MORPHOLOGY, SYSTEMATICS, EVOLUTION

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

JUAN-CARLOS NAVARRO1, 2 AND SCOTT C. WEAVER2


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) (Karakatsos 1985, Johnston and Peters 1996, Weaver 1997, 1998, Ferro et al. 2003, Weaver 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

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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), Anopheles (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 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°00'44.5" 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 Saúde Publica-NUTEM, 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 Biología 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 Biología 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 São Paulo-NUTEM).

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.

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

<table>
<thead>
<tr>
<th>Taxon*</th>
<th>Country</th>
<th>Locality</th>
<th>Group/subgroupb</th>
<th>Sourcec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx. spissipes-VZ</td>
<td>Venezuela</td>
<td>Catatumbo</td>
<td>Spissipes/none</td>
<td>Authors</td>
</tr>
<tr>
<td>Cx. taeniopus</td>
<td>Guatemala</td>
<td>Puerto Barrios</td>
<td>Taeniopus/none</td>
<td>Authors</td>
</tr>
<tr>
<td>Cx. adamesi P81</td>
<td>Peru</td>
<td>Iquitos</td>
<td>Crybda/Pedroi</td>
<td>R. Fernandez</td>
</tr>
<tr>
<td>Cx. ribeirensis</td>
<td>Brazil</td>
<td>Pariquera-Açu</td>
<td>Crybda/Pedroi</td>
<td>R. Fernandez</td>
</tr>
<tr>
<td>Cx. pedroi s.s. (CAT73, 138)</td>
<td>Venezuela</td>
<td>Catatumbo</td>
<td>Crybda/Pedroi</td>
<td>Authors</td>
</tr>
<tr>
<td>Cx. pedroi s.s. (MIR-05)</td>
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<td>Miranda-Barlovento</td>
<td>Crybda/Pedroi</td>
<td>Authors</td>
</tr>
<tr>
<td>Cx. pedroi s.s. (COL)</td>
<td>Colombia</td>
<td>Puerto Boyaca</td>
<td>Crybda/Pedroi</td>
<td>C. Ferro</td>
</tr>
<tr>
<td>Cx. pedroi s.s. (GTM)</td>
<td>Guatemala</td>
<td>Izabal Lake</td>
<td>Crybda/Pedroi</td>
<td>Authors</td>
</tr>
<tr>
<td>Cx. pedroi-Peru form (PER4-3, PER05, PER03, PER02)d</td>
<td>Venezuela</td>
<td>Miranda-Barlovento</td>
<td>Crybda/Pedroi</td>
<td>Authors</td>
</tr>
<tr>
<td>Cx. pedroi-Peru form (MRR01)</td>
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<td>Puerto Boyaca</td>
<td>Vomerifer/none</td>
<td>C. Ferro</td>
</tr>
<tr>
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<td>Peru</td>
<td>Iquitos</td>
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<td>Brazil-Pariquera-Açu</td>
<td>Vomerifer/none</td>
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<td>Cx. sacchettae</td>
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<td>Brazil-Pariquera-Açu</td>
<td>Vomerifer/none</td>
<td>M.A. Sallum/Authors</td>
</tr>
</tbody>
</table>

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

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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’-GCCGCGGTACCATGCTTAAATTTAGGGGGTA-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’-TGAAGCCGAGCTATGCTTAAATTTAGGGGGTA-3’). Each 25-μl amplification reaction contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl2, 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 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 = 0.67 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. sacchettiae 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. pedroii sequences from five localities in...
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

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**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.
original description of Sirivanakarn and Belkin), and a new, unnamed form that occurs in Peru and Venezuela (Cx. pedroi-Peuru form). Moreover, both (Cx. pedroi s.s. and Cx. pedroi-Peuru 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. comifer 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-Peuru form.” By analyzing our voucher specimens, we confirmed a larger size of the Cx. pedroi-Peuru 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-Peuru form” taxon, because there are no other specimens with white-banded tarsomeres on their check-list.

The female of the “pedroi-Peuru 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-Peuru form” are larger than our Cx. pedroi s.s. specimens. 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-Peuru 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-Peuru 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-Peuru form” proboscis length averages 1.9 ± 0.14 mm (range, 1.73–2.11 mm) versus 1.86 ± 0.09 mm for our Cx. pedroi s.s. (range, 1.66–1.88 mm), 1.85 mm reported by Sirivanakarn and Belkin (1980), and 1.76 mm reported by Sallum and Forattini (1996).

4. The “Cx. pedroi-Peuru 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-Peuru 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 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 (Peuru form and s.s.). Because we have not collected males of the “Cx. pedroi-Peuru 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 setae—pedroi-Peuru form.

Discussion

The confused taxonomic history of the subgenus Melanocomion 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.
culex were resurrected from Cx. opisthopus and Cx.
annulipes (Belkin 1969, Galindo 1969). Later, Cx.
pedroi n. sp. was described from misidentified Cx. taenio-
opus specimens from Central America (Sirivanakarn and Belkin 1980). The geographical distribution of Cx.
pedroi remains unclear, including questionable over-
lap with Cx. taenioopus. Recently, Forattini and Sallum
(1992) detected Cx. taenioopus specimens with cibarial
armature differences from Cx. taenioopus s.s. and later
described Cx. akritos and Cx. ikelos, which belong to
the Taeniopus Group.

Our phylogenetic hypothesis showed that the Vomerifer (Cx. comerifer, Cx. portesi, Cx. gnomatos, and Cx. sacchettae) and Pedroi (Cx. pedroi s.s., Cx. ribeirensis, Cx. adamesi, and Cx. pedroi-Peuru form spe-
cies) Groups are monophyletic sister groups that are
supported by 65 and 83% bootstrap values, respect-
ively. 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 charac-
ters. However, the monophyly of the Cxybra 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 cryp-
tic 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 diver-
gence than exhibited for any other species and the
sympatry of the two forms in Miranda State, Venezu-
ela. 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 (Melano-
conion) 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 geo-
graphic populations of a species may vary in suscept-
tibility to arboviruses, the Cx. pedroi populations ex-
amined in these two studies may belong to the dif-
ferent species of Cx. pedroi s.l., which could have
important epidemiological implications.

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