CHAPTER 2

Methodology

There are three major steps in the study procedures; sample selection, cast preparation and microwear examination. Microwear features were examined by two methods: low-magnification microscropy and scanning electron microscopy. The entire process for low-magnification microscopy followed the work of Semprebon *et al.* (2004), and for scanning electron microscopy followed the work of Ungar, 1996.

2.1 Materials and methods for casting

2.1.1 Sample selection

The study materials were the Miocene proboscidean fossils that formerly classified by Thasod (2007). The age of fossils were determined by stratigraphic correlation (Chaimanee *et al*., 2004; Chavasseau *et al*., 2009) and magnetostratigraphy (Benammi *et al*., 2002) of sedimentary basins. The suitable specimens for microwear analysis were selected by the following criteria: 1). the original dental enamel was present all over the molar 2). there was at least 10×10 mm^2 occlusal area that was not affected by diagenetic processes and 3). microwear features were not interfered by fresh features made during storage. The unsuitable specimens were discarded from the analysis. Fifty-six proboscidean samples were carefully screened for microwear analysis. As a result, only twenty samples (35.7%) were deemed suitable.

The studied proboscidean fossils aged Middle to Late Miocene, including *Stegolophodon nasaiensis*, *Stegolophodon* cf. *latidens*, *Stegolophodon* cf. *stegodontoides*, *Tetralophodon* cf. *xiaolongtanensis*, *Prodeinotherium pentapotamiae* and cf. *Protanancus macinnesi*. The cleaning, molding, casting and examining of the specimens followed the procedure of Semprebon *et al*. (2004).

2.1.2 Fossil specimens

Twenty proboscidean teeth were examined. There were four specimens of *Stegolophodon nasaiensis* (NS-01a, NS-01b, M4732a1, M4732a2). The *S. nasaiensis* specimens were examined at the collections of the Department of Geological Sciences, Chiang Mai University, Chiang Mai Province. There were four specimens of *Stegolophodon* cf. *latidens* (M4733f, MMEL-3, MMEL-5 and MMEL-6). The *S. latidens* specimens are housed at the Mae Moh coal mine, Lampang Province. There were four specimens of *Tetralophodon* cf. *xiaolongtanensis* (CMn2, CMn5, CMn6 and CMn7). All *T.* cf. *xiaolongtanensis* specimens are stored at the Chiang Muan coal mine, Phayao Province. There were three specimens of cf. *Protanancus macinnesi* (NM1-17, NM1-9 and NM1-3). All cf. *Protanancus macinnesi* specimens are stored at the Sirindhorn Museum in Kalasin Province. There were three specimens of *Stegolophodon* cf. *stegodontoides* (NM1-13, RIN55, RIN534) and two specimens of *Prodeinotherium pentapotamiae* (KHO and RIN15). The specimens of NM1-13 and KHO are housed at the Sirindhorn Museum, Kalasin Province and specimens of RIN55, RIN534 and RIN15 are housed at the Northeastern Research Institute of Petrified Wood and Mineral Resources, Nakhon Ratchasima Province.

2.1.3 Cast preparation

1. The specimens were cleaned by 95% ethyl alcohol to remove surface impurities including grease, then dried at room temperature (25-30 °C)
2. The specimens were then cleaned by acetone to remove any remaining shellac that may cover dental surfaces.
3. Polyvinylsiloxane (PVS) molds were prepared by the following steps: 1). the putty and activator were mixed together in the ratio of 10:1 in 100 mL beaker. 2). the material were then spread onto proboscidean occlusal surface within 3-5 minutes (Fig. 2.1 A and Fig. 2.1 B) and 3). the molds were left for completely hardened at least 24 hours.
4. The epoxy resin and its activator were mixed together in the ratio of 2:1. The mixture was gently poured into the molds to make the castings. It will be thoroughly hardened after 24 hours (Fig. 2.1 C).

5. The transparent casts were examined under stereomicroscope for microwear analyses (Fig. 2.1 D).
6. The casts were kept in the boxes for future reference and stored at the museum of the Department of Geological Sciences, Chiang Mai University (Fig. 2.1 E). The high quality epoxy resin and polyvinylsiloxane made the casts dimensionally stable for at least three years (Beynon, 1987).

There are two reasons to use the casts for microwear analysis; 1). the large specimens could not be examined under the microscope and 2). they are impossible to carry the microscope to every studied areas (Semprebon *et al*., 2004).

2.2 Microwear observation

2.2.1 Low-magnification microscropy

The transparent-epoxy casts were examined under stereoscopic microscope using lighting technique (Foster, 1997). The occlusal surface (0.4×0.4 mm^2) was thoroughly examined under the microscope at ×35 magnification. The light was lit through the casts from varying angles. Microwear features were observed both quantitatively in number of microwear and qualitatively in size and shape.

Scratches and pits on the second transverse loph(id) were counted three times at the same position and averaged. The average scratches and pits were then compared to an extant ungulate microwear database developed by Solounias and Semprebon (2002). By the microwear database, browsers are those their scratch number range between 5 to 12 and their pit number range between 10 to 70, grazers are those their scratch number range between 17 to 25 and their pit number range between 3 to 30, the mixed-feeders have their scratch and pit number fall between browsers and grazers.

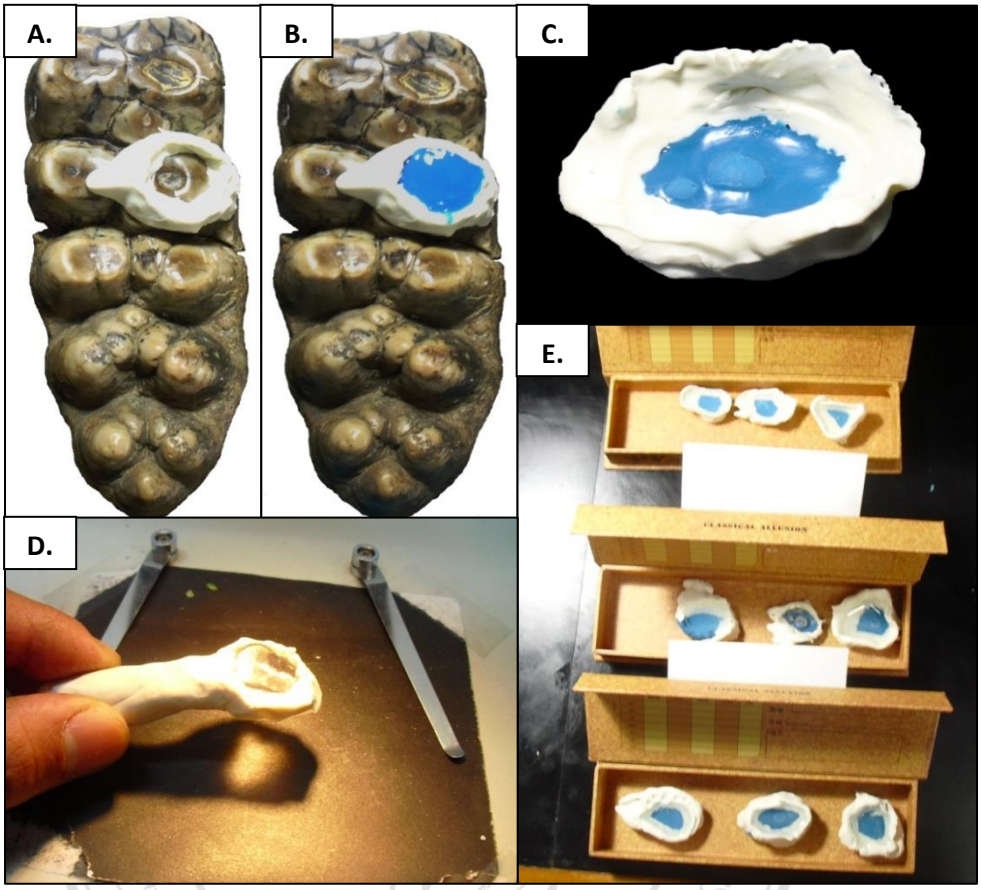

Fig. 2.1 The procedures for the preparation of the casts for microwear analyses.

A) The suitable occlusal surfaces were selected and bounded by the plasticene clay.

B) Polyvinylsiloxane impression materials were gently poured onto the occlusal surface.

C) Transparent epoxy resin was poured into the mold to create the cast.

D) Transparent casts were examined under microscope.

E) The casts were collected in the boxes for reference.

2.2.2 Scanning electron microscopy (SEM)

The transparent casts were cut into the dimension of 1×1 cm^2 in order to fit perfectly with the stub. The casts were then mounted on SEM stubs and tighten by graphite tape to ensure conductivity, and sputter-coated with a thin layer of gold. The casts were examined under scanning electron microscope (JEOL JSM5-5910LV) in the low-vacuum mode at ×60 magnification (Fig.2.2). The low-vacuum mode allow samples to be analysed without melting due to high temperature in the SEM chamber. The details of microwear features can be classified.

2.3 Classification of Microwear Features

Microwear features were observed by the contrast of bright and dark on the cast surfaces. The classification of the observed microwear features was defined by Semprebon *et al*. (2004).

Scratches are elongate scars. Their long axis is four times larger than their short axis. Size and shape of scratches depend on the type of material the proboscideans consume. Scratches were classified into three category depending on its width and light refractive properties (Fig. 2.3). Fine scratches created by objects that gently etched into the enamel surface are narrow. Coarse scratches created by objects that obviously etched into the enamel surface are wider. Both fine and coarse scratches refract light well and shiny because they are not deep. On the other hand, hypercoarse scratches are not refractive because they are very deep and trenchlike. Hypercoarse scratches are wider than fine and coarse scratches. C_4 plants which contained higher silica-content in their leaves and their first product of photosynthesis is four carbon compound, namely oxaloacetic acid tend to create coarser scratches than C_3 plants which contained lower silica-content in their leaves and their first product of photosynthesis is three carbon compound namely 3 phosphooglyceric acid. Hypercoarse scratches are usually created by silica in C_4 plants. Hence, hypercoarse scratches are characteristic of C_4 grazers.

Pits are rounded scars. Their width and length is about the same. Pits were classified into three categories (Fig. 2.3). Small pits are very shallow and refract light easily and always appear bright and shiny. Large pits are deeper, wider and consequently less refractive. Large pits are usually twice the diameter of small pits.

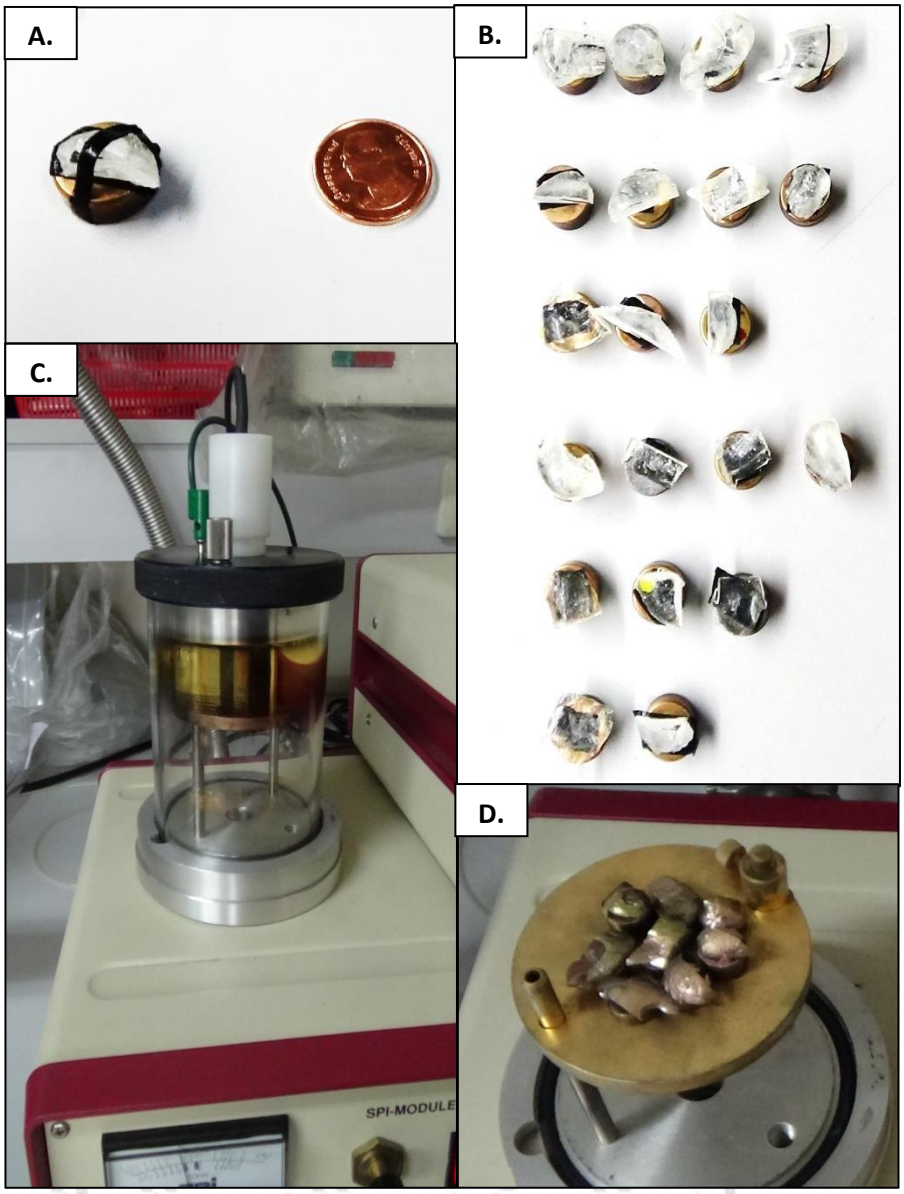

Fig. 2.2 Specimen preparation for SEM analysis.

A) The transparent casts were mounted on SEM stubs and tighten by graphite tape to ensure conductivity.

B) Twenty samples were mounted on SEM stubs.

C) The samples were sputter-coated with a thin layer of gold.

D) The gold-coated samples were then examined under scanning electron microscope.

Puncture pits were extremely large, their diameter exceeded 0.1 mm. Hard seed tends to create puncture pits.

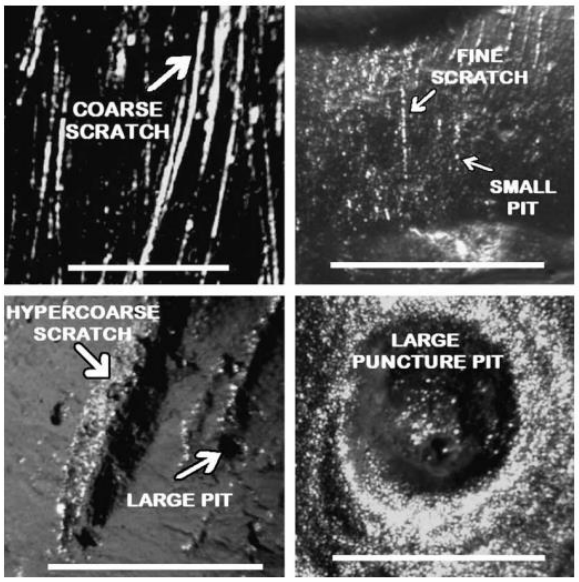

Fig. 2.3 Example of microwear features, including small, large and puncture pits, as well as fine, coarse, and hypercoarse scratches, as seen under a standard stereoscopic microscope at low magnification (50-70×). Scale bar = 0.4 mm. (Semprebon *et al*., 2004)