ungar, 2010).

Dental microwear was first studied about half a century ago (Butler, 1952; Mills, 1955), and it was proved a reliable method for paleodiet and paleoecology reconstruction of the extinct and living mammals by Semprebon *et al*. (2004). Numerous researchers have witnessed the usefulness of dental microwear in mammals from the small one, e.g. Sciuridae (squirrels) (Nelson *et al*., 2005) to the largest one on land, Proboscidea (elephants) (Todd *et al*., 2007). Rivals *et al*. (2010) combined dental microwear and mesowear, which is the larger scale transformation of teeth, to explain paleoecology of the mammoth steppe fauna in North Sea and Alaska. Additionally, dental microwear was used to interpret eating behavior in ruminants (Novello *et al*., 2010; DeMiguel *el at*., 2011). For prehistoric human diet, Soltysiak (2011) successfully utilized dental microwear on human teeth. Ungar *et al*. (2012) specified types of feeders among tragulids in East Africa. Schulz *et al*. (2013) applied industrial 3-dimensions tribology to enamel wear in hoofed mammals. Furthermore, Fahlke *et al*. (2013) finally found the reason why the ancestors of today's largest mammals on earth, whales, abandoned land to live in the water by microwear analysis.

Phytolith (silica) in plant leaves which are harder than teeth enamel can create various microscopic features (Fig.1.1). By low-magnification stereomicroscopy, microwear features can be divided into two major types, the elongate scratch and the circular pit shapes. The length and width ratio of scratch microwear exceed 1:4. They can also be further categorized into fine, coarse and hypercoarse scratches based on the width of these scars. For pit microwear, the ratio of two axes is lower than 1:4. Different types of plants cause various size and shape of pit microwear, including small pit, large pit and puncture pit. Theoretically, scratch and pit microwear are correspond to grass and woody plant, respectively (Semprebon *et al*., 2004).

In Europe and North America, Rivals *et al*. (2012) studied dental microwear in *Mammuthus*, *Palaeoloxodon*, and *Mammut* by using low-magnification stereomicroscope. They examined the occlusal area of 0.4x0.4 mm^2 to count the number of micrower features. Furthermore, the collected data were compared to the Ungulate and Proboscidea microwear database to specify types of feeder among these proboscideans. From microwear analysis, they also successfully traced back the eating habits of *Palaeoloxodon antiquus* and *Mammuthus* lineage during Pleistocene epoch.

The results show that *Palaeoloxodon antiquus* adapted themselves from being mixed feeder to leaf browser. In contrast, *Mammuthus* changed eating behavior from leaf browser in *M. meridionalis*, to mixed feeder in *M. trogontherii* and finally to grazer in *M. primigenius*, whereas Capozza (2001) specified *M. meridionalis*, in Italy, into grazers.

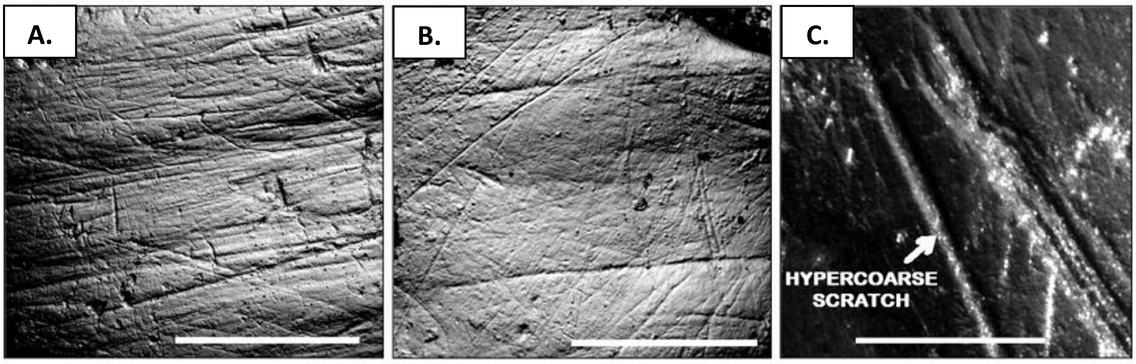


Fig. 1.1 Typical microwear features of proboscidean tooth enamel as observed by low-magnification stereomicroscope at 35 times magnification. A = *Mammuthus primigenius* from Fairbanks, Alaska (AMNH 651012-1952): B = *Mammuthus columbi* from South Carolina (AMNH 13708-0): C = *Mammut americanum* from Florida (UF 215059). Scale bar = 0.4 mm. (Rivals *et al*., 2012).

Todd *et al*. (2007) found that microwear features were not equally distributed over the dental area of *Loxodonta africana* and *Elephas maximus* from North America. Pit and scratch microwear tend to concentrated on the anterior and lingual sides of proboscidean molars. In addition, Rivals *et al*. (2012) recommended the central part of the occlusal surface for suitable area of microwear analysis because they were less interfered by taphonomic defects and less worn surface.

Green *et al*. (2005) classified *Mammut americanum* into leaf browser, as did Rivals *et al*. (2012), by using bivariate plot of average number of scratches and pits (Fig. 1.2).

For proboscideans, dental microwear studies were mostly limited to Pliocene – Pleistocene samples due to well-preserved samples in the glacial deposits (Capozza, 2001; Green *et al*., 2005; Palombo *et al*., 2005; Rivals *et al*., 2012). Two living species, *Elephas maximus* and *Loxodonta africana* are classified into mixed-feeders (Rivals *et al*., 2012).

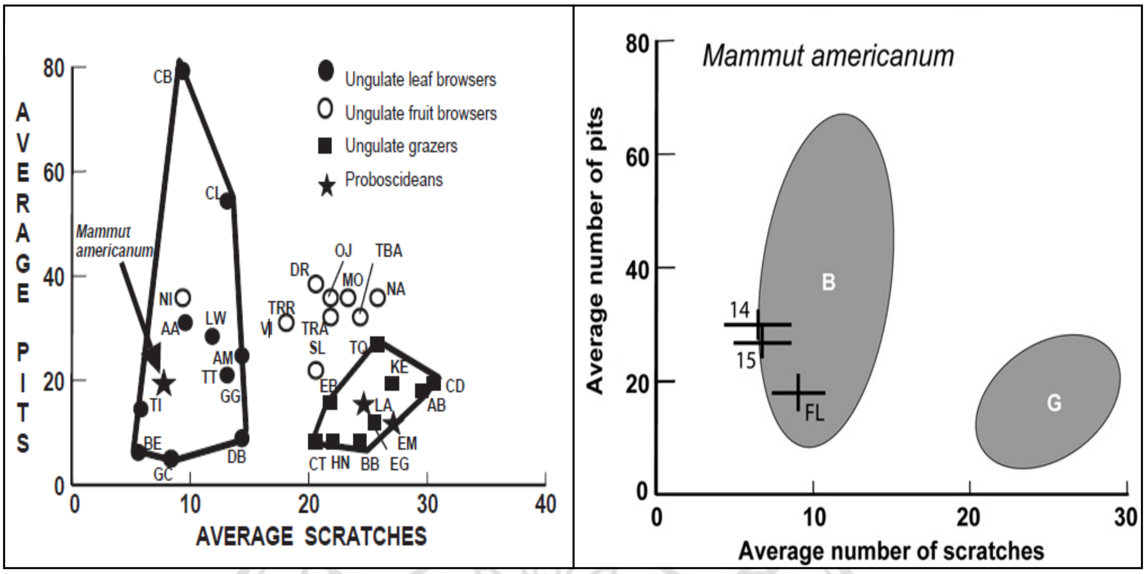


Fig. 1.2 Bivariate plot of the average number of pits versus average number of scratches for *Mammut americanum* based on Ungulata and Proboscidea database; left: convex hulls are drawn around extant leaf browsing taxa and extant grazing taxa (after Green *et al*., 2005), right: centroid are indicated for the extant browsers (B) and grazers (G) (after Rivals *et al*., 2012).

1.2 Proboscidea

Proboscidea is the order of elephants and their ancestors. At the present, there are only two living species including *Elephas maximus* and *Loxodonta africana*. In Thailand, there are four families including ten genera of proboscidean fossils. Most of them were discovered from Tha Chang sand pits, Nakhon Ratchasima Province and Cenozoic basins in northern Thailand (Thasod, 2007). The molars were most frequently found due to their robustness. On the other hand, tusks and bones were rarely found because they were generally destroyed during the transportation. One single molar is enough for the classification to the genus level.

1.3.1 Dental nomenclature

The arrangement of the cone, the thickness of the enamel and the size of the molars are all the contribution to the classification. Each species has its characteristics of molar structures. Proboscidean dental nomenclature was shown in Figs. 1.3 and 1.4. Median sulcus is the central line dividing left and right sides. The pretrite is the side which the food mastication between upper and lower molars take place. Thus, pretrite is generally more abraded than the posttrite (Thasod, 2007). It is important to note the exact position on molar for microwear examination because microwear features are not consistent all over dental surface. Microwear features tend to concentrate more on the pretrite side than the posttrite side.

1.3.2 Abbreviation of dental position

The abbreviation of four types of mammal teeth includes; I = incisor, C = canine, P = premolar and M = molar. For proboscidean, a molar composes of many lophs (for upper molar) and lophids (for lower molar). The lophs or lophids positions are specified by the number, and the upper and lower teeth are indicated by superscript and subscript those number, respectively. For example; M_3 = lower third molar, M^2 = upper second molar, and P_4 = lower fourth premolar.

1.4 Sedimentary basins of the fossil locality

There are more than 70 Cenozoic sedimentary basins in Thailand. Most of them were situated between the mountain ranges. The fossil localities are located in three Cenozoic basins of northern Thailand including Ban Na Sai, Mae Moh and Chiang Muan basins, and sand pits near the Mun River in northeastern Thailand.

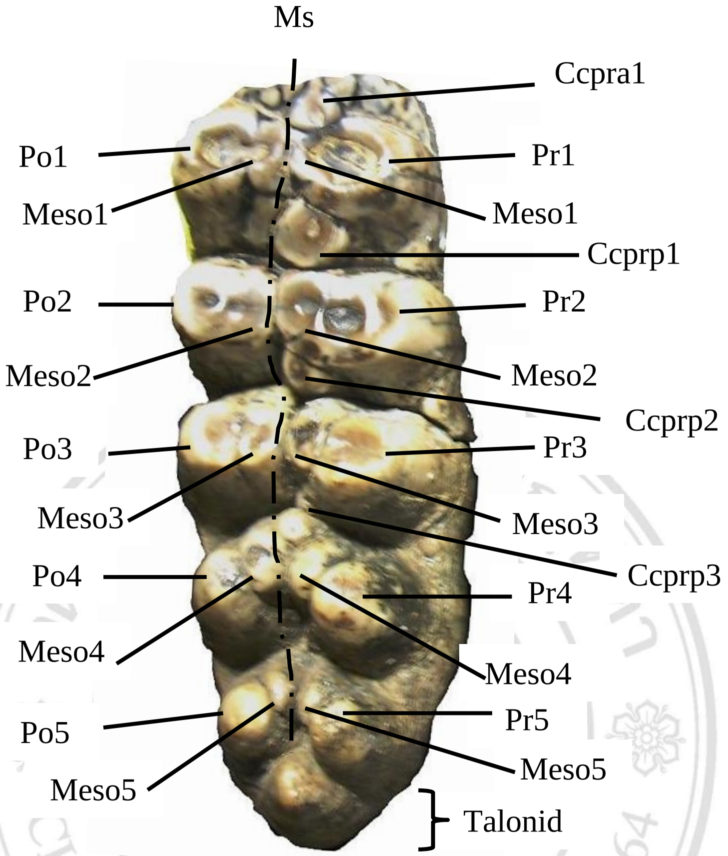


Fig. 1.3 Proboscidean dental nomenclature. Key: Po1, 2, 3, 4, 5, posttrite main cusp of 1st, 2nd, 3rd, 4th and 5th lophids; Pr1, 2, 3, 4, 5, pretrite main cusp of 1st, 2nd, 3rd, 4th and 5th lophids; Ccpra1, anterior pretrite central conule of 1st lophid; Ccprp1, 2, 3, posterior pretrite central conule of 1st, 2nd, 3rd lophids; Ms, median sulcus; Meso, mesoconelet of each half-lophid. (The dental nomenclature after Tassy, 1996)

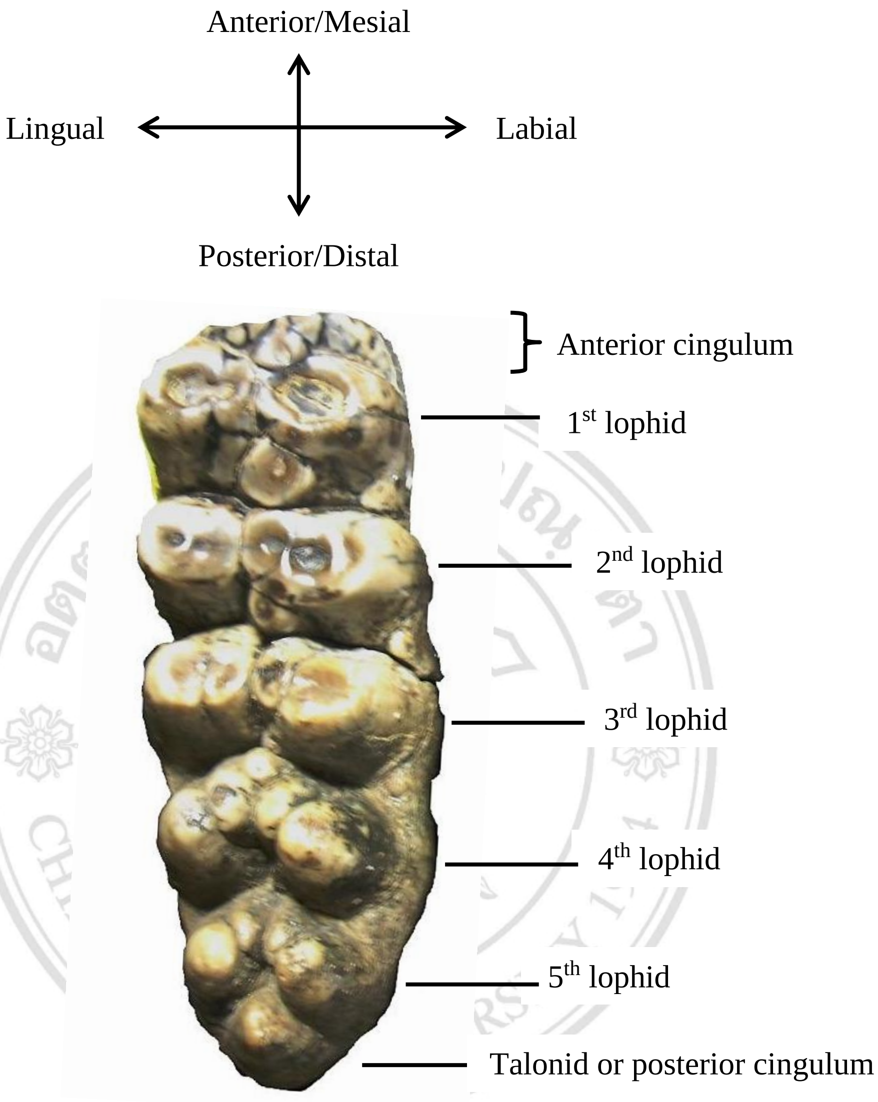


Fig. 1.4 Anatomical orientation and number of molar lophid of the right lower third molar, M_3, of *Stegolophodon nasaiensis* having X5X tooth formula. X in the front and the back of the number refer to the anterior cingulum and posterior cingulum, or talonid, respectively.

1.4.1 Ban Na Sai Subbasin

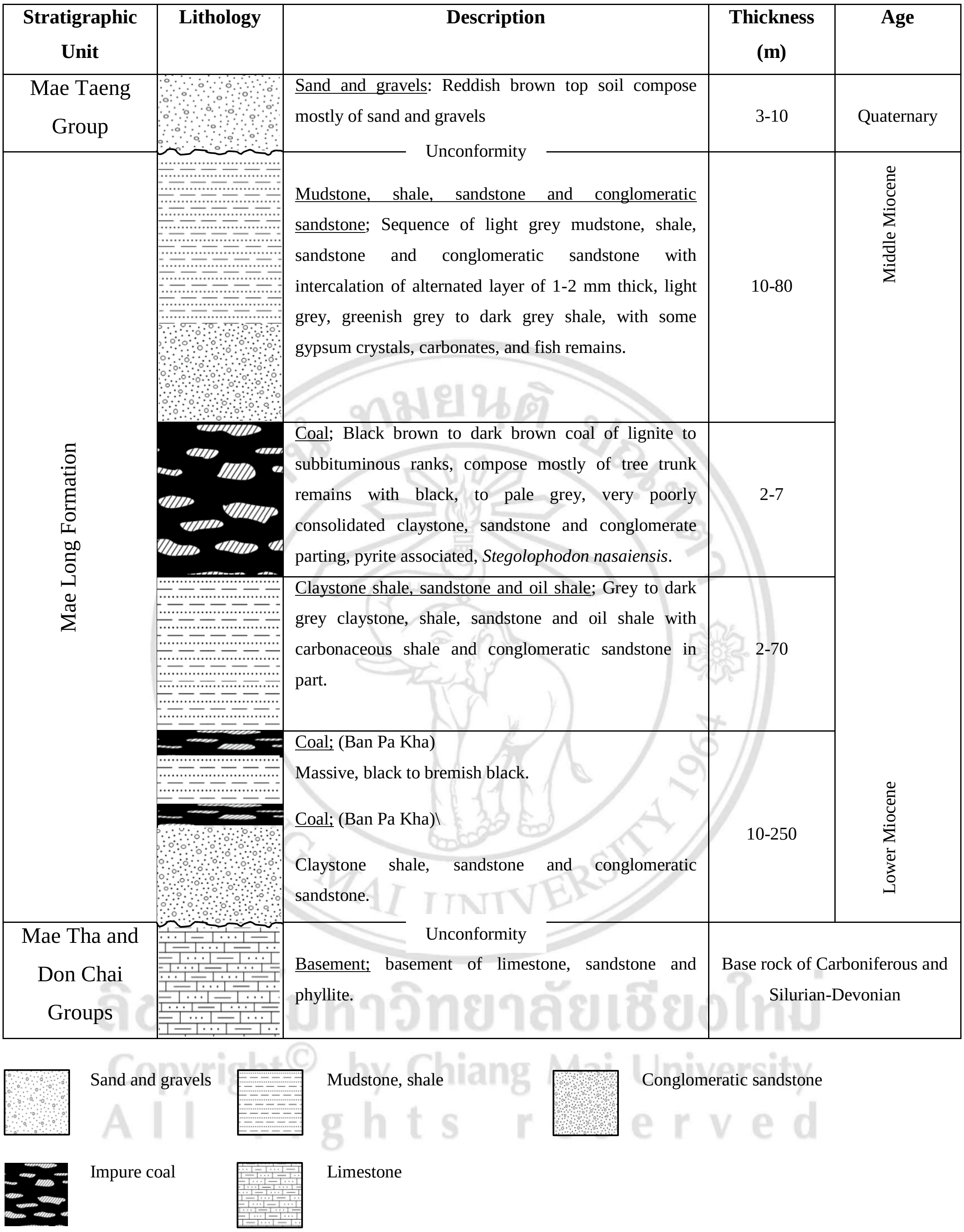
Ban Na Sai subbasin is a part of Li basin located at the south of Lamphun Province. Its stratigraphic succession includes: 1) the basement of Carboniferous and Silurian-Devonian rock of Mae Tha and Don Chai Groups, 2) Mae Long Formation and 3) the overlying Quaternary sediments of Mae Taeng Group, from the bottom to top (Fig.1.5) (Ratanasthien, 1990). The main coal seam was located at Mae Long Formation and *Stegolophodon nasaiensis* was found *in situ* in this coal seam. The age of the basin is about early Middle Miocene (Tassy *et al*., 1992).

1.4.2 Mae Moh Basin

Mae Moh basin covers the area of Mae Moh coal mine which is operated by the Electricity Generating Authority of Thailand (EGAT). This mine is the largest coal mine in Thailand with million tons of coal were already exploited. The Tertiary sediments overlaid the Triassic basement rock and was covered by the Quaternary sediments. The Tertiary sediments comprised of three formations from the bottom to top: 1) Huai King Formation, 2) Na Khaem Formation and 3) Huai Luang Formation (Fig.1.6) (Jitapankul *et al*., 1985). The Na Khaem Formation was further divided into three members. The Member III is the lowest part and contains small amount of coal seam. The most attractive coal seam is in the Member II, especially the Q and K-zone. The thick *Bellamya* bed was also found between the K-3 and K-4 Subzones. The Member I is the uppermost part of Na Khaem Formation and contains insignificant coal seams. *Stegolophodon* cf. *latidens* was collected from the K-zone. The age of this basin is about middle Middle Miocene (Tassy *et al*., 1992).

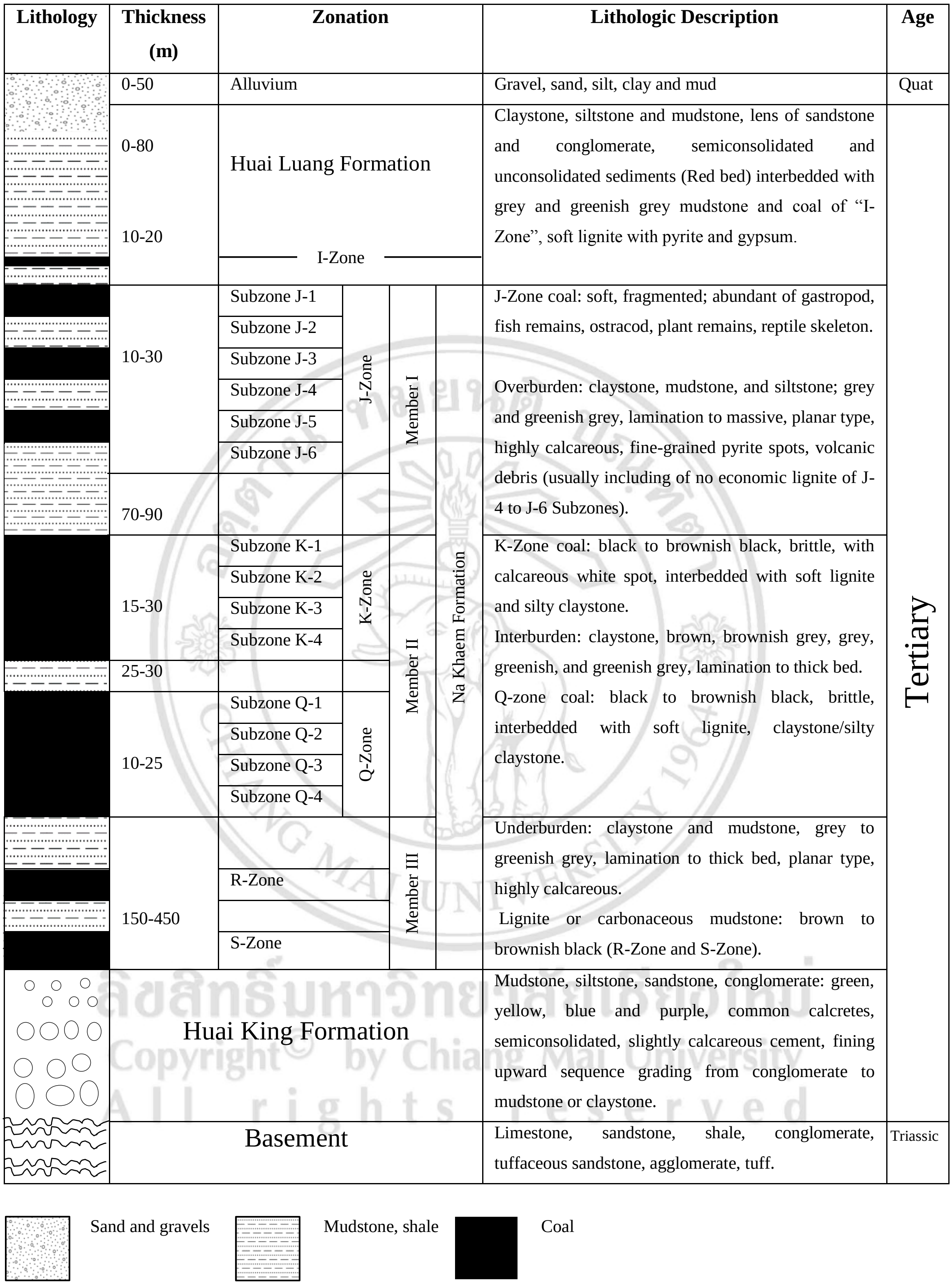
1.4.3 Chiang Muan Basin

Chiang Muan basin covers the area of Chiang Muan coal mine in Phayao Province. The stratigraphic succession comprised: 1) Cretaceous and Jurassic basement rocks, 2) Chiang Muan Formation and 3) Quaternary sediment, from the bottom to top. The Chiang Muan Formation was further divided into five Members including Sa Nua Mudstone, Sa Tai Lignite, Sa Sandstone and Conglomerate, Kon Lignite and



Stratigraphic Unit	Lithology	Description	Thickness (m)	Age
Mae Taeng Group		Sand and gravels: Reddish brown top soil compose mostly of sand and gravels	3-10	Quaternary
		Unconformity		
Mae Long Formation		Mudstone, shale, sandstone and conglomeratic sandstone; Sequence of light grey mudstone, shale, sandstone and conglomeratic sandstone with intercalation of alternated layer of 1-2 mm thick, light grey, greenish grey to dark grey shale, with some gypsum crystals, carbonates, and fish remains.	10-80	Middle Miocene
		Coal; Black brown to dark brown coal of lignite to subbituminous ranks, compose mostly of tree trunk remains with black, to pale grey, very poorly consolidated claystone, sandstone and conglomerate parting, pyrite associated, *Stegolophodon nasaiensis*.	2-7	
		Claystone shale, sandstone and oil shale; Grey to dark grey claystone, shale, sandstone and oil shale with carbonaceous shale and conglomeratic sandstone in part.	2-70	
		Coal; (Ban Pa Kha) Massive, black to bremish black. Coal; (Ban Pa Kha)\ Claystone shale, sandstone and conglomeratic sandstone.	10-250	Lower Miocene
		Unconformity		
Mae Tha and Don Chai Groups		Basement; basement of limestone, sandstone and phyllite.	Base rock of Carboniferous and Silurian-Devonian	

Fig. 1.5 Stratigraphic succession of the Mae Long Formation at the Ban Na Sai coal mine, Li Basin (modified from Ratanasthien, 1990).



Lithology	Thickness (m)	Zonation				Lithologic Description	Age
	0-50	Alluvium				Gravel, sand, silt, clay and mud	Quat
	0-80	Huai Luang Formation				Claystone, siltstone and mudstone, lens of sandstone and conglomerate, semiconsolidated and unconsolidated sediments (Red bed) interbedded with grey and greenish grey mudstone and coal of "I-Zone", soft lignite with pyrite and gypsum.	Tertiary
	10-20	I-Zone					
	10-30	Subzone J-1	J-Zone	Member I	Na Khaem Formation	J-Zone coal: soft, fragmented; abundant of gastropod, fish remains, ostracod, plant remains, reptile skeleton. Overburden: claystone, mudstone, and siltstone; grey and greenish grey, lamination to massive, planar type, highly calcareous, fine-grained pyrite spots, volcanic debris (usually including of no economic lignite of J-4 to J-6 Subzones).	
		Subzone J-2					
		Subzone J-3					
		Subzone J-4					
		Subzone J-5					
		Subzone J-6					
	70-90						
	15-30	Subzone K-1	K-Zone	Member II		K-Zone coal: black to brownish black, brittle, with calcareous white spot, interbedded with soft lignite and silty claystone. Interburden: claystone, brown, brownish grey, grey, greenish, and greenish grey, lamination to thick bed. Q-zone coal: black to brownish black, brittle, interbedded with soft lignite, claystone/silty claystone.	
		Subzone K-2					
		Subzone K-3					
		Subzone K-4					
	25-30						
	10-25	Subzone Q-1	Q-Zone				
		Subzone Q-2					
		Subzone Q-3					
		Subzone Q-4					
	150-450			Member III		Underburden: claystone and mudstone, grey to greenish grey, lamination to thick bed, planar type, highly calcareous. Lignite or carbonaceous mudstone: brown to brownish black (R-Zone and S-Zone).	
		R-Zone					
		S-Zone					
		Huai King Formation				Mudstone, siltstone, sandstone, conglomerate: green, yellow, blue and purple, common calcretes, semiconsolidated, slightly calcareous cement, fining upward sequence grading from conglomerate to mudstone or claystone.	
		Basement				Limestone, sandstone, shale, conglomerate, tuffaceous sandstone, agglomerate, tuff.	Triassic

Fig. 1.6 Stratigraphic succession of Mae Moh coal mine (modified from Jitapankul *et al.*, 1985).

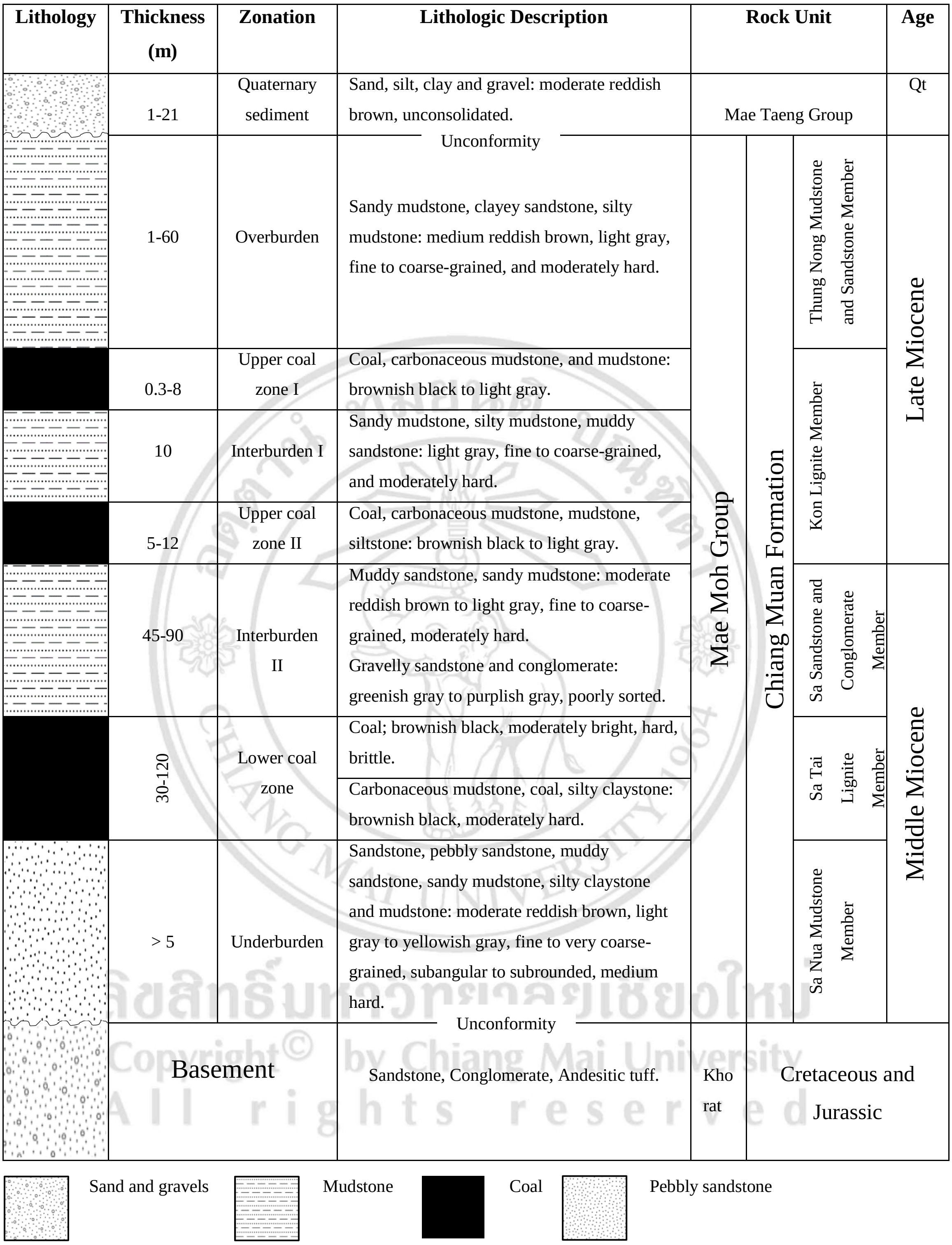
Thung Nong Mudstone and Sandstone (Fig.1.7) (Fukuchi *et al*., 2007). *Tetralophodon* cf. *xiaolongtanensis* was collected from the lower coal zone or Sa Tai Lignite Member. The age of this basin is about latest Middle Miocene (Kunimatsu *et al*., 2004).

1.4.4 Tha Chang sand pits

Tha Chang sand pits are located along the Mun River in Nakhon Ratchasima Province. The sand pits are operated by the private enterprise. The sand pit is 50 m wide and 100 m long and its depth is nearly 20 m from the ground. The precise location of the present sand pit is as follows; latitude 15° 01’ 07’’ N., longitude 102° 16’ 16’’ E. Columnar section of fossil locality is shown in Fig. 1.8. There are 5 horizons, the first horizon consists of sands and granule to pebble beds with *Elephas maximus* and stone tools. This horizon was classified to historical age. The second horizon consists of laminated fine sands with scars of roots and alternation of sand and clay. This horizon is older than the first horizon and was classified to Holocene age. The third horizon consists of cross bedded coarse grain sandstone and granule pebble conglomerate with tektite. The age of this horizon was classified to Early Pleistocene. The fourth horizon consists of granule to pebble conglomerate, lenticular beds of clays with granule conglomeratic sandstone and laminated pebble conglomerate and cross laminated conglomeratic coarse grain sandstone with many mammalian fossils. According to *Stegodon* and *Eostegodon*, this horizon was classified to late Pliocene. The fifth horizon consists of alternations of clay and laminated fine sandstone with fragment of plant and wood. *Stegolophodon* and *Mastodon* specimens were found. These indicate late early Miocene in northern Thailand (Sato, 2002). Moreover, the Miocene proboscidean fossils in this area included *Stegolophodon* cf. *stegodontoides*, cf. *Protanancus macinnesi* and *Prodeinotherium pentapotamiae* (Thasod, 2007).

1.5 Purpose of study

This research aims to specify types of feeder (paleodiet) among the Miocene mammal proboscideans, as well as to interpret the paleoecology during the Miocene in Thailand by dental microwear.



Lithology	Thickness (m)	Zonation	Lithologic Description	Rock Unit			Age
▒	1-21	Quaternary sediment	Sand, silt, clay and gravel: moderate reddish brown, unconsolidated.	Mae Taeng Group			Qt
			Unconformity				
▤	1-60	Overburden	Sandy mudstone, clayey sandstone, silty mudstone: medium reddish brown, light gray, fine to coarse-grained, and moderately hard.	Mae Moh Group	Chiang Muan Formation	Thung Nong Mudstone and Sandstone Member	Late Miocene
■	0.3-8	Upper coal zone I	Coal, carbonaceous mudstone, and mudstone: brownish black to light gray.			Kon Lignite Member	
▤	10	Interburden I	Sandy mudstone, silty mudstone, muddy sandstone: light gray, fine to coarse-grained, and moderately hard.				
■	5-12	Upper coal zone II	Coal, carbonaceous mudstone, mudstone, siltstone: brownish black to light gray.				
▤	45-90	Interburden II	Muddy sandstone, sandy mudstone: moderate reddish brown to light gray, fine to coarse-grained, moderately hard. Gravelly sandstone and conglomerate: greenish gray to purplish gray, poorly sorted.			Sa Sandstone and Conglomerate Member	Middle Miocene
■	30-120	Lower coal zone	Coal; brownish black, moderately bright, hard, brittle. Carbonaceous mudstone, coal, silty claystone: brownish black, moderately hard.			Sa Tai Lignite Member	
░	> 5	Underburden	Sandstone, pebbly sandstone, muddy sandstone, sandy mudstone, silty claystone and mudstone: moderate reddish brown, light gray to yellowish gray, fine to very coarse-grained, subangular to subrounded, medium hard.			Sa Nua Mudstone Member	
			Unconformity				
▒	Basement		Sandstone, Conglomerate, Andesitic tuff.	Khorat	Cretaceous and Jurassic		

▒ Sand and gravels ▤ Mudstone ■ Coal ░ Pebbly sandstone

Fig. 1.7 Stratigraphic succession of Chiang Muan coal mine (compiled from Chiang Muan Company Limited (unpublished document) in Songtham, 2003; Nagaoka and Suganuma, 2002; Suganuma *et al*., 2006; Fukuchi *et al*., 2007).

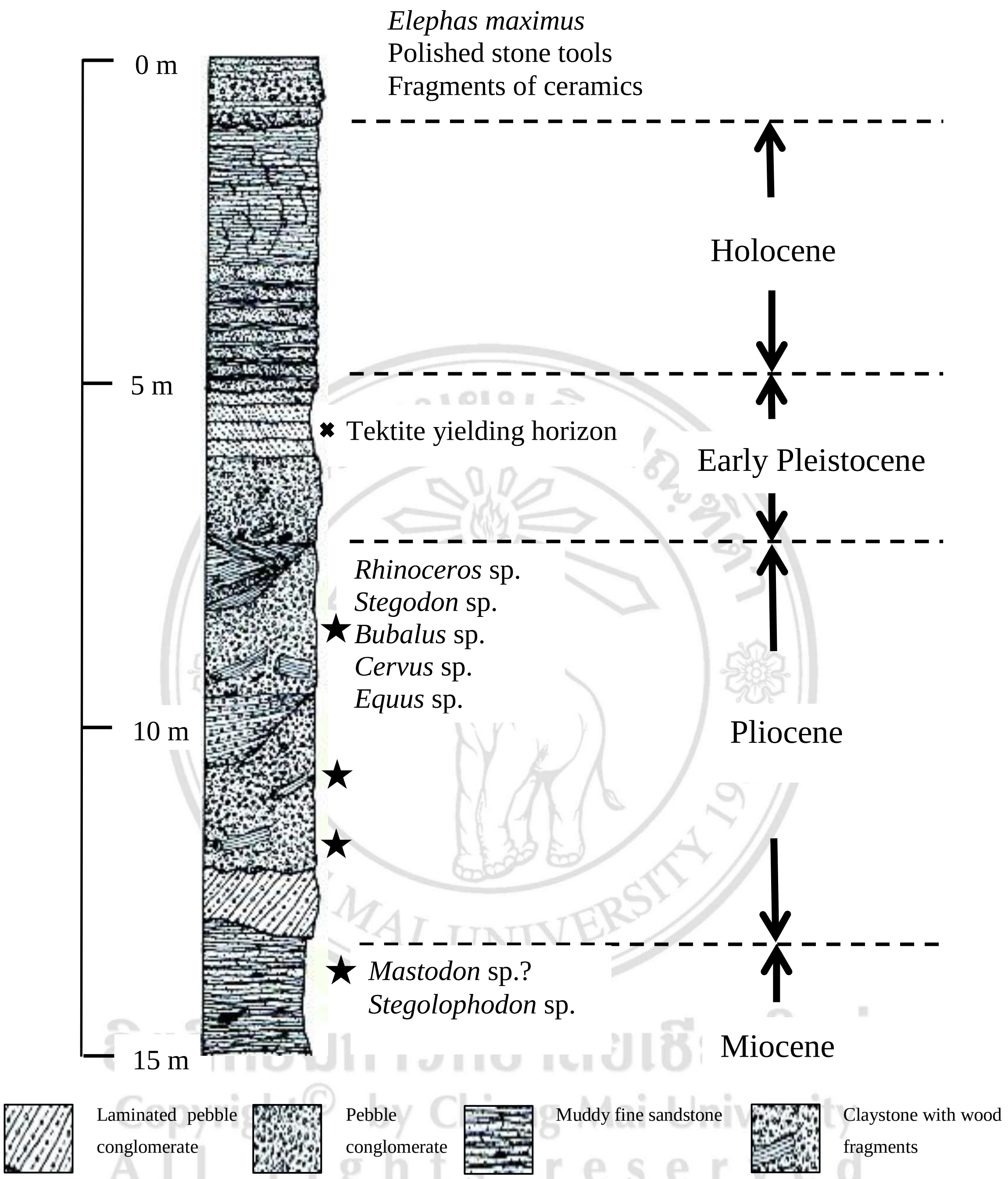


Fig. 1.8 Stratigraphic succession of the Tha Chang sand pit (after Sato, 2002).