The immature stages of *Polypedilum (Pentapedilum) nodosum* (Johannsen) and *Polypedilum (Tripodura) masudai* (Tokunaga) (Diptera, Chironomidae, Chironominae)

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Abstract

Based on associated material collected from Macau and Guangxi, the pupae and larvae of *Polypedilum (Pentapedilum) nodosum* Johannsen and *P. (Tripodura) masudai* Tokunaga are described completely for the first time. Both species are newly recorded from China. Characters for distinguishing the immature stage of these species from other allied *Polypedilum* species are noted. The previously recorded *Polypedilum (Pentapedilum) ‘K1’* of Cranston (1996) is shown to be *P. nodosum*. Information is provided on distribution and ecological tolerances.

Key words: *Polypedilum*, pupa, larva, biology, new record

Introduction

*Polypedilum* Kieffer 1912 is one of the largest genera in the tribe Chironomini, with the larvae occurring in all kinds of waterbodies (Maschwitz & Cook 2000, Epler 2001, Epler et al. 2013). At present, at least 60 species are recorded in China (Zhang 2005, R.L. Zhang pers. comm.) in 7 subgenera except subgenus *Kribionympha* Kieffer. Saether et al. (2010) recognised 8 subgenera largely in order to maintain a monophyletic sub-generic concept based only on species with known larvae. Nonetheless, there remain many unresolved problems and conflicts regarding evolutionary relationships and allocation to subgenera between life stages (Kawai et al. 2012). As is typical for the study of Chironomidae, descriptions rely on the adult stage, especially features of the male hypopygium. The association of immature stages that are valuable in environmental monitoring, with named adults has proceeded slowly. Although several works narrow the gap, e.g. Rossaro 1985 (Europe), Maschwitz & Cook 2000 (North America), Langton & Visser 2003 (western Europe), Cranston 1996, 2000 (Australia), our knowledge of the immature stages of even some common species of *Polypedilum* remains inadequate. This is especially so for taxa occurring in the subtropics and tropics including those of Asia with only few abundant and conspicuous species described (Cranston 2007, Cranston et al. 2013).

In seasonal surveys of the Macau benthos from late winter to spring, large populations emerged of two common species of *Polypedilum*, namely *Polypedilum nodosum* Johannsen 1932 and *Polypedilum masudai* Tokunaga 1938. Rearing and the sheer abundance of specimens allow us to associate the immature stages with adult forms. *P. nodosum*, an Austro-oriental taxon (Johannsen 1932, Tokunaga 1964, Sasa & Hasegawa 1983, Cranston 1996, Sasa & Suzuki 2002, Oyewo & Saether 2008, Yamamoto et al. 2012), allocated to s.g. *Pentapedilum* on the basis of the macrotrichiose wing, is recorded here for the first time from China. The previous associated immature stages of *Polypedilum (Pentapedilum) ‘K1’* of Cranston (1996) from Australia are considered
to be those of *P. nodosum*. Other than the distribution in China and Australia, the species has been reported also from Thailand and Singapore under the name of *P. (Pent.) ‘K1’* (Cranston 2007, Cranston et al. 2013).

*P. masudai* is a common species in East Asia (Tokunaga 1938, Sasa 1985, 1991, Sasa & Kikuchi 1986, Ree & Kim 1988, R.L. Zhang pers. comm.), usually occurring in eutrophic ponds and the littoral zone of rivers, perhaps more abundantly in the tropics than in temperate regions. Despite this, the species was not included in the recent keys for Chinese species of the subgenus *Tripodura* Townes (Qi et al. 2013), so this constitutes the first formal record for China.

Here we describe the larvae and pupae of *P. nodosum* and *P. masudai*. Some important immature characters are discussed, and the genus diagnosis in both larval and pupal stage is emended.

**Material and methods**

Different collection methods were use. Larvae were collected using D-frame nets in several ponds, and sorted from the substrate into presumed species in the laboratory in Guangzhou. Some mature larvae or pupae were selected to be reared individually in the laboratory to obtain associated pupal exuviae and adults. Other pupae or pupal exuviae were collected by a hand net (250 µm, 15-mins walking along the pond margins) (Raunio & Anttila-Huhtinen 2008, Bouchard & Ferrington 2011), and adults were collected by light trapping. Specimens examined were slide-mounted in Euparal. Morphological terminology and abbreviations follow Saether (1980), Maschwitz & Cook (2000) and Langton & Visser (2003). Measurements are given as ranges, with the number of observed specimens in parentheses if different from the number (n) stated at the beginning of the description.

**Abbreviations.** Morphological abbreviations of larval characters used (if pupal characters specified in parenthesis): a.l., anal lobe setal number (pupa); a.l.r, anal lobe ratio, height of anal lobe : the half width of anal lobe (pupa); AS, length of anal seta; Ant 1–5, antennal segments 1–5 length in µm; A1R, first antennal segment ratio, length of segment 1: width of segment 1 through the ring organ; A.R. length of basal segment : combined lengths of segments 2 to apex; B.l., total body length in mm; Bl, antennal blade length; BlR, blade ratio, length of blade : total length of Ant 2–5; fs.l., frontal seta length (pupa); HCR, head capsule ratio, length of postmentum : distance between setae submenti; hk., number of hook row II; Hl., head capsule length, anterior labrum to posterior margin; hr.l., length of hook row of tergite II (pupa); IPD, inter-ventromentum plates distance; L, larva; Le, larval exuviae; Le/Pe/m(f), reared adult male (female), with associated larval and pupal exuviae; masl – elevation in metres above sea level; Md, mandible length; M.w., mentum width; Mmw, median mental tooth width; P, pupa; Pe, pupal exuviae; Pm(f), pharate male (female) within pupa; PM, postmentum length; Pmd: premarginal length; ROR, ring organ ratio, distance from basal to location of ring organ : length of basal antennal segment; S4S, length of supraanal seta; SSm–SSm: distance between setae submenti; t.l., total length, from the tips of head to the end of anal lobe (pupa); V.w., ventromentum width; VmPR, width of ventromental plate : height of ventromental plate.

Specimens are conserved in the Department of Ecology, Jinan University (EJNU), Guangzhou, China, the Australian National Insect Collection (ANIC), CSIRO, Canberra, Australia, the Enns Entomological Museum (UMOC), University of Missouri-Columbia, the Natural History Museum, London, U.K. (BMNH), the Zoological Reference Collections of the Raffles Museum of Biodiversity Research (RMBR), National University of Singapore.

**Systematics**

**Polypedilum Kieffer, 1912**

**Diagnosis.** Conforms mostly in all diagnostic features to the generic description for larva (Epler et al. 2013) and pupa (Pinder & Reiss 1986). Based on the material described below, the generic diagnosis for *Polypedilum* larva and pupa should be amended as follows.

**Larva.** Frontoclypeus with or without hyaline band, site of dorsal S3 insertion with some variation among the known species. Antenna blade distinctly longer than segment 2–5 in some species (e.g., *P. cultellatum* Goetghhebuer 1931, *P. nodosum* and most members in subgenus *Tripodura*). Segment 5 usually vestigial in *Tripodura* spp., with
a minute needle-like peg suspended at the apex of segment 4. Mandible with or without dorsal teeth. If with, usually bearing 2 inner teeth, seldom with 3 (P. nodosum); if without, mandible always with 3 inner teeth (e.g., P. fallax (Johannsen 1905), P. leei Freeman 1961, P. pedestre (Meigen 1830) & P. sordens (van der Wulp 1874)).

**Pupa.** Apex of wing pad with nose in certain species, segment VIII lacking spinulation (eg. P. nodosum). Median spinulation variable in tergite II and VI.

**Polypedilum (Pentapedilum) nodosum (Johannsen, 1932)**
(FIGS 1–3)

*Polypedilum (Pentapedilum) nodosum*: Tokunaga, 1964: 597; Oyewo & Sæther, 2008: 49; Yamamoto et al., 2012: 38.  

**Material examined.** Le/Pe/♂, Macau, the Bay of St. Lazarus, near Taipa Houses Museum, 27. ii. 2013 (emerged 3. iii. 2013), other 20Pe in alcohol bottles (H.Q. Tang); 10Pe in alcohol bottles, the same place as above, 16. i. 2013; 3 P♂ and 4L, other 60L in alcohol bottle, same locality, 24. iv. 2013 (H.Q. Tang); 2L, Macau, Tapai Grande, 12. vi. 2013 (H.Q. Tang) (all EJNU).

**Additional examined material.** Holotype ♂, slide mounted, *Pentapedilum nodosum* Johannsen; INDONESIA: East Java, Klakah, Lake Lamongan, October 12 (coll. Thienemann Sunda Exped) (BMNH)  
AUSTRALIA, 14L., Queensland, Atkinson Dam, artificial stream, 27°06’S 152°27’E, 8.viii.1991 (J. McLean); Northern Territory, Kakadu N.P., Le/P♀ Magela Ck., Oenepelli Crossing, 12°34’S 132°53’E, 12.iv.1989 (P.S. Cranston); 2♂, nr Jabiru, Gulungul Ck., 12°39’S 132°53’E, 11.iv.1989 (P.S. Cranston); ♂, Magela Ck., Ranger outflow, 12°40’S 132°56’E, 5.i.1989 (P. Dostine); ♂, Ranger retention pond #1, 12°41’S 132°55’E, 31.vi.1989 (P.S. Cranston); 4Pe, Yellow Waters, 12°54’S 132°32’E, 30.v.1988; ♂, east branch West Alligator R., 2.vi.1989 (P. Dostine); ♂, 5km NNW of Cahill’s Crossing, East Alligator R., 8.vi.1973 (D.H. Colless) (all ANIC).


**Larva (n=5).** Head capsule yellow, with dark brown mental teeth and mandible teeth. Occipital margin yellow to pale brown.

- Dorsal surface of head (Fig. 1A). Frontoclypeus present, anteriorly broadened, with a very narrow hyaline strip of 3–5 µm width.
- Antenna (Fig. 1B) yellow, with reduced third segment and fourth segment about 2 times as long as third, the fifth segment subequal or longer than third, blade extending beyond the terminal of flagellum.
- Labrum. SI and SII plumose on both sides. Pecten epipharyngis with 3 scales, the lateral with 4 teeth and the median with 3 teeth. Premandible with 2 apical teeth and 1 inner tooth.
- Mandible (Fig. 1C) with 1 apical, 1 dorsal and 3 distinct inner teeth. Mola area pale yellow. Seta subdentalis reaching the apical part of most proximal inner teeth.
- Mentum (Fig. 1D) with two median teeth, first lateral mental teeth depressed to medians. Width of ventromentum less than the mentum, with the medially-directed pointed apex or slightly anteriorly directed.
- Body. Anterior claws pale golden, and posterior claws a little brown, both simple and dense. Procercus and apical setae yellowish brown. Mensural features as in Table 1.

**Pupa (n=8).** Exuviae yellow, apophyses brown. Cephalic tubercles absent (Fig. 2A). Thorax with conspicuously large granules near suture. Apex of wing pad with obvious nose (Fig. 2B).

- Length of hook row II 0.36–0.50, 0.42 times width of corresponding tergite. Tergites as in Fig. 2C & D, II–VI with obvious anterior transverse bands of spinules stronger than those of median and posterior patches. Median and posterior patch of tergites III–VI consist of 4 separated sub-patches. Median patch of tergite VII absent, only one trans-anterior band and 2 posterior bands remain. Tergite VIII without trace of spinulation. Median patch usually reduced on tergite II and VI (Fig. 3A, B & C), and sometimes two sub-median patches fused in the middle of tergite
III–V. Conjunctives present in tergite III/IV and IV/V, with points about the same size as the points of those of the anterior transverse bands. Comb of segment VIII composed of 3–4 slender small teeth (Fig. 3D). Lateral taeniae of segments IV–VIII: 0, 3, 3, 4, 4. Mensural features as in Table 2.

**TABLE 1.** Mensural features of *P. nodosum* and *P. masudai* larvae. Abbreviations as in text.

<table>
<thead>
<tr>
<th></th>
<th><em>P. nodosum</em></th>
<th><em>P. masudai</em></th>
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<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>B.l.</td>
<td>3.70–4.10, 3.87</td>
<td>4.85–5.60, 5.16</td>
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<tr>
<td>H.I.</td>
<td>355–365, 357</td>
<td>300–345, 325</td>
</tr>
<tr>
<td>PM</td>
<td>142.5–150, 147.5</td>
<td>120.0–150.0, 131.7</td>
</tr>
<tr>
<td>SSm-SSm</td>
<td>62.5–70.0, 65.8</td>
<td>55.0–70.0, 61.7</td>
</tr>
<tr>
<td>HCR</td>
<td>2.14–2.40, 2.25</td>
<td>2.08–2.18, 2.14.</td>
</tr>
<tr>
<td>Ant 1</td>
<td>48.0–52.5, 50.3</td>
<td>42.5–55.0, 49.2</td>
</tr>
<tr>
<td>Ant 2</td>
<td>15.0–17.5, 15.6</td>
<td>16.5–22.5, 19.7</td>
</tr>
<tr>
<td>Ant 3</td>
<td>3.0–5.0, 4.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Ant 4</td>
<td>8.8–10.0, 9.4</td>
<td>12.5–15.0, 13.5</td>
</tr>
<tr>
<td>Ant 5</td>
<td>5.0</td>
<td>3.0–3.5, 3.2</td>
</tr>
<tr>
<td>AR</td>
<td>1.06–1.25, 1.15</td>
<td>1.10–1.33, 1.20</td>
</tr>
<tr>
<td>A1R</td>
<td>3.3–4.0, 3.6</td>
<td>2.75–3.00, 2.86</td>
</tr>
<tr>
<td>ROR</td>
<td>0.15–0.17, 0.16</td>
<td>0.33</td>
</tr>
<tr>
<td>Bl</td>
<td>45–50, 47.5</td>
<td>50.0–60.0, 57.5</td>
</tr>
<tr>
<td>BlR</td>
<td>1.13–1.67, 1.33</td>
<td>1.25–1.48, 1.39</td>
</tr>
<tr>
<td>Pmd</td>
<td>50.0–65.0, 57.5</td>
<td>65.0–75, 69.3</td>
</tr>
<tr>
<td>Md</td>
<td>100–120, 115</td>
<td>90.5–105.0, 98</td>
</tr>
<tr>
<td>M.w.</td>
<td>75.0–87.5, 83.5</td>
<td>82.5–100.5, 90.8</td>
</tr>
<tr>
<td>Mmw</td>
<td>12.5–15.0, 14.2</td>
<td>17.5–18.0, 17.8</td>
</tr>
<tr>
<td>V.w.</td>
<td>65.0–67.5, 66.1</td>
<td>107.5–130.0, 116.7</td>
</tr>
<tr>
<td>VmPR</td>
<td>2.17–2.25, 2.19</td>
<td>1.25–1.30, 1.28</td>
</tr>
<tr>
<td>IPD</td>
<td>42.5–50.0, 45.8</td>
<td>17.5–20.0, 18.5</td>
</tr>
<tr>
<td>Striae</td>
<td>22–26, 24</td>
<td>ca. 40</td>
</tr>
<tr>
<td>AS</td>
<td>350–420, 390</td>
<td>350–420, 380</td>
</tr>
<tr>
<td>SAS</td>
<td>180–220, 205</td>
<td>200–240, 220</td>
</tr>
</tbody>
</table>

**TABLE 2.** Mensural features of *P. nodosum* and *P. masudai* pupae. Abbreviations as in text.

<table>
<thead>
<tr>
<th></th>
<th><em>P. nodosum</em></th>
<th><em>P. masudai</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>t.l.</td>
<td>2.80–3.30, 3.05</td>
<td>4.05–4.50, 4.28</td>
</tr>
<tr>
<td>b.l.</td>
<td>1.95–2.70, 2.26</td>
<td>2.75–3.35, 3.60</td>
</tr>
<tr>
<td>fs.l.</td>
<td>42.5–50.0, 46.4</td>
<td>55–80, 65</td>
</tr>
<tr>
<td>hk.</td>
<td>32–42, 36</td>
<td>60–80, 68</td>
</tr>
<tr>
<td>hr.l.</td>
<td>160–200, 175</td>
<td>260–410, 332</td>
</tr>
<tr>
<td>a.l.</td>
<td>24–30, 27</td>
<td>26–42, 33</td>
</tr>
<tr>
<td>a.l.r</td>
<td>1.25–2.14, 1.80</td>
<td>1.67–1.90, 1.78</td>
</tr>
</tbody>
</table>
Remarks. Larvae of *P. nodosum* are similar to those of *P. cultellatum* in the shape of the mentum and in the distinct antennal blade extending beyond the apex of the flagellum. These two species live together in the Macau ponds, but the latter appears to differ clearly in antennal and mandibular features, with antennal segment 3 distinctly more than half the length of the 4th segment and with 2 inner mandible teeth, in contrast to *P. nodosum* segment 3 which is about half the length of the fourth segment, and the mandible has distinctly 3 inner teeth. The less developed third antennal segment (relative to the fourth segment, not reduced as in *Tripodura*) also occurs in some species of *Uresipedilum* (e.g., *P. aviceps* Townes 1945 and *P. flavum* (Johannsen 1905)) (Epler 2001), but the latter differ significantly in well-developed ventromentum posterior lobes. We noted some characters in dorsal sclerites, such as frontoclypeus with a distinct anterior hyaline band and the S3 insertion to the posterior of lateral lobes, which resembles *P. australotropicus* Cranston 2000, but the latter appears to differ clearly in the mental teeth, with protruding median teeth and well separated 1st laterals. In contrast in *P. nodosum*, the median teeth of
the mentum are constricted basally, with small 1st laterals appressed to the medians. Among all described *Polypedilum* larvae, this is one of the *Polypedilum* larva that can be identified with confidence – it is the only one with 3 clear inner mandibular teeth, plus the interesting arrangement of the median and first lateral teeth. The three well-developed complete mandibular inner teeth with the presence of dorsal teeth is totally different from most with 2 inner teeth plus a dark-brown mola area, except few head capsules in subfossil *P. nubeculosum* (Meigen 1804) and *P. uncinatum* (Goetghebuer 1921) with developing 3 inner mandible teeth which perhaps represents the eroding mola area. (H.Q. Tang pers. obs.). Such a characteristic is unique in this genus. The depressed first mental laterals to medians also seems to be unique in *Polypedilum*: a similar arrangement can be found in *Chironomus* ‘type I’ for central mental teeth (Webb & Scholl 1985). Some *Polypedilum* species may have basally constricted median teeth, but the first laterals are relatively independent, not appressed to medians.

The pupa of *P. nodosum* resembles that of many members of subgenus *Pentapedilum*, with dominant anterior transverse bands and a prominent hook row. In the key of Oyewo and Saether (2008), the pupa of *P. nodosum* will key to *P. (Pe.) K1* Cranston if the spinulation of tergite II is ignored or *P. (Pe.) uncinatum* if not considering tergite VII, but this can be separated from the above two taxa by the tergite VII with anterior and paired posterior patches spinules, and a wing pad with a distinct nose. The occurrence of a nose on the wing pad, seemingly unique in *Polypedilum*, usually is a typical feature to distinguish many Tanytarsini from others, and seldom is seen in Chironomini.

Cranston (1996) described *P. (Pe.) K1*, noting only a small difference from our present description, including the median spinulation of tergite II and tergite VIII. Oyewo and Saether (2008) also suspected *P. (Pe.) K1* to be the true *P. nodosum*. After having reexamined the original material, it appeared that the tergite VIII is bare, with no trace of any anteromedian patch of spinules (P.S. Cranston *pers. obs.*). Thus we concluded that all those previously described as *P. (Pe.) K1* should be assigned to *P. nodosum*.
IMMATURE STAGES OF TWO POLYPEDILUM SPECIES

Distribution. Indonesia (Sumatra), Palau, Marianas Islands, Caroline Island, Australia, Singapore, Thailand, Japan, China (Macau).

Polypedilum (Tripodura) masudai (Tokunaga)

Chironomus (Polypedilum) masudai Tokunaga, 1938: 331.

Material examined. 1P♂ and 2Le, Macau, the Bay of St. Lazarus, near Taipa Houses Museum, 14. xii. 2013 (H.Q. Tang); 2Pe and 8L, same locality, 27. ii. 2013, (H.Q. Tang); 35Pe, Macau, artificial ecology park of Alto de Coloane, 18. i. 2013 (H.Q. Tang); 3P♂ and 2L, China, Guangxi, Hezhou city, Fulong, 10. viii. 2013 (J.G. Zhao) (all EJNU).
Larva (n=4). Head capsule yellow, with dark-brown postmentum. Mentum and mandible teeth dark brown. Occipital margin dark.

Dorsal surface of head (Fig. 5A). Frontoclypeus with a narrow hyaline band, surrounded the broadened apex. Antenna (Fig. 5B) pale yellow, with reduced third and fifth segment, segment 2 and 4 relative long. Antennal blade extending beyond the terminal of flagellum.

Labrum. SI and SII fine plumose. Pecten epipharyngis consists of 3 scales, each scale with 3 teeth. Premandible with 2 apical teeth and brush present.

Mandible (Fig. 5C). With 1 apical, 1 dorsal and 2 inner teeth, the length of apical tooth subequal to the combined width of 2 inner teeth. Seta subdentalis almost reaching the second inner teeth. Mola with 2–3 sharp spines.

Mentum (Fig. 5D) with 2 median teeth and 7 laterals, the location of outermost laterals relative low, the fourth laterals lower than the neighboring teeth giving the appearance of the relative greater elevation of fifth and sixth laterals. Ventromentum wider than the mentum, with the medially-directed pointed apex. Striae crowded in dense basally, 5–6 striae about 5 µm wide, total about 40 striae. Inter-plate distance about subequal to the width of two median teeth.

Body. Procercus golden brown, with 7–8 anal seta. Anal tube with a constriction in the middle. Mensural features as in Table 1.

Pupa (n=5). Pupal exuviae pale yellow, apophyses brown. Cephalic tubercles present, the height equal to the width; frontal setae long, more than 2 times as long as the height of cephalic tubercles. Mid-thorax with one row of sparse granules near suture.

Abdomen (Fig. 6C & 6D). Hook row II 0.47–0.63, 0.56 times as wide as corresponding tergite width. Tergites II–VI with obvious anterior transverse bands of points stronger than those of the posterior patch and conjunctive patch, median armament greatly reduced. Posterior patch of tergites III–VI consist of 2 separated sub-patches. Spinulation of tergite II continuous, a relative larger anterior transverse band of points fused broadly with the postero-median trapezoidal patch of small spinules. Tergite VII and VIII only with 2 anterolateral small patches, without trace of median patch.

Conjunctives present in tergite III/IV and IV/V, with spinules about the same size as that of median bands, medially usually interrupted in conjunctives III/IV or weakly connected in IV/V. The number of teeth in conjunctive III/IV varied greatly, ranging from 9–36 and 8–30 in two parts separately, the gap about half width to whole width of each part (Fig. 6C & H). Comb of segment VIII composed of 3–8 golden spines (Fig. 6E–G), often with one largest spine posteriorly. Lateral taeniae of segments IV–VIII: 0, 3, 3, 4, 4. Mensural features as in Table 2.

Remarks. Larvae of *P. masudai* cannot be separated with certainty from other members of *P. bicrenatum* group in Europe (Kiknidze et al. 1999, Klink 2002) or *P. halterale* group in North America (Epler 2001) which has a similar reduced third and fifth antennal segment. However, the heavy sclerotized dark postmentum and the relative low position of 4th mental laterals is distinctive compared to all described *Tripodura* larvae. Pupae of *P. masudai* resembles *P. digitifer* Townes 1945 in the reduced median spinulation on tergites III–VI and posterior transverse band with the relatively well separated two patches, but the latter can be separated by the reduced cephalic tubercles (Soponis & Simpson 1992).

Biology. Larvae of *P. nodosum* are found in the bottom sediment of lentic waterbodies (Johannsen 1932, Tokunaga 1964, Sasa & Hasegawa 1983). Our collections from the Macau ponds came from substrates consisting of dense detritus and fine mud, perhaps representing a mesotrophic to eutrophic condition. Other common chironomids in these ponds included *Chironomus flaviplumus* Tokunaga 1940, *Glyptotendipes tokunagai* Sasa 1979, *Harnischia longispuria* Wang & Zheng 1993, *Tanytarsus formosanus* Kieffer 1912 and *T. oscillans* Johannsen 1932. Larvae occur massively in shallow areas during colder months, but in deeper areas during the summer.

In Thailand a survey across hundreds of water bodies, including standing, running and waterfalls, larval *P. nodosum* were found only in Andaman Sea coastal pools that were created as post-impact features of the 2004 Indian Ocean tsunami (Cranston 2007, Sites & Vitheepradit 2010). Sites varied in conductivity from 45 to 1650 µS and with dissolved solids from 23 to 821 ppm. The species was not found in any unimpacted site across the whole country (Cranston 2007). Evidently the species tolerates high levels of salinity and nutrients, but also can be a pioneering or colonizing species, being found in artificial tanks and water storage reservoirs in Singapore and
artificial research channels in Queensland, Australia (P.S. Cranston *pers. obs*.). The species is found only in warm waters (Thailand 28–32°C) and is associated generally with tropical to subtropical regions of Asia and Australasia.

After checking all the published collecting sites from Okinawa to Queensland, we found that the distribution of *P. nodosum* is almost entirely restricted to the lentic habitat where the site is very close to ocean or coastal regions. All collected dates of pupae and adults indicated that the species is multivoltine, emerging from autumn to the next spring.

Specimens of *P. masudai* described here were collected from Macau ponds and the Hejiang River, Guangxi, thus it perhaps can inhabit both lentic and lotic water bodies. Those sites usually had muddy to mud-sandy substrates. Based on the seasonal investigation in Macau, it appears that *P. masudai* is one of the relatively abundant macroinvertebrate species in the cold months. Emergence records indicate that the species is multivoltine. Adults emerge from January to March in Macau and in August in Hejiang, Guangxi.

Conclusions

Pupae of *P. nodosum* can be distinguished from other *Polypedilum* by tergite VII with anterior and paired posterior spine patches, and each wing pad with a distinct nose. The larvae can be distinguished with certainty from other *Polypedilum* larvae by the clear 3 inner mandible teeth. The relative lengths of antennal segments 3 and 4 may prove to be valid characters for species separation.

Pupae of *P. masudai* can be separated from other members by the reduced median spinulation on tergites II–V and relatively well developed cephalic tubercles and frontal setae. Larvae of this species have relatively well developed segments 2 and 4, and reduced segments 3 and 5. The larvae can be readily separated from others by comparing the antenna and darker postmentum.

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