Cladistic analysis of *Simulium* (Trichodagmia) and *Simulium* (Thyrsopelma) (Diptera: Simuliidae)

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Received May 1999; revised and accepted for publication June 2000

A cladistic analysis of *Simulium* (Trichodagmia) sensu Crosskey and Howard, using 34 morphological characters of larvae (6 characters), pupa (5) and adults (23), yields nine most parsimonious trees under equal weights (length 101 steps CI 0.49 RI 0.73). Successive weighting based on the maximal rescaled consistency index preferred one of the nine (31.37 steps CI 0.62 RI 0.87 total fitcon3 = 235.8), which was also one of two trees found under implicit weights with concavity values of 3–6. The cladogram justifies the recognition of two subgenera. *Simulium* (Trichodagmia) sensu stricto (containing *S. muiscorum*, *sumapazense*, *S. wygodzinskyorum*, *S. nigrimanum*, *S. chalcocoma*, *S. huairayacu* and *S. lahillei*) is supported by the branchial tip sclerotization and the presence of cibarial teeth, larval body tegument covered with lanceolate hairs, female with simple claw, and gonapophysis size. *Simulium* (Thyrsopelma) (containing *S. scutistriatum*, *S. hirtipupa*, *S. orbitale*, *S. guianense*, *S. perplexum* and *S. itaunense*) is supported by the hypostomial teeth.

ADDITIONAL KEY WORDS: monophyly – neotropical simuliids – equal weights – successive weighting – implicit weights.

INTRODUCTION

Enderlein (1934) proposed two neotropical blackfly genera, Thyrsopelma and Trichodagmia. The type species of *Simulium* (Thyrsopelma) is *Simulium* (Thyrsopelma) brasiliense (Enderlein, 1934:284) [= *Simulium orbitale* Lutz 1910:231]. The type species of *Simulium* (Trichodagmia) [= *Simulium* (Grenieriella) Vargas & Diaz Núñera 1951:141] is *Simulium* (Trichodagmia) latitarsis (Enderlein, 1934:288) [= *Simulium* chalcocoma Knab, 1914:85]. Both species descriptions were based only on females.

Coscarón (1987, 1991) considered Thyrsopelma and Trichodagmia as two distinct taxa based on the form of the female genitalia, hairs of the larval tegument, and the arrangement of hypostomial teeth. At that time, he used the name Grenieriella instead of Trichodagmia because *Simulium lahillei* (Paterson & Shannon, 1927; type of Grenieriella) was known from all life stages. He recognized three species groups within each taxon: *scutistriatum*, hirtipupa and orbitale within *Thyrsopelma*, and lahillei, nigrimanum and municiporum within *Trichodagmia* (Coscarón, 1987). Crosskey & Howard (1997) recognized just one taxon, synonymized *Thyrsopelma* with *Trichodagmia*, and did not recognize any species groups.

Table 1. Character list and states

**Larva**
1. Larval antennae: smooth [0], with secondary annulation [1].
2. Hypostomium central and intermedial teeth: well differentiated [0] or not well differentiated [1].
3. Mandible apical tooth: not-reduced [0] or reduced [1].
4. Anal sclerite hook number: 0 [0]; 1–99 [1]; 100–149 [2]; >150 [3].
5. Anal gill diverticula number: 0 [0]; 1–5 [1]; 6–20 [2]; >21 [3].
6. Body tegument: glabrous [0]; with simple hairs [1]; with lanceolate hairs [2].

**Pupa**
7. Branchial filament number: 1–6 [0]; 7–10 [1]; 11–20 [2]; >21 [3].
8. Branchial tip form: rounded [0], sharp [1].
9. Branchial tip sclerotization: slight (clear tips) [0]; medium [1]; strong (dark tips) [2].
10. Tergites VI to IX: with a basal spine comb [0]; without a basal spine comb [1]; spine comb only in the penultimate segment [2].
11. Terminal spines: well developed [0]; reduced or absent [1].

**Female**
12. Cibarium: unarmed [0], armed [1].
13. Cibarium: with acute teeth [0]; with small tubercules [1].
14. Claw: simple [0], with a small sub-basal tooth [1], with a well-developed sub-basal tooth [2].
15. Wing length: 0–1.79 mm [0]; 1.8–2.5 mm [1]; >2.51 mm [2].
16. Subcostal vein (Sc): glabrous [0]; hairy [1].
17. Radial vein: basal sector (Rb): glabrous [0], hairy [1].
18. Paraproct form: subcircular [0], subquadrangular [1], rectangular or ventrally produced [2], digitiform [3].
19. Paraproct length/width ratio: 0–1.49 [0], >1.5 [1].
20. Paraproct microtriquies: covering the entire paraproct [0], only in the distal border [1].
21. Paraproct spicules: slender [0]; robust [1].
22. Genital fork arms: Not very expanded distally [0], very expanded distally [1].
23. Genital fork arms: with prominent internal projection [0], without internal projection [1].
24. Spermatheca: flat [0], sculptured [1].
25. Gonopophysis shape: subtriangular, apically rounded [0], subovoid [1], acuminate, apically produced [2].
26. Gonopophysis length/width ratio: length = width [0], length > width [1], length < width [2].
27. Basimere length/width ratio: 0–0.65 [0], more than 0.65 [1].
28. Distimere length/width ratio: 0–1.0 [0], 1.01–2.81 [1], >2.81 [2].
29. Distimere apex: straight [0], very bent [1].
30. Ventral plate scotadures: Inverted U shaped [0], H shaped [1].
31. Ventral plate distal border: ronly [0], crenate [1], depressed [2].
32. Endoparamere: with teeth [0], reduced or absent teeth [1].
33. Endoparamere teeth: similar in size [0], small and large teeth [1].

*Trichodagmia* (Parana, Brazil), and deposited in the School of Saúde Publica of Sao Paulo University (Brazil), as a species *inquierandae* and possibly a junior synonym of *S. pintoi*. The latter species was later synonymized with *guianense* by Crosskey & Howard (1997). After examining the syntypes of *S. albopictum*, we concluded that the scutum pattern and general colour of *S. albopictum* is sufficiently similar to *S. guianense* to maintain the synonymy of both *albopictum* and *pintoi* with *guianense*. This synonymy could be reconsidered if male, pupal and larval stages of *S. albopictum* are found. Coscarón & Miranda-Esquivel (1998) synonymized *S. (Trichodagmia) argentatum* with *S. (Inaequalium) inaequale*. These synonymies reduce the total number of species to 13.

*Thyrsopelma sensu* Coscarón occurs from the southeast of Brazil to Amazonia, the Guyanas and southern Venezuela (*guianense*), and west to Paraguay and eastern Argentina (*orbitale*). Some species of *S. Thyrsopelma*, such as *guianense* shows high anthropophily. Indeed, it is an onchocercosis vector in Amazonia; *orbitale* and *scutistriatum* are known as a nuisance to humans and cattle. *Trichodagmia* occurs throughout western South America, from Colombia to central western Argentina. *S. (Trichodagmia) nigrimanum* is con-
TRICHODAGMIA

This research attempts to solve three questions. Is Trichodagmia (sensu Crosskey & Howard, 1997) monophyletic? Are Trichodagmia and Thyrsopelma (sensu Coscarón, 1987) monophyletic? What are the relationships between Trichodagmia and Thyrsopelma (sensu Coscarón, 1987)? These questions must be answered in order to address the correct nomenclatural position of these taxa.

MATERIAL AND METHODS

In broad terms, we use the method of phylogenetic systematics as outlined in Kitching et al. (1998). Outgroup comparisons were performed following Nixon & Carpenter’s (1993). The data matrix included 2 terminal taxa: 13 species of Trichodagmia sensu Crosskey & Howard (1997) and exemplars representing the subgena Anasolen, Freemaniellum, Xenosimulium, Hearlea, Hemicnetha and the owiedo species group as an ingroup. The latter were included to test the putative sister-group relationship between Trichodagmia and Thyrsopelma. Four outgroups were included: the genus Paraustrosimulium and the subgenera Simulium (Pternaspatha), Simulium (Nevermannia) and Simulium (Pomeroeyllum).

Character codings for Paraustrosimulium were based on Paraustrosimulium anthracinum; for S. (Pter-
naspatha) on S. nemorale, S. nigristrigatum and S. diaman- 
ticum; for S. (Anasolen) on S. masabae, S. nili 
and S. dentulosum; for S. (Freemaniellum) on S. 
berghei; for S. (Xenosimulium) on S. neireti; for S. 
(Hearlea) on S. larvispinosum, S. carolinae and S.
Figure 1. Consensus of nine trees found under equal weights (101 steps, CI 0.49, RI 0.73).

capricornis; for S. (Hemicnetha) on S. mexicanum and S. paynei; for S. (Nevermannia) on S. loutetense, S. ruficorne and S. brachium; for S. (Pomeroyellum) on S. alcokei, S. shoutedeni and S. cervicornutum; for S. oviedoi on S. oviedoi and S. rivas. Terminals representing these genera, subgenera and groups were thus composite codings, based on 1–3 species.

Thirty-four morphological characters were used in the analysis. Six were based on larval morphology [0–5]; five on pupal morphology [6–10], 16 on adult female morphology [11–26]; and seven on adult male morphology [27–33]. Character codings were drawn from the original descriptions, redescriptions by Dalmat (1955), Crosskey (1960, 1969), Py-Daniel, Konrad & Gastal (1985), Coscarón (1991), Muñoz (1996) and Shelley et al. (1997) and original observations from additional specimens (Appendix). Table 1 gives descriptions of characters and states. The matrix itself appears in Table 2. All multistate characters were treated as unordered to minimize assumptions of character order.

We performed the analysis under equal weights and successive weighting with PAUP* 4.0d64 (Swofford, 1998). Tree branches were collapsed if minimal branch length was equal to zero (rule 1, Swofford & Begle, 1993; Coddington & Scharff, 1994). We used the heuristic search option in PAUP*, randomizing the taxa addition order 100 times. Tree choice was also based on successive weighting using the maximal rescaled consistency index (Farris, 1989). We used implied weights as an alternative weighting approach (Goloboff, 1993). We used Peevee version 3.0 (Goloboff, 1998) with a similar search strategy: 100 random addition sequences using time as the random seed [amb-; rseed0; bound*100; max* commands], under all concavity values between three and six. Character distribution was examined with Clados version 1.6 (Nixon, 1996) and with Winclada version 0.9.99i (Nixon, 1999) under the unambiguous reconstruction option.

To calculate Bremer support values (Bremer, 1988) we used the relative Bremer support as calculated with Nona program (Goloboff, 1998). The characters were weighted according to the final cladogram using successive weighting. To obtain Bremer support values, we used the bsupport* option with a value of 400 steps.

To test the concordance of life stages as proposed by Judd (1998), we performed three tree searches with equal weights, each one based on data from a different life stage (larva, pupa or imago), and calculated the Incongruence Index (Mickeyvich & Farris, 1981) as proposed by Judd (1998). We also calculated the Incongruence Length Difference (Farris et al., 1994) between pairs of matrices, as implemented in Winclada (Nixon, 1999), using 1000 permutations and mult*10; max* as the tree search strategy.

RESULTS AND DISCUSSION

EQUAL WEIGHTS VS. WEIGHTED DATA

PAUP* with equal weights found nine equally parsimonious trees (length 101 steps CI 0.49 RI 0.73), the strict consensus of which is shown in Figure 1. Successive weighting on this solution set chose one of the nine cladograms (Fig. 2: 31.37 steps CI 0.62 RI 0.87 total fit concav 3235.8). Implicit weights with concavity values of 3–6 all gave the same two trees, one of which was identical to Figure 2. Figure 2 is thus the only topology favoured by equal, successive, and implied weights.

Thysopelma sensu stricto is monophyletic in the strict consensus (Fig. 1), but the monophyly of Trichodagmia is not supported unambiguously by this analysis. Both taxa were supported by successive weights and in both trees under implied weights.

The support for two groups in particular is suspect. The relationship between Hemicnetha, Hearlea and the oviedoi group (Fig. 1) is supported by a claw with a small denticule (character 13), which could be considered a convergence because Nevermannia, Anasolen, Xenosimulium and many species of Trichodagmia and Thysopelma have the same character. Likewise, Hearlea and the oviedoi group (Fig. 1) are united by the basimere length/width ratio (character 27); this possibly is also a convergence.
Figure 2. Cladogram preferred by successive weights and implicit weights under concavity values 3–6 (final length 31.37, steps CI 0.62, RI 0.87, total fit $\chi^2 = 235.8$; with equal weights it has a length of 101 steps).

BRANCH SUPPORT
Relative Bremer support values (fig. 3) are comparatively high for the ingroup. The highest Bremer support values is for Thyrsopelma + Trichodagmia, the subgenus Thyrsopelma, the ‘nigrimanum species group’, and ‘hirtipupa + orbitale species groups’. The value for Trichodagmia, although low, does not necessarily indicate a non-natural group.

COMBINED VS. PARTITIONED DATA
The life stage partitions of the data all gave more ambiguous results. The larval data set gave many most parsimonious trees (length 18) and a bush as the consensus. The pupa data set was similar (length 13) and a bush as the consensus. The adult data set under equal weights produced 225 trees (length 62), the consensus of which is shown in Figure 4. Larval and pupal stage results are not consistent with the combined cladogram whereas the consensus of the adult results is congruent with the cladogram (Fig. 2). These results are expected because the adult partition has more characters than larval or pupal stages alone or combined, and most of the synapomorphies in Figure 2 are adult characters. Judd (1998) also found that the partition with more characters (in her case, the larval dataset) gave the most resolution, and was most compatible with the combined analysis.

Combined analysis cannot give a result shorter than the sum of the partitioned lengths (93 steps = 18 + 13 + 62). In fact, the minimum length for the combined data set was 101, indicating 8 steps (7.9%) attributable to incongruence, but this value is not significant using ILD with any pair of matrices (Table 3). Our analyses show that a combined analysis gives a more resolved result than any of the partitions, which indicates, as advocated by Hennig (1968), that the holomorphology is highly recommended in cladistic analysis, because one does not know a priori in which stage the synapomorphies will occur.
Figure 3. Cladogram showing relative Bremer support.

Figure 4. Consensus of 225 trees yielded for the adult partition analysis, equal weighted characters (initial trees of 62 steps CI 0.50, RI 0.78).

Table 3. Incongruence values. Incongruence Index (D) and Incongruence Length Difference (ILD) tests for all pairs of matrices. A = imago. P = pupa. L = larva. No values are statistically significant.

<table>
<thead>
<tr>
<th>Matrices</th>
<th>D</th>
<th>ILD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A – (L + P)</td>
<td>101 – (62 + 32) = 7</td>
<td>0.3337</td>
</tr>
<tr>
<td>A – P</td>
<td>80 – (62 + 13) = 5</td>
<td>0.4216</td>
</tr>
<tr>
<td>A – L</td>
<td>84 – (62 + 18) = 4</td>
<td>0.8991</td>
</tr>
<tr>
<td>L – P</td>
<td>32 – (18 + 13) = 1</td>
<td>0.9600</td>
</tr>
</tbody>
</table>

(character 26). S. (Thysopelma) perplexum has a claw with a small denticule but we consider this a reversion from the subgenus groundplan.

Although this analysis corroborates the species groups proposed by Coscarón (1987), we prefer not to maintain them nomenclaturally in order to promote homogeneity, and in light of new proposals concerning specific/supraspecific nomenclature (Pleijel, 1999; Cantino et al., 1999). The relations between the species of ‘orbitale’ species group’ require further work to solve the present soft polytomy, perhaps using chromosomal and/or molecular characters, or the discovery of the
larval stage of perplexum and the female of itaunense.

Trichodagmia is also monophyletic, based on the branchial tip sclerotization (character 8, Coscarón (1987: fig. 25E)). Coscarón (1987) used neither this character nor the branchial tip form. An additional synapomorphy is cibarial teeth in the female (character 11, Coscarón (1987: figs. 5N,O)). Shelley et al. (1997) reported that the cibarium of S. guianense and S. perplexum was unarmored, but in fact their cibaria bear small tubercules (character 12, Shelley et al. (1989: figs. 3, 4), Shelley et al. (1997: fig. 56)). Coscarón (1987) proposed that the gonapophysis length/width ratio (character 26) was also a synapomorphy of Trichodagmia, and, although a similar ratio in Anasolen and Xenosimulium does not preclude the character as an homology, we prefer not to use it.

We synonymize the 'lahillei' and 'nigrimanum species groups' proposed by Coscarón (1987) because S. wygodzinskyorum, although originally placed in the lahillei group (Coscarón and Py-Daniel, 1989), was basal to the clade.

Trichodagmia and Thyrsopelma species breed in fast-flowing creeks, also in rivers or in high-speed streams. The African taxa Xenosimulium–Anasolen–Freemaniellum live in similar places and one might expect the similarity ecologically convergent. However, there is extensive phylogenetic congruence between characters in the cladogram (see Fig. 2), and other Neotropical taxa (Hemicnetha, Chirotistilbia, Ectennaspis, or Psaroniocompsa) breed in the same places. Instead we regard these similarities as synapomorphies rather than convergences.

Crosskey (1969) proposed a possible relationship between Trichodagmia and Xenosimulium/Anasolen/Freemaniellum, but he considered Xenosimulium more likely to be related to Freemaniellum–Anasolen than to Trichodagmia. This analysis suggests that Anasolen/Xenosimulium is closer to Thyrsopelma/Trichodagmia than to Freemaniellum. Anasolen/Xenosimulium is the sister group of Thyrsopelma + Trichodagmia. Nevertheless, additional research using chromosomal and/or molecular data may support Freemaniellum as sister to Trichodagmia + Thyrsopelma. Coscarón (1987) proposed Hemicnetha as sister to Thyrsopelma + Trichodagmia, but that hypothesis fails when the Afrotropical subgenera Xenosimulium, Anasolen and Freemaniellum are included.

We found a possible sister group relationship between Nevermannia and Pomeryellum, but character support for it is weak. Craig (1987) studied the Inselliellum subgenus and found good support for Inselliellum as sister to Nevermannia, although we did not include that subgenus in our research. Nevermannia’s cladistic definition and its sistergroup relationship await further research.

**CLASSIFICATION**

Based on the relationships depicted in the cladogram we propose the following classification:

1. Subgenus Trichodagmia
   1.1. S. muiscorum
   1.1. S. sumapazense
   1.3. S. wygodzinskyorum
   1.4. S. nigrimanum
   1.5. S. chalcocoma
   1.6. S. huairayacu
   1.7. S. lahillei

2. Subgenus Thyrsopelma
   2.1. S. scutistriatum
   2.2. S. hirtipupa
   2.3. S. orbitale
   2.4. S. guianense
   2.5. S. perplexum
   2.6. S. itaunense

**ACKNOWLEDGEMENTS**

We are grateful to J. Coddington and an anonymous reviewer for valuable comments on the manuscript, to A. J. Shelley, C. Lowry and T. Howard (British museum of Natural History), who kindly loaned Afrotropical specimens. J. V. Crisci carefully read an early draft and helped with suggestions and software to improve it. J. Lynch helped to improve the perspective. N. Caligaris made microslide preparations. The first author is much in debt to Colciencias (Consejo Nacional de Ciencia y Técnica, Colombia) for its financial support.

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**APPENDIX: SPECIMENS STUDIED**

BMNH: The Natural History Museum, London, UK.

ICN: Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia.

MLP: Museo de La Plata, Universidad Nacional de La Plata, La Plata Argentina.

S. chalcocoma


S. guianense (as S. pintoi)


S. hirtipupa


S. lahillei


S. nigrimanum


S. orbitale


S. perplexum


S. scutistriatum


S. sumapazense


S. wygodzinskyorum

PERU: Junin, Estancia el Naranjal, San Ramón 1000 m. Male. 20.vi.1965 coll. P. Wygodzinsky Paratype [MLP].

S. wygodzinskyorum


S. wygodzinskyorum

PERU: Junín, Estancia el Naranjal, San Ramón 1000 m. Male. 20.vi.1965 coll. P. Wygodzinsky Paratype [MLP].

Parasimulium anthracinum


**S. (Piernaspatha) subgenus**

**Simulium nemorale**


**S. nigristrigratum (as S. bachmanni)**


**S. diamantinum**


**S. (Anasolen) subgenus**

**Simulium masabae**


**S. nili**


**S. dentulosum**


**S. (Freemanellum) subgenus**

**Simulium bergethi**


**S. (Xenosimulium) subgenus**

**Simulium neireti**


**S. (Hearlea) subgenus**

**Simulium larvispinosum**

GUATEMALA: XXII, female in slide [MLP]. XXII, male in slide [MLP].

**S. caroliniae**

GUATEMALA: Rio Santa Anita, Finca Monte de Oro, 900 m, female in slide [MLP]. Rio Santa Anita, Finca Monte de Oro, 900 m, male in slide [MLP].

**S. capricornis**


**S. (Hearlea) spp.**

Two exuvias without data [MLP].

**S. (Hemicnetha) subgenus**

**Simulium mexicanum**

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S. paynei

S. (Nevermannia) subgenus
Simulium loutetense

S. ruficorne

S. brachium

S. (Pomeroyellum) subgenus
Simulium alcocki

S. shoutedeni

S. cervicornutum

Oviedo group
S. rivasi

S. oviedo