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Elucidating the Species Limits and Range Boundaries of the African Yellow House Bats, Genus *Scotophilus*

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Abstract
Many of Kenya's vesper bat species (Vespertilionidae) are weakly distinguished from one another, resulting in uncertainty with field and museum identification. These complexities set up Scotophilus as a case study for practical application of the Biological, Morphological, and Phylogenetic Species Concepts. Clear understanding of geographic variation is needed to apply currently available species names and, where needed, to propose new names. I analyzed variation in cranial morphology using geometric morphometric analysis and quantified pelage color variation in the African Yellow house bat genus Scotophilus to examine species limits and morphological overlap among populations. These analyses identify diagnostic characters and range boundaries for these species and clarify the application of existing names to Kenyan bats. The geometric morphometric and pelage analyses conducted here are a first step in untangling Scotophilus taxonomy, although more studies are needed to classify taxa sufficiently and accurately.

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Elucidating the Species Limits and Range Boundaries of the African Yellow House Bats, Genus *Scotophilus*

by

Brittany Schweiger

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The report of the investigation undertaken as a Senior Thesis, to carry two courses of credit in the Department of Biology

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ABSTRACT

Many of Kenya's vesper bat species (Vespertilionidae) are weakly distinguished from one another, resulting in uncertainty with field and museum identification. These complexities set up *Scotophilus* as a case study for practical application of the Biological, Morphological, and Phylogenetic Species Concepts. Clear understanding of geographic variation is needed to apply currently available species names and, where needed, to propose new names. I analyzed variation in cranial morphology using geometric morphometric analysis and quantified pelage color variation in the African Yellow house bat genus *Scotophilus* to examine species limits and morphological overlap among populations. These analyses identify diagnostic characters and range boundaries for these species and clarify the application of existing names to Kenyan bats. The geometric morphometric and pelage analyses conducted here are a first step in untangling *Scotophilus* taxonomy, although more studies are needed to classify taxa sufficiently and accurately.

DEDICATION

I dedicate this thesis to my mother, Lisa Schweiger, who has supported me through all the tears and all the triumphs.

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CHAPTER 1: A LITERATURE REVIEW OF THE GENUS, *SCOTOPHILUS*

Systematics, or the study of evolutionary relationships of organisms, is one of the oldest biological disciplines. In *On the Origin of Species*, Charles Darwin (1859) defined species as a term given to a group of individuals that closely resemble one another. This is the definition of species at its most rudimentary understanding. Taken a step further, one might add that species are groups of individuals that can interbreed and share genes. Even narrower yet, species can be described as a position in the familiar taxonomic hierarchy: Domain, Kingdom, Phylum, Family, Order, Genus, Species, where species is the most basic unit of the categories (De Queiroz 2005). The author of this hierarchy is considered to be Swedish biologist, Carolus Linnaeus, who strongly influenced the field of systematics (De Queiroz 2005). However, the definitions Darwin and Linnaeus brought forth do not answer the question that has perplexed the scientific community for years: what *is* a species?

Figure 1. Hand painted copper engraving of Schreber's specimen used to describe Vespertilio nigrita, later known as Scotophilus nigrita.

Agapow et al. (2004) considered species to be the currency of biology. Species are a fundamental unit in evolutionary biology and are important in creating the taxonomic framework upon which systematics relies (De Queiroz 2005). Distinguishing and understanding species limits are important for determining biodiversity, genetic diversity, and conservation worth (Crozier 1997), as well as the evolutionary history of organisms.

In order to delineate species from one another, scientists have created many

species concepts, some more widely accepted than others. Pre-Darwinian biologists considered species to be fixed and distinct, much like Linnaeus' view (Sokal and Crovello 1970). Taxonomists in the post-Darwinian era recognized the presence of variability in a species, although some still focus on morphological similarity (Sokal and Crovello 1970). The Morphological Species Concept dates back to Linnaeus and defines species based on their morphology, making it the simplest concept (Larson 1968). The Biological Species Concept is arguably the most widely accepted species concept, generally credited to Ernst Mayr (1942). It is an attempt to accommodate several features that post-Darwinian taxonomists consider necessary in a species concept (Sokal and Crovello 1970). Under the Biological Species Concept, species are groups of individuals that are reproductively isolated from other groups and are able to interbreed within such groups. More recently, the Phylogenetic Species Concept has been put forth (Hennig 1950), where a species is defined by its evolutionary history.

I used the complex genus of African Yellow house bat, *Scotophilus,* as a case study to pursue the scientific understanding of species concepts. During their early taxonomic history, *Scotophilus* species were classified primarily using the Biological Species Concept and Morphological Species Concept criteria. However, more recent studies have used the Phylogenetic Species Concept to determine the species limits of *Scotophilus*. Nevertheless, there are complexities within the genus that a singular species concept cannot accommodate, which set up *Scotophilus* as a case study for practical application of the species concepts and by extension, a critique of the current systematics.

The Yellow house bat genus *Scotophilus* Leach, 1821 (Vespertilionidae) is riddled with a complex and unclear taxonomy at the species level. In the past, there has been considerable variation between published attempts to delineate species relationships

(Schlitter et al. 1980). Recent molecular phylogenetic studies have helped to identify genetic differences among *Scotophilus* species that can aid in species-level distinctions (Trujillo et al. 2009; Vallo et al. 2015). Morphological variation often, though not always, accompanies genetic differences (Brooks & Bickham 2014). Moreover, it is useful to have clear descriptions of morphological traits for species, as they add a level of clarity to all biological studies that depend on accurate identifications (Papenfuss & Parham 2013). Clearer resolution of genetic and morphological differences among African *Scotophilus* would assist in morphological diagnoses and the application of existing names to the genus.

Taxonomic History

The species known today in the scientific community as *Scotophilus nigrita* was first described in 1774 by the German naturalist J. C. D. Schreber (Schreber 1774). He applied the scientific name of *Vespertilio nigrita* to a bat M. Adanson collected in Senegal (Robbins 1978). It wasn't until 1821, when the English naturalist, W. E. Leach, recognized the genus as distinct from other vespertilionoid genera and transferred parts of *Vespertilio* to *Scotophilus* (Leach 1821) (Figure 1). Leach (1821) designated the type species to be *Scotophilus kuhlii*, found throughout South Asia, but did not include any further taxa.

Subsequently, many more species in the genus *Scotophilus* have been described. Presently, 15 species of *Scotophilus* are recognized, ranging from Wallacea and Southern Asia to sub-Saharan Africa and Madagascar (Brooks and Bickham 2014; Goodman et al. 2006; Jacobs et al. 2006). There has been considerable confusion and controversy surrounding the genus, beginning as far back as 1875 (Robbins 1978). Dobson (1875)

incorrectly identified a West African *S. nigrita* specimen, which led to subsequent misidentifications in the literature. According to Robbins (1978), the incorrect application of *S. nigrita* is usually given to bats with dorsal pelage that is dull-to-greenish brown and ventral pelage that is light yellow to yellow-orange, although these should be considered *Scotophilus dinganii*, according to the description of Smith (1833). Smith (1833) described the pelage of *S. dinganii* as dull olive-green dorsally and pale yellow ventrally. Another study by Robbins et al. (1985) did not find significantly measurable differences between *Scotophilus* species; this work is included in the taxonomic history. There have been many revisions to the taxonomy of *Scotophilus* since Leach (1821) described the genus. Oftentimes, these revisions have contradicted each other and led to further confusion of the taxa in the genus.

Systematic Revisions

Dobson (1878) was the first to interpret the systematics of *Scotophilus*. Dobson (1878) recognized *S. gigas* and placed *S. nigrita*, *leucogaster*, *dinganii*, *planirostris*, and *viridis* as synonyms to *S. borbonicus*. Thomas (1904) recognized two subspecies of *S. nigrita*: *S. n. colias*, formerly Dobson's *S. borbonicus*, and *S. n. nux*. In 1908, Thomas and Wroughton recognized *S. nigrita* subspecies *S. n. dinganii* and *S. n. herero* and *S. n. viridis* along with the subspecies *S. v. damarensis*. In 1939, Allen produced a complete list of all *Scotophilus* taxa recognized at the time:[1]

Scotophilus Leach, 1821
Scotophilus borbonicus (É. Geoffroy, 1806) [should be 1803]
Scotophilus gigas Dobson, 1875
Scotophilus nigrita nigrita (Schreber, 1775) [should be 1774]

[1] *Scotophilus* taxa listed here are as cited in Allen (1939).

Scotophilus nigrita colias Thomas, 1904

Scotophilus nigrita dinganii (Wahlberg, 1846) — should be A. Smith, 1883

Scotophilus nigrita herero Thomas, 1906

Scotophilus nigrita leucogaster (Cretzschmar, 1826) including *S. serratus* (Heuglin,1877)

Scotophilus nigrita nux Thomas, 1904

Scotophilus nigrita planirostris (Peters, 1852)

Scotophilus nigritellus De Winton, 1899

Scotophilus robustus Milne-Edwards, 1881

Scotophilus viridis viridis (Peters, 1852)

Scotophilus viridis damarensis Thomas, 1906

Scotophilus murino-flavus (Heuglin, 1861) including *S. flavigaster* (Heuglin, 1861) and *S. altilis* G.M. Allen, 1914

Hayman and Hill (1971) revised *Scotophilus* and recognized the following species and subspecies:

S. gigas (including *Scotophilus nigrita alvenslebeni* Dalquest, 1965)
S. nigrita
- *S. n. colias*
- *S. n. dinganii*
- *S. n. herero*
- *S. n. nigrita*
- *S. n. nux*
- *S. n. pondoensis*
- *S. n. robustus*

Synonyms o*f nigrita* are *borbonicus (part), marino-flavus,* and *flavigaster,* with *planirostris* as a synonym of *dinganii.*

S. leucogaster
- *S. 1. damarensis*
- *S. 1. leucogaster*
- *S. 1. nigritellus*
- *S. 1. viridis*

Hayman and Hill (1971) also mentioned that the Madagascar form *S. borbonicus* may represent *S. leucogaster*.

Robbins (1978) found *S. nigrita sensu stricto* to be a synonym of *S. gigas,* and *S. dinganii* as the senior name for subspecific taxa formerly allocated to *S. nigrita.* Robbins

(1978) recognized the following:

> *S. borbonicus* and *S. robustus* from the Mascarenes and Madagascar, and
> *S. nigrita, S. dinganii, S. nigritillus, S. leucogaster* and *S. viridis* occurring over various parts of the African continent. The relationship between *S. dinganii* of southern Africa and *S. leucogaster* of the sub-Saharan areas is still unclear.

Robbins et al. (1985) used multivariate statistics to analyze the morphology of the recognized taxon. Their results recognized six species of *Scotophilus* on the African mainland:

> *S. dinganii*
> *S. leucogaster (including S. l. leucogaster and S. l. damarensis)*
> *S. nigrita*
> *S. nucella*
> *S. nux*
> *S. viridis*

Grubb et al. (1998) found that *S. nigritellus* is distinct from *S. viridis.* Simmons (2005) recognized 7 species of African *Scotophilus* and cited their IUCN status*:*

> *S. borbonicus* – Critically Endangered. May be extinct.
> *S. dinganii* – Lower Risk.
> *S. leucogaster* – Lower Risk.
> *S. nigrita* – Lower Risk.
> *S. nucella* – Not evaluated.
> *S. nux* – Lower Risk.
> *S. robustus* – Lower Risk.
> *S. viridis* – Lower Risk.

Jacobs et al. (2006) examined the differences in echolocation frequency of South African *S. dinganii* to demonstrate the existence of a cryptic species, *S. mhlanganii*. However, *S. mhlanganii* does not appear to have been widely recognized as a valid *Scotophilus* taxon. Goodman et al. (2005) and Goodman et al. (2006) revised *Scotophilus* on Madagascar and recognized the following species in addition to those previously mentioned:

> *S. tandrefana*

S. marovaza

Trujillo et al. (2009) conducted a molecular phylogenetic study of the 15 currently recognized species of *Scotophilus* and found that three additional taxa should be added based on their mitochondrial DNA (and Y-chromosome DNA for the *S. dinganii*-like species), although the lineages were not named. The new clades and their distributions are as follows:

S. dinganii-like (eastern Africa)
S. dinganii-like (Ghana to Western Kenya)
S. viridis-like (eastern Africa)

Vallo et al. (2011) reevaluated the taxonomy of the *Scotophilus* from the Arabian Peninsula using genetic and morphological analyses. Their results demonstrated two mitochondrial lineages that clustered the East African *S. dinganii* and the West African *S. leucogaster*. *S. dinganii* populations within Yemen and Ethiopia exhibited morphological similarity to the type specimen of *S. nigrita colias* from Kenya. They suggested that members of this lineage should be elevated to species-status and be recognized as *S. colias*, although it is still recognized as the subspecies, *S. dinganii colias*. Brooks and Bickham (2014) examined the lineages of the three unnamed species set forth by Trujillo et al. (2009) and compared skull and body measurements to test for significant morphological differences. Based on their findings, four new species of *Scotophilus* were described:

S. andreweborii
S. livingstonii
S. ejetai
S. trujilloi

This taxonomic history accounts for the species and subspecies of African *Scotophilus* that are recognized today. There is obviously much confusion surrounding this genus. Its complex taxonomy arose from its variable morphology and the

misidentification of *S. nigrita* that resulted in subsequent publication as a new species. The following are the current systematics and distribution (Trujillo et al. 2009; Brooks & Bickham 2014) of *Scotophilus*, with those studied for this thesis in bold[2]; those marked with an asterisk represent forms described from Kenya although none of the studied specimens bore these names:

**S. andreweborii* – sub-Saharan Africa
S. borbonicus – endemic to Reunion Island
S. celenbensis – endemic to Sulawesi
S. collinus – Java and Bali
S. dinganii – sub-Saharan Africa
    S. d. colias
    S. d. dinganii
    S. d. herero
S. ejetai – sub-Saharan Africa
S. heathii – throughout India and Southeast Asia
S. kuhlii – throughout India and Southeast Asia
S. leucogaster – sub-Saharan Africa
    S. l. damarensis
    S. l. leucogaster
    S. l. nigritellus
**S. livingstonii* – sub-Saharan Africa
S. marovaza – endemic to Madagascar
S. nigrita – sub-Saharan Africa
S. nigritellus – sub-Saharan Africa
S. nucella – sub-Saharan Africa
S. nux – sub-Saharan Africa
S. robustus – endemic to Madagascar
S. viridis – sub-Saharan Africa
    S. v. nigritellis
**S. tandrefana* – endemic to Madagascar
S. trujilloi – sub-Saharan Africa

At present, literature supports the distinction of 19 *Scotophilus* species. There is still much more that needs to be examined to be able to apply scientific names accurately when in the field and to apply or revise names to specimens within collections. Future work is needed to untangle the complicated phylogenetic history and the relationships

[2] The species in bold were selected for study because these were the specimens available in the collections at the Field Museum of Natural History in Chicago, IL.

within the genus to prevent future misidentifications. The use of multiple species concepts can further complicate the taxonomy of *Scotophilus*. It is important to understand how the various concepts can alter the systematic organization of the genus. Early literature focused on the Biological Species Concept and the Morphological Species Concept, while more recent studies have used the Morphological Species Concept and Phylogenetic Species Concept to determine the species limits of *Scotophilus*.

Species Concepts

Biological Species Concept.—The Biological Species Concept (BSC) is perhaps best attributed to Ernst Mayr (1942, 1963), although many authors have articulated similar ideas (*e.g.* Poulton 1903; Jordan 1905; Huxley 1940; Wright 1940; Dobzhansky 1940). Mayr (1942) defined species as groups of natural populations that interbreed and are reproductively isolated from other populations. Species are population systems where the gene exchange is limited through one or more reproductive isolating mechanisms (Dobzhansky 1970).[3] Genetic relationships define a species rather than differences in morphology.

Mayr (2000) discussed the application of the BSC to species and subspecies. Populations are assigned species status based on local situations where reproducing groups encounter one another and remain distinct and separate. Empirical evidence is essential. The decision to assign species status is not based upon the degree of difference, but rather the presence or absence of interbreeding. When studying populations using the

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[3] This explanation is often considered the foundation of the Genetic Species Concept (Bradley and Baker 2001).

BSC, the most basic difficulty is that each isolated population is potentially an independently evolving gene pool within the overarching species (Mayr 2000). Therefore, each isolated population must be given consideration that it could be a transitional species. However, when determining this possibility, it is necessary to observe whether the BSC criteria are met by the respective populations. Ergo, two populations are not combined into one species because they are similar; they are similar *because* they belong to the same species.

Some populations might evolve negligible morphological differences and be reproductively isolated, while others might be distinctive morphologically, yet have no reproductive isolation. Differences in morphology, between a population and closely related populations, can be indicative of evolutionary changes due to natural selection or genetic drift that are independent of other populations, even without complete reproductive isolation. In these ambiguous situations, it can be beneficial to rank allopatric populations as subspecies. Wilson and Brown (1953) consider genetically distinct and geographically separate populations that are derived from the same species to be worthy of the taxonomic rank of subspecies. Mayr and Ashlock (1991) detailed the importance of subspecies rank. The trinomial conveys the closest evolutionary relationship with the specific epithet and then their allopatry with the subspecies name. This information can be valuable and can suggest that reproductive isolation or ecological compatibility has yet to evolve.

Based on the confusion of *Scotophilus* systematics and the lack of clear morphological differentiation, the PGSC would appear to be the most beneficial species concept for clarifying the genus. The BSC is not conducive for determining species limits considering empirical data is a necessity and there is a lack of literature on the

reproductive habits of *Scotophilus.* The methods to study the behaviors of individual bats are limited due to their small size and nocturnal tendencies, which often restrict bat ecology and behavior studies to using population data (Barclay 1985). The difficulty in collecting empirical evidence that would support the BSC is one reason *Scotophilus* is a great case study. The overlap in distribution of the African *Scotophilus* taxa additionally confounds the ability to determine reproductive isolation.

The dependence on empirical evidence can make practical application of the BSC more difficult, although ornithologists have used the BSC for almost a century (McKitrick 1988). Non-ornithologists, however, have been the primary critics of the BSC because of the difficulties associated with its application to sympatric populations (McKitrick 1988). If determining reproductive isolation is not feasible, previous studies have demonstrated that other data can be used in species classifications. Behavioral data, such as foraging habits or diet preferences, might be collected to analyze differences between suspected species. The echolocation call frequencies of bats or a molecular analysis of their genes might illuminate species limits. However, these types of data are no longer using BSC criteria; instead, these use a variation of the MSC that uses phenotypic clustering, such as the echolocation calls, or the Genetic Species Concept.

Based on niche and competitive exclusion theories, Fenton and Thomas (1980) predicted that the species of bats would demonstrate some resource partitioning. However, their study found overlap among *Scotophilus* species for various ecological behaviors, such as habitat use and activity patterns. Monadjem et al. (2010) observed roost selection in *dinganii* and *S. viridis* and found that both prefer to roost in *Combretum imberbe* trees. Without clear morphological differences or niche preferences between the species, it is difficult to infer the existence of reproductive isolating

mechanisms.

Despite the lack of information on *Scotophilus* interbreeding, examination of morphological variation might be useful for determining subspecies rank within the genus. The more recent literature appears to have the most confusion at this level (*e. g.* Jacobs et al. 2009; Vallo et al. 2011; Brooks and Bickham 2014). Even with the inability to categorize *Scotophilus* from the lack of data on reproductive isolation, the arguments for the importance of maintaining subspecies ranks remain valid. Future research should attempt to discern reproductive isolation among the various species of *Scotophilus* to further clarify species limits.

Phylogenetic Species Concept.—The Phylogenetic Species Concept (PGSC) was first discussed by Hennig (1950; 1966) who emphasized the necessity of a temporal dimension when classifying species. The PGSC assumes that evolution has occurred and relies on those evolutionary steps to delimit species. According to Hennig (1950), species consist of all the individuals that are connected through parent-offspring relationships between individuals in a reproductive community. Willmann (1985) added that species originate from the termination of a stem species during a speciation event and will cease to exist because of extinction or giving rise to daughter species. Meier and Willmann (2000) argued that the reproductive gap between the most inclusive population and its next of kin is important in determining species limits. This reproductive gap prevents gene flow between a species and its sister species. The PGSC emphasizes ancestry and descent when delimiting species boundaries (De Queiroz 1998). As with the BSC, species under the PGSC cannot exchange genetic information. This results in the new population being reproductively isolated from the bigger population and allows it to become the founding population for its own clade.

Wheeler and Platnick (2000) predicted that, under the PGSC, a population considered a subspecies under the BSC will be elevated to species status because of its evolutionary history. The PGSC provides the operational units for cladistic analysis and thereby elucidates the details of evolutionary mechanisms. Baum and Shaw (1995) used the most recent common ancestor to delineate species; two individuals belong to the same species if their genes coalesce within the group instead of outside the group.

The PGSC would likely result in clarification of *Scotophilus* taxonomy. Differentiation of morphological characteristics and studies on the reproductive behavior of the genus are not necessary to determine the relationships of species under the PGSC. Molecular studies often provide an initial basis to apply the PGSC as they can shed light on the populations and likely yield clearer species boundaries and range limits, although the genetic differentiation required to justify species designation is ultimately subjective.

Previous studies have already identified clades within *Scotophilus* that have guided the systematic revisions. A phylogenetic study by Trujillo et al. (2009) showed that *S. kuhlii* was the most basal species, followed by *S. nux*. An examination of the Malagasy taxa suggests independent colonizations from the African mainland (Trujillo et al. 2009). *S. dinganii*, a taxon at the center of recent disagreement over species limits, was shown to consist of two cryptic species based on genetic divergence results (Trujillo et al. 2009). Vallo et al. (2015) examined the phylogeny of *S. nigrita* and found it to be paraphyletic to *S. colias*, *S. dinganii*, *S. nigritellus*, and *S. viridis*. Their results contradicted published findings regarding *S. nigrita* in Kenya and raised questions about its taxonomic affiliation (Vallo et al. 2015). The phylogenetic study of Brooks and Bickham (2014) added four new sub-Saharan African *Scotophilus* species (Figure 2).

The PGSC might be best suited for clarifying the evolutionary history of

Scotophilus. However, it does not resolve the issue of applying names to bats in hand. Genetic information is not accessible in the field, rendering the technology useless for purposes of identifying field specimens. However, for the specimens already in

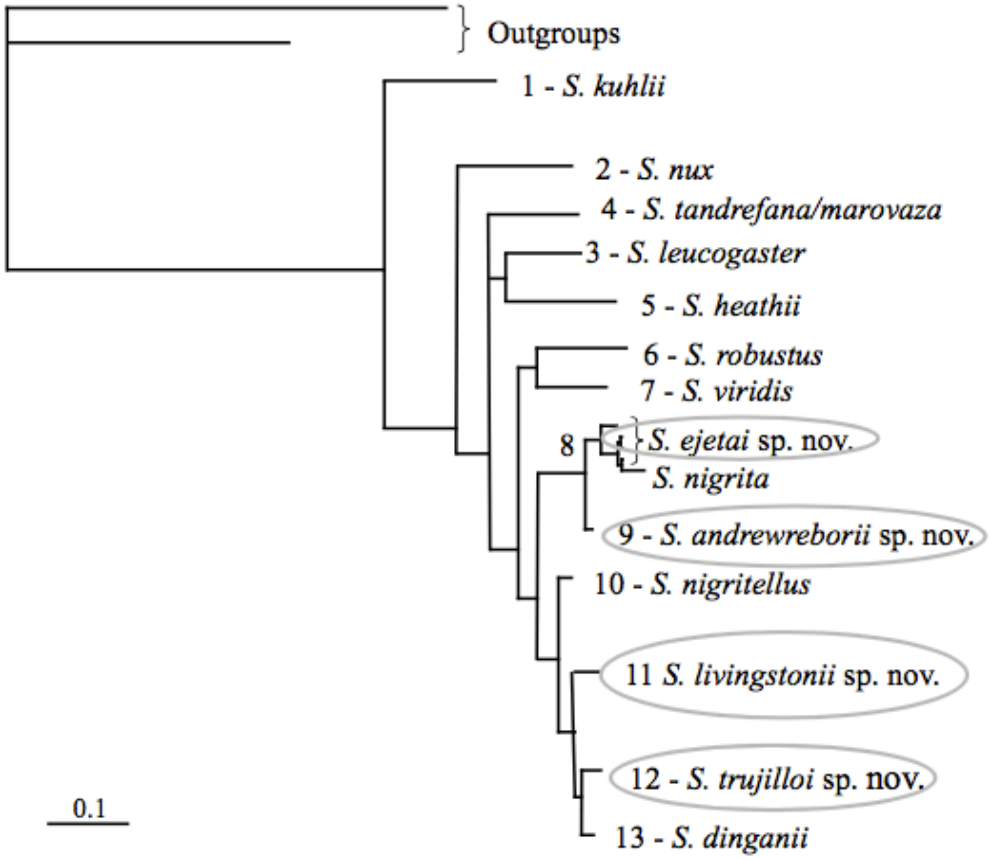

Figure 2. Bayesian phylogram showing clades and species of Scotophilus that were studied by Brooks and Bickham (2014). The grey circles represent Scotophilus species newly described in that study.

collections, the PGSC might be the best option for understanding the relationships of the species and if yet another systematic revision is needed.

Morphological Species Concept.—The Morphological Species Concept (MSC) dates back to Aristotle and Linnaeus (Goerke 1973). As far back as the fourth century B.C., Aristotle had begun to classify organisms by typology (Goerke 1973). The Linnaean Species Concept, precursor to the MSC, stems from the observation that, in nature, some individuals resemble each other more than others (Larson 1968). Larson (1968) summarizes the Linnaean Species Concept as fixed, where species are defined as unchanging, differentiated marks. Throughout the Linnaean period and in Darwin's

writings, morphological differences were the main criteria for determining species (Mayr 1963). According to Mayr (1963), the MSC is the simplest and most widely recognized species concept.

Derived from the Latin word *specere*, meaning to look at, species under the MSC fit the definition "of a different kind." When using the MSC, morphological difference and subjectivity are aspects of the MSC that are interdependent when deciding species status (Mayr 1963). Cronquist (1978) defined species as the smallest group of individuals ordinarily distinguishable and consistently distinct. However, the MSC is generally secondary to the BSC, which is better reflected in present-day taxonomy.

Without empirical evidence for reproductive isolation, the MSC is regularly used to delineate species boundaries. However, often, morphological variation is subtle or minute and not easily distinguished. When Thomas (1904) described a new species of *Scotophilus*, he noted that bats classified as *S. nigrita* that are from different geographic locations are separable by their ventral pelage color. According to Vallo et al. (2011), African *Scotophilus* differ morphologically in size and pelage color but have similar external appearances. Because of the morphological uniformity of the genus and apparently overlapping geographic ranges, delineating *Scotophilus* systematics using the MSC is difficult. Schlitter et al. (1980) assessed the non-geographic variation in African *Scotophilus* in an attempt to establish morphometric parameters for *S. dinganii* and *S. viridis*. They found differences in cranial morphology and forearm length between the two species. *S. dinganii* is characterized by a paler and duller brown ventral pelage than *S. viridis*, which is bright yellow to dull yellow-brown. The morphological characteristics of *Scotophilus* may or may not be good taxonomic characters for classifying *Scotophilus* taxa.

Based on the findings in the literature, it is clear that morphology might not be the best indicator for distinguishing *Scotophilus* species limits. However, most studies did find significant morphological differences between recognized species. While the MSC should not be used on its own to discern the evolutionary history of *Scotophilus*, it does appear useful for applying identifications in the field and for biological studies. When combined with genetic differences and ecological behavior data, differences in morphology can illuminate a more complete understanding of differences between the taxa.

The application of Species Concepts to Scotophilus.—Clearly, no single species concept is uniformly applicable to the genus *Scotophilus*. Ideally, a combination of the three outlined species concepts will provide the most in-depth, accurate depiction of the evolutionary history and species boundaries. If future research finds that the various taxa are indeed reproductively isolated, then the BSC is most likely the best method of classification for *Scotophilus*. However, if hybridization and introgression are found in sympatric *Scotophilus* populations, then it is possible that the BSC will not be sufficient in assigning species names. If this is the situation, then the PGSC would likely be the most effective means of classification. The PGSC will help aid in clarifying the existing nomenclature of *Scotophilus* as the evolutionary relationships become clearer. As molecular phylogenetic studies and DNA analyses discover further distinctions within the clades, there will need to be organization and standardization for determining whether the rank of the newly identified taxa should be at the species or subspecies level or as an unnamed clade.

The MSC is the most useful for identifying bats in the field. Having clear distinctions of the morphological differences between the species will reduce

misidentification and ease the confusion of applying scientific names. If possible, combining the criteria of the BSC, PGSC, and MSC might help to better understand the taxonomy of *Scotophilus*. This will be beneficial for conducting future research studies, establishing conservation efforts, and clarifying already identified specimens. Without clear guidelines for the application of new taxa, confusion and misidentification will continue to grow within *Scotophilus*.

CHAPTER 2: INTRODUCTION TO THE GEOMETRIC MORPHOMETRIC AND PELAGE ANALYSIS

It is clear from the taxonomic history of *Scotophilus*, its many systematic revisions, and the need for consistent criteria for species definition that more research needs to be done to define species limits and range boundaries of *Scotophilus.* Disagreements about the correct organization of taxa were evident soon after the genus was first described (Leach 1821; Smith 1833; Dobson 1878). My thesis is an attempt to clarify geographic variation for objective application of currently available names and for determining groups that would warrant the proposal of new names.

I aim to answer various questions that have plagued this genus since its discovery. As evidenced by the many systematic revisions (*e.g.* Allen 1939; Robbins et al. 1985; Trujillo et al. 2009; Brooks & Bickham 2014), there is a need to determine what nomenclature is truly appropriate for *Scotophilus*. Furthermore, it is necessary to determine morphological, ecological, and genetic distinctions between subspecies. Additionally, it must be determined if subspecies is in fact an appropriate taxonomical ranking. Finally, the results of my data analysis will need to be compared to current systematics to determine if they are applicable or if further revisions are needed.

To answer these questions, I analyzed variation in cranial morphology using geometric morphometric analysis and quantified pelage color variation in *Scotophilus* to examine species limits and morphological overlap among populations. Geometric morphometrics are used to analyze differences in shape. It removes non-shape variation and allows for the quantification and analysis of morphological shape (Adams et al. 2004). Bookstein et al. (1985) detailed the importance of morphometrics in evolutionary biology. The methods and findings of these analyses are outlined in the following chapters. These analyses identify diagnostic characters and range boundaries for *S.*

dinganii ssp., *S. leucogaster, S. marovaza, S. nux, S. robusuts,* and *S. viridis* and will help to clarify the application of existing names to the Kenyan house bats.

If the geometric morphometric results show distinct groupings based upon cranial differences, it would confirm that each grouping is a unique *Scotophilus* taxon and identify putatively diagnostic characters for each taxon. Furthermore, each group should then correspond with the descriptions set forth by previous studies and species descriptions. Similarly, analysis of pelage color may yield distinct groupings that may or may not align with the geometric morphometric groupings as well as the descriptions of the recognized species. In Kenya, the names *S. d. dinganii* and *S. d. colias* have been applied with little information about supposed differences between them. If geographic variation influences morphology, I expect to find significant differences in morphology between bats from different regions of Kenya. If these characters are found to be phylogenetically informative, then they will be useful for assigning taxonomic classification in the field.

However, if the geometric morphometric and pelage analysis results fail to demonstrate distinct groupings, then conclusions are more complicated. Either or both could be phylogenetically uninformative and these two morphological traits may or may not vary in parallel. Further, neither might be indicative of reproductive boundaries. In this event, I would argue that these characters might be subject to adaptive or convergent evolution. Within population variation will be greater than between population variation if there is a lack of distinct grouping after analysis. Considerable overlap for the subspecies *S. d. dinganii* and *S. d. colias* would suggest that these subspecies names may require reevaluation. In general, failure to find significantly differentiated groupings will indicate a need to further revise the current systematics of the *Scotophilus* genus using a

combination of species concepts.

The currently applied scientific names of the *Scotophilus* specimens examined will be scrutinized and revised as necessary. Additionally, those specimens that were not previously classified to species level will be identified based on the findings of this and earlier studies. Finally, I will discuss the results and their implications for the current systematics of *Scotophilus* and the need for future studies.

CHAPTER 3: METHODS

Geometric Morphometric Methods

Specimens examined.—Crania from 140 individual *Scotophilus* (75 male, 65 female) representing 6 or 7 species were examined. The *Scotophilus* taxa examined included African bats identified as: *S. dinganii colias* (59), *S. dinganii dinganii* (26), *S. dinganii herero* (1), *S. leucogaster* (9), *S. marovaza* (11), *S. nux* (5), *S. robustus* (20), and *S. viridis* (9). Samples came from 15 countries: Angola (2), Ivory Coast (4), Kenya (57), Madagascar (31), Malawi (1), Mali (1), Namibia (1), Rwanda (1), Senegal (1), South Africa (6), South Sudan (4), Sudan (1), Tanzania (8), Uganda (10), Zaire (1), and Zimbabwe (11) (Table A1 and Figure A1). Only specimens that had intact crania and intact mandibles were included in the present study. All specimens are housed in the Recent Mammal Collection of the Field Museum of Natural History, Chicago.

Landmark data.—A total of 139 ventral, 137 dorsal and 140 mandibular images were analyzed. Images were taken using a Konica Minolta DiMAGE Z6 digital camera (x12 optical zoom, 6.0-megapixel resolution, supermacro function) mounted on a copy stand. I captured each image under standardized conditions. *Scotophilus* crania were photographed in dorsal and ventral views and the mandibles were photographed in a lateral view. For each specimen, 17 dorsal, 17 ventral, and 10 mandibular landmarks (Figure A2) were digitized using the TPS program series by F. J. Rohlf (http://life.bio.sunysb.edu/morph/). The landmarks were chosen to represent overall cranial and mandibular variability.

Geometric Morphometric Analysis

The geometric morphometric analysis (GMA) aimed to address three goals. The

first goal was to determine if skull shape distinguishes *Scotophilus* species. The second was to see if skull shape distinguishes *S. dinganii* subspecies. Finally, GMA aimed to assign uncertainly identified specimens to taxa.

Prior to GMA, 47 *S. dinganii* specimens from Kenya had not been identified to subspecies, either *S. dinganii colias* or *S. dinganii dinganii*. In order to assign a subspecies rank to these specimens, I carried out a discriminant function analysis. The morphometric software CoordGen8 by H. D. Sheets (http://www3.canisius.edu/~sheets/morphsoft.html) was used to calculate the Bookstein Coordinates (BC) of these incompletely identified specimens. For the primary geometric morphometric analysis, BC were then used in PCAGen8 (H. D. Sheets) to calculate the principal components (PC) 1 and 2 for all *S. dinganii dinganii* and *S. d. colias* specimens. A forward stepwise discriminant function analysis (DFA) was performed using the software STATISTICA (StatSoft 2005) to obtain the discriminant function that best separated the two groups. Variables initially included in the analysis were country of origin, sex, PC1, PC2, latitude, and longitude. A forward stepwise DFA found PC1 to be significant and therefore analysis was restricted to only this variable. PC1 was used to assign incompletely identified specimens to subspecies. The resulting discriminant classifications produced by STATISTICA were then used for further analysis.

For all subsequent geometric morphometric analyses, MorphoJ version 1.06d was used to analyze the landmark data sets (C. P. Klingenberg, distributed freely at http://www.flywings.org.uk/morphoj_page.htm). Outliers were found using the deviation from the average for each dataset, and specimens that were outliers were closely examined for skeletal damage. Damage to the crania or mandible that prohibited the proper placement of a landmark resulted in the exclusion of that specimen from the

dataset. Each dataset was classified by species; specimens identified as *S. dinganii*, *S. dinganii dinganii*, or *S. dinganii colias* were pooled as "*S. dinganii* complex." Second- and third-order classifiers were added, specifying the country of origin and sex for each specimen within each dataset. A full Procrustes fit was performed to superimpose the landmark coordinates and project the data to a tangent space (Dryden and Mardia 1998), an operation done separately for the dorsal, ventral, and mandibular datasets. For each dataset (dorsal, ventral, and mandibular), PCA was performed to examine the variation of *Scotophilus* species and the level of shape differences between recognized *Scotophilus* species. For each view, a covariance matrix was generated after the Procrustes superimposition as the basis for the principal component analysis (PCA).

I conducted DFA to gauge the differences between pairs of species (Klingenberg 2011). DFA was performed comparing each continental African species to determine the level of shape difference between recognized species of *Scotophilus*. Another DFA was conducted to compare the level of difference of *S. dinganii* complex to the level of difference between full species to examine if the level of difference was equivalent or weaker.

To examine morphological variation between *S. dinganii colias* and *S. d. dinganii*, another group of datasets was created. Classifiers by species, country, and sex were included. A full Procrustes fit was performed for the dorsal, ventral, and mandibular datasets. A Canonical Variate Analysis (CVA) was performed to determine differences in shape features (Klingenberg 2011) between the two subspecies and repeated for each of the cranial and mandibular datasets. The CVA was used to investigate correlation between the subspecies (French et al. 2008). A second CVA was performed to examine geographic variation within Kenya in the morphology of the *S. dinganii* complex. I

incorporated classification by county into the analysis. The dataset consisted of ventral landmark data from Kenyan specimens classified as *S. dinganii*. I conducted DFA for each dataset to assess the differences between the taxa *S. d. dinganii* and *S. d. colias*, proposed as distinct species by Vallo et al. (2011).

Pelage Color Methods

Specimens examined.—Skins from 279 *Scotophilus* (141 male, 137 female, 1 unknown) were examined (Table A2). The skins were either dry or preserved in alcohol. Their current identifications in the Field Museum of Natural History's collection were assumed to be correct. The *Scotophilus* taxa represented included: *Scotophilus species indet.* (22), *S. dinganii* (120), *S. dinganii colias* (36), *S. dinganii dinganii* (11), *S. dinganii herero* (11), *S. leucogaster* (32), *S. marovaza* (8), *S. nux* (5), *S. robustus* (17), and *S. viridis* (17). Specimens represented 18 African countries: Angola (6), Ethiopia (3), Ghana (3), Ivory Coast (6), Kenya (137), Madagascar (24), Malawi (1), Mali (3), Namibia (11), Rwanda (3), Senegal (10), South Africa (6), Sudan (17), Tanzania (25), Uganda (10), Zaire (2), Zambia (1), and Zimbabwe (11). Of the 280 specimens sampled, 157 had crania that were measured in the geometric morphometric analysis. Only specimens that were in good condition (i.e. not bleached or damaged) were included. All specimens are housed in the Recent mammal collection of the Field Museum of Natural History, Chicago.

Color gradient.—A color gradient was created using the website, http://www.perbang.dk/rgbgradient/ (Figure 3). Each color along the 8-step gradient was numbered 0-7, with 0 being the darkest color. Once the gradient was chosen, several skins were selected at random. I compared their ventral pelage to the gradient and the

corresponding color category was recorded to ensure the color gradient encompassed the variety of pelage colors. Ten specimens were selected at random and set aside to be reanalyzed after a few hours for accuracy in replication.

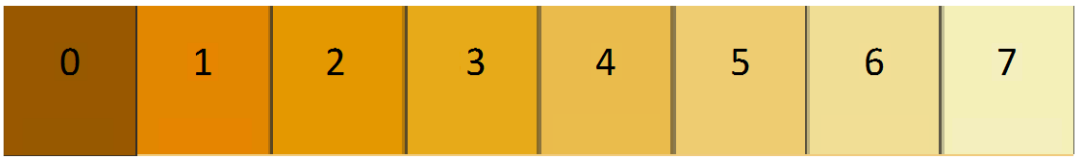

Figure 3. Pelage color gradient used to analyze FMNH specimens for ventral pelage color.

Pelage categorization.—Once the color gradient was set, each skin was analyzed and assigned to a category along the gradient. To analyze dry specimens, I placed the skin under a lamp and held the color gradient over the ventral pelage. I assigned the specimen a pelage category based on its closeness to one of the gradient categories. I compared each dry specimen under these standardized conditions.

Fluid-preserved specimens were removed from their preservation jar with forceps and placed into a separate jar filled halfway with 75% ethanol and uniformly illuminated. The specimen was manipulated with the forceps so that its ventral pelage was sufficiently lit within the jar to eliminate shadows or other confounding factors. The color gradient was held to the side of the jar and the pelage category selected based on the closeness to one of the gradient steps. Every 20 specimens, the ethanol in the jar was removed and replaced to maintain its clarity. To validate that there was not a systematic difference in color scoring, a wet specimen was observed under the lighting conditions without the ethanol jar to ensure replicability in color category.

Histograms.—After each specimen was assigned to a pelage color category, I generated histograms using Microsoft Excel. Histograms were organized by species to examine variation. To examine morphological differences based on geography, histograms of pelage category were generated by country of origin (Figure A3). I created

a third set of histograms to examine the variation among the Kenyan samples using Kenyan provenance as the discriminating factor.

Combined pelage and skull shape.—Of the 279 observed skins, 140 had skulls that were included in the geometric morphometric analysis. Based on the findings of the geometric morphometric studies, pelage was added as a fourth-order classifier to the dorsal morphometric data for all *Scotophilus* species. After adding the categorical pelage classifications, another Procrustes fit was performed. I used MorphoJ version 1.06d to perform a principal components analysis. The principal component values were exported into Microsoft Excel for further analysis. Pelage was also added as a classifier to the dorsal morphometric analysis of the *S. dinganii* complex, using the identifications determined by the discriminant function analysis. Again, a Procrustes fit and a principal components analysis were performed. The data were also exported into Microsoft Excel.

To determine if there is correlation between morphometric variation and ventral pelage color, pelage was plotted against PC1 for all species. To determine if pelage can be used to discriminate species, the PC1 coordinates were plotted against pelage category for all *Scotophilus* specimens. For the *S. dinganii* complex, pelage was plotted against PC1 to observe if there were distinct pelage differences between the different taxa. To determine if pelage color can separate *S. dinganii* spp. within Kenya, the pelage of *S. dinganii* complex specimens from Kenya were plotted against PC1.

Pelage analysis.—I used a Kruskal-Wallis non-parametric *H* test to test for significant differences in pelage color between recognized species of *Scotophilus*. *S. nux* was not included in the test because of the small sample size (n=4). I used a Mann-Whitney U test to test for significant difference in pelage color between *S. d. colias* and *S. d. dinganii* using the classifications as determined by the discriminant function

analysis. For all *S. d. colias* (n=59) and *S. d. dinganii* (n=26) specimens. I also used a Mann-Whitney U test to examine the dorsal pelage differences between *S. d. colias* and *S. d. dinganii* within Kenya.

Echolocation Call Frequencies

Recently, Brooks and Bickham (2014) described four new *Scotophilus* species, three of which are found in Kenya. These species were described in continuation of the studies by Trujillo et al. (2009) and Jacobs et al. (2006), which had included echolocation call frequency data of the Kenyan species. To determine if any of the Kenyan *S. dinganii* complex specimens in the present study could be identified under one of these new species and if call frequencies help separate the two subspecies, the echolocation call frequencies of these specimens were included as a classifier in another geometric morphometric analysis. Only specimens collected in the field by Drs. Bruce Patterson and Paul Webala were used in the echolocation call frequency GMA. Dr. Webala recorded the echolocation call frequency for these bats.

CHAPTER 4: RESULTS

Geometric Morphometric Results

All species.—To quantify the differences in cranial morphology for presently recognized species of *Scotophilus*, I conducted a geometric morphometric analysis. GMA of the dorsal and ventral data for all species showed that PC1 explained 38.9% and 33.3%, respectively, of the overall variance. The PCA of the mandibular dataset revealed that PC1 explained 26.15%. Species clusters were identifiable in the PCA of ventral and dorsal datasets, but no clear species clusters were evident in the PCA of mandibular landmarks (Figure 4; Figure A4, A5). I interpreted PC1 to be associated with species and PC2 to be associated with country of origin based on the PC matrix.

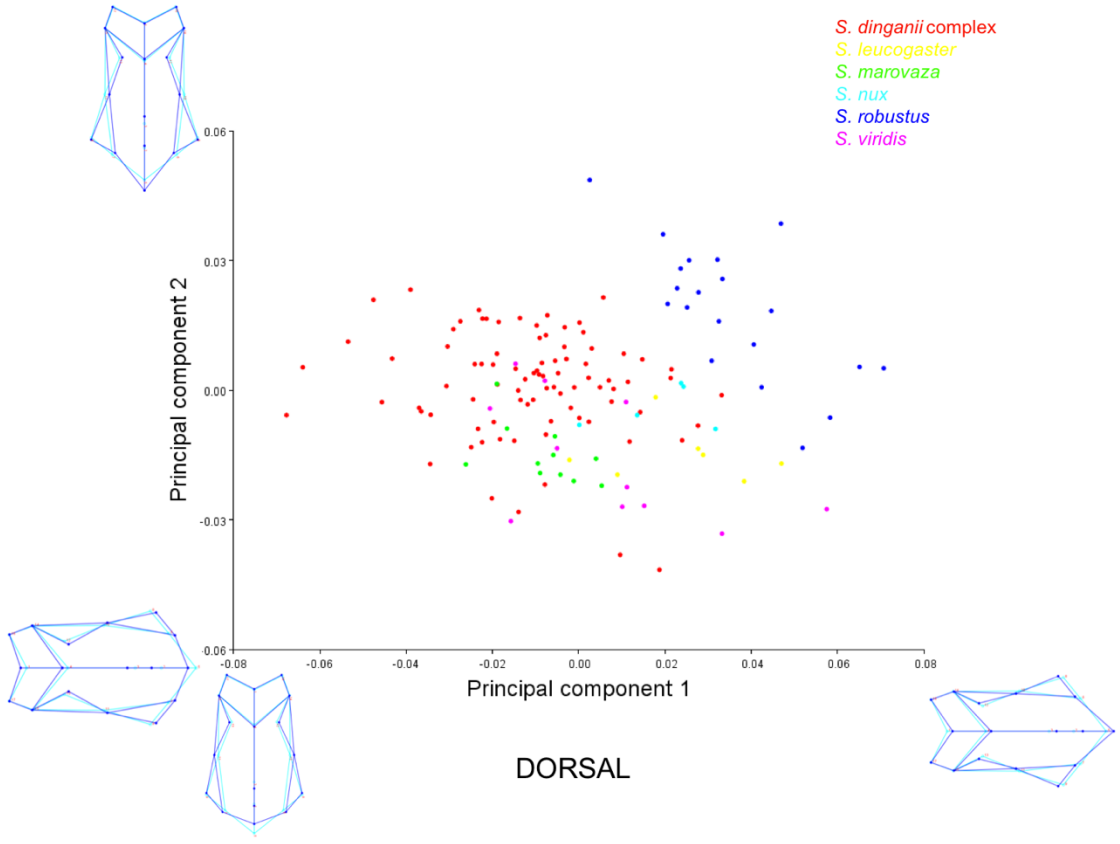

Figure 4. The Principal Component Analysis resulting from the geometric morphometric analysis of the dorsal landmark data. Species clusters are apparent, although there is clear overlap between species and wide variation within species.

I conducted a Discriminant Function Analysis of the PCs for each cranial dataset to determine statistical differences in morphology for *Scotophilus* taxa. The DFA of continental African *Scotophilus* with the dorsal dataset suggested significant differences between all continental African species except for *S. leucogaster* and *S. nux* (p=0.1300; Table 1; A4). DFA using the ventral dataset suggests significant differences between the species analyzed (Table A5; A6). The DFA with the mandibular dataset did not identify significant differences between *S. nux* and the *S. dinganii* complex (p=0.142) or between *S. nux* and *S. viridis* (p=0.121) (Table A7; A8).

	***Dinganii* complex**	***Leucogaster***	***Nux***
S. leucogaster	<.0001		
S. nux	<.0001	0.1300	
S. viridis	0.0030	0.0010	0.0060

Table 1. Dorsal dataset p-values from permutation tests (1000 permutation rounds) for Procrustes distances among groups.

S. d. colias & *S. d. dinganii.*—Next, I conducted a Canonical Variates Analysis to examine the morphological variation between the subspecies of *S. d. colias* and *S. d. dinganii.* The CVA results of morphological differentiation between specimens identified from the DFA analysis in STATISTICA as *S. d. colias* and *S. d. dinganii* showed CV1 as an indicator of overall size and explained 34.7% of the variance, with *S. d. colias* displaying higher CV values than *S. d. dinganii* in both the dorsal and ventral datasets (Figure 5; Figure A6). The mandibular dataset showed *S. d. colias* displaying smaller loadings (Figure A7). While species clusters were not entirely separate, *S. d. colias* had a lower mean value in the dorsal dataset on CV2 and a higher mean value along CV2 in the ventral dataset. *S. d. colias* also had a higher mean value along CV1 in the dorsal dataset (Figure 6; Figure A8). In the mandibular dataset, there were no clear clusters of the two species (Figure A9).

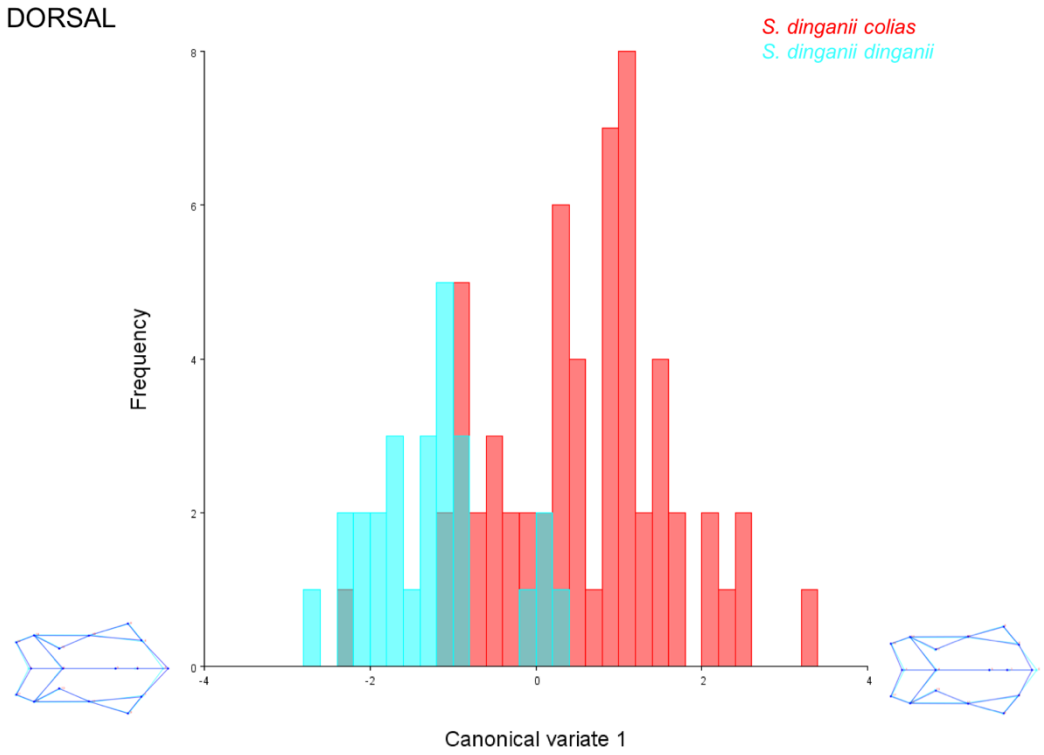

Figure 5. Histogram of CA1 for S. dinganii *species examined in the dorsal dataset, where CV1 represents overall size.*

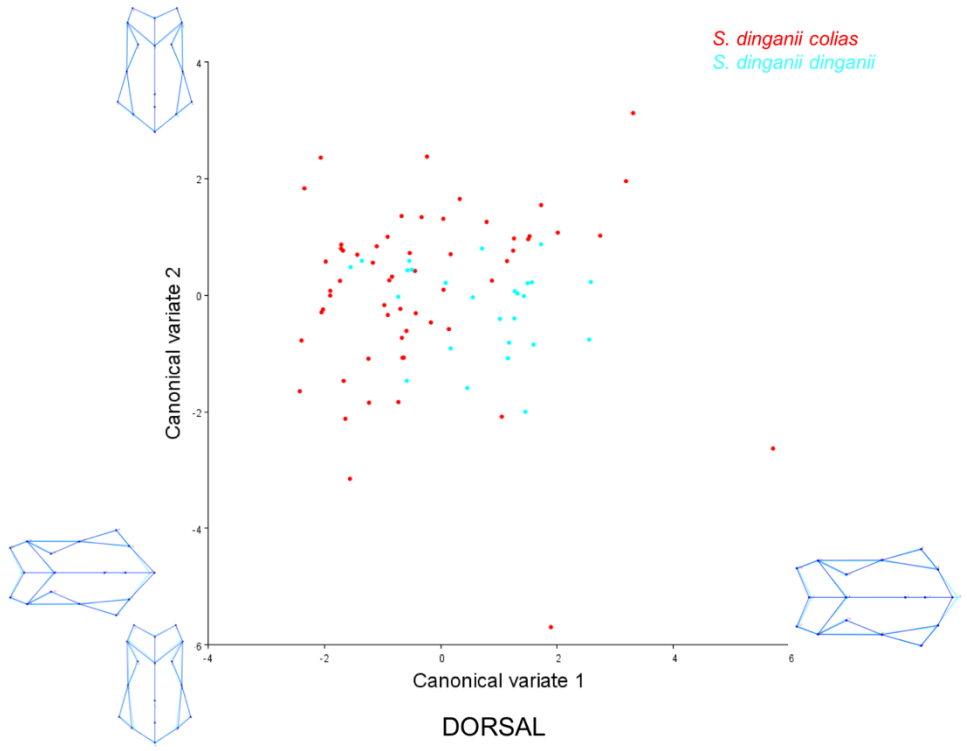

Figure 6. Scatterplot of CVA for the dorsal dataset of the S. dinganii complex. CV2 was interpreted to be associated with country of origin.

The CVA results involving *S. dinganii* ventral morphology showed clear evidence of geographic structure (Figure 7; Table 2). I interpreted CV1 to be associated with overall size, explaining 40.9% of the variance, and CV2 to be associated with geographic structure. The Kenyan counties of Meru, located in central Kenya, and Kilifi, on the eastern coast, had more distinct clusters with significant Procrustes distances between the two (p=0.0103). Kaijado, located west of Kilifi, had significant differences in Procrustes distances when compared to samples in Meru and Kilifi (p=0.0145 and p=<.0001, respectively). Samples originating in West Pokot, located in northwestern Kenya were also distinctly clustered in the CVA. Samples from West Pokot also differed significantly from samples from Meru and Kilifi (p=0.0013 and p=0.0275, respectively).

	Meru	Kilifi
Kilifi	0.0103	
Kaijado	0.0145	<0.0001
West Pokot	0.0013	0.0275

Table 2. Ventral dataset p-values from the CVA analysis among Kenyan counties.

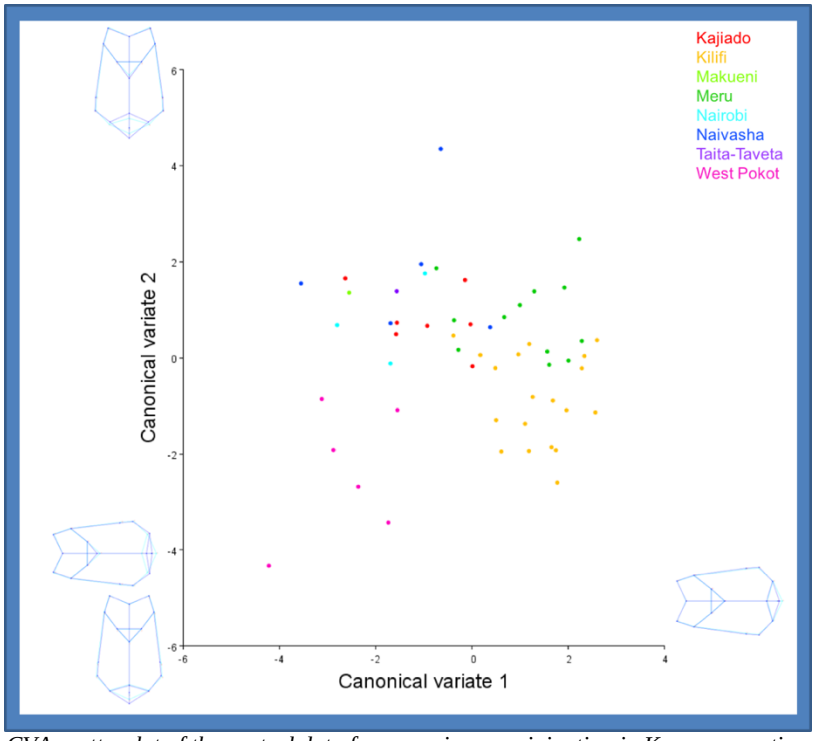

Figure 7. CVA scatterplot of the ventral data from specimens originating in Kenyan counties.

Next, I conducted a Discriminant Function Analysis to determine if the principal components calculated in the PCA could be used to correctly assign dinganii specimens to the correct subspecies. The DFA of *S. dinganii colias* and *S. d. dinganii* produced a Procrustes distance and T-square value of 0.0162 and 62.3950 (p=0.001), respectively, in the dorsal dataset; 0.0178 and 59.1587 (p=<.0001) in the ventral dataset; and 0.0116 and 37.5960 (p=0.059) in the mandibular dataset. The DFA for the dorsal dataset correctly classified 71.2% of the records to *S. d. colias,* and 84.6% of those for *S. d. dinganii* (Table 3). In the ventral dataset, 71.7% of *S. d. colias* and 75.9% of *S. d. dinganii* were classified correctly (Table A9). The DFA correctly classified 79.7% of *S. d. colias* and 80.0% of *S. d. dinganii* for the mandibular dataset (Table A10).

Classification/misclassification tables – cross-validation			
	S. d. colias	*S. d. dinganii*	Total
S. d. colias	42	17	59
S. d. dinganii	4	22	26

Table 3. Cross-validation of S. d. colias and S. d. dinganii in the dorsal dataset.

Morphological Observations

Geometric morphometric analyses of the ventral, dorsal, and mandibular views indicate differences between *S. d. dinganii* and *S. d. colias* that correspond to my observations. The midline point of the palate between the central incisors is shallower in *S. d. colias* than *S. d. dinganii*. *S. d. colias* also has a wider anterior point of interior orbit. The distance between landmark 2 and landmark 3 is less in *S. d. colias.* In *S. d. colias,* the foramen magnum is larger in the ventral view, especially along the posterior margin, compared to *S. d. dinganii*. *S. d. colias* also has a slightly narrower braincase and is smaller at the maximum curvature of the posterior margin of the zygomatic process. The tip of the palatal process is closer to the anterior midline point of the palate in *S. d. colias*, as well. Morphological variations of the mandible into an elevated posterior most point

on the canine alveolus, a smaller dorsal-most extension of the coronoid process, and a wider and larger dorsal tip of the angular process in *S. d. colias*. Overall, *S. d. colias* is slightly wider and shorter than *S. d. dinganii*, especially with respect to the braincase. Visual observation of *S. d. colias* and *S. d. dinganii* crania qualitatively corroborate the findings of the geometric morphometric analysis in detail.

During a visual observation, I noted additional morphological differences between *S. dinganii colias* and *S. d. dinganii*. It was observed that *S. d. dinganii* had much more pronounced sutures when compared with the naked eye. These sutures correspond to the dorsal landmarks 2, 3, and 4. The *S. d. colias* specimens also featured less prominent and shorter posterior projections of the occiput than *S. d. dinganii*; during geometric morphometric analysis, the posterior occiput projection was chosen as landmark 5 of the dorsal view. In *S. d. colias* specimens, this projection appears slightly narrower and shorter than in *S. d. dinganii*. In dorsal views, the braincases of *S. d. dinganii* appear slightly broader than the *S. d. colias* braincases. Broader brain cases are also evident in ventral view. The differences were most apparent along the anterior and posterior margin of the mastoids. Ventral landmarks 6, 7 denoted the posterior margin and landmarks 8, 9 denoted the anterior mastoid margin.

A visual comparison of the ventral views of the crania observed the distance between the posterior edges of the palatal process to be less on the crania of *S. d. colias* than *S. d. dinganii*, which were observed during analysis with ventral landmarks 12 and 13. It appears that the distance between the auditory bullas was wider in *S. d. dinganii*, although different landmarks are needed to test this. In *S. d. colias*, it was observed that the distance between the midpoint of the supraoccipital suture and the posterior margin of the foramen magnum was less than the distance in *S. d. dinganii*.

The upper and lower canines were thicker at the base in *S. d. colias* than *S. d. dinganii*. For the upper canine, landmarks 16, 17 was placed at the anterior-most point of the premaxilla and for the lower canine, landmark 2 was located at the posterior-most point of the canine alveolus which would be useful for observing any differences. When the mandibles of each species were compared, it was observed that *S. d. dinganii* mandibles were wider at the ramus and longer than mandibles of *S. d. colias*. The depth between the last molar and the anterior-most point on the border of the mandible also appeared greater on the *S. d. dinganii* mandibles. Mandibular landmarks 3 and 8 would be sufficient in analyzing this difference.

Pelage Results

Geometric morphometrics.—After analyzing the ventral pelage for the *Scotophilus* specimens, I included pelage category as a classifier in the geometric morphometric analysis to assess its value for determining species limits. The PCA of the dorsal GMA that included pelage as a classifier for all examined species of *Scotophilus* interpreted PC1 to be associated with species and PC2 to be associated with pelage based on the PC matrix data. PC1 explained 39.9% of the overall variance and that PC2 explained 13.8% of the variance. Addition of pelage as a classifier sharpens distinctions between the species clusters, despite some overlap (Figure 8). Figure 8 shows clearer distinctions between *S. d. colias* and *S. d. dinganii* than Figure 4, but there is still variation that results in overlap of crania shape between *S. d. colias* and *S. d. dinganii*. Species clusters were identifiable in the PCA of the dataset (Figure 9A), but when classified by country of origin, there was no clear clustering (Figure 9B).

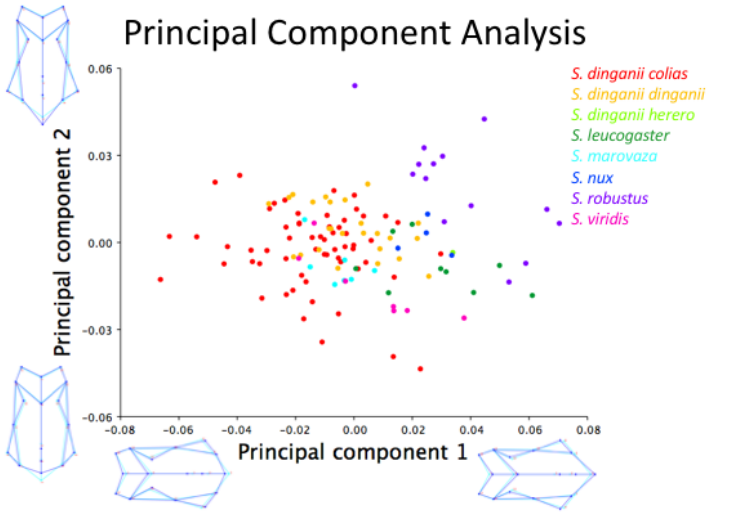

Figure 8. Bivariate scatterplot of the PCA for all Scotophilus species in the dorsal dataset that included pelage as a classifier. Clustering is more distinct with the addition of pelage.

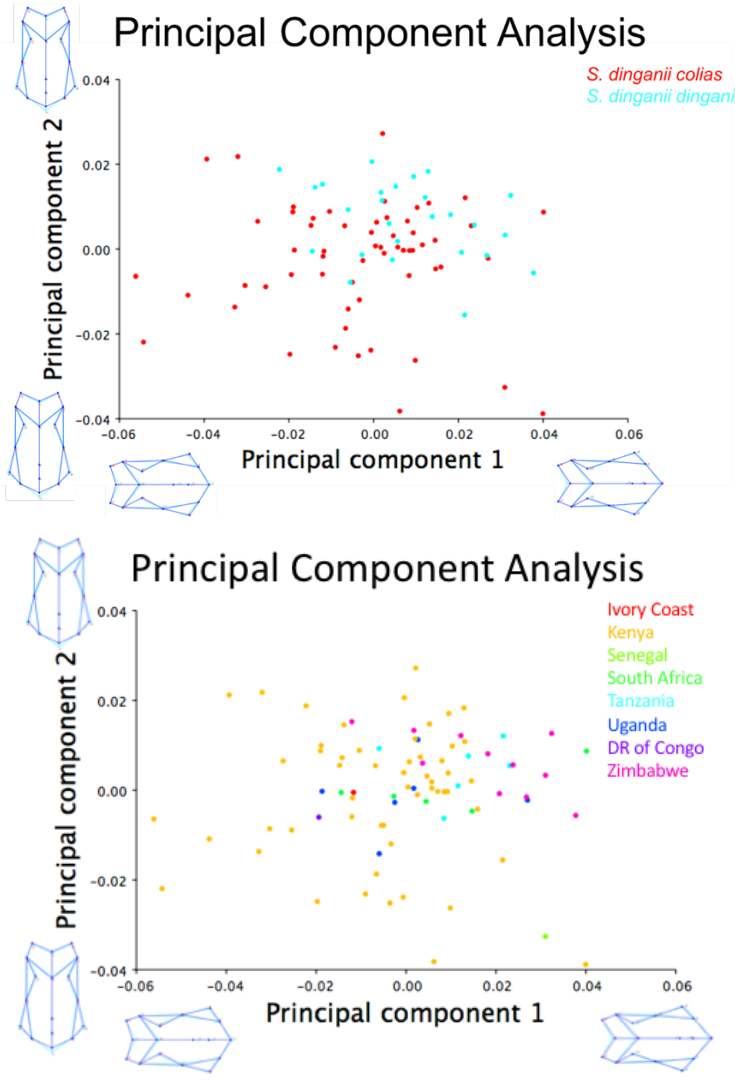

Figure 9. Bivariate scatterplots of PCA for all examined Scotophilus dinganii specimens. 9A (above graph), categorized by species. 9B (below graph), categorized by country of origin.

Pelage analysis.—Next, to analyze statistical differences in ventral pelage between *Scotophilus* species, I ran a Kruskal-Wallis *H* test. The statistical test showed that there was a statistically significant difference in pelage color between recognized species of *Scotophilus* ($\chi^2(_3)$ =7.81, p<0.001). *S. leucogaster* had a mean rank score of 31.9 and mean pelage category of 6; *S. marovaza* had a mean rank of 17 and mean pelage category of 2; *S. viridis* had a mean rank of 25.4 and mean pelage category of 4; and *S. robustus* had a mean rank of 8.5 and mean pelage category of 0. Figure 10 shows PC1 plotted against pelage category for all examined *Scotophilus* species. The histograms demonstrate the distribution of pelage color for the specimens within each species. Pelage color is not a clear distinguishing characteristic between recognized species of *Scotophilus* because overlap still occurs (Figure 11).

Finally, I ran a Mann-Whitney *U* test to examine statistical differences in pelage color between *S. dinganii* subspecies. This showed that there was a statistically significant difference in pelage color between *S. d. colias* and *S. d. dinganii* (z=-2.203, p=0.0122). The median pelage color category for *S. d. colias* was 4 (IQR=1.5) and for *S. d. dinganii* was 3 (IQR=1). A Mann-Whitney *U* test of the Kenyan *S. dinganii* samples showed that pelage color of *S. d. colias* and *S. d. dinganii* did not differ significantly (z=-1.191, p=0.1056). Figure 12 shows the distribution of pelage color for the *S. dinganii* complex. Figure 13 breaks down the distribution of pelage color amongst Kenyan counties for the *S. dinganii* specimens from Kenya examined.

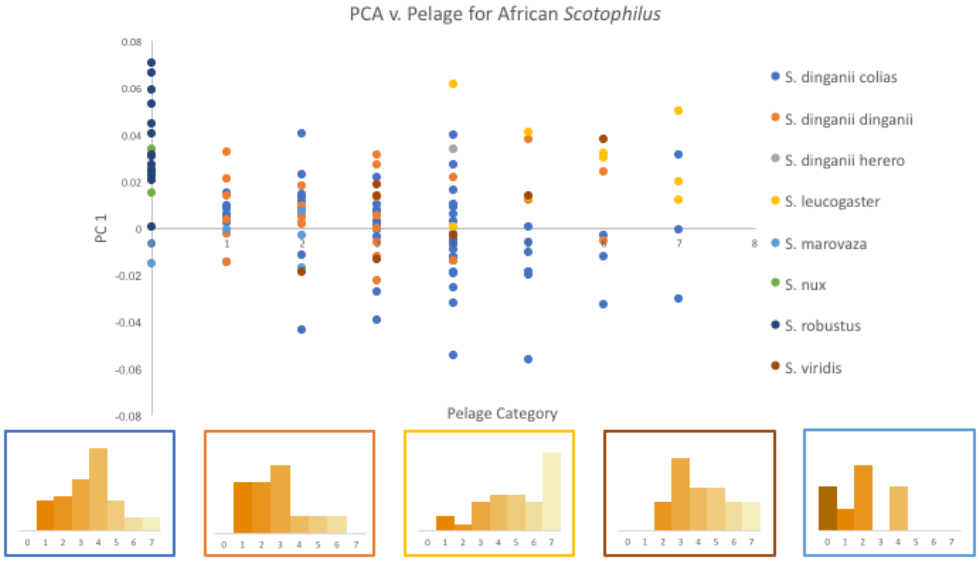

Figure 10. PC1 v. Pelage category for all examined species of Scotophilus in the pelage analysis.

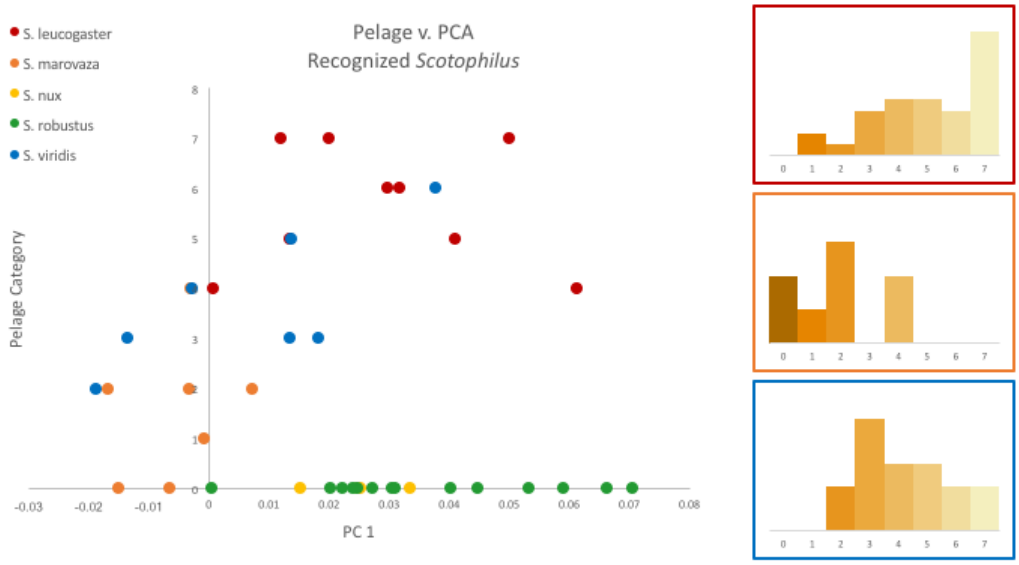

Figure 11. Pelage v. PC1 for recognized species of Scotophilus*. Results suggest pelage is not useful as a parameter on its own for assigning species names.*

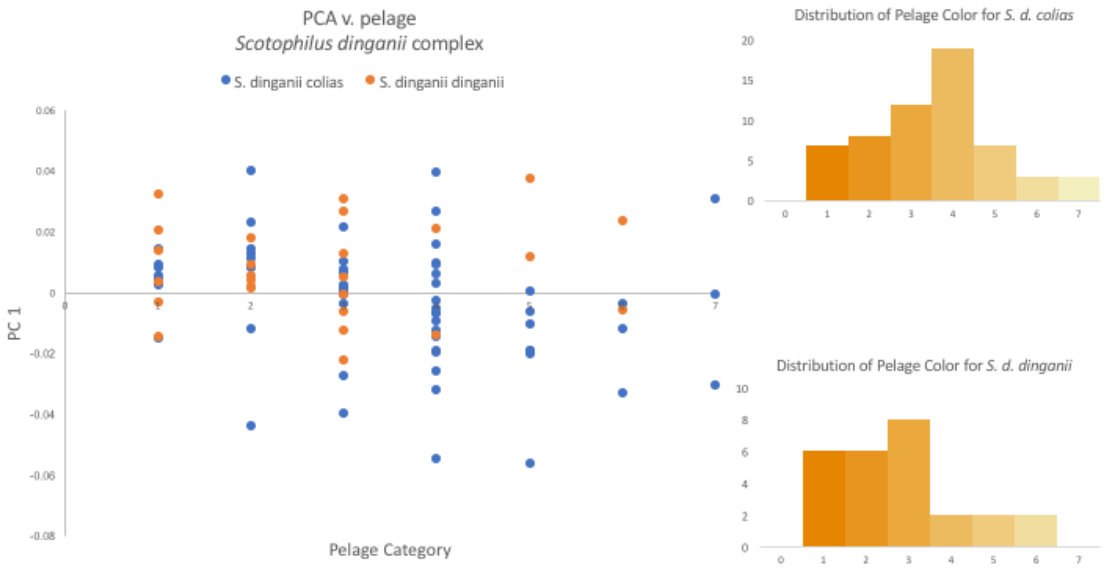

Figure 12. Variation in pelage color among S. dinganii colias *and* S. dinganii dinganii. *Scatterplot of pelage v. PC1 and histograms depicting pelage color distribution of the two species.*

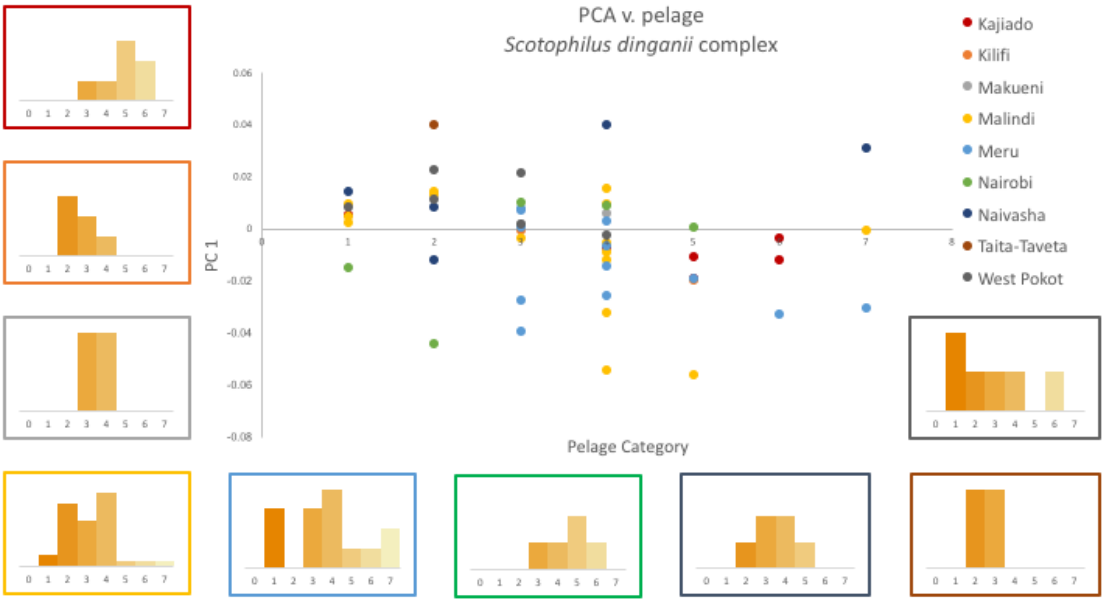

Figure 13. Scatterplot of pelage category v. PC1 for Kenyan S. dinganii *specimens. Histograms depict distribution of pelage for each Kenyan county.*

Echolocation Results

As an added layer to the morphological differences between *S. dinganii* specimens, I included echolocation call frequency data in the geometric morphometric analysis. The findings correspond with the previous GMAs in that there are species-specific clusters present, but there is broad overlap between the species (Figure 14). A statistical analysis of the echolocation call frequencies of *S. d. colias* and *S. d. dinganii* did not find a significant difference in call frequency (df=41, t=-0.207, p=0.849; Figure 24).

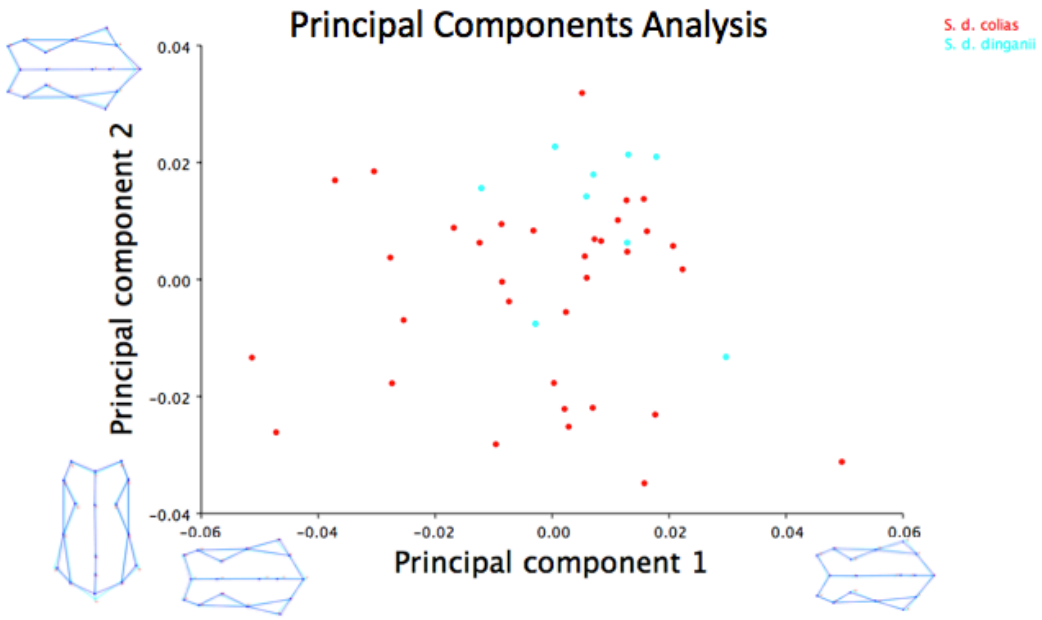

Figure 14. Bivariate scatterplot of PCA that includes echolocation call data as a geometric morphometric classifier for S. dinganii *complex specimens from Kenya.*

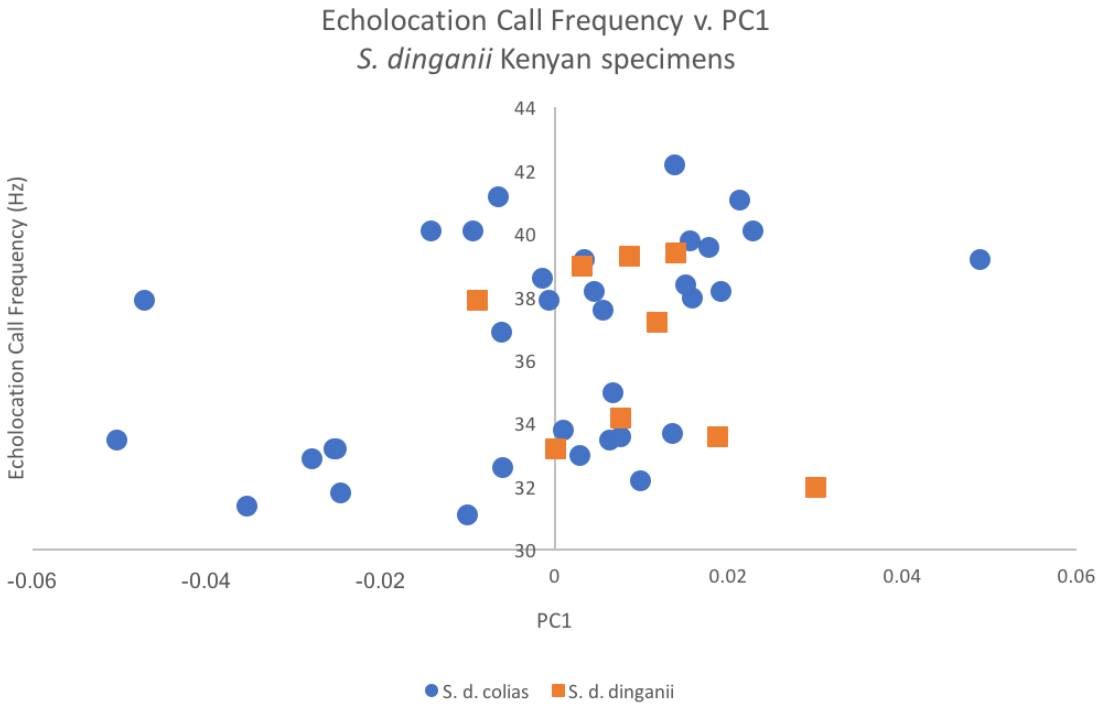

Figure 15. Scatterplot of echolocation call frequency data v. PC1 for S. dinganii *complex Kenyan specimens.*

CHAPTER 5: DISCUSSION

Geometric morphometrics.—Despite 200 years of scientific attention, *Scotophilus* taxonomy remains complicated and incompletely resolved. The geometric morphometric analysis of the crania and mandibles of *Scotophilus* species has helped to identify morphological variations that can aid in the classification of *Scotophilus*. GMA also identified variations between two subspecies of *S. dinganii*: *S. d. colias* and *S. d. dinganii*. My findings support the morphological distinctions between African *Scotophilus* taxa. Additionally, my results demonstrate that *S. d. colias* skulls are slightly shorter and narrower than *S. d. dinganii*. GMA conducted in this study have helped to quantify and solidify the morphological differences between presently defined species of *Scotophilus*. This study also illuminates previously unexamined morphological differences between *Scotophilus dinganii* subspecies. However, the findings of this analysis do not offer clear distinctions between *S. d. colias* and *S. d. dinganii* because they are so imprecise.

Cranial differences between *Scotophilus* species noted in prior taxonomic studies are also reflected in my geometric morphometric analyses (Figure 7, A8, A9). *S. leucogaster* is characterized by a smaller cranium and a wider and higher foramen magnum than *S. dinganii* (Vallo et al. 2011). *S. nux* is generally larger in size than *S. dinganii*, with a wider and less rounded braincase (Robbins et al. 1985), which was also supported by my analysis. *S. nux* can also be distinguished from *S. leucogaster* and *S. viridis* by its larger overall skull size. *S. leucogaster* and *S. dinganii* are also distinguished from *S. viridis* by their larger overall skull sizes. *S. nux* can also be distinguished from *S. leucogaster* and *S. viridis* by its larger overall skull size. *S. leucogaster* and *S. dinganii* are also distinguished from *S. viridis* by their larger skull sizes. In general, *S. robustus* can also be identified from the mainland species due to its large size and locality in

Madagascar. While *S. marovaza* is similar in size to the other *Scotophilus,* it can also be distinguished on geographic grounds as it is endemic to Madagascar.

The cranial differences between recognized species of *Scotophilus,* as described in the literature, are supported in my geometric morphometric analysis. Amongst the continental African *Scotophilus,* morphological differentiation among species is more pronounced than the types of variation observed between forms identified as *S. d. colias* and *S. d. dinganii*. Many species of *Scotophilus* can be discriminated by skull size, with many noticeably distinct in size, such as between *S. robustus* and *S. viridis*. However, the morphological differences in *S. dinganii* subspecies are less apparent, primarily observed with a much closer examination of the skulls.

Many landmarks were placed at locations on the cranium and mandible that highlighted the variations observed with the naked eye. However, a few observed differences were not bracketed by landmarks. Additionally, some of the selected landmarks did not reflect qualitative differences noted during the visual observation. Overall, the chosen landmarks highlighted most observable differences between the two forms of *S. dinganii,* perhaps exceeding the number of landmarks necessary for a geometric morphometric comparison of the species.

Based on my findings, I think the distinction between *S. d. colias* and *S. d. dinganii* is useful because the GMA found shape differences between the classified specimens. While these differences are rather minute and with overlap, the two subspecies demonstrate differences, such as braincase width and length of skull. The morphological characters that distinguish between the two subspecies would aid in identifying an unknown specimen in a laboratory setting.

Pelage and echolocation.—Since the discovery of *Scotophilus kuhlii* (Leach

1821) there have been misidentifications of specimens in the literature, often due to described pelage characteristics (Dobson 1875; Robbins 1978). According to Robbins (1978), many *S. dinganii* specimens that have light yellow to yellow-orange ventral pelage have been incorrectly classified as *S. nigrita*. Based on the taxonomic description of Smith (1833), these specimens should fall under *S. dinganii* classification. *S. dinganii* ventral pelage is described as pale yellow (Robbins 1978). A recent review of African *Scotophilus* (Goodman et al. 2005) describes the ventral pelage of *S. leucogaster* to be white to dirty brown; *S. nux* and *S. robustus* as medium brown, and *S. viridis* as white, grayish-white to yellowish. *S. dinganii* is described as having ventral pelage that is white to yellowish-orange. Jacobs et al (2006) described the ventral pelage of *S. dinganii* to be yellow and *S. viridis* to be colored white, gray, or brown ventrally. Their study, which identified a cryptic species in *S. dinganii*, suggests that morphology alone is not enough to distinguish the species. A previous study found that ventral pelage fails to diagnose *S. dinganii* from *S. leucogaster* (Vallo et al. 2011). Understandably, such confusions of morphologic characteristics result in the incorrect application of nomenclature and misidentifications.

My statistical analysis demonstrated significant differences between *S. leucogaster*, *S. marovaza*, *S. viridis*, and *S. robustus*. These differences are consistent with the morphologic descriptions as *S. robustus* has medium brown ventral pelage and *S. viridis* and *S. leucogaster* generally range from white to pale brown. A visual analysis of ventral pelage for recognized species of *Scotophilus* also agreed with previous studies taxonomic descriptions of pelage. The overlap present in the pelage analysis highlights the variation present in recognized species of *Scotophilus*. This variation in morphology has undoubtedly contributed to the complicated taxonomic history the genus has

experienced. The results of the pelage analysis presented here, when combined with the geometric morphometric findings, add another filter to the already recognized species in the *Scotophilus* genus.

The findings from the analysis from the specimens in the *S. dinganii* complex, however, paint another picture. A statistical analysis of classified *S. d. dinganii* and *S. d. colias* specimens indicate that there are significant differences in ventral pelage. Despite the statistically significant difference, there is too much overlap to assign a specimen to one or the other subspecies reliably based only on pelage color. Ventral pelage alone likely could not be used for correct application of a scientific name to an *S. dinganii* spp. bat when used in isolation alone. For the Kenyan *S. dinganii* complex specimens, there were not significant differences between the ventral pelage of *S. d. dinganii* and *S. d. colias*. It is possible that the small sample of *S. d. dinganii* specimens (n=10) prevented an accurate statistical analysis of pelage for Kenyan specimens. On the other hand, the significance found from the analysis of all *S. d. colias* and *S. d. dinganii* specimens might be due to pelage differences in these subspecies from another geographic locality. These results suggest that between species differences in morphology, such as ventral pelage, occur, but variation because of geographic locality is more pronounced than variation between the two subspecies.

The findings of the geometric morphometric and pelage analyses and the results of the echolocation call frequency data for Kenyan *S. dinganii* specimens do not suggest clear species boundaries. Jacobs et al. (2006) found 2 cryptic species of *S. dinganii* based on differences in echolocation call frequencies. Both of these cryptic species have yellow ventral pelage. Based on their findings, I expected to find significant differences in echolocation between *S. d. colias* and *S. d. dinganii* from Kenya, considering Jacobs et al.

(2006) demonstrated that echolocation might be a character for delineating species. However, no significant differences were statistically detected. Similar to the geometric morphometric studies, the results of echolocation demonstrated that wide variation within each subspecies and overlap between each subspecies was present. The study conducted here is a preliminary glance into echolocation data and species limits. Future studies should combine echolocation call data and location as classifiers into geometric morphometric analysis.

Conclusions.—The geometric morphometric findings agreed with previous studies that described *Scotophilus* taxon. Additionally, the results of the pelage analysis suggest discernible differences between *S. d. colias* and *S. d. dinganii,* although the wide variation within species makes it difficult to diagnose species in the field using ventral pelage as the preferred taxonomic character. The echolocation call frequency analysis does not significantly delineate *S. d. colias* and *S. d. dinganii* in Kenya, although inclusion of more samples may clarify these results.

Clearly, there is a need to further elucidate the species limits and range boundaries of *Scotophilus* taxa in continental Africa. Genetic studies will help to resolve the evolutionary relationships between species, but field identification still proves difficult. The species concepts will need to be used to decide on applicable scientific names for these bats. The PGSC should illuminate evolutionary relationships in the laboratory, but the MSC should be more useful in the field. If methods or technology are developed for studying the reproductive compatibility of bats in the field more readily, then the BSC would likely be of greater use in the field. More studies on the ecological behaviors of *Scotophilus* would help to identify interbreeding between populations of suspected species. The range of morphological variation due to geography of *Scotophilus* does not

simplify the process of determining application of scientific names, but continuing to quantify the differences will be useful in deciphering which morphological characters are most important. Mosaic evolution, the idea that different aspects of the phenotype evolve at different rates, should also be considered when applying species-level classifications (King 2006). Systematic revisions and future application of species names will need to be based on a combination of species concepts to avoid further confusion in the literature of the genus.

Including forearm lengths, echolocation call frequencies, and mitochondrial haplotypes in *Scotophilus* studies will help in identifying various characteristics to separate populations. Brooks and Bickham (2014) included specific skull measurements in their analysis of new *Scotophilus*, such as greatest skull length, zygomatic breadth, and braincase breadth. However, their measurements examine a single specimen, making application of their described species to the bats in this study impossible. Goodman et al. (2005) included wing measurements in their review of *Scotophilus.* Inclusion of skull and wing measurements aid in quantifying taxonomic character differences between species. Future studies should incorporate these characteristics in addition to the geography and morphology when characterizing different populations to determine the applicable scientific name. Application of the most appropriate scientific name would be useful for inferring evolutionary history, identifying ecological and genetic connections, determining conservation concerns, and recognizing public health concerns. While more genetic, morphometric, and environmental information is needed to classify these bats sufficiently and accurately, the geometric morphometric analysis conducted here is an important step in untangling *Scotophilus* taxonomy.

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APPENDIX: TABLES

Table A1. ***Specimens examined from the Field Museum of Natural History's collection in the geometric morphometric analysis.***

Species	Country of Origin	M	F
Scotophilus dinganii colias	DR of Congo	0	1
	Ivory Coast	1	0
	Kenya	25	19
	Senegal	0	1
	South Africa	0	2
	Tanzania	0	4
	Uganda	3	3
Scotophilus dinganii dinganii	Kenya	6	4
	South Africa	2	1
	Tanzania	1	1
	Zimbabwe	9	2
Scotophilus dinganii herero	Angola	1	0
Scotophilus leucogaster	Angola	1	0
	Kenya	2	0
	Namibia	1	0
	South Sudan	2	2
	Sudan	0	1
Scotophilus marovaza	Madagascar	7	4
Scotophilus nux	Kenya	0	1
	Uganda	1	3
Scotophilus viridis	Ivory Coast	2	1
	Malawi	0	1
	Mali	1	0
	Rwanda	0	1
	South Africa	1	0
	Tanzania	1	1
Combined Total		**75**	**65**

Table A2. ***Descriptions of landmarks used in the geometric morphometric analysis.***

DORSAL	1	Anterior midline point of palate between central incisors
	2	Junction of interparietal, parietal, and sagittal sutures
	3	Junction of frontal, parietal, and sagittal sutures
	4	Junction of frontal, nasal, and sagittal sutures
	5	Posterior projection of occiput
	6, 7	Posterior projection of supraoccipital margin
	8, 9	Mastoid point of maximum curvature
	10, 11	Posterior limit of interior orbit
	12, 13	Anterior point of interior orbit
	14, 15	Midpoint between posteriormost cheektooth
	16, 17	Anterior most point of premaxilla
VENTRAL	1	Anterior midline point of palate
	2	Anterior margin of foramen magnum
	3	Posterior projection of the palatine
	4	Midpoint of the supraoccipital suture
	5	Posterior margin of foramen magnum
	6, 7	Posterior margin of mastoid
	8, 9	Most anterior margin of mastoid
	10, 11	Maximum curvature of posterior margin of zygomatic
	12, 13	Tip of palatal process
	14, 15	Midpoint between posteriormost cheektooth
	16, 17	Midpoint between anteriormost cheektooth
MANDIBLE	1	Anteriormost point on incisor alveolus
	2	Posteriormost point on canine alveolus
	3	Glenoid fossa on last molar alveolus
	4	Dorsal most extension of the coronoid process
	5	Anterolateral tip of condilar process
	6	Dorsal tip of angular process
	7	Posterior-most point on the baseline perpendicular to the landmark 5
	8	Anterior-most point on the baseline perpendicular to the landmark 3
	9	Dorsal-most point on the border of the mandible
	10	Anterior lower border of the mandible

Table A3. ***Specimens examined from FMNH collection in the pelage analysis***.

Species	Country of Origin	M	F	Unknown
Scotophilus	Kenya	9	6	
	Tanzania	5	2	
Scotophilus dinganii	DR of Congo	1	1	
	Ghana	1	1	
	Ivory Coast	1	0	
	Kenya	54	42	1
	Senegal	3	6	
	South Africa	0	2	
	Tanzania	3	4	
Scotophilus dinganii colias	Kenya	9	12	
	South Africa	2	1	
	Tanzania	1	5	
	Uganda	3	3	
Scotophilus dinganii dinganii	Zimbabwe	9	2	
Scotophilus dinganii herero	Angola	3	2	
	Namibia	1	5	
Scotophilus leucogaster	Angola	0	1	
	Ethiopia	0	3	
	Ivory Coast	0	2	
	Kenya	2	1	
	Namibia	2	3	
	South Sudan	7	4	
	Sudan	4	2	
	Zambia	1	0	
Scotophilus marovaza	Ghana	1	0	
	Madagascar	3	4	
Scotohphilus nux	Kenya	0	1	
	Uganda	1	3	
Scotophilus robustus	Madagascar	6	11	
Scotophilus viridis	Ivory Coast	2	1	
	Malawi	0	1	
	Mali	3	0	
	Rwanda	0	3	
	Senegal	0	1	
	South Africa	1	0	
	Tanzania	3	2	
Combined Total		**141**	**137**	**1**

Table A4. ***Procrustes distances among groups from DFA for all continental* Scotophilus *specimens in the dorsal dataset.***

	***Dinganii* complex**	***Leucogaster***	***Nux***
S. leucogaster	0.04976265		
S. nux	0.03927849	0.02453956	
S. viridis	0.02496233	0.03972987	0.03204723

Table A5. ***Ventral dataset p-values from permutation tests (1000 permutation rounds) for Procrustes distances among groups.***

	***Dinganii* complex**	***Leucogaster***	***Nux***
S. leucogaster	<.0001		
S. nux	<.0020	0.0360	
S. viridis	<.0001	0.0740	0.0560

Table A6. ***Procrustes distances among groups from DFA for all continental* Scotophilus *specimens in the ventral dataset.***

	***Dinganii* complex**	***Leucogaster***	***Nux***
S. leucogaster	0.03281484		
S. nux	0.03193502	0.02153838	
S. viridis	0.02885138	0.01916200	0.02140623

Table A7. ***Mandibular dataset p-values from permutation tests (1000 permutation rounds) for Procrustes distances among groups.***

	***Dinganii* complex**	***Leucogaster***	***Nux***
S. leucogaster	<.0001		
S. nux	0.1420	0.0120	
S. viridis	<.0001	0.0690	0.1210

Table A8. ***Procrustes distances among groups from DFA for all continental* Scotophilus *specimens in the mandibular dataset.***

	***Dinganii* complex**	***Leucogaster***	***Nux***
S. leucogaster	0.03430926		
S. nux	0.02020638	0.03553258	
S. viridis	0.02944580	0.02350747	0.02605897

Table A9. ***Cross-validation of* S. d. colias *and* S. d. dinganii *in the ventral dataset.***

Classification/misclassification table - Cross-validation			
	S. d. colias	*S. d. dinganii*	Total
S. d. colias	43	17	60
S. d. dinganii	7	22	29

Table A10. ***Cross-validation of*** **S. d. colias** ***and*** **S. d. dinganii** ***in the mandibular dataset.***

Classification/misclassification tables – cross-validation			
	S. d. colias	S. d. dinganii	Total
S. d. colias	47	12	59
S. d. dinganii	5	20	25

APPENDIX: FIGURES

Figure A1. ***Map of specimens examined from FMNH for the geometric morphometric analysis.***

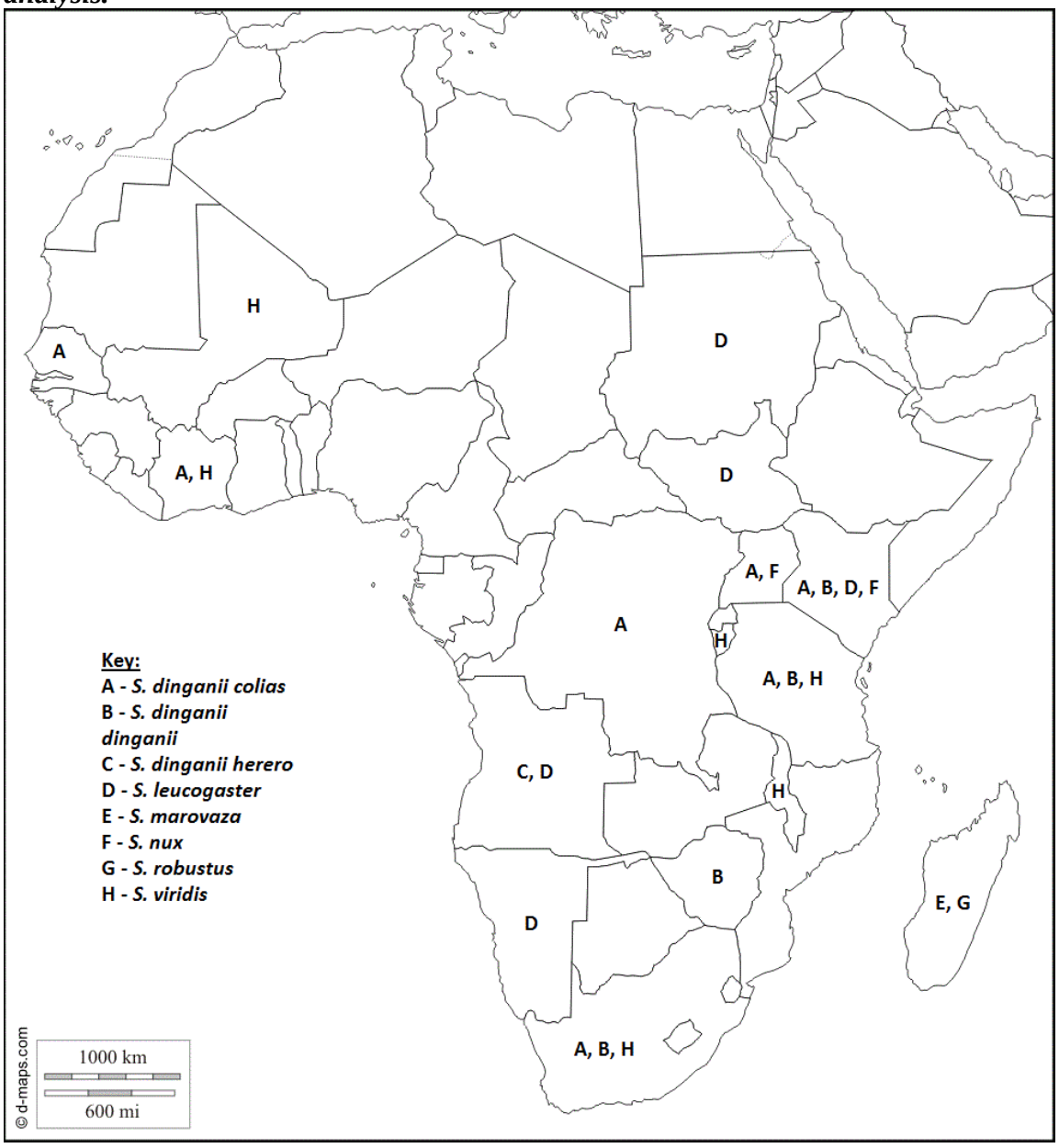

Figure A2. ***Landmark locations used in the geometric morphometric analysis.***

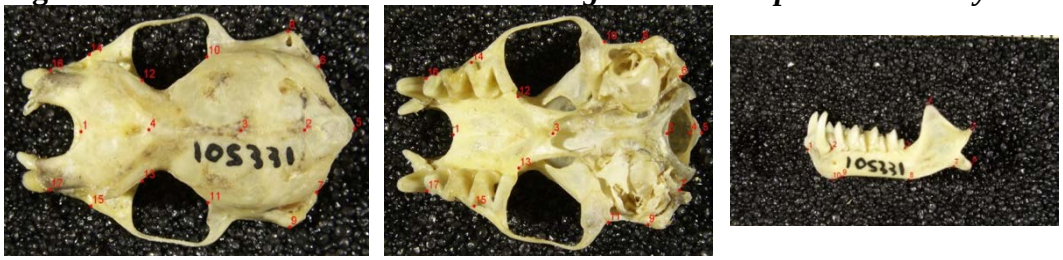

Figure A3. ***Map of country of origin for specimens examined in the pelage analysis. Histograms represent pelage categorization for specimens from that country.***

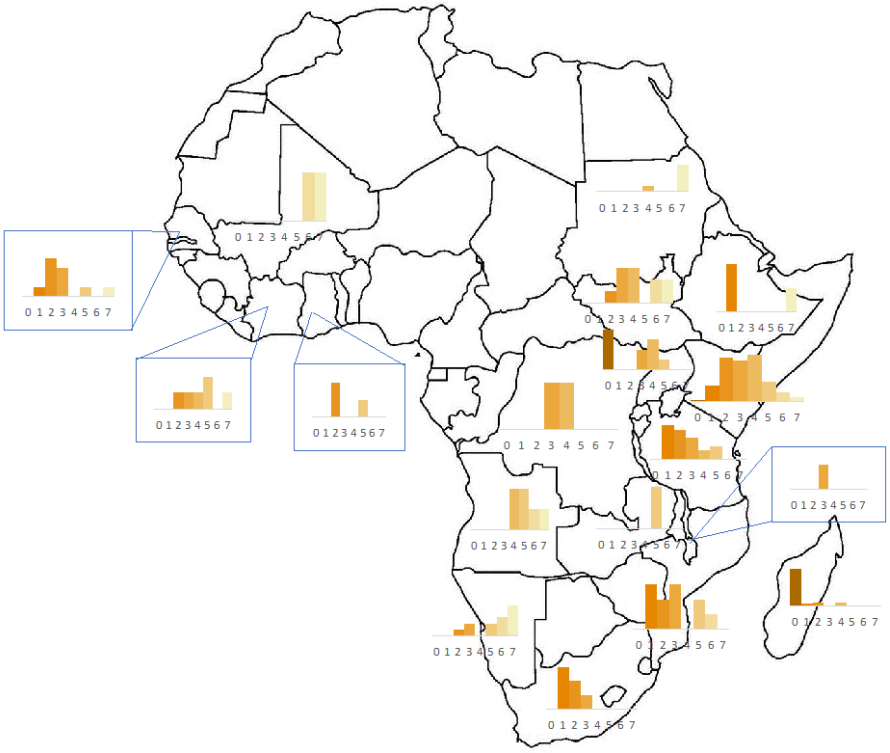

Figure A4. ***Bivariate scatterplots of PCA for all examined species of*** **Scotophilus,** ***ventral dataset.***

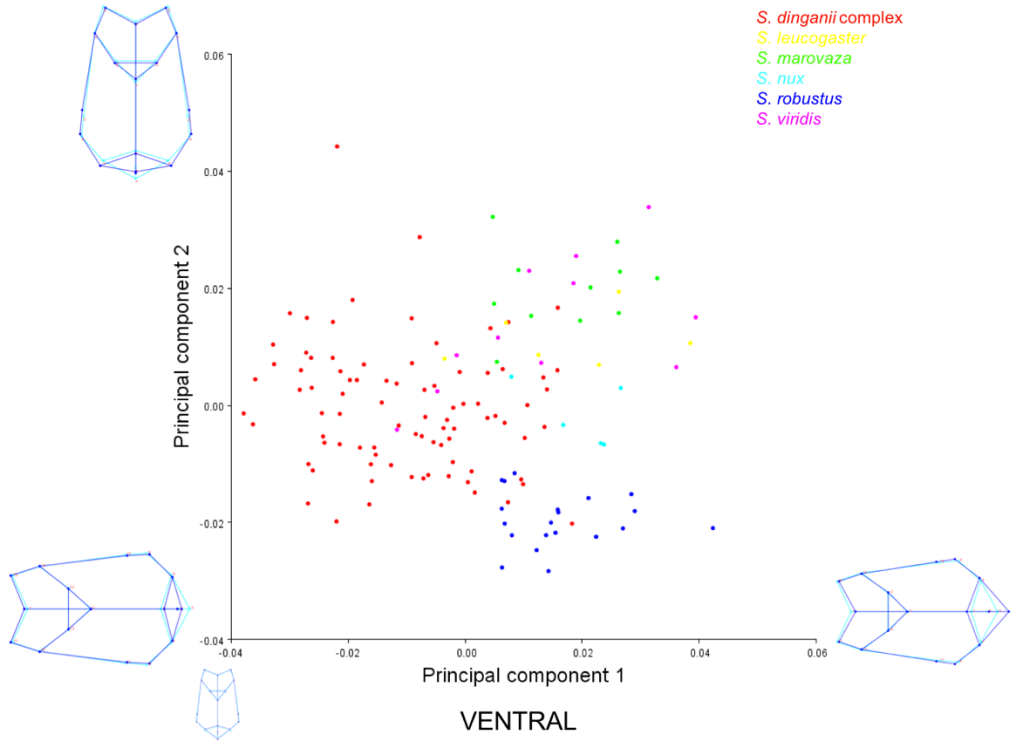

Figure A5. ***Bivariate scatterplots of PCA for all examined species of*** **Scotophilus,** ***mandibular dataset.***

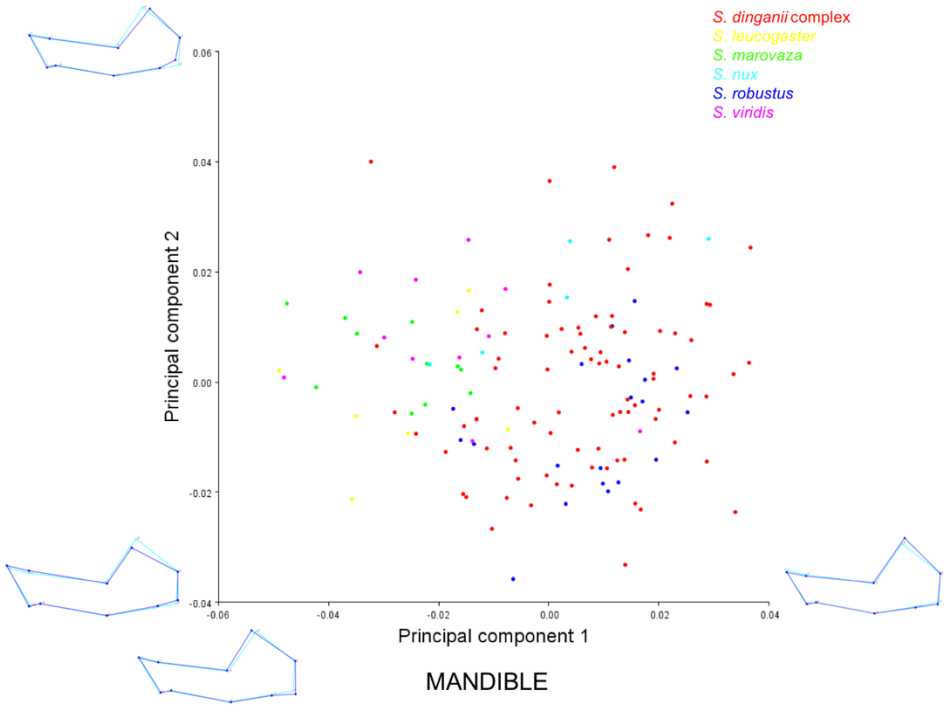

Figure A6. ***Histogram of CVA for*** **S. dinganii** ***species examined in the ventral dataset.***

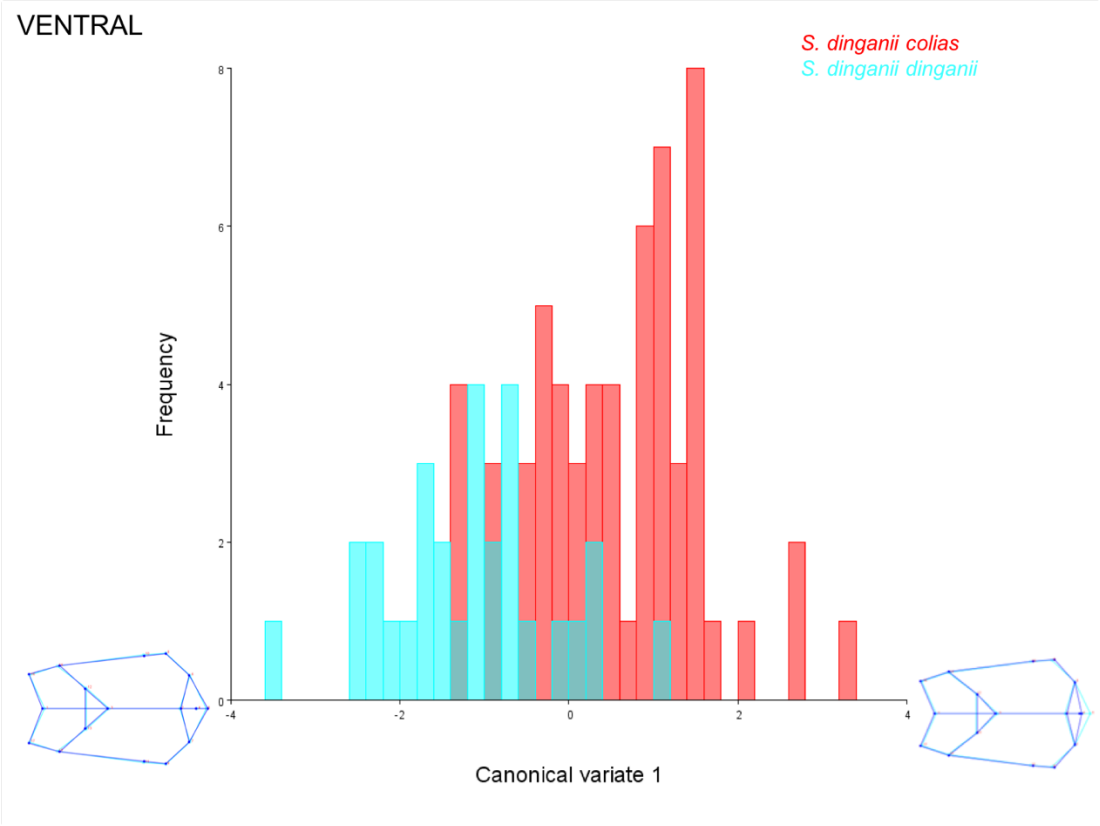

Figure A7. ***Histogram of CVA for*** **S. dinganii** ***species examined in the mandibular dataset.***

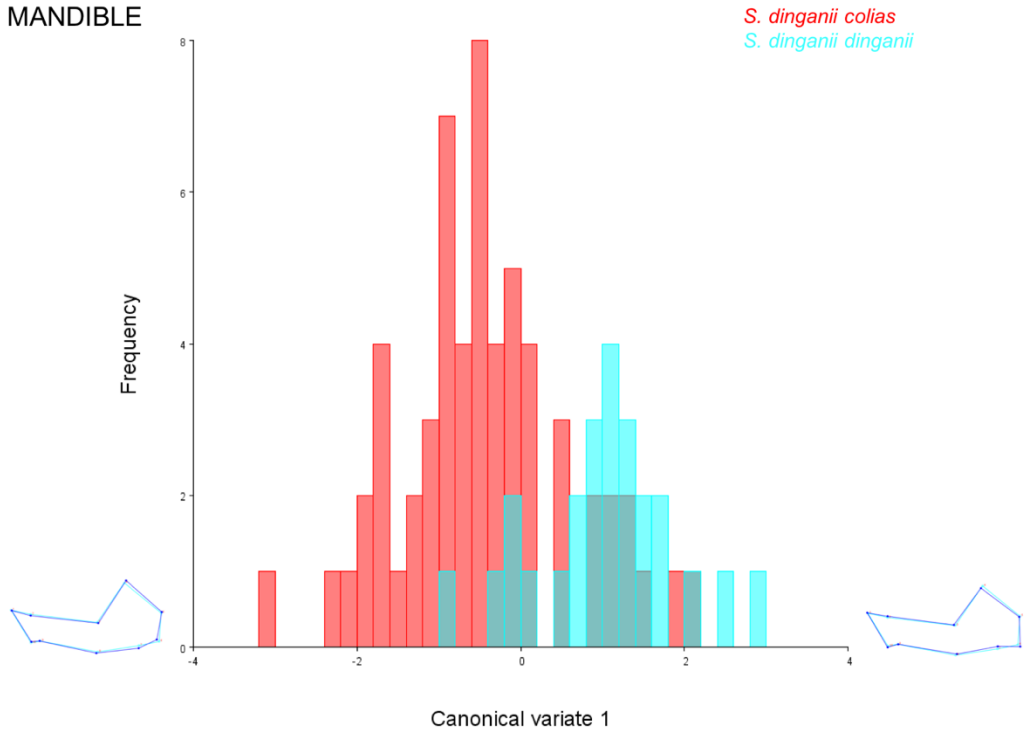

Figure A8. ***Scatterplots of CVA for ventral dataset of the*** **S. dinganii** ***species complex.***

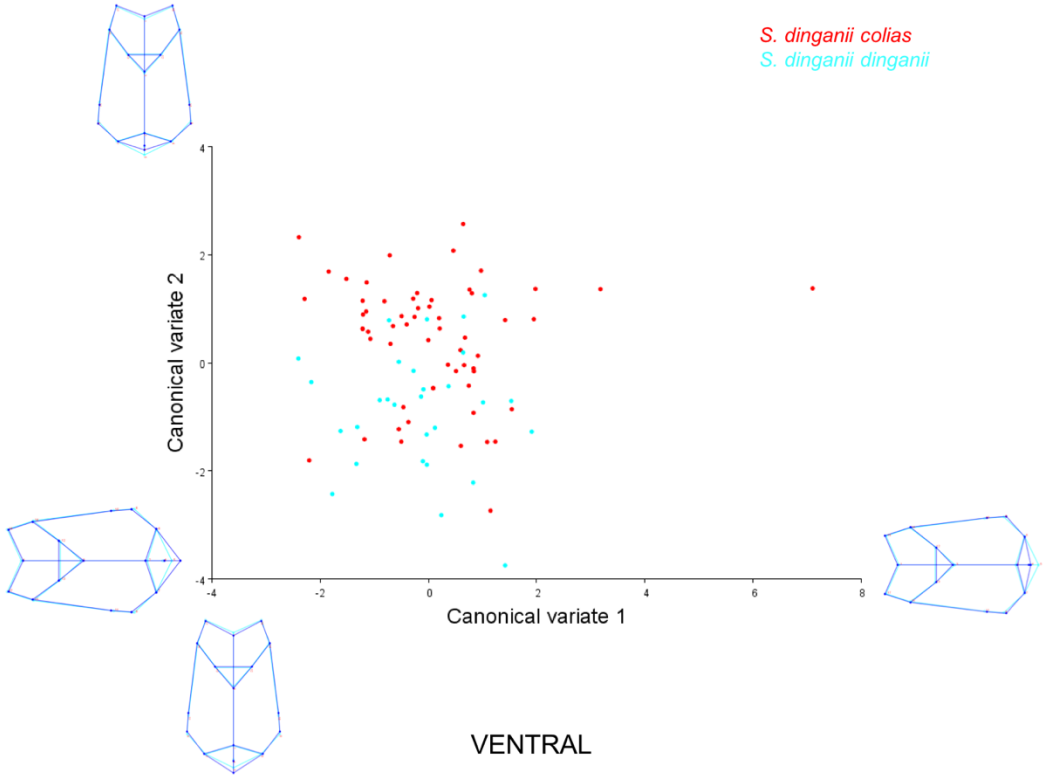

Figure A9. ***Scatterplots of CVA for mandibular dataset of the*** **S. dinganii** ***species complex.***