A new putatively cryptobiotic midge, *Polypedilum ovahimba* sp. nov. (Diptera: Chironomidae), from southern Africa

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Abstract A second species of *Polypedilum* (Diptera: Chironomidae) is inferred to tolerate desiccation in an African rock pool habitat similar to the well-known anhydrobiotic *Polypedilum vanderplanki* Hinton. The species, *Polypedilum ovahimba* sp. nov., is described and illustrated as new to science from Namibia.

Key words anhydrobiosis, cryptobiosis, new species, taxonomy.

INTRODUCTION

The ability to suspend metabolism, termed cryptobiosis, is known among certain invertebrate animals notably bdelloid rotifers, tardigrades, some nematodes, artemiid brine shrimps and in only one insect. That this insect, *Polypedilum vanderplanki* Hinton (Diptera: Chironomidae), withstands dehydration has been known since Howard Hinton (1960) experimented with larvae from the natural environment in West Africa. In an anhydrobiotic state, larval *P. vanderplanki* tolerates extreme temperatures (high and low), anoxia, pure ethanol, vacuum, pressure and radiation (Watanabe *et al.* 2006; Horikawa *et al.* 2009). The chironomid midge develops typically for a holometabolous aquatic insect – oviposition occurs into small pools on granitic outcrops, and larval development commences with the larva grazing on the substrate and building tubes ('nests') into which larvae retreat when not feeding. Development conforms to that of all other chironomids in that there are four instars prior to pupation in which metamorphosis is rapid, followed by emergence at the water surface.

In long-lived and permanent water bodies, the life-cycle of chironomids is continuous with or without synchronisation, and with the possible intervention of environmentally induced diapause (cessation of development with reduced metabolism). Especially in ephemeral or intermittent water bodies, larvae of some species encase themselves in cocoons within which their body water content may be reduced, but the larvae are not highly desiccated. Aspects of this phenomenon were reviewed, as aestivation, by Cranston *et al.* (2007) in describing over-summer survivorship in a winter–spring seasonally inundated anabranch of the Sacramento River, California.

Resistant cocoons are quite widespread in the Chironomidae (e.g. Jones 1975; Grodhaus 1980; Kondo 1996). Tolerance of loss of up to 70% water loss in Australian *Paraborniola tonnoiri* Freeman was reported by Jones (1975). Although drought tolerance in Chironomidae was reviewed by Frouz *et al.* (2003), the physiological processes involved in cocoon-building midges remains poorly known. In contrast, mechanisms for drought tolerance in cryptobiosis via anhydrobiosis are increasingly well understood, notably due to studies by the Anhydrobiosis Laboratory of Takashi Okuda, Tsukuba, Japan (<http://www.nias.affrc.go.jp/anhydrobiosis/Sleeping%20Chironimid/e-index.html>, for example, Kikawada *et al.* 2005; Sakurai *et al.* 2008; Cornette & Kikawada 2011, Gusev *et al.* 2011). However, thus far, this phenomenon remains known only for *Polypedilum vanderplanki*.

The discovery of a second species of African *Polypedilum* inferred to resist seasonal desiccation of its rockpool habitats may be important to allow physiological comparisons with *P. vanderplanki*. The species is described as new to science here, is distinguished from *P. vanderplanki*, and notes are provided suggesting desiccation tolerance.

MATERIALS AND METHODS

While seeking Chironomidae for molecular phylogenetic studies, especially of the podonomine genus *Archaeochlus* (Cranston *et al.* 2010), ephemeral southern African rock pools were examined for their insect inhabitants. The Waterberg, an isolated massif in central north-central Namibia (20°39'S 17°35'E) is a rocky plateau with many hollows that fill with seasonal rains to form ephemeral pools. On 1–2 January 2004, the first seasonal rains fell as thunderstorms, making access difficult but filling with rainfall the numerous holes on the plateau above the escarpment. Several pools, although water

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filled for maximally 3 days, already contained actively wriggling bright red chironomid larvae. Specimens were vialled individually, and individual rearing attempted. No pupal exuviae were found on pool surfaces nor were adults encountered on the surrounding low vegetation.

The same area was visited in late December 2005, prior to onset of seasonal rain. Some contents of 10 dry former pools in a close-by location were aggregated into a container and returned unwetted to Rhodes University, Grahamstown, Eastern Cape, South Africa. Water was added to a subsample of the dry material, which was maintained hydrated for 2 weeks. Daily inspections were made and live biota removed.

All Chironomidae specimens were preserved in molecular grade ethanol. Microscope slides were made according to standard protocols (Andersen *et al.* 2013) and are preserved in the British Museum (Natural History) – Natural History Museum, London, UK. Photographs were obtained using a Leica® DMRX compound microscope with Nomarski® interference optics. Photographs were taken with an Automontage™ system, allowing automated retention of focused parts of a sequence of exposures at different focal depths. Minor subsequent manipulations were made in Adobe® Photoshop™. Drawings were made by hand using a drawing tube – some redundancy is due to expressed user disagreement over the value of each source of images.

Morphological measurements, unless otherwise stated, are in micrometre rounded to the nearest 5 µm except if measurement at maximum magnification (oil immersion, x1000) provided accuracy to ±1 µm. Abbreviations: AR, antennal ratio (ultimate flagellomere divided by combined preceding flagellomeres); BMNH – British Museum, Natural History, London, UK; Larva; Le, Larval exuviae; Le/Pe/m, reared adult male (female) with associated larval and pupal exuviae; LR, leg ratio: Tarsomere 1 length: Tibia length; MV, molecular voucher; P, pupa, Pe, Pupal exuviae.

Grid references and elevations are derived from Google™ Earth.

RESULTS

Larvae collected live in 2004 from the Waterberg rock pools belong to *Polypedilum* v.d. Wulp, as defined and diagnosed (Pinder & Reiss 1983; Epler *et al.* 2013). Rearing of some larvae to pupa and adult (or pharate adult, incompletely emerged within pupa) revealed that pupae conform to the diagnosis of Pinder and Reiss (1986), as did the adult male to the diagnosis of Cranston *et al.* (1989). The adult male hypopygium, which provides generally consistent identificatory features resembled no described species from the Afrotropical region, and differing strongly from that of *P. vanderplanki* (Freeman 1958; Hinton 1960).

The dried sample collected late in 2005 was wetted on 31 December 2005. Initially, activity of nematodes and oribatid mites were observed, but on the 10th day, few red chironomid larvae were seen. One pharate pupa and live larvae had to be

sacrificed as it could not be taken through biosecurity on imminent departure from Africa. Slide mounted larvae were identical to those collected the year previously, and measurements of the mentum width showed they belonged to the fourth instar. Fine sieving and high-power inspection of much remaining dry material revealed no larvae (desiccated, in cocoons or otherwise), and none were seen after addition of water to the remaining parts (in Japan, by T Okuda, pers. comm. 2005).

Specimens sent to Davis, California (under code MV NBPP) did not provide amplifiable DNA (G Morse pers. comm. 2005).

SYSTEMATICS

Polypedilum ovahimba sp. nov. (Figs 1–3)

ZooBank registration: <http://zoobank.org/urn:lsid:zoobank.org:pub:C87BCDFC-F7D5-4448-B886-C01D1FA47F7A>

Types. *Namibia.* Holotype: Le/Pe/m, Waterberg Plateau, Mountain View, above campground/resort, ex-rock-pool, 3.i. 2004, 20°30'17'S 17°14'27'E, 1620 m. asl, Cranston, BMNH. Paratypes, 2 L, Le/Pm, Pm, as holotype; L/Pf, *Namibia*, Waterberg Plateau, Otjosongombe Farm, 20°28'35'S 17°16'137'E, 1600 m asl., 31.x.2005-9.i.2006. BMNH.

Description. Conforms in morphology of all stages to the generic diagnoses for larva (Epler *et al.* 2013), pupa (Pinder & Reiss 1986), adult males (Cranston *et al.* 1989) and adult females (Saether 1977).

Male ($n = 1 + 2$ pharate). Body length 4 mm, brown without darker vittae. Wing unmarked, bare, length 1.5 mm ($n = 1$). Flagellomeres 1–12, 330–370, flagellomere 13, 360–400, AR 1.05–1.10. Frontal tubercles absent. Head with 10–13 verticals + postorbitals linearly aligned, 12–15 strong clypeals, palp segment lengths 2–5, 40; 60–68; 60–65; 88–125. Thorax without anteprenotals, with six acrostichals, five to six dorsocentrals, three to six prealars, six to nine scutellars. Legs pale, unmarked, leg ratios (inc. pharate within sheaths) foreleg 1.1–1.15, mid-leg 0.35–0.38, hind leg 0.43–0.50; foretibial apex with well-developed triangular lobe terminating in darkened spine (Fig. 2a), length 43, mid-leg with narrow inner comb and broad outer comb with spur, length 36; hind leg with broad inner comb, narrower outer comb with long spur, length 45. Wing setation: R with 8–10, R₁ with six, R₄₊₅ with four to five subapical, six to seven squamals.

Genitalia (Fig. 1a–f) with tergite IX bands weak, meeting at base of anal point, three to five median dorsal tergal setae arising from pale area(s); posterior margin of tergite IX with 18–21 marginal setae each side of anal point. Anal point (Fig. 1a,b,e) arising from sub-posterior margin of tergite IX, dark at base, pale hyaline apically, spatulate in dorsal view (very narrow basally, dilate distally), 65 long. Inferior volsella (Fig. 1a,b) club shaped, curved dorsally; basally and ventrally microtrichiose, with increasing number and density of strong setae on dorsal and lateral dilate apical section ('club-head'), lacking a distinctive strong apical seta. Superior

Fig. 1. *Polypedilum ovahimba* new species, male genitalia: (a) hypopygium, dorsal, slightly flattened; (b) anal point, superior and inferior volsellae, detail; (c) gonocoxite and gonostylus; (d) gonostylus, lateral; (e) anal point, lateral; (f) superior volsella, detail.

volsella (Fig. 1f) large, subrectangular/subovoid, densely microtrichiose with medio-basal cluster of five to six setae and near hyaline, medially directed digitiform extension tapering to blunt point, without associated setae. Gonocoxite 110; gonostylus 150, narrow basally, broadened medially and tapering slightly to round apex, without any specific internal mesal-directed setae on subapex (Fig. 1c).

Female ($n=1$, pharate). Body length c 3 mm. Colour undeterminable. Flagellomeres 1–6: 20, 34, 34, 30, 23, 50, antennal ratio 0.36. Head with 10–11 verticals + postorbitals linearly aligned, 25–27 strong clypeals, palps teneral. Thorax without anteprenotals, with five acrostichals, five to six dor-

socentrals, four prealars, eight scutellars. Genitalia typical for *Polypedilum*.

Pupa ($n=3$, partly with pharate adult within). Length c. 6 mm, pale, with apophyses indistinct. Cephalothorax. Frontal tubercles absent, frontal seta not detectable. Thorax weakly creased, non-rugose. Thoracic horn hyaline, three or four branched, non-spinose, base simple, small, circular.

Abdomen. Tergal armament as in Figure 3a, tergite I without sternal or tergal armament. Hook row comprising 56 hooks in mostly uniserial row, extending 56–60% of width of tergite II. Tergites II–VI with anterior transverse band of spines connected to medial spine areas only on VI; II no median or

Fig. 2. *Polypedilum ovahimba* new species: (a) fore-tibial spur, male; (b) posterolateral comb, *P. ovahimba*, pupa; (c) posterolateral comb, *P. vanderplanki*, pupa; (d) antenna, larva; (e) mandible, larva; (f) mentum, larva.

posterior spines, III very sparse medial spine patch, IV and V with median patches and medially divided posterior transverse area. Conjunctive III bare; and IV partially aligned multiserial rows of spines. Caudolateral spur of VIII (Figs 2b,3b) of three to four basally fused spines, one much stronger than the others. Anal lobe bare, without dorsal seta, with uniserial fringe of 30–36 taeniae. Pedes spurii A not visible. Pedes spurii B very well developed on II, absent on III. Taeniate lateral setae conventional for genus – 3,3,4,4 (V–VIII).

Larva ($n = 1-2$). Length c. 6 mm, head capsule length c. 450–480, pale yellow, with teeth of mentum, apex of mandible and all teeth golden-brown, occipital margin pale yellow, labral margin and premandible pale yellow. Dorsal surface of head

(Fig. 3c). Fronto-clypeal apotome anteriorly broadened, with cephalic seta S3 inserted subterminally, posterior to 10 wide hyaline area. Antenna (Figs 2d,3d) with segment lengths, 45: 12: 10: 12–13: 5, AR 1.1–1.2; Lauterborn organs slender, opposite, seven to eight long; style slender 10 long; blade length 50, not extending beyond apical segment. Mandible (Figs 2e,3e): length 105–110, with dark outer tooth as long as apical, two inner teeth; inner darkened area (innermost, ‘third tooth’) is fused with mola, close to base of long simple seta subdentalis; mola with three to four inner spines. Labrum (Fig. 3c); SI and SII setae finely plumose, pecten epipharyngis comprises three distinctly separated scales, each with two to three blunt teeth; premandible 80, with two short teeth. Mentum (Figs 2f,3f); width 100, with rather bulbous protrud-

Fig. 3. *Polypedilum ovahimba* new species: (a) abdominal tergites, pupa; (b) posterolateral comb; (c) dorsal head and labrum, larva; (d) antenna, larva; (e) mandible, larva; (f) mentum, larva.

ing median teeth, small first laterals, second laterals tall but not extending to median teeth height, remainder decreasing in size to a cluster of outer teeth with somewhat projecting fifth, distinct sixth and small but clearly delimited seventh. Ventromental plate with c. 40 striae, median five to six only extending 50% of depth of plate; plate width 100, depth 40, medially with medially directed pointed apex.

Abdomen. Anterior parapod claws pale golden, simple, dense. Procercus and apical setae pale mid-brown.

Etymology. From the people native to northern Namibia, a group of Hereros living as does this new species of *Polypedilum*, sustainably, in an arid area with erratic rainfall. To be treated as a noun in apposition.

Differentiation of *P. vanderplanki* from *P. ovahimba*

Male hypopygium. Tergite IX without central strong setae; anal point bare, elongate, tapering to parallel-sided distally, blunt apically, bare beyond; superior volsella broad basally, abruptly tapering at mid-point to narrow, tapering apex.

Pupa. Tergite II with shagreen over most of tergite, pedes spurii B insignificant; tergite III with strong posterior transverse spinule band with outermost spines directed antero-medially; tergite VI with anterior are comprising few scattered spines scarcely formed into band; posterolateral corner of VIII with a single triangular spine with few marginal spinules (Fig. 2c)

Larva. With similar antenna and mandible; mentum has only six pairs of lateral teeth, with the large second slightly higher than the median pair, fifth elevated from the line of slope; the ratio of width to height of the ventromental plates ranges between 2.8 and 3.

DISCUSSION

Globally, *Polypedilum* is very diverse with more than 400 species, but there are rather few fully associated life history stages. Larval morphological diversity is low, with features of the mentum and antennal lengths and ratios providing some means of broad differentiation. Pupal morphological variation is higher, with variants in pupal armament and comb structure: within regions species-level identifications can be made on this life stage. Differentiation of species of *Polypedilum* in the Afrotropical region, as elsewhere in the world, depends almost entirely on the adult male, notably by features of the hypopygium. Members of the subgenus *Pentapedilum* Kieffer can be eliminated from consideration, as all have setose/microtrichiose wings. Otherwise allocation to any subgenus (of the seven described) requires assessment of features from each life stage (Sæther *et al.* 2010). *Tripodura* Kieffer can be eliminated on the basis of its anal point structure and the lack of dorsal setae in its pupal anal lobe. *Cerobregma* Sæther and Sundal has an unusual larval head shape (small, triangular), the known larvae of *Probolum* Andersen and Sæther have first lateral mental teeth only modestly lower than the median pair and the larva of *Tripedilum* Kieffer has alternating Lauterborn organs. Differentiation of *Uresipedilum* Oyewo and Sæther from *Polypedilum* s.s. depends in the adult male upon the very broad-based superior volsella and nearly universal absence of a spur on the anterior tibial apex. Larval *Uresipedilum* have the inner apex of the ventromental plates curved anteriorly linking to a median cluster of four mental teeth. By elimination therefore, this new species belongs to the nominotypic subgenus, *Polypedilum* s.str., although monophyly of this clade is not assured (Sæther *et al.* 2010).

Within the Afrotropical region, no described species have a superior volsella like that of *P. ovahimba* (Fig. 1f) – the closest described structure may be that of *P. abyssiniae* (Freeman 1958: Fig. 1f), but this African species has an anal point with characteristic taeniate marginal setae, quite unlike that of *P. ovahimba*.

Although free-flying adults have not been observed for *P. ovahimba*, there are some morphological clues to their behaviour. The low foreleg ratio with tarsomere I only slightly longer than the tibia is unusual, and in combination with the short mid- and hind-leg segments, and robust genitalia (Fig. 1) especially the gonostylus and inferior volsella, suggests a potential substrate (ground or water surface) mating behaviour. However, there is no genitalic rotation, the wing shape is conventional, and there is no morphological indication of loss of flying ability.

Finally, the evidence for desiccation tolerance in *P. ovahimba* needs consideration. In the Brazilian Pantanal

Fissimentum desiccatum Cranston and Nolte tolerates protracted periodic droughts in a cellophane-like envelope (Cranston & Nolte 1996). European *Polypedilum tritum* Walker survives summers in dry temporary pools if soil water content remains above 20% (Dettinger-Klemm 2002) and Australian *Paraborniella tonnoiri* Freeman survives 70% body water loss, protected in cocoons (Jones 1975). Thus far the evidence for *P. ovahimba* being more than drought tolerant, as in the aforementioned, remains circumstantial.

Larvae were found in newly water-filled rock pools. According to limited Namibian meteorological data and local people the rain 4 days prior to collection was the onset of seasonal rains with no precipitation experienced since the end of previous seasonal rains, a period of at least 6 months. Larvae all were in the fourth instar stadium, and no species is known to develop so rapidly from eggs (Nolte 1995). No desiccated larvae were found next year in dry soil collected again prior to seasonal rains. However in an adjacent close-by pool habitat, viable larvae were observed within 10 days of wetting soil. One of these pupated to a pharate female. The inference is that a second *Polypedilum* species, described here as *P. ovahimba*, can withstand desiccation in a habitat in which water retention is temporary, with summer temperatures of over 35 °C. Further physiological studies will be required to determine if *P. ovahimba* is desiccation resistant and uses an avoidance strategy by limiting water loss from its body, or if it is a new case of tolerance to complete desiccation (i.e. cryptobiosis), as observed in *P. vanderplanki*.

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REFERENCES

- Andersen T, Ekrem T & Cranston PS. 2013. Introduction. In: *Chironomidae of the Holarctic Region: Keys and Diagnoses, Part 1:*

- Larvae* (eds T Andersen, PS Cranston & JH Epler), pp. 7–12. Systematic Entomology Ltd, Lund, Sweden. *Insect Systematics and Evolution Supplements* **66**, 1–571 pp.
- Cornette R & Kikawada T. 2011. The induction of anhydrobiosis in the sleeping chironomid: current status of our knowledge. *IUBMB Life* **63**, 419–429.
- Cranston PS, Benigno GM & Dominguez M. 2007. *Hydrobaenus saetheri* Cranston, new species, an aestivating, winter-emerging chironomid (Diptera: Chironomidae) from California. In: *Contributions to the Systematics and Ecology of Aquatic Diptera – A Tribute to Ole A. Sæther* (ed. T Anderson), pp. 73–79. The Caddis Press, Ohio, USA.
- Cranston PS, Dillon M, Pinder LCV & Reiss FR. 1989. Keys and diagnoses of the adult males of the subfamily Chironominae (Diptera, Chironomidae). *Entomologica Scandinavica Supplement* **34**, 353–502.
- Cranston PS, Hardy NB, Morse GE, Puslednik L & McCluen SR. 2010. When morphology and molecules concur: the ‘Gondwanan’ midges. (Diptera: Chironomidae). *Systematic Entomology* **35**, 636–648.
- Cranston PS & Nolte U. 1996. *Fissimentum*, a new genus of drought-tolerant Chironomini (Diptera: Chironomidae) from the Americas and Australia. *Entomological News* **107**, 1–15.
- Dettinger-Klemm P-MA. 2002. Drought-tolerance and the impact of the photoperiod on growth and adult emergence in *Polypedium tritum* (Walker, 1856) (= *Polypedium uncinatum* Goetghebuer, 1921 syn. nov.). *Deutsche Gesellschaft für Limnologie – Tagungsbericht* 2001 (Kiel), Tutzing: 681–686.
- Epler J, Ekrem T & Cranston PS. 2013. The larvae of Chironominae (Diptera: Chironomidae) of the Holarctic region – keys and diagnoses. In: *Chironomidae of the Holarctic Region: Keys and Diagnoses, Part 1: Larvae* (eds T Andersen, PS Cranston & JH Epler), pp. 387–556. Systematic Entomology Ltd, Lund, Sweden. *Insect Systematics and Evolution Supplements* **66**: 1–571 pp.
- Freeman P. 1958. A study of the Chironomidae (Diptera) of Africa south of the Sahara. Part IV. *Bulletin of the British Museum (Natural History)*, *Entomology* **6**, 261–363.
- Frouz J, Matěna J & Ali A. 2003. Survival strategies of chironomids (Diptera: Chironomidae) living in temporary habitats: a review. *European Journal of Entomology* **100**, 459–465.
- Grodhaus G. 1980. Aestivating chironomid larvae associated with vernal pools. In: *Chironomidae. Ecology, Systematics, Cytology and Physiology* (ed. DA Murray), pp. 315–322. Pergamon Press, New York, USA.
- Gusev O, Cornette R, Kikawada T & Okuda T. 2011. Expression of heat shock protein-coding genes associated with anhydrobiosis in an African chironomid *Polypedium vanderplanki*. *Cell Stress and Chaperones* **16**, 81–90.
- Hinton HE. 1960. A fly larva that tolerates dehydration and temperatures of -270°C to $+102^{\circ}\text{C}$. *Nature* **188**, 336–337.
- Horikawa DD, Iwata KI, Kawai K *et al.* 2009. High hydrostatic pressure tolerance of four different anhydrobiotic animal species. *Zoological Science* **26**, 238–242.
- Jones RE. 1975. Dehydration in an Australian rockpool chironomid larva, (*Paraborniola tonnoiri*). *Journal of Entomology Series A, General Entomology* **49**, 111–119.
- Kikawada T, Minakawa N, Watanabe M *et al.* 2005. Factors inducing successful anhydrobiosis in the African chironomid *Polypedium vanderplanki*: significance of the larval tubular nest. *Integrative and Comparative Biology* **45**, 710–714.
- Kondo S. 1996. Life cycle of *Hydrobaenus kondoi* Sæther (Chironomidae) at the middle reaches of the Kiso River, Japan. *Hydrobiologia* **318**, 79–84.
- Nolte U. 1995. From egg to imago in less than seven days: *Apedilum elachistum* (Chironomidae). In: *Chironomids: From Genes to Ecosystems* (ed. PS Cranston), pp. 225–232. CSIRO, Melbourne, Australia.
- Pinder LCV & Reiss F. 1983. The larva of Chironominae (Diptera: Chironomidae) of the Holarctic region. Keys and diagnoses. In: *Chironomidae of the Holarctic Region. Keys and Diagnoses. Part 1. Larvae* (ed. T Wiederholm), pp. 293–435. Entomologica Scandinavica, Lund, Sweden. *Entomologica Scandinavica Supplement* **19**.
- Pinder LCV & Reiss F. 1986. The pupae of Chironominae (Diptera: Chironomidae) of the Holarctic region. Keys and diagnoses. In: *Chironomidae of the Holarctic Region. Keys and Diagnoses. Part 2. Pupae* (ed. T Wiederholm), pp. 299–456. Entomologica Scandinavica, Lund, Sweden. *Entomologica Scandinavica Supplement* **28**.
- Sakurai M, Furuki T, Akao K *et al.* 2008. Vitricification is essential for anhydrobiosis in an African chironomid, *Polypedium vanderplanki*. *Proceedings of the National Academy of Sciences* **105**, 5093–5098.
- Sæther OA. 1977. Female genitalia in Chironomidae and other Nematocera: morphology, phylogenies, keys. *Bulletin of the Fisheries Research Board of Canada* **197**, 1–211.
- Sæther OA, Andersen T, Pinho LC & Mendes HF. 2010. The problems with *Polypedium* Kieffer (Diptera: Chironomidae), with the description of *Probolum* subgen.n. *Zootaxa* **2497**, 1–36.
- Watanabe M, Sakashita T, Fujita A *et al.* 2006. Biological effects of anhydrobiosis in an African chironomid *Polypedium vanderplankii*, on radiation tolerance. *International Journal of Radiation Biology* **82**, 587–592.

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