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Polypedilum parthenogeneticum (Diptera: Chironomidae): a new parthenogenetic species from **Eryngium** L. (Apiaceae) phytotelmata

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***Polypedilum parthenogeneticum* (Diptera: Chironomidae): a new parthenogenetic species from *Eryngium* L. (Apiaceae) phytotelmata**

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All life stages of *Polypedilum parthenogeneticum* sp. n. are described and figured. The larva lives in the water held in the leaf axils of *Eryngium pandanifolium* Chamb and Schlecht (Apiaceae). The species is considered to be parthenogenetic as only females were obtained by rearing in the laboratory. Preliminary notes on the biology of *P. parthenogeneticum* are given.

Keywords: Chironominae; parthenogenesis; Neotropics; systematics

Introduction

The genus *Polypedilum* Kieffer, 1913 belongs to the tribe Chironomini of the subfamily Chironominae. This is a cosmopolitan genus, occurring in all zoogeographical regions except Antarctica. *Polypedilum* is a heterogeneous group, and the larvae occur in all standing and flowing waters, except at high altitude and latitude (Vårdal et al. 2002). *Polypedilum* larvae are most commonly found in sediments, with a few species mining wood or grazing on epilithic surfaces (Cranston et al. 1989). Larvae of some species co-inhabit pupal retreats of *Cheumatopsyche* caddisflies (Sæther and Sundal 1999). The genus is quite well known in the Neotropics (Edwards 1931; Townes 1945; Fittkau and Reiss 1979; Roback and Coffman 1983; Sublette and Sasa 1994; Bidawid and Fittkau 1995; Bidawid-Kafka 1996; Spies and Reiss 1996; Sæther and Sundal 1999) with approximately 63 described species but several more awaiting description (Sæther and Sundal 1999).

Eryngium L. (Apiaceae) is a cosmopolitan genus of perennial, rhizomatous herbs, comprising more than 220 species in temperate and tropical regions (Cabrera and Zardini 1978). Twenty-five species are known from Argentina, 17 of which occur in Buenos Aires Province (Cabrera and Zardini 1978). Some species of *Eryngium* known from Argentina and Brazil are able to catch and hold water in their leaf axils. The water held in the leaf axils constitutes a particular habitat named phytotelmata; some terrestrial plants are also able to impound water in other structures such as modified leaves, flowers, stem holes or depressions, open fruits, and fallen leaves (Fish 1983). In the Neotropics, few species of Chironomidae are known to inhabit phytotelmata (Epler and Janetzky 1999; Mendes et al. 2003; Donato and Paggi 2005; Pinho et al. 2005).

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Larvae of *Polypedilum* collected from the leaf axils of *Eryngium pandanifolium* Chamb and Schlecht (Apiaceae) were reared in the laboratory. All the imagines obtained were females; in addition, no male adults were collected in the vicinity of the plants. These facts prompted the hypothesis that this species might be parthenogenetic. In order to verify this assumption new material was collected and reared, and the female imagines were kept alive after emergence. Approximately 24 hours later, the females were observed to oviposit, and hatching occurred one day later. The larva, pupa, and female imagines were compared to the known *Polypedilum* species. It was concluded that this entity deserved the category of species on the basis of characters derived from both pupa and female imago. Therefore, the goals of this study are to describe the new species *Polypedilum parthenogeneticum* and to provide preliminary notes on its biology.

Materials and methods

Collected larvae were reared following the procedure outlined in Donato and Paggi (2005) at room temperature (20–25°C). In order to obtain information about the biology of the new species, one *Eryngium pandanifolium* plant was collected from the field and dissected, and the *Polypedilum* larvae held were separated and counted. All the larvae were reared, and after emergence and oviposition of the female imagines, the egg-masses obtained were transferred into Petri dishes. The Petri dishes were checked daily for developmental stages. Larvae, pupae, and adults were fixed in 70% ethanol and mounted on microscope slides in Canada balsam. Morphological nomenclature follows Sæther (1980). Counts, measurements and ratios are given as ranges, followed by the mean. The number of specimens measured (*n*) is given in parentheses. Measurements are in μm except when otherwise stated.

The holotype and paratypes are deposited at Museo de La Plata (MLP) (Buenos Aires, Argentina) and Instituto de Limnología “Dr. Raúl A. Ringuelet” (ILPLA) (Buenos Aires, Argentina).

Taxonomy

Polypedilum parthenogeneticum sp. n.

Type material. Holotype ♀ with larval and pupal exuviae, ARGENTINA: Buenos Aires, Mar de Ajó, 27- I- 2006, leg. M. Donato (MLP).

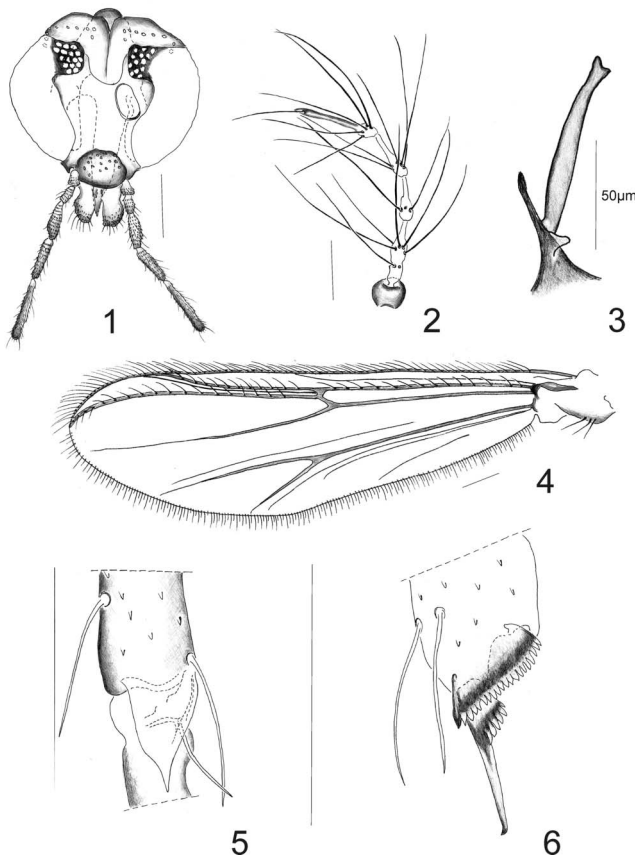
Paratypes. ARGENTINA: 2 ♀ with larval and pupal exuviae, Buenos Aires, Mar de Ajó, 27- I- 2006, leg. M. Donato (MLP); 1 ♀ with larval and pupal exuviae, Buenos Aires, road to Punta Lara and Municipal Dump, 34° 51.09' S– 57° 57.543'W, 26- IX- 2004, leg. M. Donato (ILPLA); Buenos Aires, Punta Lara, Boca Cerrada, 19- VIII- 2004 (ILPLA); 3 ♀ with larval and pupal exuviae, 1 ♀ with larval exuviae, Buenos Aires, road to Punta Lara and Municipal Dump, 34° 51.09' S– 57° 57.543'W, 4- VII- 2004, leg. M. Donato (MLP); 1 larval and 1 pupal exuviae, same data (ILPLA); 1 ♀ with pupal exuviae, same data except 26- IX- 2004 (ILPLA); 8 ♀ with pupal exuviae, 1 ♀, 1 ♀ with larval exuviae, and 2 larval exuviae, Buenos Aires, Mar de Ajó, 27- I- 2006, leg. M. Donato (MLP); 2 ♀ with larval and pupal exuviae, 8 ♀ with pupal exuviae, 1 ♀ with larval and pupal exuviae, 1 larval and 1 pupal exuviae, same data (ILPLA); 1 ♀, Buenos Aires, Magdalena, Ea. El Destino, 35° 07.637' S– 57° 23.105' W, 3/4- X- 2004, leg. M. Donato (MLP); 1 ♀ with larval exuvia and 1 pharate ♀, Argentina, Buenos Aires, Sauce Grande, 13- XII- 2005, leg. R. Campos (MLP); 1 ♀ with pupal exuviae (MLP), Argentina, Buenos Aires, A° Buñirigo, 35° 08'

37.4''S– 57° 34' 13.6''W, 13- XI- 2006, leg. M. Donato (MLP); 1 ♀ with pupal exuviae, same data (ILPLA). URUGUAY: 1 ♀ with larval and pupal exuviae, Colonia del Sacramento, Balneario Ferrando, 10- X- 2004, leg. M. Donato (MLP); 1 ♀ with pupal exuviae, Colonia del Sacramento, Balneario Ferrando, 10- X- 2004, leg. M. Donato (ILPLA); 1 ♀ with pupal exuviae, Uruguay, Sierras de Aceguá, 31° 51.429' S– 54° 19.230' W, leg. M. Donato (MLP).

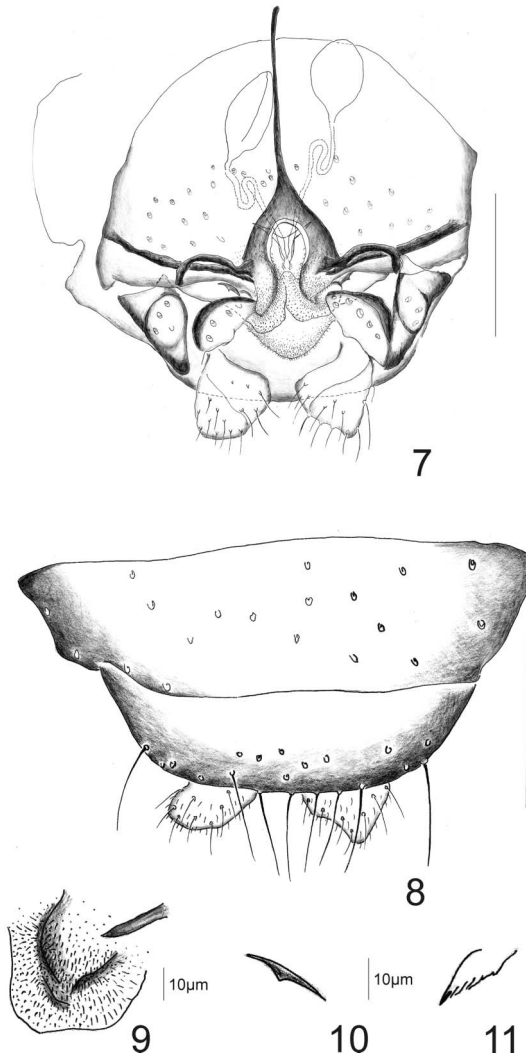
Female imago ($n = 27$, except when otherwise stated) (Figures 1–11)

Total length 2.70–3.97, 3.08 (25) mm, wing length 1.84–2.73, 2.11 mm; width 0.58–0.75, 0.64 mm. Total length/wing length 1.36–1.62, 1.43 (26). Wing length/length of profemur 1.84–2.12, 1.97. Colouration blackish brown with legs whitish.

Head (Figure 1). AR 0.44–0.57, 0.51 (26). First two flagellomeres fused (among 27 specimens examined, two specimens with five flagellomeres, the remaining specimens with flagellomeres I and II fused) (Figure 2). Length of flagellomeres: I 30–46, 36 (2); II 100–123, 111 (2); I + II 150–208, 182 (25); III 115–150, 128 (27); IV 113–150, 131 (27);



Figures 1–6. *Polypedium parthenogeneticum* sp. n. Female imago (1) head, frontal view; (2) antenna; (3) tentorium and stipes; (4) wing; (5) foreleg scale; (6) hind leg spur. Scale bar = 100 µm, except when otherwise stated.



Figures 7–11. *Polypedilum parthenogeneticum* sp. n. Female imago (7) genitalia, ventral view; (8) genitalia, dorsal view; (9) dorsomesal lobe; (10) apodeme lobe; (11) ventrolateral lobe. Scale bar = 100 μm , except when otherwise stated.

V 195–280, 224 (26). Temporal setae 8–13, 10 (25). Clypeus with 11–31, 17 (22) setae. Tentorium and stipes as in Figure 3. Tentorium 120–225, 166 (25) long; 20–38, 26 (25) wide. Length of palp segments: 28–45, 38 (26); 31–63, 47; 80–143, 101; 100–138, 110; 153–200, 174 (23).

Thorax. Dorsocentrals 6–10, 8 with 2–6, 4 on humeral area; acrostichals 7–13, 10; prealars 2–4, 3. Scutellum with 5–8, 6 setae.

Wing (Figure 4). VR 1.04–1.15, 1.1. Brachiolum with 1 seta. R with 12–23, 17; R_1 with 8–17, 12; R_{4+5} with 18–34, 26. Squama with 2–5, 3 setae.

Legs. Scale of foreleg as in Figure 5. Spur of middle tibia: 23–48, 33 (16) and 68–93, 81; of hind tibia: 21–98, 40 (26) and 78–105, 88 (26) μm long (Figure 6). Width at apex of foretibia 60–75, 67; of middle tibia 60–78, 68; of hind tibia 70–90, 78. Lengths (in μm) and proportions of legs in Table 1.

Genitalia (Figures 7–11). Gonocoxite IX rounded at end with 2–4, 3 setae. Gonapophysis VIII with dorsomesal lobe large and rounded (Figure 9), ventrolateral lobe very difficult to see and not conspicuously brush-like (Figure 11). Apodeme lobe slightly curved and partially covered by dorsomesal lobe (Figure 10), Sternite VIII with 31–51, 40 (26) setae. Segment IX with 17–32, 23 (21) setae. Segment X with 3–6, 4 (26) setae on each side. Cercus 100–175, 136 (25) μm long. Seminal capsule 80–120, 96. Notum 185–268, 218 μm long.

Pupa (*n* = 25 except when otherwise stated) (Figures 12–15)

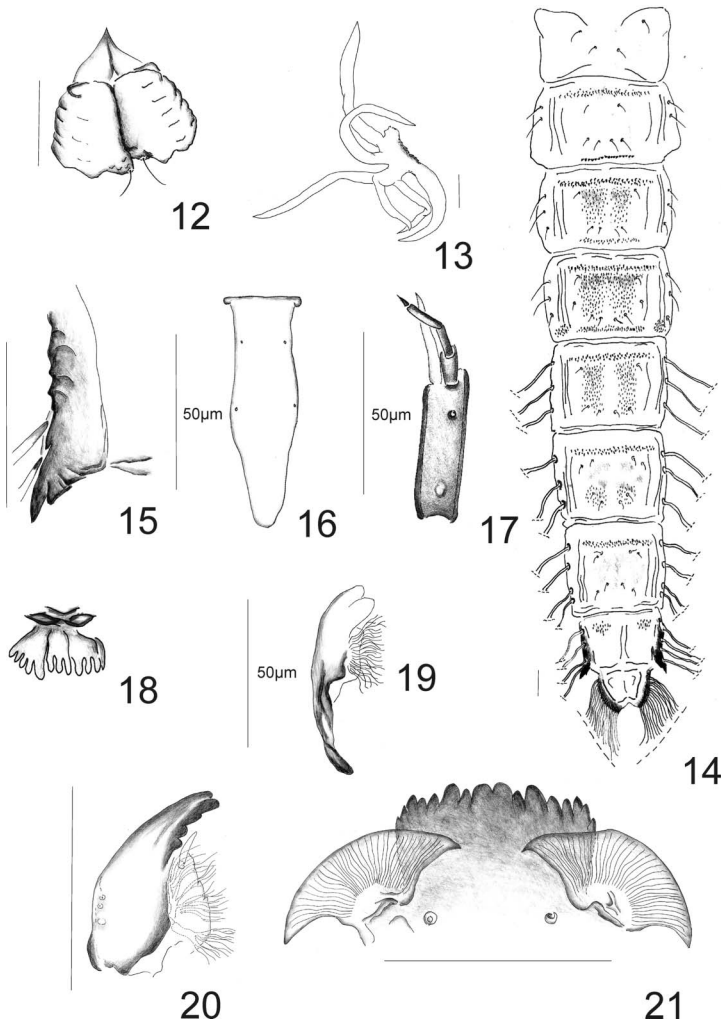
Total length 4.49–6.35, 5.74 (5) mm; length of cephalothorax 1.06–1.49, 1.24; length of abdomen 3.39–4.91, 4.44 (5). Exuviae hyaline.

Cephalothorax. Frontal apotome with cephalic tubercles and frontal setae short (Figure 12). Prealar tubercle absent. Thoracic horn with four main branches with only one branch subdivided (Figure 13). Precorneals 2, length of one precorneal 20–43, 30 (5), lateral anteprenotals 1, median anteprenotals 1 Distance between Dc1 and Dc2 1–8, 3 (24); between Dc2 and Dc3 219–295, 258 (24); between Dc3 and Dc4 1–10, 5 (24). Length of one Dc 25–90, 47 (9).

Abdomen. Tergite I bare; tergites II–VII with strong anterior band of spinules. Shagreen as shown in Figure 14. Tergite II with a single row of 39–54, 47 (5) caudal hooklets. Conjunctives III–IV and IV–V with spinules. Pedes spurii A on IV. Pedes spurii B well developed on II. Spur of Tergite VIII as in Figure 15. Anal lobe length 228–356, 308 (7), genital sac length 188–329, 261 (7). Anal lobe with dorsal setae inwards the fringe.

Table 1. Lengths (in μm) and proportions of legs of *Polypedilum parthenogeneticum* sp. n. (*n* = 24–27).

	Fe	Ti	Ta ₁
P ₁	912–1317, 1069	557–861, 646	1089–1393, 1232 (<i>n</i> = 24)
P ₂	963–1368, 1113	760–1165, 887	507–735, 555 (<i>n</i> = 24)
P ₃	1089–1596, 1261	887–1317, 1025	735–988, 825 (<i>n</i> = 24)
	Ta ₂	Ta ₃	Ta ₄
P ₁	661–864, 742 (<i>n</i> = 24)	478–610, 520 (<i>n</i> = 24)	336–437, 375 (<i>n</i> = 24)
P ₂	275–386, 310 (<i>n</i> = 24)	203–285, 230 (<i>n</i> = 24)	122–173, 142 (<i>n</i> = 24)
P ₃	376–539, 429 (<i>n</i> = 24)	366–509, 411 (<i>n</i> = 24)	214–295, 242 (<i>n</i> = 24)
	Ta ₅	LR	BV
P ₁	153–203, 172 (<i>n</i> = 24)	1.57–2.1, 1.93 (<i>n</i> = 24)	0.62–0.69, 0.65 (<i>n</i> = 24)
P ₂	81–122, 102 (<i>n</i> = 24)	0.51–0.8, 0.64 (<i>n</i> = 24)	1.21–1.42, 1.56 (<i>n</i> = 24)
P ₃	102–153, 127 (<i>n</i> = 24)	0.76–0.97, 0.81 (<i>n</i> = 24)	0.96–1.17, 1.02 (<i>n</i> = 24)
	SV		
P ₁	1.25–1.65, 1.37 (<i>n</i> = 24)		
P ₂	2.79–4.31, 3.51 (<i>n</i> = 24)		
P ₃	2.36–2.9, 2.75 (<i>n</i> = 24)		



Figures 12–21. *Polypedilum parthenogeneticum* sp. n. Pupa (12) frontal apotome with cephalic tubercle, ventral view; (13) thoracic horn; (14) abdomen, dorsal view; (15) spur of tergite VIII. Larva (16) frontal apotome; (17) antennae; (18) pecten epipharyngis; (19) premandible, ventral view; (20) mandible, ventral view; (21) mentum and ventromental plates, ventral view. Scale bar = 100 μm , except when otherwise stated.

Larva ($n = 14$ except when otherwise stated) (Figures 16–21)

Total length 5.09–6.81, 6.17 (4) mm. Head capsule 350–408, 380 μm long. Colouration uniformly red.

Head. Frontal apotome as in Figure 16, frontal warts well developed, cephalic tubercles weakly developed bearing a frontal seta at base of frontal apotome. Lengths of antennal segments (in μm): 55–68, 61 (13); 18–28, 23 (12); 11–15, 13 (12); 13–23, 18 (12); 2–10, 7 (12). AR 0.89–1.28, 1.01 (12). Basal antennal segment 20–28, 24 (13) μm wide; distance from base to ring organ 8–25, 14 (10); to basal mark of seta 30–50, 40 (12); blade 30–50, 38 (9) (Figure 17). Setae I plumose; pecten epipharyngis with serrate platelets, lateral platelets

with 4–11, 5 (11) teeth and median platelet with 3 (11) teeth (Figure 18). Premandible as in Figure 19. Mandible (Figure 20) 148–175, 160 μm long, with 3 inner teeth and one dorsal tooth; seta sub-dentalis well developed; setae interna with 4 branches. Mentum (Figure 21) with first lateral teeth lower than median and second lateral teeth. Postmentum 208–264, 238 μm long.

Discussion

Biology

Forty-two *Polypedilum parthenogeneticum* specimens in L2-L3, L4 and pupae stages were found in the *Eryngium pandanifolium* plant. Twenty-eight L4 were reared and 24 L4 reached adult stage. These adults were checked daily and their ovipositions were collected. The new generation was followed up to L1.

The larval case is a tube that may be simple or bifurcate and horizontally and/or vertically positioned. It is built from material trapped in the silk net constructed by the larva and material collected by the latter from the surrounding substratum. The larva of *P. parthenogeneticum* is a typical collector-gatherer, and the material collected is used both as food and for case building.

The pupae of *P. parthenogeneticum* remain in the larval case until emergence of the imago and approximately 24 hours later reached adult stage.

The reared females laid their egg-masses 12–24 hours after eclosion on the wall of the glass tube, near the water surface. Eggs are laid as a sinuous single row in a gelatinous sheath. *P. parthenogeneticum* is capable of producing up to three egg-masses. Twenty-four females laid egg-masses as follows: 2 ovipositions, 50% ($n = 12$); 1 oviposition, 37.5% ($n = 9$); 3 ovipositions, 8.33% ($n = 2$); no oviposition, 4.16% ($n = 1$). Total number of eggs per oviposition averaged 88 (min. 37, max. 123).

The first-instar larvae hatched three days (min. 1, max. 5) after oviposition. The larvulae spent several hours within the mass feeding on the gelatinous sheath and attempting to escape from it. Once free, the larvulae moved toward the substratum and rapidly started to build a larval case.

Distribution and ecological features

Polypedilum parthenogeneticum occurs in Buenos Aires province, Argentina, and in Uruguay, where it inhabits the phytotelmata of *Eryngium pandanifolium* Cham & Schlecht. The water impounded in *E. pandanifolium* is characterised by having pH 5.6–6.5; temperature range between 9°C and 12°C in winter, and 21°C and 23°C in spring, with a mean of 26°C in summer; relatively low concentrations of O₂ and high concentrations of dissolved CO₂ (Vucetich and Rossi 1980). Accompanying fauna that also occurred in *Eryngium* phytotelmata included *Metriocnemus eryngiotelmatus* Donato and Paggi (2005, p. 3) (Chironomidae: Orthocladiinae); *Culicoides charruus* Spinelli and Martinez (1991, p. 176) (Diptera: Ceratopogonidae); *Culex hepperi* Casal & Garcia, *Culex renatoi* Lane & Ramalho, and *Culex castroi* Casal & Garcia (Diptera: Culicidae) (Campos and Lounibos 1999); and an undetermined Psychodidae larva (Diptera).

Systematics

The genus *Polypedilum* has been divided into six subgenera: *Asheum* Sublette and Sublette; *Cerobregma* Sæther and Sundal; *Pentapedilum* Kieffer; *Polypedilum s. str.*, with two species

groups, the *nubeculosum* and the *nubifer* groups; *Tripodura* Townes; and *Uresipedilum* Sasa and Kikuchi (Sæther and Sundal 1999). The species belonging to this genus may either fall into one of these subgenera at all stages without any ambiguity, or key to one subgenus at one stage and a different one at another stage. Because of this, the different stages of *P. parthenogeneticum* will be discussed separately.

The larvae of *P. parthenogeneticum* keys out in the dichotomy of the subgenus *Polypedilum* s. str. in the subgenera key of Sæther and Sundal (1999). The only member of this subgenus present in the Neotropical region for which the larval stage has been described is *Polypedilum (Polypedilum) corniger* Sublette & Sasa (1994). This species differs from *P. parthenogeneticum* by having higher AR (1.72) and setae sub-dentalis hooked at the tip. *P. parthenogeneticum* keys in the dichotomy identifying *Polypedilum (Polypedilum) trigonus* Townes and *Polypedilum (Pentapedilum) tritum* (Walker) in the key of Epler (2001). *P. (P.) trigonus* is easily distinguishable from *P. parthenogeneticum* by its 4th lateral tooth of mentum which is lower than the 3rd and 5th ones, and by its large and distinct Lauterborn organs.

The pupa of *P. parthenogeneticum* keys out *Polypedilum* s. str. in the key to subgenera by Sæther and Sundal (1999). These authors describe bands of spinules on conjunctives III–IV and V–VI for subgenus *Polypedilum* s. str. Nevertheless, *P. parthenogeneticum* has bands of spinules on conjunctives III–IV and IV–V, similarly to the condition in the subgenus *Uresipedilum*. The pupa of *P. parthenogeneticum* is closely related to the *Polypedilum* sp. 8 pupa of Wiedenbrug (2000) sharing all of its characters except the thoracic horn and abdominal shagreen pattern. The thoracic horn in the latter species consists of two main branches, one of them with one subdivision and the other with two successive subdivisions.

Sæther and Sundal (1999) have listed several features to be used in the separation of subgenera for *Polypedilum* adult females. These authors pointed out that because very few female imagines are described and associated, it is not known whether the characters they mentioned are of taxonomic value. The female genitalia of *P. parthenogeneticum* clearly fits in the description of *P. (Cerobregma)*, the dorsomesal lobe is rounded, the gonocoxapodeme straight and ending at the level of the dorsomesal lobe, the ventrolateral lobe reduced and very difficult to see, and gonocoxite IX bears few setae.

Parthenogenesis

Parthenogenesis is the development from an egg without paternal genetic contribution. There are many modes of parthenogenesis that are associated with a wide variety of cytological mechanisms (tychoparthenogenesis, apomictic parthenogenesis, automictic parthenogenesis, gynogenesis, hybridogenesis). Generally speaking, parthenogenesis in Chironomidae is a strategy adopted to survive under extreme environmental conditions, mainly cold habitats that freeze (e.g. high altitudes and latitudes) (Edward 1963; Downes 1965; Cranston 1985; Crafford, Scholtz and Chown 1986; Langton 1999; Delettre, Frenot, Vernon and Chown, 2003; Jones, Chown, Webb and Gaston 2003; Lencioni 2004; Nondula, Marshall, Baxter, Sinclair and Chown 2004) and temporary environments (Dettinger-Klemm and Boehle 1996).

Clonal reproduction has clear advantages over sexual reproduction. The growth rate of clonal lineages should exceed that of sexual lineages because all individuals are able to reproduce unlike in the case of sexual lineages, which contain males. Clones should also have a colonisation advantage (Baker 1955), because populations can be established from single individuals, unlike sexual lineages where the group of colonisers has to include

both males and females (Jokela et al. 2003). These features could explain the occurrence of *P. parthenogeneticum* in the phytotelmata of *Eryngium pandanifolium*. The axils of this plant catch and accumulate rainwater and floodwater when the surrounding terrain is inundated. As a characteristic of this particular environment, the standing water varies seasonally and tends to dry up in summer (Campos and Lounibos 1999). Future studies will elucidate the strategy adopted by *P. parthenogeneticum* to survive in these extreme environmental conditions.

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