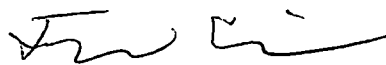


To the Graduate Council:

I am submitting herewith a dissertation written by Charles Gregory Anderson entitled "Continuous Morphological Data in Phylogenetic Analyses: An Example from the Otters (Carnivora: Lutrinae)." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.



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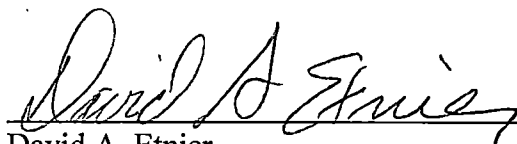


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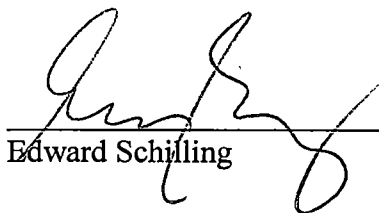
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**CONTINUOUS MORPHOLOGICAL DATA
IN PHYLOGENETIC ANALYSES:
AN EXAMPLE FROM THE OTTERS
(CARNIVORA: LUTRINAE)**

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Charles Gregory Anderson
May 2001

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Dedication

This dissertation is dedicated to my wife

Karen Rankin Anderson

and my mother

Dr. Judith W. McMahon, Ph.D.

who have both provided unwavering support and love through the years.

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I am very grateful to many people that have helped me complete this dissertation, without whom its completion would have been impossible. I owe much to the faculty and graduate students at the University of Tennessee. In particular I would like to thank my advisor, John Gittleman for his advice, use of equipment, and his mentorship over the last 7 years. I thank the other members of my Committee, Sandy Echternacht, Dave Etnier, and Ed Schilling for their support and cooperation. Their willingness to work with me made a long distance defense far less harrowing than it could have been. The Department of Ecology and Evolutionary Biology and the now defunct Department of Zoology and also deserve thanks for funding, support, and awards over the years.

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Lastly, I would like to give the greatest thanks to my wife, Karen. Without her never ending love, faith, encouragement, and support I could never have reached this goal. I can't express my thanks strongly enough. Thank you, with all my love.

Abstract

In this study, otter (Carnivora: Lutrinae) postcranial osteological measurements were used in a phylogenetic analysis. Previous systematic analyses of the otters (based primarily on discrete cranial characters) yield conflicting results leading to uncertainty regarding the evolutionary history of this group. Continuous postcranial trait data were analyzed using several approaches and techniques. In Chapter 2, continuous data from postcranial morphological measurements were converted into discretely coded characters using several methods. Two types of gap coding (Gap A and Gap B) and two segment coding approaches (12 and 6 segments) were applied and analyzed using a maximum parsimony reconstruction program. The resulting phylogenies appeared to be size related, grouping otters of similar size together. To remove the size related component, the continuous trait data were entered into a maximum likelihood analysis program without coding in Chapter 3. Regression residuals produced from a regression of body size and character measurements were analyzed using the same program. Results from this analysis were mixed. Uncoded data also yielded size related groupings, whereas the regression residuals appeared to have little biological relevance. Results from all the analyses were combined in Chapter 4 to produce a consensus phylogeny. To construct a consensus, data combining, tree combining, and consensus methods were employed. The resulting consensus tree essentially mirrored the majority of results from the source phylogenies. Overall, although the resulting phylogenies were robust and well supported by bootstrap analyses, the essentially size related input data (except regression residuals) resulted in size related phylogenies, and this approach should be undertaken with caution.

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Part 1

Otter Systematics: Statement of the Problem and Approaches

Introduction

The phylogeny of the otters (subfamily Lutrinae) is the focus of this study because this group is a good test case for investigating the use of several continuous data analysis techniques on postcranial data. The otters provide a systematic group with various competing evolutionary hypotheses, heterogeneous characters, and available samples for character measurement. Several different approaches were examined in an effort to determine the utility of continuous character trait data in phylogenetic analyses. Different methods of data treatment were combined with various methods of analysis.

Phylogenetic trees produced from different discrete coding techniques in a maximum parsimony analysis were compared for congruence in Chapter 2. In Chapter 3, cladograms produced by a maximum likelihood analysis (utilizing uncoded continuous trait data and regression residuals) were compared with the maximum parsimony analysis results from Chapter 2. In Chapter 4, consensus and combined data phylogenies were constructed by combining the phylogenies, cladograms, and data matrices produced in Chapters 2 and 3. The results were then compared with the previous phylogenetic hypotheses described in detail below.

Previous phylogenetic hypotheses

The most widely accepted current taxonomy of the Lutrinae is shown in Table 1-1 (with the addition of the genus *Hydrictis*). There have been at least 10 systematic studies of the Lutrinae in the last 30 years (Table 1-2). As shown by the results of these studies (Table 1-3), there are few points of consensus among these analyses (van Zyll de Jong 1987; Koepfli and Wayne 1998). Except for the subfamily name, there have been disagreements over almost every classification level of the otters; some appear systematic but are actually nomenclatural (see discussion of *Hydrictis* below).

The focus of this study was to reevaluate these systematic positions and produce a phylogenetic picture of the Lutrinae based on new lines of evidence. New continuous trait data were analyzed using various phylogenetic approaches and methodologies for dealing with continuous traits to clarify otter evolutionary patterns. This multifaceted approach provides results which were used to test both the many otter phylogenetic hypotheses proposed to date, as well as proposed outgroup species.

In addition to uncertainty regarding the evolutionary history of the Lutrinae, there are also several proposed sister taxa hypotheses. In an effort to clarify some of these disagreements, putative species purported to be closely related to the otters were examined. Various authors propose different outgroup species to the otter. Several recent studies (see discussion below) propose the skunks as the sister taxa to the otters, an interesting proposal in view of the recent controversial familial elevation of the skunks. Otters comprise the subfamily Lutrinae within the family Mustelidae (weasels and allies), which until recently also contained the Mephitinae (skunks).

Table 1-1. Otter and outgroup species with current distributions. Taxa not used in this study due to a lack of specimens are indicated as not available (modified from Mason 1990, and Wozencraft 1993).

Species Name Nomenclature Source	Common name	Current Distribution
<i>Amblonyx cinereus</i> (Illiger, 1815)	Asian small-clawed otter	Asia
<i>Aonyx capensis</i> (Shinz, 1827)	Cape clawless otter	Central and southern Africa
<i>Aonyx congicus</i> (not available) (Lönnberg, 1910)	Congo clawless otter	Equatorial Africa
<i>Enhydra lutris</i> (Linnaeus, 1758)	Sea otter	Pacific North America, Russia
<i>Hydrictis maculicollis</i> (Lichtenstein, 1835)	Spot-necked otter	Sub-Saharan Africa
<i>Lontra canadensis</i> (Schreber, 1777)	North American river otter	Canada, United States
<i>Lontra felina</i> (not available) (Molina, 1782)	Marine otter	Pacific South America
<i>Lontra longicaudis</i> (Olfers, 1818)	Neotropical otter	Mexico, Central and South America
<i>Lontra provocax</i> (not available) (Thomas, 1908)	Southern river otter	Southern Argentina and Chile
<i>Lutra lutra</i> (Linnaeus, 1758)	Eurasian river otter	Eurasia, North Africa
<i>Lutra sumatrana</i> (not available) (Gray, 1865)	Hairy-nosed otter	Southeast Asia
<i>Lutrogale perspicillata</i> (Geoffroy, 1826)	Indian smooth-coated otter	Asia
<i>Pteromura brasiliensis</i> (Gmelin, 1788)	Giant otter	South America
<i>Mustela vison</i> (Schreber, 1777)	American mink	Canada, United States
<i>Mephitis mephitis</i> (Schreber, 1776)	Striped skunk	Canada, United States, Northern Mexico
<i>Ictonyx striatus</i> (Perry, 1810)	Zorilla	Africa

Table 1-2. Previous Systematic and Taxonomic Studies of the Lutrinae.

Source	Method	Problems
Harris, 1968	Taxonomy	No phylogenetic implications, only nomenclatural proposals
van Zyll de Jong, 1972	Phenetic	Analysis phenetic in nature, using Mahalanobis distances and UPGMA clustering Focus on New World taxa only
Sokolov, 1973	Evolutionary Taxonomy	Analysis to genus level only, with emphasis on suprageneric groupings Evolutionary taxonomy approach based on Recent and limited fossil taxa
Davis, 1978	Phylogenetic	No critical character analysis; subjective analysis of vocal characters No synapomorphies identified Character choice and coding deemed questionable by most subsequent workers Oversimplification of numerous characters
de Muizon, 1982	Phylogenetic	Few characters and genera analyzed, deals with relationship of otters to seals Methods of analysis unclear
van Zyll de Jong, 1987, 1991	Phenetic	Analysis based on phenetics Few characters analyzed
Holmes, 1987	Phylogenetic	Of 72 synapomorphies listed, 42 are actually autapomorphies Method of phylogenetic analysis not specified No consistency in traits used across taxa
Willemssen, 1992	Taxonomic and Phylogenetic	Emphasis on fossil forms No clear analysis, use of evolutionary systematics instead of phylogenetics
Bryant et al., 1993	Phylogenetic	Emphasis on subfamilial relationships Only four genera of Lutrinae included, with no resolution within the Lutrinae
Koepfli and Wayne, 1998	Phylogenetic	Gene trees are not always representative of species trees Hierarchical relationships of some genera poorly supported Monophyly of otters obscured by possible long branch attraction of <i>Pteromura</i> Incomplete data set hindered interpretation of some results
Bininda-Emonds et al., 1999	Phylogenetic consensus	Use of several of the above systematic analyses to produce the consensus renders it susceptible to the same weaknesses in those studies

Table 1-3. Previous systematic hypotheses of the Lutrinae.

Phylogenetic Question	Hypotheses and Sources
Relationship between Old World and New World river otters	Monophyletic: Davis (1978), Bryant et al. (1993), Bininda-Emonds et al. (1999) Diphyletic: van Zyll de Jong (1991), Koepfli and Wayne (1998)
Relationship between African clawless and Asian small-clawed otters	Monophyletic: Koepfli and Wayne (1998), Bryant et al. (1993), Diphyletic: Sokolov (1973) Holmes (1987), van Zyll de Jong (1991), Willemsen (1992), Bininda-Emonds et al. (1999)
Relationship of African clawless and Asian small-clawed otters to Old and New World otters	Allied with Eurasian otter: Koepfli and Wayne (1998) African forms allied with New World otters, Asian small-clawed basal to sea and New World river otters: van Zyll de Jong (1991) Allied with giant/smooth-coated/spot-necked otter clade: Holmes (1987) Undetermined: Bininda-Emonds et al. (1999)
Relationships of India smooth-coated otter	Allied with spot-necked otter: Holmes (1987) Allied with giant and spot-necked otter: van Zyll de Jong (1991), Bininda-Emonds et al. (1999)
Relationships of spot-necked otter	Sister taxon to smooth-coated otter: Holmes (1987) Allied with giant and smooth-coated otter clade: van Zyll de Jong (1987; 1991), Bininda-Emonds et al. (1999)
Relationships of sea otter	Basal to all other forms: Holmes (1987), van Zyll de Jong (1991), Willemsen (1992), Bininda-Emonds et al. (1999) Allied with Eurasian/spot-necked/clawless/small-clawed otters: Koepfli and Wayne (1998)
Relationships of giant otter	Basal to all other forms: Koepfli and Wayne (1998) Allied with <i>Lutra</i> /spot-necked/smooth-coated otter clade: van Zyll de Jong (1991), Bininda-Emonds et al. (1999) Allied with <i>Lutra/Lontra</i> clade: Sokolov (1973) Allied with spot-necked and smooth-coated otters: Holmes (1987)

Outgroup species

Hypotheses regarding which subfamily the sister group to the Lutrinae vary widely. Authors have concluded in various studies based on a wide variety of molecular, morphological, and fossil data, that the subfamilial sister group to the otters is variously the Melinae (badgers) (Willemsen 1992; Bryant et al. 1993), Mustelinae (weasels) (Wayne et al. 1989; Koepfli and Wayne 1998), or the recently extra-familial Mephitidae (skunks) (Hunt 1974; Holmes 1987; Wozencraft 1989; Bryant et al. 1993; Wyss and Flynn 1993; Dragoo and Honeycutt 1997; Bininda-Emonds et al 1999; Wolsan 1999).

The recently posed elevation of skunks to familial status (Mephitidae) from the subfamily Mephitinae within the Mustelidae is a controversial proposal that is slowly gaining tacit approval (Dragoo and Honeycutt 1997; Wilson 1999; but see Wolsan 1999). In some studies a somewhat enigmatic African mustelid, the zorilla (*Ictonyx striatus*), has also been proposed to be the sister taxon to the otters (Dragoo and Honeycutt 1997; Koepfli pers. comm.). Because of this debate, two within-family and one extra-familial outgroup species were chosen: Mustelidae: American mink (*Mustela vison*) and zorilla (*Ictonyx striatus*), Mephitidae: striped skunk (*Mephitis mephitis*). These outgroups were used to test conflicting sister group hypotheses and attempt to clarify the systematic position of the Lutrinae relative to these species. An interesting but unintended result of the inclusion of the striped skunk (*Mephitis mephitis*) as an outgroup may be clarification of the recent systematic and taxonomic elevation of the family Mephitidae. If skunks were placed as sister taxa to otters, their familial elevation was questionable, because the otters were consistently placed within the family Mustelidae.

Tribe

Within the Lutrinae, at least five tribes have been proposed: Aonyxini, Aonychini, Lutrini, Hydriactini, and Enhydrini (Sokolov 1973; Davis 1978; Willemsen 1992).

Support for these tribes varies, as do the species comprising them. Some advocate only two tribes (Lutrini and Aonyxini), with Enhydrini subsumed within the Aonyxini (van Zyll de Jong, 1991). Davis (1978) proposes three tribes (Aonychini, Lutrini, Hydriactini), Hydriactini being comprised solely of the spot-necked otter *Hydriactis maculicollis* and the Lutrini comprised of the genus *Lutra* (sensu Davis, which included all members of the genus *Lontra*). Davis' Aonychini is comprised of the genera *Aonyx* (which contains *Amblonyx*), *Pteronura*, *Lutrogale*, and *Enhydra*. Davis' classification is not widely accepted due to a lack of character analysis, priority of Sokolov's Aonyxini over Davis' Aonychini, and the disparate nature of species placed within that tribe (van Zyll de Jong 1991; Willemsen 1992). Willemsen (1992) follows the tribal classification of Sokolov (1973) for extant otters, but differs in placement of fossil forms. Results from a recent molecular analysis seem to support three tribes (Koepfli and Wayne 1998).

In a cytochrome *b* mitochondrial DNA analysis, Koepfli and Wayne (1998) show three major clades within the Lutrinae which, due to an incomplete data set (only 9 of 13 species), were not proposed by the authors (Koepfli and Wayne 1998; Koepfli pers. comm.). These clades, while not proposed as tribes, fall out at the tribal level and are composed of taxa differing from previously described tribes (Sokolov 1973; Davis 1978; Willemsen 1992). Decisions regarding species comprising these higher level groupings and agreement with previous hypotheses should be viewed as tentative.

Genus level

Authors have recognized between four and eight genera of extant otters (Harris 1968; Davis 1978; Mason and Macdonald 1986; van Zyll de Jong 1987; 1991; Foster-Turley et al. 1990; Nowack 1991; Wozencraft 1993). Most recent authors recognize seven genera (Wozencraft 1993; Russell et al. 1998; Bininda-Emonds et al. 1999). Others have retained a more conservative taxonomic approach recognizing only six genera (Holmes 1987; Willemsen 1992; Koepfli and Wayne 1998) differing in their composition of taxa. Willemsen (1992) in recognizing six genera, asserts that *Lontra* and *Hydrictis* are junior synonyms of *Lutra*, and that *Lutrogale* should be separated, as should *Aonyx* and *Amblonyx*. Two genera are generally undisputed (*Enhydra* and *Pteronura*) and are recognized by Willemsen. The generic status of *Lutrogale* has not been recognized in all recent classifications and it is sometimes subsumed into the genus *Lutra* (Honacki et al. 1982; Corbet and Hill 1991; Nowack 1991). Based on differences illustrated by several studies, most current authors recognize *Lutrogale* as valid (van Zyll de Jong 1991; Corbet and Hill 1992; Willemsen 1992; Wozencraft 1993; Sivasothi and Nor 1994; Koepfli and Wayne 1998; Russell et al. 1998; Bininda-Emonds et al. 1999).

Koepfli and Wayne (1998) find support for the separation of *Lontra* and *Lutra* in accordance with others (van Zyll de Jong 1972; 1987; 1991; Kellnhauser 1983), but not for the separation of *Aonyx* and *Amblonyx*. A possible reason for this result is that no samples of *Aonyx congicus* were available, hence a presumably closely related *Amblonyx* might tend to fall out as a congener of *Aonyx* rather than as sister taxon to a monophyletic *Aonyx* group. Analyses with a complete sample set may provide different results.

Koepfli and Wayne (1998) find evidence for the separation of the subgenus *Hydrictis* from the genus *Lutra*, and although their evidence was strong, they withheld recommending that taxonomic split pending analysis of more species. Harris places *Hydrictis* as a subgenus of *Lutra* (Harris 1968), an approach that has had little support. In two recent phylogenies (Koepfli and Wayne 1998; Bininda-Emonds et al. 1999), the genus *Lutra* was found to be polyphyletic. This is due to the use of the generic appellation *Lutra* for the spot-necked otter (*Hydrictis maculicollis*). The separation of *Hydrictis* from the Eurasian otter (*Lutra lutra*) in their analyses results in a polyphyletic *Lutra*. If the generic appellation *Hydrictis* is maintained, *Lutra* remains monophyletic and separate from the monotypic *Hydrictis*. The recognition of the genus *Hydrictis* is maintained here in agreement with several authors (Davis 1978; Zachary and McDonald 1981; Duplaix 1984; Holmes and Hoffmann 1986; Holmes 1987).

Species level

At the species level, the controversies are less critical and are centered on the validity or separation of a few species. One such difference concerns the separation of *Aonyx capensis* and *A. congicus* (Foster-Turley et al. 1990), with most authors favoring their separation. There has also been some debate regarding the separation of *Lutra sumatrana* and *Lutra lutra* (Davis 1978; Chanin 1985) and, apart from the two works cited, most authors recognize *L. sumatrana* as distinct (van Zyll de Jong 1991; Corbet and Hill 1991; 1992; Wozencraft 1993; Koepfli and Wayne 1998).

Davis (1978) attempts to synonymize all species of the New World genus *Lontra*

(i.e. *L. longicaudis*, *L. provocax*), with *Lontra canadensis* (with the exception of *Lontra felina* which he said showed "...great geographical variation." Davis 1978, p. 21). This hypothesis never received much support (Chanin 1985 is one of the few to follow Davis' 1978 taxonomy) and is rarely even cited in germane literature except for a brief refutation by Willemsen (1992). There has recently been a new species described from Japan, *Lutra nippon* (Imaizumi and Yoshiyuki 1989), but to date there has been no independent confirmation as to its validity, and most authors have chosen to either ignore this species completely or synonymize it with *Lutra lutra* (Wozencraft 1993). No samples of *Lutra nippon* were available for analysis in this study.

Techniques and methods used in this study

Searching for consensus amid incongruity

With the competing phylogenetic hypotheses (based primarily on discrete cranial trait characters) shown in Table 1-3 and discussed above, the search for congruity based on other lines of evidence becomes paramount. By using new data in new ways, new hypotheses can be proposed that may provide support for, and contribute to, a consensus regarding the evolutionary history of otters.

In a recent study by Bininda-Emonds et al. (1999), a phylogenetic carnivore supertree (Sanderson et al. 1998) of all 271 extant species (sensu Wozencraft 1993) was constructed. By employing a matrix representation using parsimony analysis (MRP) (Baum 1992; Ragan 1992), 177 source phylogenies were combined in an unprecedented way, postulating several alternative carnivore evolution hypotheses (Bininda-Emonds et

al. 1999). As part of this comprehensive phylogeny, a composite tree of the otters was constructed by combining five previous phylogenies (Figure 3, Bininda-Emonds et al 1999), three of which were resolved above the level of genus. The composite phylogeny was essentially identical to one of the species level source trees (Holmes 1987), except several nodes resolved in that phylogeny were transformed into soft polytomies. This combination via associated trees demonstrates how one method (MRP) can generate new hypotheses by combining data (see also Bininda-Emonds and Bryant 1998). In the following chapters, different approaches (some new) are taken using postcranial data and then combined to investigate the evolutionary history of the otters.

Coded continuous data in a parsimony analysis

In Chapter 2, continuous trait data taken from postcranial measurements are transformed into discrete trait data using different coding procedures. The application of discrete coding to continuous data is a highly debated topic (Crisp and Weston 1987; Pimentel and Riggins 1987; Cranston and Humphries 1988; Farris 1990; Quicke 1993). Critics claim that the discretization of continuous data is a subjective approach that results in information loss and inaccurate phylogenetic estimates (Felsenstein 1988a; 1988b). Advocates claim some methods can reduce or eliminate these problems and allow data to be included in parsimony analyses that would otherwise be omitted (Archie 1985; Goldman 1988). Two types of “gap coding” and two “segment coding” analyses are performed and the four results are compared using tree statistics. The efficacy of the methods are discussed and related to previous otter systematic hypotheses.

Continuous and regression residuals data in a maximum likelihood analysis

Because of the controversy regarding the discretization of continuous trait data, a second analysis using log transformed original postcranial data was performed. One of the factors that influence the type of data that can be used in a phylogenetic investigation is the method of analysis chosen. Two of the most frequently used types of analysis are the most parsimonious reconstruction (MPR) parsimony approach and maximum likelihood (ML). Current parsimony-based programs cannot use continuous trait data as input, thus restricting the types of analysis available using that method. Maximum likelihood differs in the evolutionary assumptions used in its model, but allows continuous data as input. This permit a comparison of the two methods (parsimony and maximum likelihood), and their ability to resolve phylogenetic problems using different data derived from the same source. An interesting result from the analysis in Chapter 2 was a previously unreported size-based phylogeny.

Another ML approach used regression residuals (character trait measurements were regressed on body size to remove the effect of size) as data input in a phylogenetic analysis. The analyses using this novel approach were unprecedented, and pose numerous questions regarding possible biogeographic implications which are briefly addressed. The results from the two ML analyses (continuous and regression residuals) are then combined to produce a consensus maximum likelihood phylogeny, and compared with the MPR analyses from Chapter 2.

Consensus and combined data analysis

As contentious as the debate surrounding the methods of discretization (or even whether to do so) of continuous data seem, they pale when compared with those surrounding how (or whether) to combine data in phylogenetic analyses. In an effort to garner the most information from the postcranial data set and to test hypotheses regarding the combination of data, an overall consensus phylogeny was constructed. Data matrices and the trees and cladograms they produced by the methods described above were combined separately, and then brought together to produce a phylogenetic estimate based on the greatest possible amount of information from the data at hand.

Due to the nature of the various data sets, the results of their analysis, and the approaches used, several techniques had to be used to combine either data or trees. This allowed a comparison of the various combining and consensus methods. Comparisons are also made with results from the data handling approaches and phylogenetic analysis methods in Chapters 2 and 3. The overall combined/consensus phylogeny is then examined in light of the previous phylogenetic hypotheses described above. Various levels of support and contradiction of previous systematic and taxonomic works are combined with new testable hypotheses regarding otter evolution. It is hoped that these new hypotheses provide an impetus for further investigation into the history of this fascinating group described by the eminent paleontologist Bjorn Kurten as "...the most elegant solution to the problem of constructing an amphibious carnivore that Nature has so far effected." (Kurten 1968).

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Part 2

Otter Phylogenetics and the Discrete Coding of Continuous Characters

Introduction

Most previous attempts to reveal the evolutionary relationships of the otters (Carnivora: Lutrinae) have relied primarily on discrete cranial and dental traits, with the singular exception of Koepfli and Wayne (1998). Overall, the results of these previous investigations have yielded little congruence in the phylogenies they produced and more often produced contradictions. The methods used to analyze these discrete data have ranged from evolutionary taxonomy and phenetics to phylogenetics. One of the primary criticisms of these types of analyses has been that the use of discrete character sets and that the somewhat subjective nature of coding these characters may not reveal the true phylogeny, and may be biased by the choice of characters used and the coding methodology itself. How then, can continuous data be incorporated in a phylogenetic analysis?

Although the type of data used is very important, the analytical approach employed with these characters is also critically influential. To incorporate continuous characters in phylogenetic analyses (as opposed to numerical taxonomy or phenetic clustering techniques) several authors have proposed various coding approaches in an attempt to utilize continuous characters in a world dominated by analytical programs that require discrete data as input. Here, using the phylogeny of the otters as an example, a comparison of these approaches was carried out to determine which, if any, of these techniques provide a suitable method for including continuous morphological data effectively in a phylogenetic analysis.

Rationale for the use of postcranial material

The use of postcranial skeletal material in phylogenetic studies has been limited by several factors: relatively small sample sizes of postcranial specimens housed in museums (van Zyll de Jong 1987); an historical bias towards the use of cranial and dental characters (due in part to the preponderance of "skin and skull only" specimens in museums); predominance of comparable fossils composed mainly of teeth or skull fragments; and the mistaken belief (Wyss 1988, 1989; Sanchez-Villagra and Williams 1998) that postcranial elements are prone to higher levels of homoplasy due to convergence than are cranial or dental traits.

Davis (1978, p. 15) stated of the Lutrinae: "Because of a high degree of convergence and adaptive radiation within the group, a study limited to cranial morphology does not yield the true relationships between species." But, probably owing to the impediments given above for the use of postcrania, there have been no systematic studies of the Lutrinae using more than a few postcranial morphological traits, mostly based on soft tissue or external morphology (Pocock 1921; Sokolov 1973; de Muizon 1982; van Zyll de Jong 1987; 1991; Bryant et al. 1993). Most morphological systematic studies are in fact based on a very small subset of available traits, namely dental or cranial characteristics. The inclusion of a broader array of morphological data in phylogenetic analyses has been called for by several authors (Bisaillon et al. 1976; Munson 1992; Flynn 1995; Bininda-Emonds et al. 1999), and there has recently been a movement towards the production of more inclusive data sets by combining different types of data (Bull et al. 1993; Bininda-Emonds et al. 1999; Liu and Miyamoto 1999). It is generally

postulated that the more characters employed in a phylogenetic analysis, the more accurate the phylogeny estimation will be as long as there is not an excessive amount of homoplasy in those added characters (Simpson 1961; Nei et al. 1983; Fitch and Atchley 1987; Rohlf and Wooten 1988; Brooks and McLennan 1991; Charleston et al. 1994; Hillis et al. 1994a, 1994b; Otto et al. 1996). An exception to this general rule is long branch attraction in a maximum parsimony approach, where the addition of more characters may actually increase support for an incorrect tree (Felsenstein 1978; Kim and Burgman 1988; Huelsenbeck and Hillis 1993).

Incorporating a large number of postcranial morphological characters in a phylogenetic analysis, and using discrete coding methods purported to provide a solid methodology for converting continuous traits, it is possible to provide a robust and accurate estimate of the evolutionary history of the otters. A correlated problem that comes with this approach is that there is an increase in the levels of homoplasy when more and more characters (and taxa) are added to an analysis (Quicke 1993). This can result in inaccurate results if homoplasy is not accounted for, or when there is an excessive number of symplesiomorphic, autapomorphic, or uninformative characters (Siebert 1992). With these considerations in mind, there is still a firm belief that you can never have too many characters, illustrated by Brooks and McLennan (1991, p. 66).: “...stop adding characters when you stop getting different answers or resolutions...”

Discrete character coding approaches

The practice of coding continuous data into discrete characters is highly contentious and has been criticized and rejected by several authors, thus the methodology has been infrequently used in recent phylogenetic studies (Pimentel and Riggins 1987; Crisp and Weston 1987; Cranston and Humphries 1988; Farris 1990; Quicke 1993). Not all critics object to the use of continuous characters *per se*; Felsenstein (1988a, 1988b) only objected to the discretizing and in turn suggested that quantitative characters are useful phylogenetically when left on a continuous scale. The problem with this solution is that most phylogenetic computer programs and algorithms cannot deal with continuous morphological data that have not been coded into discrete characters. Felsenstein's (1993) program PHYLIP is equipped to handle continuous data with a maximum-likelihood approach (ML). Nonetheless, theoretical justification for the use of the ML approach is generally applied only to molecular analyses (nucleotide sequence, restriction site, allozyme and allele frequency; DeBry and Slade 1985; Swofford and Berlocher 1987; Rohlf and Wooten 1988; Swofford and Olsen 1990). However, its usefulness for morphological characters is advanced by Felsenstein (1993) and considered in Chapter 3.

Maximum likelihood assumes that evolutionary change is a random process and each phylogeny has a certain probability of being correct based on the given data set (Quicke 1993). This differs from maximum parsimony in which evolutionary change is viewed as an extremely unlikely event, and so the optimum phylogeny is the one which requires the fewest number of changes (shortest tree length) thus approximating the "true" phylogeny (Swofford and Olsen 1990; Wiley et al. 1991). Tree comparisons

between these methods can therefore be difficult because tree constructions are based on different algorithms and evolutionary assumptions (Felsenstein, pers. comm.). An evaluation of the ability of PHYILP to recover phylogenetic information from continuous character data is presented in the next chapter, and results are compared with those from the discrete coding techniques and approaches used in this chapter.

Among the most widely advocated discrete character coding approaches are segment coding (as proposed by Chappill 1989), homogeneous subset coding (Simon 1983), gap coding (Thorpe 1984), and generalized gap coding (Archie 1985). Some techniques vary only slightly in the statistical criteria to delineate character states, while others are based on completely different assumptions. A brief synopsis of each method follows.

Segment coding

Segment coding, as applied by Chappill (1989), is a variant of the range-coding methods of Kendrick (1964), Colless (1980), and Thorpe (1984). Segment coding as proposed by Thorpe (1984) employs binary character assignments instead of multistate character codes. This approach was criticized by Chappill (1989, p. 224) who said binary character assignment of continuous characters "...would produce gross distortion of relationships, since a pair of taxa in the middle of a range may be scored as one unit different from each other when they are not significantly different." Similarly, taxa at the extremes of the range of values, possibly many standard deviations apart, would be scored as only one unit different. For these reasons, all of the segments coded in this

study were assigned multistate character codes to improve the information provided from the matrix and increase the overall power of the analysis.

The basic elements of segment coding are to divide character state range values into equal non-overlapping segments (a priori), then assign codes to the taxa based on segment membership. Segment assignment is established by placing values into the segments into which they fall and assigning ordinal alphanumeric codes based on the number of segments. The number of assigned segments is recommended to be a function of the number of taxa under investigation and the level of their divergence, a somewhat ad hoc approach reminiscent of classical taxonomy (Baum 1988; Quicke 1993).

Detractors of this meristic approach cite several weaknesses including: bias and subjectivity related to the a priori segmentation of the characters; inability to discern taxa that may differ by several standard deviations from those that differ only slightly (which results in the possibility of statistically different taxa being scored with the same code); and the circularity of using preconceived notions about divergence and relatedness of taxa prior to analysis to determine these same factors (Archie 1985; Chappill 1989; Farris 1990). Even though there may be basic and profound philosophical issues in predetermining the relative amounts of divergence or convergence among clades, the data ultimately provide the means of character state assignment. In other words, the number of segments may be arbitrary and subjective, but where the data fall is objective and not affected by manipulation or predeterminations. The lingering problem lies in the rationale of dividing a character value range in a relatively arbitrary fashion.

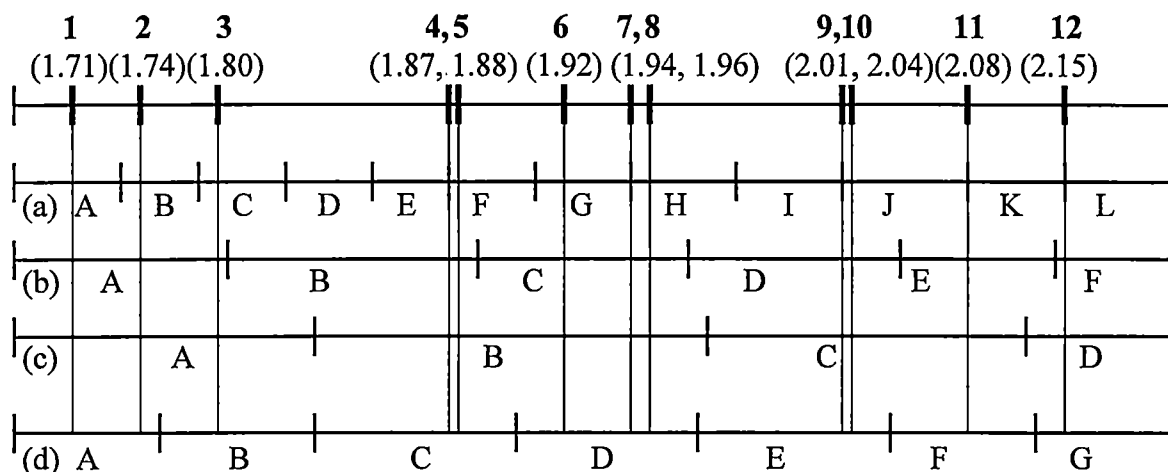
Homogeneous subset coding

Simon (1983) proposed homogeneous subset coding to overcome deficiencies (such as potential subjectivity and the possible loss of taxon differentiation due to a lack of statistical discrimination) in the segment coding method of Kendrick (1964) and Colless (1980) which were subsequently addressed by the segment coding method of Chappill (1989) (the method used here). Whereas segment coding divides (a priori) the character state range into equal segments and then assigns codes, homogeneous subset coding is based on a posteriori multiple comparison tests followed by subset assignment of homogeneous groups. This approach was preferred by Simon (1983) because it retains (and uses) all of the statistically significant information in the data, avoiding possible information loss from segment coding. Information is lost when clear gaps are not present, or statistically indistinguishable groups are created (Archie 1985).

Some impediments in using homogeneous subset coding include: sample sizes must be large enough to perform a posteriori tests (sum of squares simultaneous test, $GT-2$, or T method procedures) and sample sizes must be equal and have equal variances. These requirements may not be met by some data sets, including those used in this study. In the postcranial measurement data set used in this study sample sizes were often small (from a low of $N=1$ to a high of $N=16$), of unequal size, and not all characters had equal variance. For these reasons, homogeneous subset coding was inappropriate for this study. However, there are, several aspects of homogeneous subset coding ensconced in the gap coding procedures of Archie (1985) described below.

Gap coding approaches

The next approach is “gap coding” (Mickevich and Johnson 1976). An illustration of how this and other coding methods used here would be applied is shown in Figure 2-1. Several versions of gap coding have been proposed (Thorpe 1984; Archie 1985; Goldman 1988). In general, these procedures involve ordering species character means according to magnitude, then defining “gaps” using various statistical protocols. A refined version by Mickevich and Johnson (1976) is “generalized gap coding” (Archie 1985), also called “gap coding A” (termed “Gap A” here) which was inspired by the homogeneous subset coding of Simon (1983). However, as pointed out by Farris (1990) the use of the term “gap coding” is somewhat misleading. Farris demonstrates that the “critical gap size” used to differentiate and code continuous traits is “...not the size of a gap at all, but instead a limit on the range within subsets; and ‘generalized gap coding’ has nothing whatever to do with gaps.” (Farris 1990, p. 97). Farris bases the distinction between these terms on the definition of critical gap size as the “pooled within-taxa standard deviation”, a value based on limiting the range *within* and not *between* subsets (Archie 1985; Farris 1990). In the coding procedure proposed by Thorpe (1984), generally termed “gap coding B” (termed “Gap B” in this paper), the largest gap among population means is used as the “gap” as long as that gap exceeds 0.5 standard deviations.



Taxon codes:	1	2	3	4	5	6	7	8	9	10	11	12
Coding method	Resultant codes											
(a) 12-segment coding	A	B	C	F	F	G	H	H	J	J	K	L
(b) 6-segment coding	A	A	A	B	B	C	C	C	D	D	E	F
(c) Gap A coding	A	A	A	B	B	B	B	B	C	C	C	D
(d) Gap B coding	A	A	B	C	C	D	D	D	E	E	F	G

Taxon codes

1) *Ictonyx*, 2) *Mustela*, 3) *Mephitis*, 4) *Hydrictis*, 5) *Amblonyx*, 6) *Lontra longicaudis*, 7) *Lutra*, 8) *Lontra canadensis*, 9) *Lutrogale*, 10) *Aonyx*, 11) *Pteronura*, 12) *Enhydra*

Figure 2-1. Coding technique effects on character codes, as applied to the character tibia length (TL). Log character means in parentheses below taxon codes and indicated by bold lines. Vertical lines indicate coding for each taxon and coding method. Results are shown below the diagram. Not drawn to scale.

In the Gap A coding approach of Archie (1985) gaps between subsets are determined by multiple comparison tests of group means and generally based on standard deviations of one or more (Archie 1985; Goldman 1988), but the number of standard deviations is subjective. The magnitude of these “gaps” is left up to the investigator, with the premise that the larger number of standard deviations (i.e. 1-2 or more), the more non-overlapping divisions will be found (Archie 1985). An adverse aspect of this is that distinct but closely related taxa may not be separated by multiple standard deviations.

Both Gap A and Gap B coding protocols were used in this study, utilizing gaps of one standard deviation for the Gap A analysis and one half standard deviation for the Gap B analysis.

By performing two types of segment coding and Gap analyses, the efficacy of the methods can be evaluated and compared. Not only can the different methods be compared, but versions of each method can also be evaluated. In this way a rough cost/benefit analysis can be performed which may help resolve questions about which method (if any) is superior, and whether more complex coding methods yield results that are more robust. If simple methods produce comparable results, they may be preferred.

Materials and Methods

Postcranial skeletal materials of otter and outgroup species were examined at the following museums: National Museum of Natural History (USNM) Washington, D.C.; American Museum of Natural History (AMNH) New York City, New York; Field Museum of Natural History (FMNH) Chicago, Illinois; Harvard Museum of Comparative Zoology (MCZ) Cambridge, Massachusetts; and the Yale Peabody Museum (YPM) New Haven, Connecticut. See Appendix 2-1 for specimen lists, catalogue numbers, specimen information, and collection data. Specimens were examined for completeness and general condition to determine overall suitability prior to analysis. A total of 106 specimens comprising 13 species was measured for this study (see Table 2-1 for species sample sizes). All possible efforts were made to obtain five adult males and five adult females of each species, and use specimens collected from the same geographic locality

Table 2-1. Species specimen sample sizes and sex (Missing otter species: *Aonyx congicus*, *Lontra provocax*, *Lutra sumatrana*.)

Ingroup Species	Male	Female	Unknown	Totals
<i>Amblonyx cinereus</i>	7	6	3	16
<i>Aonyx capensis</i>	0	4	0	4
<i>Enhydra lutris</i>	5	2	4	11
<i>Hydrictis maculicollis</i>	5	4	2	11
<i>Lontra canadensis</i>	5	5	0	10
<i>Lontra longicaudis</i>	6	6	1	13
<i>Lontra felina</i>	0	1	0	1
<i>Lutra lutra</i>	1	3	2	6
<i>Lutrogale perspicillata</i>	0	2	1	3
<i>Pteromura brasiliensis</i>	5	2	1	8
Outgroup Species				
<i>Ictonyx striatus</i>	1	2	0	3
<i>Mephitis mephitis</i>	5	5	0	10
<i>Mustela vison</i>	5	5	0	10
Totals 13 species	45	47	14	106

or belonging to the same subspecies. Larger sample sizes were not generally possible due to the relative paucity of postcranial material. Data were collected on males and females to determine if there was significant sexual dimorphism. Evidence for taxa with sufficient sample sizes indicated sexual dimorphism was not an important factor. Specimens of unknown sex were included in cases where overall sample sizes were below the target sample size of ten, and subadults were sometimes included when full adult specimens were not available. The collection locality criterion was used to reduce possible confounding effects of geographic or clinal variation.

Excluded and omitted data

Postcranial material was not available in North American museums for three otter species: Hairy-nosed otter (*Lutra sumatrana*), Southern river otter (*Lontra provocax*), and Congo clawless otter (*Aonyx congicus*). Additionally, only a single specimen of Marine otter (*Lontra felina*) was available, and there were fewer than ten specimens of six other species (see Table 2-1). Captive animal specimens from zoological parks or other facilities were used only when wild caught specimens were not available and species sample size was small. Captive specimens were generally excluded since there are often severe osteological deformations related to dietary deficiencies and reduced mobility. Specimens with excessive bone deformations were omitted from analysis except in cases when there were no other available materials. In cases where specimens were only mildly osteologically distorted, these specimens were included, but only those measurements which were unaffected by the deformations were taken. Characters with bone deformations affecting measurement accuracy were entered as missing data. Including parts of slightly deformed specimens increased available data for overall measurement precision and mean values.

Continuous morphological data

After determining specimen suitability, postcranial measurements were taken with digital calipers (Fowler Max-Cal) and recorded on data sheets and transcribed to spreadsheets, or entered directly into a laptop computer. Measurements were taken

bilaterally, or on available osteological material when specimens were either incomplete or damaged in some way (see Appendix 2-2 for abbreviations and measurements).

A total of 101 measurements was taken on each of 106 specimens for approximately 21,800 total measurements (10,908 x 2; measurements taken bilaterally when available). The actual number was slightly lower due to incomplete, damaged, or otherwise unusable specimens. When only partial specimens were available, measurements were taken on applicable postcranial elements in to achieve a more meaningful species mean value. Some measurements were dropped from analysis because they were not available across all taxa used in this study or had large variances that precluded their use. As a result, 18 character measurements were used from the scapula, 21 from the humerus, 6 from the radius, 24 from the ulna, 11 from the tibia, and 7 from the femur. Differences in the number of characters generally reflected relative bone complexity (i.e. the relatively simple radius yielded only 6 usable characters while the more complex ulna yielded 24). Averaged head and body length measurements were taken from the literature and included in the analyses.

Characters were tested for possible sexual size dimorphism (SSD) (Holmes 1987; van Zyll de Jong 1987). Results from van Zyll de Jong (1987) showed that intraspecific SSD was not significant when compared to interspecific differences and did not noticeably effect his canonical variates analysis. Species mean values were calculated and log transformed to reduce potential non-linear effects of size changes (Harvey 1982), since the sizes of the species under study vary in mass by two orders of magnitude (from 0.42 kg in the zorilla to 45 kg in the sea otter) and vary in length in one order of

magnitude (between 0.35 meters for the zorilla and 1.5 meters for the giant otter; Harris 1968; Hayssen et al. 1993; Dorst and Dandelot 1995).

Statistical analyses for character variance resulted in the removal of 13 characters from the data set since some procedures for discretizing continuous data require equal or similar variances. The remaining 88 characters were initially used in all analyses, and only when coding procedures resulted in some of these being classified as parsimony uninformative characters, were they removed from that analysis.

A number of specimens were incomplete, damaged, or had anatomical anomalies and were omitted, for a total of 86 specimens comprising 13 species, thus restricting analysis of several species. Species with small sample sizes were retained in the interest of maintaining the most inclusive set of species for analyses. The phylogenetic positions of these taxa should be viewed with caution, although the use of single specimens in phylogenetic analyses is not uncommon (usually due to costs in studies that are molecular in nature, i.e. Koepfli and Wayne 1998).

One species (*Lontra felina*) was deleted from the analysis since only a single specimen was available. This deletion was also precipitated by the fact that PAUP* cannot handle more than 12 taxa in exhaustive searches due to the inordinate number of possible trees. For a data set containing 12 taxa, the number of possible trees examined in an exhaustive search is 1.37×10^{10} and the addition of one more taxon increases that number to approximately 3.1×10^{11} , an intractable number for most computers and programs (Felsenstein 1978; Hillis et al. 1996; Stone and Repka 1998). Due to the inordinate number of calculations, exhaustive searches for more than 11 taxa are not

recommended (Kitching 1992; Swofford et al. 1996). Despite these warnings and considerable computation times (up to 25 hours) the significant increase in the accuracy of phylogeny estimation warranted the exhaustive search for the 12 selected taxa.

Statistical analyses employed StatView (version 4.01, Abacus Concepts Inc.) and JMP (version 3.1.1, SAS Institute Inc.). The phylogenetic software used was PAUP* (version 4.0b4a; Swofford, 2000). Trees were scored for tree lengths, Consistency Indices (CI), Retention Indices (RI), Rescaled Consistency Indices (RC) and Homoplasy Indices (HI). In cases where PAUP* uncovered more than one parsimony uninformative character (which were omitted) the adjusted CI was also recorded.

Segment coding

Segment coding to discretize the continuous data set was performed in two ways. The a priori division of the character value range in the first analysis was based on the total number of taxa (12) under study (see Appendix 2-3 for matrix). The second analysis was based on more conservative higher level taxonomic divisions: Families, Subfamilies, and Tribes (6 segments) (Appendix 2-4). To produce 12 segments, the range of each normalized character measurement was divided by twelve and the result added to the lowest mean score, the divided range value was then added to that score and each successive mean to produce 12 segmented steps. Similar procedures were also used to construct six divisions. Species mean values were assigned to segments and coded using alphanumeric codes (A-L for the 12-segment analysis, A-F for 6-segments). Letters were used due to the 12-segment analysis (PAUP* only recognizes numeric characters 0-9 with

two digit numbers interpreted as repeats), and to maintain consistency in codings.

Additive binary coding (Goldman 1988) was not used because additive binary codes are less informative than multistate codes when they can be used (Chappill 1989).

Gap coding

Gap coding was performed in accordance with the protocols outlined in Archie (1985) for Gap A coding using log normalized character means and one standard deviation as the gap. The number of character states in the data matrix varied from 3 to 5 and is shown in Appendix 2-5. The Gap B data matrix was constructed using the gap coding method described by Thorpe (1984) applying one half of a standard deviation to determine the gap. The Gap B matrix is shown in Appendix 2-6. Characters had between 6 and 9 character states with this coding method.

Measures of fit and tree statistics

In most studies evaluating competing phylogenetic hypotheses, tree lengths are used to compare results. Overall tree lengths are not an appropriate or satisfactory measure in this case because of the variation in the number of character states produced by different coding methods due to differing amounts of discretization. It has been shown that the number of character states affects tree length. Since each character state change incurs a step, more character states result in longer branch and tree lengths (Wiley et al. 1991; Siebert 1992; Quicke 1993; Swofford et al. 1996). In this study, comparing tree lengths is misleading since the number of possible steps differs among the approaches.

The Consistency Index (CI) is a measure of how the data matrix “fits” with the tree topology and is calculated as the ratio of the minimum number of changes that might occur, divided by the number that actually do occur. This measure provides a measure of homoplasy, but does not reveal how homoplastic characters affect the tree topology, and can be inflated by uninformative characters. For these reasons, other methods are considered to be more appropriate. The Retention Index (RI) is the ratio of apparent synapomorphy to actual synapomorphy, a measure of relatedness that excludes homoplasy. The RI accounts for how well a particular tree represents a given data set (ignoring uninformative characters) without inflation from autapomorphies or symplesiomorphies. The value of the RI is high when most changes occur at internal nodes, and lower when they are more abundant or concentrated along terminal branches. Some consider the RI to be a better measure of support for a given tree than the CI (Siebert 1992) since it can somewhat account for long branch attraction. With evidence for long branch attraction in the otters from the work of Koepfli and Wayne (1998), an index incorporating the RI was preferred.

A revised index, the Rescaled Consistency Index (RC), uses the Retention Index (RI) to correct some of the deficiencies in the CI and affords characters that are only partially homoplastic the chance to contribute to the overall fit of the tree. The RC is the product of the original CI and the RI, and excludes characters that do not contribute significantly to the tree topology. Since the RC excludes totally homoplastic characters and autapomorphies, as well as retaining the positive aspects of both the CI and RI, the RC was chosen to be the most judicious method for result comparisons in this study. The

choice of the RC is appropriate for this study because of the large number of characters and since it has sometimes been postulated that morphological characters may be more homoplastic than other traits (Wyss 1988; 1989; Sanchez-Villagra and Williams 1998).

Results

Segment coding results

In the 12 segmented analysis not all partitions contained species, resulting in discontinuous gaps between some coded segments. In the second analysis (6-segment), this segment breaching occurred only twice in the data set due to the relatively coarser subdivision of segments. Data matrices were constructed using the segment codes as characters in NEXUS format for use in PAUP* 4.0b4a (Swofford 2000). Analysis of these matrices was performed using the maximum parsimony search criteria, after removing *Lontra felina* from the analysis. Three outgroup species were defined: zorilla (*Ictonyx striatus*), American mink (*Mustela vison*), and striped skunk (*Mephitis mephitis*).

Twelve segment results

After eliminating the marine otter (*Lontra felina*) from the analysis and defining the three outgroup species, PAUP* found 9 parsimony uninformative characters which were then excluded from the analysis: ML, DFW, SFL, ArcT, LTL2, GDD, AcL, AcW, and VBLc (see Appendix 2-2 for character abbreviations). The remaining 79 characters were unordered, equally weighted, and employed in an exhaustive search using the default parsimony search settings in PAUP*. The exhaustive search evaluated 654,729,075 trees (the same number for all searches), producing 30 equally parsimonious

trees (length 708 steps). Tree scores for these and all other subsequent trees produced in this study are presented in a table following the tree figures. Most positions of the Lutrinae were similar in these 30 trees, with one exception. There was uncertainty regarding the positions of the Neotropical (*Lontra longicaudis*), and Eurasian otter (*Lutra lutra*). These species each differed in one position, reversing in 15 trees. Another disagreement occurred in outgroup relationships, as well as internal disagreements among the outgroups (5 arrangements of 3 taxa, creating 15 different subtrees). Reversals of otter positions with shifting outgroups resulted in 30 equally parsimonious trees (2 otter taxa shifts times 15 outgroup subtrees). A strict consensus tree was constructed from those trees (Figure 2-2). Bootstrap analysis (1000 replications) was used to indicate support. In the consensus tree, most otter taxon positions remained unequivocal. Bootstrap support for the consensus arrangement was high (95% or above). Two exceptions were moderate support for positions of two species: *Lontra canadensis* (77%), and *Hydrictis maculicollis* (84%). While support for the positions of these taxa was lower, bootstrap analysis still provided good confidence in their placement.

Six segment results

An exhaustive search was performed on the 6 segment matrix, with the same species, ordering, weighting, and search settings. The only difference was there were no parsimony uninformative characters, resulting in a total of 88 characters. A single tree with a length of 466 steps was produced (Figure 2-3). Bootstrap support from 1000 replicates provided strong support (99% or above) for all nodes except *Lutra lutra* (75%) and *Lontra canadensis* (52%). Resolution in the positions of several taxa increased

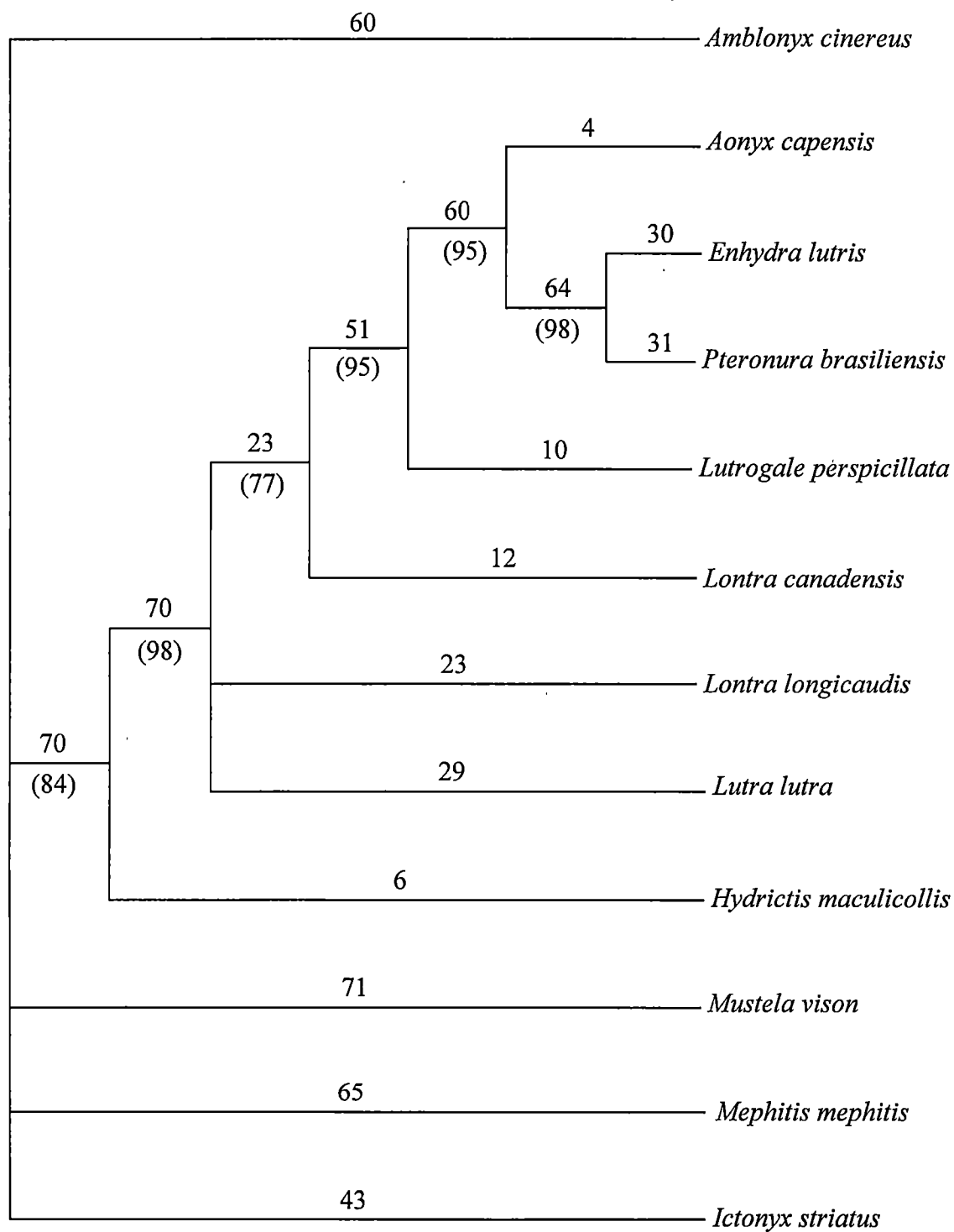


Figure 2-2. Consensus tree of 30 equally parsimonious trees from 12-segment data. Branch lengths are above lines, bootstrap support in parentheses below (tree length 708; CI = 0.950; RC = 0.734).

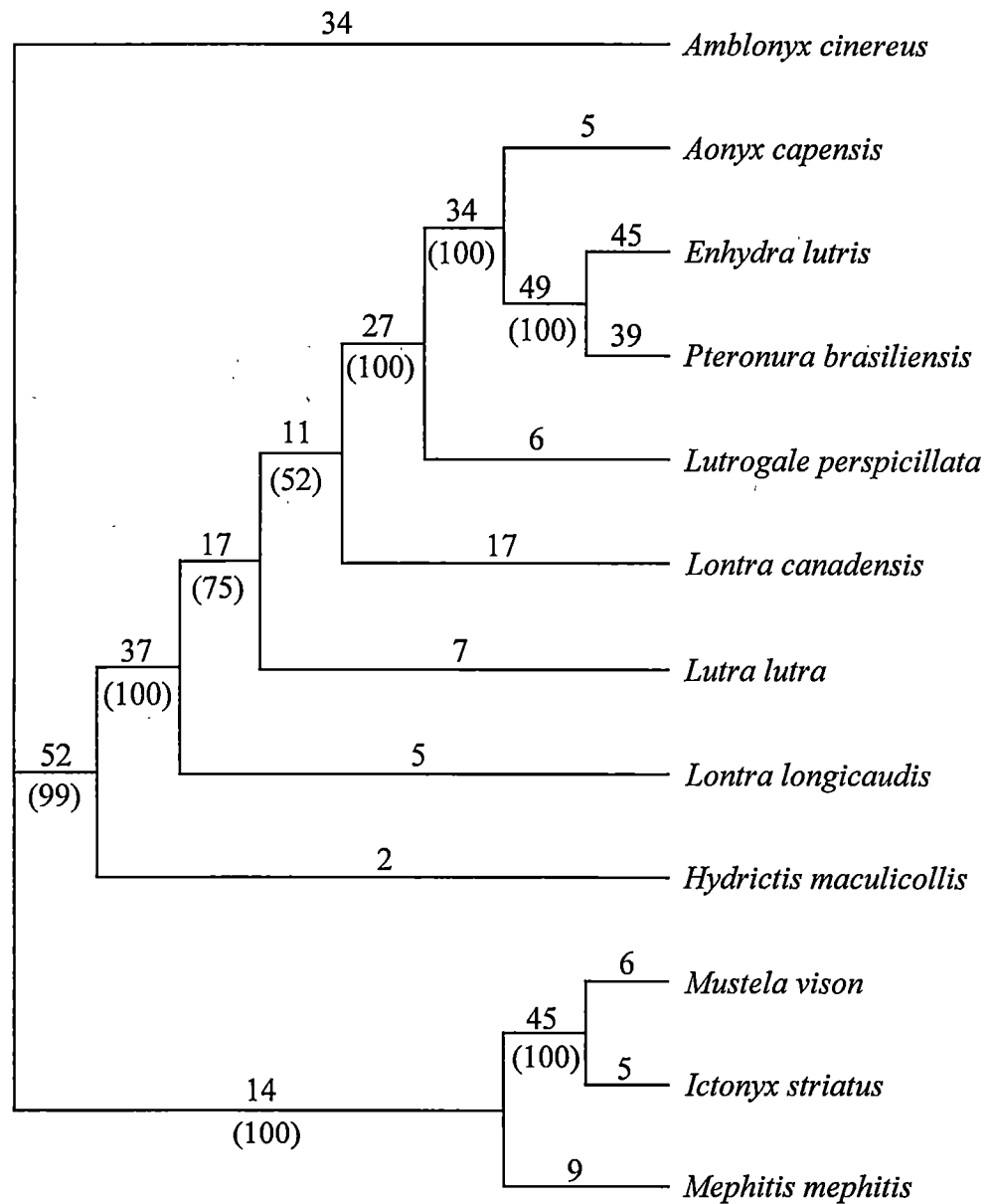


Figure 2-3. Single most parsimonious tree derived from 6-segment data. Branch lengths are shown above lines, bootstrap support in parentheses below (tree length 466; CI = 0.903; RC = 0.762).

compared with the consensus of the 30 trees derived from the 12 segmented data.

Ambiguity was reduced in outgroup species positions due to the creation of a single tree, with good bootstrap support (100%) for joining members of the Mustelidae (zorilla and American mink) and separation of Mephitidae (striped skunk). Likewise, the ambiguous bifurcation formed by the consensus tree (Figure 2-2) regarding the placement of *Lontra longicaudis* and *Lutra lutra* was resolved with excellent to fair bootstrap support for their positions (100% and 75% respectively). However, bootstrap support for placement of *Lontra canadensis* was significantly lower (52%) than that in the 12 segmented tree (75%). In both the 12 segmented coded consensus tree and the single most parsimonious 6 segmented tree (as well as all other trees in this study) the Asian clawless otter (*Amblonyx cinereus*) was placed basal to all other otter species.

Gap A coding results

The Gap A data produced three equally parsimonious trees (length 304 steps) shown in Figure 2-4 below. Topological differences between trees were due to shifts of three otter taxa: *Lontra canadensis*, *Lontra longicaudis*, and *Lutra lutra*. The most unstable placement of the three taxa was *Lontra canadensis* which had three position shifts among the trees. The other two taxa were relatively stable in their positions, *Lutra lutra* and *Lontra longicaudis* each had only a single position shift in the three trees. The positions of the other otter species were unambiguously resolved, agreeing with trees from the segment coding (Figures 2-2 and 2-3). Outgroup positions were similar to those seen in Figure 2-3.

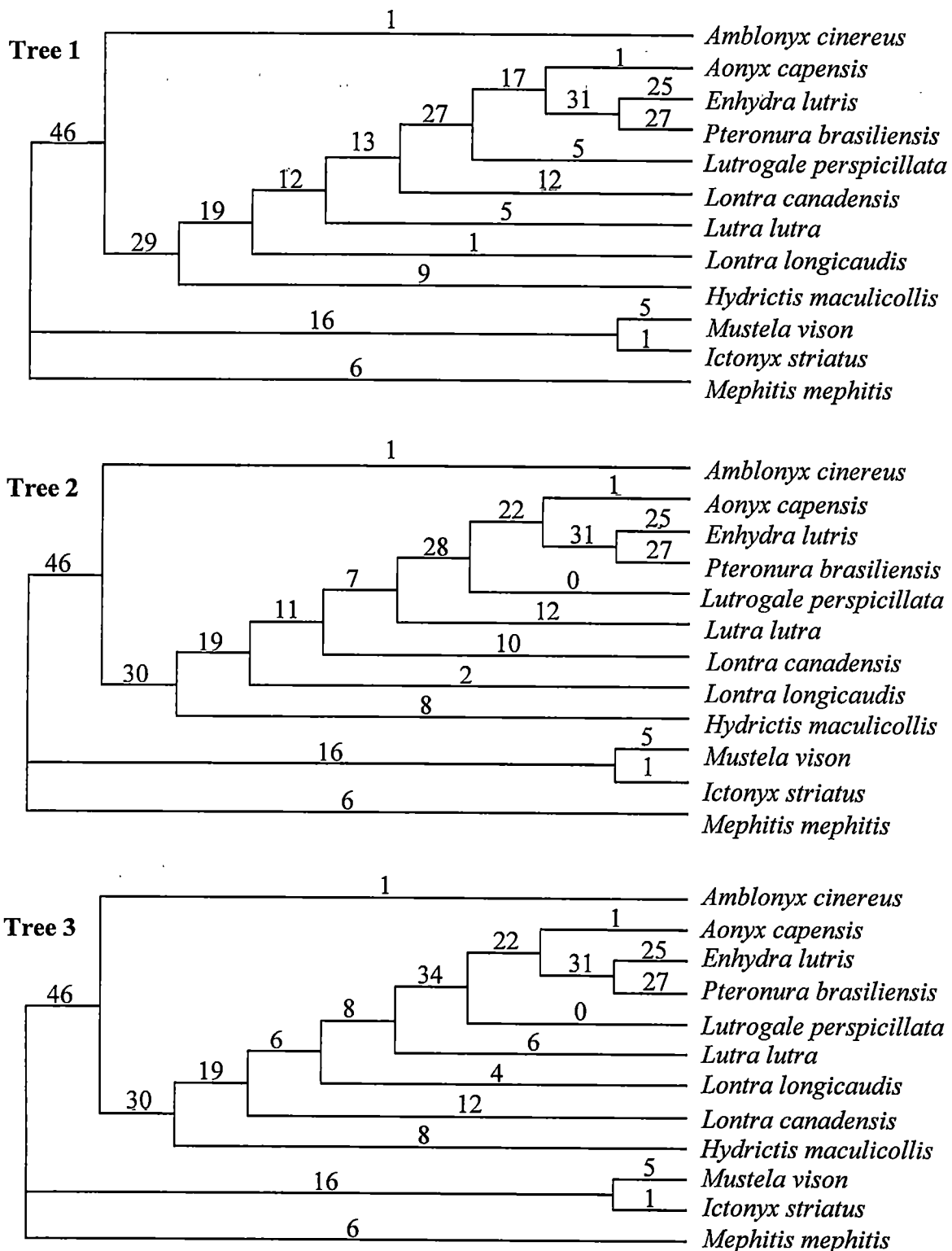


Figure 2-4. Equally parsimonious trees from Gap A coding, branch lengths above lines (all tree lengths =304; CIs = 0.852; RCs = 0.745).

A 50% majority-rule consensus tree (shown in Figure 2-5 below) was constructed from the trees in Figure 2-4. The 50% majority-rule was computed in order to preserve the relationships of the three variable taxa in the equally parsimonious trees. A strict consensus tree would have resulted in an uninformative polytomy for *Lontra canadensis*, *Lontra longicaudis*, and *Lutra lutra*, obscuring relationships provided by the equally parsimonious trees. Bootstrap support (1000 replicates) was strong (100%) for all resolved nodes in the consensus tree except the branch connecting *Lutra lutra* which only garnered moderate to poor support (52%). Tree topology agreed with previous trees (Figures 2-2 and 2-3) in regards to the basal position of *Amblonyx cinereus*, the four taxon clade consisting of *Aonyx*, *Enhydra*, *Pteronura*, and *Lutrogale*, and the internally basal position of *Hydrictis*. Resolution was reduced at the outgroup node containing the Musteline clade (zorilla and American mink) and Mephitine clade (striped skunk), and at the node containing *Lontra canadensis* and *Lontra longicaudis*. The tree differed in placing *Lutra lutra* just basal to the four taxon clade discussed above.

Gap B coding results

The Gap B analysis produced a single tree (length 526 steps) shown in Figure 2-6. No uninformative characters were found, resulting in 88 characters used. Bootstrap support was strong (98% or above) except for the area defining *Lontra canadensis* and *Lutra lutra*. Bootstrap support for *Lontra canadensis* as basal to the four taxon clade (*Aonyx*, *Enhydra*, *Pteronura*, and *Lutrogale*) was weak (52%). Support for the position of *Lutra lutra* was moderate (60%), and stronger for *Lontra longicaudis* (100%).

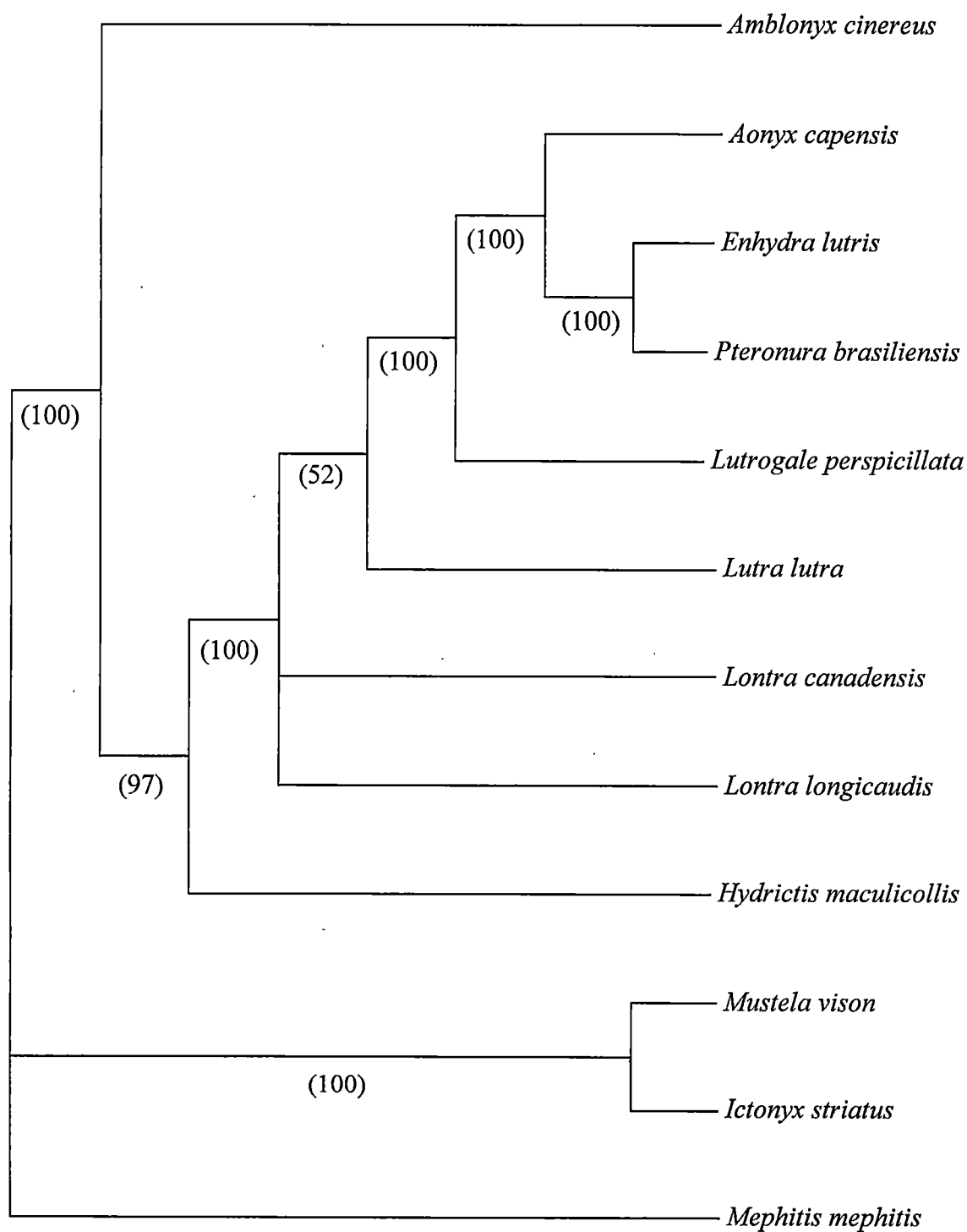


Figure 2-5. 50% majority consensus tree from the three equally parsimonious Gap A coding trees in Figure 2-4. Bootstrap support values in parentheses below lines.

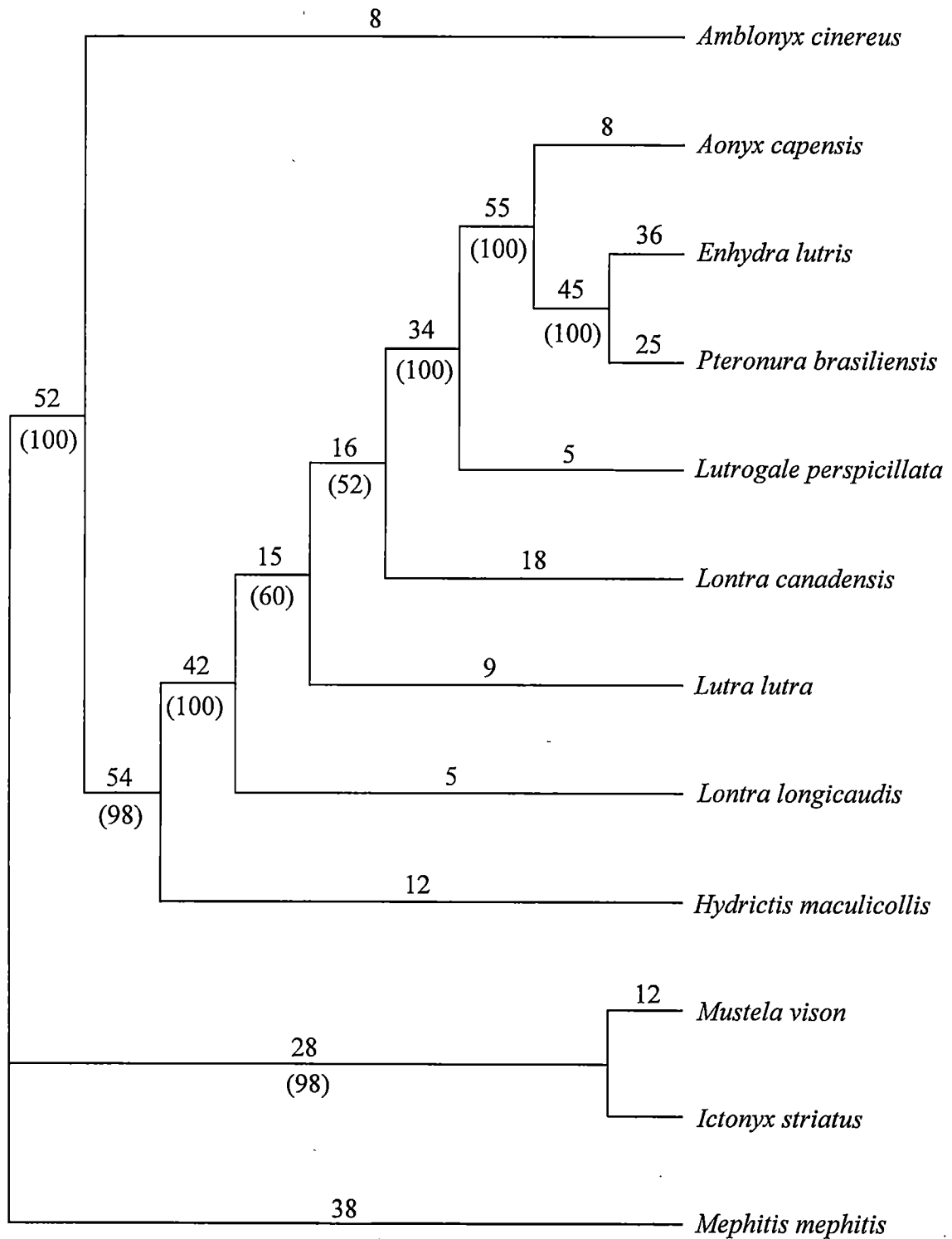


Figure 2-6. Single most parsimonious tree from Gap B data. Branch lengths given above lines, bootstrap support in parentheses below (tree length 526; CI = 0.918; RC = 0.769).

Resolution in the Musteline-Mephitine clade was reduced. The musteline clade remained intact with 98% support. Topologically, the tree in Figure 2-6 was identical to Gap A Tree 1, of Figure 2-4.

Tree statistics and consistency indices

A summary table showing tree scores for all coding methods is presented in Table 2-2 below. Tree scores were identical in those analyses which produced more than one most parsimonious tree (i.e. Gap A coding produced 3 trees and 12-segment coding produced 30 trees), consequently only a single value is shown for those analyses. For the 12-segment analysis, which had a significant number of parsimony uninformative characters removed, the non-adjusted (uninformative characters included) tree scores are included. Non-adjusted tree scores did not differ significantly from adjusted tree scores ($p = 0.01$). Overall tree scores for the various coding techniques were comparatively quite good, with the Consistency Index (CI) ranging from a high of 0.951 (12-segment coding) to a low of 0.852 (Gap A coding). The Retention Index (RI) results were also reasonably high, ranging from 0.874 for Gap A coding to 0.772 for 12-segment coding. The Rescaled Consistency Index (RC), which was chosen to best represent the results in this study had values ranging from 0.769 (Gap B coding) to 0.734 (12-segment coding). Homoplasy Indices (HIs) were generally quite low ranging from 0.049 (12-segment coding) to a high of 0.148 (Gap A coding). These tree statistics convey relatively good confidence in the results, CIs and other measures of fit were respectably high, and the HIs were correspondingly low.

Table 2-2. Tree scores showing coding method, number of characters used, number of trees produced, tree length, Consistency Index (CI), Retention Index (RI), Rescaled Consistency Index (RC), and Homoplasy Index (HI). Non-adjusted scores (for uninformative characters) in parentheses.

Coding Method	Character Number	Number of trees	Tree length	CI	RI	RC	HI
12-segment	79 (88)	30	708	0.951 (0.956)	0.772	0.734	0.049 (0.044)
6-segment	88	1	466	0.903	0.844	0.762	0.097
Gap A	87	3	304	0.852	0.874	0.745	0.148
Gap B	88	1	526	0.918	0.837	0.769	0.082

Tree topologies and phylogenetic implications

The phylogenetic results from this study (summarized in Table 2-3 below) can be used to test a series of previous phylogenetic hypotheses. Tree results and bootstrap support indicated a close relationship between the giant otter (*Pteronura brasiliensis*) and sea otter (*Enhydra lutris*) (98-100%). These two taxa were then linked to the African clawless otter (*Aonyx capensis*) (bootstrap support 95-100%), these three taxa are then linked with the Indian smooth-coated otter (*Lutrogale perspicillata*) to form a four taxon clade (bootstrap 95-100%).

Results for the New and Old World river otters were somewhat mixed. In four of the trees (12-segment (Figure 2-2), 6-segment (Figure 2-3), Gap A Tree 1 (Figure 2-4), and Gap B (Figure 2-6)) the North American river otter (*Lontra canadensis*) was weakly placed just outside the four taxon clade described above (bootstrap support 52-77%). In three other trees (Gap A Trees 2 and 3 (Figure 2-4), and consequently the Gap A 50% majority rule consensus tree (Figure 2-5)) this position was occupied by the Eurasian river otter (*Lutra lutra*) (bootstrap 52%). It is slightly misleading to include the Gap A

Table 2-3. Phylogenetic hypotheses of the Lutrinae from this study.

Phylogenetic Question	Hypotheses
Relationship between Old World and New World river otters	Monophyletic or paraphyletic
Relationship between African clawless and Asian small-clawed otters	Diphyletic
Relationship of African clawless and Asian small-clawed otters to Old and New World otters	African clawless allied with sea and giant otters Asian small-clawed basal to all other otters
Relationships of India smooth-coated otter	Allied with giant/sea/African clawless otters
Relationships of spot-necked otter	Basal to all otters except Asian small-clawed
Relationships of sea otter	Allied with giant otter
Relationships of giant otter	Allied with sea otter and African clawless otter

consensus tree as a separate analysis, since it was primarily the product of Trees 2 and 3 from Figure 2-4. The Eurasian river otter was placed basal to the North American river otter in three trees (Figures 2-3, 2-4 (Tree 1), and Figure 2-6) and its position was unresolved in two other trees, due to its ambiguous relationship with the Neotropical otter (*Lontra longicaudis*) as shown in Figures 2-2 and 2-4.

The placement of the Neotropical otter (*Lontra longicaudis*) was equivocal, lying basal to the groups discussed above in four trees (Figures 2-3, 2-4 (Trees 1 and 2), and 2-6) (bootstrap 98-100%), and forming an unresolved node with either *Lutra lutra* (Figure 2-2) or *Lontra canadensis* (Figure 2-5). This ambiguous placement is slightly overemphasized since the consensus tree in Figure 2-5 is based on 3 trees, two of which maintain the position of *Lontra longicaudis* as basal to the taxa previously discussed.

The spot-necked otter (*Hydrictis maculicollis*) was consistently placed as basal to all of the otters except the Asian small clawed otter (*Amblonyx cinereus*) in all trees evaluated (bootstrap 84-98%). This position was never equivocal, and had very strong bootstrap support (97-99% in all cases except Figure 2-2 at 84%).

Another otter, the Asian small-clawed otter (*Amblonyx cinereus*) was also never equivocal in its position, being placed basal to all other otters in every tree (Figures 2-2 to 2-6). Since all trees were unrooted in this study, bootstrap values are not shown for this basal node, however, in a post hoc analysis artificially rooting the tree, bootstrap support for this placement was consistently established at 100% in every tree.

Discussion

The effect of various character coding approaches on tree topologies

Topologically, all methods used in this study recovered many of the same subtrees as discussed below. The various methods mainly differed in their ability to separate the three species of river otter (*Lutra lutra*, *Lontra canadensis*, and *L. longicaudis*) and to a lesser degree, the placement and separation of outgroups. These differences were due to the coarseness of the divisions made by the approaches, and the formation of polytomies in consensus trees for those methods which produce numerous trees (i.e. 12-segment coding). With coarser subdivisions of the data, closely related species may not have gained enough resolution for consistent separation, resulting in polytomies.

Tree statistics

Since the use of tree length to compare results from this study is inappropriate, the other tree statistics presented in Table 2-2 were used to determine overall confidence in trees and measures of fit. Interestingly, the various measures of tree support in this study (Table 2-2) each provide different indications as to which is the best method of character discretization. On the basis of the CI alone, 12 segment coding ($CI = 0.951$) would be judged as best, based on the RI, Gap A coding provides the best score ($RI = 0.874$) and when the RC is considered, Gap B coding appears slightly superior ($RC = 0.769$). While never scored "best", 6-segment results were very close to those found in Gap B coding.

In an effort to better evaluate and understand the overall capability of the methods to produce the best supported tree, the results from Table 2-2 were scored according to the number of best, worst, and intermediate results (Homoplasy Index (HI) was omitted since it is essentially a product of the CI). Best performance for a given index was scored as a 4, second best a 3, third a 2, and the worst a 1. Scores were then totaled and results mirrored those from the RC scores in Table 2-2: Gap B was scored best overall (9), followed by 6-segment coding (8), Gap A coding (7), and 12 segment coding (6). The same order is found in the results from the RC scores alone: Gap B coding (0.769), 6-segment coding (0.762), Gap A coding (0.745), and 12 segment coding (0.734).

These findings indicate support for the use of the RC as the best measure for tree confidence in this data set. As a result, the tree produced by the Gap B coding method is posited to present the best estimate of otter phylogeny based on this postcranial data set, closely followed by 6-segment coding. The RC allows partially homoplastic traits to

contribute to the phylogeny (excluding totally homoplastic characters) and accounts for long branch attraction, making it a useful measure of confidence in this study.

Segment coding

The difference in the number of parsimony uninformative characters between the 12 and 6 segmented data (nine versus zero) was most likely the result of a reduced number of character states in the 6-segment matrix and reduction in the number of discontinuous coding gaps. The overall ability of these two methods to recover a well supported phylogeny also differed widely. Whereas the 6-segment analysis produced only a single tree, the 12-segment coding produced 30 equally parsimonious trees, the most of any analysis. The 6-segment coding scored very well in all measures of confidence, with all measures extremely close to those of the best scored method (Gap B) whereas 12-segment coding fared quite poorly, being scored the worst method in the study.

These results support the contention that overdefining the number of subsets can increase the amount of information loss. As discussed by Goldman (1988), the choice of segment number is critical in maintaining the maximum amount of information. A single segment results in taxa all receiving the same code, an uninformative effect that does not allow variation between taxa to be exposed. Conversely, choosing too many segments could result in each taxon receiving a unique code, thus overemphasizing the variation and again resulting in information loss and less informative analyses. Based on this study using segment coding, it is clear that one must provide justifiable reasons (preferably

based on some a priori knowledge of the taxa under study) for carefully dividing the data set into segments that provide a reasonable means of preserving as much information for the analysis as possible. This appears to confirm the supposition that problems can arise when data are under or over divided, and it seems advisable to chose a median number of subdivisions when segment coding.

Gap coding

Differences between the phylogenies produced by the two gap coding methods (Gap A and Gap B) were slight, in fact the two methods recovered the same tree in at least one case (Figure 2-6 and Figure 2-4, Tree 1). Slight differences in resolving the three river otter species were the only topological variation between the two methods. These differences are probably not due to the deletion of a single character (tibia styloid length, SL) from the Gap A analysis, but more likely result from the variation in the number of segments produced by the two methods. The Gap A analysis was scored the worst for CI (and consequently HI), and third worst for RC. The only confidence index in which Gap A scored well was RI, where it was best. Only the 12-segment data scored worse than Gap A coding overall, while the other gap coding method (Gap B) was scored best overall.

Tree topology

In all trees, the four largest sized species (*Aonyx capensis*, *Enhydra lutris*, *Pteronura brasiliensis*, and *Lutrogale perspicillata*) consistently formed a clade that was

recovered in all of the searches and analyses. Second, the basal position of *Amblonyx cinereus* was recovered in all trees, as was the internally basal position of *Hydrictis maculicollis* (the two smallest sized otter species in this study). An interesting problem occurred regarding *Amblonyx* when the trees were rooted and outgroups were undefined; *Amblonyx* became the outgroup, and the three outgroup species were linked more closely to the otters. Although it is possible that this paraphyletic arrangement of the otters may indicate an incredible amount of divergence for this taxon, more likely it represents the diminutive size of this otter (more in line with the outgroups than otters). The ambiguity in the placement of *Lontra canadensis*, *Lontra longicaudis* and *Lutra lutra*, is probably because these species are very close in size.

Outgroup relationship results closely linked the two musteline taxa American mink (*Mustela vison*) and zorilla (*Ictonyx striatus*). This occurred in all trees except the 12-segment consensus tree (Figure 2-2), where the three outgroup species formed an unresolved tritomy. Placement of the extra-familial skunk (*Mephitis mephitis*) as basal to the other outgroup species was consistent in all trees except the 12-segment consensus tree (Figure 2-2, where it formed a polytomy) and the 6-segment tree (Figure 2-3, where it was linked in a three taxon clade with the mustelids). This indicates that the closest related outgroup to the otters are the mustelids, as represented here by the American mink and zorilla. This supports some previous results (Wayne et al. 1989; Koepfli and Wayne 1998). Further resolution indicating which of these two taxa is most closely related to the otters is not possible based on this information.

Based on these results, the skunk appears to be more distantly related to the otters

than the other outgroup species, but the evidence was inconsistent. The polytomy in the consensus tree in Figure 2-2 is uninformative, Figure 2-3 shows a non-lutrine clade including all three outgroup species with no implications for any detailed determinations, while the trees in Figures 2-4, 2-5, and 2-6 (including the consensus tree as separate is a slight inflation) imply a more distant association for the mephitine group. Based on these trees, two are congruent in their placement of the skunk as more distant (Figures 2-5 and 2-6), one is equivocal (Figure 2-2), and one is uninformative (Figure 2-3). While these results do not confirm the validity of the family Mephitidae, they lend support to the theory that skunks may be more distantly related to the otters than previous workers have indicated (Hunt 1974; Holmes 1987; Wozencraft 1989; Bryant et al. 1993; Wyss and Flynn 1993; Dragoo and Honeycutt 1997; Bininda-Emonds et al 1999; Wolsan 1999).

Biogeographical implications

The character coding approaches used in this study all resulted in trees which essentially ordered the taxa according to size. While a size based phylogeny has recently been found in felids based on biochemical compound analyses (Janczewski et al. 1995; Bininda-Emonds et al. in press) this result has not previously been reported in otters. Despite these unusual findings, support for this arrangement is very good, with high support indices for all of the coding methods. Biogeographically these results are hard to justify, with the most consistent and best supported clade composed of one otter from eastern South America (*Pteronura*) and the other from western North America and eastern Eurasia (*Enhydra*). Given the large number of morphological, behavioral,

ecological, and reproductive differences between these species it appears that the only factor they have in common is their large size. This odd combination is made even more improbable with the addition of *Aonyx* (an otter from sub-Saharan Africa) and *Lutrogale* (Indian subcontinent and Southeast Asia) which are also quite different in virtually all aspects except for large size.

Results for the river otters (*Lontra canadensis*, *L. longicaudis*, and *Lutra lutra*) provided ambiguous resolution to their relationships and seem poorly matched to their geographical ranges. Three trees (including the two best trees Gap B, Figure 2-6 and 6-segment, Figure 2-3) indicated that the North American river otter (*Lontra canadensis*) is most closely related to the amalgamation of the four taxa discussed above, again posing some seriously questionable dispersal assumptions. In no cases do the two congeners (*Lontra* spp.) form a clade, with the exception of polytomies formed by consensus trees. In most cases the Eurasian otter (*Lutra lutra*) lies between the two, making them paraphyletic.

Another geographic problem arises when the position of the African spot-necked otter is evaluated. It was consistently placed internally basal, linked most closely to the Neotropical otter from South America (*Lontra longicaudis*). This result seems somewhat practicable based on the prior connection of those continents until at least 115 mya (Brown and Gibson 1983), and when possible taxonomic implications of recognizing *Hydrictis* as a genus separate from *Lontra* or *Lutra* are considered. So there are possible answers to the seemingly problematic position (disregarding size) of this taxon. One problem is that the oldest known otter ancestor (*Mionictis*) is not known prior to the

Lower Miocene, 23 mya. However, this genus was widespread, found in deposits in Europe and North America (Willemsen 1992). This does not preclude other progenitor dispersal after the breakup of Gondwanaland, but this would seem to indicate a very different phylogenetic arrangement than the size related ordering seen here.

Conclusions

The alignment of the taxa used in this study resulted in the species being primarily categorized by size. If the evolutionary history of these species has a primarily size related component, these results are phylogenetically informative (a result seen in some felid studies). If, however, the results were obtained because this type of data (continuous postcranial measurements), regardless of log transformation or the coding procedure employed; principally provide only size related information not associated with any evolutionary history, then the phylogenetic relationships are questionable. One problem with these results is that while the data provide strong confidence through the tree scores, biogeographically they are quite puzzling. Consistency indices were reasonably high, even very high in some cases. Is the confidence provided by these indices because the data are allometrically correlated, providing false confidence in their evolutionary connectivity? This enigma from these coding approaches does not appear easy to resolve.

While the different approaches in discretizing used in this study purport to provide differing amounts of information, the overall results did not differ significantly in either tree topology or tree statistics. Minimal differences in the consistency indices (CI and RC) are most likely due to the varying number of discrete codes produced by these

methods and do not reflect major differences in the amount of phylogenetic information provided by different coding methodologies. It also appears that a number of simple statistical analyses would result in the same information regarding the alignment of the taxa based on the characters used in this study, and could provide greater and more useful information than the resultant data matrix and trees produced from them.

As for the comparison of these results with previous phylogenetic hypotheses, the following conclusions are supported: The relationship between the New and Old World river otters (and even among the New World otters alone) was found to be paraphyletic (or polyphyletic), a result not previously presented or acceptable by current rules of systematic classification. African clawless and Asian small-clawed otters were found to be diphyletic, in agreement with Sokolov (1973) Holmes (1987), van Zyll de Jong (1991) and Willemssen (1992). The African clawless otter is allied with the sea otter and giant otter, another previously unreported result (most likely due to their large sizes since they seem to have nothing else in common). The Asian small clawed otter was found to be basal to all other otters (probably owing to its extremely small size), a result close to those from van Zyll de Jong (1991) who found them basal to New World river otters and the sea otter. The India smooth-coated otter was placed just outside the clade formed by the sea, giant, and African clawless otters, only van Zyll de Jong (1991) found a similar link with the giant otter. The spot-necked otter was found basal to all otters except the Asian small-clawed, another unreported result (it is also smaller than all otters except the Asian small-clawed otter). The giant and sea otters were always closely linked in all trees in this study (again probably due to their similar large sizes). This result is totally

inconsistent with all previous studies, and its uncertainty is compounded by their linkage with the African clawless otter (the third largest taxon in this study).

These size-related results may indicate an inherent problem in the information provided by measurement data, and the way in which the data were coded. This result is not surprising, but has not been addressed by any of the proponents of the various coding approaches applied here. While size can be an important character in phylogenetic and evolutionary analyses (Gould 1966), multiple characters which are different measures of size only present excessive redundancy and will give unjustified and disproportionate weighting to this singular trait. Except in unusual cases, large animals will have correspondingly larger anatomical features. In cases where there are adaptive shifts in the relative appendage size (Allen's Rule), there may be justification and evolutionary significance uncovered by using this type of data, but generally its usefulness as a primary source of phylogenetic information seems unwarranted. A data set using residuals from a regression analysis to offset this problem is considered in the next chapter.

The size related ordering of these taxa is disconcerting. Results may indicate that the methods of coding continuous trait data into discrete variables examined here provide a less than satisfactory means of producing useful phylogenetic data or uncovering meaningful evolutionary trends, despite the high levels of confidence provided by the various consistency indices. Papers addressing the problem of character state delineation and the use of continuous data have variously labeled the solution of this problem as "the Holy Grail" (Thiele 1993) and the "bête noire" (French for "bugbear", defined as an object of obsessive dread) (Pogue and Mickevich 1990), with understandable reason.

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Appendices

Appendix 2-1. Specimens examined.

Museum abbreviations: National Museum of Natural History (USNM), American Museum of Natural History (AMNH), Field Museum of Natural History (FMNH), Harvard Museum of Comparative Zoology (MCZ), Yale Peabody Museum (YPM), Boston Society of Natural History (BSNH)

Zoological Park Abbreviations: Central Park Zoo (CPZ), Chicago Zoological Society = Brookfield Zoo (CZS), Lincoln Park Zoo (LPZ), National Zoological Park (NZIP), New York Zoological Park (NYZP), Rotterdam Zoological Gardens (RZG).

Scapula specimens:

Amblonyx cinereus

USNM #49929; F; Indonesia: Rhio Arch., Pulo Setoko. USNM #198054; F; Indonesia: Borneo; Sungai Karangan. USNM #151879; F; Indonesia: Borneo; Pulo Laut. USNM #396645; F; NZP (Accession #105644). USNM #574897; F; NZP (Accession #106641). USNM #573965; M; NZP (Accession #105587). USNM #314979; M; NZP (Accession #18564). FMNH #62868; M; Philippines, Palawan, Iwahig, Gen Luna (left 4th metacarpal broken in life, left humerus broken proximally postmortem). AMNH #101639; M; Indonesia: Java; Cheriban (left scapula broken). AMNH #106549; M; Indonesia: Java; Cheriban (left scapula broken). AMNH #238084; M; CPZ. AMNH #135149; Unk.; (M?) NYZP. AMNH #244367; Unk.; (F?) NYZP. AMNH #244368; Unk.; (F?) NYZP.

Aonyx capensis

FMNH #73732; F; "Africa"(could be Paraonyx) "Congo drainage", Wild caught CZS sub-adult. AMNH #51853; F; Zaire: Faradje. AMNH #51837; F; Zaire: Faradje. AMNH #186955; F; Liberia: Wild caught; NYZP (sub-adult).

Enhydra lutris

USNM #11794; Unk.; Alaska, Kodiak. USNM #20966; Unk.; Alaska (sub-adult). USNM #263313; Unk.; Alaska, Amchitka. USNM #263315; M; Alaska, Kavalga. USNM #263316; M; Alaska, Ogluiga. USNM #265075; M; Alaska, Cordova. USNM #271749; M; Alaska, Amchitka. MCZ #9345; Unk.; (F?) Alaska. From Smithsonian Institution via the BSNH. FMNH #78763; F; Alaska; Aleutian Isls., Amchitka, Game Management Unit 10 (right humerus broken, left scapula missing). FMNH #129314; F; Alaska; Aleutian Isls., Amchitka, Game Management Unit 10.

Hydrictis maculicollis

FMNH #127292; F; "Africa"; CZS, 16 yrs., 5.4 kg (835/275/110/18). FMNH #127291; F; "Africa"; CZS, 5.5 kg (331/129/97/16). FMNH #124577; F; "Africa"; "Zoo specimen", Young adult, (a little cartilage on vertebral border). FMNH #58828; M; "Africa"; CZS; Young adult (a little cartilage on vertebral border). FMNH #129493; M; CZS (PL 626) Zoo born, (vertebral border cartilage almost ossified). FMNH #127290; M; CZS; (vertebral border cartilage almost ossified). FMNH #125385; Unk.; (F?) CZS;

Appendix 2-1. Continued

sub-adult. Most bone sutures not fused, right tibia broken, very young. FMNH #153719; Unk.; (F?) CZS. Slightly osteopathic, not bad. AMNH #239613; M; Liberia: Wild caught. AMNH #51827; M; Zaire: Niapu.

Lontra canadensis

USNM #484773; F; Alabama; Bullock Co. USNM #484837; F; Alabama; Barbour Co. USNM #484852; F; Alabama; Barbour Co. USNM #484864; F; Alabama; Russell Co. USNM #484869; F; Alabama; Russell Co. USNM #484794; M; Alabama; Bullock Co. USNM #484803; M; Alabama; Barbour Co. USNM #484809; M; Alabama; Barbour Co. USNM #484823; M; Alabama; Barbour Co. USNM #484824; M; Alabama; Russell Co.

Lontra felina

USNM #512791; F; Peru: San Andres.

Lontra longicaudis

FMNH #89790; M; "S. America"; Wild caught, CZS. Adult. FMNH #69582; M; Colombia: Choco; Unguia. Adult. AMNH #80092; M; Brazil: Wild caught; NYZP (sub-adult). MCZ #37845; M; Ecuador: Oriente; Rio Jatun Yacu; (*L. l. enudris*). YPM #5013; M; Guatemala: La Blanca, Naranja River. (not quite mature-old sub-adult). AMNH #63958; Unk.; (F?) Panama. AMNH #205902; F; Uruguay: Lavalleya (scapulae broken). AMNH #214498; F; Colombia: Wild caught; NYZP. AMNH #133950; F; Brazil: Gayaz; Anapolis (skin still attached on parts). AMNH #207729; F; Locality unk.; Wild caught; NYZP. MCZ #28637; F; Paraguay: Iguazu River below Numbucu. (*L. l. longicaudis*). Left half only.

Lutra lutra

FMNH #97838; F; Iran; Azerbaijan; 19 km SW of Rezaiyeh (Street Expedition #331). MCZ #24829; F; China: Hainan, Nodua (*L. l. chiniensis*, sub-adult). MCZ #51512; Unk.; (F?) Turkey: Murat Drainage, near Elazig (*L. l. lutra*). YPM #3221; Unk.; (F?) RZG, Holland; (good for a captive). FMNH #99384; M; Sri Lanka: Bandaragana W.P. (right scapula crushed).

Lutrogale perspicillata

FMNH #63799; F; "Asia", Wild caught; LPZ. AMNH #99610; Unk.; India.

Pteronura brasiliensis

USNM #304663; M; NZP (Accession #16364) almost mature. USNM #319226; M; NZP (Accession #18898), very osteopathic. FMNH #70768; M; Colombia: Caqueta; Rio Mecaya. Young adult. AMNH #30190; M; Venezuela: Bolivar; Caura, Rio Mocho. AMNH #77735; M; from Brooklyn Museum. AMNH #30191; F; Venezuela: Bolivar; Caura, Rio Mocho. AMNH #214394; F; NYZP (bad shape -osteopathic). MCZ #27868; Unk.; (F) Brazil: Upper Rio Manacapuru (purchased).

Appendix 2-1. Continued.

Mustela vison ingens

USNM #256983; F; Alaska; Echo Isl., Kasheverof Passage. USNM #256978; F; Alaska; Bluff Isl., Snow Pass. USNM #256986; F; Alaska; Thorne Isl., Kasheverof Passage. USNM #188250; F; Canada: Quebec; MacKenzie, Godbout. USNM #256980; F; Alaska; Bluff Isl., Snow Pass. USNM #546175; M; Alaska; 2nd Judicial Division, Nutuvukti Lake, C. Brooks Range. USNM #256981; M; Alaska; Shrubby Isl., Snow Pass. USNM #256982; M; Alaska; Shrubby Isl., Snow Pass. USNM #256984; M; Alaska; Echo Isl., Kasheverof Passage. USNM #256985; M; Alaska; Thorne Isl., Kasheverof Passage.

Mephitis mephitis nigra

USNM #188996; F; New York; Lewis Co., Locust Grove. USNM #188997; F; New York; Lewis Co., Locust Grove. USNM #240474; F; New York; Rochester. USNM #260921; F; West Virginia; Cabell Co., Barboursville. USNM #20973; F; Massachusetts; Woods Hole. USNM #87236; M; New York; Essex Co., Severance. USNM #87237; M; New York; Essex Co., Severance. USNM #240473; M; New York; Rochester. USNM #240911; M; New York; Rochester. USNM #397234; M; Maryland; Montgomery Co., Germantown.

Ictonyx striatus

USNM #344875; F; South Africa: Orange Free State; 6 miles NW of Bethsville. USNM #573967; F; NZP; (Accession #101357). USNM #399275; M; NZP; (Accession 102180).

Arm specimens:

Amblonyx cinereus

USNM 198054; F; Indonesia: Borneo; Sungai Karangan. USNM #151879; F; Indonesia: Borneo; Pulo Laut, sub-adult. USNM #396645; F; NZP (Accession #105644). USNM #574897; F; NZP (Accession #106641). USNM #573965; M; NZP (Accession #105587). USNM #314979; M; NZP. (Accession #18564). USNM #155324; M; Indonesia: Java; Depok. FMNH #62868; M; Philippines: Palawan, Iwahig, Gen Luna. AMNH #101639; M; Indonesia: Java; Cheriban. AMNH #106549; M; Indonesia: Java; Cheriban. AMNH #238084; M; CPZ. AMNH #135149; Unk.; (M?) NYZP. AMNH #244367; Unk.; (F?) NYZP. AMNH #244368; Unk.; (F?) NYZP. AMNH #106626; F; Indonesia: Sumatra; Boekit Sanggoel, Benkoelen.

Aonyx capensis

FMNH #73732; F; "Africa" (Paraonyx); CZS; sub-adult; Right supracondyloid foramen broken. AMNH #51853; F; Zaire: Faradje. AMNH #51837; F; Zaire: Faradje. AMNH #186955; F; Liberia: Wild caught; NYZP (sub-adult).

Appendix 2-1. Continued.

Enhydra lutris

USNM #11794; Unk.; Alaska; Kodiak. USNM #20966; Unk.; Alaska (subadult).
USNM #263313; Unk.; Alaska; Amchitka Isl. USNM #263315; M; Alaska; Kavalga.
USNM #265075; M; Alaska; Cordova, articulated. USNM #271749; M; Alaska;
Amchitka Isl., sub-adult. USNM #271748; M; Alaska; Amchitka Isl. Rt. humerus only.
MCZ #9345; Unk.; (F?) Alaska. USNM via BSNH. FMNH #78763; F; Alaska; Aleutian
Islands, Amchitka (rt. humerus broken). FMNH #129314; F; Alaska; Aleutian Islands,
Amchitka.

Hydrictis maculicollis

FMNH #127292; F; "Africa"; CZS, 16 yrs., 5.4 kg (835/275/110/18). FMNH #127291;
F; "Africa"; CZS, 5.5 kg (331/129/97/16). FMNH #124577; F; "Africa"; Young adult.
FMNH #58828; M; "Africa"; CZS; Young adult. FMNH #129493; M; CZS (PL 626)
Zoo, 2.5 yrs., 3.34 kg. FMNH #127290; M; CZS; (930/367/116/18) Testis 19x10.
FMNH #125385; Unk.; (F?) CZS; sub-adult. FMNH #153719; Unk.; (F?) CZS. Slightly
osteopathic. AMNH #51825; F; Zaire: Faradje (young adult). AMNH #239613; M;
Liberia: Wild caught. AMNH #51827; M; Zaire: Niapu.

Lontra canadensis

USNM #484773; F; Alabama; Bullock Co. USNM #484837; F; Alabama; Barbour Co.
USNM #484852; F; Alabama; Barbour Co. USNM #484864; F; Alabama; Russell Co.
USNM #484869; F; Alabama; Russell Co., right humerus broken and healed at
mediodistal end. USNM #484794; M; Alabama; Bullock Co. USNM #484803; M;
Alabama; Barbour Co. USNM #484809; M; Alabama; Barbour Co. USNM #484823;
M; Alabama; Barbour Co. USNM #484824; M; Alabama; Russell Co. USNM #271804;
M; Washington; Clallam Co.; Forks; Calawah River.

Lontra felina

USNM #512791; F; Peru: San Andres.

Lontra longicaudis

USNM #255133; M; Peru: Chanchamayo. FMNH #89790; M; "S. America" Wild
caught, CZS. Adult. FMNH #69582; M; Colombia: Choco; Unguia. Adult. AMNH
#80092; M; Brazil: Wild caught; NYZP (sub-adult). MCZ #37845; M; Ecuador:
Oriente, Rio Jatun Yacu. *L. l. enudris*. YPM #5013; M; Guatemala: La Blanca, Naranja
River. (not quite mature-old sub-adult). AMNH #63958; Unk.; (F?) Panama. AMNH
#205902; F; Uruguay: Lavalleja. AMNH #214498; F; Colombia: Wild caught; NYZP.
AMNH #133950; F; Brazil: Gayaz; Anapolis (skin attached on parts). AMNH #207729;
F; Loc. Unk.; Wild caught) NYZP. 4.9 kg. MCZ #28637; F; Paraguay: Iguazu River
below Numbucu. *L. l. longicaudis*. Left half of specimen only.

Appendix 2-1. Continued.

Lutra lutra

FMNH #97838; F; Iran: Azerbaijan; 19 km SW of Rezaiyeh. sub- adult. MCZ #24829; F; China: Hainan, Nodda. (*L. l. chiniensis*, sub-adult). MCZ #51512; Unk.; (F?) Turkey: Murat Drainage, near Elazig (*L. l. lutra*). YPM #3221; Unk.; (F?) RZG, Holland. FMNH #99384; M; Sri Lanka: Bandaragana W.P.; Adult (*L. l. nair*).

Lutrogale perspicillata

USNM #344998; F; NZP (Accession #20924). FMNH #63799; F; "Asia", Wild caught, LPZ (right humerus missing). AMNH #99610; Unk.; India.

Pteronura brasiliensis

USNM #304663; M; NZP (Accession #16364) almost mature. USNM #319226; M; NZP (Accession #18898), very osteopathic. FMNH #70768; M; Colombia: Caqueta; Rio Mecaya. Young adult-sub-adult. AMNH #30190; M; Venezuela: Bolivar; Caura, Rio Mocho. AMNH #77735; M; Brooklyn Museum. AMNH #30191; F; Venezuela: Bolivar; Caura, Rio Mocho. AMNH #214394; F; NYZP (very bad shape -osteopathic). MCZ #27868; Unk.; (F) Brazil: Upper Rio Manacapuru (purchased).

Mustela vison ingens

USNM #256983; F; Alaska; Echo Isl., Kashevarof Passage. USNM #256978; F; Alaska; Bluff Isl., Snow Pass. USNM #256986; F; Alaska; Thorne Isl., Kasheverof Passage. USNM #188250; F; Canada: Quebec; MacKenzie, Godbout. USNM #256980; F; Alaska; Bluff Isl., Snow Pass. USNM #546175; M; Alaska; 2nd Judicial Division, Nutuvukti Lake, C. Brooks Range. USNM #256981; M; Alaska; Shrubby Isl., Snow Pass. USNM #256982; M; Alaska; Shrubby Isl., Snow Pass. USNM #256984; M; Alaska; Echo Isl., Kashevarof Passage. USNM #256985; M; Alaska; Thorne Isl., Kasheverof Passage

Mephitis mephitis nigra

USNM #188996; F; New York; Lewis Co., Locust Grove. USNM #188997; F; New York; Lewis Co., Locust Grove. USNM #240474; F; New York; Rochester. USNM #260921; F; West Virginia; Cabell Co., Barboursville. USNM #20973; F; Massachusetts; Woods Hole. USNM #87236; M; New York; Essex Co., Severance. USNM #87237; M; New York; Essex Co., Severance. USNM #240473; M; New York; Rochester. USNM #240911; M; New York; Rochester. USNM #397234; M; Maryland; Montgomery Co., Germantown.

Ictonyx striatus

USNM #344875; F; South Africa: Orange Free State; 6 mi. NW of Bethsville. USNM #573967; F; NZP; (Accession #101357) 30 June, 1987. USNM #399275; M; NZP.

Appendix 2-1. Continued.

Leg Specimens:

Amblonyx cinereus

USNM #198054; F; Indonesia: Borneo; Sungai Karangan, left leg articulated with foot. USNM #151879; F; Indonesia: Borneo; Pulo Laut, sub-adult. USNM #396645; F; NZP (Accession #105644). USNM #574897; F; NZP. AMNH #106626; F; Indonesia: Sumatra; Boekit Sanggoel; Benkoelen. USNM #573965; M; NZP (Accession #105587). USNM #314979; M; NZP (Accession #18564). USNM #155324; M; Indonesia: Java; Depok. FMNH #62868; M; Philippines: Palawan; Iwahig, Gen Luna. AMNH #101639; M; Indonesia: Java; Cheriban (right side only). AMNH #106549; M; Indonesia: Java; Cheriban. AMNH #238084; M; CPZ. AMNH #135149; Unk.; (M?) NYZP. AMNH #244367; Unk.; (F?) NYZP. AMNH #244368; Unk.; (F?) NYZP.

Aonyx capensis

FMNH #73732; F; "Africa" could be Paraonyx "Congo drainage", Wild caught; CZS sub-adult. AMNH #51853; F; Zaire: Faradje. AMNH #51837; F; Zaire: Faradje. AMNH #186955; F; Liberia: Wild caught; NYZP (sub-adult).

Enhydra lutris

USNM #286368; F; Alaska, Amchitka Isl. (left tibia/fibula only). FMNH #78763; F; Alaska; Aleutian Isls., Amchitka. FMNH #129314; F; Alaska; Aleutian Isls., Amchitka; Young adult. USNM #263315; M; Alaska, Kavalga, left leg articulated. USNM #265075; M; Alaska, Cordova, articulated with left foot, tibia/fibula fused distally. USNM #271749; M; Alaska, Amchitka Isl., sub-adult. USNM #271748; M; Alaska, Amchitka Isl. USNM #11794; Unk.; Alaska, Kodiak Isl. USNM #20966; Unk.; Alaska (right & left tibia/fibula fused proximally). USNM #263313; Unk.; Alaska, Amchitka Isl. (left tibia only). MCZ #9345; Unk.; (F?) Alaska, USNM via BSNH.

Hydrictis maculicollis

FMNH #127292; F; "Africa"; CZS; Right fibula fused to tibia, very osteopathic. FMNH #127291; F; "Africa"; CZS, 5.5K kg; (331/129/97/16). FMNH #124577; F; "Africa"; "Zoo specimen". AMNH #51825; F; Zaire: Faradje (young adult). AMNH #239613; M; Liberia: Wild caught-captive; deformed on proximal surfaces. AMNH #51827; M; Zaire: Niapu. FMNH #58828; M; "Africa"; CZS. FMNH #129493; M; CZS (PL 626) Zoo born, 2.5 yrs., 3.34 kg. FMNH #127290; M; CZS; (930/367/116/18) Testis 19x10. FMNH #125385; Unk.; (F?) CZS; sub-adult; Right tibia broken, not used. FMNH #153719; Unk.; (F?) CZS.

Lontra canadensis

USNM #484773; F; Alabama; Bullock Co. USNM #484837; F; Alabama; Barbour Co., (right leg only), tibia/fibula fused proximally. USNM #484852; F; Alabama; Barbour Co., (right only), tibia/fibula fused proximally, styloid broken distally. USNM #484864; F; Alabama; Russell Co. (right only). USNM #484869; F; Alabama; Russell Co., (right

Appendix 2-1. Continued.

leg only), tibia/fibula fused proximally. USNM #484794; M; Alabama; Bullock Co. USNM #484803; M; Alabama; Barbour Co., both legs fused proximally. USNM #484809; M; Alabama; Barbour Co. USNM #484823; M; Alabama; Barbour Co., (left only). USNM #484824; M; Alabama; Russell Co., (left only). USNM #271804; M; Washington, Clallam Co., Forks, Calawah River.

Lontra felina

USNM #512791; F; Peru: San Andres.

Lontra longicaudis

AMNH #205902; F; Uruguay: Lavalleya. AMNH #214498; F; Colombia: Wild caught; NYZP. AMNH #133950; F; Brazil: Gayaz; Anapolis (skin attached on parts). AMNH #207729; F; Loc. Unk.; Wild caught; NYZP. 4.9 kg. AMNH #23494; F; Colombia: Magdalena; Bonda. MCZ #28637; F; Paraguay: Iguazu River below Numbucu (*L. l. longicaudis* 1/2 specimen). USNM #255133; M; Peru: Chanchamayo. FMNH #89790; M; "S. America" Wild caught; CZS; Adult. FMNH #69582; M; Colombia: Choco; Unguia. Adult. AMNH #80092; M; Brazil: Wild caught; NYZP (sub-adult). MCZ #37845; M; Ecuador: Oriente, Rio Jatun Yacu. (*L. l. enudris*). YPM #5013; M; Guatemala: La Blanca, Naranja River (*L. l. annectens*). AMNH #63958; Unk.; (F?); Panama.

Lutra lutra

FMNH #97838; F; Iran: Azerbaijan; 19 km SW of Rezaiyeh. MCZ #24829; F; China: Hainan, Nodoo -young adult (*L. l. chiniensis*). FMNH #99384; M; Sri Lanka: Bandaragana W.P. USNM #259466; Unk.; China: Szechwan, Wen Chuan (partial) (*L. l. chiniensis*). MCZ #51512; Unk.; (M?) Turkey: Murat drainage, near Elazig (*L. l. lutra*). YPM #3221; Unk.; (F?) Holland: RZG (captive, great shape) ("*L. vulgaris*").

Lutrogale perspicillata

USNM #344998; F; NZP (Accession #20924). FMNH #63799; F; "Asia", Wild caught, LPZ. AMNH #99610; Unk.; India.

Pteronura brasiliensis

AMNH #30191; F; Venezuela: Bolivar; Caura, Rio Mocho. AMNH #214394; F; NYZP (very bad shape -osteopathic). USNM #304663; M; NZP (Accession #16364) almost mature. USNM #319226; M; NZP (Accession #18898) adult, very osteopathic. FMNH #70768; M; Colombia: Caqueta; Rio Mecaya. Young-sub-adult. AMNH #30190; M; Venezuela: Bolivar; Caura, Rio Mocho. AMNH #77735; M; Brooklyn Museum. MCZ #27868; Unk.; (F?) Brazil: Upper Rio Manacapuru (purchased).

Appendix 2-1. Continued.

Mustela vison ingens

USNM #256983; F; Alaska; Echo Isl., Kashevarof Passage. USNM #256978; F; Alaska; Bluff Isl., Snow Pass. USNM #256986; F; Alaska; Thorne Isl., Kasheverof Passage. USNM #188250; F; Canada: Quebec; MacKenzie, Godbout. USNM #256980; F; Alaska; Bluff Isl., Snow Pass. USNM #546175; M; Alaska; 2nd Judicial Division, Nutuvukti Lake, C. Brooks Range. USNM #256981; M; Alaska; Shrubby Isl., Snow Pass. USNM #256982; M; Alaska; Shrubby Isl., Snow Pass. USNM #256984; M; Alaska; Echo Isl., Kashevarof Passage. USNM #256985; M; Alaska; Thorne Isl., Kasheverof Passage.

Mephitis mephitis nigra

USNM #188996; F; New York; Lewis Co., Locust Grove. USNM #188997; F; New York; Lewis Co.,. USNM #240474; F; New York; Rochester. USNM #260921; F; West Virginia; Cabell Co., Barboursville. USNM #20973; F; Massachusetts; Woods Hole. USNM #87236; M; New York; Essex Co., Severence. USNM #87237; M; New York; Essex Co., Severence. USNM #240473; M; New York; Rochester. USNM #240911; M; New York; Rochester. USNM #397234; M; Maryland; Montgomery Co., Germantown.

Ictonyx striatus

USNM #344875; F; South Africa: Orange Free State; 6 mi. NW of Bethsville. USNM #573967; F; NZP; (Accession #101357) 30 June, 1987. USNM #399275; M; NZP.

Appendix 2-2. Measurement abbreviations used in data sheets. 101 continuous data traits were measured, the 88 characters used in most analyses are shown in bold typeface. Character code numbers used in the data matrices are indicated.

Head and Body (not measured, taken from the literature)

1. **HB-** **head and body length**

Tibia (13 characters measured, 11 used)

2. **TL-** **total length**
3. **ML-** **medial length**
4. **PW-** **proximal width**
5. **LTW-** **lateral tuberosity width**
6. **MTW-** **medial tuberosity width**
7. **PTC-** **proximal thickness with crest**
8. **DW-** **distal width**
9. **DT-** **distal thickness**
10. **SW-** **styloid width**
11. **SL-** **styloid length**
12. **SD-** **styloid depth**
PND- popliteal notch depth
DPW- dorsal projection width

Femur (12 characters measured, 7 used)

13. **FL-** **femur length**
14. **PFW-** **proximal femur width**
15. **DFW-** **distal femur width**
16. **SFL-** **shaft of femur length**
17. **HW-** **head width**
18. **NW-** **neck width**
19. **LTrW-** **lesser trochanter width**
HGtNd- head/greater trochanter notch depth
PSW- patellar surface width
PSL- patellar surface length
IFd- intertrochanteric fossa depth
LTL- lesser trochanter length

Humerus (24 characters measured, 21 used)

20. **HuL-** **humerus length**
21. **PW2-** **proximal width**
22. **HDW-** **humerus distal width**
23. **ArcB-** **arc height to bottom of humerus (posterior)**
24. **ArcT-** **arc to top of humerus (posterior)**

Appendix 2-2. Continued.

Humerus (continued)

25. BGW-	bicipital groove width
26. BGD-	bicipital groove depth
27. HdL-	head length
28. HdW1-	head width
29. LTW2-	lesser tuberosity width
30. LTL2-	lesser tuberosity length
31. MEW-	medial epicondyle width
32. MEL-	medial epicondyle length
33. OFW-	olecranon fossa width
34. OFL-	olecranon fossa length
35. OFD-	olecranon fossa depth
36. DGW-	distal articular surface greatest width
37. DLW-	distal articular surface least width
38. TW-	trochlear width
39. CW-	capitulum width
40. LW-	least width of trochlea/capitulum
ArcL-	arc length when placed on posterior
SCFL-	supracondyloid foramen length
SCFW-	supracondyloid foramen width

Radius (10 characters measured, 6 used)

41. TLP-	total length parallel
42. TLT-	total length tilted
43. PW-	proximal width
44. PT-	proximal thickness
45. GDW-	greatest distal width
46. GDD-	greatest distal thickness depth
PTN-	proximal thickness at notch of articular surface
ASW-	articular surface width
GASD-	greatest articular surface depth
ASDS-	articular surface depth at styloid

Ulna (24 characters measured, 24 used)

47. UPL-	ulnar posterior length
48. UAL-	ulnar anterior length
49. SCL-	length from styloid knob to coronoid
50. PUW-	proximal ulnar width
51. PUT-	proximal ulnar thickness
52. PSN-	proximal width of semilunar notch
53. DSN-	distal width of semilunar notch
54. OH-	olecranon height

Appendix 2-2. Continued.

Ulna (continued)

55. SND-	semilunar notch depth
56. DT2-	distance between tips of semilunar notch
57. NT-	semilunar notch thickness
58. PNW-	proximal width of semilunar notch
59. DNW-	distal width of semilunar notch
60. NW2-	semilunar notch width
61. RNW-	radial notch width
62. RNT-	radial notch thickness
63. NP-	narrowest point of styloid process
64. SPW-	styloid process width
65. LSW-	least width of styloid process tip
66. STW-	styloid process tip width
67. SW2-	styloid process width
68. SSW-	smaller styloid width
69. SSL-	smaller styloid length
70. TT-	styloid process tip thickness

Scapula (20 characters measured, 18 used)

71. AcL-	acromion length
72. AcW-	acromion width
73. MtW2-	metacromion width
74. MtL-	metacromion length
75. SSVBH-	scapular spine vertebral border height
76. SSCH-	scapular spine center height
77. SSVH-	scapular spine ventral border height
78. SSTubW-	scapular spine tuberosity width
79. SSTubL-	scapular spine tuberosity length
80. IANW-	inferior angle notch width
81. IANL-	inferior angle notch length
82. VBLs-	vertebral border length (straight)
83. VBLc-	vertebral border length (curved)
84. AxBL-	axillary border length
85. SBLc-	superior border length (curved)
86. AnBl-	anterior border length
87. SND2-	scapular notch depth
88. SNL-	scapular notch length
AcAng-	acromion angle away from scapular spine
MetAng-	metacromion angle from scapular spine and acromion

Appendix 2-3 Data matrix produced with 12-segment coding techniques.

	1	10	20	30	40	44
<i>Amblonyx cinereus</i>	•	•	•	•	•	•
<i>Aonyx capensis</i>	•	•	•	•	•	•
<i>Enhydra lutris</i>	•	•	•	•	•	•
<i>Hydrictis maculicollis</i>	•	•	•	•	•	•
<i>Lontra canadensis</i>	•	•	•	•	•	•
<i>Lontra longicaudis</i>	•	•	•	•	•	•
<i>Lutra lutra</i>	•	•	•	•	•	•
<i>Lutrogale perspicillata</i>	•	•	•	•	•	•
<i>Pteromura brasiliensis</i>	•	•	•	•	•	•
<i>Mustela vison</i>	•	•	•	•	•	•
<i>Mephitis mephitis</i>	•	•	•	•	•	•
<i>Ictonyx striatus</i>	•	•	•	•	•	•
<i>Amblonyx cinereus</i>	•	•	•	•	•	•
<i>Aonyx capensis</i>	•	•	•	•	•	•
<i>Enhydra lutris</i>	•	•	•	•	•	•
<i>Hydrictis maculicollis</i>	•	•	•	•	•	•
<i>Lontra canadensis</i>	•	•	•	•	•	•
<i>Lontra longicaudis</i>	•	•	•	•	•	•
<i>Lutra lutra</i>	•	•	•	•	•	•
<i>Lutrogale perspicillata</i>	•	•	•	•	•	•
<i>Pteromura brasiliensis</i>	•	•	•	•	•	•
<i>Mustela vison</i>	•	•	•	•	•	•
<i>Mephitis mephitis</i>	•	•	•	•	•	•
<i>Ictonyx striatus</i>	•	•	•	•	•	•

Appendix 2-4 Data matrix produced with 6-segment coding techniques.

	1	10	20	30	40	44
<i>Amblyonx cinereus</i>	•	•	•	•	•	•
<i>Aonyx capensis</i>	BBBCBBB	ADCBBC	CCCCBB	BBB	BBB	CCBACBBCCBB
<i>Enhydra lutris</i>	DDDDDD	DECC	EDDF	DEE	DEDD	DEEFDDEEEED
<i>Hydrictis maculicollis</i>	EEFF	FF	FF	FF	FF	FF
<i>Lontra canadensis</i>	CB	CCCC	CB	BB	CC	CC
<i>Lontra longicaudis</i>	CC	CC	CC	CB	CC	CC
<i>Lutra lutra</i>	CC	CC	CC	CC	CC	CC
<i>Lutrogale perspicillata</i>	CC	CC	CC	CC	CC	CC
<i>Pteromura brasiliensis</i>	CC	CC	CC	CC	CC	CC
<i>Mustela vison</i>	CC	CC	CC	CC	CC	CC
<i>Mephitis mephitis</i>	CC	CC	CC	CC	CC	CC
<i>Ictonyx striatus</i>	CC	CC	CC	CC	CC	CC
	45	50	60	70	80	88
<i>Amblyonx cinereus</i>	•	•	•	•	•	•
<i>Aonyx capensis</i>	AB	CC	BB	BB	BB	BB
<i>Enhydra lutris</i>	DE	EE	DD	DE	DE	DD
<i>Hydrictis maculicollis</i>	EE	FF	EE	FF	EE	FF
<i>Lontra canadensis</i>	BC	BB	CB	CC	CC	CC
<i>Lontra longicaudis</i>	CC	CC	CC	CC	CC	CC
<i>Lutra lutra</i>	CC	CC	CC	CC	CC	CC
<i>Lutrogale perspicillata</i>	CC	CC	CC	CC	CC	CC
<i>Pteromura brasiliensis</i>	CC	CC	CC	CC	CC	CC
<i>Mustela vison</i>	CC	CC	CC	CC	CC	CC
<i>Mephitis mephitis</i>	CC	CC	CC	CC	CC	CC
<i>Ictonyx striatus</i>	CC	CC	CC	CC	CC	CC
	45	50	60	70	80	88
<i>Amblyonx cinereus</i>	•	•	•	•	•	•
<i>Aonyx capensis</i>	AB	CC	BB	BB	BB	BB
<i>Enhydra lutris</i>	DE	EE	DD	DE	DE	DD
<i>Hydrictis maculicollis</i>	EE	FF	EE	FF	EE	FF
<i>Lontra canadensis</i>	BC	BB	CB	CC	CC	CC
<i>Lontra longicaudis</i>	CC	CC	CC	CC	CC	CC
<i>Lutra lutra</i>	CC	CC	CC	CC	CC	CC
<i>Lutrogale perspicillata</i>	CC	CC	CC	CC	CC	CC
<i>Pteromura brasiliensis</i>	CC	CC	CC	CC	CC	CC
<i>Mustela vison</i>	CC	CC	CC	CC	CC	CC
<i>Mephitis mephitis</i>	CC	CC	CC	CC	CC	CC
<i>Ictonyx striatus</i>	CC	CC	CC	CC	CC	CC

Appendix 2-5. Data matrix produced with Gap A coding techniques.

	1	10	20	30	40	44
<i>Amblyonx cinereus</i>	•	•	•	•	•	•
<i>Aonyx capensis</i>	BBBBBBBB	AABBBB	BBBBBB	BBBBBB	AABBBB	AABBBB
<i>Enhydra lutris</i>	CCCCCCCC	CCCCCD	CCCCCC	CCCCCC	CCCCCC	CCCCDD
<i>Hydrictis maculicollis</i>	DDDDDD	DDDEDD	DDDDDD	DDDDDD	DDDDDD	DDDDDD
<i>Lontra canadensis</i>	BBBCCB	BBBBA	BBBBA	BBBBA	BBBBA	BBBBA
<i>Lontra longicaudis</i>	BBBCCB	BBBBA	BBBBA	BBBBA	BBBBA	BBBBA
<i>Lutra lutra</i>	BBBBBB	BBBBB	BBBBB	BBBBB	BBBBB	BBBBB
<i>Lutrogale perspicillata</i>	BBBBBB	BBBBB	BBBBB	BBBBB	BBBBB	BBBBB
<i>Pteromura brasiliensis</i>	CCCCCC	CCCCC	CCCCC	CCCCC	CCCCC	CCCCC
<i>Mustela vison</i>	DDDDDD	DDDDDD	DDDDDD	DDDDDD	DDDDDD	DDDDDD
<i>Mephitis mephitis</i>	AAAAAA	AAAAA	AAAAA	AAAAA	AAAAA	AAAAA
<i>Ictonyx striatus</i>	AAAAAB	AAAAA	AAAAA	AAAAA	AAAAA	AAAAA
	45	50	60	70	80	88
<i>Amblyonx cinereus</i>	•	•	•	•	•	•
<i>Aonyx capensis</i>	AABBBB	ABBA	ABBA	ABBA	ABBA	ABBA
<i>Enhydra lutris</i>	CCCCCC	CCCCC	CCCCC	CCCCC	CCCCC	CCCCC
<i>Hydrictis maculicollis</i>	DDDDDD	DDDDDD	DDDDDD	DDDDDD	DDDDDD	DDDDDD
<i>Lontra canadensis</i>	BBBCCB	BBBBA	BBBBA	BBBBA	BBBBA	BBBBA
<i>Lontra longicaudis</i>	BBBCCB	BBBBA	BBBBA	BBBBA	BBBBA	BBBBA
<i>Lutra lutra</i>	BBBBBB	BBBBB	BBBBB	BBBBB	BBBBB	BBBBB
<i>Lutrogale perspicillata</i>	BBBBBB	BBBBB	BBBBB	BBBBB	BBBBB	BBBBB
<i>Pteromura brasiliensis</i>	CCCCCC	CCCCC	CCCCC	CCCCC	CCCCC	CCCCC
<i>Mustela vison</i>	DDDDDD	DDDDDD	DDDDDD	DDDDDD	DDDDDD	DDDDDD
<i>Mephitis mephitis</i>	AAAAAA	AAAAA	AAAAA	AAAAA	AAAAA	AAAAA
<i>Ictonyx striatus</i>	AAAAAB	AAAAA	AAAAA	AAAAA	AAAAA	AAAAA

Appendix 2-6 Data matrix produced with Gap B coding techniques.

	1	10	20	30	40	44
<i>Amblyonx cinereus</i>	•	•	•	•	•	•
<i>Aonyx capensis</i>	B	C	C	C	C	C
<i>Enhydra lutris</i>	E	F	F	F	F	F
<i>Hydrictis maculicollis</i>	E	G	H	H	F	F
<i>Lontra canadensis</i>	C	C	D	D	D	D
<i>Lontra longicaudis</i>	C	D	E	E	E	E
<i>Lutra lutra</i>	C	D	D	D	D	D
<i>Lutrogale perspicillata</i>	C	D	D	D	D	D
<i>Pteronura brasiliensis</i>	D	E	E	E	E	E
<i>Mustela vison</i>	F	F	F	F	F	F
<i>Mephitis mephitis</i>	A	A	A	A	A	A
<i>Ictonyx striatus</i>	A	B	B	C	B	B
	45	50	60	70	80	88
<i>Amblyonx cinereus</i>	•	•	•	•	•	•
<i>Aonyx capensis</i>	B	B	C	C	C	C
<i>Enhydra lutris</i>	E	F	F	F	F	F
<i>Hydrictis maculicollis</i>	F	F	G	G	G	G
<i>Lontra canadensis</i>	C	C	C	C	C	C
<i>Lontra longicaudis</i>	D	D	D	D	D	D
<i>Lutra lutra</i>	D	D	D	D	D	D
<i>Lutrogale perspicillata</i>	D	D	D	D	D	D
<i>Pteronura brasiliensis</i>	D	E	E	E	E	E
<i>Mustela vison</i>	G	G	F	F	F	F
<i>Mephitis mephitis</i>	A	A	A	A	A	A
<i>Ictonyx striatus</i>	B	B	B	C	B	B

Part 3

Continuous Trait and Regression Residuals Phylogenies

Introduction

There are essentially two types of trait data used in morphological phylogenetic analyses: continuous and discrete. Discrete data predominates due to the nature of phylogenetic computer programs in use. While continuous morphological data is collected by a wide variety of researchers, it is rarely directly applied in systematics due to difficulties involved in analyzing it within a computer-based phylogenetic framework.

In this paper, otter postcranial measurements were used to construct uncoded continuous trait and regression residuals data sets. These data sets were compared with the discretely coded methods from the previous chapter, which resulted in a size-related ordering of the species. The regression residuals data set was constructed in an attempt to determine if there was underlying phylogenetic data that was being overwhelmed by the allometric component in the data. Data sets were analyzed using PHYLIP (Phylogeny Inference Package, version 3.5c, Felsenstein 1993, University of Washington), which is able to use continuous trait data and employs a maximum likelihood (ML) phylogeny approach. Results from these two analyses were compared with results from the previous section in which continuous traits were coded under different coding methods and analyzed using PAUP* (Swofford 2000), using a most parsimonious reconstruction (MPR) procedure. The ML and MPR comparison was performed to determine if significant differences result from these two approaches using uncoded and coded data from the same data set. The otter data sets used here were useful for this comparison since the size-related results from the previous chapter have not previously been reported, and different methods using continuous data have not been evaluated.

Body size was used in the regression analysis and residuals from postcranial characters were used in a parallel examination of the ML approach. Results from these two maximum likelihood analyses were compared for congruity and then compared with the MPR results from the previous chapter. The use of a residuals data set was incorporated in an attempt to remedy the apparent size-dependent ordering found in results from coded data set analyses in Chapter 2. The use of regression residuals in a phylogenetic analysis appears unprecedented, and its efficacy has not yet been demonstrated.

Maximum likelihood approach

The phylogenetic program PHYLIP (Felsenstein 1993) applies a maximum likelihood approach in the analysis of phylogenetic hypotheses. There are several evolutionary assumptions inherent in the maximum likelihood (ML) model: lineages evolve independently and after lineages split their characters change independently (the Markov model), and characters drift independently (Felsenstein 1993). The basic supposition of this evolutionary model is that Brownian motion is the primary evolutionary driving force, whereby genetic drift is the mechanism of character change (Felsenstein 1988). With the maximum likelihood method, phylogeny estimation is approached as a statistical problem, and the construction of cladograms is based on a probabilistic model of evolution (Felsenstein 1983; Siebert 1992). The statistical basis of phylogenetic assessment often produces estimates with the lowest variance and results in estimates that are least affected by sampling error (Swofford et al. 1996).

Detractors of this probabilistic approach to phylogeny cite several difficulties with

this method: the need for a priori knowledge regarding the nature of the evolutionary process (Farris 1983), that an incorrect model of evolution will yield an analysis that is subject to systematic error (Swofford et al. 1996), the seemingly reversed proposition that a tree is produced which has the highest probability that a given data set can be derived from it (instead of the tree being derived from a given data set), and probably most important for this study, that a realistic probability model of morphological evolutionary change may not currently be possible with this method (Felsenstein 1988; Siebert 1992). If character changes arise via mutation instead of genetic drift, the model will provide inaccurate estimates of phylogeny. An additional problem with ML is that rate heterogeneity (characters evolving at different rates) can make ML underestimate the number of multiple changes (Felsenstein 1978).

The Gene Frequencies and Continuous Characters Maximum Likelihood (CONTML) subprogram of PHYLIP applies a restricted maximum likelihood algorithm (REML) based on the Brownian motion model of evolution (Felsenstein 1973) from the work of Edwards and Cavalli-Sforza (1964) and Cavalli-Sforza and Edwards (1967). The continuous character mode of CONTML regards input data as taxon coordinates considered in N dimensions, and assumes that characters have been transformed to remove correlations and variances standardized (Felsenstein 1993).

Maximum parsimony approach

The basic assumption of the most parsimonious reconstruction (MPR) approach to phylogeny is to presuppose that evolutionary change is an extremely unlikely event. Only those trees which have the smallest number of changes between states (shortest branch

lengths) are considered, subsequently the shortest (most parsimonious) tree is viewed as the best estimate of the true phylogeny. There are several versions of parsimony methods, some with more restrictive criteria and constraints for transformations allowed. A brief synopsis of some assumptions displays the diversity of various parsimony methods.

Camin-Sokal parsimony assumes evolution is an irreversible process; characters may not revert back to former states (Camin and Sokal 1965). This assumption requires directed characters (polarity must be defined prior to analysis). True reversals cannot actually occur, so in fact all reversals are actually new apomorphies (Wiley et al 1991). As a result, Camin-Sokal parsimony is rarely employed (Kitching 1992). Dollo parsimony assumes partial irreversibility, (character polarity is again predetermined), homoplasy is accounted for by reversion to the plesiomorphic state first (multiple origins of derived states are not allowed), and characters cannot be lost and subsequently regained (Farris 1977; DeBry and Slade 1985; Swofford et al 1996). In Wagner parsimony, transitions from one state to another must occur through all intermediate states (Kluge and Farris 1969; Farris 1970) resulting in tree lengths which are independent of the position of the root for additively coded characters (Wiley et al. 1991; Kitching 1992). Fitch parsimony (Fitch 1971) on the other hand, allows state transitions directly from one to another for non-additively (unordered) coded characters.

These parsimony variants are usually placed under the term "generalized parsimony" (Swofford and Olsen 1990). With generalized parsimony, various restrictions are employed when they best fit the data at hand. Combinations of restrictions may be used on a data set composed of different characters (i.e. a data set made up of a

combination of morphological and molecular traits). By treating characters and their evolution differentially as a result of some knowledge regarding their transformations (morphological data may indicate ordering based on ontogeny while molecular traits may indicate an independent and therefore unordered arrangement), the resultant tree may tend to be more robust and better supported by highly weighted characters (Wiley et al. 1991). The ability to treat characters differentially based on specific properties of those characters enhances analyses and subsequent phylogenetic hypotheses. In all parsimony approaches, the basic tenet is to assign costs for any character state change, and as a result, the "least costly" tree (most parsimonious) is regarded as the optimum.

Comparisons of maximum likelihood and parsimony approaches

Basic differences in the assumptions and evolutionary models used in the ML approach in PHYLIP versus those in the MPR approach of PAUP* can cause difficulties when comparing results from these two types of analyses. Direct comparisons of ML and MPR may be complex, and has even been termed "apples and oranges" (Felsenstein, pers. comm.), but resulting cladograms can still be examined for topological similarities and contrasts, testing both the techniques and the evolutionary models employed.

One complication in comparing results from ML and MPR is that tree length is often viewed as the best indicator of optimality (Wiley et al. 1991). But as has been discussed, the basic premise for maximum likelihood approaches is statistical congruence, which may not always produce the shortest tree, while parsimony is primarily based on finding the shortest tree. Another problem lies in the resultant trees themselves. The output from an ML analysis is a statistical classification based on

probabilistic models of a specific evolutionary hypothesis, not direct estimation of evolutionary history. In discussing the problem of understanding the results of a maximum likelihood analysis in a phylogenetic light, Siebert said of ML cladograms: "Interpretation of the cladograms as phylogenetic trees is possible once the most parsimonious cladogram has been obtained." (Siebert 1992, p. 81). Such results are available for this data set from analyses in Chapter 2, which employed an exhaustive search, ensuring the most parsimonious reconstruction using coded continuous data.

Materials and Methods

The postcranial morphological data set used in this section was the same as that used in Chapter 2. The number of characters used (88) for the continuous data set and all other aspects of the data remained unchanged from Chapter 2. The number of characters used in the residuals data set was reduced to 87, due to the necessary removal of the character head-body length (HB). Head-body length was deleted from the data set because it was used in the regression analysis as the dependent variable, and regressing that character on itself as the independent variable would result in residual values of zero for all taxa, an uninformative outcome. Given the large number of morphological characters used in the data set, a single character deletion (1 character = 1.14% of the total number characters) should not have a significant effect on results or tree topology.

The same twelve taxa from Chapter 2 were used in this study (see Table 2-1) despite the fact that the limitations on the number of taxa in the exhaustive search of PAUP* do not apply to PHYLIP. This was done to maintain continuity of taxa used in each analysis and facilitate the comparison of resultant trees. Phylogenetic analyses were

performed using the CONTML (Gene Frequencies and Continuous Characters Maximum Likelihood) program in PHYLIP. The treefiles produced in PHYLIP were converted for use by PAUP*, since PHYLIP has limited tree graphing capabilities. Consensus cladograms were produced from results of each data set (continuous and residuals) and an overall consensus cladogram was produced by combining the results of all analyses.

Continuous data set

The postcranial continuous data set from Chapter 2 was used as the input file for PHYLIP without coding procedures. The data set remained log normalized, and had equal variances as required by PHYLIP (Felsenstein 1993). The data set was converted from spreadsheet to text and fitted to conform with PHYLIP infile data matrix protocols and is shown in Appendix 3-1. The Gene Frequencies and Continuous Characters Maximum Likelihood (CONTML) subprogram in PHYLIP was used with the continuous character option mode of the "Gene frequencies or continuous characters?" query applied.

Analyses were run separately using three outgroup species: American mink (*Mustela vison*), striped skunk (*Mephitis mephitis*), and zorilla (*Ictonyx striatus*) for rooting purposes. The outgroup rooting option of the CONTML subprogram only allows a single outgroup assignment at a time so outgroup rooting was alternated between the three outgroup taxa in identical runs of the program. An additional analysis was also run without an assigned outgroup. With this option exercised, the default outgroup was the first species listed in the infile (Asian small-clawed otter, *Amblonyx cinereus*). There is no option to remove the outgroup option all together in PHYLIP, and setting the outgroup at species zero caused the program to crash. It should be noted that species were listed

alphabetically in the data set, and coincidentally the first taxon (*Amblonyx cinereus*) was consistently found to be basal to all other otters in previous analyses (see Chapter 2). This coincidence was preferred to assigning a more internally placed taxon (from the analyses in Chapter 2) as the outgroup and so was accepted without reservations.

Species additions were assigned using the J (Jumble) option of the “Randomize input order of species?” query with 1000 re-orderings. This option was used because in the PHYLIP tree construction programs, search details are dependent on the order of input, which can affect results and cladogram topology (Felsenstein 1993). To ameliorate this possibly detrimental effect, the option exists to change species input order with a random number generator and multiple searches, with a suggested minimum of 100 iterations (Felsenstein 1993). The program prompts the user for a “seed” for the random number generator (an odd integer), the number 13 was arbitrarily chosen and used for all analyses, and the orders jumbled 1000 times. Treefiles produced from the analyses were transferred to PAUP* to be used in a NEXUS format treefile to print the cladograms. This was done to produce cladograms which were more comparable to those produced in the PAUP* analysis from Chapter 2 in to facilitate tree topology comparisons.

Regression analysis and residuals data set

Head-body length and postcranial trait data were log normalized prior to the regression analysis. A simple regression was then performed in which species body size (head-body length, HB) was plotted against each of the postcranial measurements used in this study, and the residuals recorded. The regression residuals data set was truncated to three decimal places for matrix display purposes and is shown in Appendix 3-2. Head-

body measurements taken from the literature were averaged for high and low values.

Residuals from the regression plot were used as characters to produce a data set (again, with the exception of head-body length). This resulted in 87 residual characters used as input versus 88 characters in the previous continuous data set analysis. Data were again converted from spreadsheet to text file and used to construct an infile. Analyses were performed identically to those in the continuous data set. Settings of the CONTML subprogram of PHYLIP remained the same, using identical parameters, starting seeds for the random number generator (13), and jumbling the species addition 1000 times.

Support indices

Two problems with the continuous character mode of CONTML are that there is no way to perform bootstrap support analyses or to produce meaningful branch lengths. For this reason, neither bootstrap support analysis nor branch lengths are available.

Results

Due to outgroup assignment restrictions in the CONTML subprogram of PHYLIP, analyses resulted in the production of four cladograms from each of the two data sets. Outgroups settings were: zorilla (*Ictonyx striatus*), striped skunk (*Mephitis mephitis*), American mink (*Mustela vison*), and the default setting of "Species 1" (the first taxon listed in the data set), the Asian clawless otter (*Amblonyx cinereus*). Consensus cladograms were then constructed from the four resultant cladograms from each analysis, and a consensus cladogram constructed combining all 8 cladograms from both analyses.

Continuous trait analysis

Four cladograms were produced using the continuous character data and are shown below. The number of trees evaluated was the same for each analysis (1437) and the natural log likelihood (Ln. Likelihood) was also identical for all continuous trait analyses (1894.66). The cladogram produced with the outgroup species assigned as the zorilla (*Ictonyx striatus*) is shown in Figure 3-1; the striped skunk (*Mephitis mephitis*) in Figure 3-2; the American mink (*Mustela vison*) in Figure 3-3; and the Asian clawless otter (*Amblonyx cinereus*) is shown in Figure 3-4. The analyses also produced four treefiles in a subset form of the Newick standard format (Felsenstein 1993) which were then used to produce the consensus cladogram shown in Figure 3-5.

Continuous trait regression residuals analysis

The data set produced with regression residuals produced four cladograms, due to the four varying outgroup species as in the continuous trait analysis above. All residuals cladograms had a natural log likelihood of 2237.78 with 22,525 trees examined. Meaningful branch lengths are not produced in MLA cladogram searches and are therefore not available in the cladograms presented here. The cladogram produced with the outgroup species assigned as the zorilla (*Ictonyx striatus*) is shown in Figure 3-6; the striped skunk (*Mephitis mephitis*) in Figure 3-7; the American mink (*Mustela vison*) in Figure 3-8; and the Asian clawless otter (*Amblonyx cinereus*) in Figure 3-9. The four Newick standard format treefiles produced from these four analyses were then used to produce the 50% majority rule consensus cladogram shown in Figure 3-10, which does not have an outgroup assigned due to the combination of the four outgroup cladograms.

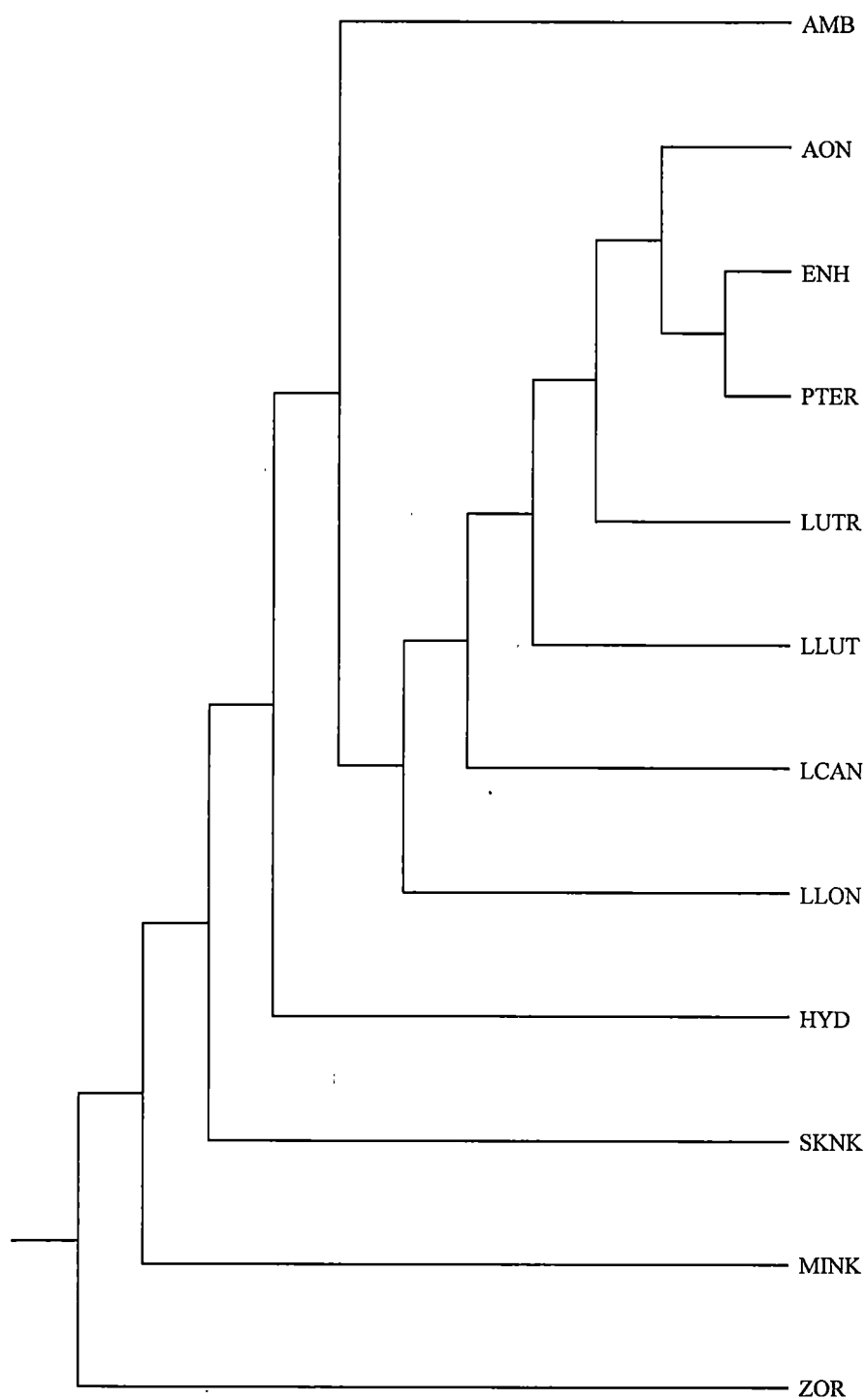


Figure 3-1. Maximum likelihood continuous data cladogram with outgroup specified as zorilla (*Ictonyx striatus*). Ln. Likelihood = 1894.66, 1437 trees examined.

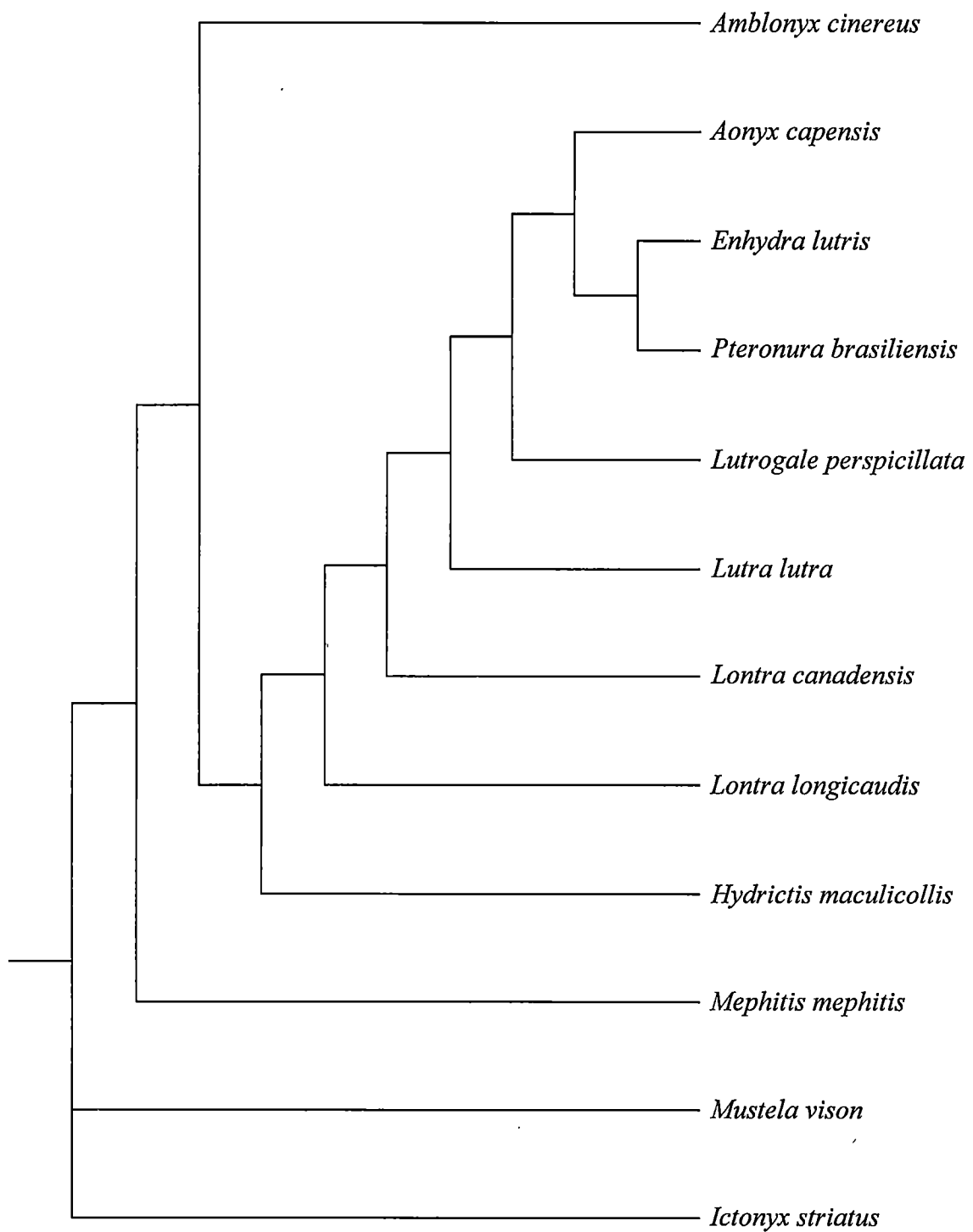


Figure 3-3. Maximum likelihood continuous data cladogram with outgroup specified as American mink (*Mustela vison*). Ln. Likelihood = 1894.66, 1437 trees examined.

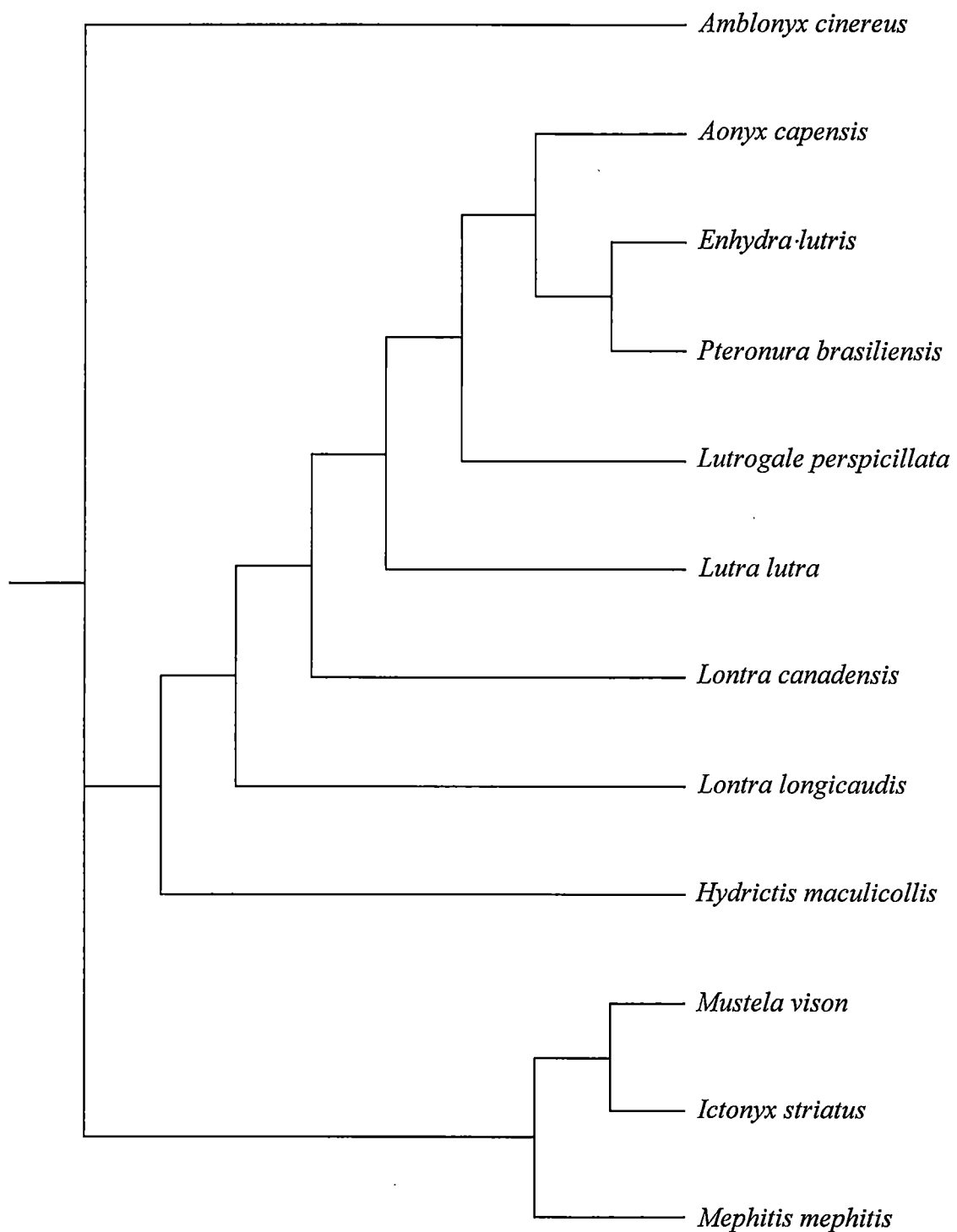


Figure 3-4. Maximum likelihood continuous data cladogram with outgroup specified as Asian clawless otter (*Amblyonyx cinereus*). Ln. Likelihood = 1894.66, 1437 trees examined.

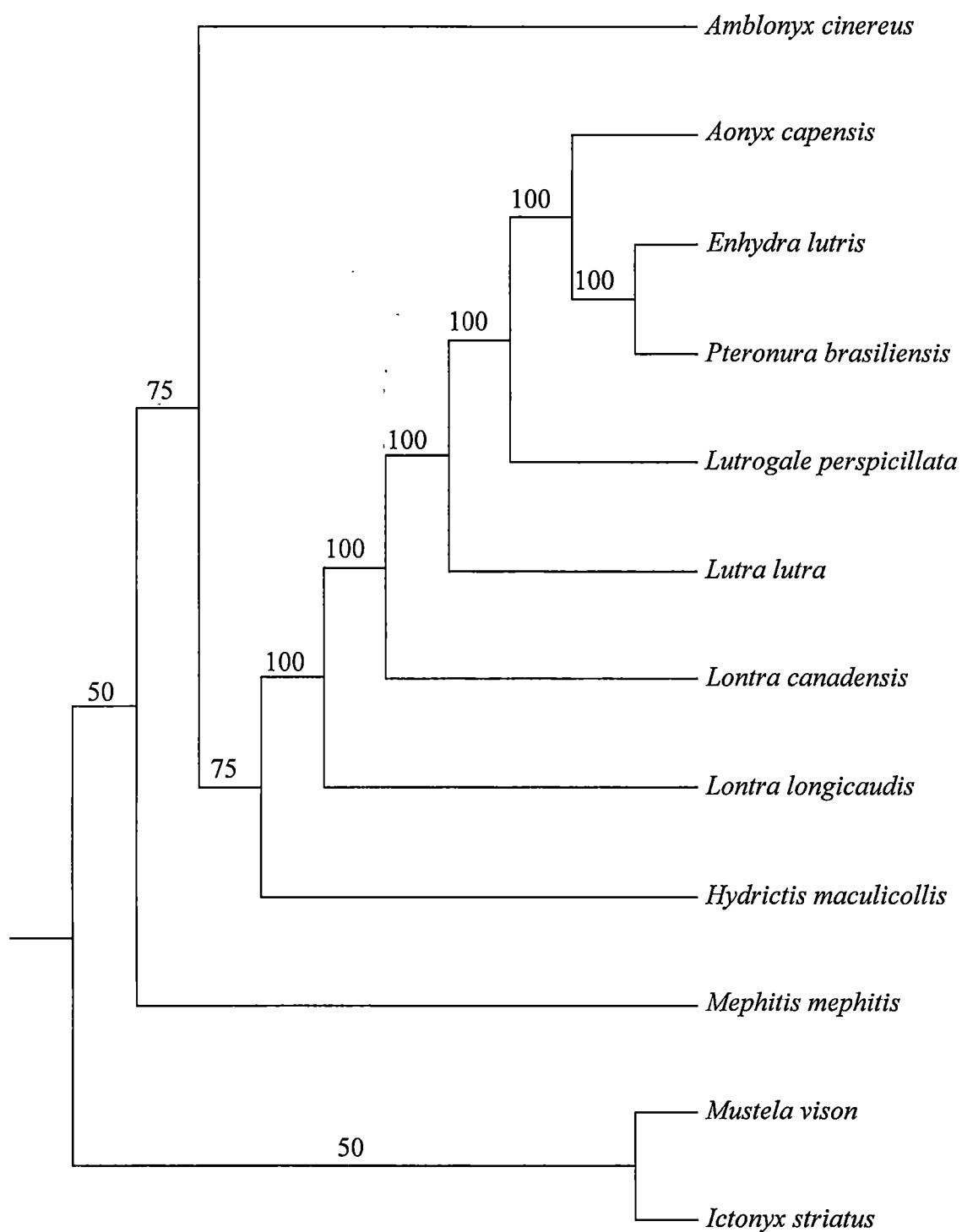


Figure 3-5. 50% Majority Rule consensus cladogram for the four maximum likelihood continuous data cladograms. Bipartition frequencies from the 4 trees are indicated above branches including compatible groupings of 50%.

Consensus tree from combined continuous and residuals data set results

By combining the eight treefiles produced by the continuous data and residuals data sets in a single Nexus format treefile, a consensus cladogram was produced combining the results of both analyses and is shown in Figure 3-11 below. In order to preserve the greatest amount of topological information (since many of the residuals results were unique), a 50% majority rule consensus tree was constructed that included bipartition frequencies of branching patterns that occurred in less than 50 percent of trees. This accounts for multiple branching occurrences better than other consensus methods.

Discussion

Continuous trait analysis

The basic result from the continuous trait data analysis was a grouping of the taxa according to size, similar to the coded continuous data results from Chapter 2. As in the coded trait data, similarities are bound to occur based solely on the proximity of the sizes of the features measured. Small taxa will be viewed as similar, as will large species, with intermediate species being placed between them in order of their magnitude. This result seems to indicate that regardless of the underlying evolutionary models or approaches applied to this type of data, the results will tend to group the species based on size. In the maximum likelihood cladograms produced from the continuous trait data matrix, the topology of the branching pattern in the otters was identical in three of the four cladograms (Figures 3-2, 3-3, and 3-4) and differed by only one position in the other (Figure 3-1). The cladogram shown in Figure 3-1 which placed the zorilla (*Ictonyx*

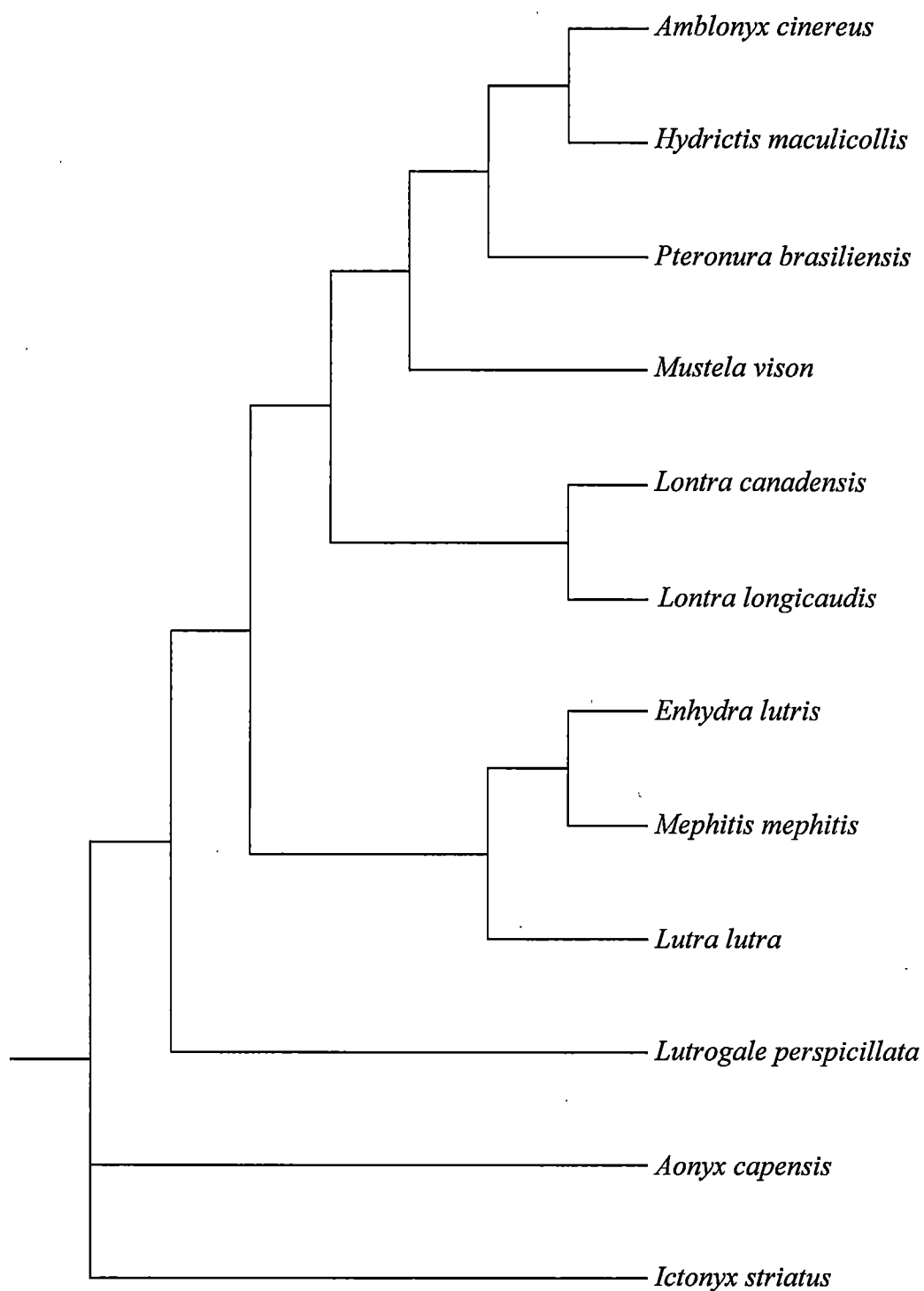


Figure 3-6. Maximum likelihood residuals data cladogram with outgroup specified as zorilla (*Ictonyx striatus*). Ln. Likelihood = 2237.78, 22525 trees examined.

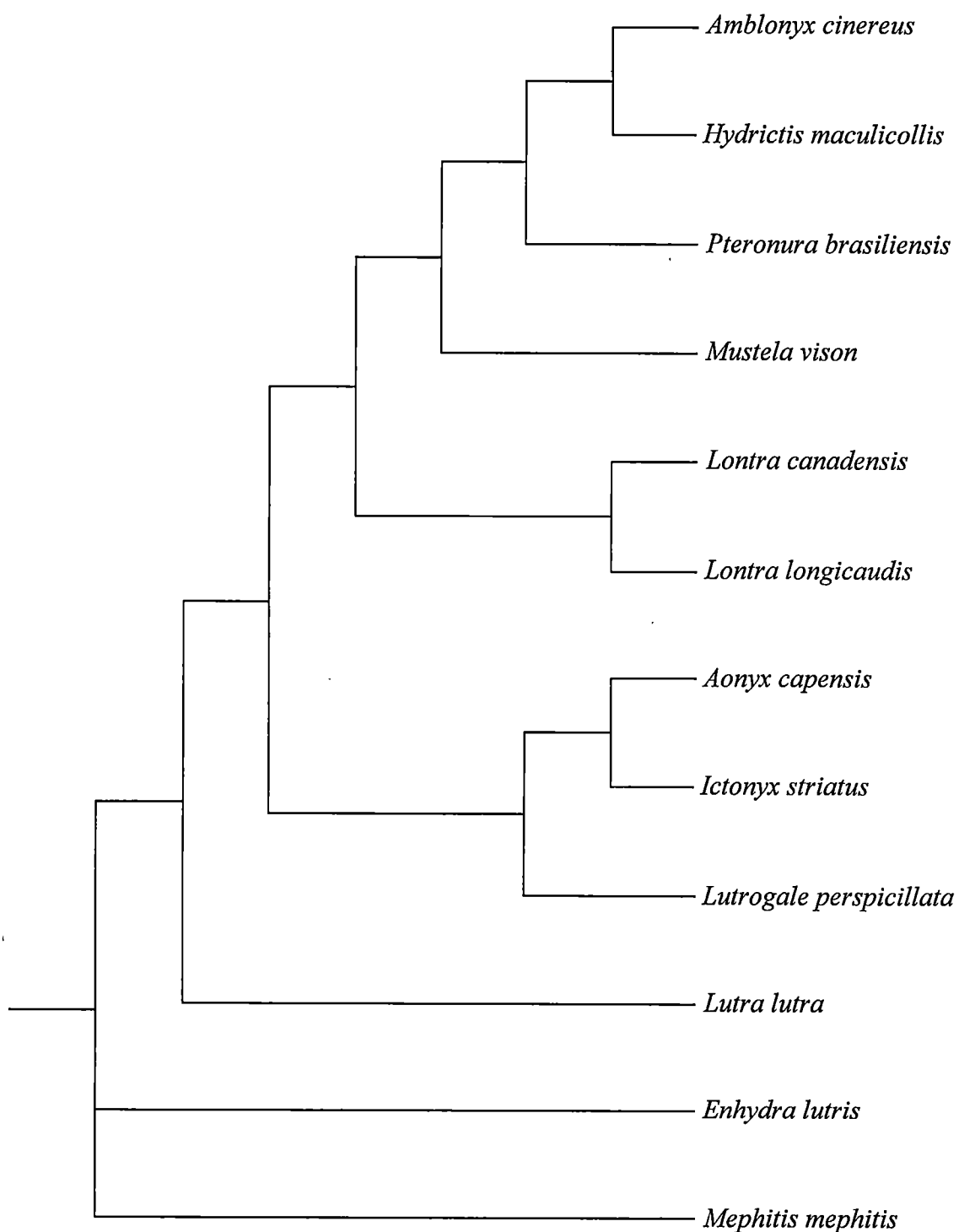


Figure 3-7. Maximum likelihood residuals data cladogram with outgroup specified as striped skunk (*Mephitis mephitis*). Ln. Likelihood = 2237.78, 22525 trees examined.

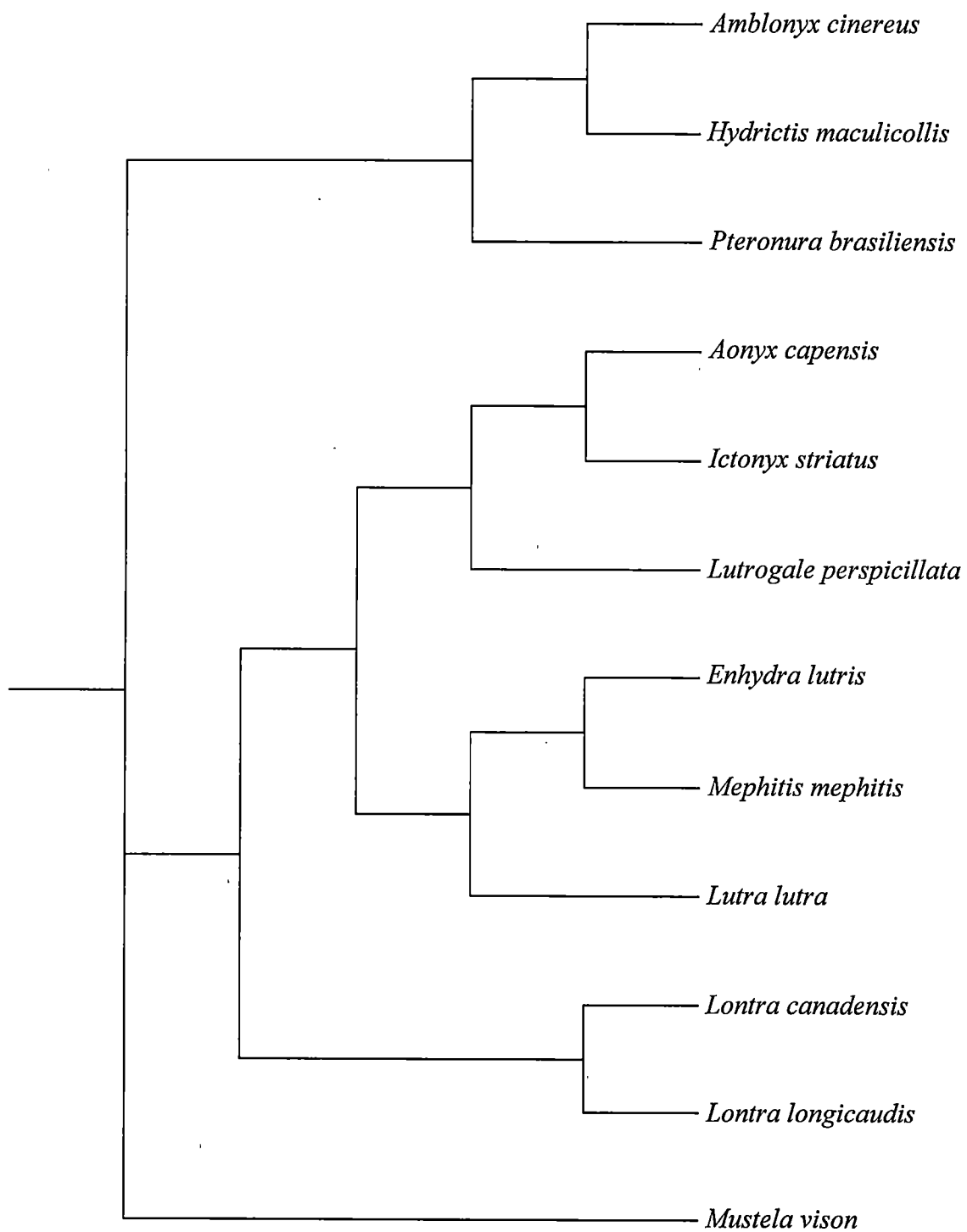


Figure 3-8. Maximum likelihood residuals data cladogram with outgroup specified as American mink (*Mustela vison*). Ln. Likelihood = 2237.78, 22525 trees examined.

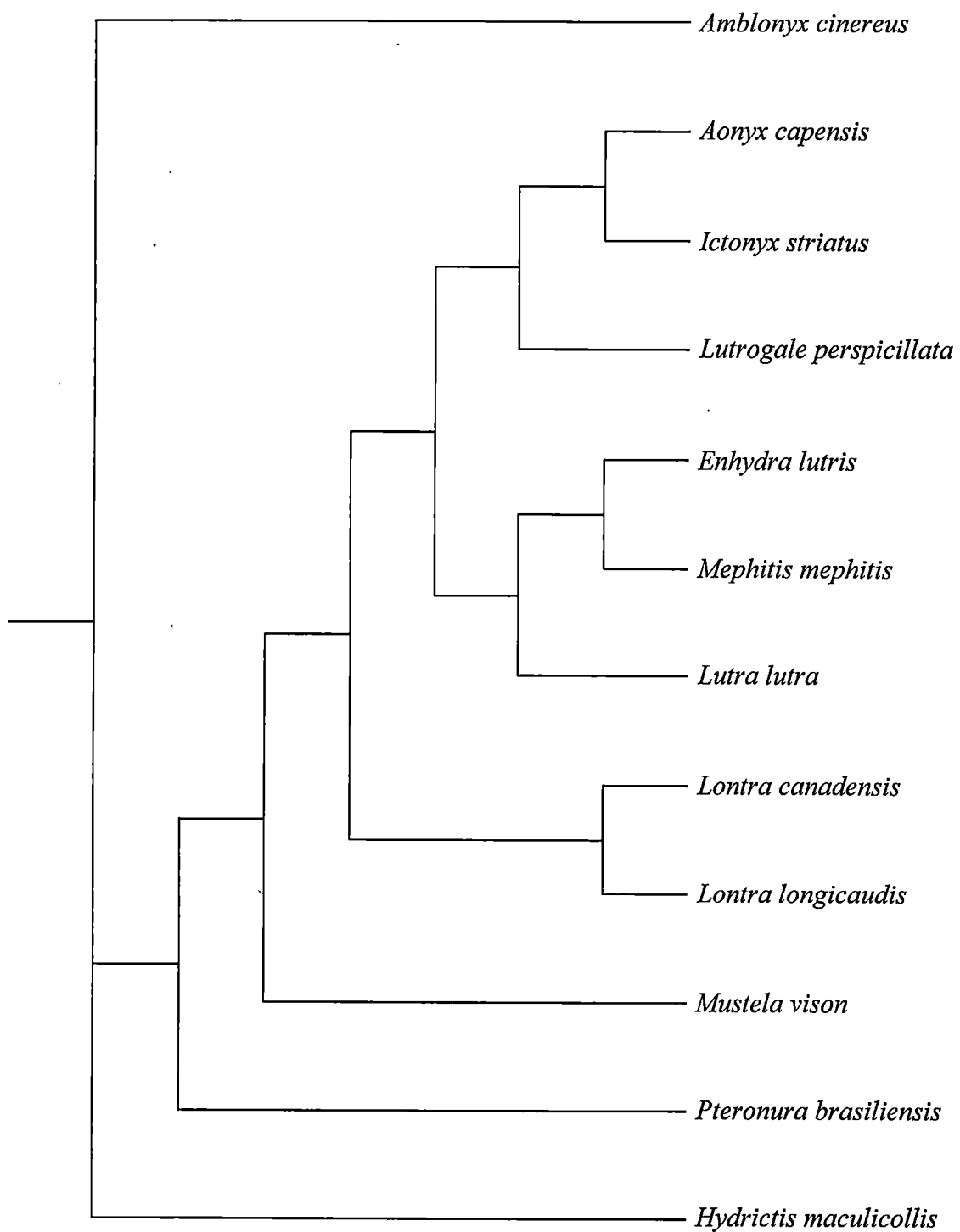


Figure 3-9. Maximum likelihood residuals data cladogram with outgroup specified as Asian clawless otter (*Amblonyx cinereus*). Ln. Likelihood = 2237.78, 22525 trees examined.

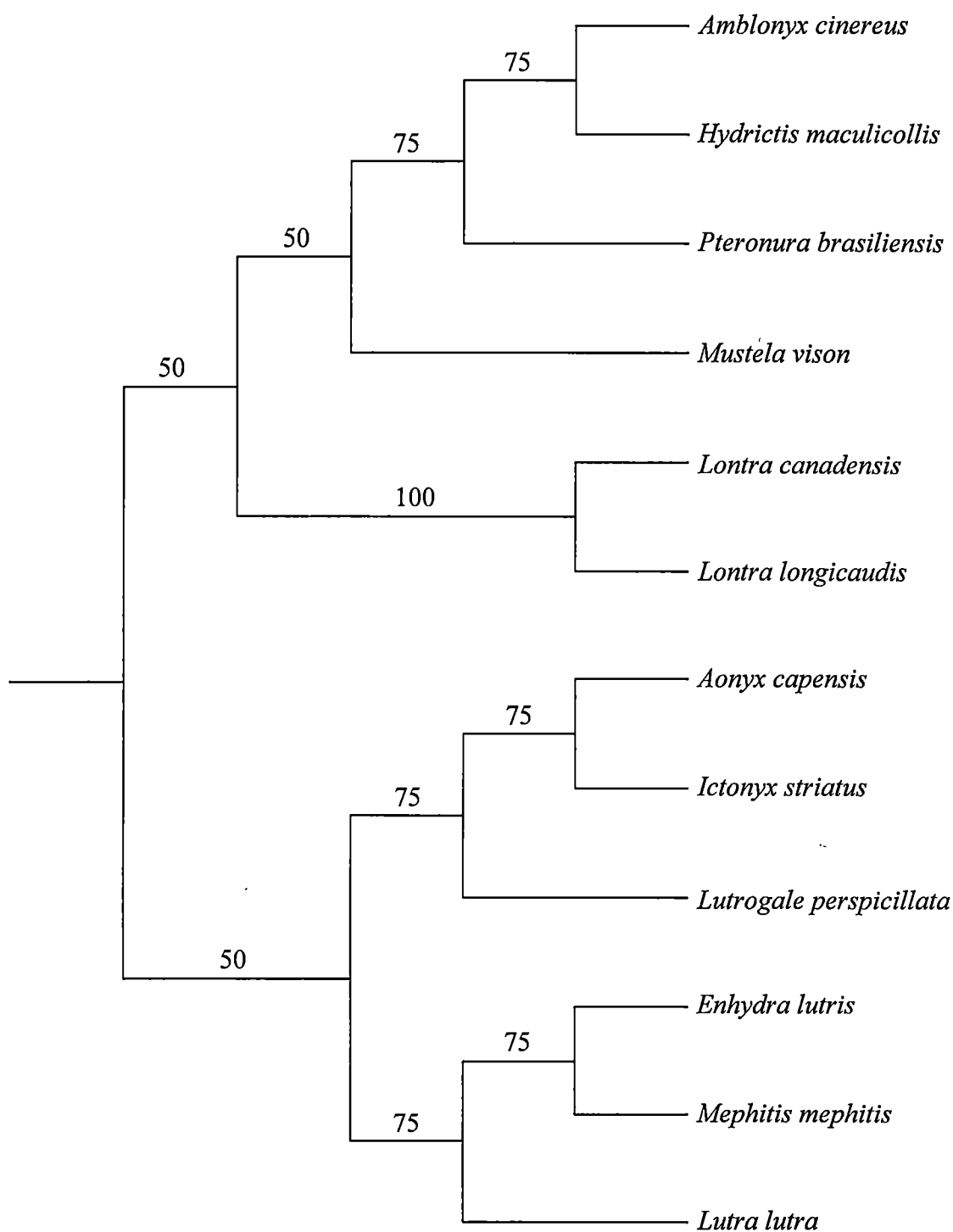


Figure 3-10. 50% Majority Rule consensus cladogram for the four Maximum likelihood regression residuals data cladograms. Bipartition frequencies from the 4 trees are indicated above branches including compatible groupings of 50%.

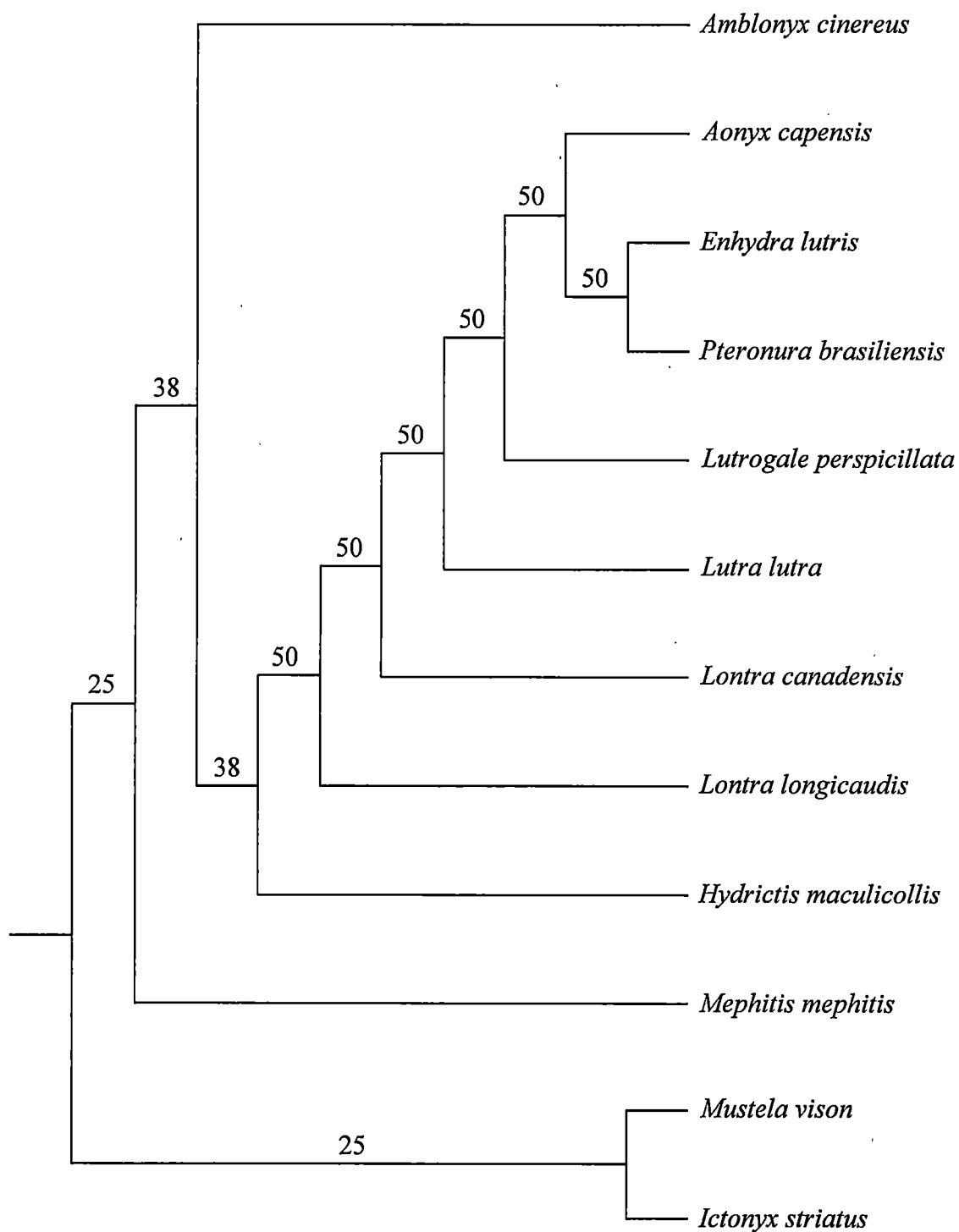


Figure 3-11. 50% Majority Rule consensus cladogram for the eight (4 continuous and 4 regression residuals) cladograms. Bipartition frequencies from the 8 trees are indicated above branches including groupings of less than 50%.

striatus) as the outgroup differed only in its positioning of the African spot-necked otter (*Hydrictis maculicollis*) as basal to all other otters. This differed from the results of the other three cladograms with other outgroup assignments (Figures 3-2, 3-3, and 3-4) where the Asian clawless otter (*Amblonyx cinereus*) was placed basal to all other otters, and the African spot-necked otter was placed as the internally basal otter. This predominating pattern was identical to Tree 2 in the Gap A coding analysis (Figure 2-4) in Chapter 2. The only other differences in tree topology were due to the variation in outgroup assignment with topology reflecting those assignments.

The different outgroup assignments resulted in four different outgroup arrangements. In cladograms which did not specify either the zorilla (*Ictonyx striatus*) or American mink (*Mustela vison*) as the outgroup (Figures 3-2 and 3-4), these two species were linked together in a musteline clade. When forced apart due to their alternating assignments as outgroups (Figures 3-1 and 3-3), they were linked as sister taxa (Figure 3-1) and formed the only polytomy in the analysis (Figure 3-3). This polytomy indicates that their positions were equivocal in regards to their relationships with the striped skunk (*Mephitis mephitis*), the other outgroup species. The position of the skunk was the most mutable in placement of all the taxa. The striped skunk was placed as sister taxon to the otters in the two cladograms assigning the mustelines as the outgroup (Figures 3-1 and 3-3). When the Asian clawless otter was assigned as the outgroup, the striped skunk was linked as sister taxon to the mustelines (Figure 3-4). In the cladogram assigning the striped skunk as the outgroup, a mephitine-musteline polytomy formed, indicating ambiguity in the assignment of the sister taxon between those two groups.

Regression residuals analysis

Results from the regression residuals analysis were enigmatic. Although the objective of removing the size dependent grouping of otters was achieved, the resulting cladograms were exceptionally perplexing, and seemingly without biological justification. The cladograms contained paraphyletic groupings for all of the subfamilies involved (Mephitinae/Mephitidae, Lutrinae, and Mustelinae). Although the groupings were aberrant, there was an exceptional amount of congruence among the cladograms, with all groupings (clades) except one being recovered in at least three out of the four cladograms (another was found in only half of the cladograms).

A single grouping was found in all four cladograms (and is also the only one that appears to have any systematic validity) containing the North American river otter (*Lontra canadensis*) and the Neotropical otter (*Lontra longicaudis*). These are the only two congeneric species in the data set. The single grouping found in only half of the cladograms contained two outgroup species which were each pulled out of the clade due to assignment as the outgroup, resulting in the break up of that clade in those two cladograms. There were essentially four clades recovered in this analysis containing the following species (which are presented in modified Newick format since relationships between clades are evanescent): Clade 1=(((*Amblonyx*, *Hydrictis*) *Pteronura*) *Mustela*); Clade 2=(*Lontra canadensis*, *Lontra longicaudis*); Clade 3=((*Enhydra*, *Mephitis*) *Lutra*); Clade 4=((*Aonyx*, *Ictonyx*) *Lutrogale*).

In evaluating these four clades, it is readily apparent that there is little biological, anatomical, or evolutionary justification for these alignments (again with the exception of

Clade 2). In Clade 1 the Asian clawless otter (*Amblonyx*) was most closely allied with the African spot-necked otter (*Hydrictis*), which was then linked to the giant otter of South America (*Pteronura*) and finally those three taxa were linked (2 out of 4 times) with the American mink (*Mustela*). This arrangement, like most from this analysis, combined species that are both biologically and geographically disparate having little or no morphological or behavioral features in common.

Clade 2 was the only arrangement that has any reasonable evolutionary or biological basis, and was the only clade that excluded non-lutrine members. The linkage of the two members of *Lontra* was also the only grouping uncovered in all four cladograms, and combines the only two congeneric species in the analysis, a singularly positive result from this analysis. This is most likely the result of their extreme similarity in size, and therefore similar residual magnitude from the regression line.

Clade 3 is another amalgam of species which closely links the almost exclusively marine dwelling sea otter (*Enhydra*) with the completely terrestrial striped skunk (*Mephitis*), and then links these two taxa with one of the most "otter-like" of the lutrines, the Eurasian river otter (*Lutra lutra*). One interesting sidebar to this odd arrangement is the linkage of the only mustelid lacking "must" or scent glands (sea otter) with one in which the scent glands are one of its primary hallmarks (striped skunk).

Clade 4, while still very implausible, at least combines two forms found on the same continent (Africa). The zorilla (*Ictonyx*) is one of three small African weasels, with strong claws, well developed scent glands (very similar to the skunks), and strictly terrestrial (even desert) habitat preferences. The Cape clawless otter (*Aonyx*) is a very

large semi-aquatic (and as the name implies) clawless otter with virtually no webbing between its toes, that generally preys on aquatic crustaceans. These two vastly incongruous species are then linked with the Indian smooth-coated otter which has webbed feet, is strongly aquatic, and found primarily between India and Borneo.

The cladogram with the outgroup assignment of zorilla (*Ictonyx striatus*), shown in Figure 3-6, contains three of the four consistent clades (a pattern found in all of the cladograms, due to the break up of at least one clade in each due to shifting outgroup assignments). Clades 1-3 are present, Clade 4 (*Aonyx*, *Ictonyx*, *Lutrogale*) is disassociated due to the assignment of one member (*Ictonyx*) as the outgroup. This resulted in the placement of *Aonyx* in a basal polytomy with *Ictonyx*, and *Lutrogale* as internally basal to the other clades. Topologically these clades were linked in the following way: Clade 1 (*Amblonyx*, *Hydrictis*, *Pteronura*, *Mustela*) is linked with Clade 2 (*Lontra canadensis*, *Lontra longicaudis*) this grouping is then allied with Clade 3 (*Enhydra*, *Mephitis*, *Lutra*). Internally basal to these three clades is the Indian smooth-coated otter (*Lutrogale*), and basal to the above listed taxa are *Aonyx* and *Ictonyx* in a polytomy.

The second residuals cladogram (Figure 3-7) in which the striped skunk (*Mephitis*) was assigned to the outgroup position contained Clades 1, 2, and 4. Clade 3 (*Enhydra*, *Mephitis*, *Lutra*) is disassociated by the outgroup assignment of the skunk, an internally integral member of that clade tied closely with the sea otter (*Enhydra*). Because of this close association with *Enhydra*, a polytomy forms with *Mephitis* and *Enhydra* having equally basal positions to the remaining taxa. The Eurasian river otter

(*Lutra*) is the third member of the clade and placed in the internally basal position of the cladogram. Clade relationships place Clade 1 (*Amblonyx*, *Hydrictis*, *Pteronura*, *Mustela*) with Clade 2 (*Lontra canadensis*, *Lontra longicaudis*) which are then joined by Clade 4 (*Aonyx*, *Ictonyx*, *Lutrogale*). Members of the disbanded Clade 4 are then placed in the manner described above.

In Figure 3-8 the American mink (*Mustela vison*) was the outgroup. This outgroup placement resulted in a somewhat unique result among the four cladograms. Here, virtually all Clades (1-4) are represented with only the American mink being pulled off of Clade 1, which did not result in the erosion of the remaining associations in that clade. The clade relationships are also somewhat unique, with the remaining intact taxa from Clade 1 (*Amblonyx*, *Hydrictis*, *Pteronura*) placed as a basal clade (no other cladogram contains an intact clade as basal). Clade 4 (*Aonyx*, *Ictonyx*, *Lutrogale*) and 3 (*Enhydra*, *Mephitis*, *Lutra*) are linked with Clade 2 (*Lontra canadensis*, *Lontra longicaudis*) as internally basal to that group. The single species outgroup (another unique result) to this group was the American mink (*Mustela*).

In the final cladogram produced in this analysis (Figure 3-9) the default outgroup setting was the default "Species 1", the Asian small-clawed otter (*Amblonyx*), an internal and integral member of Clade 1 (*Amblonyx*, *Hydrictis*, *Pteronura*, *Mustela*). The outgroup assignment of *Amblonyx* resulted in the disbanding of Clade 1. This outgroup assignment also resulted in a unique topology with essentially two outgroup species. The assigned outgroup species (*Amblonyx*) and the spot-necked otter (*Hydrictis*) are both placed as outgroups in this analysis. The remaining members of Clade 1 were placed as

internally basal to the other groups, with the American mink (*Mustela*) placed just inside of the giant otter (*Pteronura*). Clade 4 (*Aonyx*, *Ictonyx*, *Lutrogale*) is linked with Clade 3 (*Enhydra*, *Mephitis*, *Lutra*), which are then combined with Clade 2 (*Lontra canadensis*, *Lontra longicaudis*).

Regression residuals consensus cladogram

The cladogram produced by combining all four of the treefiles from the regression residuals analyses was the only one in which all Clades (1-4) remained intact (Figure 3-10). This was most likely because there was no outgroup assignment, which is not required in PAUP* (used to combine the data). An interesting aspect of the topology of this consensus cladogram is that it was quite symmetric, with two major clades each containing two of the smaller clades described above. The upper clade in Figure 3-10 was composed of Clades 1 (*Amblonyx*, *Hydrictis*, *Pteronura*, *Mustela*) and 2 (*Lontra canadensis*, *Lontra longicaudis*). The lower clade contained Clade 4 (*Aonyx*, *Ictonyx*, *Lutrogale*) and Clade 3 (*Enhydra*, *Mephitis*, *Lutra*). This resulted in both major clades containing 6 species each, presenting a uniquely symmetrical topology not found in the other cladograms which presented a more step-like topology.

Comparisons with discretely coded continuous trait phylogenies

In comparing the results from the two maximum likelihood analyses (continuous traits and regression residuals) from this chapter with the maximum parsimony analysis of coded continuous traits in Chapter 2, there is a great deal of similarity between those

using continuous data. The results from regression residuals analysis were not comparable to either continuous data analyses, with no clades or arrangements in common.

Coded continuous traits versus straight continuous traits

When comparing the coded trait MPR data from Chapter 2 with the continuous trait ML data, the congruence of the results shows a high degree of similarity. Both approaches tended to group the species by size alone which, although a previously unreported result, is not unknown in other taxa such as Felids. The same patterns are observed linking the three largest species (*Enhydra*, *Pteronura*, and *Aonyx*) with the next largest species (*Lutrogale*) followed by the intermediate species (*Lutra*, *Lontra spp.*). Internally basal to these taxa is the second smallest otter (*Hydrictis*) and externally basal to all other otters is the smallest (*Amblonyx*).

Outgroups vary in small ways, but usually the mustelines (*Mustela*, *Ictonyx*) are linked, and the skunk (*Mephitis*) (except when forced into the outgroup position) is placed more closely to the otters. This is the only major departure between the ML and MPR analyses. In the parsimony analysis, the skunk was generally placed as the most distant outgroup, with the mustelines placed closer to the otters. Another slight difference concerns the arrangement of the three river otter species (*Lutra lutra*, *Lontra canadensis*, *Lontra longicaudis*). The relative positions of these otters was quite equivocal in the parsimony analysis, having five different arrangements among seven trees. In the ML analysis, these taxa were consistently placed in the same order in all four

cladograms (five including the consensus cladogram), with no ambiguity. So overall, the results using continuous (coded or straight) data closely mirrored one another, with only a few minor incongruities that do not greatly affect the overall topology.

Coded continuous traits versus regression residuals

When comparing the topology of the ML regression residuals cladograms with the MPR coded continuous trait phylogenies, it becomes immediately clear that these results have virtually nothing in common. The arrangements found in the regression residuals cladograms seem completely haphazard, and are based on the distance from the regression line of body size to the trait in question. While the importance of size has been eliminated, so has any apparent biological significance. Of all the relationships demonstrated by the topology of the regression residuals, only two come even remotely close to the results from the coded trait analysis. The arrangement of taxa in Clade 2 (*Lontra canadensis*, *Lontra longicaudis*), while never identical, is approached in three of the seven trees presented in Chapter 2. In Clade 4 ((*Aonyx*, *Ictonyx*) *Lutrogale*), two species found in the large otter clade from the coded trait analysis (((*Enhydra*, *Pteronura*) *Aonyx*) *Lutrogale*) are almost linked, but not in the same manner.

Conclusions

From these results it would appear that size based regression residuals are not useful as characters in phylogenetic analyses. The cladograms produced using this method (while removing the size-related component) appeared to be uninformative in

regards to otter evolution. The pervasive construction of paraphyletic groupings violates a number of systematic tenets and rendered the results largely uninterpretable. While the removal of the size component is viewed as a favorable result, the overall conclusions produced are unacceptable and of little phylogenetic use. The continuous trait analyses yielded results very similar to those in the coded trait analysis of Chapter 2. While this congruence seems to indicate support for those results, the size component must again be considered. In using straight continuous trait data (as in the coded trait analysis) the grouping of taxa strictly on the basis of size must be viewed with a degree of skepticism. Character traits with similar magnitudes in their measurements (whether coded or not) will naturally tend to fall out together, regardless of the evolutionary significance (or insignificance) of that particular character. The results between the coded MPR analyses and the uncoded continuous ML analyses yielded virtually the same topology in their groupings (in fact ML was less ambiguous in at least one area). And given that coding the data can be a rather laborious undertaking, for this type of data it would be advised to use the maximum likelihood approach and PHYLIP to perform this type of analysis. There are a few problems in the use of PHYLIP which should also be considered: the loss of useful branch lengths, a great reduction in the number of confidence statistics available, and the problem of directly comparing results of ML and MPR phylogeny estimates.

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Literature Cited

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Appendices

Appendix 3-1. Log normalized continuous postcranial trait data matrix composed of 88 characters.

	1	10										18						
<i>Amblonyx cinereus</i>	6.30	1.88	1.86	1.22	0.97	1.00	1.16	1.06	0.92	0.48	0.55	0.87	1.83	1.27	1.21	1.64	0.95	0.76
<i>Aonyx capensis</i>	6.73	2.04	2.01	1.42	1.17	1.21	1.34	1.31	1.17	0.66	0.77	1.10	2.01	1.48	1.42	1.82	1.16	0.92
<i>Enhydra lutris</i>	6.83	2.15	2.11	1.55	1.41	1.34	1.45	1.45	1.24	0.85	1.05	1.22	2.06	1.62	1.56	1.78	1.30	1.11
<i>Hydrictis maculicollis</i>	6.44	1.87	1.84	1.28	1.09	1.05	1.18	1.17	1.01	0.57	0.68	0.99	1.78	1.28	1.29	1.57	0.98	0.70
<i>Lontra canadensis</i>	6.48	1.97	1.94	1.33	1.13	1.08	1.21	1.21	1.02	0.56	0.71	1.03	1.88	1.36	1.33	1.66	1.04	0.93
<i>Lontra longicaudis</i>	6.47	1.92	1.89	1.31	1.07	1.07	1.20	1.20	1.01	0.57	0.75	0.95	1.86	1.34	1.30	1.64	1.04	0.78
<i>Lutra lutra</i>	6.45	1.94	1.91	1.31	1.07	1.09	1.22	1.18	1.05	0.54	0.86	0.82	1.89	1.34	1.30	1.68	1.04	0.78
<i>Lutrogale perspicillata</i>	6.59	2.01	1.98	1.38	1.19	1.17	1.30	1.27	1.12	0.70	0.79	1.05	1.94	1.41	1.37	1.73	1.10	0.87
<i>Pteronura brasiliensis</i>	7.00	2.08	2.04	1.51	1.25	1.26	1.39	1.40	1.21	0.76	0.96	1.15	1.99	1.54	1.50	1.76	1.22	0.99
<i>Mustela vison</i>	5.90	1.74	1.72	1.06	0.82	0.90	0.97	0.91	0.79	0.38	0.78	0.43	1.70	1.09	1.05	1.53	0.80	0.58
<i>Mephitis mephitis</i>	5.94	1.80	1.78	1.14	0.94	0.89	1.08	1.00	0.89	0.46	0.75	0.43	1.78	1.23	1.13	1.59	0.96	0.74
<i>Ichtonyx striatus</i>	5.80	1.72	1.69	1.00	0.72	0.79	0.93	0.87	0.72	0.43	0.71	0.29	1.67	1.06	0.98	1.51	0.75	0.52
	19	20	30										36					
<i>Amblonyx cinereus</i>	0.61	1.82	1.14	1.23	0.89	1.18	0.74	0.21	1.07	1.00	0.58	0.83	0.93	0.58	0.82	0.58	0.36	1.04
<i>Aonyx capensis</i>	0.85	2.01	1.37	1.47	1.11	1.41	1.00	0.54	1.40	1.25	0.77	1.05	1.13	0.73	1.09	0.83	0.64	1.28
<i>Enhydra lutris</i>	0.82	2.05	1.47	1.54	1.26	1.58	1.08	0.63	1.36	1.29	1.01	1.20	1.13	0.90	1.14	0.82	0.62	1.39
<i>Hydrictis maculicollis</i>	0.50	1.81	1.23	1.33	0.99	1.29	0.83	0.29	1.19	1.11	0.62	0.90	0.97	0.68	0.88	0.62	0.39	1.16
<i>Lontra canadensis</i>	0.49	1.88	1.29	1.39	1.06	1.37	0.83	0.36	1.14	1.14	0.76	0.98	0.97	0.78	0.96	0.64	0.46	1.19
<i>Lontra longicaudis</i>	0.64	1.89	1.28	1.38	1.04	1.35	0.93	0.34	1.26	1.16	0.71	0.96	1.00	0.76	0.96	0.70	0.43	1.20
<i>Lutra lutra</i>	0.58	1.89	1.28	1.39	1.03	1.36	0.89	0.32	1.26	1.16	0.75	0.97	1.00	0.75	0.95	0.69	0.39	1.20
<i>Lutrogale perspicillata</i>	0.78	1.94	1.27	1.41	1.07	1.35	0.88	0.40	1.30	1.19	0.71	0.97	1.07	0.74	0.99	0.74	0.50	1.22
<i>Pteronura brasiliensis</i>	0.85	2.02	1.47	1.56	1.16	1.50	1.04	0.65	1.42	1.34	0.97	1.19	1.25	0.87	1.15	0.85	0.58	1.36
<i>Mustela vison</i>	0.38	1.68	1.03	1.11	0.77	1.05	0.74	-0.300	0.99	0.92	0.45	0.68	0.77	0.56	0.67	0.38	0.19	0.95
<i>Mephitis mephitis</i>	0.42	1.73	1.12	1.21	0.67	1.13	0.68	-0.01	1.10	0.97	0.65	0.82	0.85	0.58	0.80	0.59	0.30	1.05
<i>Ichtonyx striatus</i>	0.29	1.65	0.99	1.07	0.67	0.97	0.58	-0.160	0.95	0.88	0.34	0.64	0.77	0.42	0.71	0.46	0.17	0.90

Appendix 3-1. Continued.

	37	40	50													54		
<i>Amblyonyx cinereus</i>	0.83	0.91	0.85	0.70	1.73	1.73	0.85	0.71	0.92	0.81	1.83	1.78	1.69	0.92	0.77	0.96	0.96	0.91
<i>Aonyx capensis</i>	1.07	1.11	1.12	0.99	1.92	1.91	1.09	0.93	1.20	1.08	2.03	2.00	1.88	1.12	0.95	1.21	1.23	1.11
<i>Enhydra lutris</i>	1.12	1.16	1.14	0.96	1.93	1.92	1.15	1.02	1.28	1.12	2.05	2.02	1.90	1.23	1.03	1.28	1.32	1.18
<i>Hydrictis maculicollis</i>	0.90	0.97	0.94	0.78	1.66	1.65	0.94	0.80	1.03	0.91	1.80	1.77	1.61	1.08	0.80	1.06	1.06	1.03
<i>Lontra canadensis</i>	0.94	1.03	0.94	0.82	1.73	1.72	0.99	0.83	1.07	0.93	1.87	1.84	1.67	1.12	0.85	1.13	1.12	1.13
<i>Lontra longicaudis</i>	0.95	1.03	0.98	0.85	1.73	1.72	1.00	0.84	1.10	0.94	1.85	1.81	1.66	1.09	0.83	1.10	1.12	1.08
<i>Lutra lutra</i>	0.97	1.01	1.02	0.84	1.74	1.74	1.01	0.87	1.07	0.94	1.89	1.86	1.71	1.14	0.90	1.10	1.10	1.10
<i>Lutrogale perspicillata</i>	0.98	1.03	1.05	0.85	1.79	1.78	1.02	0.89	1.13	1.00	1.91	1.88	1.73	1.12	0.95	1.14	1.15	1.15
<i>Pteronura brasiliensis</i>	1.13	1.22	1.19	1.03	1.89	1.87	1.17	1.04	1.35	1.20	2.01	1.98	1.81	1.35	1.09	1.35	1.32	1.25
<i>Mustela vison</i>	0.77	0.68	0.80	0.56	1.53	1.52	0.73	0.59	0.83	0.70	1.65	1.62	1.44	0.85	0.66	0.85	0.84	0.87
<i>Mephitis mephitis</i>	0.89	0.85	0.86	0.67	1.65	1.62	0.81	0.66	0.97	0.77	1.75	1.73	1.55	0.82	0.78	0.90	0.92	0.95
<i>Ictonyx striatus</i>	0.79	0.66	0.75	0.55	1.54	1.53	0.71	0.54	0.86	0.68	1.64	1.61	1.44	0.72	0.63	0.79	0.75	0.80
	55				60										70		72	
<i>Amblyonyx cinereus</i>	0.60	0.85	0.71	0.73	0.82	0.51	0.80	0.47	0.65	0.77	0.58	0.61	0.55	0.44	0.50	0.37	0.76	0.29
<i>Aonyx capensis</i>	0.81	1.14	0.98	1.00	1.12	0.76	1.07	0.76	0.82	1.03	0.82	0.85	0.89	0.62	0.65	0.59	0.97	0.52
<i>Enhydra lutris</i>	0.95	1.04	1.02	1.10	1.14	0.90	1.07	0.82	0.96	1.12	0.84	0.86	0.96	0.72	0.76	0.65	0.98	0.73
<i>Hydrictis maculicollis</i>	0.66	0.92	0.76	0.80	0.95	0.57	0.89	0.53	0.68	0.89	0.68	0.71	0.69	0.53	0.53	0.47	0.67	0.23
<i>Lontra canadensis</i>	0.70	0.94	0.91	0.89	0.97	0.65	0.93	0.57	0.84	0.95	0.79	0.81	0.67	0.50	0.53	0.46	0.91	0.45
<i>Lontra longicaudis</i>	0.70	0.96	0.90	0.90	0.95	0.65	0.92	0.56	0.78	0.92	0.70	0.75	0.71	0.50	0.52	0.48	0.84	0.37
<i>Lutra lutra</i>	0.74	0.98	0.78	0.92	0.98	0.70	0.93	0.58	0.80	0.96	0.74	0.76	0.81	0.62	0.64	0.51	0.89	0.34
<i>Lutrogale perspicillata</i>	0.73	1.00	0.90	0.93	0.99	0.75	0.95	0.65	0.82	1.02	0.79	0.78	0.81	0.66	0.72	0.51	0.96	0.45
<i>Pteronura brasiliensis</i>	0.83	1.21	1.14	1.11	1.16	0.90	1.13	0.78	1.02	1.17	0.97	0.96	0.94	0.80	0.87	0.72	1.11	0.61
<i>Mustela vison</i>	0.42	0.70	0.59	0.63	0.75	0.41	0.69	0.33	0.51	0.67	0.44	0.51	0.46	0.31	0.32	0.23	0.60	0.05
<i>Mephitis mephitis</i>	0.55	0.77	0.64	0.76	0.83	0.51	0.77	0.40	0.61	0.69	0.53	0.56	0.64	0.26	0.26	0.35	0.72	0.21
<i>Ictonyx striatus</i>	0.38	0.70	0.50	0.56	0.71	0.41	0.65	0.37	0.42	0.59	0.42	0.45	0.40	0.22	0.33	0.25	0.60	0.05

Appendix 3-1. Continued.

	73	75	80	85	88											
<i>Amblonyx cinereus</i>	0.75	0.92	0.49	0.87	0.91	0.09	1.05	0.63	0.98	1.53	1.64	1.51	1.27	1.22	0.62	1.17
<i>Aonyx capensis</i>	0.91	1.24	0.73	1.04	1.06	0.35	1.34	0.90	1.29	1.71	1.83	1.77	1.50	1.39	0.82	1.40
<i>Enhydra lutris</i>	1.14	1.17	0.78	1.20	1.25	0.48	1.50	1.05	1.49	1.93	2.03	1.90	1.68	1.59	1.00	1.62
<i>Hydrictis maculicollis</i>	0.64	0.99	0.45	0.90	0.87	0.24	1.16	0.69	1.00	1.63	1.74	1.47	1.40	1.11	0.72	1.31
<i>Lontra canadensis</i>	0.85	1.20	0.74	1.03	1.11	0.36	1.23	0.93	1.36	1.73	1.82	1.56	1.36	1.39	0.70	1.32
<i>Lontra longicaudis</i>	0.83	1.14	0.57	0.97	0.97	0.27	1.23	0.79	1.15	1.71	1.80	1.58	1.47	1.18	0.75	1.31
<i>Lutra lutra</i>	0.89	1.00	0.57	1.00	0.98	0.34	1.24	0.65	1.01	1.65	1.76	1.59	1.46	1.35	0.69	1.28
<i>Lutrogale perspicillata</i>	0.91	1.21	0.60	1.04	1.06	0.33	1.31	0.76	1.18	1.68	1.82	1.62	1.45	1.37	0.77	1.31
<i>Pteromura brasiliensis</i>	0.97	1.27	0.80	1.13	1.12	0.54	1.37	1.13	1.38	1.90	1.99	1.74	1.57	1.46	0.82	1.51
<i>Mustela vison</i>	0.77	0.77	0.35	0.66	0.58	-0.11	0.70	0.36	0.84	1.42	1.49	1.30	1.22	0.77	0.50	1.13
<i>Mephitis mephitis</i>	0.61	0.80	0.46	0.68	0.61	0.00	0.00	0.49	0.88	1.35	1.41	1.41	1.21	1.43	0.52	1.09
<i>Ictonyx striatus</i>	0.58	0.89	0.24	0.63	0.67	0.04	0.77	0.39	0.75	1.17	1.22	1.32	1.22	1.22	0.52	1.11

Appendix 3-2. Log-normalized postcranial regression residuals data set (truncated to 3 decimal places for display).

	1	5	10	14										
<i>Amblyonyx cinereus</i>	-0.010	-0.005	-0.022	-0.046	-0.022	0.000	-0.053	-0.048	-0.056	-0.205	0.100	0.000	-0.017	-0.03
<i>Aonyx capensis</i>	0.001	0.010	-0.012	-0.048	0.009	0.006	-0.009	0.020	-0.031	-0.067	-0.018	0.050	0.012	-0.01
<i>Enhydra lutris</i>	0.084	0.075	0.073	0.142	0.093	0.079	0.078	0.052	0.126	0.188	0.027	0.063	0.111	0.081
<i>Hydricitis maculicollis</i>	-0.065	-0.066	-0.026	0.007	-0.033	-0.034	-0.013	-0.010	-0.021	-0.105	0.107	-0.095	-0.068	-0.01
<i>Lontra canadensis</i>	0.016	0.020	0.008	0.032	-0.021	-0.018	0.014	-0.020	-0.043	-0.084	0.115	-0.010	-0.003	0.015
<i>Lontra longicaudis</i>	-0.028	-0.029	-0.013	-0.026	-0.029	-0.028	0.001	-0.030	-0.032	-0.036	0.042	-0.025	-0.023	-0.01
<i>Lutra lutra</i>	-0.002	0.002	-0.004	-0.018	-0.003	0.004	-0.011	0.020	-0.057	0.068	-0.073	0.011	-0.012	-0.01
<i>Lutrogale perspicillata</i>	0.023	0.022	0.013	0.039	0.029	0.026	0.013	0.035	0.059	-0.022	0.051	0.019	0.002	0.007
<i>Pteronura brasiliensis</i>	-0.046	-0.049	-0.040	-0.098	-0.055	-0.054	-0.046	-0.049	-0.018	0.062	-0.181	-0.048	-0.05	-0.05
<i>Mustela vison</i>	-0.008	-0.010	-0.007	-0.015	0.040	-0.025	-0.018	-0.006	-0.026	0.095	-0.029	-0.011	-0.028	-0.01
<i>Mephitis mephitis</i>	0.036	0.034	0.057	0.094	0.015	0.070	0.056	0.074	0.037	0.060	-0.060	0.056	0.091	0.056
<i>Ictonyx striatus</i>	-0.002	-0.005	-0.027	-0.062	-0.024	-0.026	-0.011	-0.039	0.061	0.046	-0.082	-0.011	-0.015	-0.03

	15	20	25	28										
<i>Amblyonyx cinereus</i>	0.009	-0.034	-0.001	0.063	-0.008	-0.063	-0.069	-0.036	-0.063	-0.073	0.025	-0.092	-0.079	-0.07
<i>Aonyx capensis</i>	0.084	0.004	-0.019	0.093	0.037	-0.003	-0.005	-0.026	-0.030	0.024	0.014	0.074	0.009	-0.07
<i>Enhydra lutris</i>	0.019	0.104	0.134	0.023	0.047	0.053	0.030	0.081	0.091	0.068	0.030	-0.009	0.010	0.125
<i>Hydricitis maculicollis</i>	-0.09	-0.063	-0.120	-0.113	-0.068	-0.027	-0.025	-0.001	-0.021	-0.030	-0.004	-0.028	-0.013	-0.09
<i>Lontra canadensis</i>	-0.02	-0.016	0.094	-0.140	-0.004	0.021	0.020	0.050	0.043	-0.047	0.038	-0.084	-0.003	0.034
<i>Lontra longicaudis</i>	-0.03	-0.010	-0.053	0.010	0.001	0.013	0.012	0.038	0.031	0.055	0.027	0.033	0.024	-0.01
<i>Lutra lutra</i>	0.011	-0.006	-0.043	-0.042	0.007	0.015	0.035	0.031	0.043	0.024	0.009	0.040	0.022	0.041
<i>Lutrogale perspicillata</i>	0.030	0.003	-0.002	0.092	0.014	-0.040	-0.004	0.006	-0.026	-0.037	-0.005	0.028	0.004	-0.06
<i>Pteronura brasiliensis</i>	-0.04	-0.039	-0.047	-0.029	-0.043	-0.011	-0.020	-0.099	-0.070	-0.031	-0.078	-0.015	-0.005	0.005
<i>Mustela vison</i>	-0.01	-0.025	-0.022	0.023	-0.007	-0.010	-0.018	0.038	-0.010	0.079	-0.175	-0.014	-0.002	-0.01
<i>Mephitis mephitis</i>	0.044	0.116	0.122	0.041	0.029	0.064	0.061	-0.074	0.052	0.003	0.082	0.077	0.035	0.168
<i>Ictonyx striatus</i>	-0.01	-0.032	-0.043	-0.021	-0.004	-0.014	-0.018	-0.008	-0.040	-0.035	0.037	-0.009	-0.001	-0.07

Appendix 3-2. Continued

	29	30	35	40	42									
<i>Amblonyx cinereus</i>	-0.05	-0.08	-0.012	-0.062	-0.041	-0.017	-0.080	-0.083	-0.011	-0.084	-0.054	0.032	0.043	-0.061
<i>Aonyx capensis</i>	-0.02	-0.07	0.015	0.036	0.055	0.099	-0.012	0.028	-0.005	0.038	0.056	0.083	0.082	0.004
<i>Enhydra lutris</i>	0.077	0.063	-0.022	0.045	0.012	0.034	0.068	0.051	-0.003	0.020	-0.010	0.058	0.055	0.025
<i>Hydrictis maculicollis</i>	-0.05	-0.03	-0.026	-0.061	-0.046	-0.039	-0.016	-0.049	-0.018	-0.036	-0.028	-0.090	-0.09	-0.026
<i>Lontra canadensis</i>	0.019	0.062	-0.037	0.001	-0.040	0.011	0.006	-0.021	0.030	-0.06	-0.007	-0.03	-0.03	0.005
<i>Lontra longicaudis</i>	0.004	0.045	-0.005	0.012	0.022	-0.014	0.012	-0.014	0.036	-0.009	0.023	-0.029	-0.029	0.021
<i>Lutra lutra</i>	0.018	0.037	-0.004	0.010	0.015	-0.044	0.021	0.015	0.015	0.037	0.020	-0.006	-0.003	0.035
<i>Lutrogale perspicillata</i>	-0.04	-0.02	0.021	-0.010	0.016	0.017	-0.012	-0.014	-0.017	0.015	-0.022	-0.008	-0.007	-0.003
<i>Pteronura brasiliensis</i>	-0.005	-0.03	0.035	-0.017	-0.024	-0.070	-0.028	0.008	-0.019	0.006	-0.008	-0.042	-0.048	-0.02
<i>Mustela vison</i>	-0.03	0.035	-0.020	-0.047	-0.089	-0.034	-0.010	-0.026	-0.060	0.011	-0.033	-0.038	-0.032	-0.02
<i>Mephitis mephitis</i>	0.100	0.044	0.042	0.061	0.100	0.066	0.072	0.082	0.088	0.055	0.064	0.066	0.052	0.04
<i>Ictonyx striatus</i>	-0.02	-0.06	0.014	0.03	0.020	-0.011	-0.021	0.023	-0.037	0.002	-0.001	0.006	0.002	0.001
	43	45	50	55	56									
<i>Amblonyx cinereus</i>	-0.054	-0.102	-0.06	0.008	-0.009	0.054	-0.072	-0.049	-0.066	-0.061	-0.095	-0.027	-0.039	-0.06
<i>Aonyx capensis</i>	-0.017	0.003	0.018	0.064	0.064	0.084	-0.076	-0.021	-0.006	0.008	-0.045	0.000	0.070	0.002
<i>Enhydra lutris</i>	0.035	0.041	0.016	0.057	0.075	-0.017	0.029	0.016	0.047	-0.009	0.105	-0.070	-0.003	0.059
<i>Hydrictis maculicollis</i>	-0.026	-0.051	-0.026	-0.069	-0.066	-0.077	0.021	-0.065	-0.026	-0.032	-0.030	-0.027	-0.024	-0.07
<i>Lontra canadensis</i>	-0.005	-0.03	-0.016	-0.010	-0.007	-0.030	0.040	-0.028	0.028	0.016	0.064	0.003	-0.017	0.059
<i>Lontra longicaudis</i>	0.005	0.008	-0.01	-0.024	-0.035	-0.034	0.011	-0.040	0.006	0.020	0.012	0.005	0.005	0.053
<i>Lutra lutra</i>	0.040	-0.016	-0.003	0.019	0.018	0.018	0.071	0.034	0.008	0.006	0.035	0.049	0.023	-0.06
<i>Lutrogale perspicillata</i>	0.009	-0.004	0.002	-0.005	0.000	-0.004	-0.008	0.035	-0.009	-0.007	0.042	-0.014	-0.012	-0.01
<i>Pteronura brasiliensis</i>	-0.013	0.047	0.024	-0.052	-0.050	-0.085	0.028	0.033	0.010	-0.032	0.002	-0.092	0.035	0.028
<i>Mustela vison</i>	-0.010	-0.030	-0.007	-0.035	-0.029	-0.045	0.046	-0.017	0.007	0.005	-0.005	-0.041	-0.024	0.017
<i>Mephitis mephitis</i>	0.050	0.091	0.045	0.056	0.062	0.054	-0.005	0.092	0.038	0.064	0.063	0.075	0.030	0.053
<i>Ictonyx striatus</i>	-0.014	0.042	0.023	-0.01	-0.003	-0.009	-0.038	-0.005	-0.006	-0.036	-0.034	0.035	0.022	-0.02

Appendix 3-2. Continued

	57	60	65	70										
<i>Amblonyx cinereus</i>	-0.079	-0.088	-0.087	-0.057	-0.050	-0.046	-0.076	-0.067	-0.063	-0.114	-0.025	0.004	-0.055	-0.031
<i>Aonyx capensis</i>	-0.004	0.050	-0.023	0.042	0.057	-0.067	-0.025	-0.018	0.002	0.038	-0.050	-0.056	0.003	0.007
<i>Enhydra lutris</i>	0.032	0.076	0.008	0.076	0.026	0.014	-0.032	-0.025	0.061	0.008	0.008	0.025	-0.020	0.16
<i>Hydrictis maculicollis</i>	-0.072	-0.005	-0.082	-0.023	-0.049	-0.074	-0.019	-0.023	-0.021	-0.036	0.000	-0.035	-0.005	-0.17
<i>Lontra canadensis</i>	-0.002	-0.003	-0.018	0.005	-0.021	0.065	0.024	0.071	0.069	-0.073	-0.049	-0.055	-0.030	0.04
<i>Lontra longicaudis</i>	0.016	-0.022	-0.015	-0.04	-0.030	0.008	-0.004	-0.018	0.007	-0.022	-0.044	-0.061	-0.009	-0.16
<i>Lutra lutra</i>	0.039	0.017	0.035	0.009	-0.010	0.039	0.042	0.026	0.024	0.078	0.084	0.066	0.026	0.036
<i>Lutrogale perspicillata</i>	-0.013	-0.024	0.032	-0.02	0.006	0.002	0.041	0.025	-0.009	0.025	0.066	0.083	-0.026	0.052
<i>Pteronura brasiliensis</i>	-0.008	-0.014	0.005	-0.01	-0.033	0.015	-0.016	0.018	0.004	-0.034	0.006	0.037	0.022	0.040
<i>Mustela vison</i>	-0.012	-0.003	-0.014	-0.012	-0.025	0.002	0.021	-0.025	-0.003	-0.022	0.041	0.009	-0.041	-0.028
<i>Mephitis mephitis</i>	0.106	0.065	0.066	0.058	0.023	0.079	0.016	0.048	0.032	0.136	-0.035	-0.072	0.067	0.076
<i>Ictonyx striatus</i>	-0.030	-0.006	0.026	-0.01	0.056	-0.049	-0.019	-0.004	-0.017	-0.036	-0.002	0.073	0.022	0.012

	71	75	80	85	87												
<i>Amblonyx cinereus</i>	-0.01	-0.03	-0.08	-0.01	0.033	-0.1	0.07	-0.03	-0.06	-0.03	-0.002	-0.01	-0.09	-0.03	-0.05	-0.080	
<i>Aonyx capensis</i>	-0.00	-0.03	0.06	0.02	-0.04	-0.04	-0.06	-0.02	-0.04	-0.01	-0.09	-0.08	0.06	-0.02	-0.005	0.0	-0.021
<i>Enhydra lutris</i>	0.17	-0.06	0.030	0.07	0.100	0.10	0.03	0.04	0.06	0.14	0.07	0.06	0.156	0.12	0.15	0.15	0.161
<i>Hydrictis maculicollis</i>	-0.15	-0.19	-0.07	-0.12	-0.043	-0.08	-0.02	0.06	-0.06	-0.12	0.002	0.01	-0.10	-0.01	-0.2	0.01	0.003
<i>Lontra canadensis</i>	0.06	0.01	0.12	0.15	0.07	0.14	0.08	0.10	0.16	0.21	0.07	0.064	-0.04	-0.06	0.07	-0.02	0.008
<i>Lontra longicaudis</i>	-0.02	-0.01	0.06	-0.02	0.01	0.00	-0.01	0.01	0.02	0.006	0.06	0.05	-0.01	0.05	-0.13	0.02	-0.017
<i>Lutra lutra</i>	-0.04	0.05	-0.07	-0.01	0.04	0.02	0.07	0.13	-0.11	-0.13	0.01	0.02	0.005	0.04	0.04	-0.03	-0.046
<i>Lutrogale perspicillata</i>	-0.01	0.02	0.09	-0.05	0.02	0.03	-0.01	0.08	-0.08	-0.03	-0.03	0.01	-0.02	-0.01	0.02	0.003	-0.104
<i>Pteronura brasiliensis</i>	-0.06	-0.06	-0.03	-0.02	-0.09	-0.12	0.02	-0.24	0.03	-0.06	-0.05	-0.08	-0.09	-0.05	-0.03	-0.09	-0.025
<i>Mustela vison</i>	-0.04	0.13	-0.06	0.01	-0.03	-0.08	-0.09	0.09	-0.05	0.03	0.09	0.09	-0.04	0.01	-0.35	-0.01	0.041
<i>Mephitis mephitis</i>	0.09	-0.04	-0.05	0.10	-0.02	-0.07	-0.01	-0.65	0.06	0.04	-0.005	-0.02	0.06	-0.01	0.30	-0.01	-0.023
<i>Ictonyx striatus</i>	0.013	-0.02	0.1	-0.07	-0.003	0.06	0.10	0.25	0.04	-0.01	-0.100	-0.12	0.03	0.04	0.14	0.04	0.056

Part 4

A Consensus Phylogeny of the Lutrinae

Introduction

Phylogeny resolution relies both on character traits and the analysis used to investigate those traits. Otters show this fact well, and it is clear when previous otter phylogenetic analyses are compared. To compare trees effectively, branch lengths and tree topology are often considered. However, branch lengths can be affected by numerous factors (and are not available for all trees considered here) and tree topology has a special importance since it indicates the ordinal relationships between taxa. Using several methods, tree topologies can be compared without regard for easily influenced branch lengths. By looking at previous phylogenies, the characters used, and how the results from each analysis differ, we can evaluate the hypotheses proposed and determine their probable veracity. Previous phylogenetic hypotheses are presented, and compared with the hypotheses generated by this study. Consensus and combined data phylogenies are constructed by combining data and trees from previous analyses using several methods and the results compared.

One approach that has been successfully applied to data or tree combining studies is that of Lapointe and Legendre (1990; 1992a; 1992b). This method allows for a comparison of tree bifurcation sequences without regard for differences in hierarchy fusion levels (i.e. branch and tree lengths). This approach is not useful here since it should only be used to "...compare classifications that are based on different datasets, and not to compare different methods of classification..." (Lapointe and Legendre 1990, p. 12). The trees used here are all derived from the same dataset, differing only in the treatment of the data (coded, uncoded, and regression residuals) and method of

phylogenetic analysis (parsimony and maximum likelihood). Alternative methods were therefore used to combine and compare results from the previous analyses.

Tree combining techniques versus data combining techniques

There are differences between tree and data combining techniques. These major types of combination approaches are generally termed character congruence for data combining (also called the total or combined evidence approach (Kluge 1989)), while tree combining is termed taxonomic congruence (Mickeyvich 1978; 1980). The major difference between these techniques is that character congruence employs simultaneous analysis of all available character data (Miyamoto 1985; Kluge 1989; Eernisse and Kluge 1993; Chippindale and Wiens 1994) while taxonomic congruence requires data partitioning for separate analyses followed by consensus formation based on the produced trees (Jones et al. 1993; Chippindale and Wiens 1994). The primary difference being that total evidence combines multiple datasets in a new analysis, while taxonomic congruence performs separate analyses on partitioned datasets then combines the results using consensus methods. There are several excellent reviews on combining data versus separate analysis in phylogenetics (de Quieroz et al. 1995; Miyamoto and Fitch 1995; Huelsenbeck et al. 1996). Another tree combining method, MRP "matrix representation using parsimony" (Ragan 1992) (not to be confused with MPR), combines trees from different sources based on their topologies without separate analyses.

A third approach, termed the "prior agreement approach" (Chippindale and Wiens 1994) attempts to blend aspects of both tree and data combining in order to garner the

benefits and reduce the disadvantages of each (Bull et al. 1993; de Quieroz 1993; Chippindale and Wiens 1994; Wiens and Chippindale 1994). Proponents of prior agreement object to combining data sets that are either significantly dissimilar in nature or strongly supported but with conflicting results (Bull et al. 1993; de Quieroz 1993). Chippindale and Wiens (1994) suggest that many of these problems could be rectified by using differential character weighting, a solution criticized by Huelsenbeck et al. (1994)

In prior agreement, separate analyses are performed and data with significantly different results are not combined (Bull et al. 1993; de Quieroz 1993; Wiens and Chippindale 1994). Some argue that data should be combined, but weighted to allow data sets to contribute equally (Chippindale and Wiens 1994; Wiens and Chippindale 1994). This approach has been criticized as simplistic and difficult to apply without prior knowledge of the phylogeny or history (and heterogeneity) of data sources (Huelsenbeck et al. 1994). Here, a modified prior agreement approach is used since the datasets are derived from the same source (postcranial measurements), do not require differential weighting, and cannot be combined due to differences in format (discrete and continuous).

Materials and Methods

By using different methods to produce consensus trees and combine data, variations in results from these approaches can be compared. Relevant trees and data

Table 4-1. Sources for trees and cladograms used in consensus tree construction. Tree numbers are from Figure 4-1. Table indicates original tree source and tree type, tree production technique and analysis methods, and whether the resultant tree is a consensus or single most parsimonious tree.

Tree Number in Figure 4-1	Tree Source Chapter, Figure, and Type	Tree Production and Analysis Method	Single Tree or Consensus
Tree 1	Chapter 2, Figure 2-1 Phylogenetic tree	12 segment coding; Parsimony	Consensus (from 30 trees)
Tree 2	Chapter 2, Figure 2-3 Phylogenetic tree	6 segment coding; Parsimony	Single
Tree 3	Chapter 2, Figure 2-5 Phylogenetic tree	Gap A coding; Parsimony	Consensus (from 3 trees)
Tree 4	Chapter 2, Figure 2-6 Phylogenetic tree	Gap B coding; Parsimony	Single
Tree 5	Chapter 3, Figure 3-10 Cladogram	Regression residuals; Maximum Likelihood	Consensus (from 4 trees)
Tree 6	Chapter 3, Figure 3-5 Cladogram	Continuous trait data; Maximum Likelihood	Consensus (from 4 trees)

from the previous phylogenetic analyses in Chapters 2 and 3 (see Table 4-1 below) were used. When more than one cladogram or phylogenetic tree was produced by a particular technique, the consensus tree from that analysis was adopted as representative. The use of consensus trees avoids over-weighting the analysis with multiple results from techniques that produced numerous trees. This resulted in six trees or cladograms (shown in Figure 4-1 below) and their corresponding data matrices for use in combining and consensus analysis.

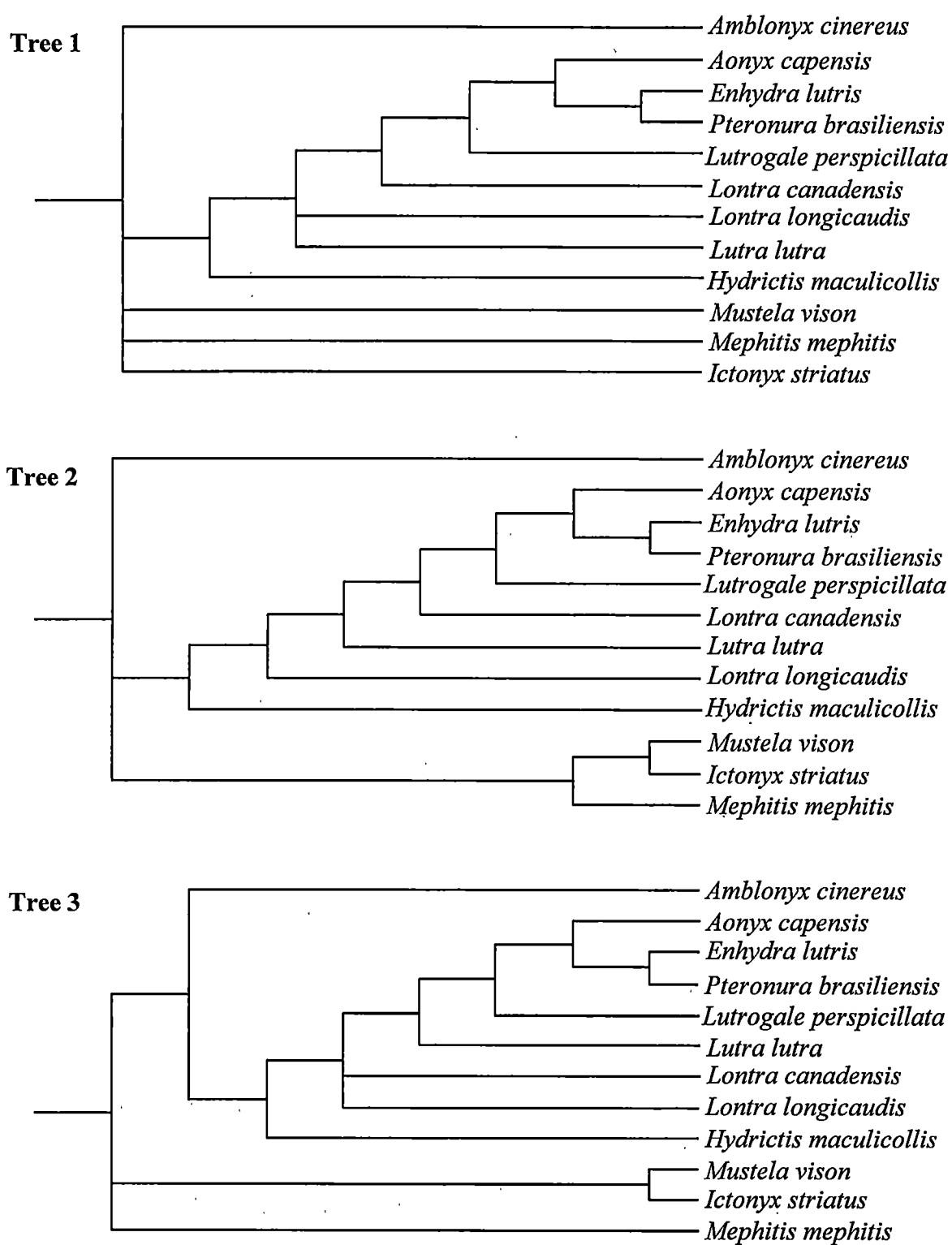


Figure 4-1. Cladograms used to construct consensus trees: 12-segment (Tree 1), 6-segment (Tree 2), and Gap A coding (Tree 3) (continued on next page).

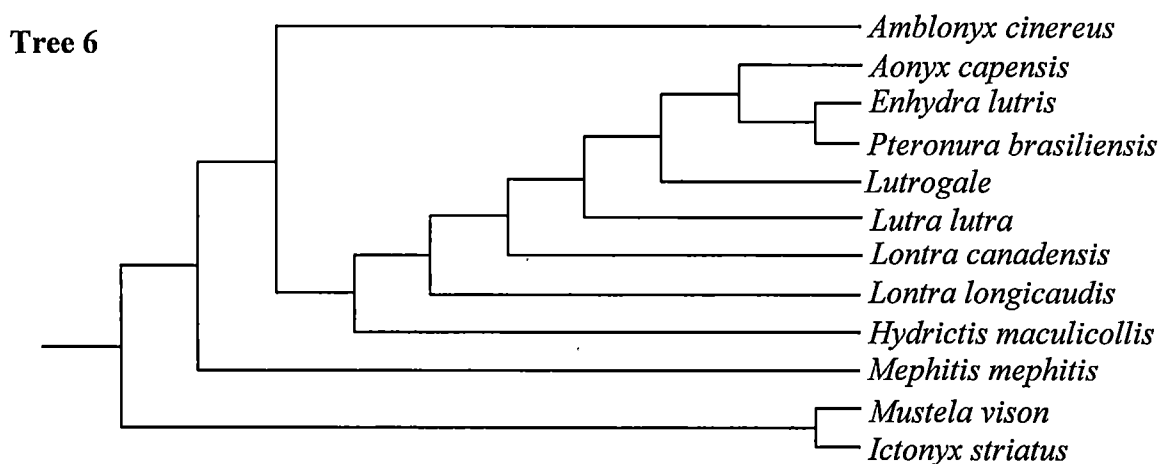
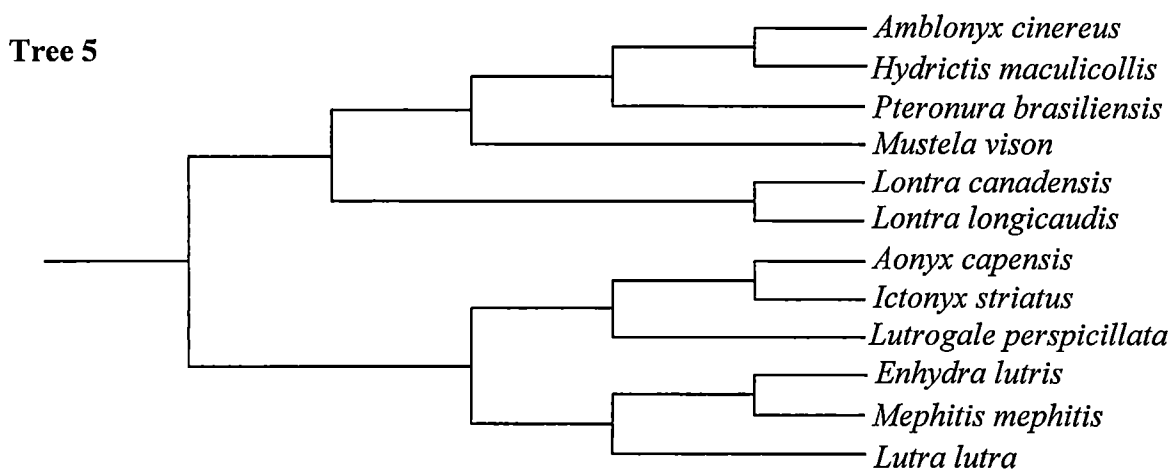
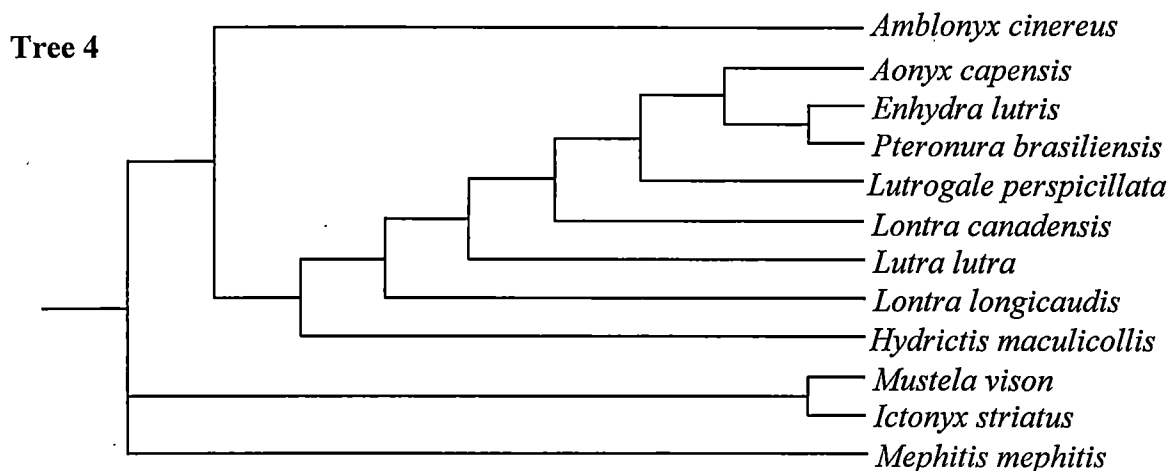


Figure 4-1 (continued). Cladograms used to construct consensus trees: Gap B coding (Tree 4), Regression residuals (Tree 5), and Continuous trait data (Tree 6).

Combining data versus combining results

The primary goal of this paper is to determine the evolutionary patterns of otters based on the greatest amount of information from the data set at hand. While the aim is not to compare various data combining or consensus methods, it is an unavoidable and useful consequence, since there is considerable debate regarding which methods are superior (taxonomic or character congruence, combining data or consensus techniques) (de Quieroz et al. 1995; Miyamoto and Fitch 1995; Huelsenbeck et al. 1996; Wiens 1998a; 1998b). Several techniques were used, as well as a hybrid approach (modified total evidence/taxonomic congruence). Comparing results from these differing approaches, while mindful of their advantages and drawbacks, may provide the means to accomplish the primary goal of this paper, as well as assess various methodologies.

Combining and Consensus Methods

Treefile combining consensus method

The first method used to integrate results from the analyses listed in Table 4-1 was treefile combining. This new tree combining approach was developed to solve several problems encountered in combining trees from different analyses and as a comparison with MRP. Some problems arose from differences in output formats (cladograms or phylogenetic trees). Other problems involve the type of information provided by the search method (tree and branch lengths or log likelihood). This combining method uses treefiles constructed with Newick Standard format. Newick standard treefiles were constructed using the topology of the trees and cladograms in Figure 4-1. Tree topology.

was used without regard for branch lengths (which were not available for all analyses). The six resultant treefiles were then incorporated into a NEXUS format file (Appendix 4-1) and analyzed using PAUP* (Swofford 2000). A dummy data matrix containing a single invariant character for all twelve taxa was created to allow treefile analyses independent of data set influence. The six resultant trees were then used to produce an overall consensus tree. Due to the large number of topological differences between most trees and the regression residuals tree (Figure 4-1, Tree 5), a strict consensus produces rather uninformative results (i.e., a 12 taxon polytomy). Therefore a 50% majority consensus tree with other compatible groupings was constructed. This approach maintains ordinal relationships that would normally be lost using strict consensus. Bootstrap support analysis was used to indicate node and branch support with 1000 replicates. While bootstrap support analyses are not appropriate for all combining and consensus methods, they were included when suitable.

Matrix representation using parsimony method

The next technique used to combine trees was matrix representation using parsimony (MRP) (Ragan 1992). This method is an extension of work done by Brooks (1981; 1990) to combine cladograms composed of different taxa (Baum 1992). It should be noted that MRP is a tree combining technique rather than a consensus method (Bininda-Emonds and Bryant 1998). The MRP method was developed independently by Baum (1992) and Ragan (1992) to overcome problems with combining trees containing different taxa (Ragan 1992; Baum and Ragan 1993). Baum (1992) and Ragan (1992)

developed this approach in response to what they felt was an inherent loss of information in consensus methods. Developing a graph-theoretical relationship between trees and matrices permits results to be combined without this information loss (Ragan 1992).

The "Matrix Representation" option of the "Trees" submenu in PAUP* was used to create six representative tree matrices (see Appendix 4-2). These matrices are not composed of characters, but rather matrix elements of a given tree. The essential difference is that characters are *properties of taxa*, while matrix elements are *tree products* that reflect hypothetical taxon relationships. Matrices were combined and used in an exhaustive parsimony search. The total number of matrix elements from the six MRP constructs was 130. The number of elements varied between 19 and 23 per matrix ($\bar{x} = 21.7$) due to differences in tree resolution and complexity of tree topology. Seventy eight of the 130 elements were deemed parsimony uninformative by PAUP* and removed from the analysis (6 of the 78 elements were also invariant). The remaining 52 elements were unordered and equally weighted. In order to test for effects of removing the invariant and uninformative characters, the analysis was also performed with all 130 matrix elements included.

Exhaustive searches were conducted using default PAUP* settings for both the complete and truncated element matrices. Exhaustive searches were feasible due to the small size of the data matrices and number of taxa ($n=12$). Bootstrap support analysis is not considered appropriate for MRP trees due to the lack of independence of matrix elements (Felsenstein 1985; Purvis 1995; Bininda-Emonds and Bryant 1998; Bininda-Emonds et al. 1999) and was therefore omitted.

Modified total evidence/taxonomic congruence approach

Another combining method which has been widely advocated (and debated) in phylogenetic studies is "total evidence" (Kluge 1989). Total evidence is a data rather than tree combining or consensus technique (Baum 1992; de Quieroz et al. 1995; Ronquist 1996). This method involves combining multiple original data matrices and performing simultaneous combined data analyses. One problem with this approach is that data in different formats or from different sources (i.e. distance data vs. discrete) cannot always be combined (de Quieroz et al 1995; Ronquist 1996). Most (four out of six) of the data matrices used here are in the same format (parsimony) and derived from the same data set (coded discrete data). The two remaining matrices are similar in format (maximum likelihood) and derived from the same data (continuous trait data). The problem of combining dissimilar data sets only becomes relevant when combining parsimony (Figure 4-1, trees 1-4) and ML data (Figure 4-1, trees 5 and 6).

To use aspects of a total evidence approach and combine disparate data, a hybrid approach similar to taxonomic congruence was used. The first step employed total evidence methods. Four data matrices used in discrete coded data parsimony analyses (Appendices 2-3, 2-4, 2-5, and 2-6) were combined (Appendix 4-3) and used in a total evidence parsimony analysis. Characters were unordered, unweighted, and with no outgroup assigned. Ten characters (out of 352) were parsimony uninformative and removed. All other options were left as the default PAUP* exhaustive search settings.

Similarly, the maximum likelihood analysis matrices (Appendices 3-1 and 3-2) were combined (Appendix 4-4) and used in a total evidence ML analysis. The resulting

175 character matrix was used in an ML analysis as described in Chapter 3. Results from the two total evidence analyses were then combined using MRP techniques. Combined matrices are shown in Appendix 4-5. This represents three combination steps. The first combines gap and segment coding data to produce a parsimony tree, the second combines continuous and regression residuals data to produce an ML tree, and the third combines the parsimony and ML trees. This approach differs slightly from taxonomic congruence in that MRP is used instead of consensus methods to combine trees (Jones et al. 1993).

This approach is not a strict total evidence approach either (Kluge and Wolf 1993), although it does combine compatible data in the same way. This method gains many of the benefits of total evidence, and avoids the problem of data loss found in congruence methods and the difficulties of combining incongruous data. While the use of MRP methods after the data analysis diverges slightly from total evidence ideology, the departure is minimal compared to its overall adherence. This small indiscretion is minor when compared to the major benefit of being able to combine results which would otherwise have to be left disassociated.

Results

Treefile combining results

Using six NEXUS format treefiles (shown in Appendix 4-1) and an invariant single character dummy matrix, a 50% Majority Rule consensus tree was constructed (Figure 4-2). A bootstrap support analysis was performed (indicated below the bipartition frequencies). Topologically, this consensus tree is identical to the tree recovered in

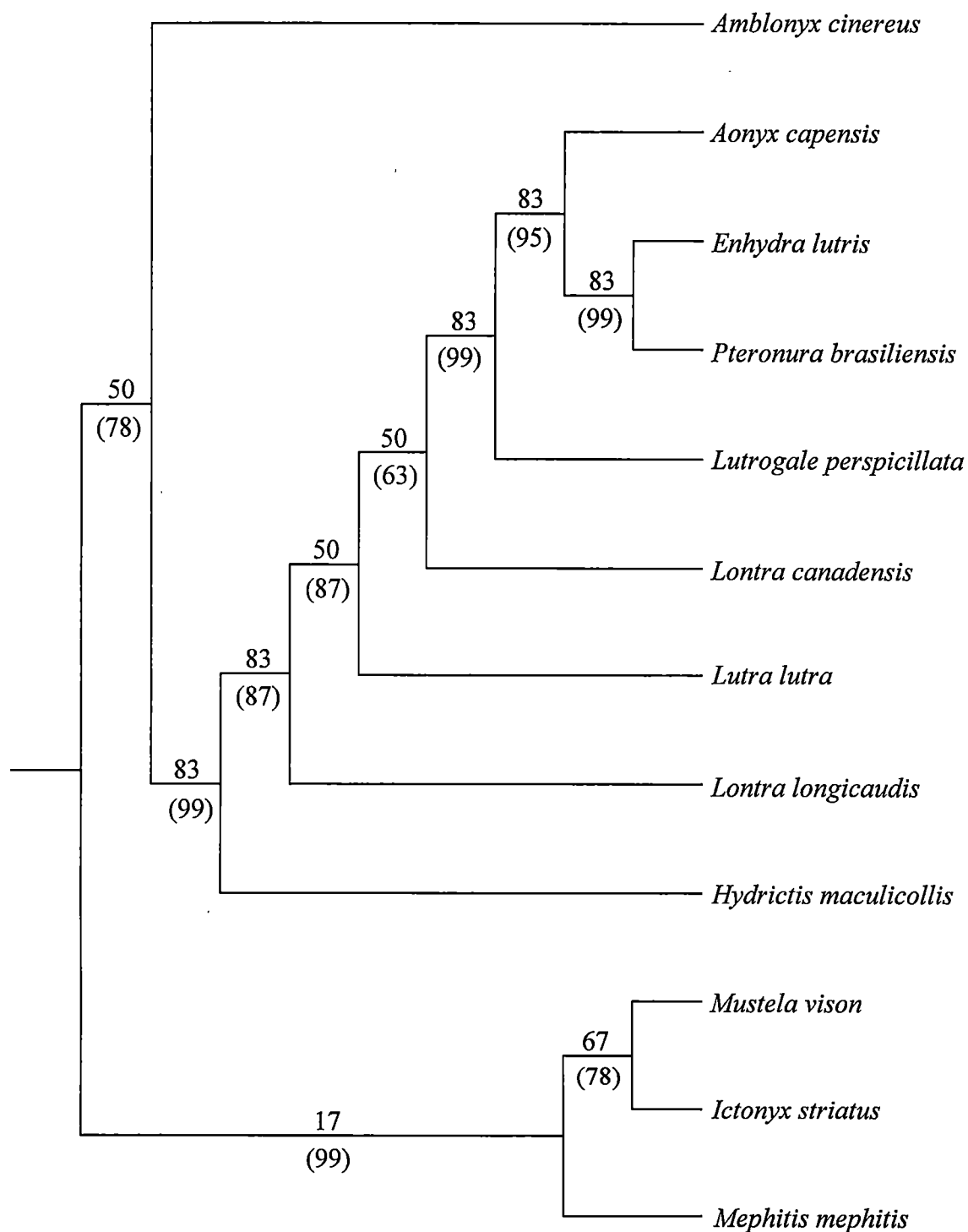


Figure 4-2. 50% Majority Rule consensus tree (with bipartitions less than 50%) from the treefile combining method for Figure 4-1 trees. Bipartition frequencies are shown above branches, bootstrap support values in parentheses.

Figure 2-3, which was based on 6 segment coding techniques. Taxon groupings are essentially size-based, with the largest otters grouped together in a clade followed in a step-like manner by otters of decreasing size. The two largest species, the sea otter (*Enhydra lutris*) and giant otter (*Pteronura brasiliensis*), are linked closely with the next largest (African clawless otter, *Aonyx capensis*). The fourth largest taxon (Indian smooth-coated otter, *Lutrogale perspicillata*) is connected to this clade, followed by the remaining taxa in approximately descending size (North American river otter, *Lontra canadensis*; Eurasian river otter, *Lutra lutra*; and Neotropical otter, *Lontra longicaudis*). Internally basal to this group is the spot-necked otter (*Hydrictis maculicollis*), in a manner seen in virtually all previous analyses. Basal to all other otters is the Asian small-clawed otter (*Amblonyx cinereus*), the smallest otter species. An outgroup clade composed of the two musteline species, American mink (*Mustela vison*) and zorilla (*Ictonyx striatus*), are linked with the mephitine striped skunk (*Mephitis mephitis*).

Bootstrap support for this consensus tree was very strong with over 80 percent for most nodes. Exceptions to this high bootstrap confidence include: the outgroup node splitting the mustelines and mephitines (17%), the node separating the outgroup species from the lutrines (50%), and the nodes positioning the Eurasian river otter (*Lutra lutra*) and North American river otter (*Lontra canadensis*). These taxa with reduced clade support are those which were equivocal in most of the previous analyses. Their proximity in size is most likely the cause for this ambiguity in their placement. The low bootstrap support for the outgroup species was also commonly seen in previous analyses and it is not surprising to see it repeated here.

Matrix representation using parsimony (MRP) results

The six matrices used in the MRP analysis contained a total of 130 matrix elements, 78 of which were deemed parsimony uninformative by PAUP* and removed from the analysis. The remaining 52 elements were used in an exhaustive search and produced a single most parsimonious tree (Figure 4-3). Another analysis was performed including all 130 matrix elements. The only difference in the results of these analyses was tree length (due to the difference in the number of matrix elements). Results from the all inclusive analysis are not shown. Bootstrap support analyses are uninformative for MRP trees, and were not performed.

Topologically, the lutrine clade in Figure 4-3 is identical to Tree 2 in Figure 2-4, which was one of three trees based on Gap A coding techniques. The only difference lies in the formation of the outgroup species clade in Figure 4-3. In Figure 2-4 (Tree 2) the two musteline species (*Mustela vison* and *Ictonyx striatus*) are linked in a clade with the mephitine (*Mephitis mephitis*) separate and basal. Figure 4-3 and Figure 4-2 differ in only two positions. The same size-based arrangement previously discussed is seen here. A single disparity between these trees lies in the placement of two species which are very close in size, the Eurasian river otter (*Lutra lutra*) and North American river otter (*Lontra canadensis*). These two species are reversed in their positions in Figures 4-2 and 4-3. In Figure 4-2 *Lontra canadensis* is linked with the Indian smooth-coated otter (*Lutrogale perspicillata*), while in Figure 4-3 this position is taken up by *Lutra lutra*.

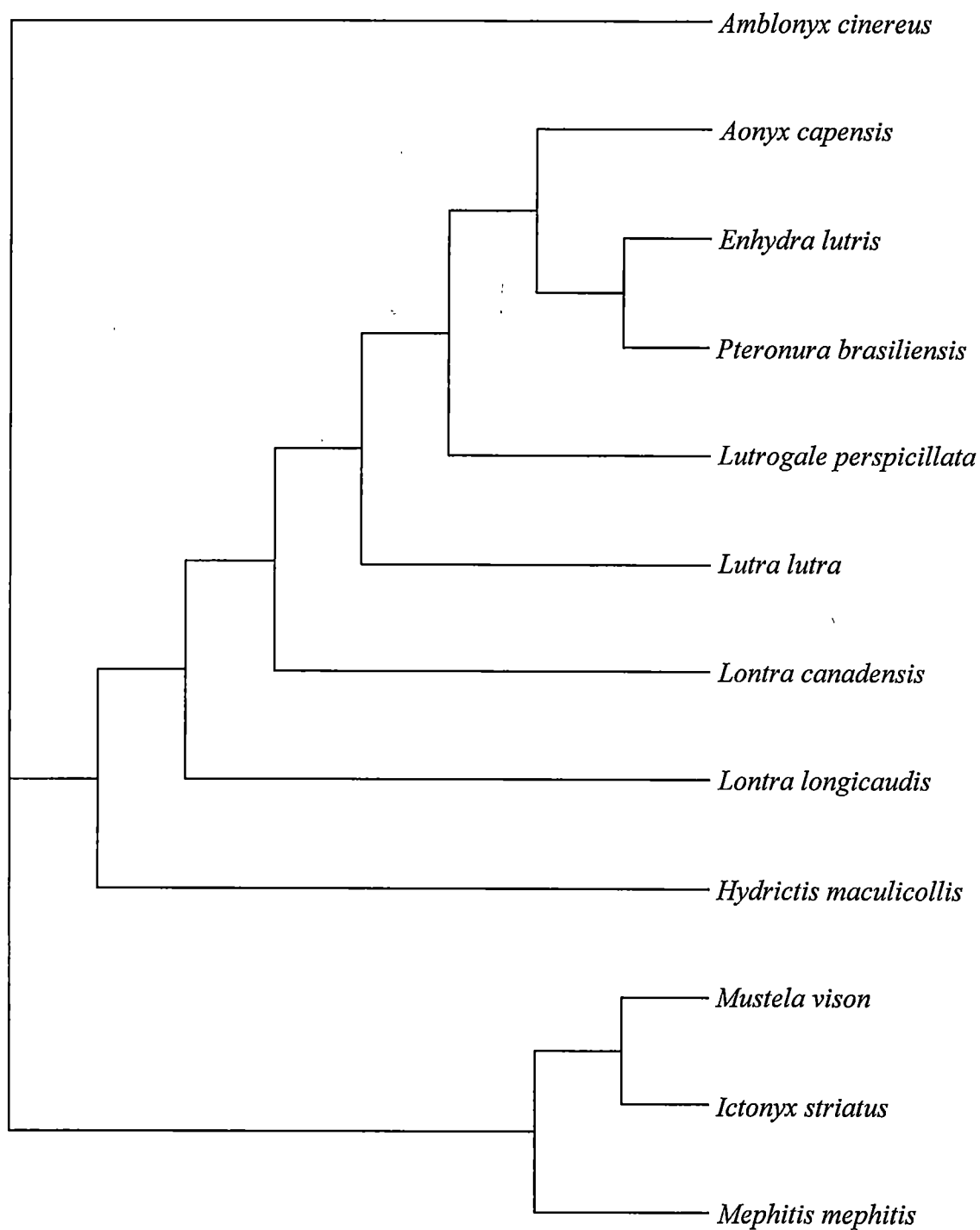


Figure 4-3. Combined tree constructed using the MRP method on the six trees from Figure 4-1.

Modified total evidence/taxonomic congruence results

The modified total evidence/taxonomic congruence approach constructs separate trees using the total evidence method followed by the construction of an MRP combined tree. Total evidence analyses were performed using the combined coded continuous parsimony data matrix (Appendix 4-3, composed of: Appendices 2-3, 2-4, 2-5, and 2-6) and the combined maximum likelihood matrix (Appendix 4-4, composed of: Appendices 3-1 and 3-2). The resulting trees were then combined using MRP techniques.

The first tree was constructed from the combined matrix of four previous parsimony analyses using coded continuous data (Appendix 4-3). The 352 character, 12 taxon matrix was used in an exhaustive parsimony search in PAUP*. A total of 645,729,075 trees was evaluated and a single most parsimonious tree was recovered (Figure 4-4). This tree was then used in conjunction with the matrix produced from the ML tree discussed below to produce an MRP matrix (Tree 1 matrix in Appendix 4-5).

The second total evidence matrix was based on maximum likelihood (ML) analyses (Appendix 4-4) and contained 175 characters. This combined ML matrix was used in a PHYLIP ML analysis which evaluated 2647 trees and produced a total evidence ML tree with a ln likelihood of 3910.13 (Figure 4-5). The resultant treefile was then transferred to a PAUP* NEXUS file in combination with the treefile from the tree shown in Figure 4-4. The Matrix Representation function in PAUP* was used to produce an MRP combination tree from the total evidence analyses (Figure 4-6). This last step was virtually unnecessary since both trees (Figure 4-4 and 4-5) are topologically identical and produced a consensus tree that did not differ from either source tree (Figure 4-6).

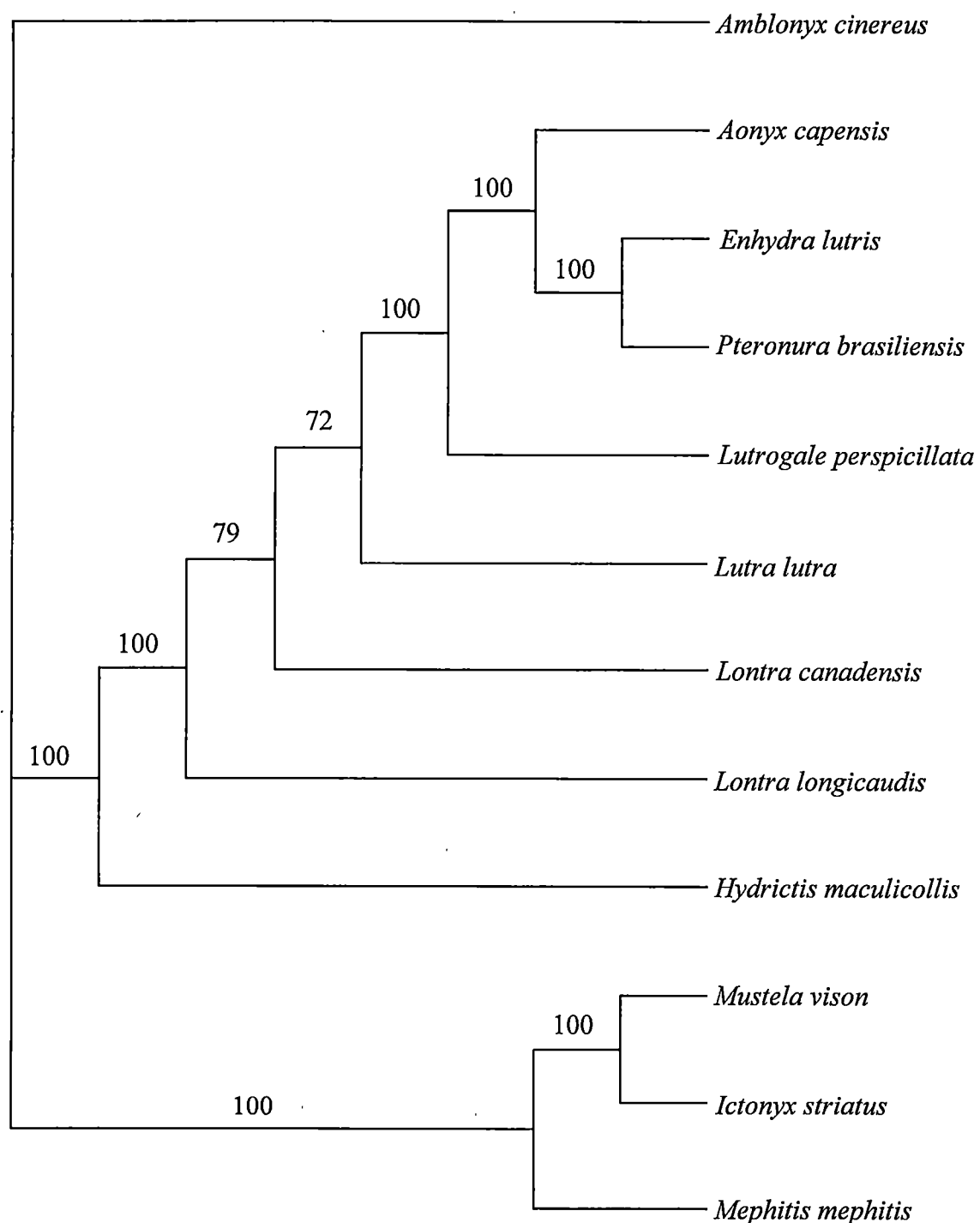


Figure 4-4. Total evidence tree constructed using the combined matrices from parsimony analyses shown in Table 4-1. Bootstrap support indices from 1000 replicates indicated above branches.

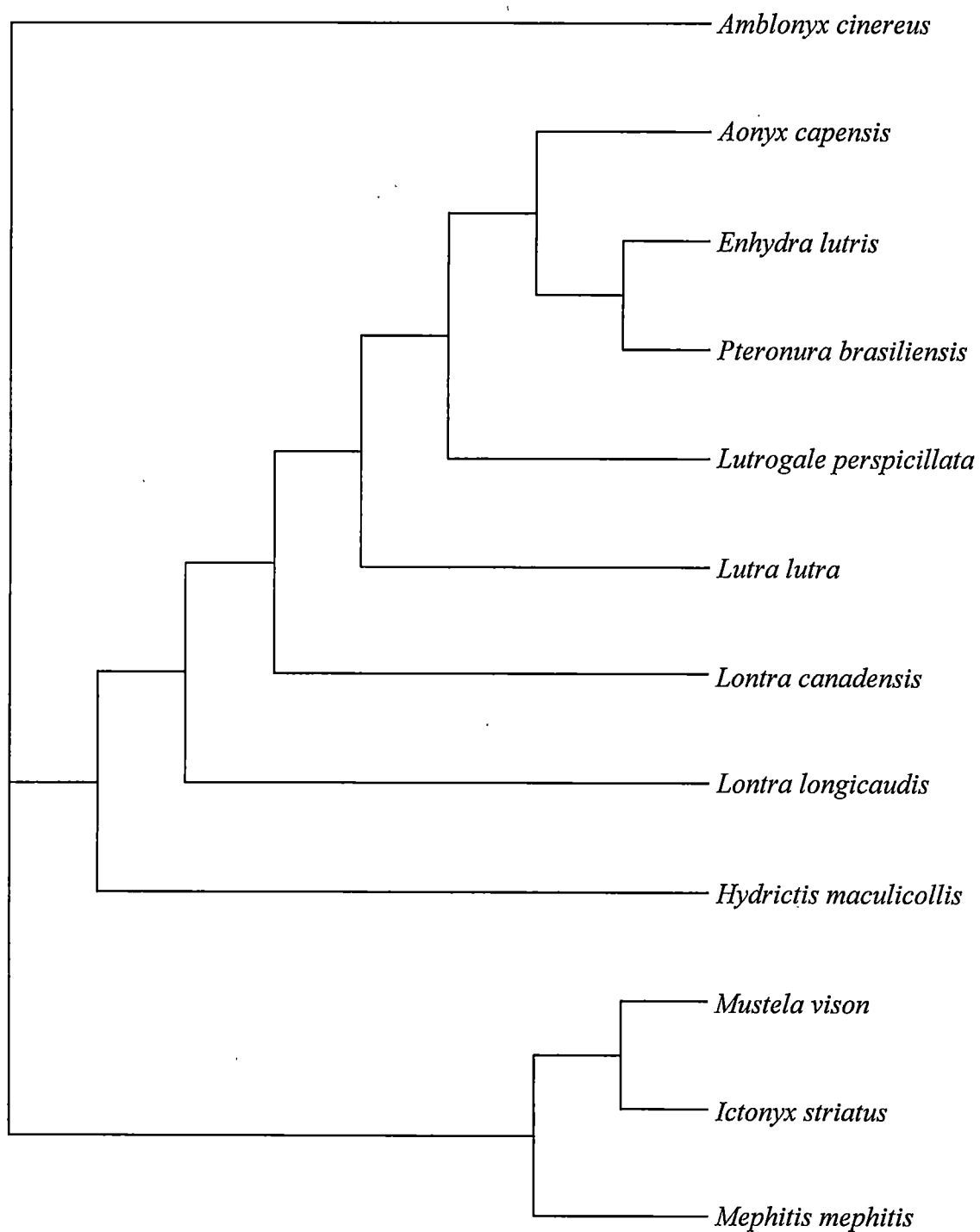


Figure 4-5. Total evidence maximum likelihood tree produced by combining the two maximum likelihood matrices indicated in Table 4-1.

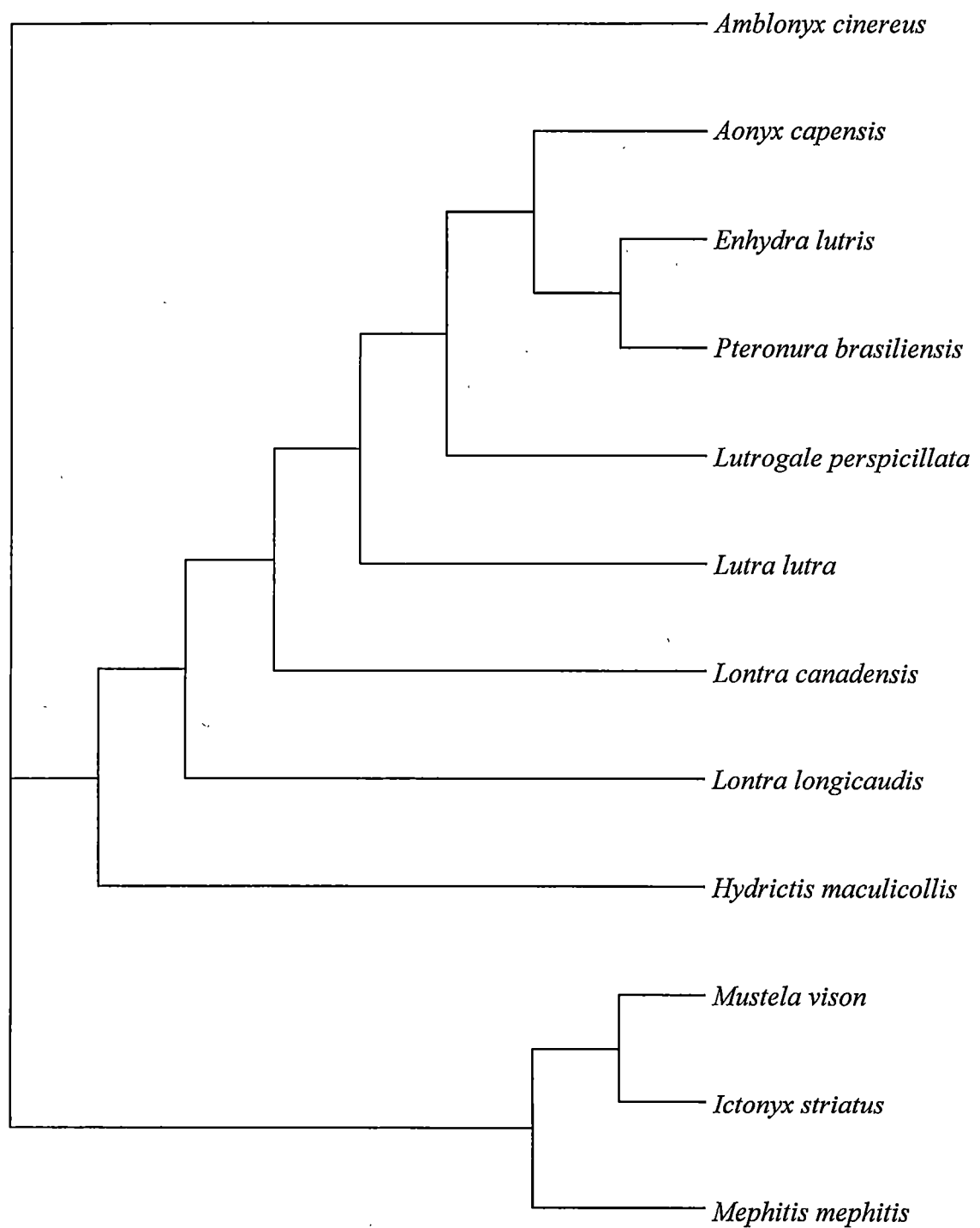


Figure 4-6. Modified total evidence MRP tree composed of tree elements combined from the parsimony and maximum likelihood trees shown in Figures 4-4 and 4-5 respectively.

Discussion

One of the most striking aspects of the results from the combined data/tree and consensus analyses was the almost complete agreement. Regardless of the method used to combine either data or trees, the overall topology was the same (with the exception of a single position reversal of two taxa in a single tree: Figure 4-2). These combined data and consensus trees were also in agreement with the primary topology of the trees produced in virtually all of the separate analyses (Figure 4-1). The size-related topology discussed in Chapters 2 and 3 persisted in the combined and consensus analyses (as might be expected given the source trees), but with some added resolution.

The combining techniques consistently grouped the outgroup species in a separate clade, a result not seen in most source trees. This “outgroup clade” result occurred in only a single source tree (Figure 4-1, Tree 2), yet was consistently recovered using tree or data combining and consensus methods. The outgroup clade was composed of a musteline clade American mink (*Mustela vison*) and zorilla (*Ictonyx striatus*) linked with the mephitine striped skunk (*Mephitis mephitis*). The linkage of the two mustelines was prevalent in the majority of source trees, two exceptions were Figure 4-1 Tree 1 (a consensus of 30 trees) and the anomalous regression residuals tree (Figure 4-1 Tree 5). The consistent linkage of this group with the skunk was somewhat unexpected given the general lack of this result in previous separate analyses.

The formation of an outgroup clade from sources which do not generally contain it supports the contention that combining data may result in signal enhancement (de Quieroz et al. 1995; Nixon and Carpenter 1996; Bininda-Emonds et al. 1999), or produce

novel combinations (Bininda-Emonds and Bryant 1998). This effect can occur when weak signals present in small source data sets are combined, and are then able to overcome “phylogenetic noise” and produce novel results (Barrett et al. 1991; de Quieroz 1995). It has been postulated by several workers (Barrett et al. 1991; de Quieroz 1993; de Quieroz et al. 1995) and shown in simulation studies (Huelsenbeck and Hillis 1993) that the more characters used in an analysis (excluding homoplastic ones), the more accurate the phylogeny estimate will become. This result could be viewed as unrepresentative of the data, producing trees that do not accurately estimate the true phylogeny (Barrett et al. 1991) or produce “positively misleading” results (Felsenstein 1978). Many have regarded the arguments against combining and consensus methods as having little merit, and advocate the judicious application of various combining and consensus techniques (Barrett et al. 1991; de Quieroz 1993; Nelson 1993; de Quieroz et al 1995).

With regards to the lutrine clade, the modified total evidence/taxonomic congruence trees (Figures 4-4, 4-5, and 4-6) were all topologically identical with the combined MRP tree shown in Figure 4-3. These trees differed from the tree combining result (Figure 4-2) by a single position switch of *Lontra canadensis* and *Lutra lutra* (discussed in MRP results above). Interestingly, this single position shift occurred between two groups (*Lontra* and *Lutra*) that have been variously separated (van Zyll de Jong 1991; Koepfli and Wayne 1998) and combined (Bryant et al. 1993; Bininda-Emonds et al. 1999) and are very similar in size. The majority of the results here (Figures 4-3, 4-4, 4-5, and 4-6) supported a placement of the Old World *Lutra lutra* that avoids a paraphyletic grouping of the New World *Lontra* group.

Comparison with previous studies

As was discussed in Chapter 1, results from previous phylogenetic and systematic studies of the otters have produced numerous conflicting hypotheses. In a table modified from Chapter 1 (see Table 4-2 below), these hypotheses are presented with results from this chapter (including outgroup hypotheses). Results from data and tree combining methods and consensus techniques produced trees that only differed by one position shift. Regarding the phylogeny of the lutrines, the combined data/tree and consensus trees also did not differ significantly from the topology in the majority of analyses in Chapters 2 and 3. Excluding the anomalous results from the regression residual analysis (Figure 4-1 Tree 5 and Figures 3-6 to 3-10), the only position differences were either the placement or ambiguity of placement of *Lontra canadensis* and *Lutra lutra*. The increased resolution in the combining and consensus trees presented in this chapter are interesting but somewhat puzzling. Most likely there is some form of signal enhancement occurring, with the uncertainty previously seen in the placement of members of the genus *Lontra* and *Lutra* (see Figure 4-1) being resolved with a larger number of characters.

Systematic and taxonomic implications

Using the combined data and consensus results from this chapter, there are a number of undetermined systematic and taxonomic questions that can be addressed. Several of the systematic problems produce or contribute to taxonomic problems, both of which are discussed below.

Table 4-2. Systematic hypotheses of the Lutrinae (results from this study in bold). Phylogenetic hypothesis sources superscripted and listed below.

Phylogenetic Question	Hypotheses and Sources
Relationship between Old and New World river otters	1. Monophyletic ^{1, 2, 3} 2. Diphyletic ^{5, 7, 8, 9} 3. Monophyletic¹² or paraphyletic¹³
Relationship of African clawless and Asian small-clawed otters	1. Monophyletic ^{2, 5} 2. Diphyletic ^{1, 4, 6, 8, 9, 11} 3. Diphyletic^{12, 13}
Relationship of African clawless and Asian small-clawed otters to Old and New World otters	1. Allied with Eurasian otter ⁵ 2. African and New World otters allied, Asian small-clawed basal to sea and New World otters ⁹ 3. Allied with giant/smooth-coated/spot-necked otters ⁴ 4. Undetermined ¹ 5. African clawless allied with sea and giant otters, Asian small-clawed basal to all other otters^{12, 13}
Relationships of India smooth-coated otter	1. Allied with spot-necked otter ⁴ 2. Allied with giant and spot-necked otter ^{1, 9} 3. Allied as sister taxon to <i>Lutra</i> and <i>Lontra</i> ¹¹ 4. Allied with giant/sea/African clawless clade^{12, 13}
Relationships of spot-necked otter	1. Sister taxon to smooth-coated otter ⁴ 2. Allied with giant and smooth-coated otter clade ^{1, 8, 9} 3. Sister taxon to African clawless/Asian small-clawed/Eurasian otter clade ⁵ 3. Basal to all otters except Asian small-clawed^{12, 13}
Relationships of sea otter	1. Basal to all other forms ^{1, 4, 9, 11} 2. Allied with Eurasian/spot-necked/African clawless/Asian small-clawed otters ⁵ 3. Allied with giant otter^{12, 13}
Relationships of giant otter	1. Basal to all other forms ⁵ 2. Allied with <i>Lutra</i> /spot-necked/smooth-coated otters ^{1, 9} 3. Allied with <i>Lutra</i> / <i>Lontra</i> clade ⁶ 4. Allied with spot-necked/smooth-coated otters ⁴ 5. Allied with sea otter and African clawless otter^{12, 13}
Outgroup relationships	1. Melinae (badgers) ^{2, 11} 2. Mustelinae (weasels) ^{5, 10} 3. Mephitidae (skunks) ^{1, 3, 4, 6} 4. Musteline/Mephitine clade^{12, 13}

1. Bininda-Emonds et al. (1999); 2. Bryant et al. (1993); 3. Davis (1978); 4. Holmes (1987); 5. Koepfli and Wayne (1998); 6. Sokolov (1973); 7. van Zyll de Jong (1972); 8. van Zyll de Jong (1987); 9. van Zyll de Jong (1991); 10. Wayne et al. (1989); 11. Willemsen (1992); 12. All analyses in this chapter except tree combining method; 13. Tree combining method from this chapter.

New and Old World river otters

Prior to the work of van Zyll de Jong (1972; 1987; 1991), the New World and Old World river otter genera were synonymized under the generic appellation *Lutra*. After extensive analysis of cranial features, van Zyll de Jong proposed splitting the New World river otters into the genus *Lontra*, leaving Old World species under the genus *Lutra* (van Zyll de Jong 1972). This proposed change was not widely accepted at first, but has recently gained strong support (Kellnhauser 1983; Wozencraft 1993; Koepfli and Wayne 1998; Larivière 1998; 1999a; 1999b; Larivière and Walton 1998; Ceballos-G. 1999). The systematic and therefore taxonomic separation of *Lontra* and *Lutra* is strongly supported here with only a single analysis (Figure 4-2) using consensus methods indicating paraphyly.

African clawless and Asian small-clawed otters

African clawless otters (*Aonyx* spp.) and Asian small-clawed otters (*Amblonyx cinereus*) also have a history of systematic and taxonomic debate. Much of the debate regarding their synonymy is based on non-systematic taxonomy checklists (Osgood 1932; Ellerman and Morrison-Scott 1951; Coetzee 1977) with a single exception (Koepfli and Wayne 1998). Most recent systematic work favors their separation (Sokolov 1973; Holmes 1987; van Zyll de Jong 1987; 1991; Willemsen 1992; Wozencraft 1993; Bininda-Emonds et al. 1999). Koepfli and Wayne (1998) proposed the synonymy of *Aonyx* and *Amblonyx* and mistakenly attribute Willemsen (1992) with supporting the monophyly of *Aonyx* and *Amblonyx*. In fact, while Willemsen proposed a close relationship between

these taxa, he consistently maintained their generic separation (Pp. 9, 81, 89, Willemsen 1992). The systematic and taxonomic separation of *Aonyx* and *Amblonyx* is strongly and consistently supported here.

African clawless, Asian small-clawed and Old and New World otters

Among the evolutionary questions regarding the otters, few are more unsettled than the relationships of the African clawless (*Aonyx* spp) and Asian small-clawed otters (*Amblonyx*) to the Old (*Lutra*) and New World otters (*Lontra*). At least three hypotheses have been proposed: African and Asian forms are monophyletic and allied with Eurasian otter (Koepfli and Wayne 1998); African forms are allied with New World river otters, Asian small-clawed otter are basal to sea and New World otters (van Zyll de Jong 1987; 1991); Asian and African forms are closely related and allied with a giant/India smooth-coated/spot-necked otter clade (Holmes 1987).

Due to the ambiguous placement of these taxa in studies used as source trees, an MRP analysis of the Carnivora was unable to resolve these relationships (Bininda-Emonds et al. 1999). The results from this study do little to clarify the ambiguity, and in fact add two new hypotheses to the mix: strong and consistent placement of the African clawless otter with a clade composed of sea and giant otters, with similarly strong and consistent placement of the Asian small-clawed otter as basal to all other otters. While this does not provide systematic support to any previous hypotheses, it lends strong support for the valid systematic and taxonomic separation of the genus *Aonyx* from *Amblonyx*.

Indian smooth-coated otter

There are essentially two hypotheses regarding the placement of *Lutrogale*. One set of hypotheses places *Lutrogale* with the spot-necked otter (*Hydrictis maculicollis*) and the giant otter (*Pteronura brasiliensis*) as sister taxon to them (Holmes 1987; van Zyll de Jong 1987, 1991), or in an unresolved clade composed of all three taxa (Bininda-Emonds et al 1999). The second hypothesis links *Lutrogale* as the sister taxon to a *Lutra* and *Lontra* clade (Willemsen 1992). Koepfli and Wayne (1998) misinterpreted the results of van Zyll de Jong (1987, 1991) and consequently misrepresent the position of *Lutrogale* proposed there, placing it instead in a clade with *Lutra* (Figure 1, Koepfli and Wayne 1998). In this study, *Lutrogale* is consistently allied with a giant otter/sea otter/African clawless otter clade. As has already been discussed, this grouping is strongly correlated with size, but does show support for the proposed connection of *Lutrogale* with *Pteronura*. This systematic result also lends support for the taxonomic separation of *Lutrogale* as valid and distinct.

Spot-necked otter

There are several divergent hypotheses regarding the placement of the spot-necked otter (*Hydrictis maculicollis*) and correlated debate over the validity of the generic appellation. One hypothesis places *Hydrictis* as the sister taxon to *Lutrogale* (Holmes 1987), while another allies the spot-necked otter with a giant and smooth-coated otter clade (van Zyll de Jong 1987; 1991), or an unresolved clade containing all three (Bininda-Emonds et al. 1999). Koepfli and Wayne (1998) did not have access to samples of the

India smooth-coated otter (Koepfli, pers comm.) and placed *Hydrictis* as the sister taxon to an African clawless/Asian small clawed/Eurasian otter clade. This previously unreported result is interesting, and lends strong support for the validity of the generic separation of *Hydrictis* (although they withheld this taxonomic recommendation pending further analysis with more samples). In this study, *Hydrictis* was virtually invariant in its placement as basal to all otters except Asian small-clawed (a singular exception is the anomalous regression residuals analysis in Chapter 3). This placement also lends strong support for the taxonomic validity of the genus *Hydrictis*.

Sea otter

The numerous unique morphological and behavioral adaptations of the sea otter (*Enhydra lutris*) have generally placed it basal to all other forms (de Muizon 1982; Holmes 1987; van Zyll de Jong 1987; 1991; Willemsen 1992; Bryant et al. 1993; Bininda-Emonds et al. 1999). In a mitochondrial DNA (cytochrome *b* gene) analysis, Koepfli and Wayne (1998) placed the sea otter in a position basal to a Eurasian otter/spot-necked otter/African clawless otter/ Asian small-clawed otter clade. Results from this study consistently placed the sea otter in a clade with the second largest otter species, the giant otter (*Pteronura brasiliensis*). Linked as the sister taxon to this two-species clade was the African clawless otter. This size based arrangement was present in all analyses in this study, again with the exception of the maximum likelihood regression residuals analysis in Chapter 3.

Giant otter

Placement of the somewhat enigmatic giant otter (*Pteronura brasiliensis*) has been very contentious, and resulted in numerous phylogenetic hypotheses. Koepfli and Wayne (1998) place the giant otter basal to all other forms, another previously unreported result from their cytochrome *b* analysis. As discussed in the relevant sections above, the giant otter has also been allied with a *Lutra*/spot-necked/smooth-coated otter clade (van Zyll de Jong 1987; 1991; Bininda-Emonds et al. 1999), or as sister taxon to a spot-necked/smooth-coated otter clade (Holmes 1987). Another study (Sokolov 1973) allied *Pteronura* with a *Lutra*/*Lontra* clade, a result that has not been replicated or supported to date. Here, as previously reported, the giant otter was unfailingly linked in a clade with the sea otter, with the African clawless otter as sister taxon to these two largest species.

Outgroup relationships

One of the surprisingly consistent results from both the combining and consensus methods was the formation of a three taxon outgroup clade. The clade was composed of a musteline group containing the American mink (*Mustela vison*) and zorilla (*Ictonyx striatus*), with the mephitine striped skunk (*Mephitis mephitis*) linked as a sister taxon. Interestingly, this outgroup clade was only present in one of the six source trees used in this study, but was invariably recovered regardless of the combining or consensus method applied. The recovery of what is essentially a combination musteline/mephitine clade as an outgroup provides no support for the proposed elevation of the skunks to full familial

status (Dragoo and Honeycutt 1997) and instead points toward the validity of the subfamilial status Mephitinae within the Mustelidae.

There have been a number of other groups proposed as outgroups or sister taxa to the otters. Based on fossil evidence (Willemsen 1992) and cranial morphological analysis (Bryant et al. 1993), the badgers (Melinae) have been placed as sister taxa to the otters. Molecular analyses generally place the weasels (Mustelinae) as the sister group to the otters (Wayne et al 1989; Koepfli and Wayne 1998). In one molecular investigation (Dragoo and Honeycutt 1997), the zorilla in particular was placed as the closest relative to the otters, skunks were elevated to familial status, and mustelids were proposed to be paraphyletic. Dragoo and Honeycutt (1997) also found the otters (Lutrinae) to be the only monophyletic subfamily within the family Mustelidae.

Another frequently cited otter outgroup is the skunks (Mephitinae or Mephitidae). Most of the research leading to this conclusion is morphological in nature and dominated by cranial (Sokolov 1973; Holmes 1987; Wozencraft 1989) or basicranial character analyses (de Muizon 1982). Bininda-Emonds et al (1999) also placed the skunks as sister taxa to the otters using multiple analyses trees joined with MRP techniques. A recent analysis of fossil material opposes the elevation of skunks to familial level, but strongly supports the Mephitinae as the sister group to the Lutrinae (Wolsan 1999). The formation of a three taxon outgroup clade in this study cannot be used to determine whether otters are more closely related to weasels or skunks based on the clade topology. This clade does indicate that skunks may be more closely related to weasels than otters, contrary to the cranial-based morphological studies above.

Conclusions

Using the data provided from the previous chapters, various combining and consensus methods were used to construct a consensus phylogeny of the otters. Results were highly consistent regardless of the method employed, and support most of the previous findings from Chapters 2 and 3. This is not surprising given that the source trees are derived from analyses in those chapters. The surprising results were the persistent formation of a three taxon outgroup (which was present in only a single source tree) and the consistent recovery of previously poorly defined nodes concerning the placement of the genera *Lontra* and *Lutra*.

The debate regarding various methods used to combine different data for phylogenetic analyses falls essentially into two main camps, with an intermediate position between them. On one side are the "total evidence" advocates who espouse the direct combination of all available data (either trees or matrices) in a simultaneous analysis (Kluge 1989; Barrett et al. 1991; Eernisse and Kluge 1993; Jones et al. 1993; Kluge and Wolf 1993). Others promote the use of consensus techniques (Shaffer et al. 1991; Nelson 1993; Miyamoto and Fitch 1995). The intermediate group propose combining data only when they do not strongly support opposing results (so called "prior agreement") (Bull et al. 1993; de Quieroz 1993; de Quieroz et al. 1995).

Parallel to the debate surrounding whether or how to combine data or results is how to compare the trees themselves. Many phylogenetic studies that either produce or employ several trees in an analysis need to compare these trees for various levels of agreement. Historically many have used the Kishino-Hasegawa test (Hasegawa and

Kishino 1989; Kishino and Hasegawa 1989), a method which has recently been criticized for its widespread misapplication (Shimodaira and Hasegawa 1999; Goldman et al. 2000). Problems with the common application of the Kishino-Hasegawa test include: cases where tree topologies are specified *a posteriori*, one or more topologies are selected with reference to the same data used for testing, or one of the topologies is a maximum likelihood estimate of the phylogeny (the latter two situations being true for the cladograms used in this study).

Goldman et al. (2000) propose the use of the non-parametric Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999) or the parametric SOWH test (named for the authors that originally described it) (Swofford et al. 1996). Another parametric likelihood-based test for computing simultaneous significance levels of multiple topologies was recently proposed by Bar-Hen and Kishino (2000). A version of PHYLIP that implements the Shimodaira-Hasegawa test is currently being developed (Felsenstein pers. comm.) and future studies using this data set will employ and compare results from these various tests. The consistency of results here provide no reason to support one combining or consensus method over another.

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Literature Cited

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Appendices

Appendix 4-1. Nexus format Treefile constructed using the treefile combining method developed here. Topologies from the cladograms in Figure 4-1 were used to produce the Newick Standard format treefiles listed below the taxon labels. Tree numbers correspond to descriptions in the “Begin trees” block, taxon numbers used in the treefiles correspond to those in the “Translate” block. An invariant dummy matrix (not shown) was used to employ treefile inputs.

#NEXUS

Begin trees; [Treefile saved Tuesday, August 8, 2000 11:19]
 [tree PAUP_1: 12-segment consensus, tree PAUP_2: 6-segment consensus, tree PAUP_3:
 Gap A consensus, tree PAUP_4: Gap B tree, tree PAUP_5: Regression Residuals
 consensus, tree PAUP_6: Continuous trait data consensus]
 [! Data file = consmat, trees input to PAUP as user defined trees]

Translate

1 *Amblonyx cinereus*,
 2 *Aonyx capensis*,
 3 *Enhydra lutris*,
 4 *Hydrictis maculicollis*,
 5 *Lontra canadensis*,
 6 *Lontra longicaudis*,
 7 *Lutra lutra*,
 8 *Lutrogale perspicillata*,
 9 *Pteronura brasiliensis*,
 10 *Mustela vison*,
 11 *Mephitis mephitis*,
 12 *Ictonyx striatus*

;

tree PAUP_1 = [&R] (1,(((((((3,9),2),8),5),6,7),4)),10,11,12);
 tree PAUP_2 = [&R] (1,(((((((3,9),2),8),5),7),6),4)),(11,(10,12)));
 tree PAUP_3 = [&R] (((1,(((((((3,9),2),8),7),5,6),4)),(10,12))11);
 tree PAUP_4 = [&R] (((1,(((((((3,9),2),8),5),7),6),4)),(10,12))11);
 tree PAUP_5 = [&R] (((((1,4),9),10),(5,6)),((2,12),8),(3,11),7));
 tree PAUP_6 = [&R] (((1,(((((((3,9),2),8),7),5),6),4)),11),(10,12));

End;

Appendix 4-2. Matrices produced by the matrix representation function in PAUP* for the six cladograms used in this study.

Node numbers indicated to the left of each matrix. Taxon labels across columns correspond to taxa shown below.

Tree 1 (Elements 1-19)												Tree 2 (Elements 20-41)												Tree 3 (Elements 42-62)												Tree 4 (Elements 63-84)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
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A=*Amblyonyx cinereus*; B=*Aonyx capensis*; C=*Enhydra lutris*; D=*Hydrictis maculicollis*; E=*Lontra canadensis*; F=*L. longicaudis*; G=*Lutra lutra*; H=*Lutrogale perspicillata*; I=*Pteromura brasiliensis*; J=*Mustela vison*; K=*Mephitis mephitis*; L=*Ictonyx striatus*

Appendix 4-2. Continued.

Tree 5 (Elements 85-107)

Regression residuals data

Taxa	ABCD	E	FGH	I	J	K	L
23	1	1	1	1	1	1	1
17	1	0	0	1	1	0	0
15	1	0	0	1	0	0	1
14	1	0	0	1	0	0	0
13	1	0	0	1	0	0	0
1	1	0	0	0	0	0	0
4	0	0	0	1	0	0	0
9	0	0	0	0	0	0	1
10	0	0	0	0	0	0	1
16	0	0	0	1	1	0	0
5	0	0	0	1	0	0	0
6	0	0	0	0	1	0	0
22	0	1	1	0	0	1	1
19	0	1	0	0	0	1	0
18	0	1	0	0	0	0	0
2	0	1	0	0	0	0	0
12	0	0	0	0	0	0	0
8	0	0	0	0	0	1	0
21	0	0	1	0	0	0	1
20	0	0	1	0	0	0	0
3	0	0	1	0	0	0	0
11	0	0	0	0	0	0	1
7	0	0	0	0	1	0	0

A=*Amblonyx cinereus*; B=*Aonyx capensis*; C=*Enhydra lutris*; D=*Hydrictis maculicollis*; E=*Lontra canadensis*; F=*L. longicaudis*; G=*Lutra lutra*; H=*Lutrogale perspicillata*; I=*Pteromura brasiliensis*; J=*Mustela vison*; K=*Mephitis mephitis*; L=*Ictonyx striatus*

Tree 6 (Elements 108-130)

Continuous trait data

	ABCD	E	FGH	I	J	K	L
23	1	1	1	1	1	1	1
21	1	1	1	1	1	0	1
20	1	1	1	1	1	1	0
1	1	0	0	0	0	0	0
19	0	1	1	1	1	1	0
18	0	1	0	1	1	1	0
17	0	1	0	1	1	1	0
16	0	1	1	0	0	1	1
15	0	1	1	0	0	1	1
14	0	1	1	0	0	0	1
2	0	1	0	0	0	0	0
13	0	0	1	0	0	0	1
3	0	0	1	0	0	0	0
9	0	0	0	0	0	1	0
8	0	0	0	0	0	1	0
7	0	0	0	0	1	0	0
5	0	0	0	1	0	0	0
6	0	0	0	0	1	0	0
4	0	0	0	1	0	0	0
11	0	0	0	0	0	0	1
22	0	0	0	0	0	0	1
10	0	0	0	0	0	0	1
12	0	0	0	0	0	0	0

Appendix 4-3. Total evidence matrix for coded continuous parsimony data. 352 total characters.

[illegible]

Aonyx capensis CCCCCCDBCCECCDCCDCCCCCCCCCCCCCCCDDCCCCDDCCCC
CCDCCCCCCCCCCCCCCCCCCCCCCCCCBDCCEEFGFEFFEDFFFF
GFEGFFFFFEEFGFFFGFFGFEFFFEFFFEFFFEFFFEFFFE
EGEEFFFDGEEDDDDECCCEEDDFDEEDEDDEEEDDDDEEFDDDEEEDD
DEEEDDDDEDEEDDDDEDDDDCEEDDDDEDDDDDDCJJKKIKKLGLLJ
KMJJMLKKJJLLMKIJJILMMKLLMCKKJKMMMIIJJLKKLJLKJLJ
IJJLJJJJKIKII

Enhydra lutris CDDDDDDDDDEDDDDCCDDDDDDDDCCDDDDDDCCDDDDCCDC
CDDCCCDCCDDCCDCCCDDDDCCDDDDCCDDDDDEGGHHFFGHIG
GHGFHFGGFGFHGGFGGFGGFGGFGGFGGFGGFGGFGGFGGF
FEGHEGGGHGGHGGEFFFFFEFFFEFFFEFFFEFFFEFFFEFF
FEEEFEEEEFEEDDEFEEDDFEDEFEEFFFEFFFKMMMMMMMM
MMMMLMMMMMMLLMJMMMMMMLLLMMMMLLMMMKLLMLMIKMML
MLLKMLKLJMJJMMMLMLMMMMMM

[illegible]

Lontra canadensis BBBCBBB BBBCBBB CCCBCCB CBBCBBBCB BBBCBBB BBBCB
BBBCB CCCB BBBCB BBBCB CCDCB CCBBB BBBCB BBBCB BBBCB BBBCB
DDFCDEEE DECEFC DDDEDE DEDEDE DEDEDE DEDEDE DEDEDE DEDEDE
DDEGEFE GEFCCG DCDCDC CCBBDC CDCCDD DDCCDD CCDDCC DDCCDD
CCDDCC CCDDCC DDCCDD CCDCDD CCCEED DECEDE CBDCCH HHHH
HFEKHHIGH JFHHIIG JHHIIG HHGHHG HHGHHG HHGHHG HHGHHG
GLLJKJK JJKJGKFG

[illegible][illegible][illegible]

[illegible]

Mustela vison

Mephitis mephitis A A A A B A B A B A A B A A A A A A B A A B B A B A B B A A A A A A
A A A A A B A B A A A A A A A A A A A A A A A D A A B B B C B C B D A
B C B B C C B B B A B B C B C C C B B B B B B B B B C A B C B B A C B B B C A
A B B B A C A A B A B B B B A G A A A B B B A B B A B A B B B B B B B A B A B B A C B A B B C B B
B B B B B B B A A B B A B A B B B B A B A B B C A A B B B A A C A A A A B B A E A A C D D E
E D E D E C F C E E E F F D D E E B D D E D G E D E E G E E F D E E D D E C E E D C E D E E C F E D D
C E D D E G B A E D D B B F C B C A D D D D A K B A

ICTONYX STRIATUS
 AAAAAAAAAABAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAABAAAAAAAAAAAC
 AAAAAAAAAAAAAAAAAABAAAAAAAAAAAAAAAAAA
 AAAAAAAAAABABEAAAAAAAAABAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABCAAAAA
 CAAAAAAAACEAAAAAAAAAAAAAAAAABBAABAAAAA
 AAABAAAAABAAAAACBABDAACDHBAAABBBB

Appendix 4-4. Maximum likelihood total evidence matrix produced by combining continuous trait data matrix and regression residuals matrix. This matrix is in PHYLIP Maximum Likelihood input file format. The number of taxa (12) and characters (175) are shown at the beginning of the file. Taxon names are in the standard 10 character format required by PHYLIP and described as follows: AMBLONYXCI = *Amblonyx cinereus*; AONYXCAPEN = *Aonyx capensis*; ENHYDRALUT = *Enhydra lutris hydrictism*; LUTRALUTRA = *Lutra lutra*; LERSPICIL = *Lutrogale canadensis*; LLONGICAUD = *Lontra longicaudis*; MUSTELAVIS = *Mustela vison*; MEPHITISME = *Mephitis mephitis*; PTERONURAB = *Pteromura brasiliensis*; MUSTELAVIS = *Mustela vison*; MEPHITISME = *Mephitis mephitis*; ICTONYXSTR = *Ictonyx striatus*

12 175

AMBLONYXCI 6.301 1.879 1.856 1.222 0.973 1.002 1.157 1.063 0.919 0.484 0.553 0.874 1.834 1.274 1.208 1.644 0.949
0.762 0.612 1.818 1.137 1.226 0.887 1.179 0.736 0.213 1.067 0.995 0.577 0.828 0.932 0.581 0.821 0.577 0.359 1.038 0.829
0.911 0.847 0.701 1.732 1.732 0.852 0.71 0.921 0.81 1.827 1.778 1.685 0.924 0.766 0.957 0.962 0.914 0.599 0.849 0.705 0.734
0.818 0.51 0.799 0.472 0.646 0.77 0.577 0.608 0.548 0.437 0.504 0.368 0.759 0.285 0.747 0.921 0.493 0.873 0.908 0.088 1.048
0.629 0.98 1.529 1.642 1.508 1.274 1.222 0.617 1.167 -0.0096 -0.0050 -0.0219 -0.0457 -0.0216 -0.00016 -0.05324 -0.0475 -0.056 -0.205
1.00 -0.00033 -0.0167 -0.0298 0.0093 -0.0343 -0.00072 0.632 -0.0081 -0.0626 -0.0692 -0.03630 -0.0631 -0.0729 0.0251 -0.0923 -0.07900 -0.0655 -
0.0538 -0.077 -0.0119 -0.0617 -0.041 -0.017 -0.0802 -0.0830 -0.0110 -0.0840 -0.0543 0.0317 0.0428 -0.06079 -0.0538 -0.1024 -0.0649 0.0081 -
0.00910 0.0535 -0.0720 -0.0485 -0.0656 -0.0607 -0.0951 -0.027200 -0.0392 -0.0598 -0.0789 -0.0875 -0.0868 -0.0573 -0.0498 -0.0457 -0.0755 -0.0673
-0.0629 -0.114 -0.02523 0.040 -0.0548 -0.0305 -0.0152 -0.0338 -0.081 -0.0256 -0.0029 0.0333 -0.0998 0.072 -0.032 -0.0654 -0.0258 -0.0015 -0.0059 -
0.0875 -0.0296 -0.04606 -0.0846

AONYXCAPEN 6.733 2.037 2.011 1.422 1.173 1.213 1.337 1.313 1.167 0.655 0.774 1.099 2.013 1.484 1.423 1.82 1.159 0.915
0.846 2.011 1.37 1.466 1.107 1.413 0.996 0.535 1.402 1.251 0.767 1.054 1.125 0.732 1.093 0.829 0.644 1.275 1.069 1.112 1.124
0.986 1.924 1.914 1.089 0.928 1.2 1.078 2.031 1.998 1.876 1.123 0.945 1.21 1.232 1.114 0.806 1.138 0.979 0.998 1.123 0.755
1.07 0.758 0.821 1.029 0.815 0.848 0.89 0.616 0.651 0.593 0.972 0.523 0.905 1.244 0.729 1.044 1.064 0.348 1.343 0.896 1.29
1.708 1.828 1.765 1.498 1.393 0.82 1.402 0.011 0.0095 -0.0120 -0.0482 0.0090 0.00558 -0.00941 0.0200 -0.031 -0.067 -0.018 0.050354 0.0115
-0.0088 0.840 0.0036 -0.01879 0.930 0.369 -0.0027 -0.0053 -0.02554 -0.0302 0.0237 0.138 0.0736 0.00900 -0.0725 -0.0219 -0.074 0.0153 0.0360
0.054 0.99 -0.0124 0.283 -0.0053 0.0382 0.0560 0.0826 0.0815 0.00442 -0.0166 0.0029 0.0180 0.0639 0.06368 0.0841 -0.0763 -0.0212 -0.0060 0.0082
-0.0449 0.000097 0.704 0.018 -0.0044 0.0496 -0.0232 0.0422 0.0566 -0.0665 -0.0248 -0.0183 0.0017 0.038 -0.05013 -0.0558 0.0031 0.0073 -0.0011
-0.0282 0.056 0.0246 -0.0404 -0.0408 -0.0567 -0.024 -0.037 -0.0028 -0.0902 -0.0795 0.0595 -0.0239 -0.0046 0.00069 -0.0215

Appendix 4-4. Continued.

ENHYDRALUT 6.83 2.153 2.108 1.55 1.409 1.337 1.45 1.447 1.239 0.845 1.048 1.221 2.055 1.624 1.556 1.778 1.298 1.106
0.822 2.054 1.465 1.541 1.261 1.579 1.077 0.626 1.357 1.29 1.009 1.196 1.125 0.903 1.141 0.822 0.617 1.393 1.121 1.158 1.141
0.959 1.931 1.92 1.148 1.02 1.277 1.117 2.052 2.024 1.903 1.228 1.029 1.275 1.316 1.184 0.951 1.038 1.022 1.104 1.143 0.895
1.074 0.818 0.957 1.115 0.844 0.861 0.956 0.72 0.761 0.652 0.984 0.732 1.137 1.17 0.776 1.202 1.254 0.482 1.498 1.053 1.485
1.926 2.028 1.904 1.681 1.585 1.001 1.621 0.840 0.749 0.734 1.424 0.925 0.7946 0.7830 0.515 1.26 1.88 0.27 0.63461 1.1107
.0807 0.192 1.039 1.3380 0.231 0.467 0.534 0.301 0.8148 0.907 0.680 0.300 -.0094 0.1028 1.252 0.765 0.63 -.0219 0.448 0.12
.034 0.676 0.514 -.0032 0.204 -.0103 0.579 0.553 0.2484 0.348 0.409 0.155 0.516 0.5662 0.751 -.0169 0.287 0.156 0.471 -.0086
.104747 -.0699 -.0029 0.590 0.319 0.761 0.077 0.763 0.256 0.144 -.0318 -.0247 0.61 0.0809 0.077 0.246 -.0201 1.576 1.696 -
.059 0.299 0.708 0.976 0.286 0.43 0.59 1.367 0.731 0.612 1.555 1.230 1.546 1.4660 1.589

HYDRICTISM 6.438 1.87 1.84 1.278 1.09 1.048 1.178 1.169 1.014 0.565 0.679 0.99 1.78 1.28 1.285 1.574 0.975 0.697 0.501
1.805 1.228 1.326 0.989 1.285 0.831 0.29 1.185 1.114 0.619 0.897 0.97 0.676 0.877 0.621 0.391 1.156 0.904 0.966 0.944 0.783
1.655 1.649 0.941 0.795 1.028 0.908 1.797 1.768 1.605 1.081 0.798 1.058 1.055 1.027 0.656 0.921 0.763 0.801 0.954 0.572
0.888 0.53 0.68 0.893 0.681 0.706 0.686 0.527 0.531 0.471 0.67 0.225 0.639 0.986 0.454 0.899 0.867 0.239 1.157 0.691 1.634
1.738 1.466 1.401 1.109 0.723 1.309 -.0653 -.0655 -.0262 0.071 -.0328 -.03443 -.01262 -.0097 -.021 -.105 1.07 -.094775 -.0683
-.0143 -.0929 -.0629 -.11997 -.1126 -.0680 -.0265 -.0250 -.00065 -.0209 -.0297 -.0036 -.0279 -.01328 -.0860 -.0464 -.029 -
0.265 -.0610 -.046 -.038 -.0159 -.0488 -.0179 -.0361 -.0277 -.0901 -.0856 -.02627 -.0262 -.0505 -.0256 -.0689 -.06579 -.0774
0.206 -.0646 -.0259 -.0315 -.0296 -.027189 -.0241 -.0692 -.0720 -.0048 -.0823 -.0227 -.0488 -.0738 -.0186 -.0233 -.0205 -.036
.00010 -.0346 -.0048 -.1751 -.1462 -.1901 -.075 -.1235 -.0430 -.0806 -.0176 0.57 -.056 -.1238 0.020 0.108 -.1087 -.0114 -.1889
.01039 .0029

LCANADENSI 6.477 1.965 1.938 1.329 1.133 1.076 1.21 1.214 1.02 0.557 0.708 1.029 1.876 1.362 1.332 1.657 1.037 0.926
0.492 1.882 1.291 1.387 1.059 1.367 0.829 0.362 1.144 1.139 0.757 0.98 0.974 0.781 0.955 0.642 0.456 1.193 0.943 1.032 0.939
0.82 1.729 1.718 0.988 0.832 1.066 0.934 1.869 1.84 1.667 1.119 0.848 1.129 1.121 1.134 0.702 0.944 0.91 0.888 0.971 0.653
0.931 0.574 0.836 0.954 0.792 0.811 0.666 0.496 0.529 0.461 0.906 0.452 0.85 1.196 0.743 1.03 1.111 0.359 1.232 0.928 1.361
1.725 1.815 1.555 1.364 1.386 0.704 1.323 0.164 0.198 0.077 0.318 -.0211 -.01816 0.1376 -.0200 -.043 -.084 1.15 -.010392 -
.0027 0.151 -.0190 -.0164 0.9358 -.1400 -.0044 0.209 0.201 0.5046 0.430 -.0465 0.383 -.0842 -.00344 0.342 0.191 0.62 -.0374
.0013 -.040 0.11 0.059 -.0214 0.304 -.0551 -.0065 -.0288 -.0296 0.0522 -.0055 -.0282 -.0163 -.0103 -.00708 -.0299 0.402 -.0283
0.276 0.164 0.638 0.02587 -.0173 0.586 -.0021 -.0029 -.0177 0.049 -.0210 0.645 0.236 0.707 0.687 -.073 -.04930 -.0553 -.0299
0.451 0.606 0.071 1.19 1.487 0.692 1.426 0.829 0.97 1.56 2.148 0.710 0.639 -.0370 -.0629 0.749 -.02272 0.014

Appendix 4-4. Continued.

LLONGICAUD 6.469 1.918 1.887 1.305 1.071 1.065 1.197 1.197 1.007 0.565 0.754 0.949 1.859 1.338 1.302 1.642 1.04 0.776
 0.638 1.885 1.28 1.376 1.043 1.351 0.927 0.344 1.258 1.163 0.71 0.961 1.003 0.761 0.962 0.701 0.428 1.196 0.948 1.034 0.982
 0.846 1.726 1.716 1.001 0.839 1.099 0.937 1.853 1.809 1.66 1.086 0.834 1.104 1.121 1.079 0.701 0.963 0.9 0.903 0.949 0.652
 0.919 0.562 0.776 0.923 0.7 0.746 0.714 0.498 0.519 0.479 0.842 0.37 0.834 1.137 0.572 0.971 0.969 0.265 1.227 0.79 1.148
 1.705 1.8 1.581 1.471 1.179 0.746 1.308 -.0279 -.0286 -.0128 -.0264 -.0288 -.02793 .00058 -.0297 -.032 -.036 .042 -.025009 -
 .0234 -.0113 -.0321 -.0102 -.05325 .0098 .0014 .0131 .0123 .03833 .0307 .0546 .0265 .0329 .02367 -.0091 .0037 .045 -.0054
 .0115 .022 -.014 .0120 -.0140 .0360 -.0092 .0228 -.0292 -.0289 .02140 .0049 .0081 -.0099 -.0236 -.03535 -.0339 .0110 -.0395
 .0062 .0201 .0116 .004915 .0050 .0526 .0164 -.0218 -.0153 -.0040 -.0297 .0082 -.0035 -.0178 .0069 -.022 -.04353 -.0614 -.0088
 -.0156 -.0172 -.0061 .063 -.0188 .0140 .0048 -.0071 .099 .023 .0064 .0555 .0538 -.0074 .0471 -.1294 .02217 -.0104
LUTRALUTRA 6.454 1.939 1.913 1.307 1.072 1.085 1.223 1.178 1.05 0.535 0.855 0.822 1.891 1.343 1.295 1.682 1.038 0.78
 0.579 1.885 1.276 1.393 1.028 1.356 0.891 0.315 1.259 1.155 0.753 0.968 0.999 0.748 0.954 0.688 0.392 1.199 0.972 1.006
 1.023 0.837 1.744 1.737 1.009 0.868 1.069 0.937 1.89 1.857 1.706 1.139 0.902 1.099 1.1 1.097 0.739 0.975 0.784 0.919 0.982
 0.696 0.926 0.575 0.8 0.961 0.737 0.757 0.807 0.618 0.639 0.508 0.888 0.335 0.886 0.996 0.571 0.995 0.975 0.335 1.242 0.645
 1.005 1.653 1.755 1.587 1.46 1.347 0.685 1.276 -.0018 .0023 -.0042 -.0184 -.0025 .00412 -.01126 .0196 -.057 .068 -.073
 .011459 -.0121 -.0115 .0114 -.0063 -.04331 -.0421 .0065 .0151 .0354 .03060 .0427 .0242 .0091 .0398 .02150 .0407 .0175 .037 -
 .0036 .0095 .015 -.044 .0209 .0145 .0148 .0372 .0198 -.0063 -.0029 .03537 .0401 -.0159 -.0035 .0186 .01776 .0177 .0710 .0338
 .0079 .0061 .0348 .049155 .0233 -.0561 .0390 .0170 .0350 .0090 -.0104 .0390 .0417 .0257 .0240 .078 .08355 .0658 .0260 .0364
 -.0445 .0512 -.072 -.0134 .0453 .0188 .0704 .128 -.112 -.1280 .0120 .0180 .0052 .0417 .0437 -.03340 -.0365
LPERSPICIL 6.586 2.009 1.976 1.382 1.191 1.172 1.298 1.265 1.121 0.695 0.791 1.051 1.938 1.413 1.373 1.732 1.1 0.874
 0.775 1.938 1.274 1.407 1.067 1.349 0.88 0.403 1.299 1.189 0.714 0.967 1.074 0.739 0.988 0.737 0.504 1.218 0.983 1.034 1.048
 0.849 1.785 1.777 1.023 0.892 1.134 0.999 1.912 1.884 1.733 1.122 0.95 1.141 1.148 1.15 0.731 0.995 0.898 0.925 0.992 0.748
 0.949 0.646 0.823 1.024 0.794 0.778 0.812 0.663 0.719 0.507 0.957 0.446 0.906 1.213 0.595 1.037 1.058 0.325 1.314 0.764
 1.175 1.684 1.822 1.62 1.452 1.37 0.769 1.305 .0232 .0223 .0127 .0387 .0294 .02588 .01275 .0354 .059 -.022 .051 .019141
 .0024 .0072 .0304 .0031 -.00158 .0915 .0143 -.0398 -.0044 .00566 -.0258 -.0367 -.0048 .0281 .00417 -.0585 -.0429 -.017 .0207
 -.0097 .016 .017 -.0118 -.0139 -.0169 .0149 -.0216 -.0084 -.0067 -.00313 .0089 -.0040 .0020 -.0047 -.00023 -.0043 -.0081 .0354
 -.0092 -.0074 .0420 -.013754 -.0116 -.0069 -.0129 -.0243 .0315 -.0204 .0057 .0022 .0410 .0250 -.0086 .025 .06625 .0825 -.0260
 .0519 -.0019 .0247 .089 -.0461 .0236 .0315 -.0059 .080 -.077 -.0336 -.0314 .0043 -.0203 -.0153 .0221 .00286 -.0600

Appendix 4-4. Continued.

PTERONURAB 6.999 2.081 2.039 1.511 1.248 1.26 1.385 1.403 1.209 0.758 0.955 1.147 1.994 1.535 1.502 1.76 1.222 0.992
 0.85 2.022 1.468 1.56 1.162 1.497 1.042 0.648 1.418 1.34 0.966 1.19 1.247 0.871 1.147 0.847 0.579 1.364 1.128 1.219 1.187
 1.03 1.886 1.873 1.17 1.043 1.351 1.198 2.006 1.975 1.806 1.352 1.093 1.345 1.316 1.253 0.825 1.213 1.136 1.111 1.163 0.895
 1.132 0.779 1.023 1.166 0.968 0.958 0.936 0.798 0.871 0.715 1.113 0.605 0.968 1.272 0.802 1.125 1.124 0.54 1.366 1.131 1.383
 1.901 1.99 1.736 1.574 1.457 0.822 1.507 -.0457 -.0490 -.0400 -.0979 -.0550 -.05371 -.04635 -.0491 -.018 .062 -.181 -.047879 -
 .0495 -.0492 -.0384 -.0394 -.04713 -.0288 -.0432 -.0113 -.0198 -.09937 -.0700 -.0309 -.0784 -.0146 -.00544 .0051 -.0054 -.027
 .0353 -.0174 -.024 -.070 -.0277 .0081 -.0186 .0059 -.0077 -.0423 -.0478 -.02036 -.0129 .0469 .0241 -.0524 -.04997 -.0847 .0275
 .0334 .0100 -.0316 .0018 -.091554 .0349 .0280 -.0081 -.0138 .0051 -.0013 -.0330 .0150 -.0161 .0183 .0036 -.034 .00632 .0368
 .0222 .0403 -.0570 -.0590 -.030 -.0168 -.0878 -.1224 .0018 -.242 .030 -.0621 -.0471 -.0800 -.0875 -.0467 -.0305 -.09352 -.0223
MUSTELAVIS 5.9 1.744 1.721 1.061 0.816 0.896 0.97 0.907 0.793 0.379 0.775 0.427 1.704 1.094 1.052 1.534 0.799 0.582
 0.382 1.682 1.029 1.114 0.767 1.046 0.736 -.0296 0.988 0.916 0.447 0.676 0.77 0.556 0.674 0.383 0.185 0.951 0.767 0.681
 0.798 0.56 1.531 1.524 0.733 0.586 0.832 0.696 1.646 1.621 1.438 0.853 0.657 0.85 0.841 0.865 0.418 0.698 0.585 0.625 0.747
 0.414 0.685 0.33 0.512 0.673 0.444 0.505 0.463 0.314 0.317 0.227 0.599 0.051 0.771 0.77 0.353 0.656 0.581 -.0107 0.702 0.356
 0.844 1.421 1.487 1.298 1.22 0.768 0.504 1.129 -.0078 -.0095 -.0065 -.0147 .0398 -.02542 -.01787 -.0060 -.026 .095 -.029 -
 .010521 -.0279 -.0058 -.0067 -.0245 -.02193 .0227 -.0066 -.0099 -.0177 .03793 -.0095 .0788 -.1745 -.0143 -.00206 -.0126 -
 .0256 .035 -.0200 -.0470 -.089 -.034 -.0101 -.0255 -.0596 .0107 -.0331 -.0384 -.0321 -.02032 -.0101 -.0301 -.0072 -.0354 -
 .02944 -.0446 .0458 -.0166 .0070 .0050 -.0050 -.041393 -.0236 .0173 -.0119 -.0027 -.0143 -.0121 -.0251 .0020 .0208 -.0249 -
 .0030 -.022 .04103 .0090 -.0407 -.0278 -.0413 .1317 -.060 .0069 -.0264 -.0801 -.0934 .090 -.053 .0283 .0922 .0885 -.0381 .0074
 -.3480 -.01402 .0369
MEPHITISME 5.94 1.801 1.777 1.142 0.943 0.888 1.082 1 0.89 0.455 0.748 0.427 1.782 1.23 1.132 1.594 0.955 0.742 0.419
 1.731 1.119 1.209 0.674 1.126 0.675 0.009 1.095 0.969 0.646 0.82 0.847 0.579 0.798 0.586 0.301 1.049 0.886 0.847 0.857 0.673
 1.648 1.621 0.808 0.663 0.969 0.765 1.751 1.726 1.551 0.821 0.78 0.899 0.919 0.947 0.551 0.768 0.64 0.76 0.83 0.511 0.771
 0.395 0.607 0.687 0.534 0.556 0.638 0.257 0.255 0.35 0.719 0.206 0.609 0.797 0.464 0.682 0.613 0 0.491 0.879 1.346 1.405
 1.411 1.212 1.428 0.522 1.108 .0356 .0335 .0569 .0935 .0151 .07045 .05604 .0743 .037 .060 -.060 .055564 .0913 .0562 .0439
 .1156 .12223 .0408 .0286 .0640 .0610 -.07445 .0519 .0027 .0816 .0770 .03539 .1682 .1004 .044 .0416 .0609 .100 .066 .0723
 .0816 .0883 .0553 .0638 .0656 .0516 .03877 .0502 .0908 .0447 .0559 .06193 .0535 -.0051 .0924 .0381 .0644 .0631 .074968
 .0298 .0526 .1055 .0647 .0659 .0580 .0233 .0788 .0156 .0476 .0318 .136 -.03485 -.0721 .0668 .0759 .0930 -.0444 -.050 .1007 -
 .0197 -.0694 -.0065 -.649 .057 .0404 -.0054 -.0179 .0571 -.0154 .2985 -.01049 -.0210

Appendix 4-4. Continued.

ICTONYXSTR 5.799 1.715 1.693 0.996 0.721 0.79 0.929 0.866 0.718 0.432 0.706 0.294 1.673 1.064 0.984 1.508 0.751 0.521
 0.291 1.65 0.985 1.073 0.672 0.969 0.5837 -0.1627 0.954 0.878 0.344 0.635 0.765 0.423 0.713 0.455 0.169 0.901 0.785 0.658
 0.753 0.551 1.542 1.525 0.714 0.54 0.863 0.683 1.644 1.613 1.436 0.722 0.633 0.792 0.753 0.801 0.382 0.702 0.502 0.563 0.705
 0.412 0.653 0.369 0.415 0.585 0.421 0.45 0.404 0.223 0.333 0.251 0.598 0.053 0.581 0.885 0.236 0.631 0.672 0.039 0.77 0.387
 0.747 1.166 1.215 1.319 1.219 1.219 0.519 1.108 -.0023 -.0047 -.0271 -.0624 -.0240 -.02568 -.01067 -.0388 .061 .046 -.082 -
 .011437 -.0154 -.0285 -.0091 -.0322 -.04293 -.0205 -.0040 -.0135 -.0175 -.00816 -.0395 -.0353 .0367 -.0088 -.00078 -.0695 -
 .0212 -.063 .0137 .0328 .020 -.011 -.0205 .0226 -.0370 .0018 -.0012 .0056 .0024 .00084 -.0138 .0415 .0231 -.0027 -.00302 -
 .0091 -.0377 -.0051 -.0058 -.0360 -.0340 -.035379 .0224 -.0160 -.0296 -.0055 .0261 -.0040 .0559 -.0493 -.0185 -.0037 -.0169 -
 .036 -.00230 .0734 .0223 .0121 .0131 -.0227 .099 -.0667 -.0026 .0647 .1033 .249 .042 -.0109 -.1059 -.1217 .0277 .0439 .1371
 .03751 .0561

Appendix 4-5. Nexus file matrix representation of the combined parsimony and ML total evidence analyses.

#NEXUS

Begin data;

Dimensions ntax = 12 nchar = 21;

Format transpose;

Taxlabels

AMB
AON
ENH
HYD
LCAN
LLON
LLUT
LPERS
PTER
MINK
SKNK
ZOR;

Format transpose; Matrix

[Tree 1 Parsimony Total Evidence]	[Tree 2 Maximum likelihood Total Evidence]
node_22 011111111111	node_22 011111111111
node_19 011111111000	node_19 011111111000
node_18 011011111000	node_18 011011111000
node_17 011010111000	node_17 011010111000
node_16 011010011000	node_16 011000111000
node_15 011000011000	node_15 011000011000
node_14 011000001000	node_14 011000001000
node_2 010000000000	node_2 010000000000
node_13 001000001000	node_13 001000001000
node_3 001000000000	node_3 001000000000
node_9 000000001000	node_9 000000001000
node_8 000000010000	node_8 000000010000
node_5 000010000000	node_7 000000100000
node_7 000000100000	node_5 000010000000
node_6 000001000000	node_6 000001000000
node_4 000100000000	node_4 000100000000
node_21 000000000111	node_21 000000000111
node_20 000000000101	node_20 000000000101
node_10 000000000100	node_10 000000000100
node_12 000000000001	node_12 000000000001
node_11 000000000010	node_11 000000000010;
End;	

Part 5

Overview and Conclusions

Introduction

Postcranial morphological trait data were employed in phylogenetic analyses of the otters using various methods. One of the primary differences was how (or if) the data were treated prior to analysis. Data were coded with several techniques that convert continuous trait data into discrete characters for analysis using parsimony approaches. Some of these discretizing methods (such as segment coding) required somewhat subjective decisions about how the data were divided. Other methods were based on more objective statistical separations and partitioning (gap coding). Other approaches required virtually no manipulation of the data (maximum likelihood).

Analyses of the data were performed using Maximum Parsimony Reconstruction (MPR) as applied by PAUP* and Maximum Likelihood (ML) as applied by PHYLIP. These two approaches have different evolutionary assumptions. The primary reason both were used is that current MPR approaches cannot use uncoded continuous data as input, whereas PHYLIP can. Using more than one approach also provided a more vigorous comparative analysis. Several analyses were performed with each approach, allowing a comparison of results within each approach (MPR and ML), as well as between these approaches. With only a few exceptions, the results were consistent, robust, well supported, and somewhat surprising in their simplicity.

Chapter 2 results

In Chapter 2, four discretizing methods were used on the postcranial continuous trait data set and analyzed using PAUP* under MPR assumptions of evolution. The overall results were similar, despite differences in the number of divisions produced by the various coding regimes and the resulting matrices. The predominant result was a grouping of the otter species by size. The largest otters were placed together in persistent sister taxa relationships, with the smallest species reliably placed as basal to the other species. Intermediate species were generally consistent in their placement with only occasional ambiguity between those species extremely similar in size. This basic size pattern was recovered in virtually all trees, regardless of coding methodology.

Virtually all measures of confidence were very high, indicating that the trees were robust and well supported. Bootstrap support analyses corroborated the high indices. As would be expected, only those few nodes that were somewhat evanescent had lower measures of bootstrap support, which was moderate at its worst. The overall high support measures were consistent, regardless of the measure used (Consistency Indices > 0.85 (most above 0.90), Retention Indices > 0.77 (most above 0.84), and Rescaled Consistency Indices above 0.73).

These persuasive results propose several previously unreported phylogenetic hypotheses. Size related phylogenies have been reported in the Felidae, whether the data were molecular, biochemical, or morphological. There is, however, some biogeographical corroboration of this arrangement in felids, a situation not seen in the otters. It would take a number of convoluted and improbable biogeographical hypotheses to support the size based groupings produced in Chapter 2.

Chapter 3 results

In Chapter 3, an attempt was made to reduce or remove the size related component that appeared to dominate the results in Chapter 2. Data were analyzed without coding, and as regression residuals from a regression of character traits on body size. To perform these analyses, a different systematic program (PHYLIP) was used. This allowed for a comparison of different evolutionary assumptions as applied to the same source data since PHYLIP uses a maximum likelihood (ML) approach to evolution and PAUP* uses maximum parsimony reconstruction (MPR).

The straight uncoded continuous trait data analysis produced size related phylogenies virtually identical to those from the Chapter 2 analyses. One difference was the assignment and placement of the outgroup species, an artifact of the outgroup assignment abilities of PHYLIP. The numerical data input would tend to produce this result since those species closer in size would tend to cluster, just as in the coded data set. The regression residuals produced vastly different results than the continuous or coded analyses. Only one arrangement (the only one recovered in all of the analysis runs) seemed informative; the linking of the neotropical otter (*Lontra longicaudis*) with the North American river otter (*Lontra canadensis*). Most of these results produced relationships that appeared to have absolutely no basis in evolutionary or even biological reality. One of the most astounding results was the placement of the sea otter (*Enhydra lutris*) as the sister taxon to the striped skunk (*Mephitis mephitis*). It would be hard to imagine a more outlandish or biologically implausible arrangement. The size component had indeed been removed.

Chapter 4 results

In Chapter 4, results and data from the previous two chapters were used to produce consensus phylogenies. Several methods of combining data and phylogenies were used, and the results were compared before an overall consensus phylogeny was produced. A new technique of producing data matrices from trees incorporated Newick Standard format methods. Other methods used data matrix combining, and tree combining procedures.

Not surprisingly, the consensus phylogenies (regardless of method used) echoed the size related arrangement seen in most of the other analyses. This result was the product of using phylogenies and data sets that were either size related or produced size related trees. The singular exception was the regression residuals data set and associated trees. These non-size related data were most likely overwhelmed by the predominance of the other input data, swamping the results and minimizing the effect of regression residuals on the overall consensus.

The results from this chapter do not indicate which of the consensus or combining techniques is superior. Consequently, which ever method is easiest or most convenient should be applied. Preferences for either combining data or trees based on philosophical grounds should be used in deciding which method(s) to employ. Only the method developed here using Newick standard format treefiles to combine tree topologies produced results that differed from the other techniques. And this difference was only in the placement of a few closely related taxa.

Overall conclusions

From the results of the various analyses (coded and uncoded continuous, and regression residuals) tested under maximum likelihood and parsimony approaches it appeared that continuous postcranial morphological trait data tended to produce size related phylogenies. The various coding methods analyzed under parsimony criteria yielded results that differed little from one another or from the uncoded trait data analyzed using maximum likelihood approaches. Only the regression residuals analysis produced results which were not correlated with size in any way. These results also seemed to lack any biological or evolutionary correlation as well. Based on these analyses, it appears that one method cannot be called superior to the others in regards to the trees produced. The additional capabilities of the parsimony program PAUP* tend to make that program preferred over PHYLIP, despite the need to discretize the data prior to entry. PHYLIP has the advantage of being able to accept straight uncoded continuous trait data (given equal variances) but has the disadvantage of a lack of available a posteriori tree analyses. The differences in evolutionary assumptions between these two methods did not produce dramatically different results from the data used here.

Of the data and tree combining methods used, there was very little evidence to recommend one method over another. Whether tree or data combining techniques were used appeared to have little effect on the data employed here. Any choice of methodology should be based on ease of implementation and agreement with the philosophical nature of each approach. The efficacy of continuous character traits in systematic analyses seems questionable based on these analyses, however, refinement of the techniques and methods used here may very well prove fruitful.

Vita

Charles Gregory Anderson was born in Denver, Colorado on March 27, 1961 during a blizzard. Things could only get better. He attended schools in the public system of Chesterfield, Missouri, the St. Louis County Public School System. After a five year tour in the United States Navy he entered Meramec Community College in Kirkwood, Missouri, in August, 1985. He received his GED in November, 1985 and graduated with honors with an Associate of Arts degree in General Transfer Studies in May, 1987. He then entered the University of Missouri-Columbia in June, 1987 where he received the Bachelor of Arts degree in Biology in December, 1990. He accepted a Research Assistant position in the Division of Mammals at the National Museum of Natural History of the Smithsonian Institution in July, 1990 where he worked for two years before pursuing a graduate degree. He entered the Doctoral program in Zoology at the University of Tennessee, Knoxville in August, 1993 and switched to the Doctoral program in Ecology and Evolutionary Biology at its inception. He took a position as Assistant Professor at the Oregon Institute of Technology in January, 2000. The doctoral degree was received in May, 2001.

He is presently employed as an Assistant Professor in the Department of Natural Sciences at the Oregon Institute of Technology in Klamath Falls, Oregon where he teaches biology, ecology, and human anatomy.