Molecular Phylogeny of the Vomerifer and Pedroi Groups in the Spissipes Section of the Subgenus *Culex* (*Melanoconion*)

JUAN-CARLOS NAVARRO[1, 2] AND SCOTT C. WEAVER[2]

J. Med. Entomol. 41(4): 575–581 (2004)

ABSTRACT Members of the New World mosquito subgenus *Melanoconion* of the genus *Culex* are important vectors of many alphaviruses including eastern and Venezuelan equine encephalitis viruses (VEEV). We investigated the phylogenetic relationships among nine putative species of the Vomerifer and Pedroi Groups of the Neotropical Spissipes Section by sequencing the internal transcribed spacer 2 (ITS-2) region of ribosomal DNA and using phylogenetic analyses. Results demonstrated that, within the Spissipes Section, the Vomerifer and Pedroi Groups are monophyletic sister groups. The clade comprised by *Culex adamesi* and *Culex ribeirensis* showed a sister group relationship to the group consisting of *Culex pedroi* sequences. The monophyly of the Vomerifer Group corroborated previous suggestions that it is a natural group. However, our topology showed that there are two well-supported, divergent groups within a major clade consisting of *Cx. pedroi* sequences, suggesting the possibility of a cryptic *Cx. pedroi*–like species. This finding could have important epidemiological implications for VEEV transmission in Central and South America.

KEY WORDS cladistics, Culicidae, internal transcribed spacer 2, phylogeny, Venezuelan equine encephalitis

AMONG THE MAJOR GROUPS of New World mosquitoes, the genus *Culex* L., subgenus *Melanoconion* Theobald, is medically important because several of its species are suspected or proven vectors of arboviruses, including eastern equine encephalitis virus (EEEV) and Venezuelan equine encephalitis virus (VEEV) (Karabatsos 1985, Johnston and Peters 1996, Weaver 1997, 1998, Ferro et al. 2003, Wearver et al. 2004). Despite its public health importance, little progress has been made in the systematics of the *Culex* (*Melanoconion*) subgenus. The taxonomic status of *Melanoconion* underwent several changes in interpretation and treatment from Theobald (1903) until Rozeboom and Komp (1950), when the subgeneric status of the group became more stabilized. Much later, Sirivanakarn (1983) made the first comprehensive morphological revision of the subgenus, distinguishing three Sections and 153 species and providing larval, pupal, and adult identification keys. However, this work did not include identification keys for the species level. Pecor et al. (1992) suggested that the Ocellatus Section (previously considered within the subgenus *Microculex*) did not belong to *Melanoconion*, and it remains without subgeneric assignment. Later, Sallum and Forattini (1996) made the first revision for the Spissipes Section based on morphological characters of the adults male and female (including species identification keys). Most of these taxonomic studies have emphasized the importance of morphological characters in a traditional, noncladistic, or nonphylogenetic treatment. Evolutionary relationships among *Melanoconion* species and other members of Culicini were not treated using phylogenetic methods until the study of Miller et al. (1996) using rDNA and Navarro and Liria (2000) using morphological characters of the fourth-instar larvae. The great difficulty in identifying species and groups of the *Melanoconion* subgenus remains; thus, it is in need of a major comprehensive phylogenetic analysis using both morphological and molecular methods.

The Spissipes Section was first proposed by Galindo (1969) as the "*Culex spissipes* Group" based on feeding habits of adults and larval characters. Later, Sirivanakarn (1983) recognized this group as the Spissipes Section including 18 species. Based on narrow decumbent scales on the vertex, small or indistinct patches of broad spatulate scales on the lateral portions, a broadly sclerotized aedeagal sclerite on the adult, and a few characters on the larvae and pupae, Sirivanakarn (1983) placed these species into eight Groups and five subgroups. Following Sallum and Forattini (1996) in the last revision of the Spissipes Section and Sallum et al. (1997) with the last described species, the Spissipes Section now comprises 23 species within eight Groups and three subgroups.

The main difference between the classification proposed by Sirivanakarn (1983) and that of Sallum and

[1] Laboratorio de Biologia de Vectores, Unid. Entomologia Molecular, Instituto de Zoologia Tropical, Universidad Central de Venezuela, Apdo. 47048, Caracas 1041-A, Venezuela (e-mail: jnavarro@strix.ciens.ucv.ve).

[2] Center for Biodefense and Emerging Infectious Diseases and Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555–0609.

0022-2585/04/0575–0581$04.00/0

Table 1. *Culex* (*Melanoconion*) species used in the study and their location

Taxon[a]	Country	Locality	Group/subgroup[b]	Source[c]
Cx. spissipes-VZ	Venezuela	Catatumbo	Spissipes/none	Authors
Cx. taeniopus	Guatemala	Puerto Barrios	Taeniopus/none	Authors
Cx. adamesi	Peru	Iquitos	Crybda/Pedroi	R. Fernandez
Cx. adamesi P81	Peru	Iquitos	Crybda/Pedroi	R. Fernandez
Cx. ribeirensis	Brazil	Pariquera-Açu	Crybda/Pedroi	M. A. Sallum
Cx. pedroi s.s. (CAT73, 138)[d]	Venezuela	Catatumbo	Crybda/Pedroi	Authors
Cx. pedroi s.s. (MIR-05)	Venezuela	Miranda-Barlovento	Crybda/Pedroi	Authors
Cx. pedroi s.s. (COL)	Colombia	Puerto Boyaca	Crybda/Pedroi	C. Ferro
Cx. pedroi s.s. (GTM)	Guatemala	Izabal Lake	Crybda/Pedroi	Authors
Cx. pedroi-Peru form (PER4-3, PER, PER05, PER03, PER02)[d]	Peru	Iquitos	Crybda/Pedroi	Authors
Cx. pedroi-Peru form(MIR01)	Venezuela	Miranda-Barlovento	Crybda/Pedroi	Authors
Cx. vomerifer (COL)	Colombia	Puerto Boyaca	Vomerifer/none	C. Ferro
Cx. vomerifer (PER)	Peru	Iquitos	Vomerifer/none	R. Fernandez
Cx. gnomatos	Peru	Iquitos	Vomerifer/none	R. Fernandez
Cx. portesi	Peru	Iquitos	Vomerifer/none	Authors
Cx. sacchettae	Brazil	Brazil-Pariquera-Açu	Vomerifer/none	M.A. Sallum/Authors

[a] All specimens were legs/ethanol except *Cx. ribeirensis* that was dry-legs/pinned.
[b] *Sensu* Sallum and Forattini (1996).
[c] Collector's institutional affiliations are provided in the acknowledgements.
[d] Sequences of different specimens from the same locality.

Forattini (1996) is comprised of the hierarchal position of a few species. Sirivanakarn (1983) includes the Vomerifer, Taeniopus, and Pedroi subgroups within the Taeniopus Group and places *Cx. vomerifer* Komp, *portesi* Sevenet and Abonnenc, and *Cx. sacchettae* Sirivanakarn and Jacob in the Vomerifer subgroup. The classification of Sallum and Forattini (1996) includes Vomerifer as a group and places the Pedroi Group (*Cx. pedroi*, *Cx. adamesi* Sirivanakarn and Galindo 1980, *Cx. crybda* Dyar, *Cx. epanastasis*, and *Cx. ribeirensis*) as a subgroup within the Crybda Group. However, both classifications consider the Vomerifer and Pedroi species as groups, independent of the level classifications.

The internal transcribed spacer region two (ITS-2) of nuclear ribosomal DNA has been used to analyze and to verify relationships and delineation of species in the taxa *Culex* (*Culex*) (Miller et al. 1996, Severini et al. 1996), Anophelini (Sallum et al. 2002, Kengne et al. 2003), and Triatominae (Marcilla et al. 2001), and also to determine evolutionary relationships in Phlebotominae (Depaquit et al. 2000) and Coleoptera (Gomez-Zurita and Petitpierre 2000). We used ITS-2 sequences to examine the systematics of the Spissipes Section of the *Culex* (*Melanoconion*) subgenus. Our results supported the monophyly of both the Vomerifer and Pedroi Groups in the sense of Sirivanakarn (1983), and they estimated phylogenetic relationships among nine species of the Spissipes Section.

Materials and Methods

Mosquitoes. Mosquito specimens were collected from localities in Brazil (Pariquera-Açu, São Paulo State: 24°30′ S, 47°50′ W), Colombia (Monte San Miguel, Puerto Boyacá in the Magdalena Valley: 6°23′30″ N; 74°21′41″ W), Guatemala (Puerto Barrios: 15.7° N, 88.6° W; Izabal Lake 15°30′ N, 89.10° W), Peru (Iquitos: 3°49′ S, 73°20′ W), and Venezuela (Catatumbo Region, Zulia State: 9°0′44.5″ N, 72°41′53″ W; Barlovento Region, Miranda State: (10°13′22″ N; 66°17′56″ W) (Table 1). Animal bait, CDC-miniature lights, and Shannon traps were used for mosquito collections.

The female mosquito species were identified using morphological keys and descriptions available in Sirivanakarn (1983), and Sallum and Forattini (1996). The identifications were corroborated by comparison with museum specimens deposited in the entomological collection of the Faculdade de Saude Publica-NUPTEM, Universidade de São Paulo (and the assistance of M.A.M. Sallum), the collections of the Walter Reed Biosystematics Unit at the Smithsonian Institute (and the assistance of J. Pecor and R. Wilkerson), and Universidad Central de Venezuela (Laboratorio Biologia de Vectores at the Instituto de Zoología Tropical, Universidad Central de Venezuela).

Genomic DNA Extraction and Polymerase Chain Reaction. Genomic DNA was extracted from two legs that were removed from freshly killed individual female mosquitoes and stored in 95% ethanol as described by Crabtree et al. (1995). The remaining voucher specimens were pinned and stored in the Collection of the Laboratorio de Biologia de Vectores, Instituto de Zoología Tropical, Universidad Central de Venezuela. The DNA of *Cx. ribeirensis* was obtained from two legs of one dry-pinned specimen (University of Sao Paulo-NUPTEM).

After removal of 95% ethanol, each mosquito pair of legs were ground in 25–30 μl of ice-cold TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) with microfuge pellet pestle grinders (Kontes, Vineland, NJ), incubated at 90–95°C for 10 min, and microfuged for 2 min at 13,000 RPM.

The ITS-2 region of the ribosomal DNA was amplified by polymerase chain reaction (PCR) using the primers CP16(−) (Crabtree et al. 1995) complementary to sequence at the 5′ end of the 28S RNA subunit (5′-GCGGGGTACCATGCTTAAATTTAGGGGGTA-3′) and JCN627(+), a primer that was designed to be complementary to the conserved sequence at the 3′ end of 5.8S RNA subunit (5′-TGAAGACCGAGCTAAATG-3′). Each 25-μl amplification reaction contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM each of dATP, dCTP, dGTP, and dTTP, 10 ng each of CP16(-) and JCN627(+) primers, 1.0 U of *Taq* polymerase (Promega, Madison, WI), and mosquito DNA. The PCR reactions were heated at 96°C for 4 min and amplified for 35 cycles, consisting of 96°C for 15 s, 55°C for 30 s, 72°C for 90 s, and a final step of 72°C for 4 min. Aliquots of the PCR reaction were analyzed by agarose gel electrophoresis to assess amplification.

Cloning and Sequencing. The PCR products including conserved 5.8S and 28S rDNA subunit regions that flank the ITS-2 region were cloned into the pcr2.1 vector (Invitrogen, Carlsbad, CA) and sequenced using an Applied Biosystems (Foster City, CA) ABI 377 automated sequencer using an ABI PRISM Dye terminator cycle sequencing kit with T7 and M13 primers, following the manufacturer's instructions. At least five individuals/locality of each species were sequenced (including *Cx. pedroi* specimens from Venezuela, Colombia, Guatemala, and Peru) to examine intra- and interspecific sequence divergence (uncorrected pairwise distance). Also the ITS-2 variability within individual mosquitoes was assessed by comparison of several clones from the same amplicon.

Sequence and Phylogenetic Analysis. Contig assembly and related sequence analysis was performed using the Sequencer 3.0 (Gene Codes, Ann Arbor, MI) and MacVector (Accelrys, Madison, WI) software. DNA sequences were aligned using PILEUP (Devereux et al. 1984) in the GCG Wisconsin Package, version 8.0, with gap creation penalties ranging from three to five, gap extension penalties ranging from zero to one, and end gap penalties.

Parsimony and maximum likelihood analyses were implemented in PAUP. 4.0b10 (Swofford 2002) using the heuristic search option with a Tree Bisection Reconnection branch-swapping algorithm with stepwise addition at random using 10 replicates for each search and 100–1,000 replications per analysis. Gaps were treated both as missing data and as a fifth character state. Initially unordered, all and equally weighted characters were analyzed. The robustness of the trees was estimated using parsimony bootstrap with 500 pseudoreplicates after excluding uninformative characters (Carpenter 1996).

Results

ITS-2 sequences were generated for all mosquitoes subjected to PCR amplification. The sequences have been submitted to GenBank under accession numbers from AY633719 to AY633739. Alignments with a range of gap creation penalties from three to five and gap creation penalties zero to one generated very similar alignments and identical phylogenetic results (Fig. 1). Alignments generated 531 characters that were analyzed and treated as unordered with equal weight; 97 sites were parsimony-informative, 62 were uninformative, and 372 were invariant (133, 75, and 323 when gaps were treated as a fifth character, respectively). *Cx. spissipes* Theobald and *Cx. taeniopus* Dyar and Knab were used as outgroups based on results with different subgenera of *Culex*, including members of the Melanoconion and Spissipes Sections (unpublished data). A single parsimonious solution was obtained in the unweighted analysis using gaps as missing data, with length 328 steps, consistency index, CI = 0.76; rescaled consistency index, RC = 0.59; and retention index, RI = 0.78; two equally parsimonious solutions with L = 522, CI = 0,697; RC = 0,53; RI = 0,76 were obtained using gaps as a fifth character. The topologies of both analyses (gaps as missing data and fifth characters) were the same. Parsimony bootstrap support values are seen in Fig. 1. Results with maximum likelihood analyses were essentially identical.

The variation among sequences of clones from the same specimens was 0–0.5%; among individuals in the same species within the same locality, it was 0–3.3%, (*Cx. pedroi* in Venezuela or *Cx. pedroi*-Peru form in Peru), whereas the variation among individuals of the same species in different localities was 0–4.5% (*Cx. vomerifer:* Colombia versus Peru). The sequence divergence between outgroups was 22.6% (*Cx. spissipes* versus *Cx. taeniopus*), whereas divergence between the outgroup clade and ingroup clade ranged from 19 to 20%. The minimum divergence value between sequences included within the Vomerifer and Pedroi clades was 10.2%. Within the Vomerifer clade, genetic divergence ranged from 4 to 8%, whereas within the Pedroi Group, sequence divergence varied from 7 to 9% in the three different internal clades.

Within the Vomerifer clade (100% bootstrap value), the phylogeny depicted *Cx. sacchettae* in a basal position, whereas *Cx. vomerifer* was in a more derived position, and *Cx. gnomatos* and *Cx. portesi* were in an intermediate position. All of these species were very closely related, with only 8–10% genetic divergence. According to Sallum and Forattini (1996), the Vomerifer Group can be distinguished from other Groups in the Spissipes Section by characters of the adults and male genitalia, including the presence of a hyaline, triangular expansion near the middle of the ventral side of the gonostylus. Sallum et al. (1997) reported that, although *Cx. gnomatos* does not show this character state, it can be recognized as a member of the Vomerifer Group by possessing all of the other features. Our results suggest that the absence of this state character represents a character reversal for *Cx. gnomatos.*

The Pedroi-Group clade (96% bootstrap support) was comprised of two well-supported major sister groups, one including *Cx. ribeirensis* and *Cx. adamesi* (98% bootstrap), and one comprised of two clades containing *Cx. pedroi* sequences from five localities in

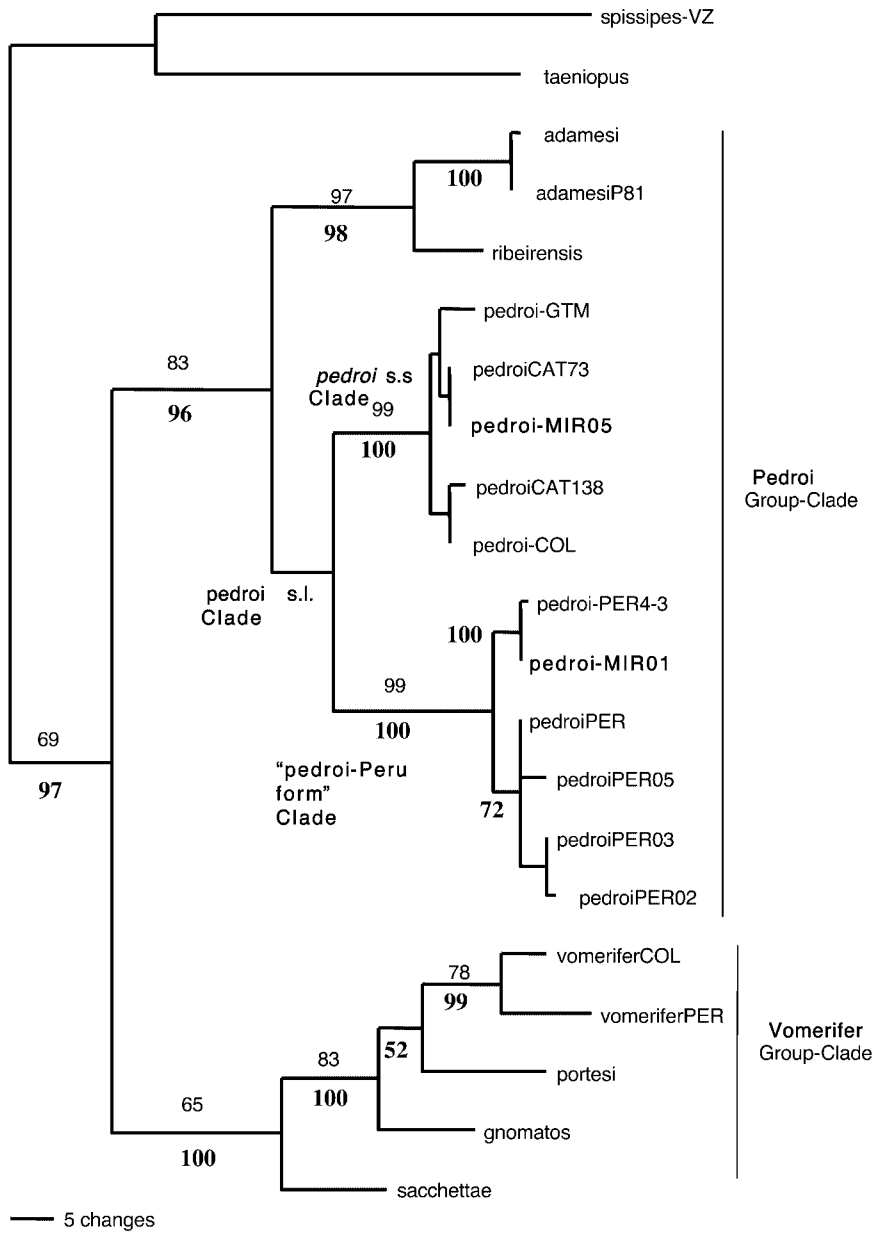

Fig. 1. The single most parsimonious tree showing the phylogenetic relationships of the Vomerifer and Pedroi Groups. There were two internal clades (*Cx. spissipes* and *Cx. taeniopus* as outgroups); the names of the clades are shown on the right side with bars. The derived clades in the Pedroi Group are shown at the base of the branches. Bootstrap values (%) indicate support levels for clades (top, numbers using gaps as missing data; bottom, bold numbers using gaps as a fifth character state). The sequences generated from the two sympatric, cryptic *Cx. pedroi* forms (from Miranda State) are printed in bold.

Guatemala, Colombia, Venezuela, and Peru (83% bootstrap). This result supports the monophyly of the Pedroi Group (including *Cx. crybda* and *Cx. epanastasis*, not included in this study). Additionally, the two well-supported *Cx. pedroi* clades (100% each bootstrap) showed genetic divergence levels greater than those of any other geographic populations of other members of the Spissipes Sections examined (8–9%), suggesting that they may be two separate species.

***Culex pedroi sensu lato* Clade.** *Culex pedroi* sequences grouped into two distinct well-supported clades (99% bootstrap values). The genetic distance among sequences clustered varied from 1 to 3%, but between those two clades, sequence (*p*) uncorrected distance varied from 8.2 to 9%. This result suggested that *Cx. pedroi* may be comprised of two cryptic species: *Cx. pedroi* s.s occurs in Venezuela, Colombia, and Guatemala (the morphological characters fit with the

original description of Sirivanakarn and Belkin), and a new, unnamed form that occurs in Peru and Venezuela (*Cx. pedroi*-Peru form). Moreover, both (*Cx. pedroi* s.s. and *Cx. pedroi*-Peru form) occur sympatrically in Miranda State, Venezuela (Fig. 1). The two forms of *Cx. pedroi* exhibited greater sequence divergence (8–9%) that that of geographic populations of any other species examined; e.g., *Cx. vomerifer* from Peru and Colombia, exhibiting the greatest variation excluding *Cx. pedroi*, differed by only 5% in uncorrected distance.

Diagnostic Morphological Characters of *Cx. pedroi sensu lato* (*Cx. pedroi* s.s. Sirivanakarn and Belkin 1980 and "*Cx. pedroi*-Peru form." By analyzing our voucher specimens, we confirmed a larger size of the *Cx. pedroi*-Peru form specimens, which was overlooked previously. Other constant differences were also observed, including chaetotaxy on the mesokatepisternum and mesepimeron and length of the proboscis, abdomen, and wings (see below). Nevertheless, other immature and adult genitalia characters should be examined after identifying larval habitats and collecting adult males. Specimens collected in the same localities from Iquitos, Peru, were reported as nominal *Cx. pedroi* by Pecor et al. (2000); however, these specimens probably belong to the "*Cx. pedroi*-Peru form" taxon, because there are no other specimens with white-banded tarsomeres on their checklist.

The female of the "*pedroi*-Peru type" species is very similar to *Cx. pedroi* s.s. in most of the external morphological characters compared with Sirivanakarn and Belkin (1980) and Sallum and Forattini (1996). However, we identified several differences. Our "voucher" specimens of "*Cx. pedroi*-Peru form" are larger than our *Cx. pedroi* s.s. from Venezuela, Colombia, and Guatemala, and here we report some comparative morphometric data from examination of >10 specimens of each population.

1. The "*Cx. pedroi*-Peru form" abdomen length averages 2.3 ± 0.14 mm (range, 2.27–2.50 mm), whereas our *Cx. pedroi* s. s. specimens (Venezuela, Colombia, and Guatemala) averaged 2.2 ± 0.16 mm (range, 1.88–2.22 mm), and the original description (Sirivanakarn and Belkin 1980) reports 2.2 mm.
2. The "*Cx. pedroi*-Peru form" wing length averages 3.3 ± 0.21 mm (range, 3.33–3.66 mm) versus 3.16 ± 0.22 mm for our *Cx. pedroi* s. s (range, 2.77–3.33 mm), 3.2 mm reported by Sirivanakarn and Belkin (1980), and 3.08 mm reported by Sallum and Forattini (1996).
3. The "*Cx. pedroi*-Peru form" proboscis length averages 1.9 ± 0.14 mm (range, 1.72–2.11 mm) versus 1.86 ± 0.09 mm for our *Cx. pedroi s.s.* (range, 1.66–1.88 mm), 1.8 mm reported by Sirivanakarn and Belkin (1980), and 1.76 mm reported by Sallum and Forattini (1996).
4. The "*Cx. pedroi*-Peru form" thorax exhibits a U-shaped pale cream-white band in the mid portion of mesepimeron, which covers 40% of the sclerite area (0.25–0.18 mm wide from upper to lower border, and a total width of the mesepimeron ranging from 0.57 to 0.64 mm). Morphologically, this band occurs in *Cx. pedroi* s.s, but it covers only 20% of mesepimeron area (the band is 0.14–0.11 mm wide, and the sclerite is 0.57–0.55 mm long).
5. The "*Cx. pedroi*-Peru form" type exhibits 21–25 posterior mesokatepisternal setae from the lower limit (inferior portion of the mesomeron or midcoxa) to the upper limit (prealar area). The *Cx. pedroi* s.s. specimens exhibit 11–18 of these setae, covering the same portion but more scattered.
6. The diagnostic white band (cream-white) was reported by Sallum and Forattini (1996) for *Cx. pedroi* as follows: "pleural integument dark brown to black, slightly lighter on median portion of mesepimeron," and the authors also point out ". . . 9–13 upper mesokatepisternal, 8–13 lower mesokatepisternal (setae). . ." The white band (cream-white) and the mesokatepisternal setae numbers reported by Sallum and Forattini (1996) agree partially with our *Cx. pedroi* s.s specimens. However, Sirivanakarn and Belkin (1980) reported ". . . posterior margin of stp (sternopleura = mesokatepisternum) with a characteristic even row of at least 20 dark setae extending from the upper corner along lower posterior border to level of midcoxa." This suggests that these previous studies may have analyzed a mixture of both pedroi species (Peru form and s.s.). Because we have not collected males of the "*Cx. pedroi*-Peru form" species in Iquitos, it was not possible to confirm if this species is conspecific with *Cx. epanastasis* (morphologically, the most similar species). Therefore, we suggest its provisional inclusion in the key of Sallum and Forattini (1996), with the addition of the above-mentioned diagnostic characters to step 7, as follows.
7. Male: palpomeres 2–4 entirely dark, palpomere 5 with small patch of white scales on base of dorsal surface—*pedroi*.
   Male: palpomeres 2–5 with distinct ring of white scales at base—*epanastasis*.
   Female: with a small white-cream band on the mid-portion of msp, diagonal shape and covering around 20% of the sclerite area. Among 11–18 posterior mesokatepisternal setae—*pedroi*.
   Female: with a wide white-cream band on the mid-portion of msp, with a U shape and covering 40% of the sclerite area. Among 21–25 posterior mesokatepisternal seta—*pedroi*-Peru form.

Discussion

The confused taxonomic history of the subgenus *Melanoconion* and the Spissipes Section demonstrates the complexity of the group and the difficulty in reaching a natural (evolutionary) and reliable classification using traditional morphological methods. Several *Cx. taeniopus*–like species (specimens with white ringed tarsomeres or identical male genitalia) were confused until the currently valid species *Cx. taeniopus* and *Cx.*

cedecei were resurrected from *Cx. opisthopus* and *Cx. annulipes* (Belkin 1969, Galindo 1969). Later, *Cx. pedroi* n. sp. was described from misidentified *Cx. taeniopus* specimens from Central America (Sirivanakarn and Belkin 1980). The geographical distribution of *Cx. pedroi* remains unclear, including questionable overlap with *Cx. taeniopus.* Recently, Forattini and Sallum (1992) detected *Cx. taeniopus* specimens with cibarial armature differences from *Cx. taeniopus* s.s. and later described *Cx. akritos* and *Cx. ikelos,* which belong to the Taeniopus Group.

Our phylogenetic hypothesis showed that the Vomerifer (*Cx. vomerifer*, *Cx. portesi*, *Cx. gnomatos*, and *Cx. sacchettae*) and Pedroi (*Cx. pedroi* s.s., *Cx. ribeirensis*, *Cx. adamesi*, and *Cx. pedroi*-Peru form species) Groups are monophyletic sister groups that are supported by 65 and 83% bootstrap values, respectively. This result supports the classifications of Sirivanakarn (1983) and Sallum and Forattini (1996), including the recently named species *Cx. gnomatos* (Sallum et al. 1997), based on morphological characters. However, the monophyly of the Crybda Group, as well as the taxonomic status of the Pedroi clade, remains to be clarified by the addition of other related species.

We present here phylogenetic evidence that a cryptic *pedroi*-like species may occur in Venezuela and Peru. The lack of specimens representing some stages of closely related *Cx. pedroi*–like species (e.g., adult females of *Cx. epanastasis* and *Cx. paracrybda*) has resulted in poor scrutiny of the use of the white ringed tarsomere character. We believe that the *Cx. pedroi*-Peru form detected by ITS-2 sequences could be an unknown or new species in the Spissipes Section, based on the greater interpopulation sequence divergence than exhibited for any other species and the sympatry of the two forms in Miranda State, Venezuela. Additional analysis of *Cx. epanastasis* DNA and of interpopulation divergence levels for ITS-2 in *Cx. pedroi* are need to further evaluate the possibility of a cryptic species of *Cx. pedroi* s.l. and to evaluate the use of ITS-2 in delineating species in the *Culex* (*Melanoconion*) subgenus. Additional mosquito genes and morphological characters should also be sought and evaluated, including characters from males and larvae, which were not available for our study.

Our phylogenetic results may explain both the poor laboratory vector competence of the Peruvian *pedroi*-like population for VEEV reported by Turell et al. (2000) and the natural transmission of VEEV by *Cx. pedroi* in Colombia (Ferro et al. 2003). Although geographic populations of a species may vary in susceptibility to arboviruses, the *Cx. pedroi* populations examined in these two studies may belong to the different species of *Cx. pedroi* s.l., which could have important epidemiological implications.

Acknowledgments

This research was funded by the NIH Fogarty International Center grant R03TW415640 to SCW, the National Institutes of Health Grants AI39800, AI49725, and AI48807, The National Aeronautics and Space Agency (NASA), the John D. and Catherine MacArthur Foundation, the Consejo de Desarrollo Cientifico y Humanistico-UCV, and Fondo Nacional de Ciencia y Tecnologia (FONACIT-S1–2001000921) to J.C.N. We thank M. A. Sallum, O. P. Forattini, I. Kakitani, and R. La Corte for the Brazilian specimens and helpful assistance from the Museum (NUPTEM-Sao Paulo) and R. Tesh (UTMB, Galveston). We thank the following individuals for technical assistance and specimens: W. Kang, K. Alwell-Warda, A. Brault, and W. Sweeney (UTMB, Galveston); H. Ateste, R. Fernandez, and D. Watts from the Naval Medical Research Center Detachment in Lima, Peru; J. Arrivillaga (BIOMED-Univ. Carabobo/UTMB); R. Barrera, J. Liria, and W. Mendez (Universidad Central de Venezuela; Division of Malariologia, Zulia State, Venezuela); P. Sanchez (Padrón Agriculture Station-INIA); and M. C. Ferro (Instituto Nacional de Salud, Bogota, Colombia).

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Received 26 February 2003; accepted 18 February 2004.