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PHYLOGENETIC RELATIONSHIPS AMONG EIGHTEEN NEOTROPICAL CULICINI SPECIES

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ABSTRACT. The subgeneric relationships among 8 generic and infrageneric taxa of the Culicini tribe were examined by cladistic analysis based on 30 larval mouthpart characters (maxillae and mandibles) of 7 subgenera of *Culex* L. and 1 of *Deinocerites* Theobald. We analyzed 18 ingroup species as well as *Deinocerites melanophylum* Dyar and Knab as a sister group, and *Aedes taeniorhynchus* Wiedemann as an outgroup. A parsimony analysis using the Nona program resulted in 2 trees each of 109 steps (consistency and retention indices = 0.88). The topologies obtained were similar to the current classification of the tribe, based on nonexplicit methods mainly including adult characters, with 2 exceptions. In the present work, the monophyly of the tribe Culicini (*Culex* + *Deinocerites*) was supported by 4 synapomorphies. The subgenus *Lutzia* Theobald formed the most basal clade in the tribe Culicini and the placement of *Phenacomyia* Harbach and Peyton as a subgenus was validated by its location as a sister group of the subgenus *Culex* and other subgenera. The subgenus *Carrollia* Lutz was the most robust taxon, supported by 5 synapomorphies, and was congruent with the infragroups of the current classification. The relationships among *Deinocerites, Anoedioporpa* Dyar, *Microculex* Theobald, and *Melanoconion* Theobald were unresolved, but were placed in the most internal clade of the tribe. The 1st exception to the accepted classification was the poorly resolved boundary between *Anoedioporpa* and *Microculex* The 2nd was the strong support (with 11 synapomorphies) for the inclusion of *Deinocerites* as a subgenus of *Culex* in the Culicini, which is proposed here.

KEY WORDS cladistics, *Culex,* Culicidae, *Deinocerites,* larval mouthparts, mandibles, maxillae, phylogeny

INTRODUCTION

Since the pioneering work of Edwards (1932) and Dyar (1923, 1928) the taxonomy and classification of the family Culicidae has been based on classical adult and immature morphologic characters. Although the use of holomorphology in systematics inference (Hennig 1966) is preferred, recent authors have found that larval mouthparts provide good morphologic characters for alpha taxonomy (Harbach and Peyton 1993) and their use in phylogenetics should be explored.

Snodgrass (1959) was the 1st to describe the morphology of some mosquito mouthparts and Knight (1971) showed the structural diversity in the mandibles of several genera. Later, Harbach and Knight (1977) reported a variety of maxillary structures and shapes that offer additional diagnostic characters for identification.

Harbach and Peyton (1993) resumed studies with some species of the tribe Sabethini and recognized the importance of these structures for the identification of generic taxa; they suggested their use to achieve more natural classifications. Recently, Perez and Navarro (1996) reported diagnostic characters for 3 subgenera of *Anopheles* Meigen, and concluded that morphology of mouthparts is an additional tool for identification of these taxa.

Despite extensive alpha taxonomy studies of Culicidae, phylogenetic relationships have not been intensively studied. The large size of the family (3,000 species) (Knight and Stone 1977) represents a challenge. The new taxonomy described by Munstermann and Conn (1997) represents a powerful approach toward obtaining a more objective and natural classification of the Culicidae. Cladistics using molecular characters has been used by Pape (1992) with chromosomal characters in *Anopheles* (*Cellia*); Wesson et al. (1992) with *Aedes*; Besansky et al. (1994) with *Anopheles gambiae,* and Miller et al. (1996) with the Pipiens Complex. All of these studies have used ribosomal DNA. However, Besansky and Fahey (1997) estimated phylogenetic relationships among 14 Culicidae species using the white-eye gene.

Using classical morphologic characters and cladistic analyses, Judd (1996) studied the tribe Sabethini and Harbach and Kitching (1998) analyzed 34 genera of Culicidae. However, despite conclusions about the higher relationships in the family, the internal (infrageneric) relationships remain largely unresolved.

We used larval mouthpart characters to infer phylogenetic relationships among taxa within the tribe Culicini. We examined species belonging to 7 of 8 subgenera reported from Venezuela, and 13 that occur in the Neotropics. We also evaluated the power and importance of larval mouthparts in the cladistic analysis in obtaining a natural classification for this medically important taxon.

MATERIALS AND METHODS

Source of specimens: We used 4th-stage-larvae belonging to the Mosquito Collection of the Laboratorio de Biología de Vectores, Museo de Biología of the Universidad Central de Venezuela (LBV, after Guimarães 1997). These specimens came from a variety of different breeding sites (Ta-

Table 1. List of species examined with data of locality in Venezuela, date of collection, and breeding site.

Species	Locality	Date	Breeding site
Culex (Carrollia) bihaicolus	Loma de Hierro, Aragua State	Feb. 1992	*Heliconia aurea*
Cx. (Car.) rausseoi	Loma de Hierro, Aragua State	Feb. 1992	Palm spathes *Euterpe* sp.
Cx. (Car.) iridescens	Hacienda Río Claro, Zulia State	Aug. 1995	Palms spathes *Euterpe* sp.
Cx. (Car.) urichi	La Azulita, Mérida State	Dec. 1981	*Xanthosoma* sp.
Cx. (Phenacomyia) corniger	Panaquire, Miranda State	Dec. 1981	Cacao husks
Cx. (Andoedioporpa) bamborum	Panaquire, Miranda State	Feb. 1986	Bamboo internodes
Cx. (Culex) dolosus	La Azulita, Mérida State	March 1995	Discarded tire/rockhole
Cx. (Cux.) nigripalpus	El Jobo (Sinamaica), Zulia State	Oct. 1997	Tree hole at ground level
Cx. (Cux.) coronator	Instituto de Zoología Tropical UCV, Caracas, D.F.	Aug. 1997	Artificial container
Cx. (Cux.) quinquefasciatus	Cementerio del Sur, Caracas, D.F.	Jan. 1981	Artificial container
Cx. (Microculex) microphylus	Guanay Tepui, Amazonas State	Feb. 1995	*Brocchinia tatei*
Cx. (Mcx.) chryselatus	Sierra de San Luis, Falcón State	Aug. 1993	*Guzmania mucronata*
Cx. (Mcx.) pleuristriatus	Cerro Santa Ana, Falcón State	Aug. 1993	*Aechmea aquilega*
Cx. (Melanoconion) albinensis	Hacienda Las Nubes, Zulia State	Oct. 1997	Ground pool with aquatic plants
Cx. (Mel.) "grupo *atratus*"	Haciendas Las Nubes/Río Claro, Zulia State	Oct. 1997	Ground pools
Cx. (Mel.) nicceriensis	Haciendas Las Nubes/Río Claro, Zulia State	Oct. 1997	Ground pools
Cx. (Lutzia) bigoti	Sierra San Luis, Falcón State	April 1994	Discarded tire
Deinocerites melanophylum	Cayo Borracho, Falcón State	Jan. 1983	Crab hole
Aedes taeniorhynchus	Guajira-Paraguaipoa, Zulia State	Oct. 1986	Ground pools in mangroves

ble 1). A total of 19 species was examined. These belonged to the subgenera *Anoedioporpa* Dyar (1 species), *Microculex* Theobald (3 species), *Lutzia* Theobald (1 species), *Culex* L. (4 species), *Melanoconion* Theobald (3 species), *Carrollia* Lutz (4 species), and *Phenacomyia* Harbach and Peyton (1 species), and also *Aedes taeniorhynchus* Wiedmann and *Deinocerites melanophylum* Dyar and Knab.

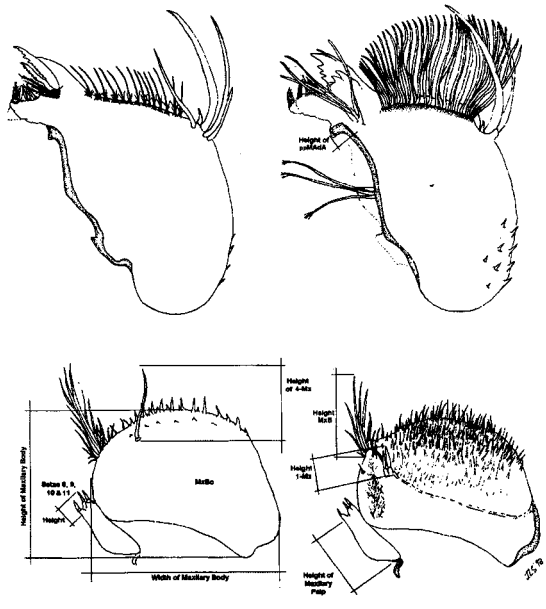

Fig. 1. Morphometric measurements for some mandibular and maxillary structures.

Mounting techniques and terminology: Larvae were stored in 80% ethanol and clarified in a 10% KOH solution, then processed as described by Harbach and Peyton (1993), with the modifications of Perez and Navarro (1996). A total of 30 characters was scored. These consisted of 16 characters associated with mandibles and 14 characters of the maxillae (Appendix 1). Characters were scored based on the nomenclature of Harbach and Knight (1980). The generic and subgeneric abbreviations followed those of Reinert (1975). Drawings of the morphometric characters used are shown in Fig. 1. A schematic of the general morphology from each supraspecific taxon examined is shown in Figs. 2a (*Lutzia*), 2b (*Phenacomyia*), 3a (*Culex*), 3b (*Carrollia*), 4a (*Deinocerites*), 4b (*Melanoconion*), 5a (*Anoedioporpa*), and 5b (*Microculex*).

Selection of characters and cladistics analysis: Characters were selected based upon the results of previous papers that also used mouthpart structures (Knight 1971, Harbach and Knight 1977, Harbach and Peyton 1993, Perez and Navarro 1996). We also included new characters (character [ch.] 13, PMnL, and ch. 15, ppMAdA) not previously studied based on structural diversity (Appendix 1). Polymorphic (multistate characters) were also included in the data set. Characters not determined in one or more taxa were treated as missing.

The genus *Aedes* Meigen (*Ae. taeniorhynchus*) was chosen as outgroup to root trees but without

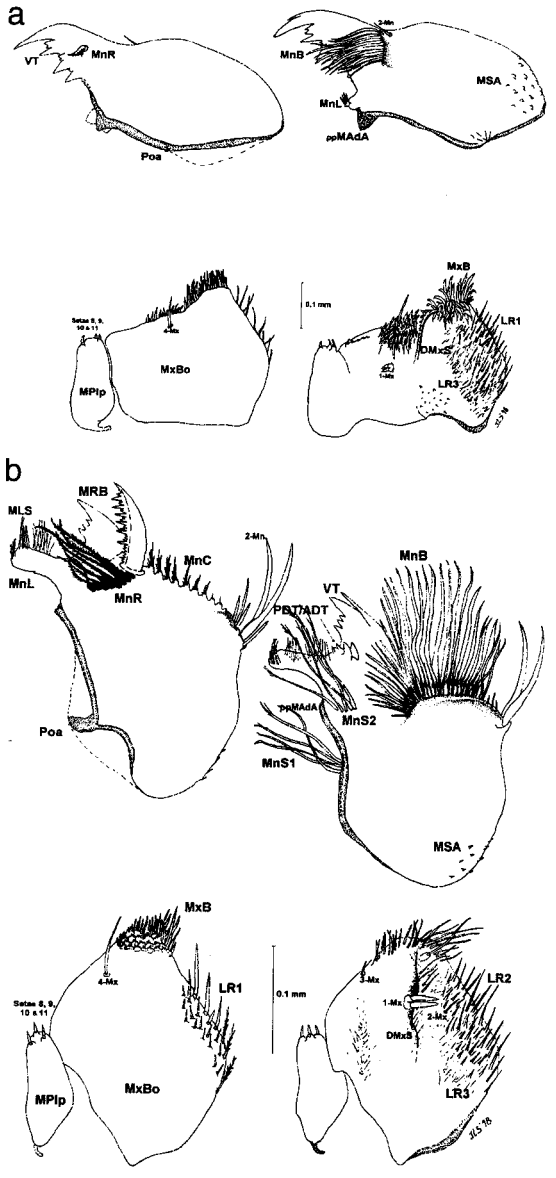

Fig. 2. a. Ventral (left) and dorsal (right) view of *Culex* (*Lutzia*) *bigoti*. Mandibles (above) and maxillae (below) are shown. b. Ventral (left) and dorsal (right) view of *Culex* (*Phenacomyia*) *corniger*. Mandibles (above) and maxillae (below) are shown.

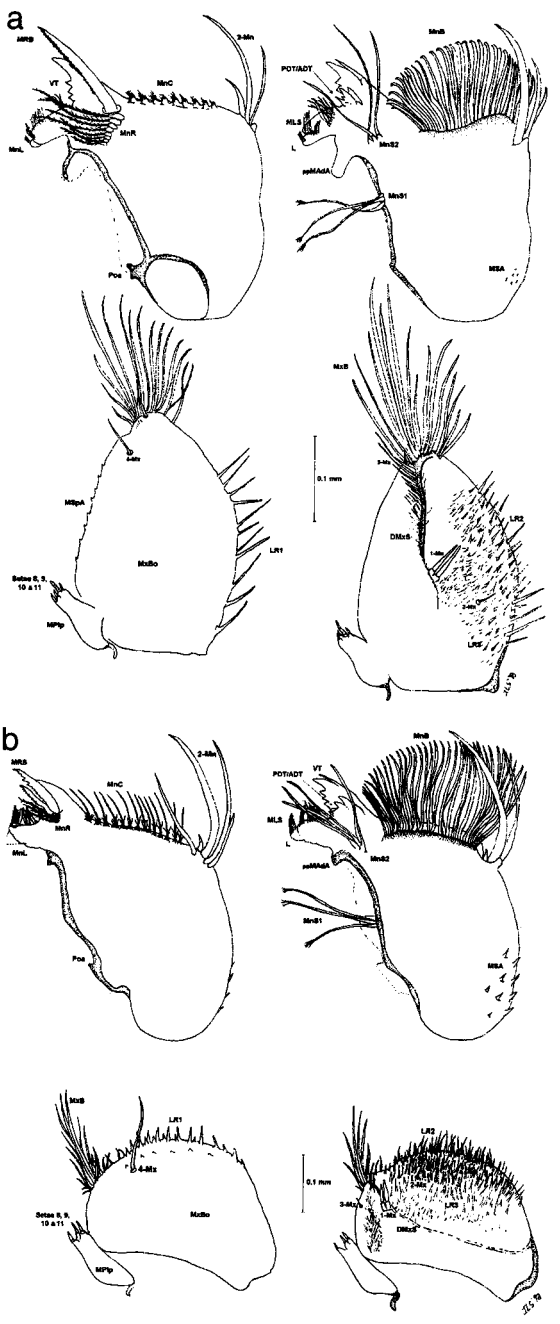

Fig. 3. a. Ventral (left) and dorsal (right) view of *Culex* (*Culex*) *dolosus*. Mandibles (above) and maxillae (below) are shown. b. Ventral (left) and dorsal (right) view of *Culex* (*Carrollia*) *bihaicolus*. Mandibles (above) and maxillae (below) are shown.

the intention of exploring the sister relationships of Culicini. Choice of this taxon as outgroup is supported by the sister relationships of *Culex* reported by Miller et al. (1997), Pawlowsky et al. (1996), Besansky and Fahey (1997), and Harbach and Kitching (1998). *Deinocerites melanophylum* was used as sister group of *Culex* in agreement with current and accepted classifications.

All characters were coded as either binary or multistate (Appendix 1) and all characters were treated as unordered (Appendix 2) (Nixon and Carpenter 1993). The Nona program (Goloboff 1996) was used to search for the most parsimonious cladograms using the Tree Bisection Reconnection (TBR) heuristic algorithm. Fifty random additions were completed using the MULT*50 option. The

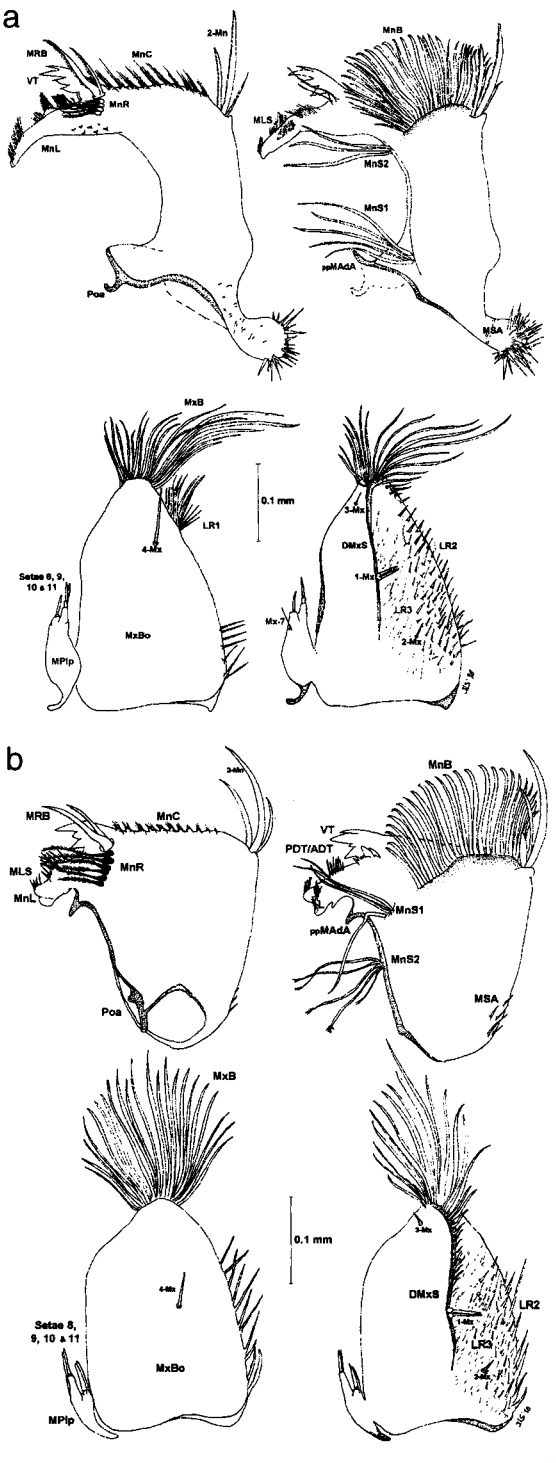

Fig. 4. a. Ventral (left) and dorsal (right) view of *Deinocerites melanophylum*. Mandibles (above) and maxillae (below) are shown. b. Ventral (left) and dorsal (right) view of *Culex* (*Melanoconion*) *nicceriensis*. Mandibles (above) and maxillae (below) are shown.

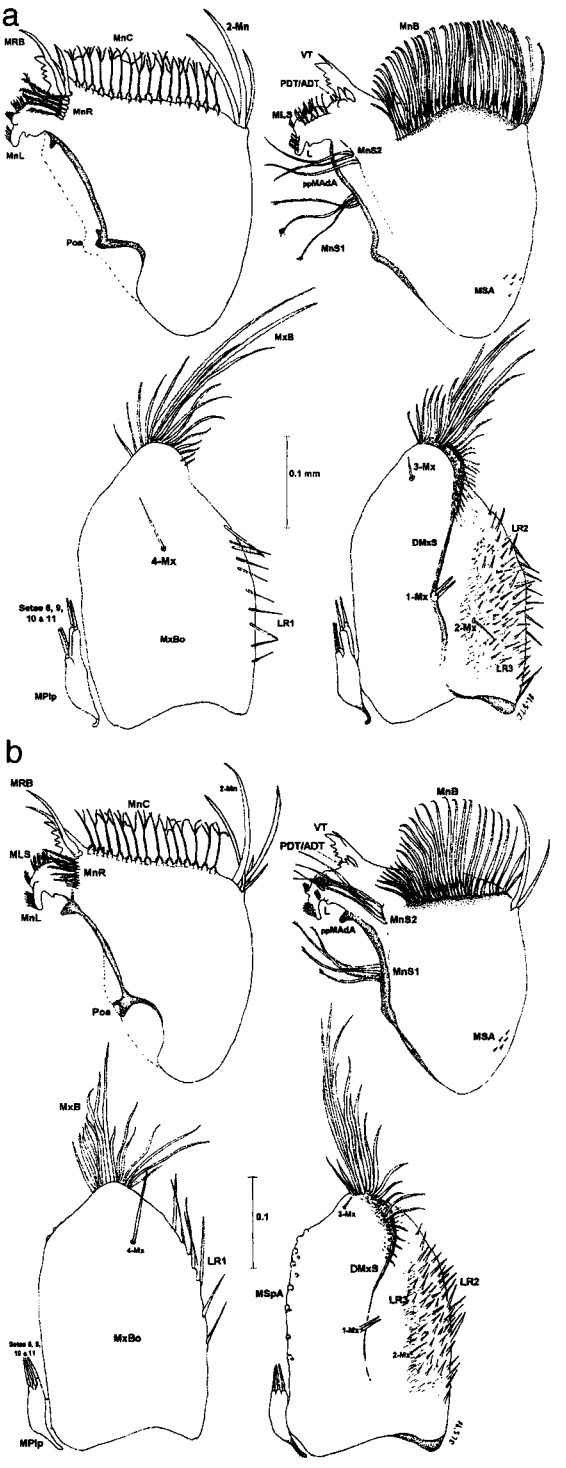

Fig. 5. a. Ventral (left) and dorsal (right) view of *Culex* (*Anoedioporpa*) *bamborum*. Mandibles (above) and maxillae (below) are shown. b. Ventral (left) and dorsal (right) view of *Culex* (*Microculex*) *chryselatus*. Mandibles (above) and maxillae (below) are shown.

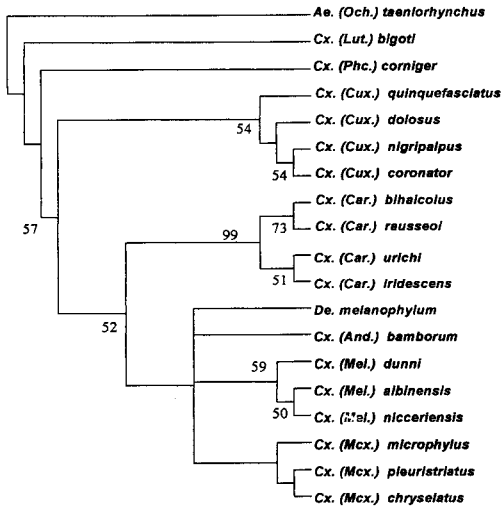

Fig. 6. Strict consensus tree of 2 most parsimonious solutions.

JUMP*1,2 option was used to perform branch swapping on all trees with a difference of 1 or 2 steps relative to the most parsimonious tree. Support for individual derived branches was evaluated by bootstrapping with 1,000 replications, after deleting the autapomorphic characters (Carpenter 1996). The options hold* and mult*20 were specified such that each tree was searched for by random additions and all of the most parsimonious trees were retained in memory.

RESULTS AND DISCUSSION

Two trees, each with 109 steps, were detected. Both trees had consistency indices (CIs) and retention indices (RIs) of 0.88 (88%). Permutation of suboptimal trees and random addition did not identify additional cladograms. The strict consensus cladogram (with bootstrapping values) and 1 of the most parsimonious trees (with synapomorphic characters) are shown in Figs. 6 and 7, respectively.

The strict consensus tree (Fig. 6) derived from the 2 most parsimonious cladograms had an unresolved node a with species of *Melanoconion, Micoculex* (both internally resolved), *Anoedioporpa,* and *Deinocerites.* The remaining basal taxa occurred within resolved clades. Bootstrap values > 50% are also shown in Fig. 6. The highest bootstrap score was found for the subgenus *Carrollia* (99%) and its internal groups, followed by *Melanoconion* (59%) and the node that included the subgenera of *Culex* (*Carrollia* [*De.* + *And.* + *Mel.* + *Mcx.*]) (52%).

Node a: Culicini

The tribe Culicini has never been in doubt, since Edwards (1932) included it as 1 of the 3 mosquito tribes (Anophelini, Megarhini [Toxorhynchitini], and Culicini), and Belkin (1962) listed it as 1 of 10 tribes. However, the internal arrangements of this tribe have not been well studied.

The monophyly of Culicini (*Culex* and *Deinocerites*) is supported by 4 synapomorphies (Fig. 7): posterior dorsal teeth (ch. 1: 1 → 0, changing from occurrence with 1 tooth and 3 smaller accessories to subsequent absence or presence with different shapes), mandibular seta no. 1 (ch. 4: 1 → 0, with loss of this character), mandibular spiculose area (ch. 24: 0 → 3, change from 5 spiculae to 9, 13, loss in *Culex quinquefasciatus* Say, and independent occurrence in *Culex nigripalpus* Theobald), and galeastipital stem (ch. 29: 0 → 1, with loss of this character).

Culex bigoti Bellardi (subgenus *Lutzia*), which has enlarged maxillary setae 8, 9, 10, and 11 (ch. 22: 0 → 1), is the basal taxon and sister to the other taxa (node b). This arrangement agrees with the traditional classification sensu Belkin (1962) of *Lutzia* as a specialized lineage that shares similarity and hence ancestry with members of the subgenus *Culex.* This result is also in concordance with the topology of Miller et al. (1996) who used sequence variation in the internal transcribed spacer of ribosomal sequences. Both results and the geographic distribution of the subgenus imply that *Lutzia* represents an ancient Gondwanian lineage.

Node f

Among the internal clades, *Culex corniger* Theobald (subgenus *Phenacomyia*) formed a sister group of node f (ch. 10: 1 → 2, occurrence of labula developed, and ch. 28: 0 → 1, maxillary palpus normal) that is located at the node c of *Culex* (subgenus) species: (*Cx. quinquefasciatus* + *Cx. dolosus* Arribalzaga + *Cx. nigripalpus* + *Cx. coronator* Dyar and Knab). Within this node c (ch. 1: 3 → 2, sequence and size of the Mn ventral teeth; and ch. 23: 0 → 1, shape of maxillary body, with reversion in *Carrollia, Deinocerites,* and *Culex pleuristriatus* Theobald) was found the following arrangement: *Cx. quinquefasciatus* (widespread species) formed a sister clade to 3 neotropically restricted species (node d), with *Cx. dolosus* representing the most related species to *Cx. nigripalpus* and *Cx. coronator* (node e). Although the Neotropical species node is not well supported, the placement of *Cx. quinquefasciatus* and *Cx. nigripalpus* is also in agreement with the topology of Miller et al. (1996), suggesting the validity of both cladistic hypotheses and also suggesting the Gondwanian origin of the Pipiens Complex.

On the other hand, the basal position of *Phenacomyia* in the tree supports the proposal of Harbach

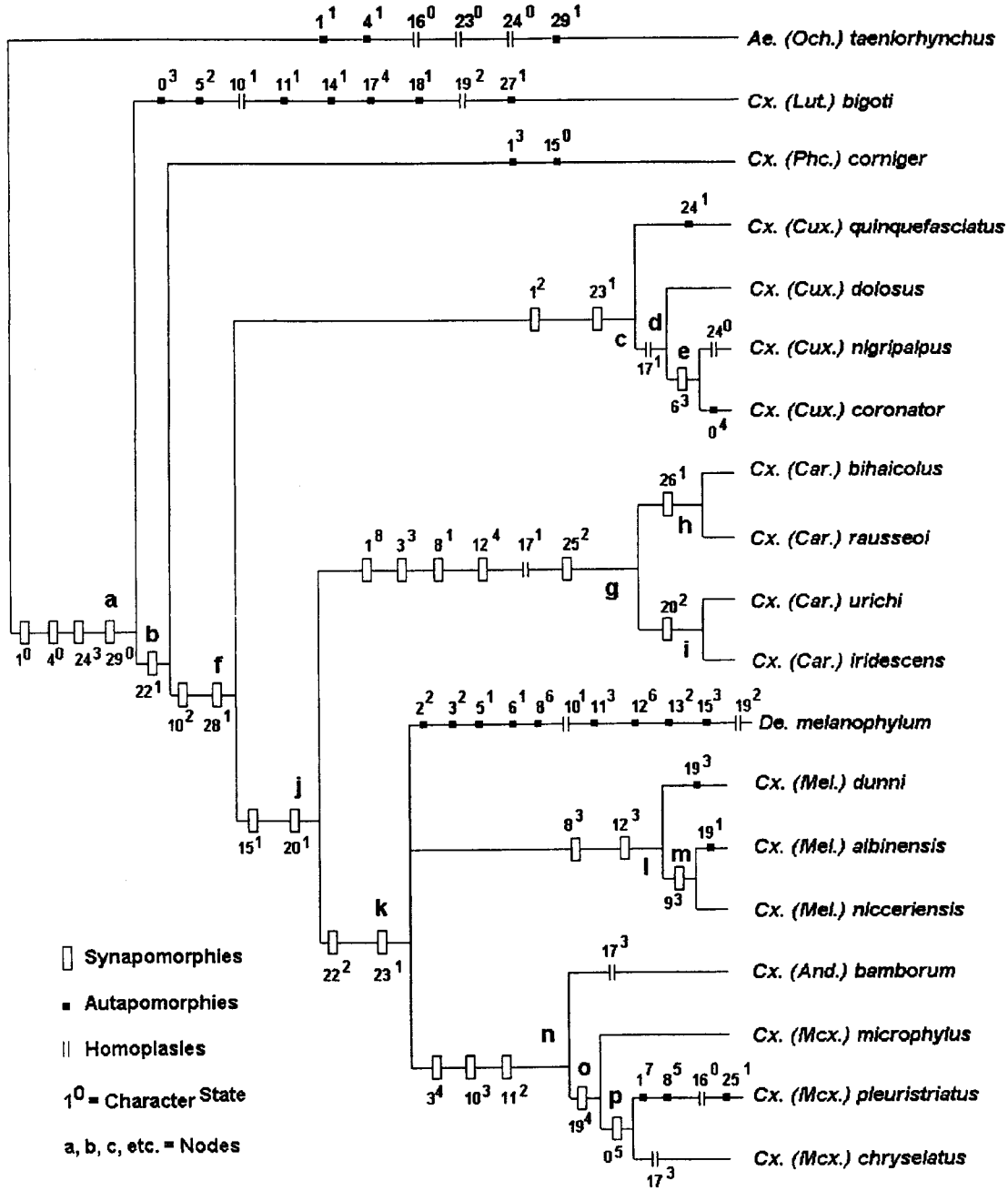

Fig. 7. One of the most parsimonious trees, showing the synapomorphies, some autapomorphies, and homoplasies.

and Peyton (1992) to split these species (*Cx. corniger* + *Culex lactator* Dyar and Knab and *Culex airozai* Lane) from the subgenus *Culex* and suggests a possible close affinity with Old World species not examined in this work. However, the autapomorphic characters ch. 10 (0) and 28 (0) not reported by these authors should be considered in the future for subgenus diagnosis.

Nodes j and k

The subgenus *Culex* node is a sister clade of the completely Neotropical node j (ch. 15: 2 → 1, reduction of posterior projection of aductor apodeme Mn; and ch. 20: 0 → 1, position of seta 4-Mx with subsequent change to state 2 in the Urichi Group and reversion in node k) where subgenus *Carrollia*

(node g) is the most basally related to the unresolved node (k) with *Mel.* + *Mcx.* + *And.* + *Deinocerites.* Within the *Carrollia* node, 2 sister taxa form the node h (*Culex bihaicolus* Dyar and Nunez-Tovar + *Culex rausseoi* Cova-Garcia, Sutil, and Pulido) and the node i (*Culex urichi* Coquillett + *Culex iridescens* (Lutz)) as a monophyletic group and a sister clade of the remaining taxa (node k). The monophyly of *Carrollia* is strongly supported by 5 synapomorphies (ch. 1, 3, 8, 12, 25) and a 99% bootstrap value, possibly representing the more recent ancestor of the fully Neotropical *Culex* subgenera.

The node k is supported by 2 synapomorphies (ch. 22: 1 → 2, enlargement of maxillary setae 8, 9, 10, 11; and ch. 23: 1 → 2, shape of maxillary body, with a regression on *Cx. pleuristriatus*). *Deinocerites* is the taxon most related to the subgenera *Anoedioporpa, Microculex,* and *Melanoconion,* but the analysis did not find any synapomorphies to establish its sister relationships. Despite the unclear relationships among these taxa, the internal relationships among *Melanoconion* and *Anoedioporpa* with *Microculex* are supported by 2 and 3 synapomorphies, respectively.

In general, the most parsimonious cladograms indicate very few homoplasic characters (Fig. 7) with CI and RI = 0.88 (88%). These homoplastic characters are 16, 17, 19, 23, and 24, and the most homoplasic character change occurs in seta 1-Mx (ch. 17), which occurs 4 times independently in the trees (CI = 0.66; RI = 0.71). This means that ch. 17 is uninformative to explain evolutionary trends, and for species or taxa diagnosis.

Congruence with the current classification

With 2 exceptions, the tree topologies found are congruent with the current classification, including monophyletic clades for *Lutzia, Phenacomyia, Culex, Carrollia,* and *Melanoconion.* Also, the monophyletic *Culex* and *Carrollia* clades are consistent with the intuitive internal groups such as the basal Pipiens Group in *Culex,* and Valencia's groups Bihaicolus (*bihaicolus* and *rausseoi*) and Urichi (*iridescens* and *urichi*) (Valencia 1973). However, 2 incongruences with current and accepted classifications occur in the clade k showing *Anoedioporpa* in the same clade with *Microculex* and the genus *Deinocerites* at the most internal branches of the Culicini clade.

Anoedioporpa, a subgenus proposed by Dyar (1923), had later hierarchic taxonomic changes and was subsequently included in the subgenera *Melanoconion* and *Tinolestes* Coquillett. In the last revision, Belkin (1968) raised *Anoedioporpa* to a subgenus comprised of 12 species (Berlin and Belkin 1980), among them *Culex restrictor* Dyar and Knab, formerly included in the subgenus *Microculex.* Nevertheless, our analysis did not permit us to propose a hypothesis about this group. We conclude that the subgenus *Anoedioporpa* should be studied further to clarify the relationships with *Microculex* species in search of the natural classification of the genus.

The 2nd and the most important exception to the current classification is the paraphyletic position of *Deinocerites melanophylum,* with internal placement in the analysis and supported by 11 synapomorphies suggesting that it is a subgenus within the genus *Culex* (sensu lato). These results are in agreement with Mallampalli (1995), who placed *Deinocerites cancer* Theobald and *Galindomyia leii* Stone and Barreto-Reyes in the most internal clade of the *Culex* topology, using mainly adult characters.

The hierarchic position of *Deinocerites* as a different genus in the tribe Culicini and family Culicidae had been largely based upon autapomorphic characters (9 included in the present study) (Theobald 1901, Belkin and Hogue 1959, Adames 1971) that do not seem to represent synapomorphies and therefore are misleading in estimating evolutionary relationships among other members of the tribe Culicini or the Culicidae sensu Belkin (1962). Belkin and Hogue (1959) commented "opinions regarding the relationships of *Deinocerites* have varied widely," including it as separate subfamily, subgenus, or the current genus level, by Mitchell, Dyar, and Edwards, respectively. Additionally, Belkin and Hogue, aware of the close relationships between *Culex* and *Deinocerites,* also said "this lack of comparative studies has been a great handicap in our attempt to determine the relationships of *Deinocerites*" and finally accepted the genus status of this taxon.

Later, in the last review of the genus, Adames (1971) concluded that "*Deinocerites* is undoubtedly a member of the tribe Culicini" and reported 3 characters of adults not shared with *Culex* to support the generic status without considering possibly homologous characters. In our case, *Deinocerites* has 9 autapomorphic characters but also has 11 synapomorphic characters that place it within the most internal clade within *Culex.*

The strictly coastal distribution of *Deinocerites* associated with crab holes and the internal position in the cladogram supported by 11 synapomorphies suggests a recent origin from "a stock of *Culex* subgenus," and not "from a common ancestral stock which separated very early from the stock that gave rise to the dominant genus *Culex*" sensu Adames (1971).

Finally, no evidence from our analysis supports the generic status for *Deinocerites* in the tribe Culicini, at least in the current classification. In contrast, our phylogenetic evidence recognizes this taxon as another member of *Culex* sensu lato. Based on comments of Zavortink (1990) and Harbach and Kitching (1998) and the studies of Judd (1996, 1998a), a natural classification of Culicidae may be achieved by recognizing subordinate infrageneric

taxa as valid genera. This could be reached in the future using cladistic methods. Nevertheless, until the boundaries for the nominal subgenus are clearly defined we propose the reduction of *Deinocerites* to subgenus status (*Dei.*). This proposal will be corroborated by additional phylogenetic studies using molecular characters.

Using a new methodological approach, with 30 larval mouthpart characters we obtained similar topological cladograms to Mallampalli (1995), who used 67 mainly adult characters. We have demonstrated that the larval structures and particulary the morphology of mouthparts are useful for the formulation of evolutionary hypotheses.

Judd (1998b) suggested that data from a single life stage "will sometimes produce an erroneous arrangement of the taxa," in agreement with other authors who followed phenetic methods (Rohlf 1963). In our case for Culicini, the trees obtained are "congruent" with those of Mallampalli (1995) and molecular data (unpublished).

Evolutionary trends of breeding sites

Our resolved phylogeny suggests that the ancestral member of this group was a ground-pool-breeding, filter-browsing organism that gave rise to 2 lineages. Members of the 1st lineage evolved structural reductions in the mandibular brush and development of mandibular teeth and a maxillar palpus that enabled predation (*Lutzia* type). The 2nd lineage retained symplesiomorphic characters allowing the independent colonization of specialized breeding places with mono- and dicotyledoneous phytotelmata in the most apomorphic environments (e.g., *Car.* and *Mcx.*). The latter morphologic modifications could explain the large radiation and diversification of some lineages (subgenera) with species-specific breeding site associations, for example, *Microculex* and bromeliads (Frank 1983, Navarro et al. 1995).

Despite the complexity of *Culex,* due to its large and taxonomically problematic groups that have been poorly studied, this study addresses questions about the monophyly of current Neotropical genera and subgenera (based on intuitive analysis), and indicates that the use of additional taxa and morphologic characters may clarify poorly supported groups and improve current classifications.

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APPENDIX 1

Character codings used in phylogenetic analysis.

(0) VT: Ventral teeth

Size: VT0 > VT1 = VT2 = VT3 > VT4, teeth 1, 2, and 3 normal (0)
Size: VT0 > VT1 = VT2 = VT3 > VT4, teeth 1, 2, and 3 thin (1)
Size: VT0 ≅ VT1 > VT2 = VT3 > VT4, teeth 1, 2, and 3 slightly sclerotized (2)
Size: VT0 > VT1 > VT2 > VT3 > VT4, teeth 1, 2, and 3 (3) not serrated (3)
Size: VT0 > VT2 > VT1 = VT3 > VT4, teeth 1, 2, and 3 normal (4)
Size: VT0 > VT3 > VT1 = VT2 > VT4, teeth 1, 2, and 3 normal (5)
Size: VT0 ≅ VT3 > VT1 = VT2 > VT4, teeth 1, 2, and 3 normal (6)

(1) PDT: Posterior dorsal tooth

Absent (0); Simple serrate, and tree accessory smaller (1); Simple not serrate, and 2 accessories (external bigger than) (2); Simple not serrate, and tree accessory (the medial is smallest) (3); Simple not serrate, and tree accessories (all same size) (4); Simple not serrate, and tree accessory (1 over a spheric surface) (5); Simple not serrate, and 4 accessory (the 2 internal smaller) (6); Simple not serrate, and several small accessories (all of these over spheric surface) (7); Simple not serrate, and 2 accessories over spheric surface (8)

(2) ADT: Anterior dorsal tooth

Simple serrate (0); Simple not serrate (1); Absent (2)

(3) MnC: Mandibular comb

Absent (0); Large: 1 spicule per each insertion (1); Large: several spicules in each insertion (2); Large: 1 spicule in central position surrounded by short spicules in each insertion (3); Large: 1 central and branched spicule surrounded by short ones in each insertion (4); Short spicules (5); Short spicules over conspicuous protuberances (6)

APPENDIX 1
Continued

(4) 1-Mn: Mandibular seta no. 1

Absent (0); Present: behind the mandibular teeth (1)

(5) 2-Mn: Mandibular seta no. 2

Several groups of setae (2a, 2b, 2c, . . . , etc) (0); Several groups of setae (2 of them serrate) (1); Only 1 reduced and behind the MnT (2)

(6) MnS: Mandibular sweeper

MnS1 with 7 filaments and MnS2 with 5 filaments (0); MnS1 6 filaments, MnS2 4 filaments, all with no branched apex (1); MnS1 and MnS2 with the same number of filaments (2); MnS1 with 8 filaments and MnS2 6 filaments (3); MnS1 and MnS2, absent (4)

(7) MRB: Mandibular rake blade

Simple serrate and curved towards the MnB (0); Simple serrate and very developed (1); Simple serrate and reduced (2); Simple serrate normal (3); Simple lightly serrate (4); Double: 1 lightly serrate and the other 1 normal (5); Double (both lightly serrate) (6); Double serrate: 1 very thin (7); Double serrate (8)

(8) MSA: Mandibular spiculose area

Dorsal spicules, large and few (0); large and abundant spicules (1); short and few spicules (2); short and thin (3); inconspicuous (4); Dorsals and ventrals large (5); Dorsals very developed (6); Dorsals, large and blunt (7)

(9) MnR: Mandibular rake

Number of filaments: Five not pectinate (0); Seven or 8 large and pectinate (1); Five to 7 large pectinate (2); Seven or 8 short pectinate (3); Five or 6 short pectinate (4); Three or 4 short pectinate (5); Absent (6)

(10) L: Labula

Lightly developed (0); Absent (1); Developed (2); Strongly developed (3)

(11) MnL: Mandibular lobe

One and one-half the width of base (0); reduced (1); One and one-half the width of base, and trilobulate (2); Four times width of base, with spicules (3)

(12) Poa: Postartis

Rectangular shape and short (0); Rectangular and large (1); Bilobulate: Both lobes equal in size (2); Bilobulate: Posterior lobe larger than anterior one (3); U-shaped, short (4); U-shaped, large (5)

(13) PMnL: Posterior mandibular lobe
Absent (0); Normal (1); Well developed (2)

(14) MnB: Mandibular brush
Normal (0); Reduced, toward MnT (1)

(15) ppMAdA: Posterior projection of mandibular aductor apodeme

Very short: 0.013–0.019 mm (0); Short: 0.025–0.038 mm (1); Large: 0.051–0.057 mm (2); Large: 0.051–0.057 mm, hooklike (3)

(16) MxB: Maxillary brush
Short: 0.140–0.178 mm (0); Short 0.152 mm, with little serrate spicules (1); Very short 0.102 mm (2); Large: 0.305–0.406 mm (3)

(17) 1-Mx: Maxillary seta no. 1

Large: 0.064–0.076 mm, thin at beginning of DMxS (0); Large: 0.064–0.076 mm, thin and beyond end of DMxS (1); Short: 0.038–0.051 mm, thin and medial to DMxS (2); Very short 0.025 mm, thin and medial to DMxS (3); Very short 0.025 mm, thick and far apart DMxS (4)

(18) 3-Mx: Maxillary seta no. 3

Present (0); Absent (1)

(19) 4-Mx: Maxillary seta no. 4

Very short: 0.038–0.064 mm, normal (0); Very short: 0.038–0.064 mm and sclerotized (1); Short: 0.089–0.095 mm (2); Short: 0.089–0.095 mm and sclerotized (3); Large: 0.102–0.114 mm (4); Very large: 0.127–0.159 mm (5); Very large: 0.127–0.159 mm and sclerotized (6)

(20) Position of 4-Mx

Anterior, beyond palp (0); Posterior, beyond LR1 (1) medial to MxBo (2)

(21) 7-Mx: Maxillary seta no. 7

Absent (0); Present (1)

(22) 8-, 9-, 10-, and 11-Mx (=MS): Maxillary setae nos. 8, 9, 10, and 11

APPENDIX 1
Continued

Very short (0); Short (1); Large (2)

(23) MxBo: Maxillary body

Approximately as large as wide, square shape (0); twice its width, rectangular shape (1); more than twice its width (2)

(24) MSpA: Maxillary spiculose area

Five dorsolateral spicules (0); Nine to 13 dorsolateral spicules (1); Fourteen dorsolateral rounded spicules (2); Absent (3)

(25) DMxS: Dorsal maxillary suture

Large and vertical (0); Large and diagonal (1); Short and diagonal (2)

(26) LR1: Laciniarastrum no. 1

Large spicules (0); Nine to 13 short spicules (1)

(27) LR2: Laciniarastrum no. 2

Present (0); Absent (1)

(28) MPIp: Maxillary palpus

Prominent (0); Normal (1)

(29) GSS: Galeastpial stem

Absent (0); Present (1)

APPENDIX 2
Data matrix used for cladistic analysis.

Taxa	Characters[1]																													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Aedes taeniorhynchus	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Deinocerites melanophylum	2	0	2	2	0	1	1	7	6	4	1	3	5	2	0	3	3	2	0	2	1	1	2	2	3	0	0	0	1	0
Culex (Lutzia) bigoti	3	0	2	0	0	2	4	2	7	6	1	1	?	0	1	2	2	4	1	2	0	0	0	0	3	0	0	1	0	0
Cx. (Carrollia) bihaicolus	0	8	1	3	0	0	2	8	1	5	2	0	4	0	0	1	0	1	0	5	1	0	1	0	3	2	1	0	1	0
Cx. (Car.) rausseoi	0	8	1	3	0	0	2	8	1	5	2	0	4	0	0	1	0	1	0	5	1	0	1	0	3	2	1	0	1	0
Cx. (Car.) urichi	0	8	1	3	0	0	2	8	1	5	2	0	4	0	0	1	0	1	0	6	2	0	1	0	3	2	0	0	1	0
Cx. (Car.) iridescens	0	8	1	3	0	0	2	8	1	5	2	0	4	0	0	1	0	1	0	6	2	0	1	0	3	2	0	0	1	0
Cx. (Culex) dolosus	0	2	1	5	0	0	2	3	4	1	2	0	2	0	0	2	3	1	0	0	0	0	1	1	3	0	0	0	1	0
Cx. (Cux.) nigripalpus	0	2	1	5	0	0	3	3	4	1	2	0	2	0	0	2	3	1	0	0	0	0	1	1	0	0	0	0	1	0
Cx. (Cux.) quinquefasciatus	0	2	1	5	0	0	2	3	4	1	2	0	2	0	0	2	3	2	0	0	0	0	1	1	1	0	0	0	1	0
Cx. (Cux.) coronator	4	2	1	5	0	0	3	3	4	1	2	0	2	0	0	2	3	1	0	0	0	0	1	1	3	0	0	0	1	0
Cx. (Anoedioporpa) bamborum	1	5	1	4	0	0	2	5	4	4	3	2	2	0	0	1	3	3	0	0	1	0	2	2	3	0	0	0	1	0
Cx. (Microculex) pleuristriatus	5	7	1	4	0	0	2	8	5	2	3	2	2	1	0	1	0	2	0	4	1	0	2	0	0	1	0	0	1	0
Cx. (Mcx.) chryselatus	5	4	1	4	0	0	2	6	4	2	3	2	2	1	0	1	3	3	0	4	1	0	2	2	2	0	0	0	1	0
Cx. (Mcx.) microphylus	1	4	1	4	0	0	2	4	4	4	3	2	2	0	0	1	3	2	0	4	1	0	2	2	3	0	0	0	1	0
Cx. (Melanoconion) albinensis	6	6	1	5	0	0	2	5	3	3	2	0	3	0	0	1	3	2	0	2	1	0	?	2	3	0	0	0	1	0
Cx. (Mel.) dunni	6	6	1	5	0	0	2	5	3	4	2	0	3	0	0	1	3	2	0	3	1	0	2	2	3	0	0	0	1	0
Cx. (Mel.) nicceriensis	6	6	1	5	0	0	2	5	3	3	2	0	3	0	0	1	3	2	0	0	1	0	2	2	3	0	0	0	1	0
Cx. (Phenacomyia) corniger	0	3	1	6	0	0	2	1	2	1	0	0	1	0	0	0	1	2	0	0	0	0	1	0	3	0	0	0	0	0

[1] ?, Could not be determined.