

MORPHOLOGY *OF THE FEMALE* *OVIPOSITOR*

as a valuable source to explain the diversification of oviposition strategies in Tachinidae (Diptera)

by Filipe Macedo Gudin

Postdoctoral researcher at the Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Laboratório de Sistemática e Biogeografia de Insecta, Rua do Matão, Travessa 14, nº 101, São Paulo, SP, CEP 005508-090, Brazil. E-mail: filipe.gudin@gmail.com

Parasitoid species usually fascinate researchers and enthusiasts of natural history because of their interactions with their hosts. Either directly or indirectly, parasitoids exhibit a wide variety of oviposition strategies to parasitize their hosts and complete development. In insects, the most representative groups of parasitoids are found first in parasitic Hymenoptera, followed by tachinid flies (Diptera: Tachinidae) (Eggleton & Belshaw 1992, Feener & Brown 1997). Parasitic hymenopterans have complex ovipositors associated with accessory glands, which give females an advantage when accessing and manipulating hosts (Feener & Brown 1997); however, hymenopteran wasps usually must have direct contact with the host to lay their eggs. In Tachinidae, on the other hand, although the host range is less broad and the oviposition strategies are less sophisticated than in Hymenoptera, the diversity of direct and indirect oviposition strategies is impressive (O'Hara 1985, Stireman et al. 2006, Nakamura et al. 2013, Dindo & Nakamura 2018).

OVIPOSITION STRATEGIES IN TACHINIDAE

Several variations in direct and indirect oviposition strategies are found in the four tachinid subfamilies (see Fig. 7.3 in Nakamura et al. 2013). The direct type involves oviparous and ovoviviparous species that lay their eggs on the host's cuticle, e.g., in Winthemini (Exoristinae) and Gymnosomatini (Phasiinae), or inject their eggs into the host's body, e.g., in Blondeliini (Exoristinae) and Phasiinae groups with piercing ovipositors. The greatest diversity of oviposition strategies in Tachinidae, however, are indirect, with ovoviviparous species that lay eggs with well-developed first instar larvae that either actively search for the host (i.e., searching type), e.g., in Dexiini (Dexiinae) and some Blondeliini (Exoristinae), or wait for the host to cross their path (i.e., waiting type), e.g., in Tachinini (Tachininae); and oviparous species that lay well sclerotized microtype eggs on the food plant to be ingested by the host, e.g., in Goniini and some Blondeliini (Exoristinae) and some Dufouriini (Dexiinae).

Parasitoid females are responsible for the success of the parasitization, as they have to precisely trace and locate the host's cues and lay their eggs in a suitable oviposition site. Therefore, females are expected to present features that reflect evolutionary adaptations during the diversification of oviposition strategies in parasitoid lineages, especially in the structures used during these processes, such as antennal and tarsal sensilla, egg/larval type, and ovipositors. In this brief review, I want to focus on the importance of the female ovipositor in the diversification of oviposition strategies in Tachinidae.

Several ovipositor characters are correlated with oviposition strategies of parasitoid species, such as ichneumonids (Belshaw et al. 2003), and of non-parasitic species as well, such as fig wasps (Elias et al. 2018), cerambycids (Lee & Lee 2020), moths (Kawakita & Kato 2016), and mantises and cockroaches (Hörnig et al. 2018). The major advantage of these studies lies in the possibility of inferring the oviposition strategies of related species that share the same characters and whose biology is unknown.

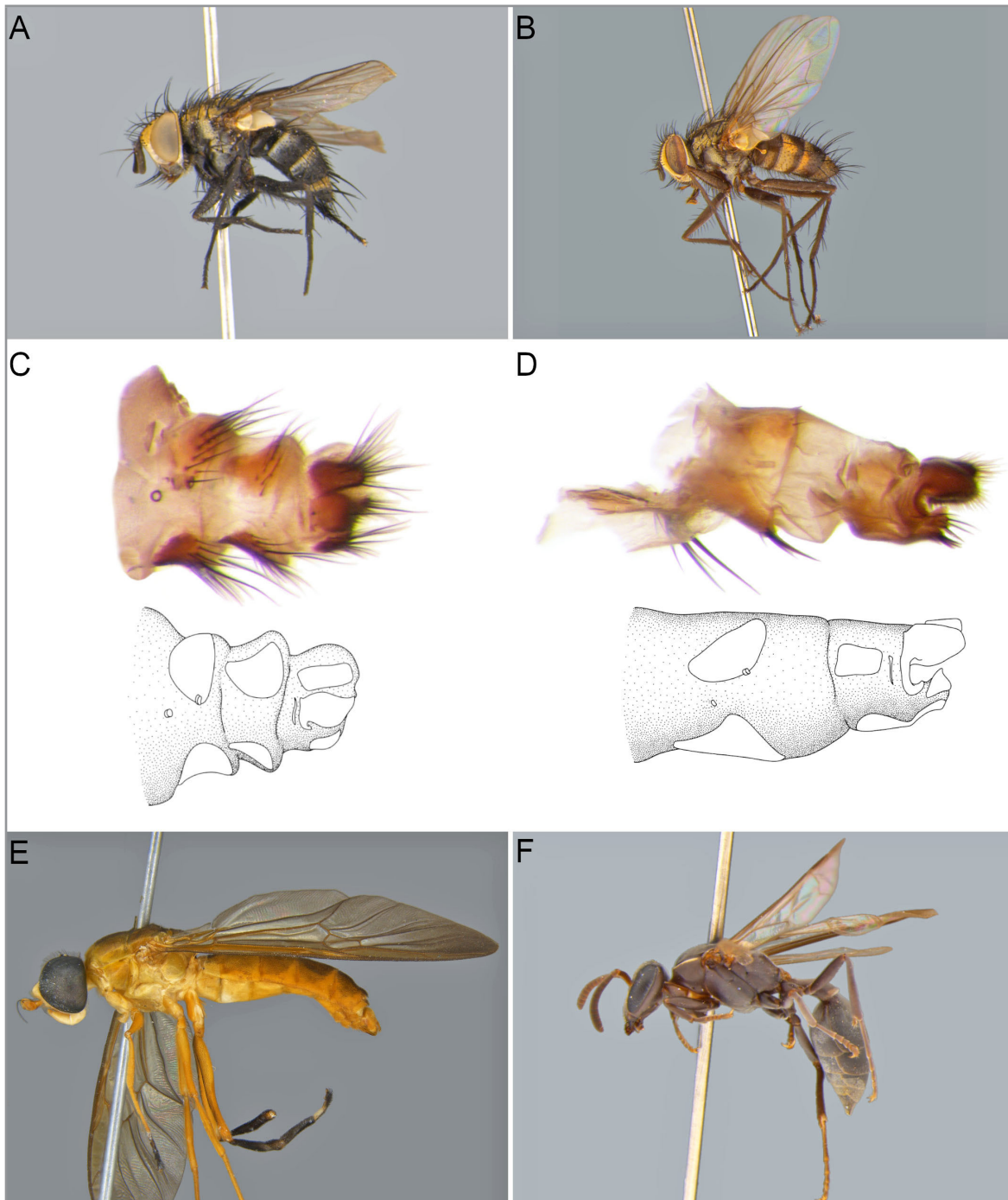


Figure 1. Comparison of ovipositors of two species of *Lixophaga* Townsend (Blondeliini) and their respective hosts. Images extracted from Gudín et al. (2022) and Gudín (2023). **A.** Female of *L. stratiophaga* Gudín. **B.** Female of *L. punctata* (Townsend). **C.** Image and line drawing of *L. stratiophaga* ovipositor in lateral view. **D.** Image and line drawing of *L. punctata* ovipositor in lateral view. **E.** Host of *L. stratiophaga*: *Plecticus testaceus* (Fabricius) (Diptera: Stratiomyidae). **F.** Host of *L. punctata*: *Polybia (Myrapetra) scutellaris* (White) (Hymenoptera: Vespidae).

For instance, in Tachinidae, most species are not accessible for direct observation, especially in tropical environments where the majority of species inhabit the higher strata of forests (Amorim et al. 2022). Curiously, studies on the comparative morphology and evolution of ovipositors in tachinid females are scarce, preventing the inference of robust explanatory hypotheses regarding the diversification of oviposition strategies in the family. Herting (1957) was the first author to summarize the diversity and homologies of female ovipositors (also called female postabdomen or female terminalia) in tachinids and other calyprate flies, arguing about the validity of these characters in explaining the diversification of Calypratae lineages. Later, Cantrell (1988) provided a comprehensive descriptive and comparative contribution on the female ovipositors of the Australasian fauna of Tachinidae. Farinets (1994, 2006, 2010, 2017, 2018) has made valuable contributions to the homology and diversity of female ovipositors in Palearctic Tachinidae as well.

In the last decade, however, the understanding of the evolution of oviposition strategies in Tachinidae has improved with the clarification of phylogenetic relationships in the family (Tachi & Shima 2010, Cerretti et al. 2014, Blaschke et al. 2018, Stireman et al. 2019). However, these inferences are based on ancestral state reconstruction or optimization of broad behavioral characters (e.g., oviparity with macrotype eggs and oviparity with microtype eggs), which provide limited explanations regarding oviposition strategies and how hosts are exploited in each group. For instance, oviposition with microtype eggs occurred independently in Tachinidae at least three times: most notably in the tribe Goniini but also in some blondeliine genera (e.g., *Anisia* Wulp) and in the dufouriine genus *Oestrophasia* Brauer & Bergenstamm (Wood 1985, de Santis & Nihei 2022). A closer analysis of ovipositor morphology shows that, although these three groups share a similar oviposition strategy, the evolutionary pathways that led to this result were different. Therefore, it is very important to clearly delimit the homologies and verify how ovipositor characters evolved in different lineages of Tachinidae.

Most taxonomic studies of Tachinidae have focused on the characters of the male terminalia because these tend to be more useful for discriminating between species than female ovipositors. However, there is evidence that the female ovipositor of tachinid flies is a valuable source of information for delimiting suprageneric groups, and sometimes species. De Santis and Nihei (2022) provided convincing evidence that ovipositor morphology has a strong phylogenetic signal in species of Dufouriini and related tribes, which is probably a result of adaptation to parasitize adult coleopteran hosts. In taxonomy, Dios & Nihei (2017, 2020) showed that the female ovipositor is informative for the delimitation of the genus and even species of Gymnosomatini; the same is true for other groups of Tachinidae, such as Ormiini (Nihei 2015, Gudin & Nihei 2019) and some Blondeliini (Burlington 2022, Gudin et al. 2022, Gudin 2023).

MY POSTDOCTORAL PROJECT

In my postdoctoral research project, I am working with the comparative morphology of ovipositors of calyprate flies, mainly Tachinidae, to provide a robust hypothesis on the evolution of oviposition strategies in these flies. I hope to delimit homologies, reconstruct the ancestral state of ovipositor characters in the main lineages of Calypratae, and improve the knowledge about host use and oviposition strategies in particular groups.

During this project, I have had the opportunity to study the taxonomy and hosts of species of the New World genus *Lixophaga* Townsend (Blondeliini), whose ovipositor morphology seems to reflect the diversity of their hosts. *Lixophaga* species are parasitoids of borers of plants and fungi and show a varied set of hosts in the orders Coleoptera, Diptera, Hymenoptera and Lepidoptera (Gudin et al. 2022, Gudin 2023). When comparing the ovipositor morphology of two species that attack different hosts, it was possible to see an impressive intraspecific variation in both the length and shape of the sclerites (Fig. 1). *Lixophaga stratiophaga* Gudin is a parasitoid of fruit-boring Stratiomyidae larvae (Gudin et al. 2022), whereas *L. punctata* (Townsend) is the only species in the genus adapted to parasitize larvae of eusocial wasps of the genus *Polybia* Lepeletier (Hymenoptera: Vespidae) (Gudin

2023). Eusocial wasps are not borers, but their larvae are reared within comb cells that have varying degrees of exposure and length. *Lixophaga* species are ovoviviparous, laying eggs with well-developed first instar larvae at the entrance of the host cavity (Waggy & Beardsley 1974, Roth et al. 1978). Therefore, it is expected that *L. punctata* females have a similar oviposition strategy, laying eggs near the entrance of the nest. However, considering the challenges of parasitizing eusocial wasps, such as aggressiveness and parental care, it is expected that the ovipositor of this species reflects a process of adaptation to explore this new kind of host. Although preliminary, these findings provide evidence that ovipositor morphology is highly informative in this genus. Further details on this discussion can be found in Gudín et al. (2022) and Gudín (2023).

If you are interested in the evolution of oviposition strategies and host use in the Tachinidae, I am happy to collaborate. There is a lot of work to be done and fascinating discoveries ahead!

REFERENCES

- Amorim, D. de S., Brown, B.V., Boscolo, D., Ale-Rocha, R., Alvarez-Garcia, D.M., Balbi, M.I.P.A., Barbosa, A. de M., Capellari, R.S., de Carvalho, C.J.B., Couri, M.S., de Vilhena Perez Dios, R., Fachin, D.A., Ferro, G.B., Flores, H.F., Frare, L.M., Gudín, F.M., Hauser, M., Lamas, C.J.E., Lindsay, K.G., Marinho, M.A.T., Marques, D.W.A., Marshall, S.A., Mello-Patiu, C., Menezes, M.A., Morales, M.N., Nihei, S.S., Oliveira, S.S., Pirani, G., Ribeiro, G.C., Riccardi, P.R., de Santis, M.D., Santos, D., dos Santos, J.R., Silva, V.C., Wood, E.M. & Rafael, J.A. 2022. Vertical stratification of insect abundance and species richness in an Amazonian tropical forest. *Scientific Reports* 12: 1734. <https://doi.org/10.1038/s41598-022-05677-y>
- Belshaw, R., Grafen, A. & Quicke, D.L.J. 2003. Inferring life history from ovipositor morphology in parasitoid wasps using phylogenetic regression and discriminant analysis. *Zoological Journal of the Linnean Society* 139: 213–228. <https://doi.org/10.1046/j.1096-3642.2003.00078.x>
- Blaschke, J.D., Stireman, J.O. III, O'Hara, J.E., Cerretti, P. & Moulton, J.K. 2018. Molecular phylogenetics and piercer evolution in the bug-killing flies (Diptera: Tachinidae: Phasiinae). *Systematic Entomology* 43: 218–238. <https://doi.org/10.1111/syen.12272>
- Burington, Z.L. 2022. A taxonomic revision of the *Eucelatoria ferox* species group (Diptera: Tachinidae). *Zootaxa* 5143: 1–104. <https://doi.org/10.11646/zootaxa.5143.1.1>
- Cantrell, B.K. 1988. The comparative morphology of the male and female postabdomen of the Australian Tachinidae (Diptera), with descriptions of some first-instar larvae and pupae. *Invertebrate Taxonomy* 2: 81–221. <https://doi.org/10.1071/IT9880081>
- Cerretti, P., O'Hara, J.E., Wood, D.M., Shima, H., Inclán, D.J. & Stireman, J.O. III 2014. Signal through the noise? Phylogeny of the Tachinidae (Diptera) as inferred from morphological evidence. *Systematic Entomology* 39: 335–353. <https://doi.org/10.1111/syen.12062>
- Dindo, M.L. & Nakamura, S. 2018. Oviposition strategies of tachinid parasitoids: two *Exorista* species as case studies. *International Journal of Insect Science* 10: 1–6. <https://doi.org/10.1177/1179543318757491>
- Dios, R. de V.P. & Nihei, S.S. 2017. Taxonomic revision of the Neotropical genus *Ectophasiopsis* Townsend, 1915 (Diptera: Tachinidae: Phasiinae). *European Journal of Taxonomy* 334: 1–27. <https://doi.org/10.5852/ejt.2017.334>
- Dios, R. de V.P. & Nihei, S.S. 2020. Taxonomic revision of the genus *Trichopoda* Berthold, 1827 (Diptera: Tachinidae: Phasiinae), with emphasis on the Neotropical fauna. *Zootaxa* 4870: 1–104. <https://doi.org/10.11646/zootaxa.4870.1.1>
- Eggleton, P. & Belshaw, R. 1992. Insect parasitoids: an evolutionary overview. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 337: 1–20. <https://doi.org/10.1098/rstb.1992.0079>
- Elias, L.G., Kjellberg, F., Farache, F.H.A., Almeida, E.A.B., Rasplus, J.Y., Cruaud, A., Peng, Y.Q., Yang, D.R. & Pereira, R.A.S. 2018. Ovipositor morphology correlates with life history evolution in agaonid fig wasps. *Acta Oecologica* 90: 109–116. <https://doi.org/10.1016/j.actao.2017.10.007>

- Farinets, S.I. 1994. Ovipositor morphology of tachinid flies of the tribe Winthemiini (Diptera). *Entomologicheskoe Obozrenie* 73: 16–21. [In Russian.] Note: English translation in *Entomological Review* 73 (9): 24–29.
- Farinets, S.I. 2006. Morphological particularities of genital organs of the females of some tribes of Tachininae (Diptera, Tachinidae). *Науковий вісник Ужгородського університету Серія Біологія* 19: 202–206. [In Ukrainian.]
- Farinets, S.I. 2010. Affinities of some genera and tribes of the subfamily Tachininae (Diptera, Tachinidae) according to the results of the morphobiological analysis of the genitalia of females and larvae of the 1st instar. *Науковий вісник Ужгородського університету Серія Біологія* 29: 33–36. [In Ukrainian.]
- Farinets, S.I. 2017. Comparative morphological analysis of postabdomen structures of tachinid females (Diptera, Tachinidae) that lay eggs under integument and in crevices of the host body. *Науковий вісник Ужгородського університету Серія Біологія* 42: 47–51. [In Ukrainian.]
- Farinets, S.I. 2018. Comparative-morphological analysis of structure of oviposition, spermathek and larvae of 1st age of females of some Voriini tribe species (Diptera, Tachinidae). *Науковий вісник Ужгородського університету Серія Біологія* 45: 45–58. [In Ukrainian.] <https://doi.org/10.24144/1998-6475.2018.45.45-58>
- Feener, D.H. Jr. & Brown, B.V. 1997. Diptera as parasitoids. *Annual Review of Entomology* 42: 73–97. <https://doi.org/10.1146/annurev.ento.42.1.73>
- Gudin, F.M. 2023. Annotated catalog of vespidae hosts (Hymenoptera: Vespidae) of Tachinidae (Diptera), with description of a new species of *Ophirion* Townsend from Brazil. *Zoological Studies* 62. [In press.] <https://doi.org/10.6620/ZS.2023.62-06>
- Gudin, F.M. & Nihei, S.S. 2019. Taxonomic revision of the Neotropical genus *Ormiophasia* Townsend, 1919 (Diptera: Tachinidae), with the description of eight new species. *Zootaxa* 4643: 1–74. <https://doi.org/10.11646/zootaxa.4643.1.1>
- Gudin, F.M., Soares, M.M.M., Fernandes, D.R.R. & Rafael, J.A. 2022. The first record of parasitism in soldier flies by tachinids: *Lixophaga stratiophaga* Gudin, sp. nov. (Diptera: Tachinidae), reared from *Ptecticus testaceus* (Fabricius) (Diptera: Stratiomyidae) in Amazon rainforest and updated catalogue of dipteran hosts of Tachinidae. *Austral Entomology* 61: 387–406. <https://doi.org/10.1111/aen.12620>
- Herting, B. 1957. Das weibliche Postabdomen der calyptraten Fliegen (Diptera) und sein Merkmalswert für die Systematik der Gruppe. *Zeitschrift für Morphologie und Ökologie der Tiere* 45: 429–461.
- Hörnig, M.K., Haug, C., Schneider, J.W. & Haug, J.T. 2018. Evolution of reproductive strategies in dictyopteran insects—clues from ovipositor morphology of extinct roachoids. *Acta Palaeontologica Polonica* 63: 1–24. <https://doi.org/10.4202/app.00324.2016>
- Kawakita, A. & Kato, M. 2016. Revision of the Japanese species of *Epicephala* Meyrick with descriptions of seven new species (Lepidoptera, Gracillariidae). *ZooKeys* 568: 87–118. <https://doi.org/10.3897/zookeys.568.6721>
- Lee, S. & Lee, S. 2020. Multigene phylogeny uncovers oviposition-related evolutionary history of Cerambycinae (Coleoptera: Cerambycidae). *Molecular Phylogenetics and Evolution* 145: 106707. <https://doi.org/10.1016/j.ympev.2019.106707>
- Nakamura, S., Ichiki, R.T. & Kainoh, Y. 2013. Chemical ecology of tachinid parasitoids. Pp. 145–167. *In*: Wajnberg, E. & Colazza, S., eds., *Chemical ecology of insect parasitoids*. 312 pp. Wiley-Blackwell, Oxford.
- Nihei, S.S. 2015. Systematic revision of the ormiine genera *Aulacephala* Macquart and *Phasioormia* Townsend (Diptera, Tachinidae). *Zootaxa* 3931: 1–26. <https://doi.org/10.11646/zootaxa.3931.1.1>
- O'Hara, J.E. 1985. Oviposition strategies in the Tachinidae, a family of beneficial parasitic flies. *University of Alberta Agriculture and Forestry Bulletin* 8(2): 31–34.
- Roth, J.P., King, E.G. & Thompson, A.C. 1978. Host location behavior by the tachinid, *Lixophaga diatraeae*. *Environmental Entomology* 7: 794–798. <https://doi.org/10.1093/ee/7.6.794>
- Santis, M.D. de & Nihei, S.S. 2022. Phylogenetic analysis of the tribe Dufouriini (Diptera: Tachinidae) using a total evidence approach based on adult and immature stages. *Arthropod Systematics & Phylogeny* 80: 1–38. <https://doi.org/10.3897/asp.80.e69618>

- Stireman, J.O. III, Cerretti, P., O'Hara, J.E., Blaschke, J.D. & Moulton, J.K. 2019. Molecular phylogeny and evolution of world Tachinidae (Diptera). *Molecular Phylogenetics and Evolution* 139: 106358.
<https://doi.org/10.1016/j.ympev.2018.12.002>
- Stireman, J.O. III, O'Hara, J.E. & Wood, D.M. 2006. Tachinidae: evolution, behavior, and ecology. *Annual Review of Entomology* 51: 525–555. <https://doi.org/10.1146/annurev.ento.51.110104.151133>
- Tachi, T. & Shima, H. 2010. Molecular phylogeny of the subfamily Exoristinae (Diptera, Tachinidae), with discussions on the evolutionary history of female oviposition strategy. *Systematic Entomology* 35: 148–163.
<https://doi.org/10.1111/j.1365-3113.2009.00497.x>
- Waggy, S.L. & Beardsley, J.W. 1974. Biological studies on two sibling species of *Lixophaga* (Diptera: Tachinidae), parasites of the New Guinea sugarcane weevil, *Rhabdoscelus obscurus* (Boisduval). *Proceedings of the Hawaiian Entomological Society* 21: 485–494.
- Wood, D.M. 1985. A taxonomic conspectus of the Blondeliini of North and Central America and the West Indies (Diptera: Tachinidae). *Memoirs of the Entomological Society of Canada* 132: 1–130.
<https://doi.org/10.4039/entm117132fv>