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Differentiation and species status of the Neotropical yellow-eared bats *Vampyressa pusilla* and *V. thyone* (Phyllostomidae) with a molecular phylogeny and review of the genus

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A systematic re-evaluation of *Vampyressa pusilla* warrants the elevation of *V. p. thyone* from subspecies to species rank based on its distinction from the allopatric *V. p. pusilla*. Morphological, mensural, chromosomal, and mitochondrial differences define each of these two taxa as divergent lineages. *Vampyressa pusilla* is endemic to the Atlantic Forest of southeastern South America and *V. thyone* is found allopatrically in northwestern South America, Central America, and southern Mexico. A molecular phylogenetic analysis of the mtDNA ND3–4 gene region using restriction endonuclease cut sites resulted in a monophyletic, although weakly supported *Vampyressa* ingroup with *Chiroderma*, and a clade of *Mesophylla* and *Ectophylla* as successive basal outgroup lineages. The phylogeny within *Vampyressa*, with the exception of *V. melissa* which is most similar to *V. thyone* based on karyotypes and morphology, had a topology of ((*pusilla* + *thyone*) + ((*brocki* + *nymphaea*) + *bidens*))).

Key words: *Chiroderma*, *Ectophylla*, *Mesophylla*, morphology, Phyllostomidae, restriction site mapping, *Vampyressa*, *Vampyressa pusilla*, *Vampyressa thyone*

INTRODUCTION

There are several species of bats that are widely distributed in the Neotropical lowlands from Central to South America but have a peculiar disjunction between southeastern Atlantic Forest and northwestern Amazonian Forest populations (e.g., *Molossops neglectus* — R. Gregorin *et. al*., In press). These two corresponding forested regions are broadly separated by distinctly drier open habitats of the Caatinga in eastern Brazil, the dry grasslands of the Cerrado in central Brazil, the dry Chaco forest in northwestern Paraguay and Bolivia, and the wetlands of the Pantanal bordering southwestern Brazil, Bolivia, and Paraguay. *Vampyressa pusilla* exhibits this pattern (Lewis and Wilson, 1987), although the distribution of this species (Fig. 1) is poorly understood given the recent discovery of *V. p. thyone* in western Amazonian Brazil (Nogueira *et al*., 1999) and Guyana (Lim and Engstrom, 2001). Extensive comparisons throughout its range are lacking and the holotype from southeastern Brazil is

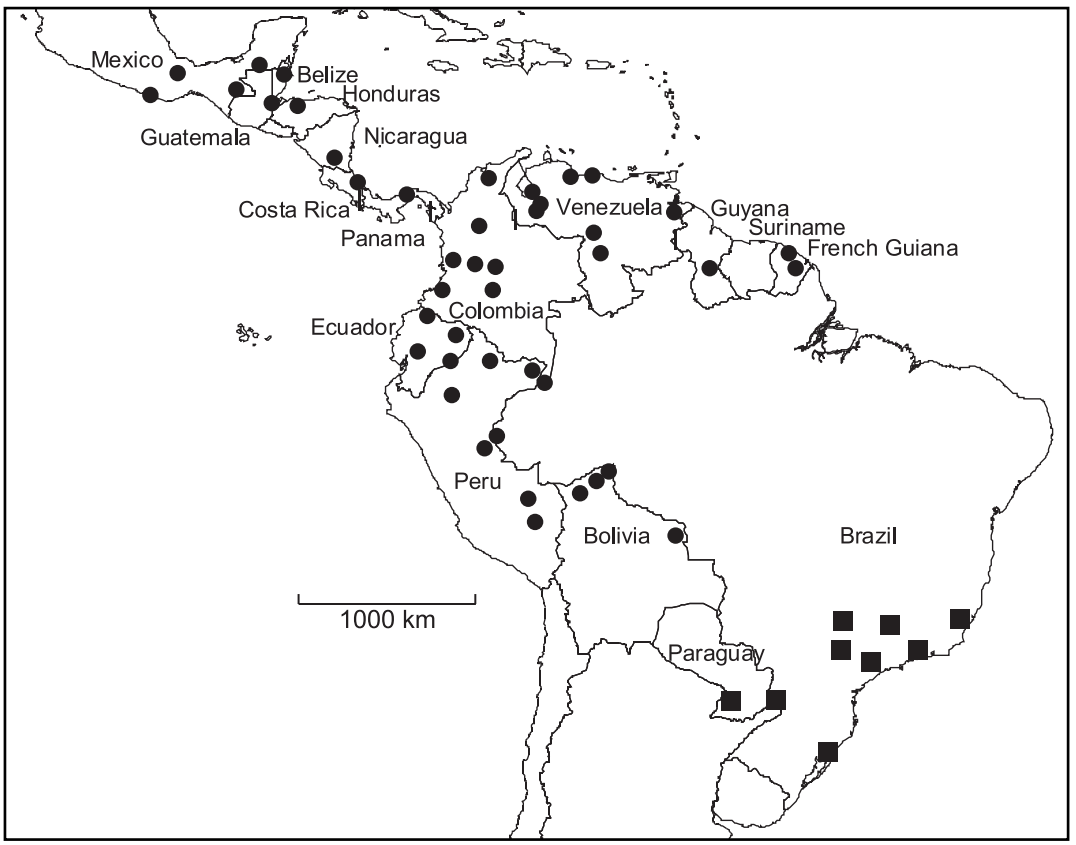

FIG. 1. Distribution map of *Vampyressa thyone* (circles) and *V. pusilla* (squares) constructed from the literature references and specimens examined in the Species Accounts section. Notice the allopatric distribution between the two species with separation of approximately 1,200 km centred around the dry Chaco and wet Pantanal of Boliva, Paraguay, and Brazil

a mounted specimen with the skull enclosed within the skin, which has hindered taxonomic evaluation. In addition, *V. p. pusilla* is also not well represented in collections with only a few specimens from several scattered localities in southeastern South America.

A series of eight specimens of *V. p. pusilla* was collected from a single locality in southern Brazil as part of a larger project on surveying bat species diversity and frugivore-plant interactions. This has afforded us the opportunity to compare and review the taxonomic and distributional status of *V. p. pusilla* and its distinction from *V. p. thyone*. In addition to morphological comparison, we also present a preliminary molecular phylogeny for the genus *Vampyressa* (with the exception of *V. melissa*) and comment on higher level relationships with *Chiroderma*, *Mesophylla,* and *Ectophylla.*

MATERIALS AND METHODS

A representative voucher collection of bats was made at Caetetus Ecological Station (22°03'S, 49°40'W, 700 m a.s.l.), São Paulo State, Brazil from 17 to 29 July 1998 during the dry season. Caetetus is a fragmented 2,178 hectare refugium of interior Atlantic Forest surrounded primarily by coffee plantations. The habitat is broadleaf semideciduous forest with areas of primary, secondary, and swamp forest. This collection will serve as comparative material for

species richness and abundance in studies on frugivore and plant interactions. Specimens will be deposited at the Universidade Estadual Paulista in Araçatuba, Brazil and the Royal Ontario Museum in Toronto, Canada. Field numbers (F) are used as a cross-reference in the text until the collection is officially distributed between these two institutions.

Thirty-five specimens representing *V. p. thyone* from northwestern South America and Central America, and 13 specimens representing *V. p. pusilla* from southeastern Brazil and Paraguay were examined for morphological and mensural study (see Species Accounts). Museum acronyms are: BMNH, British Museum of Natural History, London; MECN, Museo Ecuatoriano de Ciencias Naturales, Quito; NP, personal field number of Wagner A. Pedro, Araçatuba; ROM, Royal Ontario Museum, Toronto; UMMZ, University of Michigan, Museum of Zoology, Ann Arbor; and ZSM, Zoologische Sammlung des Bayerischen Staates, Munich. Forearm and cranial measurements similar to those reported in Goodwin (1963) were recorded as follows: forearm length; greatest length of skull; zygomatic breadth; interorbital breadth; mastoid breadth; greatest width across the upper molars; maxillary toothrow length; and mandibular toothrow length.

For molecular analysis, 17 tissue samples were used representing specimens of *V. p. pusilla*, *V. p. thyone*, *V. bidens, V. brocki,* and *V. nymphaea* with *Chiroderma trinitatum, C. villosum, Mesophylla macconnelli,* and *Ectophylla alba* employed as outgroups (see Species Accounts and Appendix I). An approximately 2,400 basepair mitochondrial (mt) DNA gene region composed of ND3, transfer (t) RNA for arginine, ND4L, and ND4 as defined with primers LGL 772 and 773 (LGL Ecological Genetics, Inc., Bryan, Texas) was used in a partial endonuclease digest mapping technique as developed by Morales *et al*. (1993) and modified by Lim and Engstrom (1998). Thirteen unique restriction enzymes (AatII, AluI, BstUI, BstZ17I, DpnII, HaeIII, HhaI, HpaII, NdeI, NlaIII, PstI, RsaI, and TaqI) from New England Biolabs, Inc. (Beverly, MA) were used to indirectly survey mtDNA variation by mapping cut sites. Phylogenetic relationships were hypothesized using a branch-and-bound parsimony algorithm with characters unordered and equally weighted as implemented in PAUP* version 4.0b9 (Swofford, 2001). Additional heuristic search settings included the simple addition sequence option and polytomies were created if the maximum branch length was zero. Branch support was summarized with bootstrap and jackknife analyses with 1,000 replications each, and a decay index was calculated using a converse constraints approach.

Results

The mean values of all external and cranial measurements were larger for *V. p. pusilla* than for *V. p. thyone* (Table 1). In

Table 1. One external and seven cranial measurements for the holotype of *Vampyressa pusilla thyone* (Goodwin, 1963), *V. p. thyone* ($n = 31$), *V. p. pusilla* ($n = 10$), and the holotype of *V. p. nattereri* (Goodwin, 1963) including range, mean, and standard deviation (in parentheses) where applicable

Character	*V. p. thyone*	*V. p. thyone*	*V. p. pusilla*	*V. p. nattereri*
Forearm length	32	30–34	33–36	35
		31.5 (1.2)	34.3 (1.1)	
Greatest length of skull	19.0	17.8–18.8	19.5–20.6	20.1
		18.4 (0.3)	20.0 (0.4)	
Zygomatic breadth	11.0	10.2–11.1	11.2–12.1	12.1
		10.8 (0.2)	11.7 (0.2)	
Interorbital breadth	–	4.4–5.1	4.8–5.3	5.2
		4.7 (0.2)	5.0 (0.1)	
Mastoid breadth	9.5	8.8–9.8	9.5–9.9	10.1
		9.2 (0.2)	9.8 (0.1)	
Greatest width across upper molars	–	7.2–8.1	8.1–8.8	8.6
		7.6 (0.2)	8.4 (0.3)	
Maxillary toothrow length	6.1	5.8–6.6	6.6–7.3	7.0
		6.2 (0.2)	6.9 (0.2)	
Mandibular toothrow length	–	5.5–6.1	6.4–6.9	6.7
		5.8 (0.2)	6.6 (0.2)	

addition, three of the seven cranial measurements (greatest length of skull, zygomatic breadth, and mandibular toothrow length) showed no overlap in range between the two populations. Within each taxon, there was no noticeable sexual dimorphism with near-overlap in range for most measurements. Qualitative morphological differences are described in the Species Accounts diagnosis.

The use of 13 restriction enzymes resulted in 94 unique cut sites for the 17-specimen data set (Appendix II). The number of cut sites for each enzyme were as follows: 0 AatII, 17 AluI, 0 BstUI, 1 BstZ17I, 13 DpnII, 15 HaeIII, 5 HhaI, 3 HpaII, 1 NdeI, 14 NlaIII, 1 PstI, 12 RsaI, and 12 TaqI. Nine characters were constant, 19 were autapomorphic, and 66 were parsimony informative. Two equally parsimonious trees of length 117 steps were found with consistency index of 0.7265 and retention index of 0.8150. The topology of one of these trees (Fig. 2) had the two specimens of *V. p. pusilla* from Brazil as the sister lineage to *V. p. thyone* with samples from Panama, Ecuador, and Guyana. The other species of *Vampyressa* were in another clade with *V. brocki* sister to *V. nymphaea*, and *V. bidens* basal to this grouping. *Vampyressa* was monophyletic relative to the outgroup with *Chiroderma* (*trinitatum* and *villosum*) branching next based on three unique synapomorphies and a clade of *Mesophylla macconnelli* with *Ectophylla alba* united by two unique synapomorphies. The only difference in the two equally parsimonious trees was the arrangement among the three samples of *Mesophylla macconnelli* with a trichotomy or two samples from Ecuador being sister.

Support for the individual clades of *V. p. pusilla, V. p. thyone, V. brocki, V. nymphaea,* and *V. bidens* was high with bootstrap, jackknife, and decay values of at least 96%, 94%, and 4 respectively (Fig. 2). Likewise, support for outgroup relationships was nearly as high. In contrast, relationships among taxa of *Vampyressa* were not well supported. The sister-grouping of *V. p. pusilla* and *V. p. thyone* collapsed with a tree one step longer, and *V. brocki* with *V. nymphaea* collapsed after two steps. Bootstrap and jackknife values were also moderate ranging from 52% to 71%. The sister-relationship of *V. bidens* with the clade of *V. brocki* and *V. nymphaea* had similarly weak support.

Discussion

Historical Perspective

As outlined by Goodwin (1963), the exact provenance of five bats collected by Johann Natterer during the early 1800's in Brazil and identified as '*Phyllostoma pusillum*' has caused some taxonomic confusion within the genus *Vampyressa*. Two were collected from 'Sapeteba' with one of them becoming the holotype for *V. pusilla*, and three were collected from Ipanema with the holotype of *V. nattereri* presumably being one of these (Goodwin, 1963). Unfortunately, only the two holotypes are extant with the other three specimens lost, destroyed in fire, or discarded. The holotype of *V. pusilla* is a dry skin with the skull enclosed of a subadult male with the wings spread out. As explained by Goodwin (1963), the original description of *V. pusilla* by Wagner (1843) was brief but a more detailed account was given in Wagner (1850). An illustration was also included with the second publication but it was of another specimen, an adult female from Ipanema, which most probably was one of the missing Natterer specimens.

Because of the lack of a description or figure for the cranial and dental characters of the holotype due to the skull remaining in the skin, Peters (1866) redescribed the holotype and had illustrations commissioned,

that were published posthumously (Thomas, 1909), of an adult male from Ipanema to represent typical *V. pusilla*. Thomas (1909) subsequently used this illustration as representing *V. pusilla*, at that time the only known species in the genus, during his description of *V. nymphaea* and *V. thyone*, and later *V. venilla* (Thomas, 1924) and *V. melissa* (Thomas, 1926). While sorting through the Natterer's notes and collection, Goodwin (1963) designated the specimen illustrated in Peters (1866) as the holotype of another new species *V. nattereri*. Peterson (1968), however, in his description

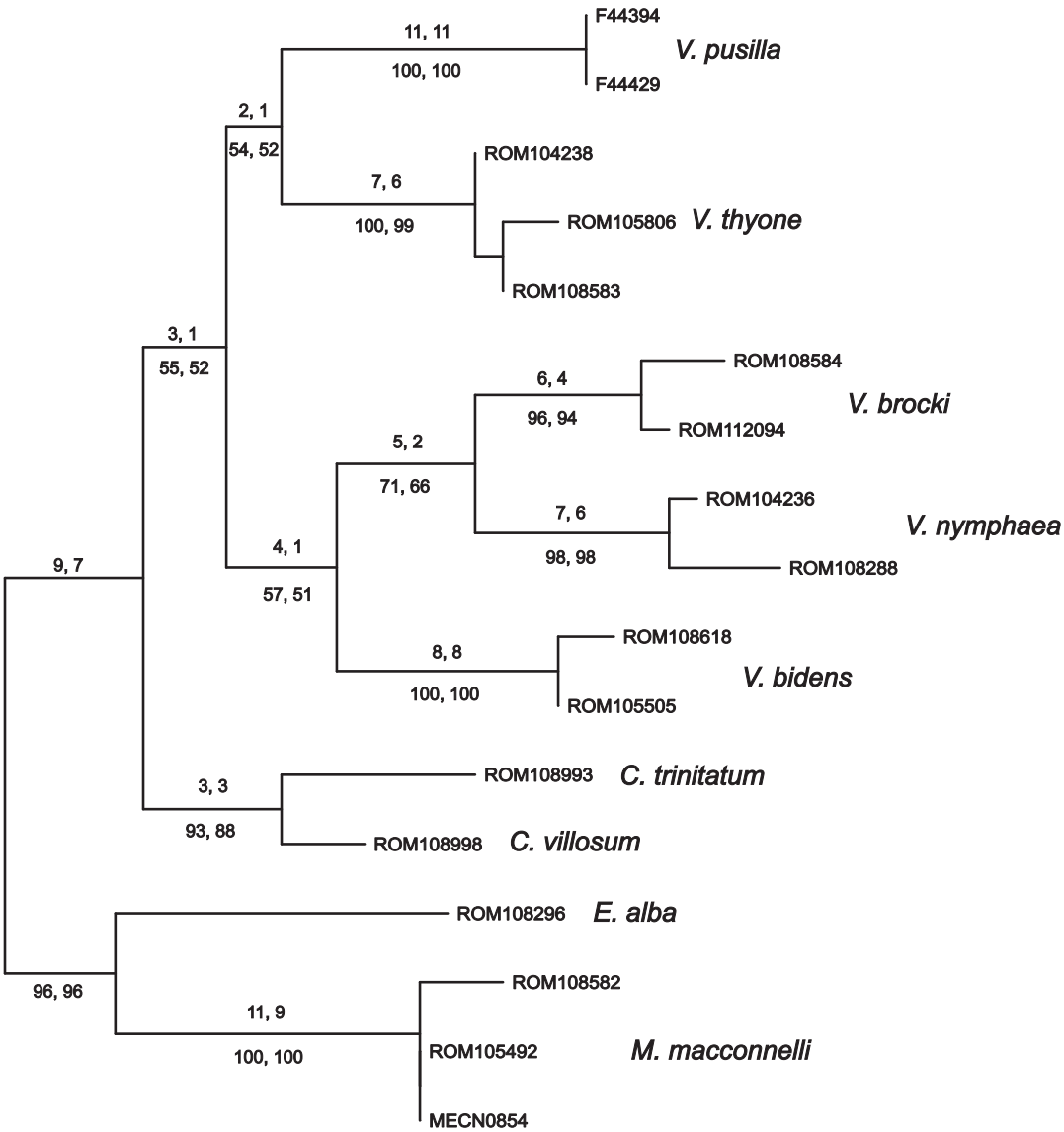

FIG. 2. Most parsimonious topology from a phylogenetic analysis of restriction sites in the ND3–4 mtDNA gene region of *Vampyressa* with *Chiroderma*, *Ectophylla*, and *Mesophylla* used as outgroups. Terminal taxa are cross-referenced in the Species Account and Appendix I. Numbers above the branches are branch lengths and decay value, and below are bootstrap and jackknife percentages from 1,000 replications. Branch lengths are also proportional to the number of character changes

of *V. brocki* considered *V. nattereri* an adult example of *V. pusilla* as Natterer had designated in his original notes, with which we concur. Curiously, Goodwin (1963: 14) stated that "the differences [Thomas] noted between *venilla* and *V. thyone* are not greater than those between subspecies of *V. pusilla*" although his only example of the latter was the subadult type specimen. This led to his designation of *venilla* and *thyone* as subspecies of *V. pusilla*. Handley (1966: 767) followed this synonymy because "the characters that are supposed to distinguish the subspecies of this species ... are age rather than geographic variables", presumably also based on the subadult holotype of *V. pusilla*. Peterson (1968: 11) concurred that "the type of *V. venilla* shows close similarity in all basic characters with *V. thyone* and is here regarded as a synonym for it", with which we agree. However, he accepted without comment Goodwin's (1963) synonymy of these two taxa with *V. pusilla* because he was not able to examine the holotype or any specimens from the Atlantic Forest.

Mensural and Morphological Differences

As first explicitly reported by Myers *et al*. (1983: 144), but without supporting data, "*Vampyressa* [*p.*] *pusilla* from Paraguay are larger than *V. p. thyone* from Central America". Our study, which included two of their five specimens from Paraguay, also found this noticeable difference in size with the mean values for the length of forearm and all seven cranial measurements larger for *V. p. pusilla* than *V. p. thyone* (Table 1). Furthermore, three of the cranial measurements did not have any overlap in range. In contrast, within *V. p. thyone* from northwestern South America (including populations from Venezuela, Ecuador, and Colombia) and Central America (Panama and Nicaragua), there was no obvious geographic variation warranting subspecies designations (Baker *et al*., 1973). They found no significant differences between these populations (n = 62) based on one external and eight cranial measurements. In addition, there was no sexual dimorphism reported in a population of *V. bidens* from Peru (Davis, 1975). The number of museum voucher specimens for *V. p. pusilla*, however, are not as numerous as for *V. p. thyone*. Other than the 10 specimens measured herein, which also included three individuals reported from southern São Paulo State (Pedro *et al*., 1997), we are only aware of published measurements for two other specimens (an adult and subadult) of *V. p. pusilla.* These two specimens are from northwestern São Paulo State (Taddei, 1979) and compared favourably with our *V. p. pusilla*, and exhibited no noticeable variation. Therefore, we concluded that quantitative mensural data support two distinct morphologically homogeneous taxa, *V. p. pusilla* and *V. p. thyone*.

We are not aware of any explicit comparison of a series of specimens for both *V. p. pusilla* and *V. p. thyone*. In our direct comparison of qualitative morphological characters, we found several cranial and external characters that distinguished these two taxa. As outlined in the Species Account diagnosis, there are differences in the orbito-rostral region, palato-nasal region, molariform teeth, extent of body hair, and colouration on the noseleaf and ears.

Molecular and Chromosomal Differences

Distinct populations were also corroborated by the molecular data (see Fig. 2). The two specimens of *V. p. pusilla* were collected sympatrically from southeastern Brazil and were identical haplotypes, which was reflected in the high support values. In contrast, the three samples representing *V. p. thyone* were from geographically distant localities (Panama, Ecuador, and Guyana) but

had nearly as high clade support. Interestingly, although each taxon is in a strongly supported monophyletic lineage, these two putative conspecifics are weakly supported as sister groups. There are, however, two unique restriction sites (one each in AluI and TaqI) that unite these two lineages.

Chromosomal data have also alluded to the divergence of *V. p. pusilla* and *V. p. thyone*. There has been one karyotype (diploid number, $2n = 20$ and fundamental number, FN = 36) reported for *V. p. pusilla* from Paraguay (Myers *et al*., 1983), which was distinct from the three lower FN chromosomal races reported for *V. p. thyone*. The latter included karyotypes of $2n = 18$ with FN = 20 from Central America, $2n = 23$ for males and 24 for females with FN = 22 from Colombia (Baker *et al*., 1973), and $2n = 22$ for males and 23 for females with FN = 22 from Peru (Gardner, 1977). Although there is chromosomal variation within *V. p. thyone*, this is not associated with any noticeable quantitative or qualitative differences in gross morphology (Baker *et al*., 1973) as seen between *V. p. thyone* and *V. p. pusilla*. Four independent data sets (mensural, morphological, chromosomal, and mitochondrial) identify distinctly divergent lineages supporting the recognition of *V. thyone* and *V. pusilla* as separate species.

Phylogeny of Vampyressa

Our molecular analysis within *Vampyressa* had strongly supported monophyletic lineages at the species level but weakly supported interspecific relationships (Fig. 2). This indicates that slightly slower evolving genes would be useful in corroborating this phylogeny. Nonetheless, a previous morphological study compared favourably. Peterson (1968) had placed *V. brocki* and *V. nymphaea* into a new subgenus *Metavampyressa* based on the combination of four lower incisors and the presence of a dorsal stripe. These two species were closely allied to *V. bidens*, which was the only representative he put in the subgenus *Vampyriscus*. This relationship is identical to the topology derived from our molecular analysis (Fig. 2). However, based on individual variation in the number of lower incisors in a population of *V. bidens* from Peru, Davis (1975) did not think *Metavampyressa* merited subgeneric status and included all three species in the subgenus *Vampyriscus*.

Unfortunately, we were unable to consistently amplify a tissue sample of *V. melissa* from Peru provided by V. Pacheco and test the hypothesis that this larger species belongs in another subgenus *Vampyressa* with the smaller *V. pusilla* and *V. thyone*. They were grouped together, however, based on similarly modified molars and the absence of a dorsal stripe (Peterson, 1968). Chromosomally, *V. melissa* has a low FN karyotype ($2n = 14$; FN = 24) similar to the chromosomal races of *V. thyone* (Gardner, 1977), but distinctly different from the $2n = 20$ and FN = 36 karyotype of *V. pusilla* (Myers *et al*., 1983).

Higher-level relationships

There has been much debate on the monophyly of *Vampyressa* and its relationship with *Chiroderma, Mesophylla,* and *Ectophylla*. Although our molecular analysis identifies a monophyletic clade for *Vampyressa*, it is not highly supported. One possible explanation is that the ND3–4 gene region did not evolve exactly with the diversification of this group so the phylogenetic signal is not strong for recovering the phylogeny. More genes need to be surveyed for a robust molecular analysis that includes all species within *Vampyressa*. Nuclear RAG2 (Recombination-Activating Gene-2) has been sequenced as part of a larger study on the systematics of Phyllostomidae (Baker *et al*., 2000) but it included only a sampling of

taxa (*V. bidens, V. thyone, M. macconnelli, E. alba, C. villosum*) that were used in our study. Their preliminary results had low resolution and grouped *V. pusilla* with *Mesophylla*, which formed a polytomy including *V. bidens, C. villosum, Uroderma,* and a clade composed of *Platyrrhinus* and *Vampyrodes*. *Ectophylla* was in a divergent sister lineage.

Another recent study on phyllostomid phylogeny used a total evidence approach, which incorporated primarily morphological data sets (Wetterer *et al*., 2000). Their results suggested that "*Vampyressa* is not monophyletic because *V. pusilla, V. nymphaea,* and *V. bidens* are successive sister taxa to *Ectophylla* and *Mesophylla*" (Wetterer *et al*., 2000: 142), with *Chiroderma* the basal lineage. Although they identified a need for inclusion of all recognized species of *Vampyressa* and cautioned against nomenclatural changes until a more comprehensive study was done, the successive basal branching of *Vampyressa* species was a novel topology. The node uniting *V. nymphaea* with subsequent terminal taxa is diagnosed by only the absence of a third lower molar. This character is, however, individually variable in *V. bidens* with 6 of 33 specimens from Peru missing a third lower molar (Davis, 1975).

Based on a consensus of combined quantitative and qualitative morphological data analysed with distance and parsimony methods, Owen (1987: 33) concluded that his resultant tree "does not support or refute *Vampyressa* as a monophyletic group. However, if this genus is in fact monophyletic, *Mesophylla macconnelli* must be included in it". His consensus topology had five unresolved lineages, three of which were composed of *V. melissa*, a trichotomy with *V. pusilla, V. brocki* and *V. bidens*, and a clade of *V. nymphaea* and *M. macconnelli*. These were all novel relationships. *Ectophylla alba* appeared in a fourth diverge lineage with *Artibeus* and *Uroderma*. The fifth lineage grouped *Chiroderma* with *Platyrrhinus*.

An alternative morphological reappraisal of stenodermatine phylogeny by Lim (1993) was at the generic level and incorporated a more traditional view of bat taxonomy with all species of *Vampyressa* character-coding the same. The most parsimonious reconstructions had *Mesophylla* and *Ectophylla* as sister-taxa, which then formed a trichotomy with *Vampyressa* and *Chiroderma*. This higher-level morphological topology did not contradict our molecular analysis (Fig. 2) where *Mesophylla* and *Ectophylla* were united as sister taxa based on two unique synapomorphies. In addition, *Chiroderma* and *Vampyressa* had three unique synapomorphies grouping them as each others closest relative. Although not coded as such at the time because of its autopomorphy at the generic level of analysis (Lim, 1993), all species of *Vampyressa* have unique faintly and unevenly bifid cusps on the inner upper incisors different from the distinctly and evenly bifid teeth of *Artibeus* and *Uroderma*. In older individuals of *Vampyressa*, the cusp distinction is obscured because of wear giving the appearance of simple pointed incisors similar to *Chiroderma, Mesophylla*, and *Ectophylla*.

Chromosomal data

Based on karyotypes, Greenbaum *et al*. (1975) concluded that the $2n = 21$ for males and 22 for females with FN = 20 karyotype (Baker and Hsu, 1970; Hsu and Benirschke, 1971; not FN = 22 as reported in Greenbaum *et al*., 1975) of *M. macconnelli* was distinctly different from *E. alba* ($2n = 30$ and FN = 56). Furthermore, the two chromosomal races ($2n = 18$ with FN = 20; $2n = 21/22$ with FN = 22) of *V. p. thyone* known at that time seemed more similar to *M. macconnelli* than to the other species of *Vampyressa*. Greenbaum *et al*. (1975) proposed

a phylogenetic tree with the high diploid and fundamental number karyotype of *Ectophylla* as the basal lineage followed by a successivly derived karyotype for *Chiroderma* and then a clade with a paraphyletic *Vampyressa* and *Mesophylla*. In contrast, Gardner (1977) proposed independent chromosomal rearrangements, within a presumed monophyletic *Vampyressa,* for each species or unique karyotype from a high diploid (26) and low fundamental number (24) hypothetical ancestral karyotype. He ascribed the chromosomal similarities of *V. p. thyone* and *Mesophylla* as karyotypic convergence as also seen in the sex chromosome systems in the divergent genera of *Choeroniscus, Carollia*, and *Artibeus* (Gardner, 1977).

Assuming our phylogeny (Fig. 2) is correct, and fully bi-armed high diploid and fundamental numbers are considered ancestral (Greenbaum *et al*., 1975), karyotype data can be mapped onto the tree under the following scenario. A karyotype similar to *E. alba* (2*n* = 30 and FN = 56) would be ancestral to the genera under study. The 2*n* = 26 and FN = 48 karyotype of all species of *Chiroderma, V. bidens,* and *V. nymphaea* would be at the node uniting these two genera. A karyotype similar to *V. pusilla* (2*n* = 20 and FN = 36) would be at the node for the remaining species of *Vampyressa*. Although we did not have *V. melissa* in our analysis, this implies that this species is sister to *V. thyone* with the common ancestor still retaining a fully bi-armed 2*n* = 14 and FN = 24 karyotype. This relationship may explain the weak support uniting *V. pusilla* and *V. thyone* in our molecular analysis because they may not be each others closest relative. Within the chromosomal races of *V. thyone*, which have most chromosomes as acrocentric, other rearrangements now appear other than autosomal fusions which reduced the diploid and fundamental numbers of the previously described species. The 2*n* = 18 and FN = 20 karyotype of Central American *V. thyone* can be derived by two centric fissions and two centromeric terminalizations. The Colombian karyotype of 2*n* = 23–24 and FN = 22 is hypothesized to evolve next with two centric and one autosomal fissions with the male having an additional small unpaired autosomal fusion. The most derived karyotype under this scenario is 2*n* = 22–23 and FN = 22 from Peru which has a unique large unpaired metacentric auto-some and a Y-chromosome translocation (Gardner, 1977).

Taxonomic Considerations

Two generic taxonomic changes have recently been recommended, which we think are premature especially considering the incongruence of many of the studies. Owen (1987) considered *M. macconnelli* as congeneric with *Vampyressa* because it was the sister species to *V. nymphaea*. Based on the critique of Lim (1993), there is no phylogenetic support of this relationship nor is there restriction site or chromosomal data to suggest that they are sister taxa. Although we agree with Wetterer *et al*. (2000) that *M. macconnelli* and *E. alba* are sister taxa well supported by several morphological and molecular synapomorphies, there are still obvious differences suggesting that these species are divergent and should retain their generic ranks. Chromosomally, *E. alba* has the proposed ancestral karyotype for this group and that of *M. macconnelli* is highly derived. However, morphologically, there are several differences in the cranium and dentition between the two taxa suggesting that *E. alba* is the most modified, including "shortened rostrum, raised nasals, and exaggerated circular and flattened last lower molar" (Starrett and Casebeer, 1968). The restriction site data also identified eight autapomorphies that defined *E. alba* and five for *M. macconnelli.* The karyological,

morphological, and molecular differences are substantial for retaining the traditional generic distinctions for *E. alba* and *M. macconnelli*.

Conclusions

Because of the conflicting topologies from the different data sets and analyses, much more work is required to confidently hypothesize the phylogeny of *Vampyressa, Mesophylla, Ectophylla,* and *Chiroderma* within the larger context of phyllostomid systematics. One emphasis for future research should be on complete species-level relationships within genera or genera-complexes. There are a growing number of examples suggesting that traditionally recognized genera are paraphyletic (e.g., Wetterer *et al*., 2000; Lee *et al*., 2002) so taxonomic sampling within genera is much more important at higher levels especially for speciose families such as Phyllostomidae. There is, however, strong evidence from quantitative mensural data, qualitative morphological characters, chromosomal differences, and mitochondrial synapomorphies to recognize *V. pusilla* and *V. thyone* as distinctly mensural species. They are also allopatrically distributed and as presently known their ranges are separated by the Chaco and Pantanal at a distance of about 1,200 km (Fig. 1).

Species Accounts

Vampyressa pusilla (Wagner, 1843)

Phyllostoma pusillum Wagner, 1843
Chiroderma pusillum Peters, 1866
Stenoderma (*Chiroderma*) *pusillum* Pelzeln, 1883
Vampyrops pusillus Thomas, 1889
Vampyrops (*Vampyressa*) *pusillus* Thomas, 1900
Vampyressa pusilla Miller, 1907
Vampyressa pusilla pusilla Goodwin, 1963
Vampyressa nattereri Goodwin, 1963
Vampyressa (*Vampyressa*) *pusilla pusilla* Peterson, 1968

Holotype — ZSM 184312, subadult ♂, skin with skull enclosed and wings spread.

Type locality — Brazil: Rio de Janeiro; Sapitiba.

Distribution — Known from the Atlantic Forest in southeastern Brazil from Minas Gerais, Rio de Janeiro, São Paulo, Rio Grande do Sul (Taddei, 1979), and Espírito Santo (Zortéa and Alves de Brito, 2000), southern Paraguay in Paraguarí (Myers *et al*., 1983), and extreme northeastern Argentina from Misiones (Barques *et al*., 1993).

Specimens examined (* indicates specimens used in the molecular analysis) — Brazil: São Paulo; Caetetus Ecological Station, 22°23'S, 49°40'W, 700 m, 3 ♀♀, 5 ♂♂ (F44370, F44371, F44375, F44382, F44386, F44394*, F44429*, F44445). Between Pariquera-Açú and Registro, 1 ♀, 2 ♂♂ (NP 0001, 0002, 0003). Paraguay: Paraguarí; Parque Nacional Ybycuí, 1 ♀, 1 ♂ (UMMZ 133730, 103731).

Emended diagnosis — Compared with *V. thyone, V. pusilla* has a larger, more robust skull and dentition. The postorbital processes are more developed in *V. pusilla* resulting in a broader appearance to the rostrum. The posterior cusp of the last upper premolar is more developed in *V. pusilla* giving it an elongated shape. The upper molars are also broader than those in *V. thyone*. In the nasal region, *V. pusilla* has a thicker anterior medial palatal extension with a central and two lateral foraminas anteriorly. This is often obscured if the cranium is not thoroughly cleaned. The palatal openings in this area are also not constricted posterolaterally. Externally, *V. pusilla* is a slightly larger bat with the length of forearm averaging longer than *V. thyone* (Table 1). *Vampyressa pusilla* is hairier with relatively more fur on the forearms and legs. In addition, the dorsal fur is longer and laxer with hairs extending noticeably beyond the interfemoral membrane. The noseleaf of *V. pusilla* is uniformly brown and the edging on the ears is faintly paler.

Measurements — All seven cranial measurements average larger in *V. pusilla* than in *V. thyone* (see Table 1). There was no overlap in range for greatest length of skull, zygomatic breadth, and mandibular toothrow length.

Vampyressa thyone Thomas, 1909

Vampyressa thyone Thomas, 1909
Vampyressa minuta Miller, 1912
Vampyressa venilla Thomas, 1924
Vampyressa thyone Hershkovitz, 1949
Vampyressa pusilla thyone Goodwin, 1963
Vampyressa pusilla Handley, 1966
Vampyressa (*Vampyressa*) *pusilla thyone* Peterson, 1968

Holotype — BMNH 97.11.7.77, adult ♂, skin in alcohol and skull removed.

Type locality — Ecuador: Bolívar; Chimbo, 1000 feet.

Distribution — Known from northwestern South America and Central America including northern Bolivia (Anderson, 1997); western Brazil (Nogueira *et al*., 1999); Peru (Thomas, 1924; Sanborn, 1953; Gardner, 1977; Ascorra *et al*., 1991; Woodman *et al*., 1991; Pacheco *et al*., 1993); Ecuador (Thomas, 1909; Goodwin, 1963); Colombia (Thomas, 1909; Hershkovitz, 1949; Baker *et al*., 1973); Venezuela (Handley, 1976; Ochoa *et al*., 1988; Ochoa, 1995); Guyana (Lim and Engstrom, 2001); French Guiana (Brosset and Charles-Dominique, 1990); Panama (Miller, 1912; Handley, 1966); Costa Rica (Goodwin, 1946; Armstrong, 1969; Gardner *et al*., 1970; Baker *et al*., 1973); Nicaragua (Starrett and de la Torre, 1964; Jones *et al*., 1971; Baker *et al*., 1973); Honduras (Valdez and LaVal, 1971; Baker *et al*., 1973); Belize (Peterson, 1965); Guatemala (Rick, 1968); and southern Mexico (Davis *et al*., 1964; Hall, 1981) as far north as Veracruz (Schaldach, 1964) and Oaxaca (Arnold and Schonewald, 1972).

Specimens examined (* indicates specimens used in the molecular analysis) — Belize: Rockstone Pond, 17°46'N, 88°22'W, 1 ♀ (ROM 33614). Colombia: Antioquia; Remedios, Finca San Martin, 07°02'N, 74°41'W, 1 ♂ (ROM 84983). Cauca; Mechenguito, 02°40'N, 77°12'W, 1 ♀ (ROM 63213). Meta; Restrepo, 04°15'N, 73°33'W, 1 ♀ (ROM 62500). Tolima; Gualanday, 04°17'N, 75°02'W, 1 ♀ (ROM 84971). Costa Rica: Limon; near Rio Barbilla, foothills Talamanca Mountains, 10°01'N, 83°23'W, 1 ♂ (ROM 94244). Ecuador: Esmeralda; 2 km S Alto Tambo, 00°54'N, 78°33'W, 1 ♂ (ROM 105806*). Napo; 37–76 km S Pompeya Sur, 00°38'S, 76°27'W to 00°50'S, 76°20'W, 13 ♀♀, 3 ♂♂ (ROM 104391, 104414, 104453, 104493, 104535–6, 104544, 105226–8, 105233, 105714–5, 105882, 105995, 106332). Guyana: Mazaruni-Cuyuni; Namai Creek, 5 km W of Pariuma, 05°48'N, 61°06'W, 1 ♂ (ROM 108145). Potaro-Siparuni; Iwokrama Forest, Cow Fly Camp, 04°22'N, 58°49'W, 1 ♀, 1 ♂ (ROM 108583*, 108622). Panama: Canal Zone, Gamboa, 09°08'N, 79°42'W, 1 ♀, 1 ♂ (ROM 78446, 91187). Chiriqui; Santa Clara, 08°52'N, 82°45'W, 2 ♂♂ (ROM 104306, 104332). Darien; La Laguna, 08°52'N 79°48'W, 1 ♀ (ROM 78447); Parque Nacional Darien, Estacion Pirre, 08°00'N, 77°43'W, 1 ♀ (ROM 104367). Panama; Parque Nacional Altos de Campana, 08°41'N, 79°56'W, 1 ♀, 2 ♂♂ (ROM 99937, 104238*, 104239).

Emended diagnosis — Compared with *V. pusilla*, *V. thyone* has a smaller, less robust skull and dentition. The postorbital processes are less developed in *V. thyone* resulting in a narrower appearance to the rostrum. The posterior cusp of the last upper premolar is less developed giving it a less elongated shape. The upper molars are also narrower than those in *V. pusilla*. In the nasal region of *V. thyone,* the anterior medial palatal extension is flattened with a single prominent anterior foraminea. The palatal

openings in this area are also constricted posterolaterally. Externally, *V. thyone* is a slightly smaller bat with the length of forearm averaging shorter than *V. pusilla* (Table 1). *Vampyressa thyone* is not as hairy with relatively less fur on the forearms and legs. In addition, the dorsal fur is shorter with hairs not extending noticeably beyond the interfemoral membrane. For the noseleaf of *V. thyone*, the outer edge is yellowish and the edging on the ears is predominately yellow.

Measurements — All seven cranial measurements average smaller in *V. thyone* than in *V. pusilla* (Table 1). There was no overlap in range for greatest length of skull, zygomatic breadth, and mandibular toothrow length.

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Appendix I

Additional specimens examined for mensural and morphological study. * — indicates specimens used in the molecular analysis. Museum acronyms are: MECN, Museo Ecuatoriano de Ciencias Naturales, Quito; and ROM, Royal Ontario Museum, Toronto.

Chiroderma trinitatum — Guyana: Potaro-Siparuni; Iwokrama Forest, Turtle Mountain, 2 ♀♀ (ROM 108993*, 108950).

Chiroderma villosum — Guyana: Potaro-Siparuni; Iwokrama Forest, Turtle Mountain, 1 ♀ (ROM 108998*).

Ectophylla alba — Costa Rica: Limon; Cano Palma Biological Station, 1 ♀ (ROM 108296*).

Mesophylla macconnelli — Ecuador: Napo; 38 km S Pompeya Sur, 2 ♂♂ (ROM 105492*, MECN 0854*). Guyana: Demerara-Mahaica; Ceiba Biological Center, 1 ♂ (ROM 113723). East Berbice-Corentyne; Mapenna River, 1 ♂ (ROM 100305). Potaro-Siparuni; Iwokrama Forest, Cow Fly Camp, 1 ♀, 1 ♂ (ROM 108582*, 108620). Upper Takutu-Upper Essequibo, Cacique Mountain, 1 ♂ (ROM 113524).

Vampyressa bidens — Colombia: Vaupes; Mitu, 1 ♀ (ROM 45277). Ecuador: Napo; 34–76 km S Pompeya Sur, 12 ♀♀, 4 ♂♂ (ROM 103994–5, 104065, 104479, 104503, 104534, 104551, 105133–4, 105505*, 105612, 105722, 105929, 105961, 106074, 106335). Guyana: Barima-Waini; Baramita, 1 ♂ (ROM 100964). Cuyuni-Mazaruni; Namai Creek, 5 km W Paruima, 2 ♀♀, 1 ♂ (ROM 108143, 108174, 108205). Potaro-Siparuni; Iwokrama Forest, Cow Fly Camp, 1 ♂ (ROM 108618); Iwokrama Forest, 25 km SSW Kurupukari, 1 ♀ (ROM 104742). Upper Demerara-Berbice; 1.6 km E Ituni, 1 ♂ (ROM 59590); 11.2 km NE Ituni, 1 ♀ (ROM 59895); Lucky Spot, 3 ♀♀ (ROM 60394, 66587, 66596). Upper Takutu-Upper Essequibo; Chodikar River, 55 km SW Gunn's Strip, 1 ♀, 1 ♂ (ROM 106592, 106616); Curary Wau, E of Shea Village, 1 ♀, 1 ♂ (ROM 37296, 37297); Kamoa River, 50

km SWW Gunn's Strip, 1 ♂ (ROM 106718); 5 km SE Surama, 1 ♂ (ROM 103043).

Vampyressa brocki — Guyana: Demerara-Mahaica; Ceiba Biological Center, 2 ♀♀ (ROM 113614, 113807). Potaro-Siparuni; Iwokrama Forest, 4 ♀♀, 2 ♂♂ (ROM 107227, 108584*, 108850, 111935, 112094*, 112095). Upper Demerara-Berbice; Arampa, 4.8 km E Ituni, 1 ♂ (ROM 59745); Wismar, 1 ♀ (ROM 68008). Upper Takutu-Upper Essequibo; Oshi Wau head near Marurawaunowa, 1 ♀ (ROM 38515, holotype).

Vampyressa nymphaea — Costa Rica: Limon; Cano Palma Biological Station, 1 ♂ (ROM 108322); Rio Barbilla, Talamanca Mountain foothills, 1 ♀, 1 ♂ (ROM 94237, 94246); Tortuga Lodge, Tortuguero, 1 ♀ (ROM 108288*). Panama: Panama; Parque Nacional Altos de Campana, 2 ♀♀ (ROM 104236*, 104270).

Appendix II

Presence (1) or absence (0) of 94 restriction cut sites in the ND3–4 mtDNA gene region for *Vampyressa* and associated outgroup taxa. Each of the following 11 restriction enzymes are listed from left to right from the 5' end and separated by a space beginning with the corresponding voucher specimen catalogue number (see Species Accounts and Appendix I): AluI, BstZ17I, DpnII, HaeIII, HhaI, HpaII, NdeI, NlaIII, PstI, RsaI, and TaqI.

F44394 — 01100100100100100 0 1111010000011 000001000101000 00110 000 0 00111010100111 0 010010000100 000110010000;

F44429 — 01100100100100100 0 1111010000011 000001000101000 00110 000 0 00111010100111 0 010010000100 000110010000;

ROM 104238 — 01000000000100100 1 1111010001001 000000010101000 00000 000 0 00011001101111 1 010000000100 001100010000;

ROM 105806 — 01000000000100010 1 1111010001001 000000010101000 00000 000 1 00011001101111 1 010000000100 001100010000;

ROM 108583 — 01000000000100100 1 1111010001001 000000010101000 00000 000 1 00011001101111 1 010000000100 001100010000;

ROM 108584 — 11000000010000101 0 1111100000001 101001000101000 01000 000 0 00011000100111 0 010001010000 011100000000;

ROM 108618 — 11000000100001001 0 1111010000000 000101000101001 00000 001 0 10011000100111 0 010000011100 001100000110;

ROM 105505 — 11000000100000001 0 1111010000001 000101000101001 00000 001 0 10011000100111 0 010000011100 001100000110;

ROM 104236 — 11000010000000000 1 1111000000000 100001000101000 00000 010 0 00111000100111 0 110101010100 000100000010;

ROM 108288 — 11000010000000100 1 1111000010000 101010000101000 00000 010 0 00111000100111 0 110101010100 000100000010;

ROM 112094 — 11000010010010000 0 1111100000001 101001000101000 01000 000 0 00011000100111 0 010001010000 011100000000;

ROM 108993 — 11000000000000100 1 1111010000101 000000100101100 00000 000 0 01011100110100 0 011000000111 001100100000;

ROM 108998 — 01001000000000000 1 1111010000001 000000100101100 00000 000 0 00011000100100 0 011000000110 000100000000;

ROM 108582 — 00000001110000010 0 1111001000001 010000001000010 00000 000 0 01010000100100 0 011000100100 101100101010;

ROM 105492 — 00000001110000110 0 1111001000001 010000001000000 00000 000 0 01010000100100 0 011000110100 101100101010;

MECN 0854 — 00000001110000110 0 1111001000001 010000001000000 00000 000 0 01010000100100 0 011000110100 101100101010;

ROM 108296 — 00010000001000110 0 1111100100001 010000100010000 10001 100 0 00010000100100 0 010000110100 000101000001