

A new anhydrobiotic midge from Malawi, *Polypedilum pembai* sp.n. (Diptera: Chironomidae), closely related to the desiccation tolerant midge, *Polypedilum vanderplanki* Hinton

Cornette R., Yamamoto N., Yamamoto M., Kobayashi T., Petrova N., Gusev O., Shimura S., Kikawada T., Pemba D., Okuda T.

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2017 The Royal Entomological Society The sleeping chironomid (*Polypedilum vanderplanki* Hinton) lives on temporary rock pools in the semi-arid tropical regions of Africa. Its larvae are able to survive the dry season in a completely desiccated ametabolic state known as anhydrobiosis. So far, *P. vanderplanki* was the only species among all insects showing demonstrated anhydrobiotic ability. Here, we show that a new related species originating from Malawi, *Polypedilum pembai* sp.n., is also anhydrobiotic and that its desiccation tolerance mechanism is probably similar to what is observed in *P. vanderplanki*. The new species, *P. pembai* sp.n., is described with special attention to the common and different morphological features, compared with *P. vanderplanki*. Phylogenetic analysis showed that both species are closely related, suggesting that anhydrobiosis evolved only once in their common ancestor about 49 Ma somewhere in Africa, before the divergence of two species, one in the sub-Saharan area and another in southeastern Africa.

<http://dx.doi.org/10.1111/syen.12248>

References

- [1] Adams, A. (1985) Cryptobiosis in Chironomidae (Diptera) – two decades on. *Antenna: Bulletin of the Royal Entomological Society London*, 8, 58–61.
- [2] Andersen, T., Ekrem, T. & Cranston, P.S. (2013) The larvae of the Holarctic Chironomidae (Diptera) – introduction. *Chironomidae of the Holarctic Region. Keys and Diagnoses – Larvae* (ed. by T. Andersen, P.S. Cranston and J.H. Elper), pp. 7–12. *Scandinavian Entomology*, Lund.
- [3] Cornette, R. & Kikawada, T. (2011) The induction of anhydrobiosis in the sleeping chironomid: current status of our knowledge. *IUBMB Life*, 63, 419–429.
- [4] Cornette, R., Kanamori, Y., Watanabe, M. et al. (2010) Identification of anhydrobiosis-related genes from an expressed sequence tag database in the cryptobiotic midge *Polypedilum vanderplanki* (diptera; chironomidae). *Journal of Biological Chemistry*, 285, 35889–35899.
- [5] Cranston, P.S. (2014) A new putatively cryptobiotic midge, *Polypedilum ovahimba* sp. nov. (Diptera: Chironomidae), from southern Africa. *Austral Entomology*, 53, 373–379.
- [6] Cranston, P.S., Dillon, M.E., Pinder, L.C.V. & Reiss, F. (1989) The adult males of Chironominae (Diptera: Chironomidae) of the Holarctic region – keys and diagnoses. *Chironomidae of the Holarctic Region: Keys and Diagnoses, Part 3: Adult Males*, *Entomologica Scandinavica Suppl.* 34 (ed. by T. Wiederholm), pp. 353–502, Entomological Society of Lund, Lund.

- [7] Cranston, P.S., Hardy, N.B. & Morse, G.E. (2012) A dated molecular phylogeny for the Chironomidae (Diptera). *Systematic Entomology*, 37, 172-188.
- [8] Cranston, P.S., Martin, J. & Spies, M. (2016) Cryptic species in the nuisance midge *Polypedilum nubifer* (Skuse) (Diptera: Chironomidae) and the status of *Tripedilum Kieffer*. *Zootaxa*, 4079, 429-447.
- [9] Elper, J.H., Ekrem, T. & Cranston, P.S. (2013) The larvae of Chironominae (Diptera: Chironomidae) of the Holarctic region – keys and diagnoses. *Chironomidae of the Holarctic Region: Keys and Diagnoses, Part 1: Larvae* (ed. by T. Andersen, P.S. Cranston and J.H. Elper), pp. 387-556. Brill Academic Publishers, Lund.
- [10] Feakins, S.J. & Demenocal, P.B. (2010) Global and African regional climate during the Cenozoic. *Cenozoic Mammals of Africa* (ed. by L. Werdelin and W.J. Sanders), pp. 45-55. University of California Press, Oakland.
- [11] Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294-299.
- [12] Freeman, P. (1958) A study of the Chironomidae (Diptera) of Africa South of the Sahara part IV. *Bulletin of the British Museum (Natural History), Entomological Series*, 6, 263-367.
- [13] 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.
- [14] Gusev, O., Cornette, R., Kikawada, T. & Okuda, T. (2010a) Expression of heat shock protein-coding genes associated with anhydrobiosis in an African chironomid *Polypedilum vanderplanki*. *Cell Stress & Chaperones*, 16, 81-90.
- [15] Gusev, O., Nakahara, Y., Vanyagina, V. et al. (2010b) Anhydrobiosis-associated nuclear DNA damage and repair in the sleeping chironomid: linkage with radioresistance. *PLoS ONE*, 5, e14008.
- [16] Gusev, O., Suetsugu, Y., Cornette, R. et al. (2014) Comparative genome sequencing reveals genomic signature of extreme desiccation tolerance in the anhydrobiotic midge. *Nature Communications*, 5, 4784.
- [17] Hinton, H.E. (1951) A new chironomid from Africa, the larvae of which can be dehydrated without injury. *Proceedings of the Zoological Society of London*, 121, 371-380.
- [18] Hinton, H.E. (1960a) Cryptobiosis in the larva of *Polypedilum vanderplanki* Hint. (Chironomidae). *Journal of Insect Physiology*, 5, 286-300.
- [19] Hinton, H.E. (1960b) A fly larva that tolerates dehydration and temperatures of -270° to $+102^{\circ}\text{C}$. *Nature*, 188, 336-337.
- [20] Horikawa, D.D., Iwata, K., Kawai, K., Koseki, S., Okuda, T. & Yamamoto, K. (2009) High hydrostatic pressure tolerance of four different anhydrobiotic animal species. *Zoological Science*, 26, 238-242.
- [21] Jacobs, B.F. & Herendeen, P.S. (2004) Eocene dry climate and woodland vegetation in tropical Africa reconstructed from fossil leaves from northern Tanzania. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 213, 115-123.
- [22] Jones, R.E. (1975) Dehydration in an Australian rockpool chironomid larva, (*Paraborniola tonnoiri*). *Journal of Entomology*, 49, 111-119.
- [23] Keilin, D. (1959) The problem of anabiosis or latent life: history and current concept. *Proceedings of the Royal Society of London, Series B: Containing Papers of a Biological Character*. Royal Society (Great Britain), 150, 149-191.
- [24] Kikawada, T., Nakahara, Y., Kanamori, Y. et al. (2006) Dehydration-induced expression of LEA proteins in an anhydrobiotic chironomid. *Biochemical and Biophysical Research Communications*, 348, 56-61.
- [25] Kikawada, T., Saito, A., Kanamori, Y. et al. (2007) Trehalose transporter 1, a facilitated and high-capacity trehalose transporter, allows exogenous trehalose uptake into cells. *PNAS*, 104, 11585-11590.
- [26] McLachlan, A.J. (1983a) Habitat distribution and body size in rain-pool dwellers. *Zoological Journal of the Linnean Society*, 79, 399-407.
- [27] McLachlan, A.J. (1983b) Life-history tactics of rain-pool dwellers. *Journal of Animal Ecology*, 52, 545-561.
- [28] Miller, P.L. (1969) On the occurrence and some characteristics of *Cyrtopus fastuosus* Bigot (Dipt., Stratiomyidae) and *Polypedilum* sp. (Dipt., Chironomidae) from temporary habitats in Western Nigeria. *Entomologist's Monthly Magazine*, 4 February, pp. 223-238.
- [29] Petrova, N.A., Cornette, R., Shimura, S. et al. (2015) Karyotypical characteristics of two allopatric African populations of anhydrobiotic *Polypedilum Kieffer* 1912 (Diptera, Chironomidae) originating from Nigeria and Malawi. *Comparative Cytogenetics*, 9, 173-188.
- [30] Pinder, L.C.V. & Reiss, F. (1986) The pupae of Chironominae (Diptera: Chironomidae) of the Holarctic region. *Keys and diagnoses. Chironomidae of the Holarctic Region. Keys and Diagnoses. Part 2. Pupae*, *Entomologica Scandinavica, Suppl.* 28 (ed. by T. Wiederholm), pp. 299-456. Entomological Society of Lund, Lund.
- [31] Saether, O.A. (1977) Female genitalia in Chironomidae and other Nematocera: morphology, phylogenies, keys. *Bulletin of the Fisheries Research Board of Canada*, 197, 1-211.
- [32] Saether, O.A. (1980) Glossary of Chironomid morphology terminology (Diptera: Chironomidae). *Entomologica Scandinavica Supplements*, 14, 1-51.

- [33] Saether, O.A. (1990) A review of the genus *Limnophyes* Eaton from the Holarctic and Afrotropical regions (Diptera: Chironomidae, Orthoclaadiinae). *Entomologica Scandinavica, Supplements*, 35, 1-139.
- [34] Sakurai, M., Furuki, T., Akao, K. et al. (2008) Vitrification is essential for anhydrobiosis in an African chironomid, *Polypedilum vanderplanki*. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 5093-5098.
- [35] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731-2739.
- [36] Tunnacliffe, A. & Lapinski, J. (2003) Resurrecting Van Leeuwenhoek's rotifers: a reappraisal of the role of disaccharides in anhydrobiosis. *Philosophical Transactions of the Royal Society of London, Series B: Biological Science*, 358, 1755-1771.
- [37] Watanabe, M. (2006) Anhydrobiosis in invertebrates. *Applied Entomology and Zoology*, 41, 15-31.
- [38] Watanabe, M., Kikawada, T., Minagawa, N., Yukuhiro, F. & Okuda, T. (2002) Mechanism allowing an insect to survive complete dehydration and extreme temperatures. *Journal of Experimental Biology*, 205, 2799-2802.
- [39] Watanabe, M., Kikawada, T. & Okuda, T. (2003) Increase of internal ion concentration triggers trehalose synthesis associated with cryptobiosis in larvae of *Polypedilum vanderplanki*. *Journal of Experimental Biology*, 206, 2281-2286.
- [40] Watanabe, M., Sakashita, T., Fujita, A. et al. (2006) Estimation of radiation tolerance to high LET heavy ions in an anhydrobiotic insect, *Polypedilum vanderplanki*. *International Journal of Radiation Biology*, 82, 835-842.
- [41] Yamamoto, N. & Yamamoto, M. (2016) The taxonomic implication of frontal tubercles in *Polypedilum* subgenera diagnoses, with re-description of *Polypedilum isigabeceum* Sasa & Suzuki (Diptera, Chironomidae). *Zootaxa*, 4193, 189-194.