Is that a Chrysotachina?

And that one also?

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What is Chrysotachina

B. & B. (Diptera: Tachinidae)?

It is well known that Tachinidae possess an extraordinary diversity not just in number of species, but also in terms of forms and life-history strategies. The family is notoriously difficult taxonomically for several reasons, some intrinsic and some due to the workers approach to them (O'Hara 2013). With over 1477 genera and 8592 species (O'Hara & Henderson 2020), it is difficult to gain a comprehensive knowledge of the family. The Tachinidae are characterized by recurrent rampant morphological homoplasies. This, along with the reliance of authors on a limited set of mainly chaetotaxic characters has led to historical taxonomic instability. Furthermore, there has been relatively little attention paid to immature stages and life histories in most taxonomic works. Despite the significant progress towards a classification that reflects the natural groupings within the family, there are still many limitations in the comprehension of the true relationships and diversity of tachinid taxa at all taxonomic levels (Wood & Zumbado 2010, O'Hara 2013).

The Polideini are a good example of the recurrent taxonomic difficulties faced in the study of this family, especially in the Neotropical Region. Revised only two decades ago, this tribe gathers together taxa previously assigned to nine different tribes (O'Hara 2002). Within the Polideini, the New World genus *Chrysotachina* Brauer & Bergenstamm is one of the most diverse genera. It has drawn the interest of several dipterists since its description in 1889 (e.g., Townsend 1912, 1919, 1931, Aldrich 1926, Curran 1939, Nunez *et al.* 2002, Nunez & Couri 2002), and despite significant progress over the last century in its classification, new taxonomic changes are needed to approach a definition of the genus that reflects its evolutionary history.

Here we provide an introduction to the diversity of the *Chrysotachina sensu lato* lineage and briefly discuss several aspects of the morphology and life history of the group. Our goal is also to use *Chrysotachina* as an example to highlight the importance of incorporating other characteristics complementary to adult morphology into tachinid systematics, such as immature stages, internal structures and natural history traits to find the "signal through the noise" (Cerretti *et al.* 2014) and better understand the evolutionary relationships of Tachinidae.



Figure 1. A small sample of representative species of *Chrysotachina sensu lato*: **a**. *Chlorohystricia reinwardtii* Wiedemann. **b**. *Chrysotachina s.l.* ("*Chlorohystricia*") n. sp. 1. **c**. *Chrysotachina s.l.* ("*Chlorohystricia*") n. sp. 6b. **d**. *Chrysotachina* n. sp. ald2. **e**. *Chrysotachina* n. sp. ery3. **f**. *Chrysotachina* n. sp. tow2. **g**. *Chrysotachina* n. sp. C3. **h**. *Chrysotachina* n. sp. W10. **i**. *Chrysotachina* n. sp. 49. (Scale bars = 1.0 mm.)

History of Chrysotachina classification

Brauer & Bergenstamm (1889) made a brief morphological description of *Chrysotachina* based on a comparison with *Gymnocheta* R.-D., a genus containing species with metallic green body color, which is distantly related and belongs to the tribe Ernestiini. Aldrich (1926, 1928) suggested a close relationship among genera of Tachinidae with metallic green color that are distributed around the world, grouping *Chrysotachina* with the ernestiine genera *Gymnocheta*, *Chrysosomopsis* Tnsd. and *Janthinomyia* B. & B.

Early work on *Chrysotachina* recognized only species with green or bluish metallic color as belonging to the genus (Townsend 1912, 1919, 1931, Aldrich 1926, Curran 1939), whereas non-metallic colored *Chrysotachina*-like species were included in other genera (e.g., Curran 1927, Aldrich 1932, Reinhard 1935). Later in the 20th Century, Guimarães (1971) included 13 species in *Chrysotachina* in his catalogue of the *Tachinidae of America South of the United States*. Guimarães included the strikingly similar non-metallic species *C. ornata* (Tnsd.) and *C. subcyanea* (v.d. Wulp) in the genus. Nunez & Couri (2002) later redescribed seven metallic-colored South American species of *Chrysotachina* and Nunez *et al.* (2002) added six new metallic-colored Neotropical species to the genus.

It was not until O'Hara's (2002) work on the delimitation of the Polideini and revision of Polideini of America north of Mexico that the boundaries of the genus were expanded to more of the non-metallic forms. Considering the similarities in the male terminalia and external morphology, O'Hara assigned to *Chrysotachina* all of the non-metallic species previously comprising the genera *Exoristopsis* Tnsd., *Helioplagia* Tnsd., *Mericina* Curran and *Neorigone* Tnsd., and described three new species for North America. In the same work, a morphological phylogenetic analysis of the Nearctic Polideini suggested that *Chrysotachina* may have affinities with *Chlorohystricia* Tnsd. (Fig. 1a–c) and *Hystricia* Macq., and the three genera were treated as the *Hystricia* genus group. No synapomorphies were found for *Chrysotachina*, raising doubts about the monophyly of this genus. However, the genus concept was maintained given the uniform appearance of the species. The cladistic analysis suggested that *Chlorohystricia* and *Hystricia* were sister taxa, a relationship supported by the presence of a globose abdomen and numerous discal setae on tergite 3. Historically, the genus *Chlorohystricia* has not received as much attention as *Chrysotachina*, perhaps because it contains only three described species. However, this limited number of species does not exclude it from the complicated taxonomic issues common in tachinid taxa (see O'Hara 2002: 83–84).

Is Chrysotachina monophyletic?

Defining Chrysotachina, following its current concept, through morphological methods based on the examination of adult specimens seems to be a task difficult to achieve. As currently defined, the genus comprises a group of remarkably similar but at the same time distinct and disjunct non-metallic (Fig. 1g-i) to metallic forms (Fig. 1d-f). Its species are characterized by the following combination of external characters: hairy eyes, bare parafacial, gena well-developed (about 0.2–0.25X head height), protruded lower facial margin, supra-alar row with 2 strong and subequal setae (in addition to other setae), metathoracic spiracle with dense and interwoven setae, wings usually hyaline or slightly infuscated, and in males proclinate orbitals are absent. In addition, they share the following combination of male terminalia characters: sternite 5 narrow and shallow, epandrium higher than long and pregonite long and curved. However, these are traits shared with other Polideini taxa (i.e., Ecuadorana Tnsd., Spilochaetosoma Smith, some Hystricia, and other undescribed Neotropical genera). Despite our detailed morphological examination, synapomorphies seem to be elusive in Chrysotachina. Some traits that are frequently used for the recognition of other tachinid genera are variable in Chrysotachina. For example, in Chrysotachina the inner vertical setae are crossed or parallel, in males the outer vertical setae are not differentiated to well-developed, palpi are usually yellow but in a few species are completely dark (almost black), prosternum is haired or bare, abdominal tergites 3 and 4 usually each bear one pair of median discal setae (but in some species these setae are missing), and in the wing, vein R₁ is usually devoid of setae but can be partially or completely haired.

Despite the absence of synapomorphies in the adults of *Chrysotachina*, JMPL's preliminary data obtained through next-generation sequencing of ultraconserved elements (UCEs), life-history traits, and previously unexplored morphological characters support the monophyly of an expanded *Chrysotachina*.

Redefining the genus Chrysotachina

The phylogenetic reconstruction of the Polideini using UCEs included only six representatives of *Chrysotachina sensu stricto*, and two representatives each of *Chlorohystricia* and *Hystricia*. However, this small representation provided a foundation for the understanding of the relationships, generic limits and diversity of these polideines. Preliminary results of the reconstructed phylogeny (Fig. 2) suggest that: a) *Chlorohystricia* species arose within the genus *Chrysotachina*, meaning that they are indeed larger and spiny *Chrysotachina*, b) the large spiny body form of *Chlorohystricia* species has arisen at least twice independently, and c) non-metallic forms are sister to the more derived metallic species. Although such results for *Chlorohystricia* species might seem shocking at first, a close relationship between these two genera was previously suggested by O'Hara (2002). However, our phylogenomic analysis did not reconstruct *Hystricia* as sister of these two genera as suggested by O'Hara. Nevertheless, results of these two phylogenetic analyses cannot be directly compared given the geographic focus of O'Hara's (2002) work on the Nearctic Polideini. These relationships uncovered by our phylogenetic analysis represent remarkable discoveries considering the historical assumption that spiky tachinids are closely related, and it is just one more example of the recurrent evolutionary convergence present across the family.





Figure 2. Left: A phylogeny of *Chrysotachina s.l.* based on ultraconserved elements. Species names in green indicate their metallic color. Right: Lateral and ventrolateral views of the abdomens of large and bristly *Chrysotachina s.l.* species included in the phylogenetic analysis: **a,b**. *Chrysotachina s.l.* ("*Chlorohystricia*") n. sp. 2, a species with sexual patches on abdominal tergites 4 and 5. **c,d**. *Chrysotachina s.l.* ("*Chlorohystricia*") n. sp. 6, a species without sexual patches.

Inclusion of *Chlorohystricia* in the genus *Chrysotachina* will reduce even more the possibility of finding synapomorphies in the adult stage to support the monophyly of a broadened *Chrysotachina*. However, despite this broader array of forms, external morphological characters and male terminalia may provide hints about the phylogenetic position of independent lineages of *Chlorohystricia* and their relationships with allied *Chrysotachina s.s.* species. We will use the term *Chrysotachina sensu lato* below for the grouping of *Chlorohystricia* and *Chrysotachina*. We are not formally synonymizing these two names here. As currently understood, *Chrysotachina s.s.* and *Chlorohystricia* comprise 29 described species (5 Nearctic, 24 Neotropical) and 3 described species (1 Nearctic/Neotropical, 2 Neotropical), respectively. However, this is only a fraction of the true diversity of *Chrysotachina s.l.* During the examination of miscellaneous Polideini material borrowed from several collections for JMPL's Ph.D. dissertation project, over 70 undescribed Neotropical species of *Chrysotachina s.l.* were found. Examining this overwhelming, but not surprising, diversity of forms of *Chrysotachina s.l.* has allowed us to obtain a more complete picture of the diversity of this clade, and to explore traits that could be used to identify patterns of diversification. However, we have no doubts that the number of species in this genus is significantly greater than the number we have seen.

Sexual patches in Chrysotachina s.l.

Several authors have recorded the presence of sexual patches in descriptions of metallic-colored *Chrysotachina s.s.* species. However, limited attention has been given to these structures due to their uniqueness within the tribe. These specialized patches are exclusively found on male individuals, and they are characterized by abdominal areas with patches of setae and microtrichia that differ in morphology from the surrounding setae and are thought to have a secretory function (Cerretti *et al.* 2015). In *Chrysotachina s.l.* sexual patches are located on the ventrolateral surface of tergite 4 (T4) and tergite 5 (T5) or only on T5. Absence or presence of the sexual patches and their anatomical form, along with the male terminalia, seem to be the key to understanding the relationships among species of *Chrysotachina s.l.* with disparate body habitus.

Although sexual patches have been recorded only for some metallic species of *Chrysotachina s.s.*, we have found them also in Chlorohystricia. They are also present in at least two non-metallic species of Chrysotachina. The development of sexual patches across metallic, non-metallic and large bristly species of Chrysotachina s.l., suggests that these organs may have developed independently multiple times across this lineage. Close examination of sexual patches across Chrysotachina s.l. reveals that these structures vary widely in the degree of area covered, lengths and densities of microtrichia and setae, degrees of punctuations at the base of the setae, and the presence of longitudinal grooves on the cuticle. Variation in the composition of these traits seems to form a continuous gradient, making it difficult to split these structures into well-defined types. However, patterns in the structure of these sexual patches seem to be conserved among closely related taxa. We have characterized four main configurations of abdominal tergites 4 and 5 in *Chrysotachina s.l.*: a) sexual patch absent (Figs. 2c,d, and 3a), b) sexual patch with strong punctures at the base of setae and grooves on the cuticle on T5 (Fig. 3b), c) sexual patch with strong punctures at the base of setae and grooves on the cuticle on T4 and T5 (Fig. 3c,d), and d) sexual patch without strong punctures at the base of setae and often with conspicuous microtrichia on T4 and T5 or only on T5 (Fig. 2a,b, and 3e-h). This last category includes the presence of a sexual patch on T4 and T5 or only on T5 since in several species there is only a slight difference in the density of setae present in the sexual patch or there is no clear definition of the sexual patch on T4. This is a temporary categorization that seems not to be exclusive to a particular group of species, but in combination with male terminalia traits it may help to cluster allied species. In considering these proposed categories for the sexual patches, it is not surprising that these organs had not been reported for *Chlorohystricia* species before, since the three described Chlorohystricia species present the last configuration of sexual patch, and the more setose and hairier body habitus of these species masks their presence. Sexual patches of some large-bodied Chrysotachina s.l. that are not evident at first glance can be recognized by the presence of slightly longer setae and a different angle orientation compared with the setae in the surrounding area. In addition, they are indicated by the presence of microtrichia covering the cuticle, albeit dense setation might interfere with their observation.



Figure 3. Ventrolateral view of male abdomens. a. Sexual patch absent, *Chrysotachina s.l.* n. sp. 8. b. Sexual patch with strong punctures at the base of setae and grooves on the cuticle on T5, *Chrysotachina willistoni* Curran. c,d. Sexual patch with strong punctures at the base of setae and grooves on the cuticle on T4 and T5. c. *Chrysotachina braueri* Tnsd. d. *Chrysotachina s.l.* n. sp. IG. e-h. Sexual patch without strong punctures at the base of setae and often with conspicuous microtrichia on T4 and T5 or only on T5. e. *Chrysotachina s.l.* n. sp. 1b. f. *Chrysotachina* n. sp. J39, microtrichia conspicuous. g. *Chrysotachina s.l.* n. sp. rwA. h. *Chlorohystricia reinwardtii* Wiedemann.

In addition to the three described species of *Chlorohystricia*, we have found about 15 large and spiny undescribed species of *Chrysotachina s.l.* displaying each of the four configurations on T4 and T5. With the combination of these sexual patch characteristics and male terminalia traits we have clustered these large-bodied species into what appears to be six groups of closely related species. This clustering of large-bodied *Chrysotachina s.l.* allows us to associate these groups with closely related but distinct-looking and smaller species.

Finding the signal: puparium traits and host associations

Despite the absence of synapomorphies to define *Chrysotachina s.l.*, we have found that some traits of the posterior spiracle discs of the puparium and host-associations provide insight into relationships within this clade. Unfortunately, for most Neotropical tachinids there is virtually no information about their immature stages nor their host associations. However, data obtained mostly from the biodiversity inventory programs at the Area de Conservación Guanacaste (Costa Rica) and Yanayacu Biological Station and Center for Creative Studies (Ecuador) are providing useful information to help improve comprehension of these polideines.

Comparison of the posterior spiracular discs of the puparia of around 20 species of *Chrysotachina s.l.* and several representatives of other polideine genera with robust and hairy bodies (i.e., *Hystricia, Pseudobombyliomia* Tnsd. and *Eucheirophaga* James) shows that *Chrysotachina s.l.* possess sinuous spiracular slits (Fig. 4a,b,d,e) in contrast to the straight or slightly curved slits present in the other genera (Fig. 4c,f). Additionally, we have found two states in these posterior spiracular discs of the puparia of *Chrysotachina s.l.* that concur with the relationships found in the UCE-based phylogenetic analysis. The more basal lineages of *Chrysotachina s.l.* (i.e., some of the non-metallic forms) possess a puparium that is posteriorly more or less evenly rounded on upper and lower surfaces with the posterior spiracular discs of the puparia of polideines could potentially support the presence of the sinuous spiracular slits in the posterior spiracular discs as a synapomorphy for the entire clade of *Chrysotachina s.l.*, and variation in it may help to resolve relationships of lineages within *Chrysotachina s.l.*



Figure 4. Posterior spiracular discs of the puparium: a. Chrysotachina urichi (Aldrich). b. Chrysotachina n. sp. subc2. c. Pseudobombyliomyia n. sp. YY. d. Chrysotachina n. sp. 5. e. Chrysotachina s.l. n. sp. CH3. f. Hystricia sp. 6. (Scale bars = 1.0 mm.)

Host associations of *Chrysotachina s.l.* can also provide evidence about their evolutionary relationships. During the initial stages of JMPL's work on the Polideini, we considered the undescribed species *Chrysotachina* n. sp. 49 (Fig.1i) and three allied taxa as a potential separate genus sister to *Chrysotachina s.l. Chrysotachina* n. sp. 49 and closely related species have slender body habitus and reduced and lightened setae ventrally. Additionally, *Chrysotachina* n. sp. 49 parasitizes *Pedaliodes montagna* Adams & Bernard (Lep.: Nymphalidae), a free-living caterpillar whereas most *Chrysotachina s.l.* attack shelter-building caterpillars of the families Hesperiidae and Mimallonidae.

However, detailed examination of the host records available for *Chrysotachina s.l.* revealed a broader array of host associations. We found that at least one complex of undescribed species of *Chrysotachina s.s.* is associated with caterpillars belonging to five genera of metalmark butterflies (Riodinidae), a family known for the associations of its free-living caterpillars with ants, and at least three species of *Chrysotachina* have records of parasitizing free-living nymphalid caterpillars. Hence, although species of *Chrysotachina s.l.* are not strictly restricted to shelter-builder caterpillars, their hosts are restricted to these four above-mentioned families of Lepidoptera. Complementary to this, *Hystricia, Pseudobombyliomia* and *Eucheirophaga* are also caterpillar parasitoids, but they are not known to parasitize caterpillars in any of the families parasitized by *Chrysotachina s.l.* Furthermore, there is no overlap in the lepidopteran families parasitized by these three genera.

Notwithstanding the external heterogeneity of *Chrysotachina s.l.*, the examination of previously poorly-explored morphological traits, puparium characters and host associations, supports the monophyly of this disparate group of tachinids as well as the hypothesized independent evolution of lineages with large and bristly bodies. This raises the question of why several lineages of polideines have experienced this "hedge-hog fly syndrome". The repeated evolution of robust, spiny bodies is an interesting pattern of morphological convergence present across several lineages of Tachinidae that have diversified extensively in the Andes. However, this pattern of diversification in tachinids remain largely unexplored.

The final steps in this study of *Chrysotachina s.l.* will include a redefinition of the genus and the description and/or redescription of its many species. Further observations and analyses are needed to better understand the evolutionary relationships within *Chrysotachina s.l.* A more detailed examination of sexual patches using SEM imaging is necessary to better understand their structure and variation across the genus, and new characters need to be found to add support for the multiple origins of the large and bristly *Chlorohystricia*-like species.

Note to the reader: If you rear caterpillars from any of the lepidopteran families mentioned above, keep an eye open for parasitized specimens because you might be a happy witness to the emergence of a *Chrysotachina* specimen.

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