

Ultrastructure of antennal sensilla in *Hydrotaea armipes* (Fallén) (Diptera: Muscidae): New evidence for taxonomy of the genus *Hydrotaea*

QI-KE WANG¹, XIAN-HUI LIU¹ PENG-FEI LU^{2,3} & DONG ZHANG^{1,3}

¹College of Nature Conservation, Beijing Forestry University, Beijing 100083, China

²The Key Laboratory for Silviculture and Conservation of Ministry of Education, College of Forestry, Beijing Forestry University, Beijing 100083, China

³Corresponding author. E-mail: ernest8445@163.com (D. Z.); lpengfei@126.com (P.-F. L.)

Qi-ke Wang and Xian-hui Liu contributed equally to this work.

Abstract

The morphology and ultrastructure of the antennal sensilla of male *Hydrotaea* (*Hydrotaea*) *armipes* (Fallén) are examined via scanning electron microscopy in order to highlight the importance of antennal sensilla as a source of morphological characters for taxonomy and phylogeny of *Hydrotaea*. Antennal scape and pedicel have only one type of sensilla, the sharp-tipped chaetic sensilla, whereas antennal funiculus possesses several types of sensilla, including trichoid sensilla, two subtypes of basiconic sensilla, coeloconic sensilla and clavate sensilla. These results are compared with previously published studies on other fly species, especially on *H. (H.) irritans* (Fallén) and *H. (Ophyra) chalcogaster* (Wiedemann), and there are possible uniquely derived characters or diagnostic characters examined on antennal pedicel and antennal funiculus, which suggests either affinities and divergence between species at subgenus level. Thus, new morphological evidence is provided, which might help to develop the much disputed taxonomy of genus *Hydrotaea* Robineau-Desvoidy in future.

Key words: *Hydrotaea armipes*, antenna, sensilla, scanning electron microscopy

Introduction

The genus *Hydrotaea* Robineau-Desvoidy is the largest and most diverse within the azeliine genera, with over 100 described species (Savage & Wheeler 2004). According to classification proposals of different taxonomists, there is still a controversy over the taxonomy of the genus *Hydrotaea*, for the morphological and biological characters of larvae and adults in *Hydrotaea* are extremely similar to those in *Ophyra* Robineau-Desvoidy. Some taxonomists suggested that *Ophyra* and *Hydrotaea* are synonyms (Pont 1989; Savage & Wheeler 2004), but others did not recognize that synonymy and treated the former as a separate genus (van Emden 1965; Skidmore 1985; Carvalho *et al.* 1993; Carvalho & Courri 2002).

Although molecular characters are nowadays widely used to reconstruct phylogenetic relationships of Diptera, novel morphological characters were recently applied with varying degrees of success as well (Yeates & Wiegmann 2005; Giroux *et al.* 2010). As the main olfactory sense organs of flies, antennae play an important role in their life history (Fernandes *et al.* 2005; Smallegange *et al.* 2008) and manage to evolve particular characters to improve their fitness in environment (Ross 1992; Sukontason *et al.* 2004; Zhang *et al.* 2012a; Zhang *et al.* 2012b). Therefore, the variety of sensilla in different species examined might be shared by groupings according to taxonomic classifications (Marshall & Lewis 1971), which indicates that antennal sensilla could serve as a source of morphological characters for taxonomy and phylogeny, and more attention should be given to sensory structures.

Hydrotaea (*H.*) *armipes* (Fallén) is a fly species of medical and forensic importance in many parts of the world (Xue & Zhao 1998), and previously, the antennal sensilla of two other species in the genus, *H. (H.) irritans* (Fallén) (Been *et al.* 1988) and *H. (Ophyra) chalcogaster* (Wiedemann) (Sukontason *et al.* 2007), have been studied. Thus,

more morphological information of re-evaluating their taxonomy, detailed comparisons are made on the antennal sensilla among *H. (H.) armipes*, *H. (H.) irritans* (Been *et al.* 1988) and *H. (O.) chalcogaster* (Sukontason *et al.* 2007).

Although no sensilla other than Ch is found on antennal pedicel of *H. armipes*, a group of quite similar unidentified sensilla are separately discovered on those of *H. irritans* and *H. chalcogaster* (Been *et al.* 1988, Sukontason *et al.* 2007). On some extent, these structures can be recognized as one type of plaques disclosed by Greenberg (1970) and Greenberg & Ash (1972), which commonly occur in Calyptratae. Nevertheless, these structures in *H. irritans* and *H. chalcogaster* differs from all the plaques described before (Greenberg 1970; Greenberg & Ash 1972; Been *et al.* 1988; Sukontason *et al.* 2007), implying that they might serve as derived character in genus *Hydrotaea* and species in two subgenera have close affinities with each other.

Among funicular sensilla, there are three distinctive features that differentiate flies in subgenus *Hydrotaea* from that in *Hydrotaea Ophyra*. More types of Ba are detected in *H. armipes* (two types) and *H. irritans* (three types) (Been *et al.* 1988) than in *H. chalcogaster* (only one type) (Sukontason *et al.* 2007). Unlike the Co with a smooth peg described in the antennal sensory complex of *H. chalcogaster* (Sukontason *et al.* 2007), the Co in *H. armipes* and *H. irritans* (Been *et al.* 1988) are characterized by deep longitudinal grooves in their walls. Besides, Cl are discovered in two species of *Hydrotaea s. str.* (Been *et al.* 1988), while none exists in the species of subgenus *Ophyra* (Sukontason *et al.* 2007).

This research provides additional information that will enhance future developments in taxonomy of the genus, except for the traditional distinction that these two subgenera of *Hydrotaea* can only be distinguished from each other by male fore femur with distinctly indentation on ventral surface facing the ventral projection on basal part of fore tibia (Savage & Wheeler 2004). However, the sensillar morphology of the taxon sample alone is far from a perfect predictor for success in phylogenetic analyses. Therefore, an exhaustive study of structural variations in the Calyptratae would probably provide more useful information for its phylogeny, and further research on the morphology of more species of the two subgenera, *Hydrotaea s.str.* and *Ophyra*, will be especially important to improve our understanding of *Hydrotaea*.

Phylogeny is always absorbing the latest technologies to solve some of the oldest problems throughout history, especially when there is a controversy over taxon relationships. The widely used molecular tools are the perfect example of this trend (Bernasconi *et al.* 2000; Kutty *et al.* 2008; Kutty *et al.* 2010; Wiegmann *et al.* 2011). However, it raises as much questions as it solves (Nirmala *et al.* 2001; Dittmar *et al.* 2006; Petersen *et al.* 2007; Kutty *et al.* 2008), leaving the classical morphological study the mainstream tool for phylogeny even today. Because of the disability in high resolution, microscopes are crippled in cases like this. As an economical, convenient, fast and precise way to observe fine structure on surface, SEM could be a valuable increment in phylogenetic analysis.

Acknowledgments

We are grateful to Adrian Pont (Oxford University Museum of Natural History) and Ms. Hui Zhang (Beijing Forestry University), who gave us invaluable help with this study. We wish to extend our sincerest thanks to Dr Silvio Nihei (Universidade de São Paulo, São Paulo, Brazil) and the anonymous reviewers for their helpful suggestions on our manuscript and assistance in proofreading the manuscript. This study was supported by National Science Foundation of China (No. 31201741, 31272347), Programme for Colleage Student of Beijing Forestry University Scientific and Technological Innovation (No. 201310), and the Program for New Century Excellent Talents in University (No. NCET-12-0783).

References

- Been, T.H., Schomaker, C.H. & Thomas, G. (1988) Olfactory sensilla on the antenna and maxillary palp of the sheep head fly, *Hydrotaea irritans* (Fallén) (Diptera: Muscidae). *International Journal of Insect Morphology and Embryology*, 17, 121–133.
[http://dx.doi.org/10.1016/0020-7322\(88\)90006-2](http://dx.doi.org/10.1016/0020-7322(88)90006-2)
- Bernasconi, M.V., Valsangiacomo, C., Piffaretti, J.C. & Ward P.I. (2000) Phylogenetic relationships among Muscoidea

- (Diptera: Calyptratae) based on mitochondrial DNA sequences. *Insect Molecular Biology*, 9, 67–74.
<http://dx.doi.org/10.1046/j.1365-2583.2000.00158.x>
- Carvalho, C.J.B. de (1993) *Micropotamia*, gen. n. of Neotropical Muscidae (Diptera, Azeliinae), with comments on allied Azeliini genera. *Revista Brasileira de Zoologia*, 9, 241–246.
<http://dx.doi.org/10.1590/s0101-81751992000200009>
- Carvalho, C.J.B. de & Couri, M.S. (2002) Part I. Basal Groups. In: Carvalho, C.J.B. de (Ed.), *Muscidae (Diptera) of the Neotropical Region: Taxonomy*. Editora Universidade Federal do Paraná, Curitiba, pp. 17–132.
- Dittmar, K., Porter, M.L., Murray, S. & Whiting, M.F. (2006) Molecular phylogenetic analysis of nycteribiid and streblid bat flies (Diptera: Brachycera, Calyptratae): Implications for host associations and phylogeographic origins. *Molecular Phylogenetics and Evolution*, 38, 155–170.
<http://dx.doi.org/10.1016/j.ympev.2005.06.008>
- Fernandes, F. de F., Freitas, E. de P.S., Linardi, P.M. & Pimenta, P.F.P. (2005) Ultrastructure of contact-chemoreceptor sensilla found among the genae of female *Gasterophilus nasalis*. *Journal of parasitology*, 91, 1218–1220.
<http://dx.doi.org/10.1645/ge-501r3.1>
- Giroux, M., Pape, T. & Wheeler, T.A. (2010) Towards a phylogeny of the flesh flies (Diptera: Sarcophagidae): morphology and phylogenetic implications of the acrophallus in the subfamily Sarcophaginae. *Zoological Journal of the Linnean Society*, 158, 740–778.
<http://dx.doi.org/10.1111/j.1096-3642.2009.00561.x>
- Greenberg, B. (1970) Species distribution of new structures on fly antennae. *Nature*, 228, 1338–1339.
<http://dx.doi.org/10.1038/2281338a0>
- Greenberg, B. & Ash, N. (1972) Setiferous plaques on antennal pedicels of Muscoid Diptera: Appearance in various species and tests of function. *Annals of the Entomological Society of America*, 65, 1340–1346.
- Gregor, F., Rozkosny, R., Barták, M. & Vaóhara, J. (2002) *The Muscidae (Diptera) of Central Europe*. Masaryk University, Brno, 280 pp.
- Hunter, F.F. & Adserballe, C.F. (1996) Cuticular structures on the antennae of *Hypoderma bovis* De Geer (Diptera: Oestridae) females. *International Journal of Insect Morphology and Embryology*, 25, 173–181.
[http://dx.doi.org/10.1016/0020-7322\(95\)00013-5](http://dx.doi.org/10.1016/0020-7322(95)00013-5)
- Kutty, S.N., Pape, T., Pont, A., Wiegmann, B.M. & Meier, R. (2008) The Muscoidea (Diptera: Calyptratae) are paraphyletic: Evidence from four mitochondrial and four nuclear genes. *Molecular Phylogenetics and Evolution*, 49, 639–652.
<http://dx.doi.org/10.1016/j.ympev.2008.08.012>
- Kutty, S.N., Pape, T., Wiegmann, B.M. & Meier, R. (2010) Molecular phylogeny of the Calyptratae (Diptera: Cyclorrhapha) with an emphasis on the superfamily Oestroidea and the position of Mystacinobiidae and McAlpine's fly. *Systematic Entomology*, 35, 614–635.
<http://dx.doi.org/10.1111/j.1365-3113.2010.00536.x>
- Marshall, A.T. & Lewis, C.T. (1971) Structural variation in the antennal sense organs of fulgoroid Homoptera (Insecta). *Zoological Journal of the Linnean Society*, 50, 181–184.
<http://dx.doi.org/10.1111/j.1096-3642.1971.tb00758.x>
- Nirmala, X., Hypsa, V. & Zurovec, M. (2001) Molecular phylogeny of Calyptratae (Diptera: Brachycera): the evolution of 18S and 16S ribosomal rDNAs in higher dipterans and their use in phylogenetic inference. *Insect Molecular Biology*, 10, 475–485.
<http://dx.doi.org/10.1046/j.0962-1075.2001.00286.x>
- Petersen, F.T., Meier, R., Kutty, S.N. & Wiegmann, B.M. (2007) The phylogeny and evolution of host choice in the Hippoboscoidea (Diptera) as reconstructed using four molecular markers. *Molecular Phylogenetics and Evolution*, 45, 111–122.
<http://dx.doi.org/10.1016/j.ympev.2007.04.023>
- Pont, A.C. (1989) Family Muscidae. In: Evenhuis, N.L. (Ed.), *Catalog of the Diptera of the Australasian and Oceanian regions*. Bishop Museum Special Publication 86. Bishop Museum Press, Honolulu, and E.J. Brill, Leiden, pp. 675–699.
- Ross, K.T.A. (1992) Comparative study of the antennal sensilla of five species of root maggots: *Delia radicum* L., *D. floralis* F., *D. antique* MG., *D. platura* MG. (Diptera: Anthomyiidae), and *Psila rosae* F. (Diptera: Psilidae). *International Journal of Insect Morphology and Embryology*, 21, 175–197.
[http://dx.doi.org/10.1016/0020-7322\(92\)90015-f](http://dx.doi.org/10.1016/0020-7322(92)90015-f)
- Savage, J. & Wheeler, T.A. (2004) Phylogeny of the Azeliini (Diptera: Muscidae). *Studia dipterologica*, 11, 259–299.
- Setzu, M.D., Poddighe, S. & Angioy, A.M. (2011) Sensilla on the antennal funiculus of the blow fly, *Protophormia terraenovae* (Diptera: Calliphoridae). *Micron*, 42, 471–477.
<http://dx.doi.org/10.1016/j.micron.2011.01.005>
- Shanbhag, S.R., Müller, B. & Steinbrecht, R.A. (1999) Atlas of olfactory organs of *Drosophila melanogaster* L. Types, external organization, innervation and distribution of olfactory sensilla. *International Journal of Insect Morphology and Embryology*, 28, 377–397.
- Skidmore, P. (1985) The Biology of the Muscidae of the World. *Entomology Series*, 29, 1–550.
- Smallegange, R.C., Kelling, R.J. & Den Otter, C.J. (2008) Types and numbers of sensilla on antennae and maxillary palps of small and large houseflies, *Musca domestica* (Diptera, Muscidae). *Microscopy Research and Technique*, 71, 880–886.

- <http://dx.doi.org/10.1002/jemt.20636>
- Sukontason, K., Methanitikorn, R., Chaiwong, T., Kurahashi, H., Vogtsberger, R.C. & Sukontason, K.L. (2007) Sensilla of the antenna and palp of *Hydrotaea chalcogaster* (Diptera: Muscidae). *Micron*, 38, 218–223.
<http://dx.doi.org/10.1016/j.micron.2006.07.018>
- Sukontason, K., Sukontason, K.L., Piangjai, S., Boonchu, N., Chaiwong, T., Ngern-klun, R., Sripakdee, D., Vogtsberger, R.C. & Olson, J.K. (2004) Antennal sensilla of some forensically important flies in families Calliphoridae, Sarcophagidae and Muscidae. *Micron*, 35, 671–679.
<http://dx.doi.org/10.1016/j.micron.2004.05.005>
- van Emden, F.I. (1965) *The fauna of India and the adjacent countries*. Government of India, Delhi, 647 pp.
- Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J.W., Lambkin, C., Bertone, M.A., Cassel, B.K., Bayless, K.M., Heimberg, A.M., Wheeler, B.M., Peterson, K.J., Pape, T., Sinclair, B.J., Skevington, J.H., Blagoderov, V., Caravas, J., Kutty, S.N., Schmidt-Ott, U., Kampmeier, G.E., Thompson, F.C., Grimaldi, D.A., Beckenbach, A.T., Courtney, G.W., Friedrich, M., Meier, R. & Yeates, D.K. (2011) Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences*, 18, 5690–5695.
<http://dx.doi.org/10.1073/pnas.1012675108>
- Xue, W.Q. & Chao, C.M. (1998) *Flies of China. Vol. I*. Liaoning Science and Technology Press, Shenyang, 1365 pp. [in Chinese]
- Yeates, D.K. & Wiegmann, B.M. (2005) Phylogeny and evolution of Diptera: recent insights and new perspectives. In: Yeates D.K. & Wiegmann B.M. (Eds.), *The evolutionary biology of flies*. Columbia University Press, New York, pp. 14–44.
- Zhang, D., Wang, Q.K., Hu, D.F. & Li, K. (2012a) Sensilla on the antennal funiculus of the horse stomach bot fly, *Gasterophilus nigricornis*. *Medical and Veterinary Entomology*, 26, 314–322.
<http://dx.doi.org/10.1111/j.1365-2915.2011.01007.x>
- Zhang, D., Wang, Q.K., Hu, D.F. & Li, K. (2012b) Cuticular structures on antennae of the bot fly, *Portschinskia magnifica* (Diptera: Oestridae). *Parasitology Research*, 111, 1651–1659.
<http://dx.doi.org/10.1007/s00436-012-3004-9>