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Phylogenetic relationships in the genus Psorophora Robineau-Desvoidy (Diptera: Culicidae), based on morphological characters

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ABSTRACT

Phylogenetic relationships in the genus Psorophora were examined based on a cladistic analysis of 66 morphological characters (fourth instar larvae, pupae, males and females) of 29 species available from the 45 species reported in the genus, representing the three subgenera. The ingroup species were: five Psorophora (10 spp total, 50%), seven Grabhamia (15 spp total, 47%) and twelve Janthinosoma (20 spp total, 60%). Two Culicini genera (Culex and Toxorhynchites), two Aedini genera (Aedes and Haemagogus), and one Mansoniini (Mansonia) were used as outgroups and sister groups respectively. A parsimony analysis using TNT resulted in 11 trees, each with 164 steps (CI = 0.66 and RI = 0.83). The analysis indicated that Aedini (Haemagogus, Aedes and Psorophora), is monophyletic group that includes Mansonia titillans (Mansoniini), suggesting natural inclusion of the Mansoniini tribe into Aedini. The genus Psorophora is monophyletic, supported by 3 synapomorphies: larvae with presence of trident-like scales in segment VIII; female with tergo and sternum VIII with rod-like structure and male genitalia with few teeth on the sternite process on segment X. The current subgeneric classification was validated by our results. The clades representing the subgenera Psorophora, Grabhamia and Janthinosoma were supported by four, three and two synapomorphies respectively. However, within Grabhamia and Janthinosoma, internal polytomies were observed leaving the internal evolutionary relationships unresolved.

Key words: Aedini, cladistics, mosquitoes, morphology, VEEV, vectors

INTRODUCTION

Mosquitoes (Diptera: Culicidae) play an important role as vectors of different pathogens, like arboviruses that cause dengue, yellow fever, equine encephalitis, Mayaro and other emerging diseases. Venezuelan equine encephalitis virus (VEEV) has caused several epizoodemics in Southern North America and South America [1-4]. The main epizootic vectors of VEEV include a wide range of mosquitoes that feed on mammals: *Aedes*, *Mansonia*, and *Psorophora*. Species of *Culex (Melanoconion)* spp. are reported as enzootic vectors [5]. Later, *Psorophora confinnis* (Lynch Arribálzaga) and *Aedes taeniorhynchus* (Wiedemann) have been reported infected and also as epizootic VEEV vectors [6], while *Ae. taeniorhynchus* has been involved in natural and experimental epizoodemic transmission [1, 7-9]. Others *Psorophora* species are probably also involved in epizootic transmission [5,10], but also several species of *Psorophora* has been implicated as vectors of Wyeomyia virus, La Crosse virus and other arboviruses [11].

The genus *Psorophora* is comprised by 45 species, subdivided in the subgenera *Grabhamia* (15 spp.), *Janthinosoma*

(20 spp.) and *Psorophora* (10 spp.), distributed from the south of Canada to Argentina. [12-14].

John Belkin and others [15; p. 116] on revision of the Culicidae of Jamaica, stated that "*We have encountered more problems in studying Psorophora that with any other genus primarily because of the very confused taxonomy of the group and the paucity of material, particularly in the subgenus Janthinosoma. It appears that undue reliance has been placed on similarity in male genitalia which has resulted in unwarranted extensive synonymy of species frequently well characterized in the larvae or pupae or even in adult ornamentation. Distinctive features in the immature stages are not always easily found because of very pronounced individual variation in chaetotaxy. The male genitalia appear to be extremely similar in many forms in the subgenera Janthinosoma and Grabhamia.*"

Recently, some papers focused on the misguided use of autapomorphic characters to define or create taxonomic hierarchies in the past. The consequence of this practice and the use of non-explicit methods is the creation of non-natural (paraphyletic) classifications that complicates the handling of identification keys and hides the probable evolutionary relationships of the groups (e.g recently [16]). Such use has been demonstrated and also, new taxa and hierarchic arrangements have been proposed by the correct use of cladistic methods in Sabethini [17,18], Culicini [19], and Anophelinae [20] using cladistic analysis, by the contrast others have been creating confusing new taxa using an incorrect interpretation of this explicit method [21].

Phylogenetic Systematics in Culicidae: The phylogenetic relationships in Culicidae have not been examined rigorously, due, among other things, to the high diversity with more than 3000 species worldwide, including 969 Neotropical species [12, 22]. However Faran [23] and Faran & Linthicum [24] approached mosquito phylogeny, particularly on the subgenus *Nyssorhynchus* of *Anopheles*, but without use cladistic methods. Later, cladistic methods were used with chromosomes on *Anopheles* (*Cellia*) [25], Aedini tribe (*Haemagogus*, *Aedes* and *Psorophora*) using rDNA-ITS [26], for *Anopheles gambiae* Giles [26], the Pipiens complex of *Culex* L., both with rDNA (ITS 1&2) [28], also using the white gene for some genera of Culicidae [29], with the Australian *Anopheles* (mtDNA) [30], and [31] with infragroups of *Culex (Melanoconion)* with rDNA (ITS-2).

On the other hand, using cladistics and morphological evidence, [17] proposed evolutionary trends of the Sabethini. Then, [32] showed the first approach for the family Culicidae. Later [33], focusing on the Series Pyretophorus of *Anopheles* using pupal and adult characters (particularly the female cibarial armature). Recently, Navarro & Liria [19] focused on 18 Neotropical Culicini species using larval mouthpart characters, and demonstrated the monophyly of the genus *Culex*, and reduce the genus *Deinocerites* subgenus of *Culex*. Sallum et al. [20] focused on 64 Anophelinae species and reduced the *Anopheles* subgenera to 3 taxa. Finally, Harbach & Peyton [18] created the genus *Onirion* (Sabethini) without using the characters of the masculine genitalia and with the information presented in previous work [32]. Later, Reinert et al. [21] to increase the Aedini genera, however that proposal have been not accepted by many authors and editors because that lacks of cladistic support. Recently, [34] in a review of Culicidae taxonomy, classification and phylogeny, concluded (p.629) "*that the application of explicit methods of phylogenetic analysis is revealing weaknesses in the traditional classification of mosquitoes, but there is strength in intuitive interpretation because the explicit methodology often confirms the monophyly of mosquito taxonomic groups that are diagnosed by unique combinations of characters*". Furthermore [35], using characters from all life stages coded for 270 Aedini species, including non-aedine genera as outgroup. However, those authors underweight high homoplasic characters to produce phylogenetic hypothesis, and culminate discussing groups (generic-level) validation based on clades that are supported (Jacknifing frequencies) by values ≥ 40, or slightly lower values in a few cases.

The internal relationships in *Psorophora* have only been studied in the context of phenetics or global similarity by Hendrickson & Sokal [36], including 29 species of Culicidae: 15 of *Psorophora* (subgenera *Psorophora, Grabhamia* and *Janthinosoma*) and 14 of *Aedes*. Using 158 adult characters, [36] consider (p.390) "*that the 3 subgenera of Psorophora are lumped in a single genus simply by a historical accident. The 3 subgenera seem quite far from one another phenetically and are as close or closer to various members of the genus Aedes than to each other.*" Furthermore Wesson et al. [26] using ITS-rDNA with *Aedes (Stegomyia), Ae. (Protomacleaya), Ae. (Aedimorphus)*, *Haemagogus* and *Psorophora*, showed a neighbor-joining tree where *Psorophora ferox* is placed in the same group with *Ae. (Pro.) triseriatus* (Say) and separate from *Hg. mesodentatus* Komp & Kumm. Wesson et al. stated that (p.266) "*Both evolutionary analysis methods grouped the three New World species separated from the other four Aedes species (representing the Stegomyia and Aedimorphus subgenera), thus distinguishing Ae. triseriatus from the others in the genus. The similarities among these three species suggest a common evolutionary*

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origin". However, Harbach & Kitching [32] consider *Ae. triseriatus* and other species to be possibly more closely related to *Haemagogus* than to any other genus, and they also pointed out a simplification due to so many uncertainties in the taxonomic status into *Aedes*, and the few number of species used in the analysis from Wesson et al.. Harbach & Kitching [32] did not make any inference regarding the relationships among *Psorophora* and the rest of the Aedini species: *Armigeres*, *Udaya*, *Eretmapodites*, *Heizmannia*, *Opifex* and *Zeugnomyia*, all of the Old World, or the worldwide distributed *Aedes* or the New World *Haemagogus*. *Psorophora* is placed in an unresolved node with *Aedes* and *Opifex*. Later on, the clade of *Psorophora* + (*Coquilletidia* + *Mansonia* = Mansoniini) is supported by two synapomorphies, and these authors state that is regrettable that taxonomic studies of Psorophora are not available, suggesting that an exhaustive revision of this genus.

Considering the limitations of phenetics in evolutionarily informative classifications [37], our aim was to test the Hendricks and Sokal proposal [36] using a cladistic methods and to infer the internal relationships within the genus *Psorophora*, using all morphological evidence available and including new characters, to propose an evolutionary hypothesis of the genus.

MATERIALS AND METHODS

Source of specimens and taxa: twenty-nine species were examined. These belong to the three subgenera of the genus *Psorophora* (*Psorophora*, *Grabhamia* and *Janthinosoma*), two additional species of Aedini: *Ae. serratus* (Theobald), *Haemagogus celeste* Dyar & Nuñez-Tovar, one species of Mansoniini: *Mansonia titillans* (Walker), and two species of Culicini: *Cx. coronator* Dyar & Knab and *Toxorhynchites theobaldi* (Dyar & Knab). The species used are listed in Table 1.

We used specimens from collections of National Museum of Natural History - Smithsonian Institution, Laboratorio de Morfología de Insectos (DERM) and Laboratorio de Biología de Vectores (see [12,22]).

Selection of characters and cladistic analysis: Sixty-six characters from three life stages were included in the analysis (Table 2): 21 from fourth-instar larvae, two from pupae and 43 from adults (including 12 of male genitalia and three of female). The genera *Culex* and *Toxorhynchites* were chosen as outgroup to root the trees supported by the sister relationships of *Psorophora* reported by [32]. *Ae. serratus*, *Hg. celeste*, and *Ma. titillans* were used as ingroup (sister) in agreement with the currently accepted classifications.

The 66 characters were codified 44 as binary and 22 as multistate. All characters were initially treated as equally weight and unordered. Characters not determined in one or more taxa were scored as missing (?). Characters were scored based on recent nomenclature [38-40]. We followed the subgeneric nomenclature of Reinert [41], but not the proposed generic classification of Aedini [21] because we do not agree with their cladistic interpretation of the classification due the weakness that explained in the introduction.

The unweighted character data set (Table 2) was analyzed under the parsimony criterion using the TNT program [42] to search for the most parsimonious cladograms, using the heuristic strategy [37,43]: Wagner trees with 100 random taxa sequence additions (mult*100), and Tree Bisection and Reconnection (TBR) at each group of trees (25 for each replication or hold/25) and all the most parsimonious trees were retained in memory. These trees were displayed (using WINCLADA [44]) with synapomorphies, autapomorphies and homoplasies; subsequently the consistency and retention indices were calculated for each character and tree. Support for individual and derived branches was evaluated by Jackknifing and Bootstrapping [37,45,46] calculated by 1,000 pseudoreplications, and heuristic searches employed with TBR branch-swapping and 25 random-addition replications per bootstrap/jackknife pseudoreplicate, after deleting autapomorphic characters [47].

RESULTS AND DISCUSSION

Morphological characters:

Larval Characters (Fourth Instar, L_4)

1. *Antenna:* Shorter than head, without surpassing their anterior border (0); shorter than head, surpassing their anterior border (1); longer than head (2).

In [48-50], the most widespread condition within Culicidae are antennas thin and shorter than head surpassing the anterior border (e.g. *Aedes serratus* and *Haemagogus celeste*), representing an exception the genera *Mansonia*, *Coquillettidia* and *Aedomyia* which the antenna is thicker and longer than cephalic capsule, and in species of *Culex* (*Melanoconion*) which the antennas are thin and longer than head. In the larval cephalic tagma of strict predators species: *Culex (Lutzia) halifaxii*, *Aedes (Mucidus) painei*, *Toxorhynchites brevipalpis*, *Tx. splendens* and *Tx. inornatus,* all these taxa the antenna are shorter without surpassing their anterior border of head [49]. The last character state also occurs in some Neotropical species, e.g., *Tx. portoricensis, Tx. haemorrhoidalis superbus, Tx. guadeloupensis, Tx. theobaldi, Cx. (Lut.) bigoti, Trichoprosopon digitatum, Runchomyia frontosa,* and *Johnbelkinia longipes* [15,48].

2. *Antenna:* Thin (0); thick (1).

3. *Mandible:* Posterior dorsal tooth (PDT) absent (0); with two equal teeth (1); with several accessory teeth (2).
In his study, [51] did not show the posterior dorsal tooth in his figures of the genera of Culicidae. However, he points out that the posterior dorsal tooth can be toothed or modified. Subsequently, [38] showed the PDT in Cx. *(Cux.) pipiens*, *Ae. (Och.) taeniorhynchus* and *An. (Ano.) crucians*, without any comparative discussion of the structure. In a recent work [52,53] and [19] report this structure for 18 species of Culicini (genus *Culex* including *Deinocerites*), highlighting the phylogenetic importance of this character. And [54] show the mandible and PDT for 12 *Psorophora* species, concluding that several accessory teeth occur in subgenera *Janthinosoma* and *Grabhamia*, while in subgenus *Psorophora* the posterior dorsal tooth is absent.

4. *Mandible:* Mandibular comb (MnC) absent (0); with short filaments (1); with long filaments (2). In all genera of Culicidae [51], except for *Uranotaenia* and predatory species (e.g., *Psorophora* s. *str*., *Culex* subgenus *Lutzia*, and the genus *Toxorhynchites),* have a mandibular comb. Later, [19] and [53] found great variation of phylogenetic value in the development of the mandibular comb in the *Culex* subgenera *Culex*, *Melanoconion*, *Carrollia*, *Microculex*, *Phenacomyia*, and *Anoedioporpa.* In the present study, the mandibular comb of species of *Janthinosoma* and *Grabhamia* has long and thin filaments (similar to those of *Mansonia titillans*), while it has long and thick filaments in *Ae. serratus* and *Hg. celeste.* According to [51], the MnC in Aedini of the genera *Armigeres*, *Eretmapodites*, *Opifex*, *Udaya*, and *Zeugnomyia* is comprised of long and thick filaments.

5. *Mandible:* MnC absent (0); formed by thin filaments (1); formed by thick filaments (2).

6. *Mandible:* Mandibular rake blade (MRB) absent (0); present (1).
The mandibular rake blade it is extremely variable in size [51], and usually serrate and larger than ventral tooth. In predator species e.g. *Cx. (Lutzia) bigoti* and *Ae. (Mucidus) alternans* the MRB is reduced, while in *Tx. theobaldi* and subgenus *Psorophora* is absent. This structure occurs in *Hg. celeste, Ae. serratus, Cx. coronator, Ma. titillans, Ps. (Jan.) sp.* and *Ps. (Gra.) sp.* [19,51]. Then [54] also report the MRB for the species of *Psorophora*, showing differences between *Janthinosoma* (single) and *Grabhamia* (double). The double MRB has been showed for the subgenera *Deinocerites, Melanoconion, Anoedioporpa* and *Microculex* of *Culex* [19].

7. *Mandible*: Mandibular Rake (MnR) absent (0); present (1).
The MnR is not presented in predator species e.g. *Tx. theobaldi* and *Psorophora (Psorophora)* [except for *Ae. (Muc.) sp.* and *Cx. (Lut.) sp.*], while the rest of the studied Culicidae [*Ma. titillans, Hg. celeste, Cx. coronator, Ae. serratus, Ps. (Jan.) sp.* and Ps. *(Gra.) sp.*] it is very variable in size and shape [19,51,53,55].

8. *Mandible:* Mandibular lobe (MnL) absent (0); without spicules and not sclerotized (1); with spicules and strongly sclerotized (2).
Within subgenera of *Anopheles*, [56] indicate differences among *Kerteszia*, *Anopheles* and *Nyssorhynchus,* later on *Culex*, a large shape variety in this structure is reported [19], e.g., *Culex (Deinocerites) sp.* it is very lengthened, in the predator species *Cx. (Lutzia) sp.* is reduced, and in the *Toxorhynchites* species is absent. Into the predators species of *Psorophora* (subgenus *Psorophora*), the MnL is reduced, sclerotized and with spicules. This observation was corroborated by [54] for *Ps. howardii* and [55] for *Ps. ciliata.*

9. *Maxilla*: Galeastipital stem (GSS) absent (0); present (1).

10. *Maxilla:* 4-Mx shorter than MxBo (0); longer than MxBo (1).
In a Culicidae maxillae descriptions, [57] report the 4-Mx very long (almost the length of the maxillary body:

MxBo) and sclerotized for: *Aedomyia, Hodgesia, Mimomyia, Mansonia, Coquillettidia, Phoniomyia* and *Sabethes*. In the remaining taxa (including *Psorophora*), the 4-Mx is variable in size (but never long than above genera) being an exceptional taxa *Anopheles, Bironella* and *Chagasia*, which it is reduced.

11. *Maxilla*: 4-Mx thin (0); thick (1).

12. *Maxilla:* 1-Mx a single sensor (0); double sensors (1).
A single sensor (or filament) occurs in: *Psorophora*, *Orthopodomyia* and *Phoniomyia*. In species of *Ae (Mucidus.), Opifex* and *Limatus*, the double of sensors are fused [57]. In *Anopheles*, [56] shown two sensors for the subgenera. In addition, the last authors reports differences in the length of 1-Mx among *Kerteszia*, *Anopheles* and *Nyssorhynchus*. The same variations was stated by [53] within *Carrollia* subgenus of *Culex*. We have observed two sensors are present for *Ps. (Jan.) cyanescens* and the species of *Ps. (Psorophora)*. In the reference taxa, *Ma. titillans, Hg. celeste*, *Ae. serratus* and *Tx. theobaldi*, the setae 1-Mx is comprised by double sensors.

13. *Maxilla:* Maxillary body (MxBo) as long as wide (0); longer than wide (1).
In a Culicidae maxillae descriptions, [57] report it as long as wide in most of the taxa, being an exception the genera *Aedomyia, Hodgesia, Mimomyia, Mansonia* and *Coquillettidia* where is longer than wide. Later, [19] find that this character is shared in the subgenera of *Culex*: *Anoedioporpa, Microculex, Melanoconion* and *Deinocerites,* being the maxillary body more than twice the width of its base.

14. *Maxilla*: Maxillar spiculose area (MSpA) absent (0); present (1).
According to [57] in the Culicidae genera, *Chagasia, Bironella, Anopheles, Aedes, Udaya, Culex, Topomyia, Malaya* and *Maorigoeldia*, these spicules occurs in the border of the maxillary body. Within different subgenera in *Culex* the spicules number represent numerous homoplasies [19]. In *Psorophora*, the spicules are present in some species of the subgenus *Janthinosoma: Ps. albipes*, *Ps. ferox, Ps. cyanescens, Ps. lutzii, Ps. melanota* and *Ps. discrucians,* and *Grabhamia: Ps. columbiae, Ps. confinnis, Ps. jamaicensis* and *Ps. pygmaea.* In all the species of *Psorophora* s. *str.* are absent. Finally, the MSpA occurs in *Ae. serratus* and *Cx. coronator* while in *Tx. theobaldi* and *Ma. titillans* is absent.

15. *Dorsomental teeth (DmT)*: With four teeth (0); with 7-8 teeth (1); with more than 11 teeth (2).
The dorsomental teeth (DmT), is shared in larvae of Diptera-Nematocera, with a serrate border in dorsomentum, some authors [15,38,49,50,58] showed the dorsomentum (and DmT) in different taxa. This character seems to be variable in number of teeth in the genera *Aedes*, *Culex*, *Uranotaenia*, Sabethini as *Wyeomyia* (New World), *Tripteroides* and *Maorigoeldia* (Old World), and few ones in *Anopheles*, *Mansonia*, *Coquilletidia*, *Mimomyia*, and *Aedomyia*.

16. *Labiohypopharynx:* Labial palpal sensors (LPS3 and LPS4) long (0); short (1).

17. *Labiohypopharynx:* Lateral premental teeth (LPT) developed (0); not developed (1).

18. *Labiohypopharynx*: Superlingua (Sl) present (0); absent (1).

19. *Labiohypopharynx:* Premental sensory (PS) not developed (0); developed (1).

20. *Labiohypopharynx*: Ventral premental processes (VPP) absent (0); abundant, with scales shape (1); not very abundant, with teeth shape (2).

The VPP are absent in *Heizmania* and *Toxorhynchites*, in some Aedini: *Opifex*, *Eretmapodites*, *Zeugnomyia*, *Udaya*, *Armigeres*, *Aedes* and *Haemagogus*, are very abundant with scales shape. In the on study *Psorophora* and *Mansonia* are not very abundant. In *Ae. serratus* and *Hg. celeste* a typical VVP showed scales shape, nevertheless in *Psorophora*, *Cx. coronator* and *Ma. titillans* was tooth type and not very numerous.

21. *VIII abdominal segment:* Combs without three-dentate scales, comprised by a single and central spine (0); with three-dentate scales (1).
The three-dentate scales in the comb of VIII is wide reported in [15,48,49,54], for mosquitoes of North American, Neotropical, South Pacific and Jamaica respectively in comparison with the other genera of Culicidae [being the

only exception Ae. *(Stegomyia) aegypti* where the comb scales are lightly similar]. On our study, all the species of *Psorophora* shown three-dentate scales in contrast with *Ma. titillans, Tx. theobaldi, Ae. serratus, Hg. celeste* and *Cx. coronator*.

22. *X-segment, abdominal*: Precratal setae absent (0); present (1).
In their revision [15], considered this character as diagnostic for *Psorophora*. None of the other genera and in the literature was found that character is shared by another taxon. Nevertheless, in the genera *Mansonia* and *Coquillettidia* some precratal setae are present, although there are not numerous as in *Psorophora*.

23. *X-segment, abdominal:* Saddle complete and forming a ring (0); with incomplete ring (1).
The sclerotized saddle of the tenth abdominal segment, it has been used in many taxonomic keys to differentiate *Aedes* from *Psorophora*. Recently, under a cladistic view [32] consider this character (p. 335) "*A saddle is almost always complete in Orthopodomyia and Culiseta, and usually complete in Culex, Psorophora and Uranotaenia. It is usually incomplete in Aedes and Mimomyia subgenus Ingramia*." The complete saddle, forming a ring around segment X (**ch. 38**. [32]) is a synapomorphy for *Psorophora* + Mansoniini (*Coquillettidia* + *Mansonia*), with a reversal of the state (saddle complete). Nevertheless, [15] showed some species of *Psorophora* without the saddle of the segment X forming a complete ring. In addition, the key of [59], *Psorophora* appears separated twice, because some Colombian species can to present this complete saddle. In our case, Ps. *(Gra.) pygmaea* and *Hg. celeste* have an incomplete ring, contrary to the other taxa with the saddle forming a ring around the tenth segment.

24. *Breathing tube:* Without modification to attach the aquatic plants (0); with this modification (1).

Pupal Characters

25. *Ventral lobes*: not developed (0); well developed (1).
In their studies about *Psorophora* pupae [15] and [60], considered the ventral lobes developed a diagnostic character for *Psorophora (Psorophora)* and *Janthinosoma*, while in *Grabhamia* there are not developed. We agree with these authors based on our analysis. The sister and outgroups analyzed (*Ae. serratus, Hg. celeste, Ma. titillans, Tx. theobaldi* and *Cx. coronator*) these lobes were not developed.

26. *Abdominal segments:* Without spicules in all segments (0); present in III-V (3); presents in IV only (1); presents in III-IV (2).
The most widespread condition in Culicidae is the absence of spicules in the pupal abdomen. However, [61] shows the presence of spicules on pupal segments IV for *Ps. forceps* and IV-VI for *Ps. discrucians*. Later, [60] to affirm that the spicules on the IV segment are characteristic of some species of the subgenera *Janthinosoma* of *Psorophora*. Later [62] in the pupa of *Ae. serratus* description shows the presence of spicules in the segments III to V. Subsequently, [15] indicate that these spicules are diagnostics for the Serratus group (*Aedes* subgenus *Ochlerotatus;* genus *sensu* [63]). Nevertheless, in *Psorophora* three patterns occurs: The absence of spicules in *Ps. cyanescens, Ps. totonaci (Janthinosoma),* species of *Grabhamia* and *Psorophora s. str.;* the presence in the segments III-IV in *Ps. (Jan.) discrucians* and *Ps. (Jan.) albigenu;* and the presence on IV for Janthinosoma species *Ps. ferox, Ps. champerico, Ps. lutzii, Ps. forceps, Ps. longipalpus, Ps. melanota* and *Ps. pseudomelanota.*

Adult Characters

27. *Head (female Cibarium):* With four palatal papillae (pp) (0); with six pp (1).
The palatal papillae (pp: Lee 1974) are four in most of the Culicidae species, except for *Psorophora (Janthinosoma), Culiseta* and *Aedes (Ochlerotatus) dorsalis* where six papillae are observable [64,65]. Particularly, in *Janthinosoma* the pp are different in size, being the anterior ones smaller, while the posterior couples are large [66].

28. *Head (female Cibarium):* short pp (0); long pp (1).
In the subgenus *Grabhamia*, the palatal papillae are long [66], in opposition of the other studied taxa and the literature reports [64,65,67].

29. *Head (female Cibarium)*: Without cibarial teeth (0); with cibarial teeth (1).
The cibarial armature or cibarial teeth (CT: [68]) are common in species of Culicini (*Culex*) and Anophelinae (*Anopheles* subgenera *Cellia*, *Nyssorhynchus* and *Kerteszia*). One Aedini (*Opifex fuscus*) and one of Sabethini (*Wyeomyia smithii*) has been reported the presence of cibarial teeth [15,64,65, 68-71]. Interestingly, in *Psorophora* this structure is also occurs [66]. It was observed in *Grabhamia* species only was located in the posterior-ventral

wall with a series of specialized teeth-like structures.

30. *Head (female Cibarium):* With 5-8 trichoidea sensilla (ts) (0); with 14-15 ts (1).
The trichoidea sensilla (ts: [67]) in most of the Culicidae there have 5-8, being an exceptional case in *Toxorhynchites* (*Tx. rutilus septentrionalis)* and members of *Psorophora* (subgenus *Psorophora*) where there are around 1 -15 sensilla. [64,65,66]

31. *Head:* Maxillary palpomere (females) with three segments (0); with four segments (1).

32. *Head:* Maxillary palpomeres (female) 3rd similar to the others (0); 3rd longer than the others (1).

33. *Head:* Occiput with white scales and a conspicuous spot of violets scales (0); only with golden scales (1); white and a small spot of dark scales (2); white spread only (3); blue (4); green (5); yellow and white (6).

In the subgenus *Janthinosoma* species three patterns were observed: The occiput is white and with a conspicuous spot (large or small) of violet scales in *Ps. albigenu, Ps. albipes, Ps. varipes, Ps. lutzii, Ps. longipalpus* and *Ps. horrida,* also previously reported by [72] and [73]. Nevertheless, in *Ps. varipes,* the spot violet rather seems something dispersed in opposition to the other taxa with a rounded shape. A golden pattern occurs in *Ps. ferox, Ps. cyanescens, Ps. discrucians* and *Ps. champerico.* The third pattern comprises dispersed scales in *Ps. johnstonii*. In the *Grabhamia* species the occiput is white with a small spot of dark scales, with the exception of *Ps. discolor.*

34. *Thorax:* Dorsocentral setae absent (0); dorsocentral setae present (1).

35. *Thorax:* Paratergite nude (0); with scales or setae (1).

36. *Thorax*: Acrostical setae absent (0); acrostical setae present (1).

37. *Thorax*: Preespiracular setae absent (0); setae present (1).

38. *Thorax*: Postspiracular setae absent (0); setae present (1).

39. *Thorax*: Lower mesepimeronn without setae (0); with setae (1).

40. *Thorax:* Mesoscutum scales copper, green on center and lateral (0); scale in longitudinal bands only (1); green or blue metallic scales (2); mixed yellow and white (3); yellow on both the sides and dark in the center (4); white on both the sides and dark in the center (5); brown on both sides and white in the center (6); mixed dark and white (7).

In the Lane's [48] revision, the descriptions are presented for the species of *Psorophora*, and in these patterns shown the mesoscutum scales. In species *Ps. (Janthinosoma) albipes* and *Ps. (Jan.) lutzii* can be appreciated lateral yellow bands and a dark wide band, on the contrary in *Ps. (Jan.) ferox* and *Ps. (Jan.) cyanescens* the mesoscutum is place setting for yellow scales that can be blended with white *(*i.e. *Ps. ferox).* In the subgenera *Psorophora* and *Grabhamia*, the first one the mesoscutum does not possess scales and just they occurs in bands, on the contrary in the second the scales varied patterns form from three white lines (*Ps. confinnis)* until borders of copper scales with shadowy mixtures of white (i.e. *Ps. cingulata*). In the genus *Aedes* varied patterns of scales are presented, being this character used thoroughly in the classification of some subgenera: *Ochlerotatus*, *Stegomyia*, *Howardina* and *Finlaya* (15,48,74). On the other hand in *Haemagogus*, the mesoscutum is covered with scales of metallic colors (mainly blue and green); this next to the development of the antepronote is the main characters diagnoses for such taxon (48,50). Concerning *Culex* patterns are presented varied in the subgenera: *Lutzia, Melanoconion, Aedinus, Anoedioporpa*, *Culex*, *Tinolestes* and *Microculex* [48,71,75]. Subsequently *Tx. theobaldi* also shows variations in the scales, although in general they are of metallic colors (mainly blue or green). Finally, *Ma. titillans* showed dispersed scales with no pattern. [48]

41. *Legs:* Femur III, dark (0); blue dark complete (1); dark with white marks (2); yellow with basal dark band and white on border (3); yellowish with dark erect scales on apex (4).

42. *Legs*: Tibia, dark (0); dark with white spots forming conspicuous white circles (1); dark with irregular white

spots (2); blue dark without spots (3); white with a few dark spots (4); dark peppered with white scales (5).
In his revision**,** Dyar [76], was the first to use the presence of conspicuous white spots in the tibia in the key of *Ps. (Grabhamia)* spp. Later, [15] report the tibia spots patterns to differentiate some Grabhamia species. In *Janthinosoma*, *Psorophora s. str., Tx. theobaldi, Cx. coronator, Hg. celeste, Ae. serratus* and *Ma. titillans*, the patterns and colors in the tibia were different from *Grabhamia* species reported.

43. *Legs:* Fore tarsomere 5th (Ta-III5) dark (0); white marked (1); half white, half dark (2).
Lanes's study [48], uses this character (in the step 8 of the *Psorophora* key), to differentiate species of *Janthinosoma*: e.g., *Ps. varipes*, *Ps. johnstonii, Ps. discrucians, Ps. ferox, Ps. melanota, Ps. circunflava, Ps. champerico, Ps. albipes, Ps. lutzii* and *Ps. forceps* presents white tarsomere, while *Ps. lanei* and *Ps. cyanescens* have it dark. Subsequently, [77] modify the Lane's key: hind 5th tarsomere with dark marking variable, and the alternative option 5th hind tarsomere white. This way differentiates two blocks of species: *Ps. albigenu, Ps. johnstonii, Ps. discrucians* and *Ps. fiebrigi* in the first block*; Ps. circunflava, Ps. lutzii, Ps. forceps, Ps. champerico, Ps. albipes, Ps. melanota, Ps. ferox, Ps. amazonica* and *Ps. albigenu* in the second one. *Grabhamia* species presents the 5th tarsomere half scaled with white and black, while in *Psorophora* species the 5th tarsomere it is dark.

44. *Legs*: Fore tarsomere 4th (Ta-III4) dark (0); marked with white (1); half white and dark half (2).
In *Psorophora,* the tarsomere III-4 is white in *Ps. albipes, Ps. albigenu, Ps. champerico, Ps. ferox, Ps. discrucians, Ps. lutzii, Ps. longipalpus, Ps. horrida, Ps. melanota* and *Ps. varipes*, while it is half white in *Ps. (Jan.) johnstonii* and the species of *Ps. (Grabhamia).* Finally, Ta-III4 is dark in *Ps. (Jan.) cyanescens* and the other studied taxa *(Tx. theobaldi, Cx. coronator, Ae. serratus, Hg. celeste* and *Ma. titillans).*

45. *Head*: Female Proboscis, with no white ring (0); with a white ring (1).
The presence of the white ring in the proboscis seems to be unique for the *Grabhamia* species, although this character is also presented in *Ma. titillans*. Differences in the white ring length occurs in *Grabhamia* subgenus, e.g., in *Ps. cingulata* and *Ps. infinis* is small, while in *Ps. confinnis*, *Ps. columbiae*, *Ps. jamaicensis* and *Ps.* discolor is larger. The white ring is absent in the subgenera *Janthinosoma* and *Psorophora*; it also lack in *Tx. theobaldi, Cx. coronator, Hg. celeste* and *Ae. serratus.*

47. *Legs:* Erect scales absent in fore leg (0); present (1). Lane´s [48] did not report this character for *Psorophora*. However, in our studied species it is well conspicuous for *Ps. (Pso.) cilipes, Ps. (Pso.) ciliata* and *Ps. (Jan.) ferox,* while in the other species (including the sister taxa) there are absent.

48. *Legs*: Simple claw (0); double (1). Lane [48] uses this character for the subgeneric distinction between *Grabhamia* and *Janthinosoma*, nevertheless in *Psorophora* s. str. and the genus *Aedes* (particularly subgenera *Ochlerotatus* and *Finlaya*); the double claws are present and also in the Old Word *Aedes*

49. *Wing:* scales with uniform color (0); with pale and dark scales (1).
Some authors [48,54], uses this character to differentiate the Confinnis group species [15] form other *Grabhamia* species. Nevertheless, in Mansoniini, dispersed scales of pale and dark colors are also presents (but thicker scales). While in the other taxa these white and dark scales are absent. However, *Anopheles*, *Aedes (Finlaya)* Kochi Group [12 species], *Orthopodomyia* [nine species], *Aedomyia* [five species], *Culex* (Culex) [six species], *Cx. (Lutzia)* [two species], *Uranotaenia* [three species] and *Ps. (Gra.) signipennis,* patterns of dark and white scales present in the costal vein, already used as characters diagnoses and for the definition of infrageneric groups [78]. In the species *Ps. (Gra.) cingulata, Ps. infinis* and members of the subgenera *Janthinosoma* and *Psorophora* (except for *Ps. pallescens)* the wings present this pattern scales. Our specimens of *Ps. (Gra.) columbiae, Ps. confinnis, Ps. jamaicensis, Ps. pygmaea* and *Ps. discolor* (Confinnis group) the wings show pale and dark scales. The last one shown the patterns are present in the veins R_5, M_1, M_2, M_{3+4}, CuA, and 1A.

50. *Wing:* With thin scales (0); with wide scales (1).

51. *Abdomen (female):* with short cerci (0); with long cerci (1).

52. *White scales on torus (females):* absent (0); present and large (1); present and small (2).
Cerqueira report this character in *Psorophora (Janthinosoma*) [79], into the key for *Ps. lutzii, Ps. albipes* and *Ps. forceps.* In *Ps. albipes* and *Ps. forceps* presents a patch of white scales in the internal face of the torus, while *Ps.*

lutzii is unscale. Subsequently, [80] in description of *Ps. lanei*, consider that the female torus is ocher without indicating the presence or absence of internal scales. Then Roth [72], indicates that the torus is spherical and with a patch of white scales in *Ps. horrida* and *Ps. longipalpus.* Later on, [81] report dark brown torus with some white scales in the internal side for *Ps. amazonica.* Subsequently, [77] reports for *Ps. albigenu and Ps. albipes* the presence of white scales in the torus. Recently, [82] it indicates for *Ps. pseudoalbipes* (male) and *Ps. pilosus* (male) a group of white scales in the internal side of the torus. Then [83], in *Ps. pseudomelanota* the torus is brown dark and it does not present scales. Lastly, in *Ps. mathesoni,* the torus presents in its expensive mesal a big patch of white scales [84]. On the other hand in *Ps. (Grabhamia),* the torus shows in its internal face some thin scales with white reflection to silver. In the mosquitoes of Jamaica revision Belkin et al. [15] indicate the presence of scales or white setae in the mesal face in *Ps. (Gra.) infinis, Ps. insularia and Ps. pygmaea.* Finally, in *Ps. (Psorophora)* and the other studied taxa these scales (thin or thick) they are not presented.

53. *Female genitalia*: Insula short (0); insula long, tongue-like (1).
In Reinert's comprehensives work [40,63,85,86] for Aedini, reports that the insula very long tongue-like with setae in *Psorophora* is unusual for the tribe, nevertheless certain similarities occur in the development of the insula for the genera *Ayurakitia* Thurman and *Haemagogus* [50]. This character occurs in *Hg. celeste*, suggesting that is not unusual as reported by Reinert.

54. *Female genitalia*: VIII sternite with no sclerotized rods (0); with sclerotized rods (1).

55. *Male genitalia*: Gonostylar claw (GC) long (0); short (1).

56. *Male genitalia:* Gonostylar claw blunt (1); sharp (1).

In *Psorophora* spp. and *Ma. titillans*, the gonostylar claw is short and sharp, while long and blunt in *Ae. serratus, Hg. celeste* and *Tx theobaldi;* in *Cx. coronator* short and blunt.

57. *Male genitalia:* Gonostyle wide and with a digit form projection (0); thin at all extension (1); thin on the base and apex, wide in the middle (2); thin almost all extension but wide on apex (3); thin at base and well and hyper-developed in apex (4).

In *Ma. titillans* and other species of the genus the gonostyle (Gs: [38]) is wide and with a projection digit form [15,48,87]. On the other hand, in *Tx. theobaldi, Cx. coronator, Ae. serratus* and *Hg. celeste* the gonostyle are thin. Then in *Psorophora*, the widespread pattern is thin and wide Gs in the rest, alone attenuating in its apex. In some cases, like *Janthinosoma* and *Psorophora s. str.* the gonostyle can only get wider in the apex until arriving to a great development [15,48,54,73, among others].

58. *Male genitalia:* Gonostylar reticulation absent (0); reticulation present (1).

59. *Male genitalia:* Gonostyle without accessory setae (0); short (1); long (2).

The accessory setae of the gonostyle are absent in *Cx. coronator, Ae. serratus, Hg. celeste* and *Ma. titillans.* In *Tx. theobaldi* and subgenera the subgenera *Grabhamia* and *Janthinosoma* (except for *Ps. melanota*, long and thin), the setae are shorts and thin. In *Psorophora s. str.* appear some variations: *Ps. ciliata, Ps. cilipes* and *Ps. saeva* have short and thick setae, *Ps. pallescens* short and thin while in *Ps. lineata* is absent. In the description for *Ps. (Jan.) pseudomelanota* [83] indicates the presence of long setae in the gonostyle, that could be suggest it relationship with *Ps. (Jan.) melanota.*

60. *Male genitalia*: Gonocoxite (Gc) long (0); short (1).

In *Psorophora*, the gonocoxite is long for *Ps. (Jan.) champerico* and *Ps. longipalpus,* while in the other species it is short.

61. *Male genitalia:* Gonocoxite without internal setae (0); internal setae very long (2); modified in scales (3); modified in hooks (2); short (1).

62. *Male genitalia*: Gonocoxite with apicodorsal lobule absent (0); present (1).

63. *Male genitalia:* with claspette stem absent (0); fused to the gonocoxite (1); separated from the gonocoxite (2).

In *Ma. titillans, Ae. serratus, Hg. celeste,* species of *Ps. (Psorophora)* and in most of *Ps. (Janthinosoma)* the claspette stem is separated from gonocoxite. In *Ps. (Jan.) cyanescens* and *Ps. (Grabhamia) spp,* the claspette is fused with gonocoxite. In the remaining taxa (*Tx. theobaldi* and *Cx. coronator*) the claspette is absent.

64. *Male genitalia:* Claspette without foliated setae (0); single setae (1); one seta with fingernail shape (2); present but accompanied by others (3); present (4).

The claspette filament (CF; [38]) it is a specialized setae, usually simple or filiform apical. In Aedini, some *Aedes* species and *Haemagogus*, the claspette presents a single filament in its apex, and on the other hand in some *Aedes* subgenera, and the genera *Armigeres* and *Opifex*, the claspette shows several setae [15,48,49,50,58,63]. In *Psorophora*, the species of *Grabhamia* present several simple setae in the claspette, while in most of the *Janthinosoma* the foliated setae is accompanied by other; a particular case happens in *Ps. (Jan.) cyanescens* in that the claspette presents several simple setae. On the other hand, in *Psorophora s. str.* the claspette presents a foliated setae accompanied by other simple ones.

65. *Male genitalia:* Aedeagus without serrate border (0); with serrate border (1).

66. *Male genitalia:* X sternite process (PSX) without apical teeth (0); with few teeth (1); with a crown of teeth (2). The PSX (*sensu* [39]) present some teeth in the apical region (genus *Psorophora*), a crown of teeth or spines is occurs in *Culex coronator,* and is absent in remain taxa analyzed. Other taxa e.g., *Hodgesia solomonis*, *Ficalbia solomonis*, *Mimomyia gurneyi*, *Coquilletidia* (5 spp.), *Mansonioides melanesiensis*, *Tripteroides* (9 spp.), presents conspicuous teeth in the process of the sternite X [15,48,49,50,54,58].

Cladistic analysis

Eleven equally parsimonious trees with 164 steps each were detected with a consistency index (CI) of 0.66 (66%) and a retention index (RI) of 0.83 (83%). Permutation of suboptimal trees and random addition did not identify additional cladograms [37,43]. One of the most parsimonious trees (with with synapomorphies and homoplasies) and the strict consensus cladogram (with Bootstrapping and Jackknife values are shown in the Fig. 1 and 2 respectively.

Node Aedini+(*Mansonia*+*Psorophora*), Fig. 1:

This node is supported by three synapomorphies (characters 15, 38 and 63): In the larvae, the dorsomentum teeth are 11 to 15 (ch.15: 0->2; Fig. 4); adult with postspiracular setae (ch.38: 0->1); and the male genitalia, the stem the claspette is separated from the gonocoxite (ch.63: 0->2, with independent occurrence in all species of the *Janthinosoma* subgenus, except *Ps. cyanescens*). Several authors, including [49] and [15], showed the *Culex* subgeneric variability in the teeth number from the dorsomenton. In *Cx. (Culex) iyengari* and *Cx. quinquefasciatus,* among others is about 12 teeth, in *Cx. (Cux.) albinervis, Cx. squamosus, Cx. bitaeniorhynchus* and *Cx. starckeae* is countless, in *Cx. (Culiciomyia) papuensis, Cx. fragilis* and *Cx. pullus* is from 13 to 17. For the previously exposed thing, this synapomorphy should be taken carefully. According to [32] the postspiracular setae are present, in *Aedes* (except the *Ayurakitia* and *Kompia* subgenera), *Heizmannia*, *Armigeres (Armigeres*), *Psorophora*, *Haemagogus*, *Eretmapodites*, *Opifex*, *Udaya* and in Mansoniini: *Coquillettidia (Rhynchotaenia)* and *Mansonia*, and in general it is absent in *Culex*, *Hodgesia*, *Zeugnomyia*, *Toxorhynchites* and Sabethini. In Aedini, the claspette and the mesal lobes are homologies structures that can be identified as such by their basomesal connection, more or less thin. Certain authors examined comparatively this structure in Culicidae, observing that in species of *Aedes* subgenus *Geoskusea* has gotten lost, while on the other hand in the subgenus *Howardina* the claspette has been developed as a spicule, other times in a lobe lengthened with a usually expanded portion. In fact, in two species of this taxon the claspette has decreased to an aedeagal guide. This particularity it also happen in species of the subgenus *Kenknightia* of *Aedes* [85]. In the revision of the Jamaican species, [15] stated for *Mansonia titillans*: *Claspette: Greater part sessile on sidepiece, including a sternal sclerotization meeting its mate on midline, a tergal sclerotization extending towards mesal basal angles of sidepiece, and a broader caudal sclerotization extending in basal mesal membrane of sidepiece*. On the other hand, for the Aedini species in the Neotropics is characterized in the larval phase, for the following character combinations [15]: Scales of the comb present, normal siphon with comb with at least a couple of teeth, and with a subventral setae near to its base, ventral setae with several couples of setae. The adults of *Aedes* and *Psorophora* posses postspiracular setae and the females have the abdomen finishing in tip; nevertheless although the genus *Haemagogus* resembles each other to the sabethines (tribe Sabethini), they can differ because the base of the third coxa is clearly ventral to the meron and the postnotum without setae. The pupal lacks tracheoid in the trumpet and they are but similar to Culisetini and Orthopodomyiini. Recently, [32] in a family analysis show that the node Aedini+Mansoniini is supported by two synapomorphies: in the adult, dorsocentral setae absent and paratergite with scales. Subsequently, they point out that these characters revert in the node 71 (*Aedes+Eretmapodites+Opifex+Psorophora* +Mansoniini) and in fact the dorsocentral seta have the same transformation (present to absent) in *Toxorhynchites* and with partial reversion until being made polymorphic in *Tripteroides*. Then the paratergite appear independently in *Chagasia*, *Aedomyia* and *Culiseta*.

In this work, the character related with the absence or presence scales/setae on the paratergite, it showed to not be informative or autapomorphic. Only an Aedini (*Haemagogus celeste*) it showed paratergite scales, while in *Ae. serratus,* this sclerite was nude. Even when in the genus *Aedes* both conditions can be presented [32]. Nevertheless, in the Aedini definition *sensu* [49] that suggest *Aedes* as a natural group, the author refers that certain members of the tribe shared affinities with higher taxa, except for Anophelinae. This tribe includes more than 1100 species in nine genera that are extremely varied, and many of them difficult to identify at generic level due to the shared characters. In fact several overlapped combinations of characters are necessary for the definition of genera and several subgenera and included species. The general characters of the tribe Aedini includes in the adult the presence of tarsal claws and the female abdomen in sharp form. Even when the tarsal claws are not represented in all species, they are not in any other tribe of Culicinae [32].

Node Aedini (*Aedes+Haemagogus*), Fig. 1:

This node is supported (bootstrap: 56%/jackknife: 70%) by four synapomorphies in the larva: MnC (ch.5: 0->2) with thick filaments, with galeastipal steam present (ch.9: 0->1; Fig. 5A), and ventral premental with numerous spicules like scales (ch.20: 0->1; Fig. 5B). The galeastipal steam is presented in aedines like *Aedes*, *Haemagogus*, *Opifex*, *Udaya* and *Zeugnomyia*, and it is absent in *Eretmapodites*, *Armigeres*, *Psorophora*, *Ae. (Muscidus)* and *Heizmannia*. Then, in the labiohypopharynx the numerous premental spicules and with form of scales are appreciated in *Aedes*, *Haemagogus*, *Opifex*, *Eretmapodites*, *Zeugnomyia*, *Udaya*, *Armigeres*, and it is different in *Psorophora* and *Heizmannia*.

In the *Haemagogus* revision, Arnell [50] suggested that *Haemagogus* is one of the derived genus in the tribe (in the sense of [15]), originated in South America or close to the Caribbean (where their occurs in a diverse way) and undoubtedly splitting from the genus *Aedes*, even when there are not elements of the present fauna in the Neotropical aedines that show obvious likeness with *Haemagogus*. More recently, [26] using the rDNA (region ITS) and [29] using the white gene, obtained trees where the species of *Aedes* forming a paraphyletic group, locate in a clade to *Ps. ferox* and *Ae. (Pro.) triseriatus,* and other with *Hg. equinus* and *Ae. triseriatus.* Subsequently, their suggest that the position of *Haemagogus* as generic range is questioned, and propose the separation of *Aedes* in two genera, one contain to *Haemagogus*, *Psorophora* and species related to *Ae. triseriatus.* Later, [32] consider that although *Ae. triseriatus* and other species can be more related to *Haemagogus* that to other species of *Aedes*, the proposal would be a simplification due to many questions without answer on the taxonomic status of many species and bigger groups. Recently, Reinert [63] using diagnostic characters (particularly in the female genitalia) and without a cladistic treatment, split the *Aedes* in two genera: *Aedes s. str* and *Ochlerotatus* Lynch Arribálzaga. This author considers that the creation of more natural groups and defined better genera are valid reasons to propose this change and overweight the initial unsuitability of the generic change of the affected species. In fact the author seems that it tried to look for characters to build the perfect key instead of studying the genus evolution and phylogeny, and additionally the its creation of new taxon using autapomorphic characters, represents inappropriate practice to the definition of natural groups. It is not in doubt the difficulties in *Aedes* and very probably, as the molecular evidence it points out it, be a paraphyletic group; however the unique phylogenetic treatment of Aedini in [21] shows *Ochlerotatus* as polyphyletic group and others taxa into *Aedes* with very low support to a stable creation of new genera.

Node *Mansonia+Psorophora*, Fig. 1:

This node is supported (bootstrap: 56%/jackknife: 63%) by four synapomorphies: In the larva 4-Mx setae are thick (ch.11: 0->1) and precratal setae are present (ch.22: 0->1; Fig. 6A), the adult legs are dark with white marks (ch.41: 0->2; this character is missed in the consensus tree) and the male genitalia presents a gonostylar claw short and sharp (ch.56: 0->1; Fig. 6B). The precratal setae in the tenth abdominal segment of the larvae are characteristics of *Psorophora* and *Mansonia*. In the first one, they are multiple setae around the border of the segment forming a continuous array, and in *Mansonia* they are few simple or double setae (not more than four). On the other hand, in *Coquillettidia* [15,49] precratal setae does not occur in the X segment. The gonostylar claw shows differences in Aedini, this way in most the classic condition of the long GC is presented and blunt, in *Opifex* and species of *Ae. (Levua)* it is short and sharp, and in *Ae. (Verralina)* is absent [49].

Recently, [32] consider that the clade formed by *Psorophora*+(*Coquillettidia*+*Mansonia*) node 48 it is supported by two synapomorphies: complete saddle, forming a ring around the segment X (ch.23: 1->2; interocular space constrained, without scales or setae extending to the postfrontal sutures (ch.38: 1->0, highly homoplastic). Later on, those authors indicate that of both characters, just the saddle (reverted in the node 75) provides enough supports for the clade (obviously discarding the homoplastic characters). The above-mentioned is interesting, because in our work the saddle of the segment X form a complete ring in all the studied species (including *Psorophora*) with two exceptions, *Ps. (Gra.) pygmaea*, and another in *Hg. celeste.*

By the other hand, the genus *Mansonia sensu* Belkin [49] and Belkin et al. [15] included to *Coquillettidia* as subgenus that later on was elevated to generic level [87,88]. Belkin points out for Mansoniini that the adults form a heterogeneous group linked *Culex* and *Aedes*, similar to this last one, but without the tarsal claws and the female truncated abdomen, reason because shows off the possible affinities of the tribe, and more toward the genus *Ficalbia* (Old World). Nevertheless, the larvae of *Mansonia* and *Coquillettidia* are clearly different to *Culex* and *Aedes* and due to this a tribe was created separated for these taxa. In his review [88] studied *Mansonia* and *Coquillettidia* characters of immature and mature, as well their biology, and its consider that [49] did not made the Mansoniini diagnosis; then these authors defined the tribe by: With acrostical and dorsocentral setae in a row across the previous region to the pre-scutelar area; development of supraalar and pronotal setae; prespiracular setae absent; postspiracular setae absent or present; high mesokatepisternal setae present; mesepimeral lower and high present; 1st tarsomere short than tibia; wing with wide scales; female abdomen with blunt apex; gonostylus with basal lobule and a terminal claw at the apex. Larvae with respiratory breathing tube transformed in to piercing organ adapted for the fixation of aquatic plants; antennas with almost so long filament that the rest of the antenna; X segment twice long as wide; pupae with respiratory trumpet adapted for the fixation of aquatic plants.

Some of these characters was used in the present analysis of *Psorophora*: Dorsocentral setae (ch.34) present in all species with exception of *Toxorhynchites theobaldi* and *Haemagogus celeste*; acrostical setae (ch.36) present in all species with exception of *Tx theobaldi* and *Hg celeste*; prespiracular setae (ch.37) absent in *Ma titillans*, *Cx coronator, Ae serratus* and *Hg. celeste*; lower mesepimeron with setae in all species with exception of *Hg. celeste*; gonostylar claw blunt (ch.56) in *Psorophora* spp. and *Ma titillans*.

Our results shows that Aedini is a monophyletic group including Mansoniini: then, two actions could be suggested: To consider *Mansonia* like a member more than the Aedini or to consider separated to *Mansonia* and *Psorophora* like a group, however by mean of a carried out cladistic studies including more number of Mansoniini species will can clarify the evolutionary relationships. Recently Reinert et al. [21] pointed out in the discussion of the all Aedini data set "*…However, the recovered relationships are still unsatisfactory. Mansonia titillans is once again included in Aedini, this time as the unambiguous sister-group of Psorophora (although it is worth noting that this clade is present in the preferred hypothesis of generic relationships recovered by Harbach & Kitching, 1998).*" Those authors also included just one taxon of Mansoniini (Ma titillans) and this taxon was placed basal to *Psorophora* but also with other Aedini as *Ochlerotatus*.

Node *Psorophora s. str.*+(*Grabhamia*+*Janthinosoma*), Fig. 1:

The genus *Psorophora* is supported (bootstrap: 66%, jackknife: 69%) by three synapomorphies: the larvae for presence of trident-like scales in the VIII abdominal segment (ch.21: 0->1; Fig. 7A), in the adult (females) with tergum and sternum VIII (ch.54: 0->1; it Fig. 7B) with structure rod-like, and in the male genitalia the sternite process of the X (ch.66: 0->1; Fig. 7C) it possesses few teeth. The scales of the VIII segment abdominal trident-like, that is with a long central thorn and two or three a little shorter, it is a character that defines to the genus *Psorophora* very well. Nevertheless, Belkin [49] for the species of the subgenus *Stegomyia* of *Aedes*, reports in *Ae. aegypti* similar scales without the typical form of *Psorophora.* Later Reinert [40] show for the female genitalia that the lateral bars (rods) strongly sclerotized and pigmented, of the tergum and sternum VIII seem to be a stable surface that gives form and it forces to the primary membranous sclerites. In some species of the subgenus *Ochlerotatus* of *Aedes: Ae. mitchellae, Ae. nigromaculis* and *Ae. sollicitans* are present similar structures, even when these they differ significantly in form.

Node *Psorophora s. str.*, Fig. 1:
The node is supported (bootstrap: 100%, jackknife: 99%) by four synapomorphies: the larval mandibular lobe presents spicules and very sclerotized (ch.8: 0->1; Fig. 8A), labiohypopharynx with developed premental sensory (ch.19: 0->1; Fig. 8B), in the adult with mesoscutum with naked longitudinal areas (ch.40: 0->3; Fig. 8C), femur III yellowish with with dark erect scales on apex (ch.41: 0->4) and aedeagus with sawed border (ch.65: 0->1; Fig. 8D). Subsequently, *Ps*. *lineata* (supported by autamorphic ch.61) is more related and basal to the node that group to *cilipes+(saeva+(ciliata+pallescens))*. This node is supported by a synapomorphy: in the adult with gonostylar accessory seta short and thick (ch.59: 0->1 Fig. 9A), in *Ps*. *pallescens* is short and thin). Finally, *Ps*. *ciliata* and *Ps pallescens* are contained based on a homoplasy (ch.11). In a revision of the Argentinean species [89], consider that the subgenus *Psorophora* diagnosis is: *big mosquitoes, legs with erect scales especially in the apex of the femurs, tibia and first segments of the posterior tarsus, female palpus biggest that the first five segment of flagellum antennal, palpus of the male exceeding the long of the proboscis for the last two segments that are enlarged and hairy, mesonotum with naked longitudinal areas, spiracular setae generally numerous and long, female tarsal claws and the male toothed. Clip (gonostylus) thin, with the expanded apex or bilobulated, some short and strong thorns in their internal face*. Later on, [14] and [90] showed for *Ps*. *saeva* their likeness with *Ps*. *cilipes*, on the base of the male genitalia, where the gonocoxite is distal conical with setae covering around the third distal of this, and a modified setae (foliform) located in the base of the hairy area. The gonostylus has a thin apex, the angle subapical it is big and in lateral and near position to the apex; the gonostylar claw is long and with several strong setae on conspicuous tubers in the internal margin of the half apical. Presently study, the larval synapomorphies increases the characters support to define the subgenus, and those presented in the genitalia they support the hypothesis of the relationships of *Ps*. *saeva* with *Ps*. *cilipes* and other near species.

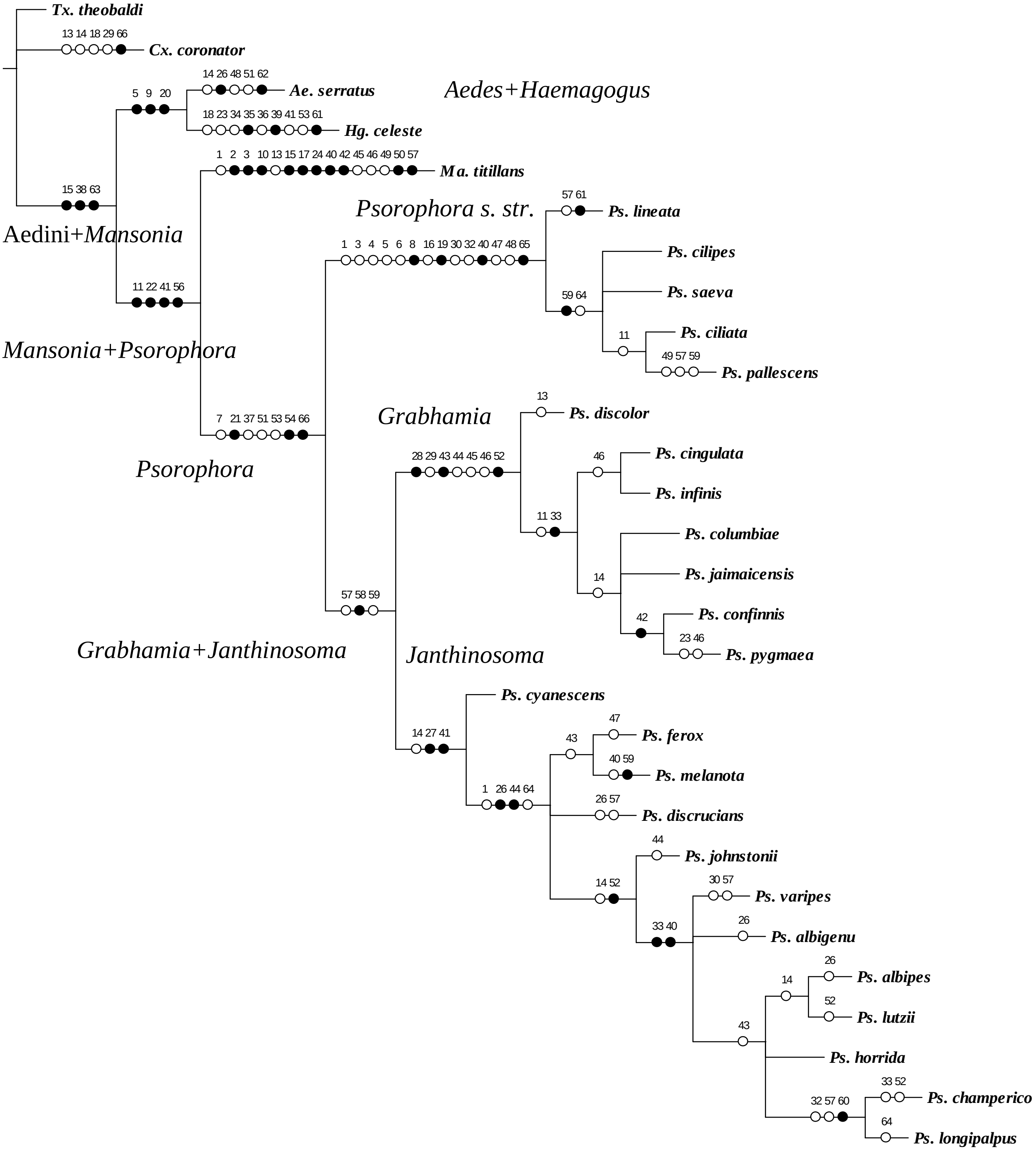
Node *Grabhamia* and *Janthinosoma*, Fig. 1:
This node is supported (bootstrap: 58%, jackknife: 60%) by one adult synapomorphic character, gonostylus reticulated (ch.58: 0->1; Fig. 10). Hendrickson and Sokal [36] in their phenetic proposal, indicates "that the three subgenera was placed in an only genus by a historical accident"; according to these authors the three taxa is far away (in a phenetics sense) to each other, and surely nearer to some members of *Aedes*. Equally, they point out that *Grabhamia* and *Psorophora* are very distinct, and related with *Ae*. *(Stegomyia) aegypti*.

Our results, shows the three subgenera into a monophyletic group, with *Psorophora* as basal taxon and sister of *Grabhamia* + *Janthinosoma* as more related taxa and derivate groups. However [21], shows an inverse relationship among these taxa, with *Grabhamia* + *Janthinosoma* as basal clade and *Psorophora* as derivate. Then, the internal relationships are equal in both hypothesis, the basal and derivate group can be forced by the rooted taxa.

Node *Grabhamia*, Fig. 1:
This node is supported (bootstrap/jackknife: 99%) by three synapomorphies in the adults: female Cibarium with developed palatal papillae (ch.28: 0->1; Fig. 11A), tarsomere III5 (ch.43: 0->2; Fig. 11B) half white and dark half and white scales in small torus (ch.52: 0->2). Inside this node it is appreciated *Ps*. *discolor* in more basal position to the group *((cingulata+infinis)+(columbiae+jamaicensis+(confinnis+pygmaea)))*. This last one supported by one adult synapomorphy, occiput with white scales and a small stain of dark scales (ch. 33: 3->2). The other nodes are supported by homoplasies, except for the group *confinnis+pygmaea* supported by a synapomorphy: fore leg (femur) with white scales that form irregular bands (ch.42: 2->3 Fig. 11C). According to [15] *Grabhamia* is differentiated at least of the other two subgenera by the presence of clear basal marks in the basal segments of all the tarsi and the absence of extensive areas without scales between the acrostical and dorsocentral areas. These authors in Jamaica, consider two different groups exist: (1) cingulata group, represented by *Ps*. *infinis* where the wing has dark scales, and (2) confinnis group, represented by *Ps*. *jamaicensis*, *Ps*. *pygmaea* and *Ps*. *insularia* (including *Ps*. *columbiae* that is present in Cuba and Great Cayman) whose the wings have white and dark scales.

In our cladistic analysis there are not evidence to accept the groups proposed by [15]; in fact, the character of the scales in the wings was used in the study (ch.49: 0->1, with independent occurrence in *Ps*. *pallescens)*, being homoplastic. Additionally, the topology of the cladogram where the arrangements type is appreciated the cingulata and confinnis groups, they are spurious because they are supported by homoplasies. On the other hand, the node that contains *Ps*. *confinnis* and *Ps*. *pygmaea* it is supported by a synapomorphies (ch.42: 2->3), femur dark with irregular white stains.

Figure 1. One of the 11 most parsimonious trees for the data matrix found with TNT, presented to show character mapping, non homoplasious (dark circles) and homoplasious (white circles).



Another interesting aspect inside *Grabhamia* clade, is the basal position of *Ps. discolor,* because the larval morphology (particularly the siphon and antennae) is distinctive within the genus. Recently, material of the *Psorophora confinnis* Complex was studied (*Ps. confinnis* and *Ps. columbiae sensu* [15] and [91] from Greg Lanzaro's collections (UC Davis) from the United States (Maryland, California, Arkansas and Florida), Colombia (Tolima and Bogotá), and Mexico (Oaxaca) and also using our specimens from Venezuela. The cladistic analysis of these data (unpublished) using morphometric and qualitative characters was conducted and several more parsimonious solutions were obtained that demonstrate the difficulty of the group, as well as the necessity of including new evidence (morphological and molecular) in the future studies.

Figure 2. Strict consensus tree of the 11 most parsimonious tress obtained with TNT (L=166 steps, consistency index = 0.66, retention index = 0.82). Number above each branch indicates the percentage of the Bootstrap (below Jackknife percentage) in which that node is supported.

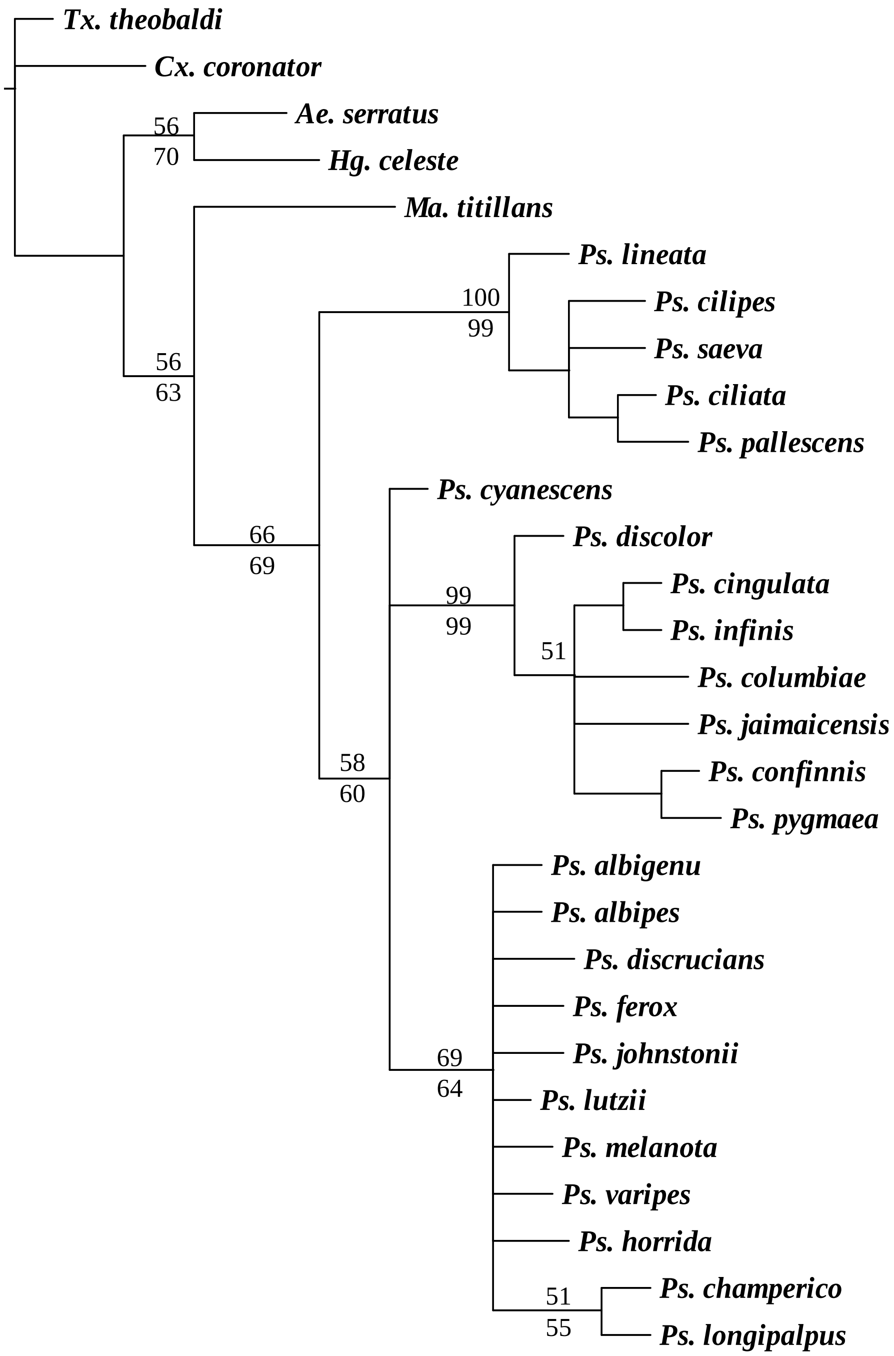


Figure 4. Synapomorphy for Aedini (including *Mansonia* and *Psopohora*), ch.15 Dorsomentum teeth are 11 to 15.

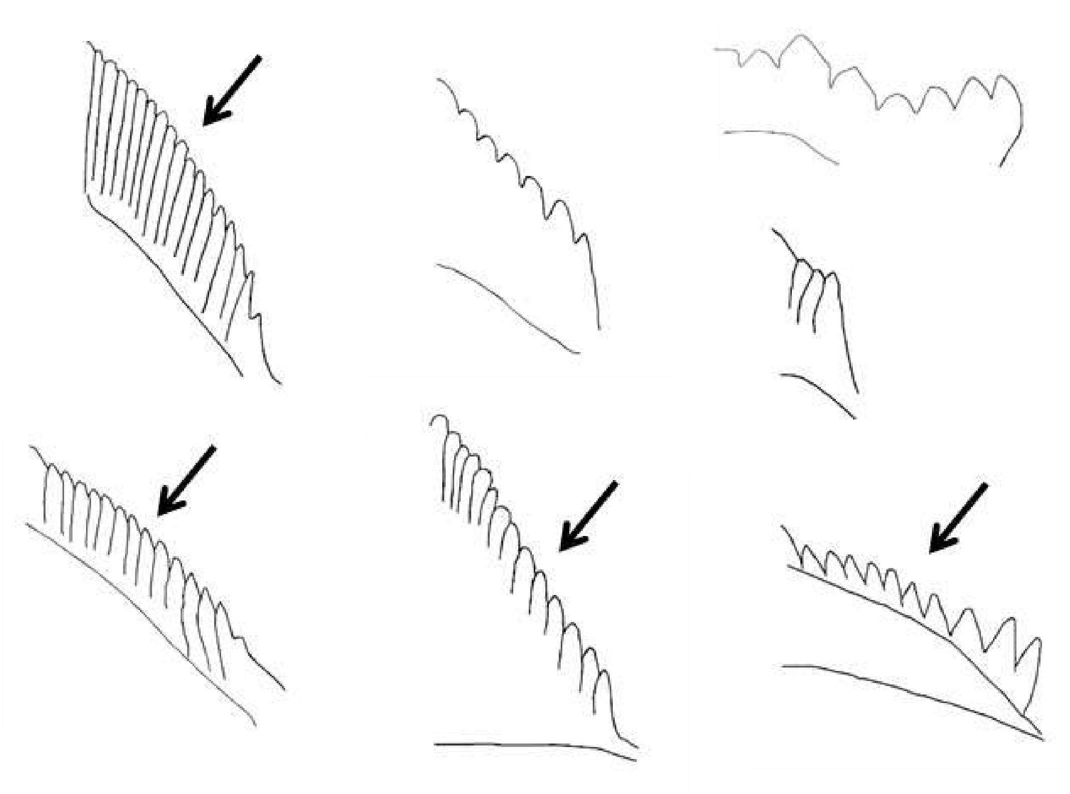


Figure 5. Synapomorphies for Aedini (*Aedes*+*Haemagogus*), ch.9 galeastipal steam present.

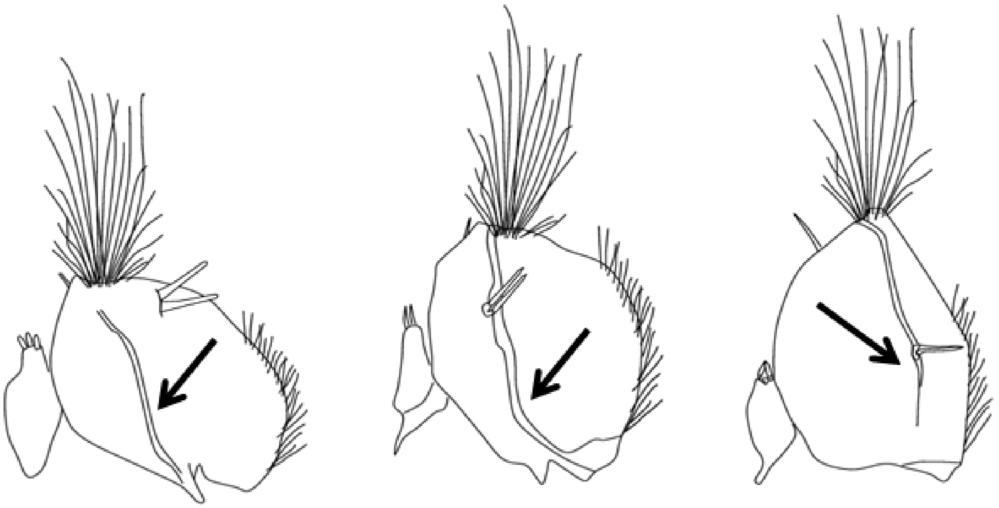


Figure 6. Synapomorphies for *Mansonia+Psorophora*, ch.22 precratal setae present (A) and ch.56 male genitalia presents a gonostylar claw short and sharp (B).

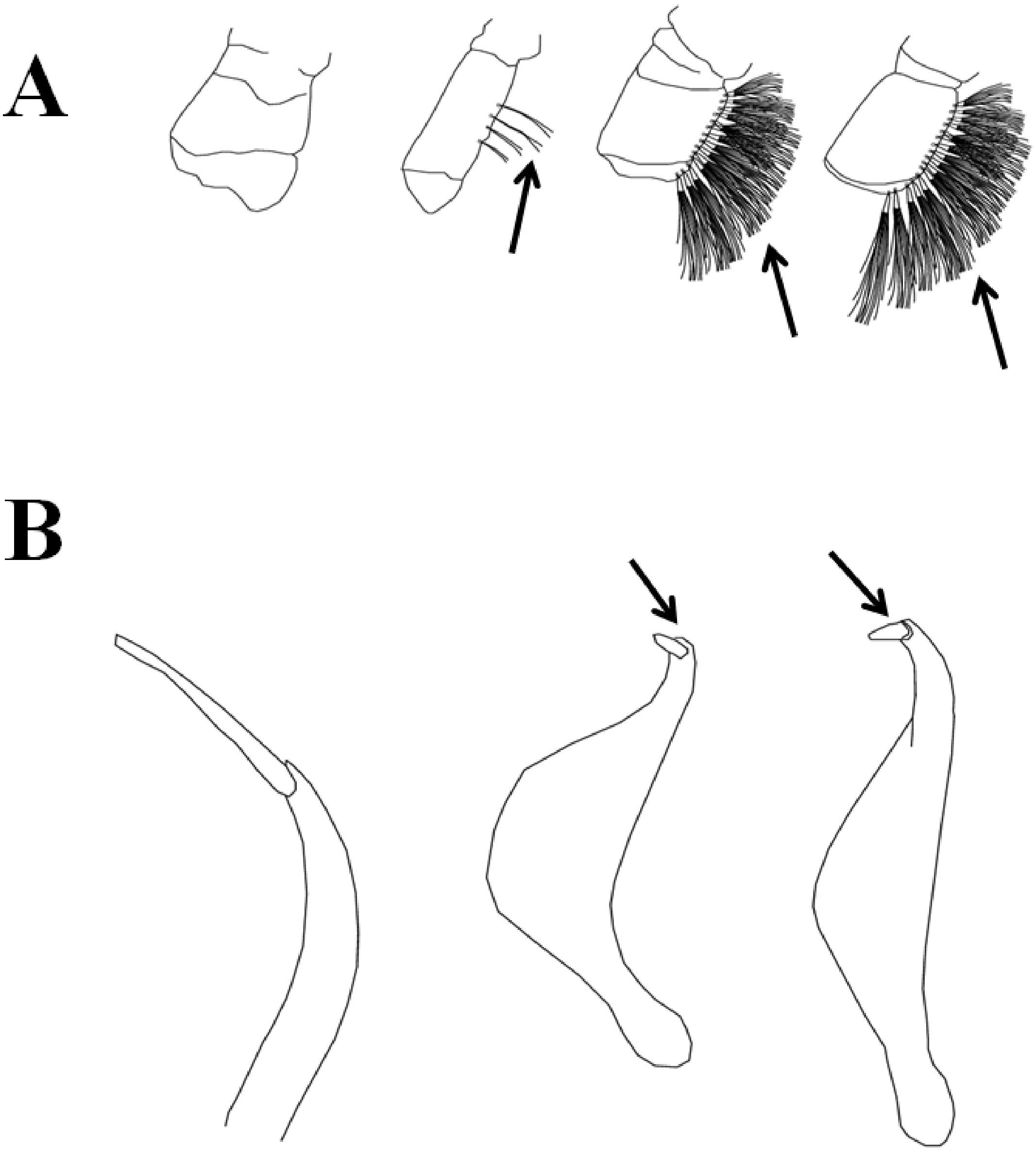


Figure 7. Synapomorphies for *Psorophora s. str.*+(*Grabhamia*+*Janthinosoma*), ch.21 larvae with trident-like scales in the VIII abdominal segment (A), ch.54 adult (females) with tergo and sternum VIII with structure rod-like (B), and ch.66 male genitalia the sternite process of the X it possesses few teeth (C).

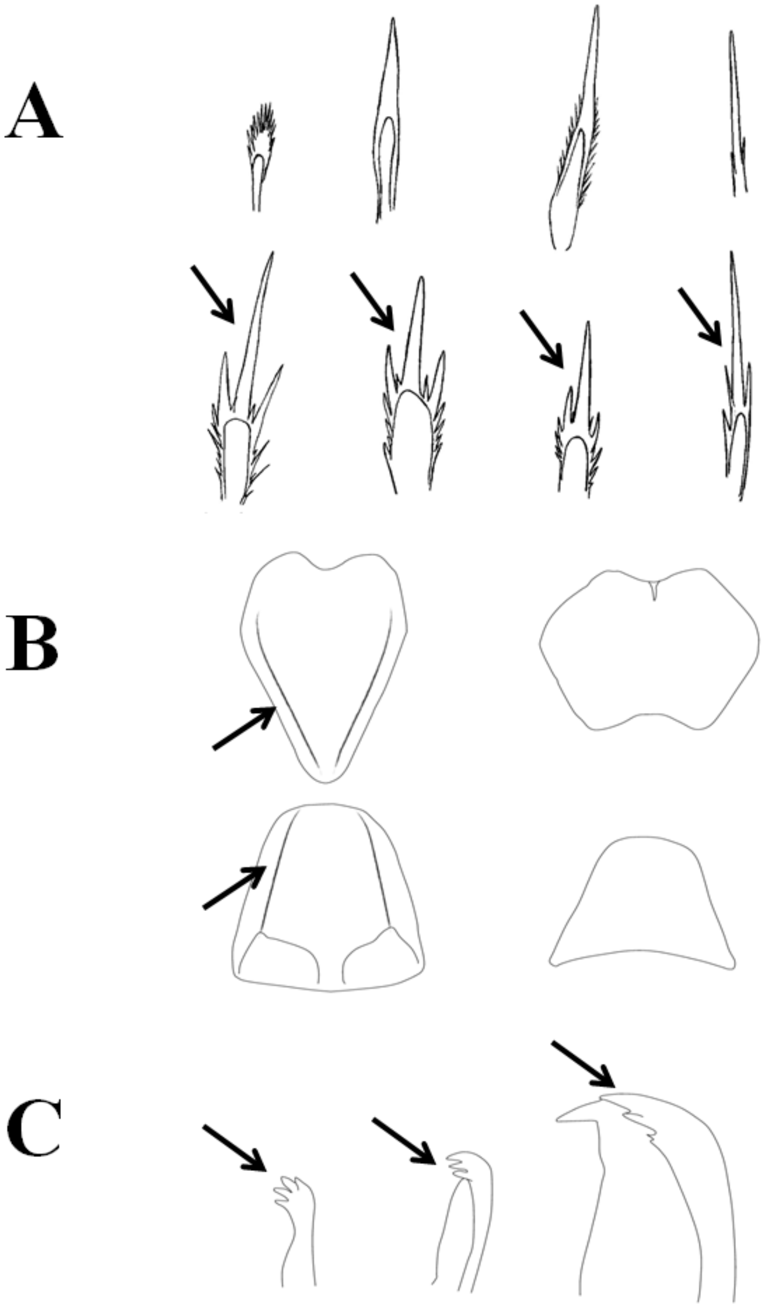


Figure 8. Synapomorphies for *Psorophora s. str.*, ch.8 the larval mandibular lobe presents spicules and very sclerotized (A), ch.19 labiohypopharynx with developed premental sensory (B), ch.40 adult with mesoscutum with naked longitudinal areas (C) and ch.65 aedeagus with sawed border (D).

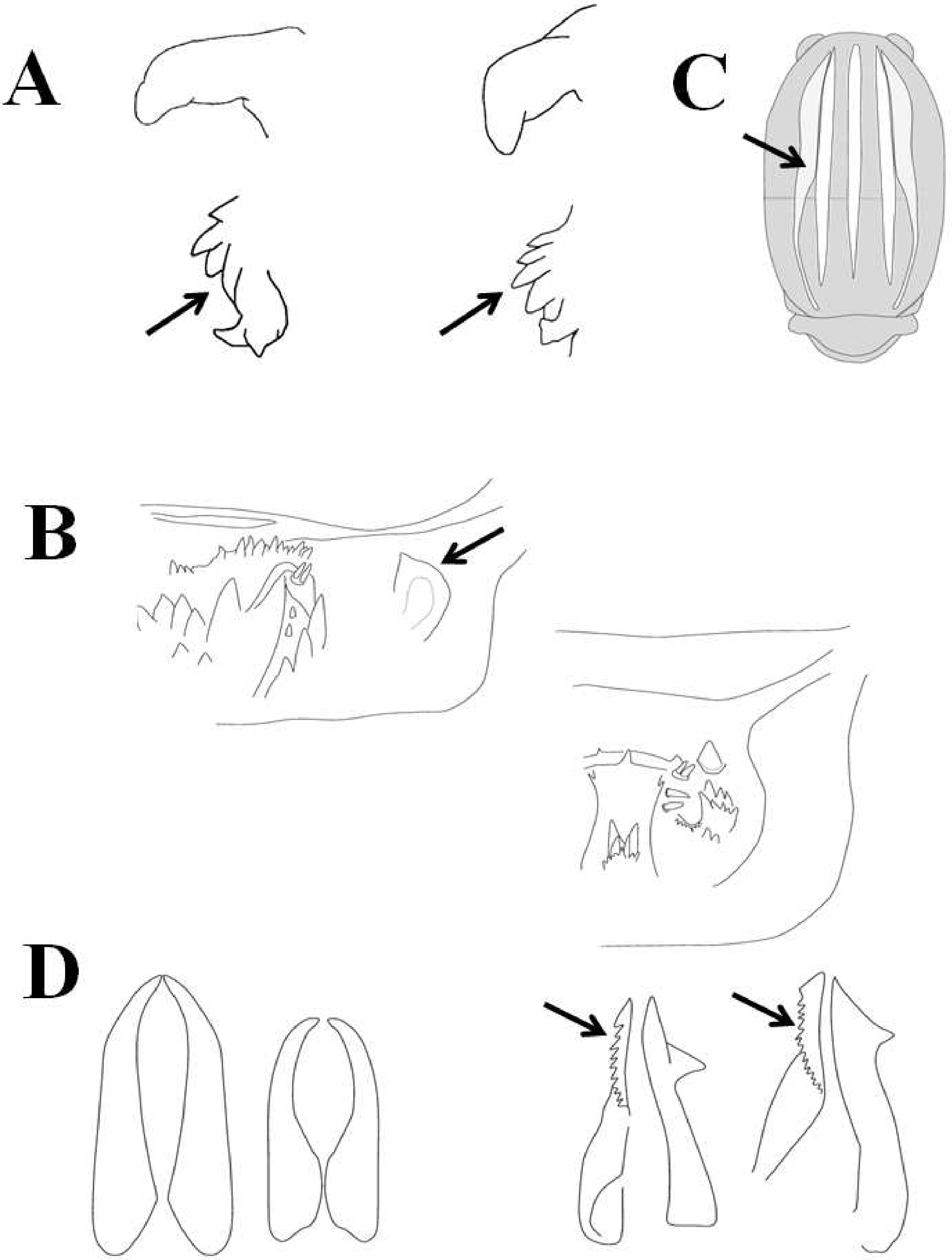


Figure 9. Synapomorphies for the group (*cilipes*+*saeva*+(*ciliata*+*pallescens*)), ch.59 adult with gonostylar accessory seta short and thick.

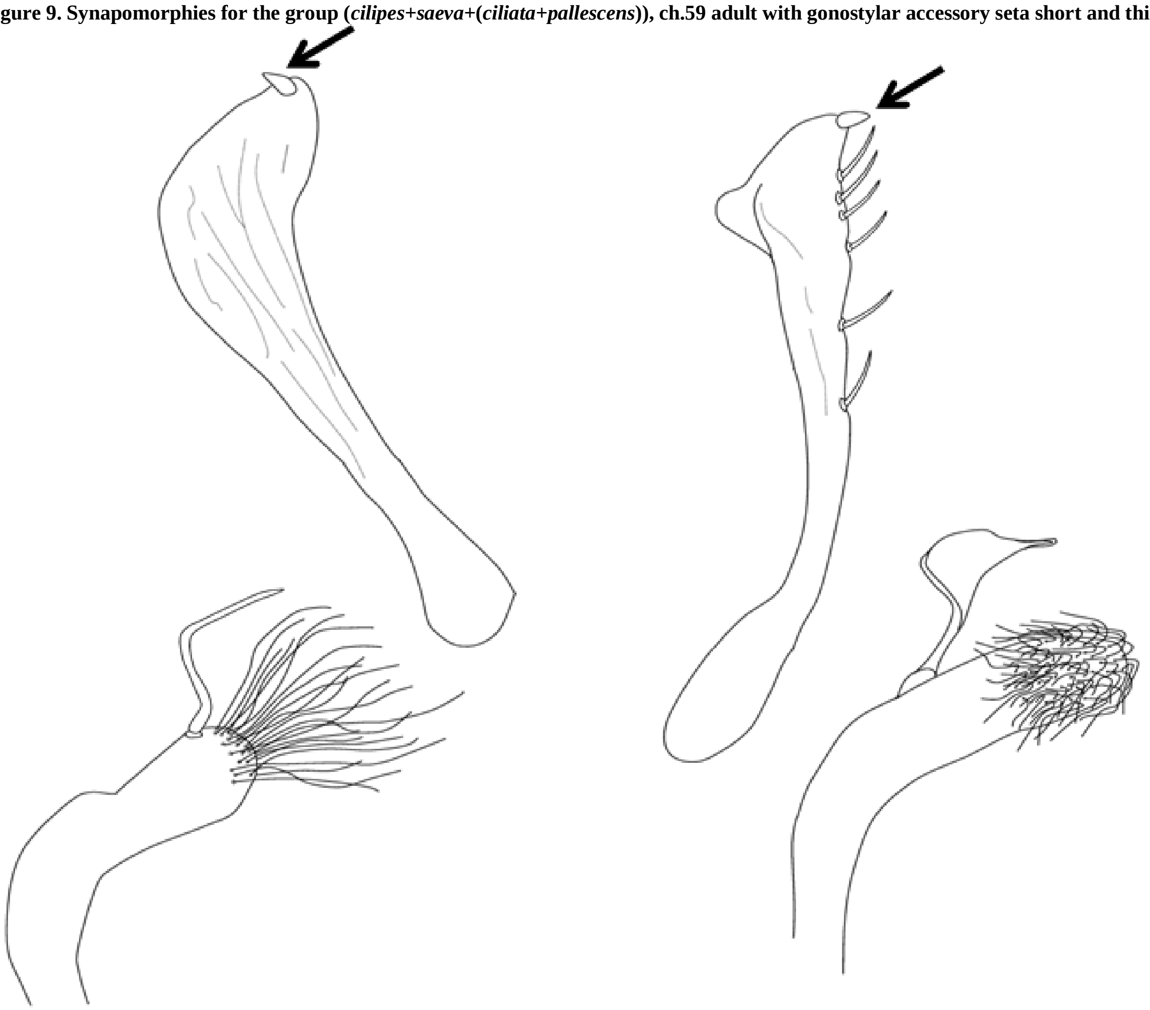


Figure 10. Synapomorphies for *Grabhamia* and *Janthinosoma*, ch.58 gonostylus reticulated.

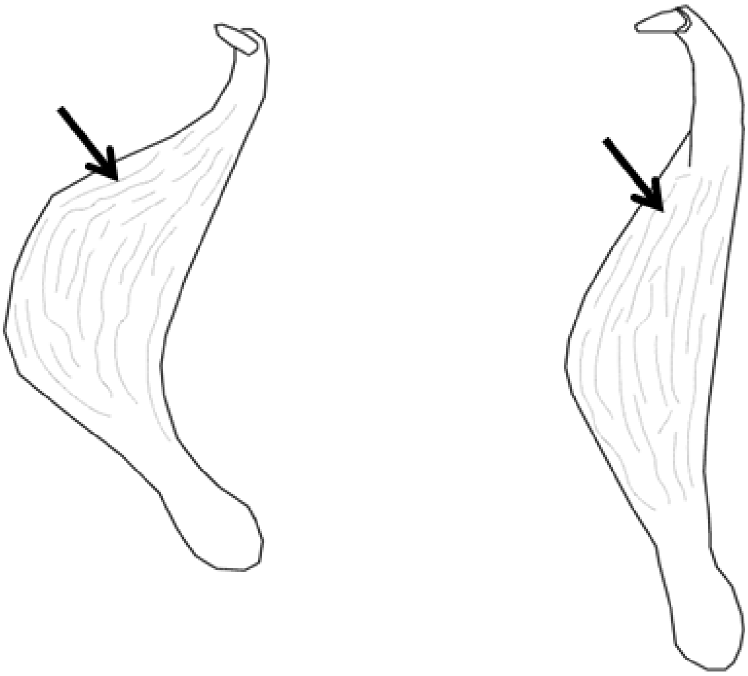


Figure 11. Synapomorphy for *Grabhamia*, ch.28 female cibarium with developed palatal papillae (A) and ch.43 tarsomere III5 half white and dark half (B).

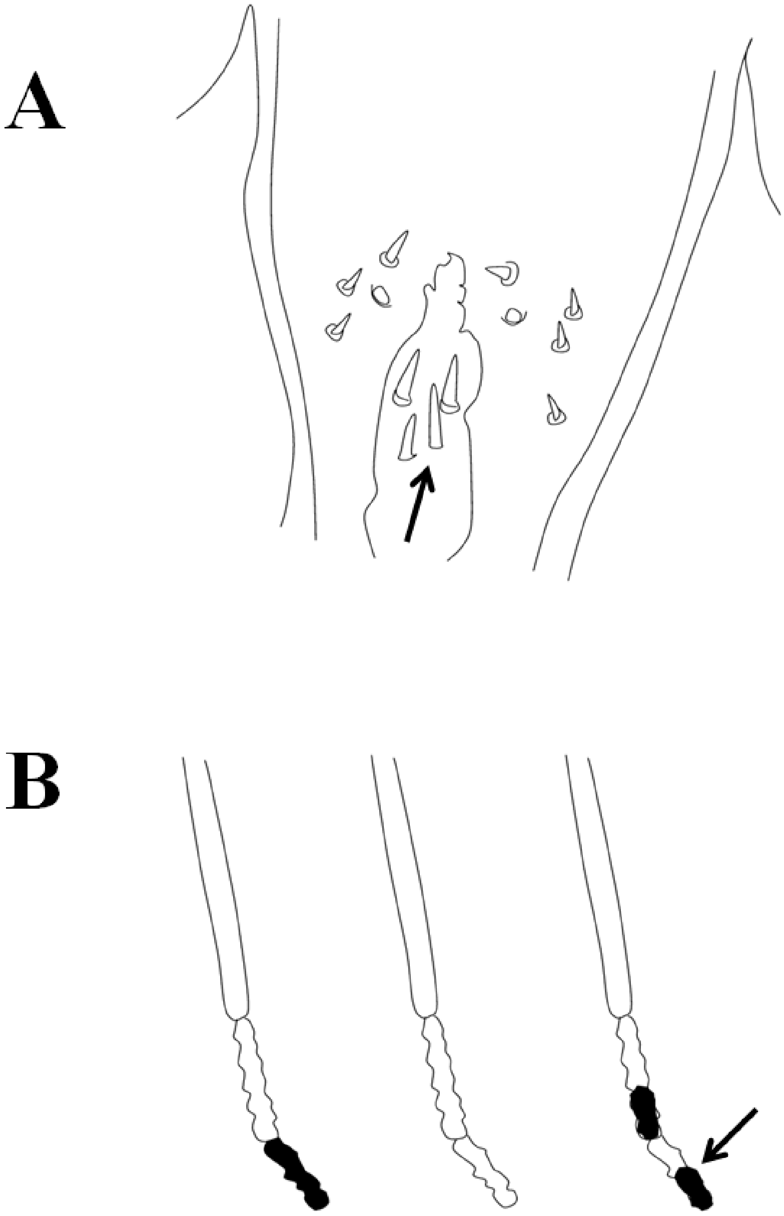


Figure 12. Synapomorphy for *Janthinosoma*, ch.27 female cibarium with six palatal papillae.

Figure 13. Synapomorphy for *Janthinosomna* (excluding *Ps. cyanescens*), ch.26 pupae with spicules of the abdomen present in the IV segment (A) and ch.44 adult with tarsomere III4 white (B).

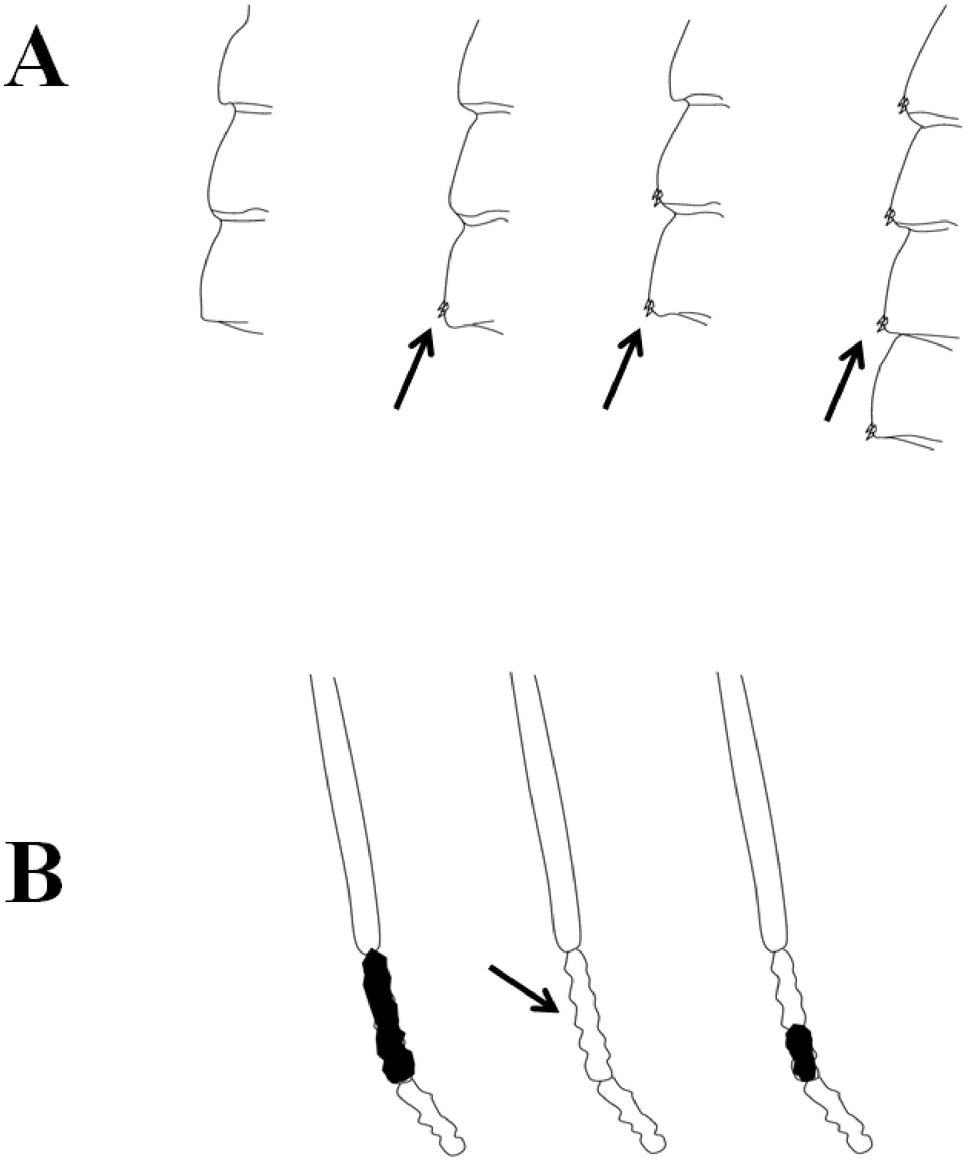


Table 1. List and geographical distribution of the species included in the cladistic analysis

Tribe	Genus	Subgenus	Species	Distribution
Culicini	*Culex*	*Culex*	*Cx. coronator* Dyar and Knab	Neotropical
	Toxorhynchites	*Lynchiella*	*Tx. theobaldi* (Dyar and Knab)	Neotropical
Mansoniini	*Mansonia*	*Mansonia*	*Ma. titillans* Walker	Neartic/Neotropical
Aedini	*Aedes*	*Ochlerotatus*	*Ae. serratus* (Theobald)	Neotropical
	Haemagogus	*Haemagogus*	*Hg. celeste* Dyar and Nuñez-Tovar	Neotropical
	Psorophora	*Psorophora*	*Ps. lineata* Humboldt	Neotropical
			Ps. saeva Dyar & Knab	Neotropical
			Ps. cilipes Fabricius	Neartic/Neotropical
			Ps. ciliata Fabricius	Neartic/Neotropical
			Ps. pallescens Edwards	Neotropical
		Grabhamia	*Ps. columbiae* (Dyar and Knab)	Neartic
			Ps. cingulata (Fabricius)	Neotropical
			Ps. confinnis (Lynch Arribalzaga)	Neartic/Neotropical
			Ps. discolor (Coquillett)	Neartic
			Ps. infinis (Dyar and Knab)	Antillan
			Ps. jamaicensis (Theobald)	Antillan
			Ps. pygmaea (Theobald)	Antillan
		Janthinosoma	*Ps. albigenu* Lutz	Neotropical
			Ps. albipes Theobald	Neotropical
			Ps. champerico Dyar & Knab	Neartic/Neotropical
			Ps. cyanescens Coquillet	Neartic/Neotropical
			Ps. discrucians Walker	Neotropical
			Ps. ferox Humboldt	Neartic/Neotropical
			Ps. johnstoni (Grabham)	Neartic
			Ps. lutzii Theobald	Neotropical
			Ps. longipalpus Randolph & O'Neil	Neartic
			Ps. horrida (Dyar & Knab)	Neartic
			Ps. melanota Cerqueira	Neotropical
			Ps. varipes (Coquillett)	Neartic

Table 2. Data matrix of 66 characters and 29 taxa used in the cladistic analysis (?, Missing data; -, inapplicable characters)

	1 5 10 15 20 25 30 35 40 45 50 55 60 65
Tx. theobaldi	000000000001001100000000000000110500010100000000000000000110311000000
Cx. coronator	102111110001111001020000000001000610100130100000000000001110014000002
Ae. serratus	102221111001012000010000030000000-10101161100000100100000110010124000
Hg. celeste	102221111001002001010010000000004010010201000000000001001100130240000
Ma. titillans	211211110111100010020101000000000-10101172500120011000010000100220000
Ps. cingulata	102211010000000200002110000011000210111113222211000011111021310011010
Ps. columbiae	102211010000001200002110000011000210111113222212001011111021310011010
Ps. confinnis	102211010000001200002110000011000210111113232212001011111021310011010
Ps. discolor	102211010010102000021100000110001101111132422120010111110213100110100
Ps. infinis	10221101000000020000211000????0021011111322221100001111102131001101
Ps. jaimaicensis	102211010000001200002110000011000210111113222212001011111021310011010
Ps. pygmaea	102211010000001200002111000011000210111113232211001011111021310011010
Ps. albigenu	20221101001000200002110012????000101111531010000001211102131002301
Ps. albipes	202211010010012000021100121000000101111531110000001211102131002301
Ps. champerico	------------------------1110000111011115311100000010??103130002301
Ps. cyanescens	102211010011012000021100101000001101111331000000001011102131001101
Ps. discrucians	20221101001001200002110012????001101111331010000001011104131002301
Ps. ferox	202211010010012000021100111000001101111331110010001011102131002301
Ps. johnstonii	20221101001000200002110011????001101111331020000001211102131002301
Ps. lutzii	20221101001001200002110011????000101111531110000001011102131002301
Ps. longipalpus	202211010010002000021100111000010101111531110000001211103130002001
Ps. horrida	2022110100100020000211001?????000101111531110000001211102131002301
Ps. melanota	20221101001001200002110011????00?10111123111000000010??102121002301
Ps. varipes	202211010010002000021100111001000101111531010000001211104131002301
Ps. ciliata	000000020001002100121100100001013101111141000011001011101011002311
Ps. cilipes	000000020011002100121100100001013101111141000011001011101011002311
Ps. lineata	000000020011002100121100100001013101111141000011001011103001202111
Ps. pallescens	000000020001002100121100100001013101111141000011101011102031002311
Ps. saeva	000000020011002100121100100001013101111141000011001011101011002311

Node *Janthinosoma*, Fig. 1:

This node is supported (bootstrap: 69%/jackknife: 64%) by two synapomorphies in the adult: female cibarium with six palatal papillae (ch.27: 0->1; Fig. 12) and femur III yellow with dark base and white basal border (ch.41: 1->3). According to [15] and [92], the metallic violet scales of the legs, labium, and abdominal tergites can identify this subgenus. Most of the species have conspicuous white marks on hind tarsomeres 4 and/or 5 and sometimes on 3. These authors consider the taxonomy of the subgenus to be very confused, due to the lack of males and associated immature stages; the larvae have been described for about half of the species. However [92] emphasizes the urgent need for more intense morphological studies of the subgenus *Janthinosoma*, and the importance of many new diagnostic morphological characters. In studies of the morphology of the cibarium in Culicidae [65], show the presence of six palatal papillae in *Ps. (Janthinosoma) ferox* and *Ps. (Jan.) varipes.* This character is also observed in *Culiseta alaskaensis, Cu. inornata, Cu. morsitans, Aedes dorsalis* and *Coquillettidia perturbans* and could possibly be involved in food palatability. The character should be more thoroughly studied, using electron microscopy and even physiological response to determine possible differences or similarities between these taxa and *Janthinosoma*. The most basal taxon of the subgenus is *Ps. cyanescens*, which is separated from the next node by two synapomorphies: pupae with spicules on abdomen segment IV (ch.26: 0->2; Fig. 13A) and adult with hind tarsomere 4 white (ch.44: 0->1; Fig. 13B). The claspette of the male genitalia of *Ps. cyanescens* is fused to the gonocoxite, as it is in species of *Grabhamia*; this is interesting, particularly because of the location of the species in the tree topology where *Ps cyanescens* seems to be a link species between both subgenera. On the other hand, it is also the only species (together with *Ps. lanei*, not included in the analysis) that presents the tarsomere III4,5 dark. In the only subdivision of this subgenus [79,81] proposed the Lutzii Series as a natural group that comprise *Ps. lutzii*, *Ps. albipes*, *Ps. forceps* and *Ps. amazonica.* Our results do not allow supporting the validity of the series, because the nodes are supported by homoplasies and the material of *Ps. amazonica* was not revised and immature of *Ps. forceps* are unknown.

CONCLUSION

In this work we found morphological diagnostic characters for the genus *Psorophora*, in the larva, abundant precratal setae in the anal segment (or tenth) and scale of the eighth segment (or comb of the VIII) trident-like. As for the adults, there were no unique characters for this taxon, nor any reason for the continued separation of *Aedes* for the combination of conventional characters. At the subgeneric level *Psorophora* s. *str.* was the evolutionary basal and most distinctive taxon, due to the presence in the larvae antennas shorter than the head without surpassing the previous border, mandibular lobes with spicules and sclerotized, labiohypopharynx with premental sensor developed; in the adult, mesoscutum with longitudinal areas without scales, fore legs with erect scales, female cibarium with 14-15 trichoid sensilla and aedeagus with sawed intern border. In *Grabhamia* and *Janthinosoma*, diagnostic characters were found in adults. In *Grabhamia*: the presence of long palatal papillae in the feminine cibarium, fore femur with a tarsomere half white and dark half, and small white scales in the internal face of the torus. *Janthinosoma* is characterized by the presence of six palatal papillae in the cibarium and a yellow fore femur with dark basal band and white border. The genera *Aedes* and *Haemagogus* showed characters shared in the larva by the presence in the maxilla of the galeastipal stem and labiohypopharynx with numerous ventral premental processes with scales-like form. *Mansonia*, presents ten diagnostic characters: In the larva, the antenna are wide and longer than the head, the jaw has the latter dorsal tooth formed by two teeth, labiohypopharinx with lateral premental teeth inconspicuous, and breathing siphon with a modification to adhere to aquatic plants. In the adult, the wings have white and dark wide scales, and in the male genitalia the gonostylus is wide and with a digitiform projection.

The genus *Psorophora* is clearly a monophyletic taxon, but Aedini (*Haemagogus*, *Aedes* and *Psorophora*), using the data produced to date, seems to be a paraphyletic group that should contain the members of Mansoniini to form a more natural classification. This proposal is supported by the results obtained in a cladistic analysis of the family Culicidae [32]. Our results plus the latter authors' hypothesis, show the Mansoniini tribe (*Mansonia* and *Coquillettidia*) placed along Aedini. However, the addition of more Mansoniini taxa and with the use of molecular data may support or to reject the hypothesis of a Mansoniini +Aedini clade as natural group.

The infrageneric classification of *Psorophora* shows that the 3 subgenera form monophyletic groups, although internal polytomies were observed in *Grabhamia* and *Janthinosoma* because the relationships among some species could not be established.

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