CHAPTER 2

Materials and Methods

2.1 Research Design, Scope and Methods

2.1.1 Mosquito collections and rearing

Mosquitoes specimens were collected as the immature stages during 2012–2014 from three sites in Chiang Mai Province: Mae Hia Agricultural Research, Demonstrative and Training Center, Mae Hia subdistrict, Chiang Mai, Thailand, 18º45ˈ21.52ˈˈ N, 98º56ˈ22.84ˈˈ E, 322 m altitude (Figure 2.1), Ban Pang Mai Daeng, Mae Taeng district, Chiang Mai, Thailand, 19º08ˈ19.78ˈˈ N, 98º12ˈ07.76ˈˈ E, 415 m altitude (Figure 2.2) and Napamaytanidol Chedi and Phra Mahatat Napaphon Bhumisiri, Doi Inthanon, Chom Tong district, Chiang Mai, Thailand, 18º33ˈ11.90ˈˈ N, 98º28ˈ47.83ˈˈ E, 2,116 m altitude (Figure 2.3). They were brought back for rearing in the insectary of Department of Parasitology, Faculty of Medicine, Chiang Mai University. The collected larvae were identified by using illustrated keys of Rattanarithikul et al. (2005) under a stereomicroscope. The larvae were reared singly provided with larvae of laboratory *Aedes aegypti* or *Cx. quinquefasciatus* as prey. A few numbers of fourth instar larvae were mounted on slides. The rests were reared to pupae and adults. The emerging adults were pinned and the associated larval and pupal exuviae preserved in 80% ethanol until they were mounted on slides with Hoyer's medium or Euparal.

Larval, pupal and adult specimens of *Lt. vorax* from Tokyo, Japan (type locality of *Lt. vorax*), were kindly provided by Dr. Y. Tsuda.

2.1.2 Morphological examination

Larval and pupal chaetotaxy with setal positions of *Lutzia* are shown in Figures 2.4 and 2.5, respectively. Setal branching counts were obtained from both left and right sides of larval and pupal exuviae using a compound microscope with a calibrated ocular micrometer. Setal lengths were measured from the longest setae for

each position of pupal exuviae. Larval comb scales were also counted. Larval integument was observed under bright field microscopy and scanning electron microscopy (SEM). To prepare the specimens for SEM, the larvae were fixed with 2.5% glutaraldehyde mixed in phosphate buffer solution (PBS) pH 7.4 at 5°C for 12 hours, followed by rinsing twice with PBS, then preserved in 80% ethanol and dehydrated with ethanol series. After specimens were dry at room temperature, they were attached to double stick tape on an aluminum stub and kept in an oven at 50°C at least 12 hours. Then, they were coated with gold in sputter-coating apparatus. The integument of larvae was examined in a JEOL-JSM6610LV Scanning Electron Microscope.

Illustrations of larval and pupal exuviae were prepared by using Adobe illustrator CS5. The adult morphology was studied by the aid of stereomicroscope using the keys mentioned above. The wing venations were photographed by using a digital camera mounted on a stereomicroscope. The morphological terminology, abbreviations, and measurements that are used in description and tables followed the anatomical Glossary of the online Mosquito Taxonomic Inventory (http://mosquito-taxonomic-inventory.info/anatomical-glossary-overview).

2.1.3 Data analysis

Setal lengths were measured from the longest setae for each position of pupal exuviae. Lengths of respiratory trumpets were tabulated as range and mean separated by species and sexes. Pupal seta branching tables were tabulated as range and mode separated by species and sexes that are calculated from fewest branching to the most branching of two sides of all specimens. The ranges and modes were calculated from fewest branching to the most branching of two sides of all specimens. The number of larval comb scales was compared by mode and mean.

Figure 2.1 Breeding sites of *Lutzia* at Mae Hia Agricultural Research, Demonstrative and Training Center, Mae Hia subdistrict, Chiang Mai, Thailand (322 m altitude).

Figure 2.2 Breeding sites of *Lutzia* at Ban Pang Mai Daeng, Mae Taeng district, Chiang Mai, Thailand (415 m altitude).

Figure 2.3 Breeding sites of *Lutzia* on Doi Inthanon, Chom Tong district, Chiang Mai, Thailand (2,116 m altitude).

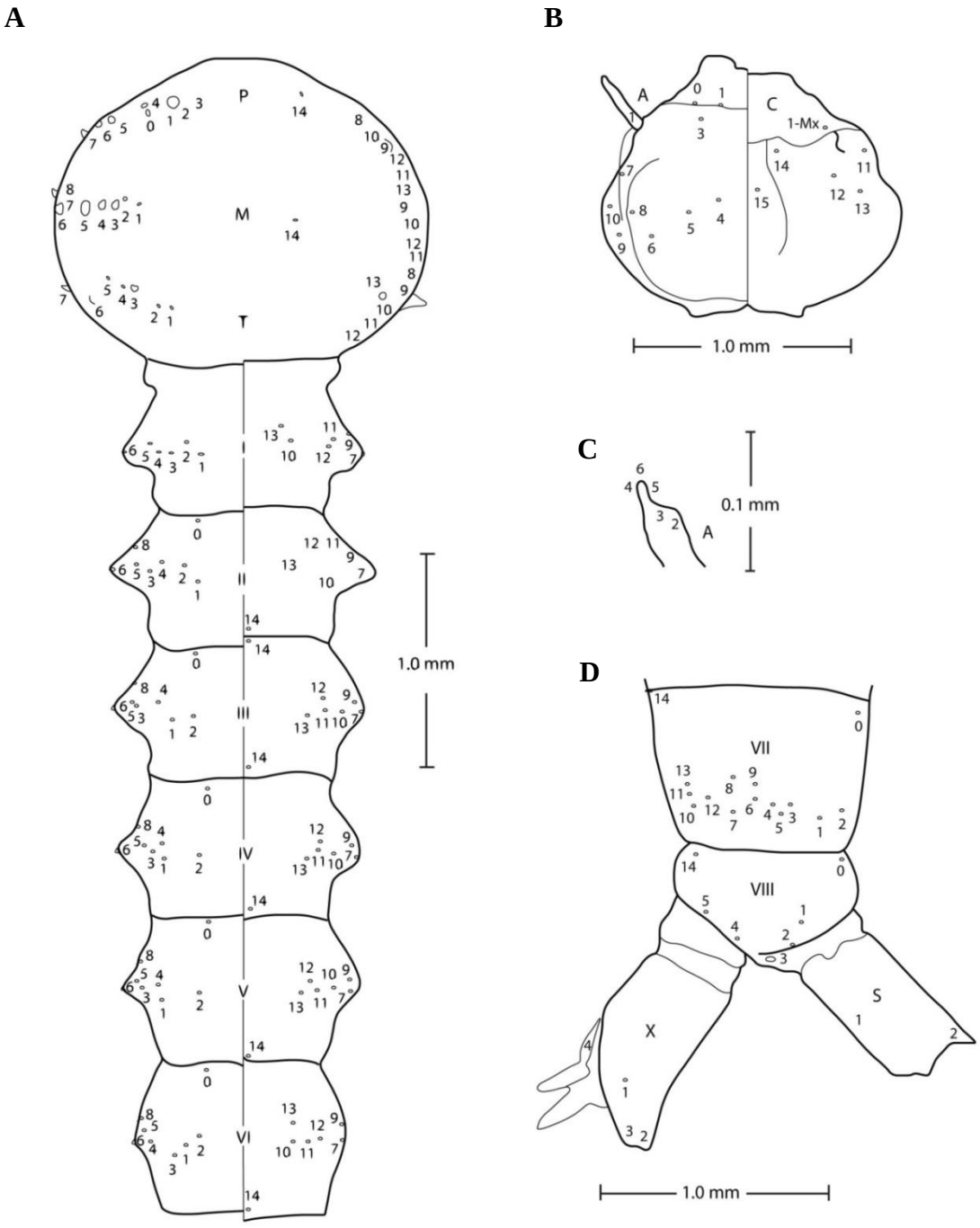

Figure 2.4 Setal positions of *Lutzia* forth-instar larva: A, dorsal view (left) and ventral view (right) of thorax and I–VI abdominal segments. B, dorsal view (left) and ventral view (right) of head capsule. C, antennal segment. D, lateral view of VII and VIII abdominal segments, siphon, and X segment. (modified from Tanaka et al., 1979).

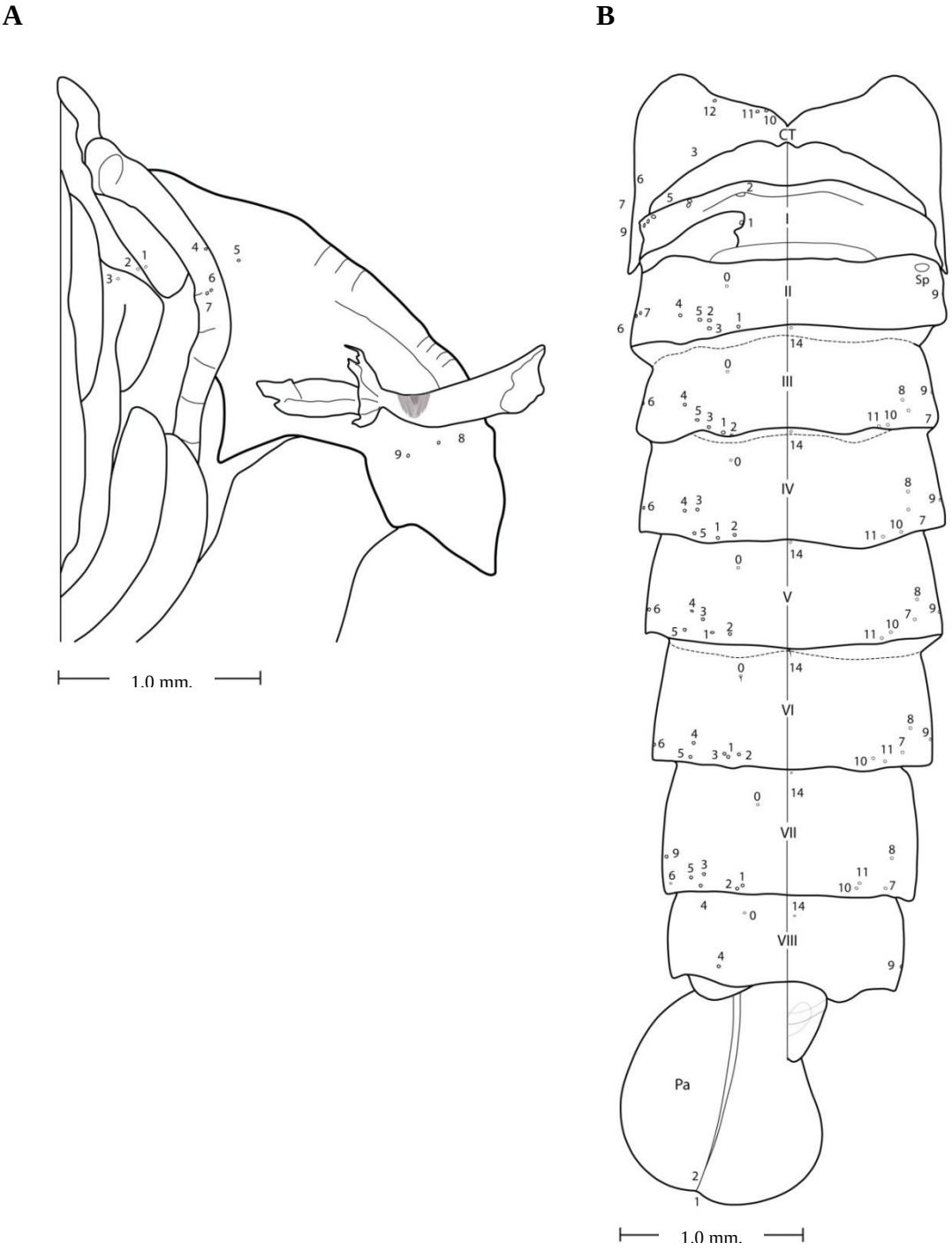

Figure 2.5 Setal positions of pupal exuviae of *Lutzia*. A, left side of cephalothorax, dorsal view. B, dorsal view (left) and ventral view (right) of metathorax and abdomen.