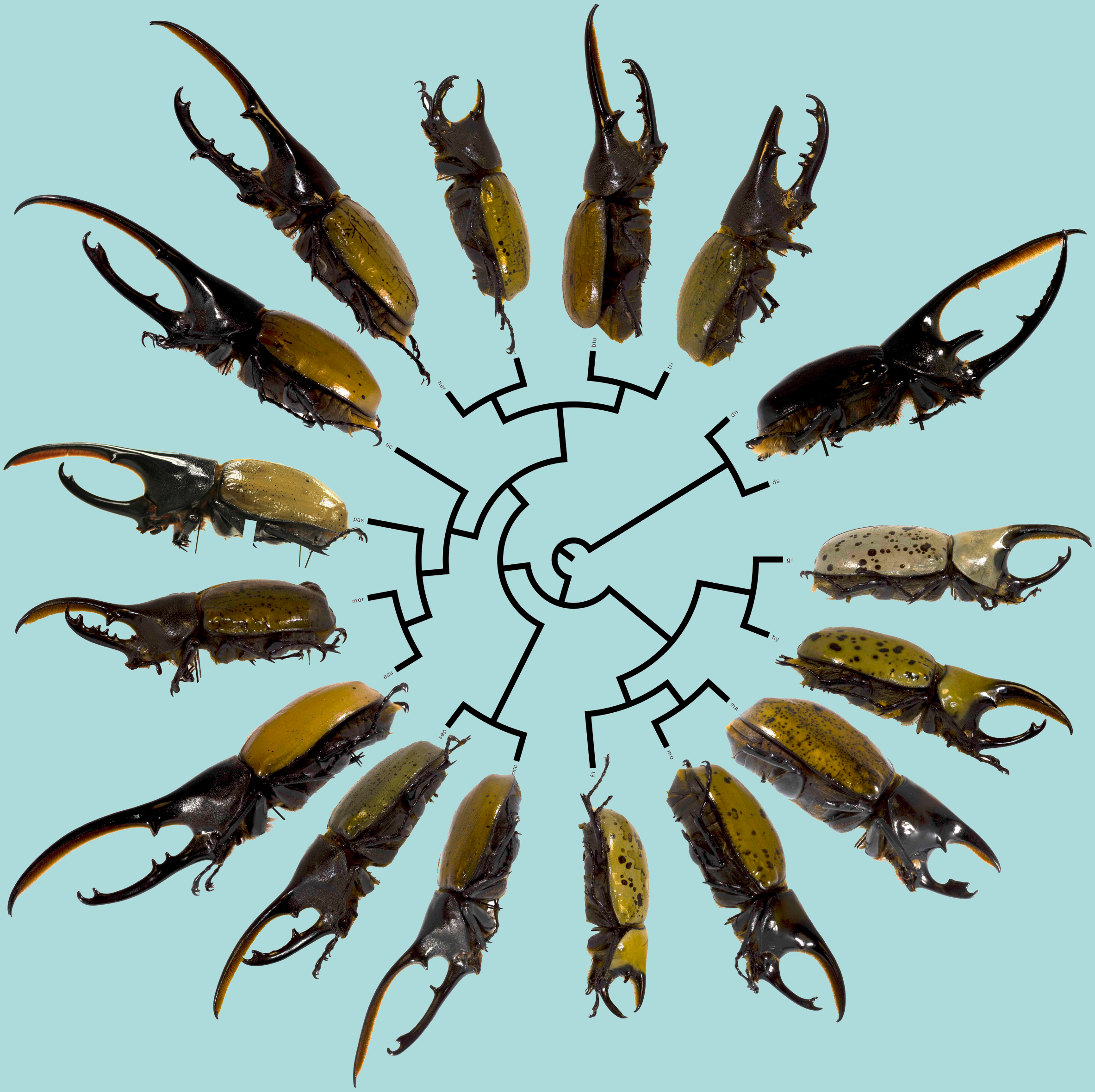
THE HERCULES BEETLES (SUBGENUS *DYNASTES*, GENUS *DYNASTES*, DYNASTIDAE): A REVISIONARY STUDY BASED ON THE INTEGRATION OF MOLECULAR, MORPHOLOGICAL, ECOLOGICAL, AND GEOGRAPHIC ANALYSES

by

JEN-PAN HUANG



MISCELLANEOUS PUBLICATIONS
MUSEUM OF ZOOLOGY, UNIVERSITY OF MICHIGAN, 206

Ann Arbor, September 15, 2017
ISSN 0076-8405

PUBLICATIONS OF THE
MUSEUM OF ZOOLOGY, UNIVERSITY OF MICHIGAN NO. 206
J. B. Burch, *Managing Editor*
Gerald R. Smith, *Editor*
Linda Garcia, *Associate Editor*

The publications of the Museum of Zoology, The University of Michigan, consist primarily of two series—the *Miscellaneous Publications* and the *Occasional Papers*. Both series were founded by Dr. Bryant Walker, Mr. Bradshaw H. Swales, and Dr. W. W. Newcomb. Occasionally the Museum publishes contributions outside of these series. Beginning in 1990 these are titled *Special Publications* and *Circulars* and each are sequentially numbered. All submitted manuscripts to any of the Museum's publications receive external peer review.

The *Occasional Papers*, begun in 1913, serve as a medium for original studies based principally upon the collections in the Museum. They are issued separately. When a sufficient number of pages has been printed to make a volume, a title page, table of contents, and an index are supplied to libraries and individuals on the mailing list for the series.

The *Miscellaneous Publications*, initiated in 1916, include monographic studies, papers on field and museum techniques, and other contributions not within the scope of the *Occasional Papers*, and are published separately. Each number has a title page and, when necessary, a table of contents.

A complete list of publications on Mammals, Birds, Reptiles and Amphibians, Fishes, Insects, Mollusks, and other topics is available. Address inquiries to Publications, Museum of Zoology, The University of Michigan, Ann Arbor, Michigan 48109–1079.

RECENT *MISCELLANEOUS PUBLICATIONS*

Huang, J. P. 2017. The hercules beetles (subgenus *Dynastes*, genus *Dynastes*, dynastidae): a revisionary study based on the integration of molecular, morphological, ecological, and geographic analyses. *Misc. Publ. Mus. Zool., Univ. Michigan*, No. 206, pp. 1-32, 36 figures, 4 tables, and 2 maps.

Smith, G. R., J. Chow, P. J. Unmack, D. F. Markle, and T. E. Dowling, 2017. Evolution of the *Rhinichthys Osculus* Complex (Teleostei: Cyprinidae) in Western North America. pp. 45-84, 17 figs., 5 tables, 5 maps and supplementary material.. *In*: Fishes of the Mio-Pliocene Western Snake River Plain and Vicinity. *Misc. Publ. Mus. Zool., Univ. Michigan*, No. 204 no.2.

Ruedas, L. A., S. M. Silva, J. H. French, R. N. Platt II, J. Salazar–Bravo, J. M. Mora, and C. W. Thompson. 2017. A Prolegomenon to the Systematics of South American Cottontail Rabbits (Mammalia, Lagomorpha, Leporidae: Sylvilagus): Designation of a Neotype for S. brasiliensis (Linnaeus, 1758), and Restoration of S. andinus (Thomas, 1897) and S. tapetillus Thomas, 1913. Misc. Publ. Mus. Zool., Univ. Michigan, No. 205. pp. i-iv, 1-67, 33 figs., 5 tables, 2 appendices, and supplementary material.

Stearley, R. F. and G. R. Smith, 2016. Salmonid fishes from Mio-Pliocene lake sediments in the Western Snake River Plain and the Great Basin. pp. 1-43, 17 figs., 4 tables, 3 maps. *In*: Fishes of the Mio-Pliocene Western Snake River Plain and Vicinity. *Misc. Publ. Mus. Zool., Univ. Michigan*, No. 204 no.1.

RECENT *OCCASIONAL PAPERS*

Kraus, Fred. 2015. A new species of the miniaturized frog genus *Paedophryne* (Anura: Microhylidae) from Papua New Guinea. *Occ. Pap. Mus. Zool., Univ. Michigan,* No. 745, pp. 1-11, 2 figs., 1 table, 1 map.

Wilkinson, M., A. O'Connor, R.A. Nussbaum. 2013. Taxonomic status of the neotropical Caeciliam genera *Brasilotyphlus* Taylor, 1968, *Microcaeilia* Taylor, 1968 and *Parvicaecilia* Taylor, 1968 (Amphibia: Gymnophiona: Siphonopidae) *Occ. Pap. Mus. Zool., Univ. Michigan,* No. 744, pp. 1-10, 2 figs., 1 table.

Smith, G.R., J.D. Stewart & N.E. Carpenter. 2013. Fossil and Recent mountain suckers, *Pantosteus*, and significance of introgression in catostomin fishes of the western United States. *Occ. Pap. Mus. Zool., Univ. Michigan,* No. 743, pp. 1-59, 12 figs., 2 appendices, supplementary material.

Lindsay, A.R. & S.C.G. Haas. 2013. DNA from feces and museum specimens confirms a first state record bird. *Occ. Pap. Mus. Zool., Univ. Michigan,* No. 742, pp. 1-10, 4 figs., 1 table.

Oldfield, R.G. 2009. Captive breeding observations support the validity of a recently described cichlid species in Lake Apoyo, Nicaragua. *Occ. Pap. Mus. Zool., Univ. Michigan,* No. 741, pp. 1-14, 6 figs., 3 tables.

Ann Arbor, Michigan 48109-1079, U.S.A.

COVER PHOTOGRAPH— by Jan-Pen Huang.

MISCELLANEOUS PUBLICATIONS
MUSEUM OF ZOOLOGY, UNIVERSITY OF MICHIGAN, NO. 206

THE HERCULES BEETLES (SUBGENUS *DYNASTES*, GENUS *DYNASTES*, DYNASTIDAE): A REVISIONARY STUDY BASED ON THE INTEGRATION OF MOLECULAR, MORPHOLOGICAL, ECOLOGICAL, AND GEOGRAPHIC ANALYSES

BY

JEN-PAN HUANG

Department of Ecology and Evolutionary Biology and Museum of Zoology
University of Michigan
Ann Arbor, Michigan 48109–1079, USA

Ann Arbor, September 15, 2017
ISSN 0076-8405

THE HERCULES BEETLES (SUBGENUS *DYNASTES*, GENUS *DYNASTES*, DYNASTIDAE): A REVISIONARY STUDY BASED ON THE INTEGRATION OF MOLECULAR, MORPHOLOGICAL, ECOLOGICAL, AND GEOGRAPHIC ANALYSES

By

Jen-Pan Huang[1]

ABSTRACT

The taxonomic treatment of Hercules beetles (subgenus *Dynastes* of the genus *Dynastes* MacLeay, 1819) is revised, with the recognition of two major species groups and 15 species. The following 10 taxa, originally designated as subspecies, have been elevated to full species status: *Dynastes moroni* Nagai, 2005 (**REVISED STATUS**); *Dynastes bleuzeni* Silvestre and Dechambre, 1995 (**NEW STATUS**); *Dynastes ecuatorianus* Ohaus, 1913 (**NEW STATUS**); *Dynastes lichyi* Lachaume, 1985 (**NEW STATUS**); *Dynastes morishimai* Nagai, 2002 (**NEW STATUS**); *Dynastes occidentalis* Lachaume, 1985 (**NEW STATUS**); *Dynastes paschoali* Grossi and Arnaud, 1993 (**NEW STATUS**); *Dynastes reidi* Chalumeau, 1977 (**NEW STATUS**); *Dynastes septentrionalis* Lachaume, 1985 (**NEW STATUS**); and *Dynastes trinidadensis* Chalumeau and Reid, 1995 (**NEW STATUS**). The use of the species name *Dynastes hercules* (Linnaeus, 1758) is restricted to only two island populations from Guadeloupe and Dominica. This taxonomic revision is accompanied by discussions of the basis for these taxonomic changes, i.e. based on differences in genetic, phenotypic, and ecological data and on estimates of geographic overlap. An updated *Dynastes* beetle phylogeny based on Illumina sequencing data is also presented for a discussion of the evolutionary relationships among the species.

---

[1] Museum of Zoology, University of Michigan, Ann Arbor, MI 48109, USA

INTRODUCTION

The species is the fundamental unit of study, so thorough taxonomic revisions are critical to a diversity of biological studies. For example, comparing the patterns of biodiversity between geographic regions requires thorough and consistent taxonomic practice for objectively studying the differences between ecological communities. Questions about the mechanisms that drive the evolution of diverse life forms cannot be answered if this diversity has not been systematically and objectively estimated (e.g. Smith et al., 2013). The revision of the Hercules beetles (subgenus *Dynastes*) from the genus *Dynastes* MacLeay, 1819, in this study is no exception. Multiple species of Hercules beetles are found in North and Central America, but only one species with multiple geographic forms unequivocally designated as subspecies (Ratcliffe et al., 2013) is found in the Neotropics. These taxonomic designations imply that the ecologically diverse and geologically highly active Neotropical region, which importantly is also the geographic origin of the genus *Dynastes* (Dutrillaux and Dutrillaux, 2013; Huang 2016a), only harbors one polytypic taxon. The center of biodiversity of Hercules beetles, though, is in North America instead of the Neotropics. Conservational priorities for the Neotropical taxon may go unrecognized if it is considered to be a geographically widespread species, with the severe threat of habitat loss it is facing.

Speciation in the Hercules beetles fits the classic mode of allopatric/parapatric diversification (Huang and Knowles, 2016), where geographic ranges rarely overlap between putative taxa. Taxonomic treatments have thus been based solely on patterns of phenotypic divergence without information on possible sympatric distribution, which is the key evidence for establishing reproductive isolation (Mayr, 1942). The conventional diagnostic characters between species and between subspecies (horn shape and elytral coloration), however, are variable not only between different taxa, but also within a taxon (Ratcliffe et al., 2013). This variability raises two questions about past taxonomic designations: 1) do different taxonomic categories correspond to different biological units (i.e. are recognized species fundamentally different from subspecies), and 2) is divergence always more apparent between putative species than between putative subspecies? The answers to these two questions have ramifications on ecological, evolutionary, and conservational studies.

An integrative approach that combined information from genetic, phenotypic, ecological, and distributional data addressed the equivalency and relative degree of divergence between species and subspecies of Hercules beetles (Huang and Knowles, 2016). The conclusions from these analyses are presented here in detail, and the integrated data and quantitative analyses form the basis for the revision of the subgenus *Dynastes*. This study is thus not a traditional morphological revision, but provides a statistical evaluation of the probabilities of taxonomic distinctiveness in addition to the diagnostic characters and descriptions of each species. Phenotypic variation within and among species is emphasized, which highlights the difficulty of separating some taxa, especially sister taxa, based on diagnostic characters. A typological view of species can thereby be avoided, and a biological species as a collective group of individuals, each with its own phenotypic/genetic properties, can be fully appreciated (Mayr, 1942, 1963). A revised phylogenetic hypothesis for *Dynastes* species (cf. Fig. S5 in Huang and Knowles, 2016) is also provided based on a molecular data set obtained using Illumina sequencing (a total of 43,205 unlinked loci; Huang, 2016b). I hope that this taxonomic revision will not only augment our knowledge and understanding of Hercules beetles, but also provoke discussion about how revisionary studies can be refined to incorporate evolutionary concepts, which can serve as a firm foundation for future biological studies.

THE GENUS *DYNASTES*

The genus *Dynastes* MacLeay, 1819 is composed of two subgenera endemic to the New World. Individuals from the subgenus *Theogenes* Burmeister, 1847, which comprises two species and one subspecies, can be easily distinguished from Hercules beetles (subgenus *Dynastes*) by their distinct tarsal morphology (Hwang, 2011). The phylogenetic split between subgenera is supported by a recent molecular phylogeny (Huang and Knowles, 2016). The genus *Megasoma* Kirby, 1825 is most closely related to *Dynastes* and is also endemic to the New World (Rowland and Miller, 2012). The distinct thoracic horn morphology and elongated frontal legs of *Megasoma* male beetles can be easily discriminated from those of *Dynastes* beetles (Hwang, 2011).

Rhinoceros beetles of the Australasian genus *Xylotrupes* Hope, 1837 and the African genus *Augosoma* Burmeister, 1841 have male horn phenotypes similar to those of the *Dynastes* beetles, which led to the unification of the three genera under *Dynastes* by Arrow (1937). These three genera, however, have their own unique morphologies, which then led to the revision by Endrödi (1947) that reestablished the three genera. A recent phylogenetic study based on combined molecular and morphological characters supported Endrödi’s treatment by determining that *Augosoma*, *Dynastes*, and *Xylotrupes* did not form a monophyletic group (Rowland and Miller, 2012). The similarity in male horn shapes across the three genera may be due to convergence or represent the ancestral state of horn shape in Dynastine beetles.

SPLITTING THE HERCULES BEETLES

The Hercules beetles have two phylogenetically distinct lineages: the White and the Giant Hercules beetles (Huang and Knowles, 2016). The White Hercules beetles from North America include five allopatrically distributed taxa that have traditionally been designated as different species (Morón, 2009; but see Ratcliffe et al., 2013). The Giant Hercules beetles are a species complex composed of >10 geographic forms distributed throughout various ecoregions of the Neotropics that have traditionally been designated as subspecies. (Chalumeau and Reid, 2002; Hwang, 2011; Huang and Knowles, 2016). Discussing the taxonomic relationships within the White and Giant Hercules lineages separately is meaningful because of the differences in geographic affinity (subtropical versus tropical), general body coloration, and the type of geographic isolation (allopatric forested habitats versus parapatric ecoregions) between these two lineages and because of the significant phylogenetic split (with approximately 4 million years of divergence; Huang and Knowles, 2016).

(1) The White Hercules beetles, whose name refers to their lighter elytral and pronotal coloration (generally whitish, grayish, or light yellow in North American species), with the following species: *Dynastes granti* Horn, 1870; *Dynastes hyllus* Chevrolat, 1843; *Dynastes maya* Hardy, 2003; *Dynastes moroni* Nagai, 2005 (**REVISED STATUS**); and *Dynastes tityus* (Linnaeus, 1763).

(2) The Giant Hercules beetles, whose name refers to their larger body and extravagant major male horn structure, includes all taxa previously classified under the *Dynastes hercules* complex: *Dynastes bleuzeni* Silvestre and Dechambre, 1995 (**NEW STATUS**); *Dynastes ecuatorianus* Ohaus, 1913 (**NEW STATUS**); *Dynastes hercules* (Linnaeus, 1758); *Dynastes lichyi* Lachaume, 1985 (**NEW STATUS**); *Dynastes morishimai* Nagai, 2002 (**NEW STATUS**); *Dynastes occidentalis* Lachaume, 1985 (**NEW STATUS**); *Dynastes paschoali* Grossi and Arnaud, 1993 (**NEW STATUS**); *Dynastes reidi* Chalumeau, 1977 (**NEW STATUS**); *Dynastes septentrionalis* Lachaume, 1985 (**NEW STATUS**); and *Dynastes trinidadensis* Chalumeau and Reid, 1995 (**NEW STATUS**).

SYSTEMATIC METHODOLOGY

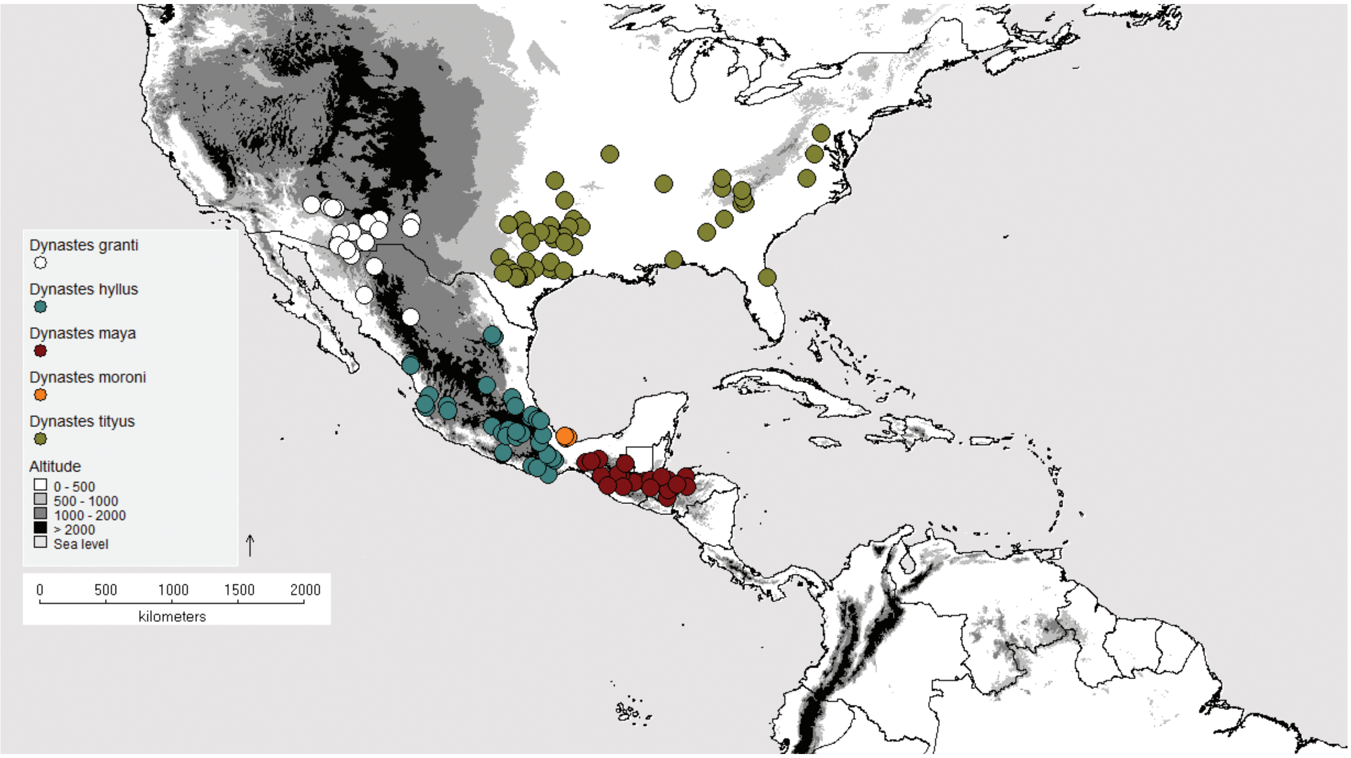
Species Concept.— The general lineage concept, which posits that a species is an evolutionary lineage independent of other such lineages (de Queiroz, 2007), has been widely applied in recent coalescent-based quantitative species delimitations and will be adopted in this revision. This concept is applied because the coalescent-based method used by Huang and Knowles (2016) statistically identified independently evolving lineages that could be detected by molecular and phenotypic data. Specifically, taxa that can be statistically supported as independently evolving lineages re designated as species in this revision.

The biological species concept, which posits that a species is a population composed of (potentially) interbreeding individuals that are reproductively isolated from other such groups, also implies that species are evolutionarily independent lineages (de Queiroz, 2007). Speciation is also acknowledged as a continuous process in the biological species concept, where divergence between independently evolving lineages can proceed along different axes, or dimensions, at different rates (Mayr, 1963; Nosil et al., 2009). For example, the divergence between evolutionarily independent lineages can range from taxa that differ genetically to those that have diverged significantly in both phenotype and ecological preference. This speciation continuum is apparent in the system of Hercules beetles (Huang and Knowles, 2016), so the biological species concept can help to categorize the extent to which divergence has accumulated between species of Hercules beetles along different axes of differentiation. The relationships between species are therefore also discussed based on their position along the speciation continuum following the biological species concept, in addition to providing descriptions for species and reasons why taxa are elevated to full species status.

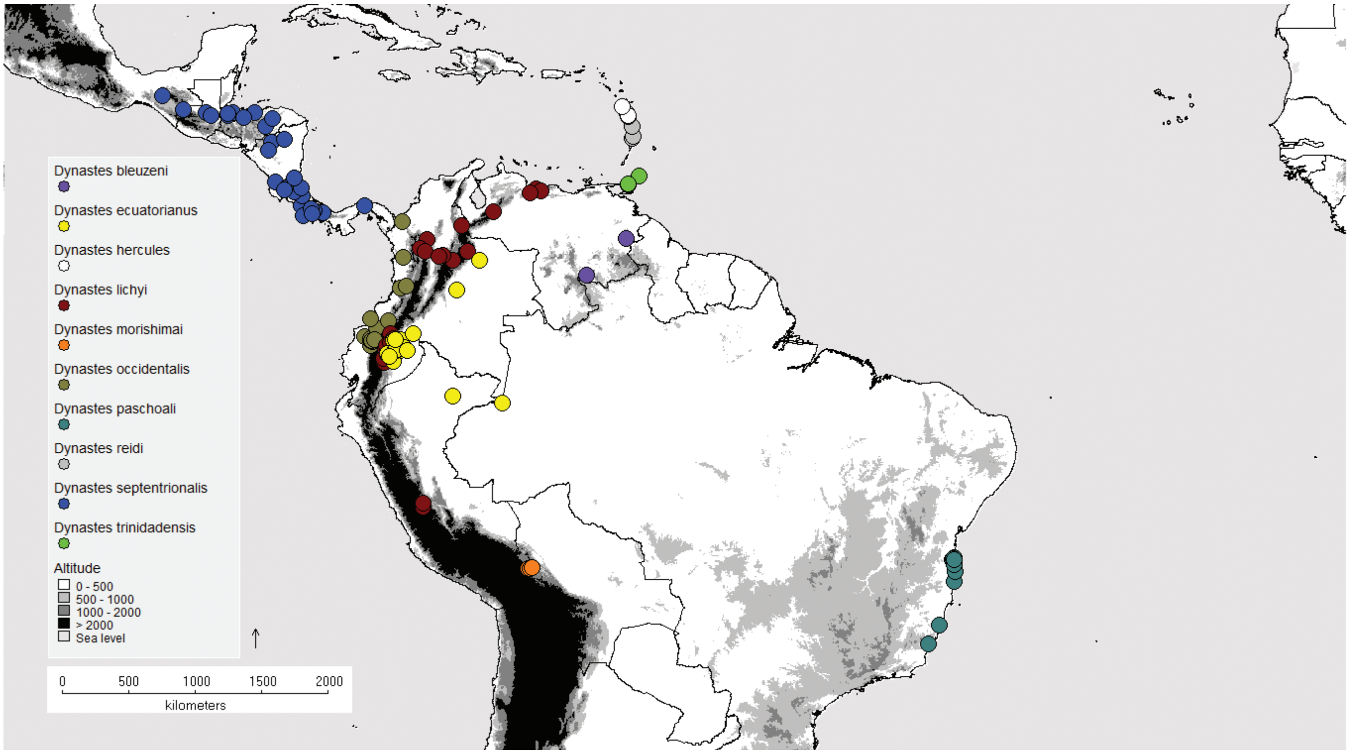
Reliance on Results from an Integrative Approach. I based the taxonomic decisions in this revision on the results published by Huang and Knowles (2016), who studied species delimitation in Hercules beetles by integrating molecular, phenotypic, ecological, and distributional analyses. Multiple axes have been taken into account for delimitating species, so the results likely capture the biological reality of the emergence of new species via different processes of diversification (e.g. geographic isolation or natural selection). The relationship between species can also be discussed using the biological species concept (see discussion in the previous section) by incorporating data that represent different possible dimensions where speciation may proceed. Lastly, the statistical equivalency of the taxa (i.e. their delimitation) is demonstrated (Huang and Knowles, 2016) by analyzing the data in a model-based Bayesian framework, which suggest that previous designations of species versus subspecies represent regional differences in taxonomic practice.

Nomenclature.— Nomenclatural details, especially for the Giant Hercules beetles, such as synonyms, have been listed and discussed extensively by Ratcliffe et al., (2013) and Ratcliffe and Cave (2015). Nomenclatural details are therefore not provided, and only the most commonly used synonyms are included for each species account in this study. Specifically, only species with a revised taxonomic status in this monograph are associated with a section of brief taxonomic notes.

Material Examined.— The majority of the material has been collected by the author during many trips to southeastern and southwestern United States and a trip to Ecuador. Additional specimens were acquired with the help of amateur entomologists and local universities and museums.



Map 1— Geographic distributions of the species of White Hercules beetles. Arrow points northward.



Map 2— Geographic distributions of the species of Giant Hercules beetles. Arrow points northward.

All records pertain to specimens currently vouchered in the Insect Division at the Museum of Zoology, University of Michigan (UMMZ). Data from digital images of the type specimens of seven species were retrieved from books by Lai and Ko (2008) and Hwang (2011) (see figures and appendix for details). Specimens collected near the type locality have been examined when images of the type specimens were not available, as noted in each species account and in the figure legends.

Distributional Map.— Collection records retrieved from Huang and Knowles (2016) were illustrated geographically using DIVA-GIS (Hijmans et al., 2002). The type locality for each species and the localities documented in published data sets are included (Maps 1, 2). In addition to distributional data, the elevation of the geographic range is reported in four categories: 0–500, 500–1000 (lowland/rainforest), 1000–2000 (highland/cloud forest), and >2000 m above sea level (ASL). All species except *Dynastes tityus* live in or near hilly/mountainous areas, but very few can live >2000 m ASL (only *Dynastes granti* and *Dynastes lichyi*).

MORPHOLOGICAL NOTES FOR DESCRIPTION AND DIAGNOSIS

The horn shapes and elytral colorations of adult males have traditionally been used as diagnostic characters to identify Hercules beetles. The same characters are also used as diagnostic characters in this revision, although geographic origin may be the most reliable information to discriminate between species, especially for minor males and females. Differences in horn shape between species may have significant evolutionary implications, because horn shape has been hypothesized to serve an anti-predation function (Jarman and Hinton, 1974) in addition to intraspecific male-male competition. Variation in elytral coloration, which may serve a camouflage function in Hercules beetles (Hinton and Jarman, 1973), can also be evolutionarily meaningful in producing or maintaining the evolutionary independence between species living in different geographic/ecological regions. Differences in characters with adaptive significance may be better candidates for diagnosis, because they not only help identify distinct species, but also suggest why there are so many species of Hercules beetles.

Genitalic structure, however, will not be discussed nor used as a diagnostic character in this revision. Specifically, the lock and key hypothesis of genitalic differences between species does not apply to the system of Hercules beetles, because different species can readily interbreed under artificial conditions (Lai and Ko, 2008), and the argument for reinforcement is itself a contentious subject in evolutionary biology (Noor, 1999). Different species of Hercules beetles are allopatrically or parapatrically distributed, where the selective pressure to develop distinct genitalic structures to prevent interspecific copulation is virtually non-existent. If species boundaries are maintained by strong physical barriers or ecological, phenological, or behavioral differences, differences in genitalic structure other than random variation between species are not expected.

Male Horn Shape.— This trait is used as the main diagnostic character in this revision. Outline analyses were applied to calculate the mean horn shapes in all available digital images examined in each species, in addition to verbal descriptions of the horn shapes in each species (see Figs. 1–25; elliptical Fourier analysis with 100 harmonics; Kuhl and Giardina, 1982). The outlines for each specimen image were drawn following the suggestions by Marquez and Knowles (2007). Principal component analysis (PCA) was applied to quantitatively investigate the differences in the outlines of horn shapes between individuals of the same and different species (Figs. 26–29). The PC1 and PC2 values of the cephalic and thoracic-horn shapes were then extracted and used in a nonmetric multi-dimensional scaling (NMDS) plot to determine whether different species had different horn shapes (Figs. 30, 31). The statistical support for significant differences in horn shape among species was then tested using a multivariate analysis of variance (MANOVA) with 1000 random permutations (Tables 1, 3). A linear discriminant function was used to test sample assignments based on the PC1 and PC2 values of both thoracic- and cephalic-horn shapes (Tables 2, 4). All analyses were performed using the R packages Momocs (Bonhomme et al., 2014), vegan (Dixon, 2003), and MASS (Venables and Ripley, 2002).

Male Body Coloration.— Elytral coloration was used as a supplementary diagnostic character in this revision. Different Hercules beetles generally have different predominant elytral colors and textures (see figures of exemplar specimens vouchered at UMMZ). Museum collections, especially historical collections, however, tend to have lighter elytral coloration, which may be due to the degradation of pigmentation with time. Pubescent structures on the elytra, which may affect the brightness of the elytral surface, can also be easily lost in historical collections. The color and general texture of the elytra are therefore described for each species following previous studies (Chalumeau and Reid, 2002; Morón, 2009) and based on the UMMZ museum collections, but quantitative methods are not used for these characters. Body coloration as a diagnostic character, especially for historical specimens, should be used with extreme caution.

PHYLOGENY OF THE GENUS *DYNASTES*

A well-resolved phylogenetic hypothesis is the basis for biological studies because it provides an evolutionary perspective. Reconstructing phylogenetic relationships between recently diverged species, such as the system of Hercules beetles, can be challenging, because lineage sorting will likely be incomplete where different gene trees are expected to

Table 1— MANOVA results for the PC1 and PC2 values for White Hercules beetle horn shapes. D.f., degrees of freedom; S.S., sums of squares; M.S., mean squares.

	D.f.	S.S.	M.S.	F	R^2	P (>F)
Species	4	0.48559	0.12140	28.948	0.81663	9.99×10^{-5}
Residuals	26	0.10904	0.00419		0.18337	
Total	30	0.59463			1	

Table 2— Results from the linear discriminant function* based on horn shapes in White Hercules beetles. *The number of individuals of a species (rows) identified with a different possible assignment (columns). #The probability that an individual of the species is correctly identified as a sample of that species.

	gr	hy	ma	mo	ty	Prob#
gr	5	2	0	1	0	0.63
hy	2	3	0	1	0	0.50
ma	0	0	5	0	0	1.00
mo	3	0	0	1	0	0.25
ty	0	0	0	0	8	1.00

Table 3— MANOVA results for the PC1 and PC2 values of Giant Hercules beetle horn shapes. D.f., degrees of freedom; S.S., sums of squares; M.S., mean squares.

	D.f.	S.S.	M.S.	F	R^2	P (>F)
Species	8	0.20247	0.02531	9.3067	0.57962	9.99×10^{-5}
Residuals	54	0.14684	0.00272		0.42038	
Total	62	0.34931			1	

Table 4— Results from the linear discriminant function* based on horn shapes in Giant Hercules beetles. *The number of individuals of a species (rows) identified with a different possible assignment (columns). #The probability that an individual of the species is correctly identified as a sample of that species.

	blu	ecu	her	lic	mor	occ	pas	sep	tri	Prob#
blu	1	0	0	1	0	0	0	0	2	0.25
ecu	1	11	0	0	0	0	0	1	0	0.85
her	0	0	3	0	0	0	0	0	0	1.00
lic	0	0	0	16	0	0	1	0	0	0.94
mor	1	0	0	0	1	0	0	0	0	0.50
occ	0	0	0	0	0	7	0	1	0	0.88
pas	0	0	0	1	0	0	2	0	0	0.67
sep	0	1	0	0	0	0	0	9	0	0.90
tri	1	0	0	1	1	0	0	0	0	0.00

have different phylogenetic patterns (Maddison, 1997). Reconstructing the phylogenetic relationship for a clade with more species will also be more difficult. Such difficulty can be identified when studying the phylogenetic relationships in Hercules beetles. Specifically, the relationship among the five species of White Hercules beetles can be confidently reconstructed using five unlinked loci; the relationships among the ten species of Giant Hercules beetles, however, are not well resolved (Huang and Knowles, 2016). The phylogenetic relationships of the Hercules beetles are discussed as a latest revisionary study based on a newly obtained data set from Illumina sequencing with a significantly increased number of genetic loci that better resolves the phylogenetic relationships (Huang, 2016b).

Molecular Data.— DNA extracted from a total of 57 Hercules beetles representing 15 species and from three individuals from two species of the subgenus *Theogenes* were prepared in two RAD-Seq libraries following a protocol modified from Gompert et al., (2012) and sequenced by an Illumina Hi-Seq machine (single end with 150 base pairs) at the Centre for Applied Genomics, The Hospital for Sick Children (Toronto, Canada). The sequencing results were analyzed using the pyRAD pipeline (Eaton, 2014) and subsequently processed using customized R scripts (details can be found in Huang, 2016b). A total of 43,205 unlinked SNPs (23,813 parsimoniously informative) had representatives from at least four species and were retained for phylogenetic reconstruction. The final data set contained a total of 48 individuals of Hercules beetles, because sequencing coverage was too low for six individuals (perhaps due to low DNA quality), which were discarded after initial sequence processing. Individuals Dmi2 and DhrSL5 (belonging to *Dynastes hyllus* and *Dynastes reidi*, respectively), which were included in the phylogenetic analyses, also had relatively small sequencing products (about 10% relative to the other individuals) and thus fewer homologous loci shared with other individuals (Huang, 2016b; about 80% missing data; the aligned unlinked SNP data are available online [dryad doi:10.5061/dryad.p4f5v]). The phylogenetic positions based on the concatenated data set for Dmi2 and DhrSL5 should thus be interpreted with caution. The individual-based phylogeny using the concatenated data set is provided (analyses based on Neighbor-Net and maximum likelihood), but these results should be used and interpreted with caution (Kubatko and Degnan, 2007). The phylogenetic interpretations should only be based on analyses of species trees (Fig. 35).

Methods of Phylogenetic Reconstruction.— A concatenated data set containing one SNP per locus, preferably a parsimoniously informative site, was compiled using a customized R script (51 beetle individuals and 43,205 sites). A Neighbor-Net analysis (Bryant and Moulton, 2004) based on LogDet genetic distance from the concatenated data set was reconstructed using SplitsTree (Huson and Bryant, 2006). A maximum-likelihood tree based on individual samples was also reconstructed using the GTRGAMMA model in RAxML (version 8.2; Stamatakis 2014). The final GAMMA-based score for the best tree was -313436.317572. Supports for the branching patterns were further evaluated using 100 bootstrap replicates with an approximate GAMMA model (GTRCAT).

A method based on maximum likelihood that reconstructs phylogenies on a population/species level based on the frequency spectra of bi-allelic loci was also used to separately estimate the phylogenies of White Hercules and Giant Hercules beetles (TreeMix version 1.12; Pickrell and Pitchard, 2012). TreeMix analyses do not allow missing data/taxa, so many more loci could be retained for estimating species trees when analyzing the data sets for White and Giant Hercules beetles separately. The concatenated SNP data set was converted into TreeMix input files using customized R scripts. The final data sets for the White and Giant Hercules beetles contained 7,758 and 4,784 unlinked loci, respectively. Finally, a coalescent-based Quartets method was used to reconstruct the species tree directly from the unlinked-SNP data set (SVDQuartets; Chifman and Kubatko, 2014). Specifically, individuals were assigned to different species (a total of 17 species), the scores of all possible species quartets (exhaustive search; a total of 178,005 quartets) were calculated (Chifman and Kubatko, 2014), and the final species tree was estimated using the Quartet FM method (Fiduccia and Mattheyses, 1982). One thousand bootstrap replicates were used to estimate supports for the branching patterns. The SVDQuartets analysis was performed in PAUP* (version 4.0a146; Swofford, 2002). The concatenated SNP data set is available online (Huang, 2016b).

SPECIES GROUPS AND SPECIES ACCOUNTS

Relationship between species groups.— The species included in the White Hercules group are generally smaller than the species in the Giant Hercules group. Females of the White Hercules group generally have a smooth elytral surface, and females in the Giant Hercules group commonly have a coarsely rugopunctate elytral surface. A dated molecular phylogeny has inferred that the two species groups diverged more than 4 million years ago (Huang and Knowles, 2016). Species from the White Hercules group are found in North and Central America, whereas species from the Giant Hercules group are found in Central America and in parts of South America and the Caribbean (Fig. 35). Species from the two groups geographically overlap in southern Mexico, Guatemala, and Honduras (Ratcliffe et al., 2013).

THE WHITE HERCULES GROUP

Figures 1–9

Map 1

Included species.— *Dynastes granti* Horn, 1870; *Dynastes hyllus* Chevrolat, 1843; *Dynastes maya* Hardy, 2003; *Dynastes moroni* Nagai, 2005; and *Dynastes tityus* (Linnaeus, 1763).

Distinctive characters.— This species group is recognized based on molecular phylogeny, male horn shape, and body coloration: adult males possess one cephalic and one thoracic horn. The major adult males lack cephalic-horn denticles (for example, cf. Figs. 2, 12). The color of the pronotum ranges from grayish-white, dark reddish-brown, to completely black. Adult male body length (including the thoracic horn) is generally 4–10 cm.

***Dynastes granti* Horn, 1870**

Figures 1, 2

Map 1

Type locality.— Fort Grant, Arizona, USA. Specimens from the type locality were not available for examination, but material from near the type locality has been included in this study (Star Valley and Pine, AZ, and Reserve, NM). Only this *Dynastes* species occurs in the vicinity of the type locality, and the figures and descriptions provided by Hwang, 2011 and Ratcliffe et al., 2013 are adequate for identification.

Diagnosis.— Males of adult *D. granti* cannot be easily distinguished morphologically from those of its sister taxon, *D. hyllus* (Figs. 26, 27, 30); the geographic origin is the best basis for identification (Map 1). Nevertheless, body coloration is generally brownish to yellowish in *D. hyllus* (Morón, 2009; Fig. 3), and the tooth on the base of the thoracic horn is often more significantly protruded forward in males of *D. hyllus* (Fig. 4). Distinct body coloration can easily separate *D. granti* from *D. moroni* and *D. maya* (Fig. 5), which generally have dark pronota and yellowish to brownish elytra. Horn shapes are significantly different between *D. granti* and *D. maya* and between *D. granti* and *D. tityus* (Figs. 26, 27, 30).

Identification.— Body length ranges from 35 to 60 mm, while captive-bred individuals can reach up to 80 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 1). Body coloration: pronotum and elytra range from white to grayish white. Very few individuals are light or brownish yellow. Elytra often have irregular black spots that vary in size and number. Pronotum and elytra can be discolored black with high humidity. A small but sharp tooth can be found near the base on either side of the thoracic horn (can be largely reduced or invisible in minor males). A pointed tooth on the dorsal side of the cephalic horn can be found in major males (Fig. 2).

Distribution.— This is the only *Dynastes* species found in southern Utah, Arizona, and western New Mexico of the United States (Map 1). In northern Mexico, it is allopatric with its sister taxon, *D. hyllus*. Specifically, *D. granti* has been reported in Chihuahua and Sonora, while the northern limit of *D. hyllus* is in Sinaloa (Ratcliffe et al., 2013). This species is most often found in highland forest (1600 m ASL).

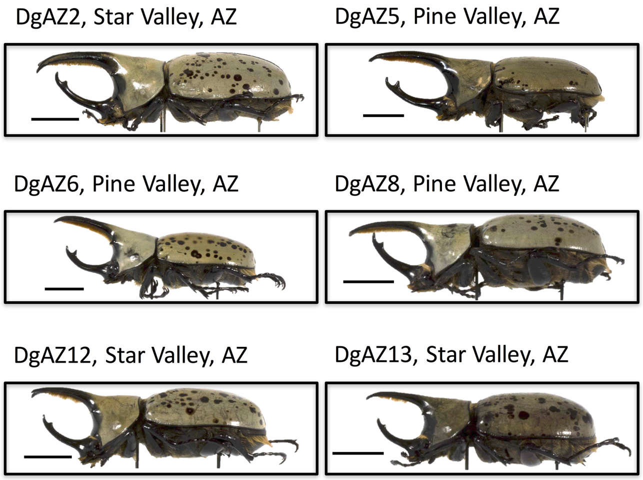


Figure 1— Exemplar specimens of *Dynastes granti* vouchered in UMMZ. Scale bar = 1 cm.

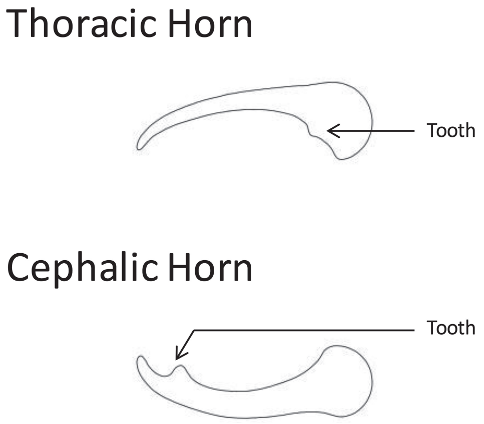


Figure 2— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes granti*. Digitized images from the following eight individuals were used: **Dg1M** and **Dg4M**, historical UMMZ collection from Payson, Arizona; **DgAZ2**, **DgAZ12**, **DgAZ13**, Star Valley, Arizona; **DgAZ5**, **DgAZ6**, and **DgAZ8**, Pine, Arizona.

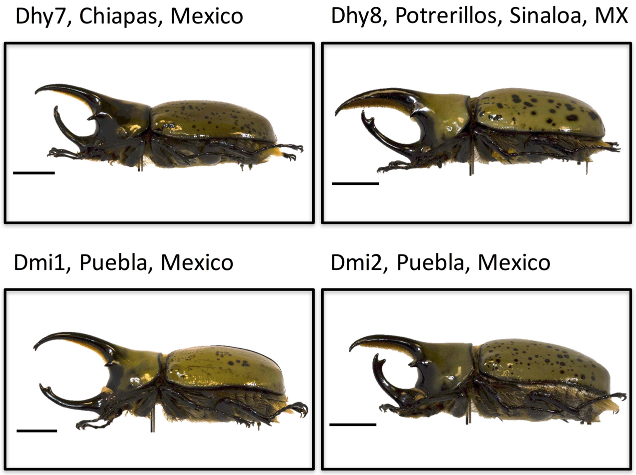


Figure 3— Exemplar specimens of *Dynastes hyllus* vouchered in UMMZ. Scale bar = 1 cm.

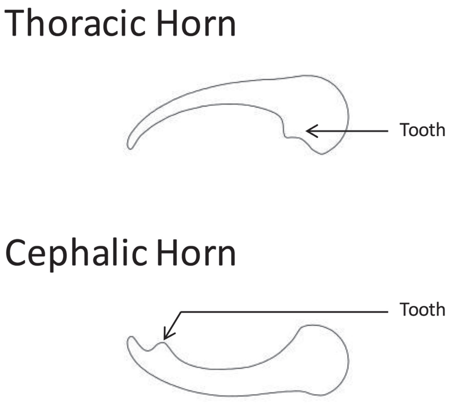


Figure 4—The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes hyllus*. Digitized images from the following six individuals were used: **Dhy7**, Chiapas, Mexico; **Dhy8**, Potrerillos, Sinaloa, Mexico; **Dmi1** and **Dmi2**, captive-bred individuals originating from Puebla, Mexico; **DmiyashitaiJL1** and **DmiyashitaiJL2**, images of wild specimens designated as *Dynastes miyashitai* by Lai and Ko (2008).

***Dynastes hyllus* Chevrolat, 1843**

Figures 3, 4
Map 1

Synonym.— *Dynastes miyashitai* Yamaya, 2004: 4. Synonymized by Morón (2009).

Taxonomic notes.— *Dynastes miyashitai* was synonymized with *D. hyllus* based on morphological characters and geographic distribution (Morón, 2009). This taxonomic treatment concurs with molecular data, where putative *D. miyashitai* individuals and *D. hyllus* individuals of various geographic origins have closely related genotypes (Huang and Knowles, 2016).

Type locality.— The region of Orizaba, Mexico (neotype in Muséum national d'Histoire naturelle, Paris). Specimens from the type locality were not available for examination, but specimens from the province of Puebla, which were originally identified as *D. miyashitai*, have been included. Only this species occurs in the vicinity of the type locality (Morón, 2009), and the figures and descriptions provided by Hwang, 2011 and Ratcliffe et al., 2013 are adequate for identification.

Diagnosis.— See Diagnosis for *D. granti*. Body coloration is distinct between *D. hyllus* and *D. moroni*, which has a dark pronotum and dark yellow-brownish elytra (Fig. 5). The horn shapes are significantly different between *D. hyllus* and *D. maya* and between *D. hyllus* and *D. tityus* (Figs. 26, 27, 30).

Identification.— Body length ranges from 35 to 80 mm, while captive-bred individuals can reach up to 90 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 3). Body coloration: pronotum and elytra range from light yellow to brownish yellow or greenish white. Very few individuals are light gray. Elytra often have irregular black spots that vary in size and number. Pronotum and elytra can be discolored black with high humidity. A tooth can be found near the base on either side of the thoracic horn (can be reduced in minor males). A tooth on the dorsal side of the cephalic horn can be found in most males (Fig. 4). The shape and size of the cephalic tooth varies between individuals (Fig. 3).

Distribution.— This is the only *Dynastes* species found in Sierra Madre Oriental and Sierra Madre de Sur of Mexico. In northwestern Mexico (Sierra Madre Occidental), it can be found in allopatry with its sister taxon, *D. granti*. Specifically, *D. granti* has been reported in Chihuahua and Sonora, while the northern limit of *D. hyllus* is in the province of Sinaloa (Ratcliffe et al., 2013; Map 1). In the southern part of Mexico (Sierra Madre de Chiapas), a different species, *D. maya*, lives in allopatry with *D. hyllus*, which can be found in Sierra Madre de Oaxaca (Hardy, 2003; Morón, 2009). There is another endemic species, *D. moroni*, living in a geographically proximate but isolated mountain range, Sierra de los Tuxtlas, with *D. hyllus* in southeastern Mexico. This species primarily lives in the highland-forest region (1000 m ASL) but can also sometimes be found at sea level (Ratcliffe et al., 2013).

***Dynastes maya* Hardy, 2003**
Figures 5, 6
Map 1

Type locality.— Lacanja, Chiapas, Mexico (holotype in the Canadian Museum of Nature, Ottawa). Specimens from the type locality were not available for examination. Only specimens from north-central Honduras and captive-bred individuals, also originating from Honduras, were used in the study by Huang and Knowles (2016). However, only this species from the White Hercules group occurs in the rainforest regions of Central America (southern Mexico, Guatemala, and Honduras; Morón, 2009, Ratcliffe personal comm.), and the figures and descriptions provided by Hwang, 2011 and Ratcliffe et al., 2013 are adequate for identification.

Diagnosis.— Body coloration is similar between *D. maya* and its sister taxon, *D. moroni*, which has a dark pronotum and dark yellow-brownish elytra (Fig. 5). They differ significantly in horn shape (Figs. 6, 7, 26, 27, 30). *Dynastes maya* has a thoracic-horn shape that is similar to *D. tityus*, which is short and stout with a very significant forward-protruding tooth at the basal position. *Dynastes maya* also has its own specific cephalic-horn shape, slab-shaped (Fig. 6), which is distinct from all other white Hercules beetle species (Fig. 27).

Identification.— Body length ranges from 35 to 80 mm, while captive-bred individuals can reach up to 99 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 5). Pronotal color ranges from dark reddish brown to completely black. Elytral color ranges from brownish yellow to light yellow. Some individuals may also have a slightly reddish brown hue. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. A significant tooth protruding forward can be found near the base on either side of the thoracic horn (can be reduced in minor males; Figs. 5, 6). Thoracic horn is short and stout even in major males. A slab-shaped tooth, or a multiple-toothed structure, on the dorsal side of the cephalic horn can be found in major males (Fig. 6). The shape and size of the cephalic tooth varies between individuals (Fig. 5).

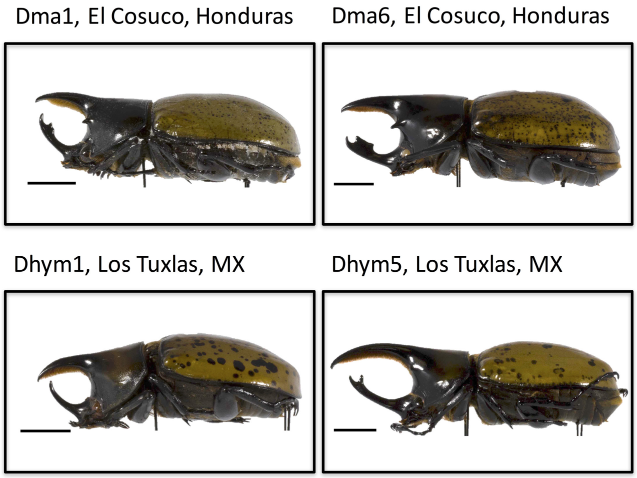


Figure 5— Exemplar specimens of *Dynastes maya* (upper panels) and *Dynastes moroni* (lower panels) vouchered in UMMZ.

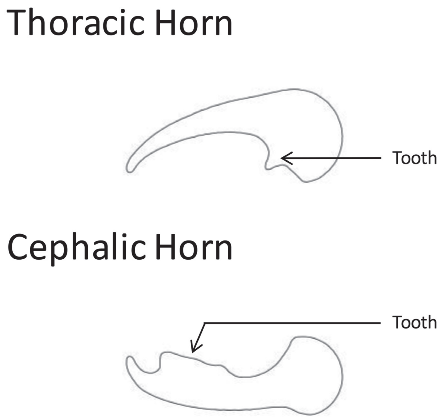


Figure 6— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes maya*. Digitized images from the following five individuals were used: **Dma1** and **Dma6**, El Cusuco, Cortez, Honduras; **Dmaya**, a captive-bred recorded individual image (99.1 mm) from the magazine BeKuwa; **DmaJL2**, image of a wild specimen from San Cristobal, Alta Verapaz, Guatemala in Lai and Ko (2008); **DmayaUNL**, an image from the online guide to Neotropical Scarab beetles hosted by the University of Nebraska State Museum.

Distribution.— This is the only White Hercules species found in the Central American rainforest (Map 1). In the southern part of Mexico (Sierra Madre de Chiapas), it lives in allopatry with *D. hyllus*, which can be found in Sierra Madre de Oaxaca (Hardy, 2003; Morón, 2009). Its sister taxon, *D. moroni*, lives in a geographically isolated mountain range, Sierra de los Tuxtlas, in southeastern Mexico. This species primarily lives in mid-altitude rainforest regions (500–1500 m ASL) but can sometimes also be found near sea level (Ratcliffe et al., 2013). A Giant Hercules species, *D. septentrionalis*, can be found co-distributed with *D. maya* but prefers cloud-forest habitat at higher altitudes.

Dynastes moroni **Nagai, 2005**

Revised Status

Figures 5, 7
Map 1

Synonym.— *Dynastes hyllus moroni* Nagai, 2005: 31.

Taxonomic notes.— This subspecies was raised to full species status by Morón in 2009 but subsequently reduced to subspecies status by Ratcliffe and colleagues in 2013. The revised full species status of *D. moroni* is supported based on the study by Huang and Knowles (2016). Specifically, both molecular and morphological (between major males) divergences indicate that *D. moroni* forms an evolutionary lineage that is independent from its sister taxon, *D. maya*, and therefore merits full species status. Additionally, the original taxonomic designation of *D. moroni* as a subspecies under *D. hyllus* (Nagai, 2005) based on selected morphological characters is phylogenetically misleading. *Dynastes moroni*, instead of being closely related to *D. hyllus*, is sister to *D. maya*.

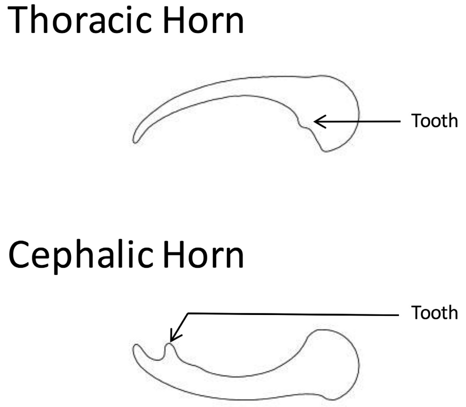


Figure 7— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes moroni*. Digitized images from the following four individuals were used: **Dhym5**, Sierra de Los Tuxtlas, Veracruz, Mexico; **Dmoroni**, a captive-bred recorded individual image (96.8 mm) from the magazine BeKuwa; **DmoroniJL1** and **DmoroniJL2**, images of a wild specimen from Cerro El Vigia, Santiago Tuxtla, Mexico and a captive-bred 94-mm sample, respectively, retrieved from Lai and Ko (2008).

Type locality.— Volcano San Martin, Veracruz, Mexico (holotype in the Entomological Laboratory, Ehime University, Matsuyama). Specimens from the type locality were available for examination. Only this White Hercules species occurs in the rainforest regions of Sierra de los Tuxtlas from southeastern Mexico (Morón, 2009; Ratcliffe et al., 2013; Map 1), and the figures and descriptions provided by Hwang, 2011 and Ratcliffe et al., 2013 are adequate for identification.

Diagnosis.— Body coloration is similar between *D. moroni* and its sister taxon, *D. maya*, which has a dark pronotum and dark yellow-brownish elytra (Fig. 5). They differ significantly in horn shapes (26, 27, 30). *Dynastes moroni* has horn shapes that are similar to *D. hyllus* and *D. granti*. The horn is characterized as an elongated and thin/slim thoracic horn, with a less significant tooth at the base of the thoracic horn, and very often a simple and pointed cephalic tooth (Fig. 7, but see Fig. 29 in Morón, 2009).

Identification.— Body length ranges from 35 to 80 mm, while captive-bred individuals can be longer than 90 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 5). Pronotal color ranges from dark reddish brown to completely black. Elytral color ranges from brownish yellow to light yellow. Some individuals may have a slightly reddish-brown hue. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. A tooth can be found near the base on either side of the thoracic horn (can be reduced or completely absent in some, particularly but not exclusively, in minor males; Fig. 5). Thoracic horn is elongated and thin/slim in major males. A pointed tooth, but in few cases can be slab-shaped (e.g. Fig. 29 in Morón, 2009), on the dorsal side of the cephalic horn can be found in major males (Fig. 7).

Distribution.— This is the only White Hercules species found in the rainforest region of Sierra de los Tuxlas (Ratcliffe et al., 2013; Map 1). In the southeastern part of Mexico (Sierra Madre Oriental), an allopatric species, *D. hyllus*, can be found in its geographic proximity (Morón, 2009). Its sister taxon, *D. maya*, also lives allopatrically in the rainforest region of Central America. A possible remnant population of a Giant Hercules taxon, *D. hercules tuxtlas*, is found co-distributed in the same mountain range with *D. moroni*. However, the collection of *D. hercules tuxtlas* in this region is extremely rare (Ratcliffe et al., 2013).

Dynastes tityus **(Linnaeus, 1763)**

Figures 8, 9
Map 1

Type locality.— New Orleans, Louisiana, USA (lectotype in Zoologisches Museum, Berlin). Specimens from the type locality were not available for examination. However, only this species of Hercules beetles occurs in southeastern United States (Morón, 2009), and the figures and descriptions

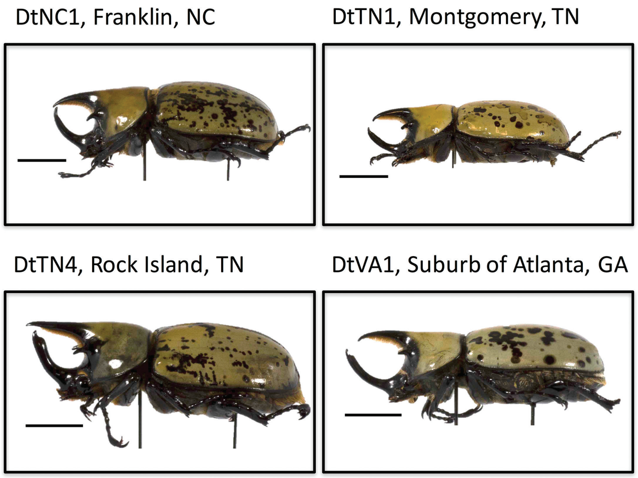


Figure 8— Exemplar specimens of *Dynastes tityus* vouchered in UMMZ. Scale bar = 1 cm.

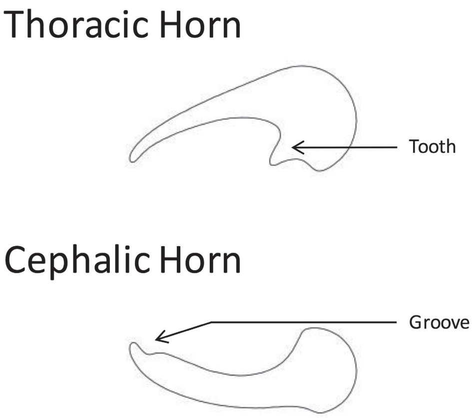


Figure 9— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes tityus*. Digitized images from the following eight individuals were used: **DtNC1**, Franklin County, NC; **DtTN1**, Montgomery, TN; **DtTN4**, Rock Island, TN; **DtVA1**, suburb of Atlanta, GA; **Dty5M**, **Dty7M**, **Dty8M**, and **Dty9M**, historical collections in UMMZ.

provided by Morón, 2009 and Hwang, 2011 are adequate for identification.

Diagnosis.— Body coloration can be similar between *D. tityus* and *D. granti*, but often *D. tityus* is light yellow, creamy yellow, or light green, while *D. granti* is light gray or white. Furthermore, they differ significantly in horn shape (Figs. 26, 27, 30). *Dynastes moroni*, *D. hyllus*, and *D. granti* have horn shapes that are significantly different from *D. tityus*, which has a short and stout thoracic horn with significant forward-protruding teeth at the base and a toothless cephalic horn. *Dynastes tityus* and *D. maya* have similar thoracic-horn shapes, but the cephalic-horn shape is distinct between them. In addition, pronotal color is often dark in *D. maya* while light in *D. tityus* (cf. Figs. 5, 8). It is the smallest species of Hercules beetle.

Identification.— Body length ranges from 30 to 55 mm, while captive-bred individuals can reach up to 65 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 8). Body coloration: pronotum and elytra range from light yellow, tan, to light green. Some individuals are light gray or whitish. Elytra often have irregular black spots that vary in size and number. Elytra and pronotum can be discolored black with high humidity. A significant tooth protruding forward can be found near the base on either side of the thoracic horn (can be reduced in minor males; Fig. 9). Thoracic horn is short and stout even in major males. An apical groove on the dorsal side of the cephalic horn can be found in major males (Fig. 9). Generally, there is no tooth-like structure formed on the cephalic horn of adult males.

Distribution.— This is the only *Dynastes* species found in southeastern United States (Map 1). Its sister lineage contains two Central American species, *D. maya* and *D. moroni*, which are isolated from *D. tityus* by the Gulf of Mexico. This species primarily lives in low-altitude forest regions.

THE GIANT HERCULES GROUP
Figures 10–25
Map 2

Included species.— *Dynastes bleuzeni* Silvestre and Dechambre, 1995; *Dynastes ecuatorianus* Ohaus, 1913; *Dynastes hercules* (Linnaeus, 1758); *Dynastes lichyi* Lachaume, 1985; *Dynastes morishimai* Nagai, 2002; *Dynastes occidentalis* Lachaume, 1985; *Dynastes paschoali* Grossi and Arnaud, 1993; *Dynastes reidi* Chalumeau, 1977; *Dynastes septentrionalis* Lachaume, 1985; and *Dynastes trinidadensis* Chalumeau and Reid, 1995.

Incertae sedis.— *Dynastes hercules takakuwai* Nagai, 2002 and *Dynastes hercules tuxtlaensis* Morón, 1993.

These two geographically isolated and endemic taxa were not included in the study by Huang and Knowles (2016); no material was available for examination in this revision. These two taxa, however, may represent well-differentiated species with respect to the other Giant Hercules beetles, because all the geographically isolated taxa in the Giant Hercules group examined merit distinct species status. Until new genetic and morphological material can be obtained for examination, the taxonomic status of the two Giant Hercules taxa is best recognized as uncertain. The species name, *D. hercules*, is not appropriate for these two taxa, because this name can only be applied to two island endemic Giant Hercules populations from the Lesser Antilles, Guadeloupe, and Dominica, which are highly unlikely sister to *D. h. takakuwai* (Rondônia, Brazil) or *D. h. tuxtlaensis* (Los Tuxtlas, Mexico).

Distinctive characters.— The major adult males can have one or multiple cephalic-horn denticles, except those from *D. paschoali* (Fig. 19). The pronotum in most species is completely black, but a dark brownish-red hue can sometimes be observed among individuals of *D. septentrionalis*. Elytral color ranges from light green, yellow, to dark brown. Adult male body length (including the thoracic horn) is generally 8–15 cm.

Dynastes reidi **Chalumeau, 1977**
New Status
Figures 10, 11
Map 2

Synonyms.— *Dynastes hercules baudrii* Pinchon, 1976: 14.

Treated as *Nomen Nudum* (Chalumeau and Reid, 2002)

Dynastes hercules reidi Chalumeau, 1977: 237.

Taxonomic notes.— The full species status of *D. reidi* is raised here based on the study by Huang and Knowles (2016). Specifically, molecular and phenotypic analyses indicated that *D. reidi* formed an evolutionary lineage independent from its sister taxon, *D. hercules*. Therefore, *D. reidi* merits full species status based on the general lineage concept.

Type locality.— Saint Lucia (lectotype in Muséum national d'Histoire naturelle, Paris). This species of *Dynastes* beetles occurs on islands of the Lesser Antilles (Martinique and Saint Lucia; Map 2), and individuals from both islands were included in the study by Huang and Knowles (2016). This is the only *Dynastes* species that can be found from these two islands.

Diagnosis.— Body coloration and texture are similar between *D. reidi* and *D. hercules*, but they differ significantly in male horn allometry (Fig. 10). Specifically, male *D. reidi* is similar to the minor male phenotype of *D. hercules*. Geographic origin is the best indicator for species identification, particularly for minor males and females. Elytral coloration and texture are different between *D. reidi* and *D. trinidadensis* from the islands of Trinidad and Tobago. Specifically, elytra are often dark yellow, brown, or olive and covered with pubescence that makes it less glossy for *D. trinidadensis* (cf. Fig. 13).

Identification.— Body length ranges from 45 to 75 mm, while captive-bred individuals can reach up to 90 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 10). Pronotal color is completely black. Elytral color is light glossy yellow. Some individuals exhibit a greenish hue on the elytra. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. A tooth can be found near the base on either side of the thoracic horn. The position of the thoracic-horn tooth varies. In larger males, it can be at the 1/4 position from the base of the thoracic horn, while it is located at the basal position in smaller males. All adult males exhibit the minor (short horn) phenotype. A pointed tooth on the dorsal side of the cephalic horn can be found in males. Cephalic-denticle number ranges between 0 (*reidi* form) and 1 (*baudrii* form). Cephalic denticle, if it exists, is pointed (Fig. 11).

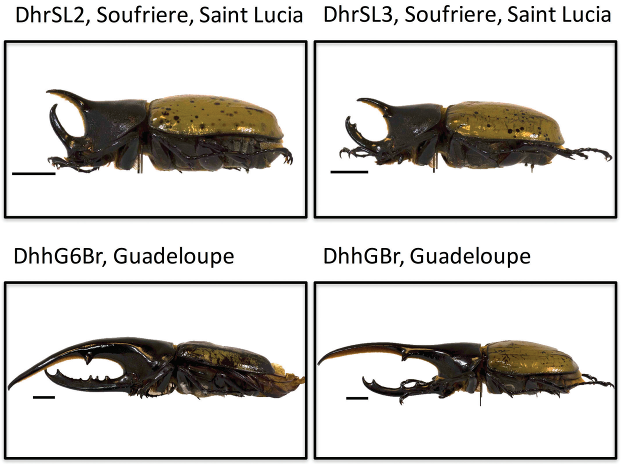


Figure 10— Exemplar specimens of *Dynastes reidi* (upper panels) and *Dynastes hercules* (lower panels) vouchered in UMMZ.

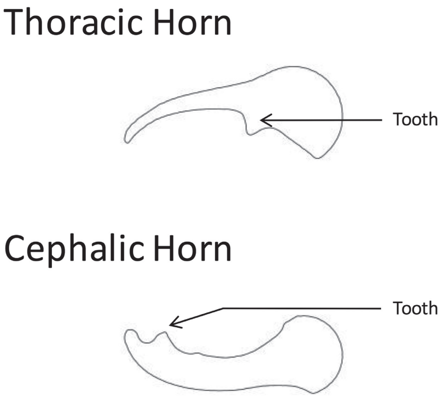


Figure 11— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes reidi*. Digitized images from the following two individuals were used: **DhrSL3**, Soufriere, Saint Lucia; **DhrMJL**, image of a wild specimen from Morne Bleue, Martinique in Lai and Ko (2008).

Distribution.— This is the only *Dynastes* species found on the islands of Martinique and Saint Lucia. Its sister species, *D. hercules*, is allopatrically distributed on the islands of Dominica and Guadeloupe.

Dynastes hercules **(Linnaeus, 1758)**
Figures 10, 12
Map 2

Taxonomic notes.— This taxon of Giant Hercules beetle is inferred as the nominate exemplar for *Dynastes hercules* (Chalumeau and Reid, 2002), and its full species status based on the study by Huang and Knowles (2016) is accordingly raised.

Type locality.— Guadeloupe (Basse-Terre) or Dominica (lectotype in the Linnean Society of London) (see discussion in Chalumeau and Reid, 2002). Newly obtained tissue

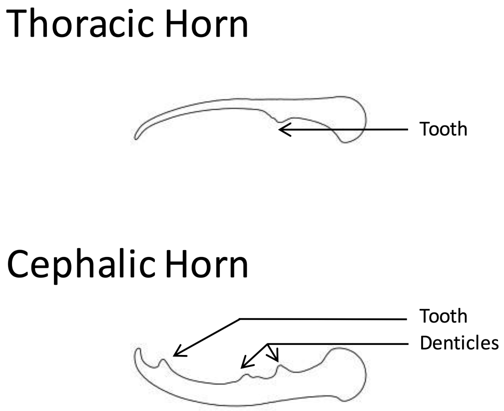


Figure 12—The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes hercules*. Digitized images from the following three individuals were used: **DhhGBr**, a captive-bred major male originating from the island of Guadeloupe; **DhhJLWild**, image of a wild specimen from Guadeloupe in Lai and Ko (2008); **DhhType**, image of the type specimen in Hwang (2011).

samples from wild-collected individuals from the possible type localities, Guadeloupe and Dominica, were included in the study by Huang and Knowles (2016). Guadeloupan individuals were obtained with help from Dr. Chalumeau and the national park of Guadeloupe. Dr. Ivie from Montana State University kindly shared Dominican individuals from the Caribbean Scarab beetle project. This is the only Giant Hercules beetle that can be found on these two islands.

Diagnosis.— See Diagnosis for *D. reidi* for details. Geographic origin is the best information to identify minor males and females. Major males exist in *D. hercules* but not *D. reidi*. Body coloration and texture are different between *D. hercules* and *D. trinidadensis*. The elytral color is brightly glossy yellow in *D. hercules* but less glossy in *D. trinidadensis*.

Identification.— Body length ranges from 50 to 170 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 10). Pronotal color is completely black. Elytral color is light and glossy yellow and sometimes light brown. Some individuals exhibit a greenish or bluish hue on the elytra. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. A tooth can be found on either side of the thoracic horn. The position of the thoracic-horn tooth varies, where in major males it can be at the 1/3 to 1/2 position from the base of the thoracic horn (Fig. 12). The thoracic-horn tooth is located at the base of the horn in minor males. There are often two pointed cephalic denticles in major males; however, cephalic-denticle number generally ranges between 1 and 4. Individuals with >5 cephalic denticles can also be found. Some cephalic denticles may merge into a large slab-shaped denticle.

Distribution.— This species occurs in forested regions from the Basse-Terre part of Guadeloupe and in Dominica (Map 2). Its closely related taxon, *D. reidi*, is found allopatrically on the islands of Martinique and Saint Lucia.

Dynastes bleuzeni Silvestre and Dechambre, 1995

New Status

Figures 13, 14

Map 2

Synonym.— *Dynastes hercules bleuzeni* Silvestre and Dechambre, 1995: 52.

Taxonomic notes.— This taxon of Giant Hercules beetle is raised to full species status based on the study by Huang and Knowles (2016). Specifically, *D. bleuzeni* can be evolutionarily independent from its sister taxon, *D. trinidadensis*. Furthermore, a strong physical barrier, an oceanic barrier, between the two sister taxa implies constant isolation in the near future. It qualifies as a species based on the general lineage concept (de Queiroz, 2007).

Type locality.— La Escalera, Santa Elena, Bolivar, Venezuela (holotype in Muséum national d'Histoire naturelle, Paris). This species of *Dynastes* beetles occurs in a mountainous cloud-forest area in the Bolivar province of Venezuela (Map 2), and two wild-caught individuals (originating from near Cerro Sarisariñama) and one captive-bred sample from the Bolivar province were included in the study by Huang and Knowles (2016). This is the only *Dynastes* species that can be found from the mountainous area southeast of the Orinoco swamp delta ecoregion (north-central Guiana Highland).

Diagnosis.— Body coloration and texture are similar between *D. bleuzeni* and *D. trinidadensis*. Individuals of *D. trinidadensis* may exhibit a yellow color, while those of *D. bleuzeni* often exhibit a brown or olive color (Fig. 13). Geographic origin is the best indicator for species identification, particularly for minor males and females. Elytral coloration and texture are different between *D. bleuzeni* and *D.* reidi from the islands of the Lesser Antilles (see Diagnosis for *D. reidi*). Body coloration can be very similar between *D. bleuzeni* and *D. lichyi*, which exhibits large variation in body coloration. However, distinct horn shapes, especially in the cephalic-horn shape with a slab-shaped cephalic tooth in *D. lichyi*, can easily help to distinguish major males between the two species (Figs. 29, 30, 31).

Identification.— Body length ranges from 65 to 140 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 13). Pronotal color is completely black. Elytral color is dark brown. Some individuals exhibit a greenish/olive hue on the elytra. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. A layer of short hair/pubescence can be found on

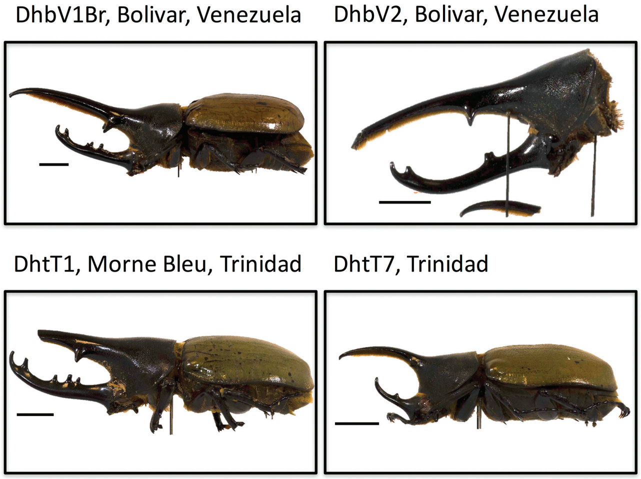


Figure 13— Exemplar specimens of *Dynastes bleuzeni* (upper panels) and *Dynastes trinidadensis* (lower panels) vouchered in UMMZ.

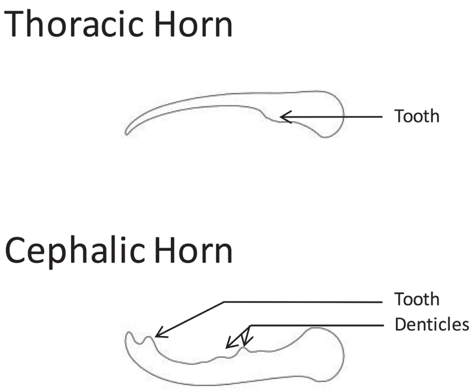


Figure 14— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes bleuzeni*. Digitized images from the following four individuals were used: **DhbV1Br**, a captive-bred major male originating from Bolivar, Venezuela; **DhbV2**, Cerro Sarisariñama, Bolivar, Venezuela; **DhbJL**, image of a wild specimen from La Escalera, Bolivar, Venezuela in Lai and Ko (2008); **DhbHwang**, image of a specimen in Hwang (2011).

the elytra. A tooth can be found on either side of the thoracic horn. The position of the thoracic-horn tooth varies, where in major males it can be at the 1/4 to 1/3 position from the base of the thoracic horn (Fig. 14). The thoracic-horn tooth is located at the base of the horn in minor males. Cephalic-denticle number ranges between 1 and 4. Cephalic denticles can be either pointed or merged into one slab-shaped denticle.

Distribution.— This is the only *Dynastes* species found in the mountain-forest region southeast of the Orinoco River (North central Guiana Shield). Its sister species, *D. trinidadensis*, is allopatrically distributed on the islands of Trinidad and Tobago. Another species that can be found in Venezuela, *D. lichyi*, is found in mountain cloud-forest regions of the northern Andes and is isolated from *D. bleuzeni* by a large area of unsuitable habitats (Los Llanos).

Dynastes trinidadensis Chalumeau and Reid, 1995
New Status

Figures 13, 15
Map 2

Synonym.— *Dynastes hercules trinidadensis* Chalumeau and Reid, 1995: 3.

Taxonomic notes.— This taxon of Giant Hercules beetle is raised to full species status based on the study by Huang and Knowles (2016). Specifically, *D. bleuzeni* is evolutionarily independent from its sister taxon, *D. trinidadensis*, supported by a data set that integrates information for both morphological and genetic variation. Furthermore, a strong physical barrier, an oceanic barrier, between the two sister taxa implies constant isolation. Therefore, they qualify as different species based on the general lineage concept (de Queiroz, 2007).

Type locality.— Morne Bleu, Trinidad (holotype in Institut de Recherches Entomologiques de la Caraïbe). This species of *Dynastes* beetle occurs on the islands of Trinidad and Tobago, and eight wild-collected individuals from the type locality, Morne Bleu, were included in the study by Huang and Knowles (2016). This is the only *Dynastes* species that can be found on these two Caribbean islands.

Diagnosis.— Body coloration and texture are similar between *D. bleuzeni* and *D. trinidadensis*, but individuals of *D. trinidadensis* tend to exhibit yellow instead of brown color. Olive and greenish elytra are common among historical museum collections of *D. trinidadensis*. Geographic origin is the best indicator for species identification, particularly

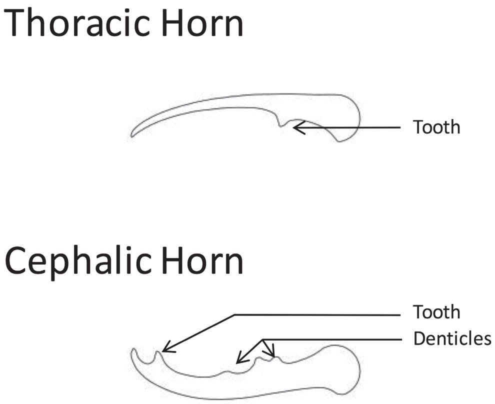


Figure 15— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes trinidadensis*. Digitized images from the following three individuals were used: **DhtT1**, Morne Bleu, Trinidad; **DhtJL1**, image of a wild major-male (130 mm) specimen from Morne Bleu, Trinidad in Lai and Ko (2008); **DhtHolotype**, image of the holotype.

for minor males and females. Elytral coloration and texture are different between *D. trinidadensis* and *D. reidi* from the islands of the Lesser Antilles.

Identification.— Body length ranges from 69 to 137 mm, while captive-bred individuals can grow to 145 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 13). Pronotal color is completely black. Elytral color is dark brown or olive. Some individuals exhibit a greenish hue on the elytra. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. Sometimes a layer of pubescence can be found on the elytra. A tooth can be found on either side of the thoracic horn. The position of the thoracic-horn tooth varies, where in major males it can be at the 1/4 to 1/3 positions from the base of the thoracic horn (Fig. 15). The thoracic-horn tooth is located at the base of the horn in minor males. Cephalic-denticle number ranges between 1 and 4 but is most often 2. Cephalic denticles can be either pointed or merged into a large slab-shaped denticle.

Distribution.— This is the only *Dynastes* species found on the islands of Trinidad and Tobago (Map 2). Its sister species, *D. bleuzeni*, is allopatrically distributed in mountain cloud-forest habitats southeast of the Orinoco delta. There are two other species of Giant Hercules beetles allopatrically distributed on islands of the Lesser Antilles (Martinique, Saint Lucia, Dominica, and Guadeloupe).

***Dynastes ecuatorianus* (Ohaus, 1913)**
New Status
Figures 16, 17
Map 2

Synonym.— *Dynastes hercules ecuatorianus* Ohaus, 1913: 131.

Taxonomic notes.— This taxon of Giant Hercules beetle is raised to full species status based on the study by Huang and Knowles (2016). Specifically, *D. ecuatorianus* has been shown to be evolutionarily independent from its sister taxa, *D. morishimai* and *D. paschoali*. It qualifies for full species status based on the general lineage concept (de Queiroz, 2007).

Type locality.— Rio Villano, Ecuador (lectotype in Museum für Naturkunde der Humboldt Universität, Berlin). Multiple newly collected individuals from near the type locality, specifically, Misahualli of Napo province, Yasuni National Park, and Loreto Canton of Orellana province from Ecuador, were included in the study by Huang and Knowles, (2016). The figures and descriptions provided by Chalumeau and Reid (2002) and Hwang (2011) are adequate for the identification of major males, and only major males from potential contact zones with *D. lichyi* were included in the study by Huang and (2016).

Diagnosis.— Body coloration and texture are unique in *D. ecuatorianus*, where brightly and lightly yellow-creamy elytra are distinct from other Giant Hercules beetles (Fig. 16). However, the body coloration does vary between individuals and can overlap with other taxa. Many captive-bred individuals of *D. morishimai* have been shown to exhibit similar bright-yellow coloration (Lai and Ko, 2008). Geographic origin is the best indicator for species identification, particularly for minor males and females. Cephalic-horn shape is significantly different between *D. ecuatorianus* and the parapatrically distributed *D. lichyi*. *Dynastes lichyi* exhibits a slab-shaped cephalic tooth with very often only one denticle, while a pointed cephalic tooth with often two denticles can be found on the dorsal side of male *D. ecuatorianus*.

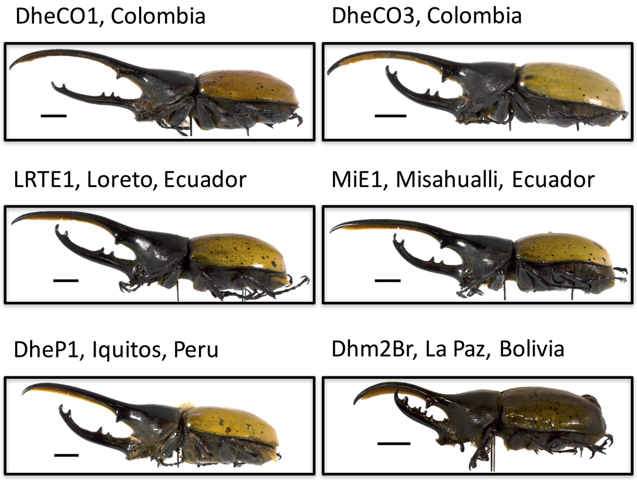


Figure 16— Exemplar specimens of *Dynastes ecuatorianus* and *Dynastes morishimai* (lower-right panel) vouchered in UMMZ.

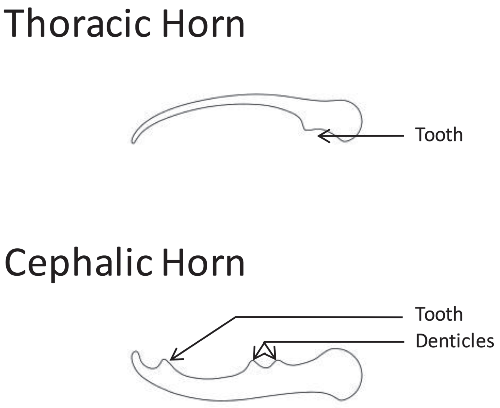


Figure 17— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes ecuatorianus*. Digitized images from the following 13 individuals were used: **DheCO1**, **DheCO3**, and **DheCO4**, Colombia; **DheE1**, **DheE2**, **MiE1**, **MiE2**, **MiE3**, and **MiE5**, Misahualli, Ecuador; **LRTE1**, Loreto, Ecuador; **DheP1**, **DheP5**, and **DheP6**, Iquitos, Loreto, Peru.

Identification.— Body length generally ranges from 65 to 145 mm, while captive-bred individuals may grow to 155 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 16). Pronotal color is completely black. Elytral color is often brightly yellow. Some individuals exhibit a brownish hue on the elytra. Elytra may have irregular black spots that vary in size and number. However, clear elytra without black spots can be found among *D. ecuatorianus* individuals. Elytra can be discolored black with high humidity. A tooth can be found on either side of the thoracic horn. The position of the thoracic-horn tooth varies, where in major males it can be at the 1/5 to 1/4 positions from the base of the thoracic horn (Fig. 16). The thoracic-horn tooth is located at the base of the horn in minor males. There are often two pointed cephalic denticles in major males (Fig. 17). Cephalic-denticle number, however, can range between 0 and 4; individuals with >4 cephalic denticles can also be found in rare cases (very often with two major large denticles and some small denticles). Some cephalic denticles may merge into large slab-shaped denticles.

Distribution.— This species occurs in rainforest regions from the eastern slopes of the Ecuadorian, Colombian, and Peruvian Andes. There is another *Dynastes* species that can be found in close proximity, *D. lichyi*, which prefers highland cloud-forest habitats. Its closely related taxa are found in geographically distant regions, *D. paschoali* in the Brazilian Atlantic forest and *D. morishimai* in the forest region of the Yungas in Bolivia.

***Dynastes morishimai* Nagai, 2002**

New Status

Figures 16, 18

Map 2

Synonym.— *Dynastes hercules morishimai* Nagai, 2002: 4.

Taxonomic notes.— This taxon of Giant Hercules beetle is raised to full species status based on the study by Huang and Knowles (2016). It is morphologically similar to, but genetically distinct from, its sister taxon, *D. ecuatorianus*. Its mitochondrial haplotypes are more closely related to taxa associated with highland cloud forests, i.e. *D. lichyi* and *D. occidentalis*, than to those that prefer lowland rainforest habitats (Appendix Fig. 8). This taxon merits distinct species status based on the unique evolutionary history of the examined individuals*.

*Only captive-bred individuals were studied by Huang and Knowles (2016)

Type locality.— Near Tipuani, La Paz, Bolivia (holotype in the Entomological Laboratory, Ehime University, Matsuyama). Only captive-bred individuals originating from near the type locality were included in the analysis by Huang and Knowles (2016). The original description by Nagai (2002) indicated that the holotype of this taxon was from an altitude of about 3000 m ASL The region near Tipuani, or the Guanay region, however, is at an elevation ranging from 500 to 1000 m (3000 feet) ASL Furthermore, an altitude of 3000 m in Bolivia characterizes a specific ecoregion of Andean Puna, which is not a typical forest habitat for *Dynastes* beetles. Because allopatric speciation and niche conservatism have been inferred in Hercules beetles as the main driver of speciation, the sister relationship between *D. morishimai* and *D. ecuatorianus* implies that *D. morishimai* may prefer similar rainforest habitats. The record of 3000 m is likely a misinterpretation of the collection data.

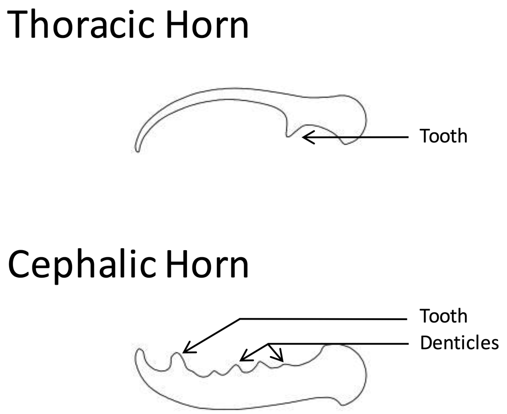


Figure 18— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes morishimai*. Digitized images from the following two individuals were used: **Dhm2Br**, a captive-bred sample from La Paz, Bolivia; **DhmType**, holotype image from Nagai (2002).

Diagnosis.— The specific cephalic-horn shape, a stout horn with many denticles, can be distinguished from that of its sister taxon, *D. ecuatorianus*. However, captive-bred individuals can exhibit similar phenotypes between *D. morishimai* and *D. ecuatorianus* (Lai and Ko, 2008). Geographic origin is the best identifier for this species.

Identification.— Body length generally ranges from 60 to 120 mm, while captive-bred individuals can reach up to 145 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 16). Pronotal color is completely black. Elytral color is light yellow in the holotype. However, captive-bred individuals often exhibit brightly yellow elytra, which is similar to the typical elytral color of *D. ecuatorianus* (Lai and Ko, 2008 and Hwang, 2011). Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. A tooth can be found on either side of the thoracic horn. The position of the thoracic-horn tooth varies, where in major males it can be at the 1/5 to 1/4 positions from the base of the thoracic horn. The number of cephalic denticles varies. A pointed tooth can be found on the dorsal side of the cephalic horn (Fig. 18).

Distribution.— This species occurs in the lower rainforest/cloud-forest regions of the Bolivian Yungas (500 to 1000 m ASL).

***Dynastes paschoali* Grossi and Arnaud, 1993**
New Status

Figure 19

Map 2

Synonym.— *Dynastes hercules paschoali* Grossi and Arnaud, 1993: 13.

Taxonomic notes.— This taxon of Giant Hercules beetle is raised to full species status based on the study by Huang and Knowles (2016)*. It is morphologically and genetically distinct from all other Giant Hercules taxa and is found in geographically isolated regions beyond the reach of other Giant Hercules beetles.

*Only captive-bred individuals were included in Hang and Knowles (2016)

Type locality.— Anchieta, Espirito Santo, Brazil (holotype in E. J. Grossi personal collection; paratypes in Museu Nacional do Rio de Janeiro and Museu de Zoologia de São Paulo). Only captive-bred individuals were included in the analysis by Huang and Knowles (2016). The tissues from individuals were obtained from a world-renowned *Dynastes* beetles breeder, J. Lai (two progeny originating from beetles bred by two famous Japanese breeders [detail information can be retrieved from dryad doi:10.5061/dryad.8p6m0]). This is the only *Dynastes* species that lives in the Brazilian Atlantic forest.

Diagnosis.— The specific cephalic-horn shape, a complete lack of cephalic denticles, is distinct from all other giant Hercules beetles (this trait state also occurs rarely in *D. ecuatorianus* and *D. lichyi*). The geographic origin can be used to definitively identify this species.

Identification.— Body length ranges from 85 to 144 mm, while captive-bred males can reach up to almost 150 mm (from the apex of the elytra to the apex of the thoracic horn; J. Lai, personal communication). Pronotal color is completely black. Elytral color is often light yellow and brown. Some individuals exhibit a greenish hue on the elytra. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. A tooth can be found on either side of the thoracic horn. The position of the thoracic-horn tooth varies, where in major males it can be at the 1/3 position from the base of the thoracic horn (Fig. 19). The thoracic-horn tooth is located at the base of the horn in minor males. There is no cephalic denticle in males (Fig. 19), but in rare cases, small punctuations may exist on the dorsal side of the cephalic horn of males. The cephalic tooth is pointed.

Distribution.— This species is endemic to the Brazilian Atlantic Forest (states of Bahia and Espirito Santo). It is completely geographically isolated from all other giant Hercules beetles.

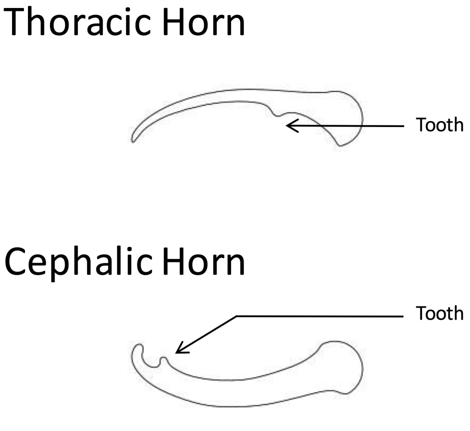


Figure 19— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes paschoali*. Digitized images from three type specimens were used: **DhpHolotype**, holotype image; **DhpParatype1** and **DhpParatype2**, images of two paratypes. All images of the types were retrieved from Lai and Ko (2008).

***Dynastes lichyi* Lachaume, 1985**
New Status

Figures 20, 21

Map 2

Synonym.— *Dynastes hercules lichyi* Lachaume, 1985: 21.

Taxonomic notes.— This taxon of Giant Hercules beetle is raised to full species status based on the study by Huang and Knowles (2016). It is morphologically and genetically distinct from all other Giant Hercules taxa, including taxa that can be found in geographic proximity. Reproductive isolation may have been established between *D. lichyi* and its parapatrically distributed species.

Type locality.— Rancho Grande, Venezuela (holotype in Muséum national d'Histoire naturelle, Paris). Specimens from the type locality (Venezuela) were not available in the study by Huang and Knowles (2016). However, this is the only *Dynastes* species that prefers cloud-forest habitats along the eastern slopes of the Ecuadorian and Peruvian Andes, and major adult males of this species exhibit a specific horn shape, which can be easily identified based on the figures and descriptions provided by Chalumeau and Reid (2002) and Hwang (2011). Recently collected wild specimens from this species were included in the study by Huang and Knowles, (2016).

Diagnosis.— The specific cephalic-horn shape, a slab-shape tooth near the apex, can be used to distinguish major males of *D. lichyi* and *D. occidentalis* from males of all other Giant Hercules taxa (Figs. 29, 31). The relative position of the tooth along the thoracic horn can be used to confidently distinguish *D. lichyi* from *D. occidentalis* (Fig. 28). The position of the thoracic-horn tooth is at the 1/3 position from the base of the thoracic horn in major males of *D. lichyi*, while it is always at the very basal position in *D. occidentalis* for both major and minor males.

Figure 20— Exemplar specimens of *Dynastes lichyi* vouchered in UMMZ. Scale bar = 1 cm.

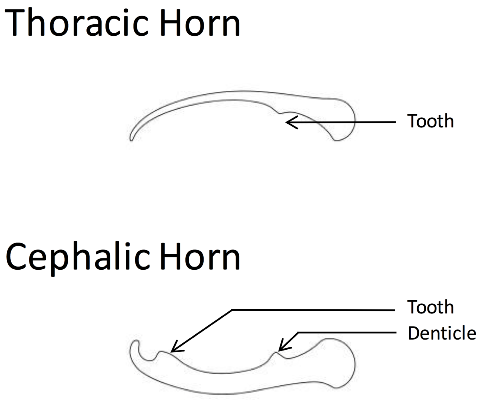


Figure 21— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes lichyi*. Digitized images from the following 17 individuals were used: **DhlCO1**, **DhlCO4**, and **DhlCO7**, La Bellela, Santander, Colombia; **DhlE3**, **DhlE4**, **CSGL3**, **CSGL5**, and **CSGL6**, Cosanga, Napo, Ecuador; **ERVL1** and **ERVL3**, El Reventador, Ecuador; **SCL1** and **SCL3**, Santa Clara, Ecuador; **SPBL2** and **SPBL3**, San Pablo, Napo, Ecuador; **DhlP2**, Selva Central, Peru; **DhlP5**, Satipo, Junin, Peru; **DhlType**, image of the type specimen in Hwang (2011).

Identification.— Body length ranges from 60 to 170 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 20). Medium-sized to large (120 to 145 mm) major males can be found frequently in this species. Pronotal color is completely black. Elytral color is highly variable but most often ranges from brown to dark brown. Few individuals exhibit a greenish hue on the elytra. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. A tooth can be found on either side of the thoracic horn. The position of the thoracic-horn tooth varies, where in major males it can be at the 1/3 position from the base of the thoracic horn (Fig. 21). The thoracic-horn tooth is located at the base of the horn in minor males. Sometimes males have reduced thoracic teeth, and in rare occasions, there can be an absence of a thoracic tooth. There is often one pointed or slab-shaped cephalic denticle in major males; however, cephalic-denticle number can range between 0 and 2. The tooth near the apex of the cephalic horn is slab-shaped (Figs. 20, 21). The apex of the cephalic horn curves inward and forms a hook.

Distribution.— This species occurs in highland cloud-forest regions along the eastern slopes of the Ecuadorian and Peruvian Andes (Map 2). It is also found in highland cloud forests of the Colombian and Venezuelan Andes (generally 500–2000 m ASL). Two distantly related Giant Hercules taxa, *D. ecuatorianus* and *D. occidentalis*, can be found in parapatry with *D. lichyi*. However, *D. ecuatorianus* prefers lowland rainforest habitats, while *D. occidentalis* is endemic to the wet Chocó ecoregion on the western slopes of the Colombian and Ecuadorian Andes.

***Dynastes occidentalis* Lachaume, 1985**

New Status

Figures 22, 23

Map 2

Synonym.— *Dynastes hercules occidentalis* Lachaume, 1985: 20.

Taxonomic notes.— This taxon of Giant Hercules beetle is raised to full species status based on the study by Huang and Knowles (2016). It is morphologically and genetically distinct from all other Giant Hercules taxa, except its sister taxon, *D. septentrionalis*. The two sister taxa differ significantly in genetic data and cephalic-horn shape, but the thoracic-horn shape is similar between them.

Type locality.— Cali, Colombia (holotype in Muséum national d'Histoire naturelle, Paris). No sample from near the type locality (Colombia) were available for examination. However, this is the only *Dynastes* species that lives in the Chocó ecoregion along the western slopes of the Colombian and Ecuadorian Andes, and major adult males of this species have a specific horn morphology, which can be easily identified based on the figures and descriptions provided by Chalumeau and Reid (2002) and Hwang (2011). Newly collected wild specimens were included in the study by Huang and Knowles (2016).

Diagnosis.— See Diagnosis in *D. lichyi* for details.

Identification.— Body length generally ranges from 70 to 140 mm, while captive-bred individuals can grow to 150 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 22). Pronotal color is completely black. Elytral color is most often dark brown, but individuals with light-yellow and light-green elytral coloration can also be found. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity.

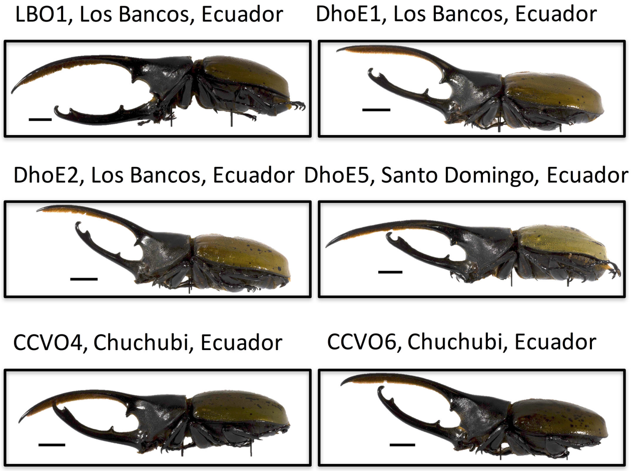

LBO1, Los Bancos, Ecuador
DhoE1, Los Bancos, Ecuador
DhoE2, Los Bancos, Ecuador
DhoE5, Santo Domingo, Ecuador
CCVO4, Chuchubi, Ecuador
CCVO6, Chuchubi, Ecuador

Figure 22— Exemplar specimens of *Dynastes occidentalis* vouchered in UMMZ. Scale bar = 1 cm.

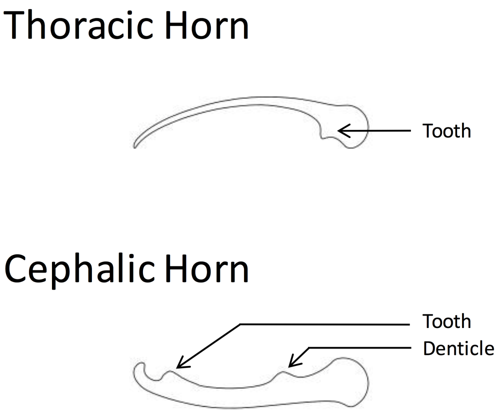


Figure 23— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes occidentalis*. Digitized images from the following eight individuals were used: **CCVO2**, **CCVO4**, and **CCVO6**, Chuchuvi, Esmeraldas, Ecuador; **DhoE1**, **DhoE2**, and **LBO1**, Los Bancos, Pichincha, Ecuador; **DhoType**, image of the type specimen in Hwang (2011).

A tooth can be found on either side of the thoracic horn. The thoracic-horn tooth is located at the base of the horn in both major and minor males (Figs. 22, 23). There is one pointed or slab-shaped cephalic denticle in major males; slab-shaped cephalic denticles may represent a merger of two closely located denticles. The tooth near the apex of the cephalic horn is often slab-shaped but can also be pointed (Fig. 23). The apex of the cephalic horn may curve inward and form a hook.

Distribution.— This species occurs in the Chocó cloud forest along the western slopes of the Colombian and Ecuadorian Andes (Map 2). A distantly related Giant Hercules taxon, *D. lichyi*, can be found in geographic proximity based on species distribution models (Fig. S3b in Huang and Knowles, 2016). Empirically, *D. lichyi* is rarely found in forests on the western slope of the Andes. There is evidence for niche divergence between these two species (Huang and Knowles, 2016). The sister taxon, *D. septentrionalis*, is distributed allopatrically in cloud-forest regions of Central America.

Dynastes septentrionalis Lachaume, 1985
New Status
Figures 24, 25
Map 2

Synonym.— *Dynastes hercules septentrionalis* Lachaume, 1985: 19.

Taxonomic notes.— This taxon of Giant Hercules beetle is raised to full species status based on the study by Huang and Knowles (2016). It is morphologically and genetically distinct from all other Giant Hercules taxa, except from its sister taxon, *D. occidentalis*. The two sister taxa differ significantly in genetic data and cephalic-horn shape, but the thoracic-horn shape is similar between them (Fig. 29). *D. septentrionalis* individuals included in Huang and Knowles (2016), which were from Costa Rica, Guatemala, Honduras, and Panama, have very closely related mitochondrial haplotypes (supplementary COI tree in Huang and Knowles, 2016 [dryad doi:10.5061/dryad.8p6m0]; Appendix Fig. 8). The *D. septentrionalis* mitochondrial haplotypes form a monophyletic lineage and are distantly related to all other Giant Hercules beetles.

Type locality.— Quixal, Alta Verapaz, Guatemala (holotype in Muséum national d'Histoire naturelle, Paris). Individuals from near the type locality (Guatemala) were examined by Huang and Knowles (2016). This is the only Giant Hercules species that lives in Central America and can be easily identified based on the figures and descriptions provided by Chalumeau and Reid (2002) and Hwang (2011).

Diagnosis.— The unique thoracic-horn shape, the position of the thoracic tooth, can distinguish *D. septentrionalis* from all other Giant Hercules beetles, except its sister taxon (Fig. 28). The cephalic-horn shape and body coloration in combination can be used to distinguish *D. septentrionalis* from *D. occidentalis*. Nevertheless, geographic origin is the best diagnosis to identify this species (except for individuals from eastern Panama, Chalumeau and Reid, 2002).

Identification.— Body length generally ranges from 70 to 140 mm (from the apex of the elytra to the apex of the thoracic horn; Fig. 24). Pronotal color is completely black. However, in rare cases, the pronotum may show a dark reddish hue. Elytral color can be dark brown or dark and light green. Elytra often have irregular black spots that vary in size and number. Elytra can be discolored black with high humidity. A tooth can be found on either side of the thoracic horn. The thoracic-horn tooth is located at the base of the

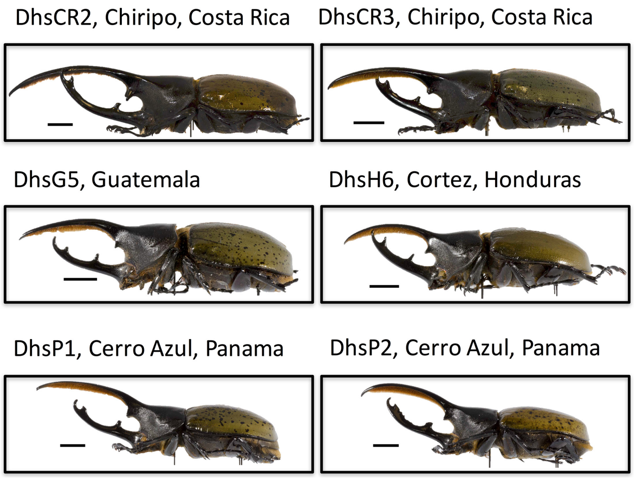


Figure 24— Exemplar specimens of *Dynastes septentrionalis* vouchered in UMMZ. Scale bar = 1 cm.

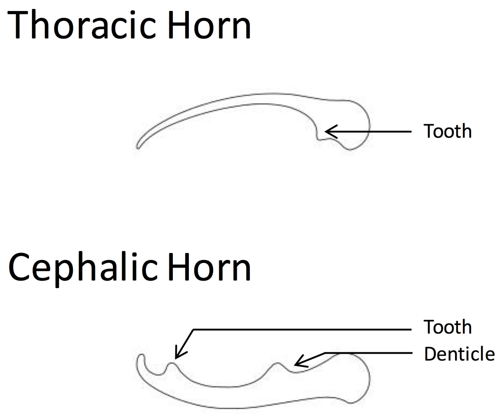


Figure 25— The estimated mean shapes of thoracic- and cephalic-horn outlines of *Dynastes septentrionalis*. Digitized images from the following 10 individuals were used: **DhsCR1**, **DhsCR2**, and **DhsCR3**, near Chiripo, Costa Rica; **DhsG5**, Finca La Firma, Sierra de Coral, Guatemala; **DhsH6**, El Cusuco, Honduras; **DhsP1** and **DhsP2**, Cerro Azul, Panama; **DhsPJL1** and **DhsPJL2**, images of two wild specimens from Chiriqui, Panama in Lai and Ko (2008); **DhsHolotype**, image of the type specimen in Hwang (2011).

horn in both major and minor males (Fig. 25). There is often one pointed or slab-shaped cephalic denticle in major males; however, the number and shape of cephalic denticles can vary greatly among individuals (Fig. 24). The shape of the cephalic tooth also varies among individuals.

Distribution.— This species occurs in cloud-forest regions of Central America (Map 2). A White Hercules taxon, *D. maya*, can be found in geographic proximity, parapatric with *D. septentrionalis*, but prefers a lowland rainforest type of habitat. The sister taxon, *D. occidentalis*, is distributed allopatrically in the Chocó ecoregion.

DISCUSSION

Phylogenetic relationships are discussed in the following section based on the reconstructed molecular species tree using the new SNP data from Illumina sequencing (specifically, the result from the SVDQuartets analysis; Fig. 35). The relationships between pairs of beetle species based on the study by Huang and Knowles (2016) are summarized by their positions along the speciation continuum following the biological species concept (Mayr, 1963). I also used the classical taxonomic hierarchies by Mayr (1963) and Amadon (1966) to categorize the relationships between species based on the observed divergences in genetic, phenotypic, and ecological axes and on the predicted amount of geographic overlap (Fig. 36).

THE WHITE HERCULES BEETLES

Sister relationships were found between *D. granti* and *D. hyllus* and between *D. maya* and *D. moroni*. Sister taxa are allopatric and tend to prefer similar ecological conditions (Huang and Knowles, 2016). For example, *D. granti* and *D. hyllus* are more frequently encountered in subtropical mountainous regions (frequently >1000 m ASL), while *D. maya* and *D. moroni* live in tropical rainforests (about 500 m ASL). Furthermore, body coloration, which is hypothesized to serve as camouflage in *Dynastes* beetles (Hinton and Jarman, 1973), is similar between sister taxa and dissimilar between distantly related taxa (Figs. 1, 3, 5). *Dynastes tityus* is sister to the lineage leading to *D. maya* and *D. moroni*, which are geographically separated from *D. tityus* by the Gulf of Mexico (Map 1, Fig. 35).

1. Allopatric sister taxa that are genetically completely divergent.

Dynastes granti versus *D. hyllus*: The phenotypic data for this pair of sister taxa do not significantly support divergence (in fact, very weakly; Fig. S7 in Huang and Knowles, 2016; also Table 2 and Fig. 30). The molecular and combined data sets, though, suggest that each of the two taxa forms an evolutionarily independent lineage, with absolute supports (Fig. 5, S6 in Huang and Knowles, 2016). Models of species distribution indicate little possibility for this pair of sister taxa to meet in sympatry, because uninhabitable geographic areas separate them (Fig. S3a in Huang and Knowles, 2016). Continuous geographic isolation should therefore be a common norm before further evolution in environmental requirements occurs for either species (or major changes in climatic conditions occur that may lead to shifts in their geographic distributions). Differences in the environmental conditions between predicted habitats for *D. granti* and *D. hyllus* can be explained by geographic autocorrelation. These geographic species have acquired enough genetic differences to be confidently delimited using neutral molecular data (i.e. different evolutionary lineages

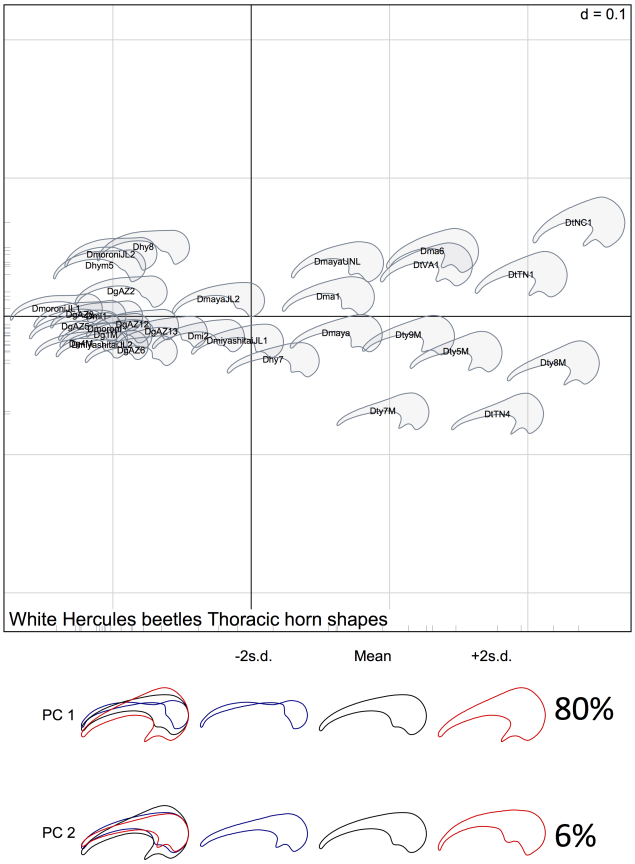


Figure 26— Results of the PCA of the outline data for White Hercules beetle thoracic horns. Information for each sample can be found in previous figure legends.

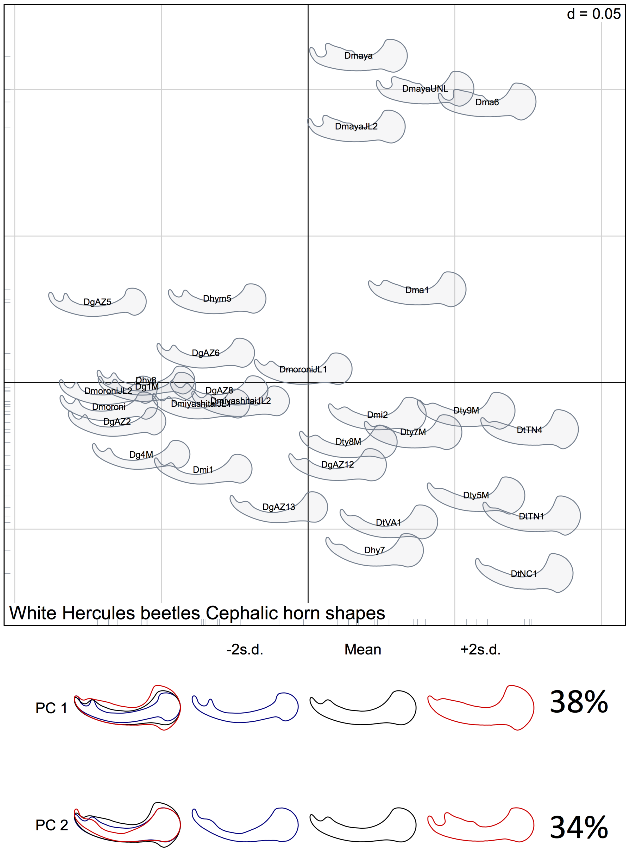


Figure 27— Results of the PCA of outline data for White Hercules beetle cephalic horns. Information for each sample can be found in previous figure legends.

that merit species status), so they may represent allopatric sibling species or two semispecies that form a superspecies by the biological species concept even though they are still highly similar morphologically (Mayr, 1942, 1963).

2. Allopatric morphological taxa with historical geographic overlap.

Dynastes maya versus *D. moroni*: These two sister taxa are allopatrically distributed, but models of species distribution indicate that they could have been sympatric during the last glacial maximum (LGM), when their habitats were connected by highly suitable areas (Fig. 3, Fig. S3a in Huang and Knowles, 2016). They can also live in the same region if rare introductions are made, because the predicted suitable habitats of *D. maya* covers the current distribution of *D. moroni* and the two species share significant niche conservatism (Huang and Knowles, 2016). Molecular data strongly support (98% of the time) their evolutionary independence, and the phenotypic data indicate that they are completely distinct (Figs. S6, S7 in Huang and Knowles, 2016). They may be an example of two allospecies that comprise a superspecies, because they are evolutionarily independent lineages that are morphologically too divergent to be classified under the same species (Mayr, 1942; Silvester-Bradley, 1954; Amadon, 1966, 1968). If the LGM geographic overlap is taken into account, these two taxa can be recognized as completely divergent biological species, because their evolutionary uniqueness has been well maintained even though they encountered each other in the past.

3. Allopatric taxa with absolute divergences in all possible axes.

Dynastes tityus versus *D. maya*: *Dynastes tityus* has a unique morphology (Figs. 26, 27, 30) and is evolutionarily independent from all other taxa in the White Hercules group (Fig. 5 in Huang and Knowles, 2016). The Gulf of Mexico, which lies between *D. tityus* and taxa of its sister lineage, e.g. *D. maya*, is neither inhabitable nor permeable to the Hercules beetles. Evidence also suggests significant niche divergence between subtropical *D. tityus* and species of its sister lineage from tropical Central America (Fig. 3 in Huang and Knowles, 2016). *Dynastes tityus*, which has been evolving along its own distinct path, has therefore remained and will continue to remain in geographically isolated regions far from all other congeneric species. It represents a good biological species.

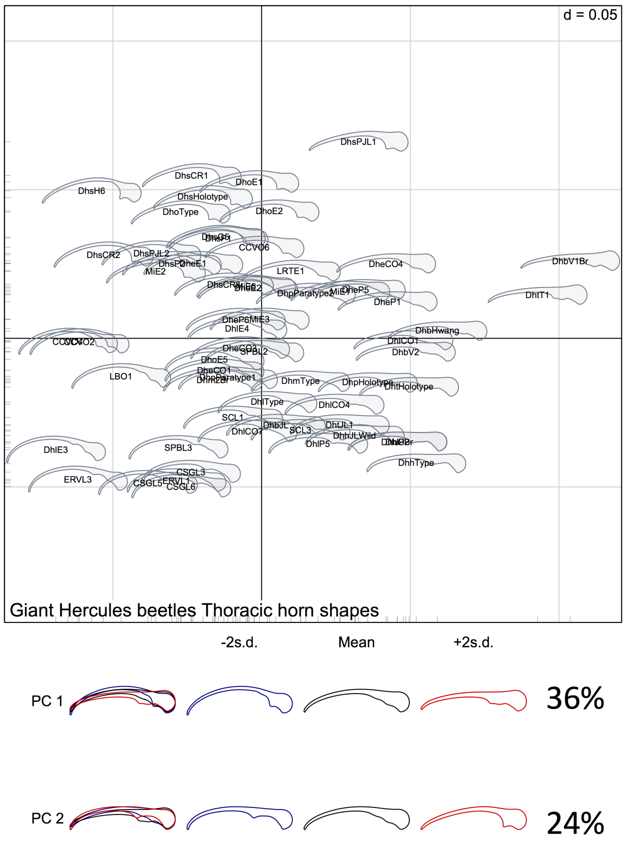


Figure 28— Results of the PCA of the outline data for Giant Hercules beetle thoracic horns. Information for each sample can be found in previous figure legends.

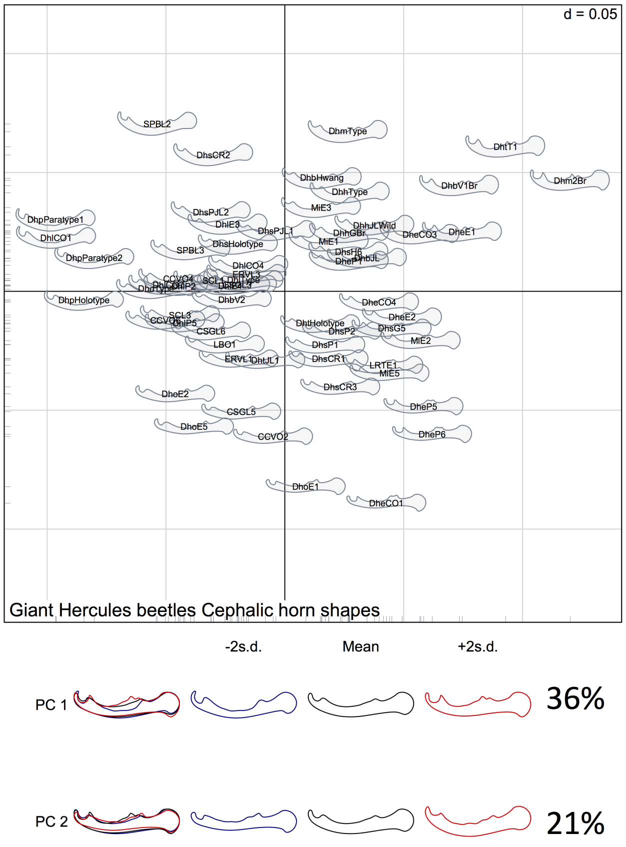


Figure 29— Results of the PCA of the outline data for Giant Hercules beetle cephalic horns. Information for each sample can be found in previous figure legends.

4. Parapatric taxa with absolute divergences based on phenotypic and molecular evidence.

Dynastes hyllus versus *D. maya*: These species are evolutionarily independent entities from evolutionarily distantly related lineages supported by both molecular and phenotypic data and have extensive predicted geographic overlaps, both currently and historically (Fig. S3a in Huang and Knowles, 2016). These two taxa have also been found in geographic proximity (Hardy, 2003). This level of interspecific divergence represents the late phase along the speciation continuum between biological species (Mayr, 1942, 1963), where reproductive isolation must have been established to help maintain the differences between taxa living in a possible contact zone.

THE GIANT HERCULES BEETLES

Sister relationships were found between *D. reidi* and *D. hercules* and between *D. bleuzeni* and *D. trinidadensis*. Together these four species form a Lesser Antilles clade (Fig. 35). Bootstrap support for a Lesser Antilles clade, however, is only moderate (> 70%). These pairs of sister taxa are allopatric and isolated by oceanic barriers. *D. bleuzeni* and *D. trinidadensis* have mitochondrial haplotypes that are closely related to that of *D. ecuatorianus* (Appendix Fig. 8). Another pair of sister taxa, *D. septentrionalis* and *D. occidentalis*, are isolated geographically by the Isthmus of Panama. They together form an ecoregional clade from Central American to Chocó-Darién. *Dynastes ecuatorianus*, *D. paschoalis*, and *D. morishimai* form a tri-species Amazonian lineage and are isolated from one another by largely unsuitable habitats. The concatenated maximum-likelihood analysis does not support a within-group relationship among the three Amazonian rainforest species, but a sister relationship between *D. morishimai* and *D. ecuatorianus* is strongly supported by genomic data and a newly developed coalescent model of species-tree reconstruction (note, however, that *D. morishimai* has mitochondrial haplotypes that are nested within that of *D. lichyi*; Appendix Fig. 8). The phylogenetic analysis suggests that *D. lichyi* is close to the Amazonian tri-species lineage, but this reconstructed relationship is on moderately supported (>70%, Fig. 35).

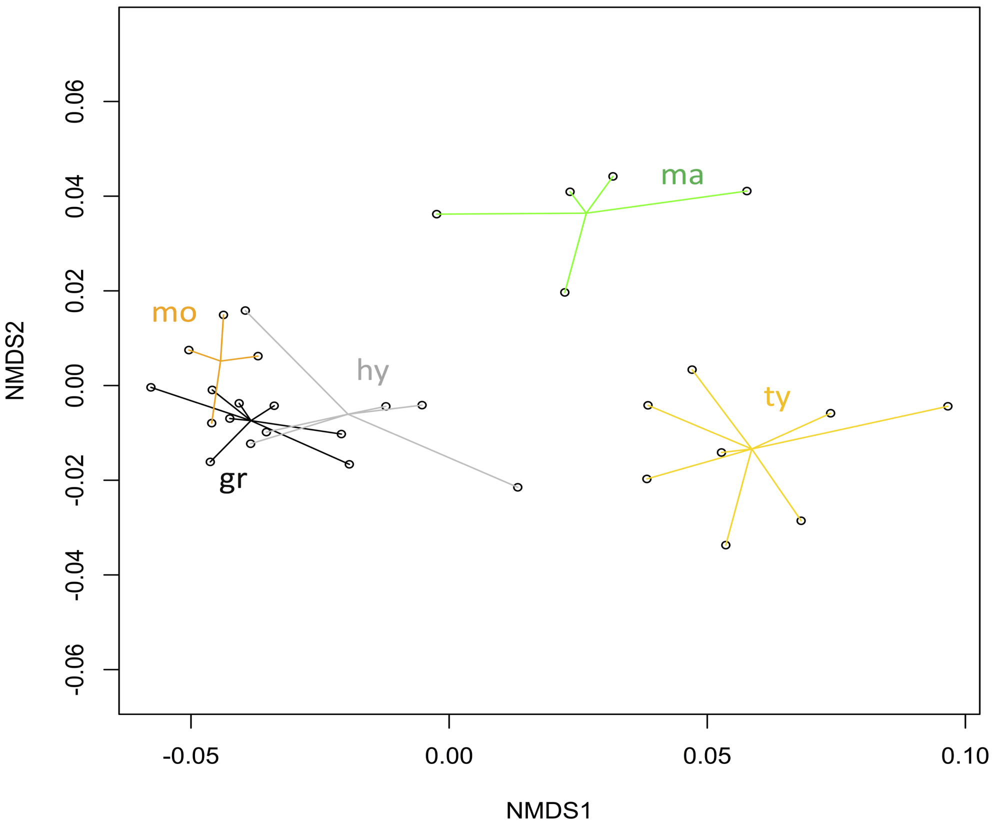


Figure 30— Result of the NMDS plot based on the PC1 and PC2 values extracted from the outline analyses using thoracic- and cephalic-horn shapes of White Hercules beetles (stress = 0.07718366). Significant groupings were obtained by 1000 permutations using a MANOVA based on Euclidean distances between individuals ($P < 9.99 \times 10^{-5}$; see Tables 1, 2 for details). gr: *Dynastes granti*, hy: *Dynastes hyllus*, ma: *Dynastes maya*, mo: *Dynastes moroni*, ty: *Dynastes tityus*.

1. Allopatric sister taxa.

Dynastes bleuzeni versus *D. trinidadensis*: These two taxa are highly evolutionarily independent, but the molecular and phenotypic data do not absolutely support their divergence. The combined data set, although strongly supporting this sister-taxa split (ca. 90% posterior probability), also does not absolutely support their divergence (Huang and Knowles, 2016). The only clear separation is their geographic distributions, which are isolated by an oceanic barrier (Map 1). They may therefore represent distinct geographic taxa at the initial stage of speciation driven by geographic isolation (they can be identified as subspecies following Mayr, 1942, 1963).

2. Allopatric sister taxa that are genetically completely divergent.

Dynastes ecuatorianus versus *D. morishimai*: The phenotypic data alone do not absolutely support their divergence (Fig. S7 in Huang and Knowles, 2016), but the combined phenotypic and molecular data sets suggest that each taxon forms an evolutionarily independent lineage (Huang and Knowles, 2016). A predicted geographic distribution for *D. morishimai* is not available due to the scarcity of occurrence data. The predicted distribution of *D. ecuatorianus* does not overlap the current range of *D. morishimai*. *D. ecuatorianus* and *D. morishimai* may thus represent two semispecies that are evolutionarily independent lineages that have diverged completely but still very highly similar to each other morphologically.

Dynastes septentrionalis versus *D. occidentalis*: The phenotypic data do not absolutely support their divergence (Fig. S7 in Huang and Knowles, 2016), but the molecular and combined data sets suggest that each taxon forms an evolutionarily independent lineage (Huang and Knowles, 2016). Additionally, *D. septentrionalis* samples have mitochondrial haplotypes that form a basal monophyletic lineage to the other Giant Hercules beetles, including *D. occidentalis*, which has mitochondrial haplotypes that are most closely related to *D. lichyi* (Appendix Fig. 8). Models of species distribution suggest past and present low possibilities of sympatry. Continuous geographic isolation should therefore be common before further evolution in environmental requirements occurs in either species. *Dynastes septentrionalis* and

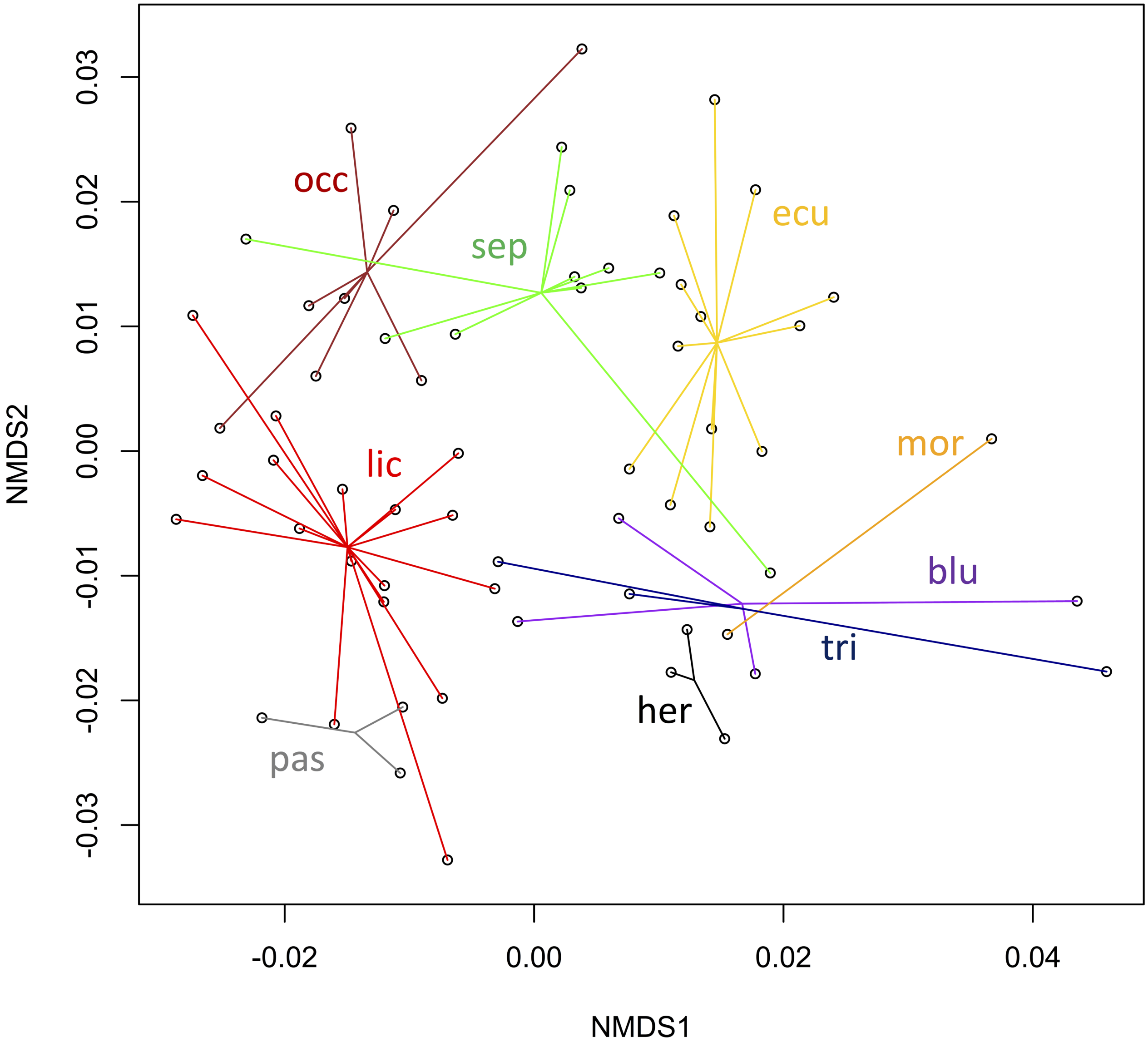


Figure 31— Results of the NMDS plot based on the PC1 and PC2 values extracted from the outline analyses using thoracic- and cephalic-horn shapes of Giant Hercules beetles (stress = 0.1949462). Significant groupings were obtained by 1000 permutations using a MANOVA based on Euclidean distances between individuals ($P < 1\times10^{-5}$; see Table 3, 4 for details). Note that individuals of *Dynastes reidi* are not included in this analysis because of their unique horn allometry. blu: *Dynastes bleuzeni*, ecu: *Dynastes ecuatorianus*, her: *Dynastes hercules*, lic: *Dynastes lichyi*, mor: *Dynastes morishimai*, occ: *Dynastes occidentalis*, pas: *Dynastes paschoali*, sep: *Dynastes septentrionalis*, tri: *Dynastes trinidadensis*.

D. occidentalis may thus also represent two semispecies that together form a superspecies.

Dynastes reidi versus *D. hercules*: The genetic data statistically support the divergence between these two taxa. The difference in horn allometry (*D. reidi* males never develop major horn phenotypes) clearly implies that these two species are under distinct selective pressures (cf. Hinton and Jarman, 1974). The current geographic distribution (isolated islands of the Lesser Antilles) also indicates that geographic overlap may have been rare. These two taxa are morphologically distinct, so they may represent two allospecies of a superspecies (Mayr, 1942; Silvester-Bradley, 1954; Amadon, 1966, 1968).

3. Allopatric taxa with absolute divergences in all possible axes.

Dynastes paschoali versus the other Amazonian Hercules beetles (*D. ecuatorianus* and *D. morishimai*): *Dynastes paschoali* has a unique and distinct horn morphology and

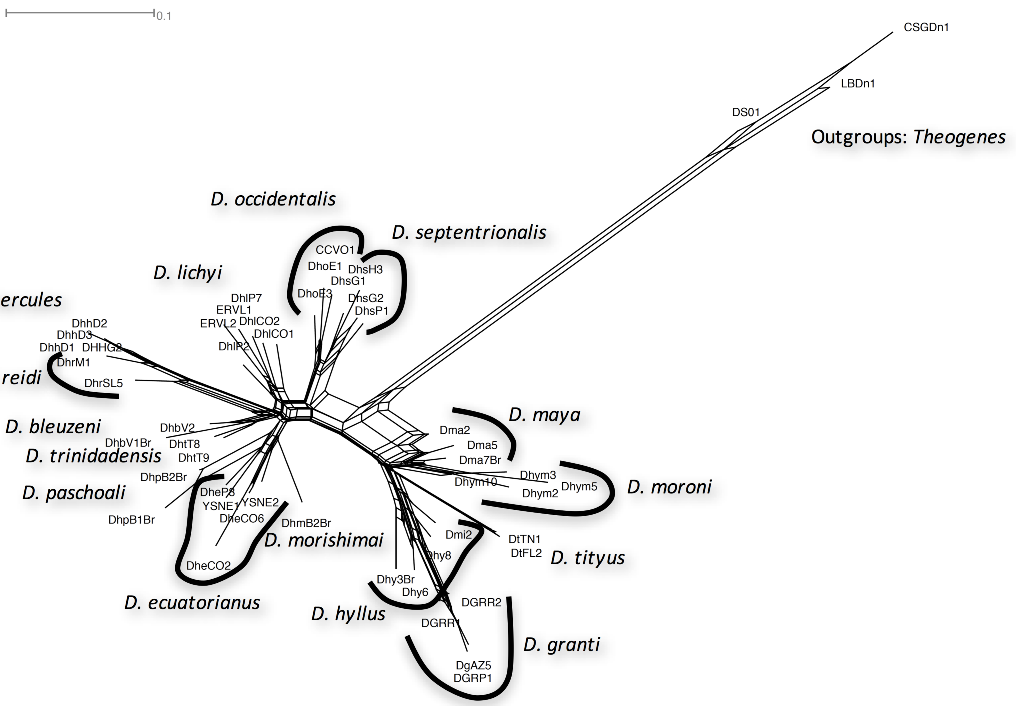


Figure 32— A Neighbor-Net based on LogDet distance using the concatenated SNP data set.

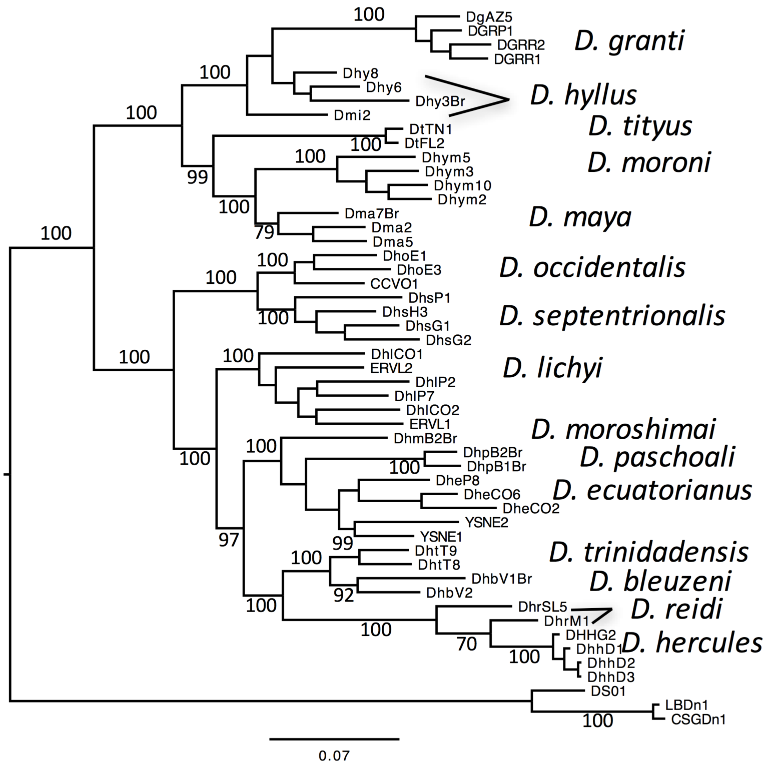


Figure 33— A maximum-likelihood tree for *Dynastes* Hercules beetles estimated from 43,205 concatenated unlinked SNPs. Numbers next to nodes are bootstrap support values (100 replicates). Individuals from paraphyletic species are indicated by solid lines. Collection information about each sample can be found in the appendix (molecular data examined).

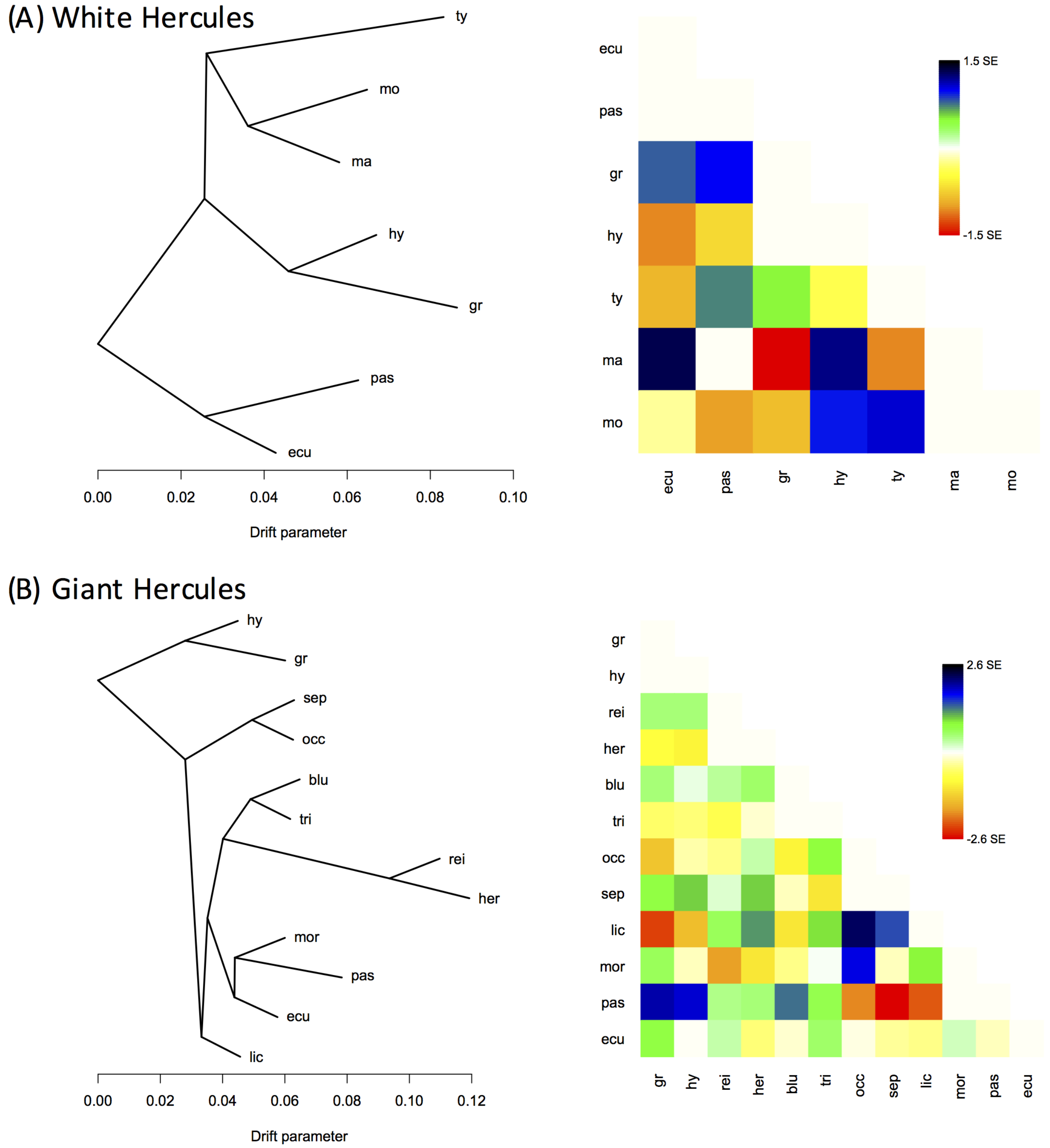


Figure 34— Maximum-likelihood species phylogenies (left panels) and the residuals from the phylogenies (right panels) for (A) the White Hercules and (B) the Giant Hercules beetles. The residuals of the ML analyses show how well the phylogenetic tree represents the molecular data set. High values of residuals, for example those in dark blue, indicate that the pair of species compared may be phylogenetically more closely related to each other than explained by the ML phylogeny (may be caused for example by post-divergence gene flow, e.g. between hy and ma and between lic and occ). The species abbreviations are defined in Figs. 30, 31 and the appendix.

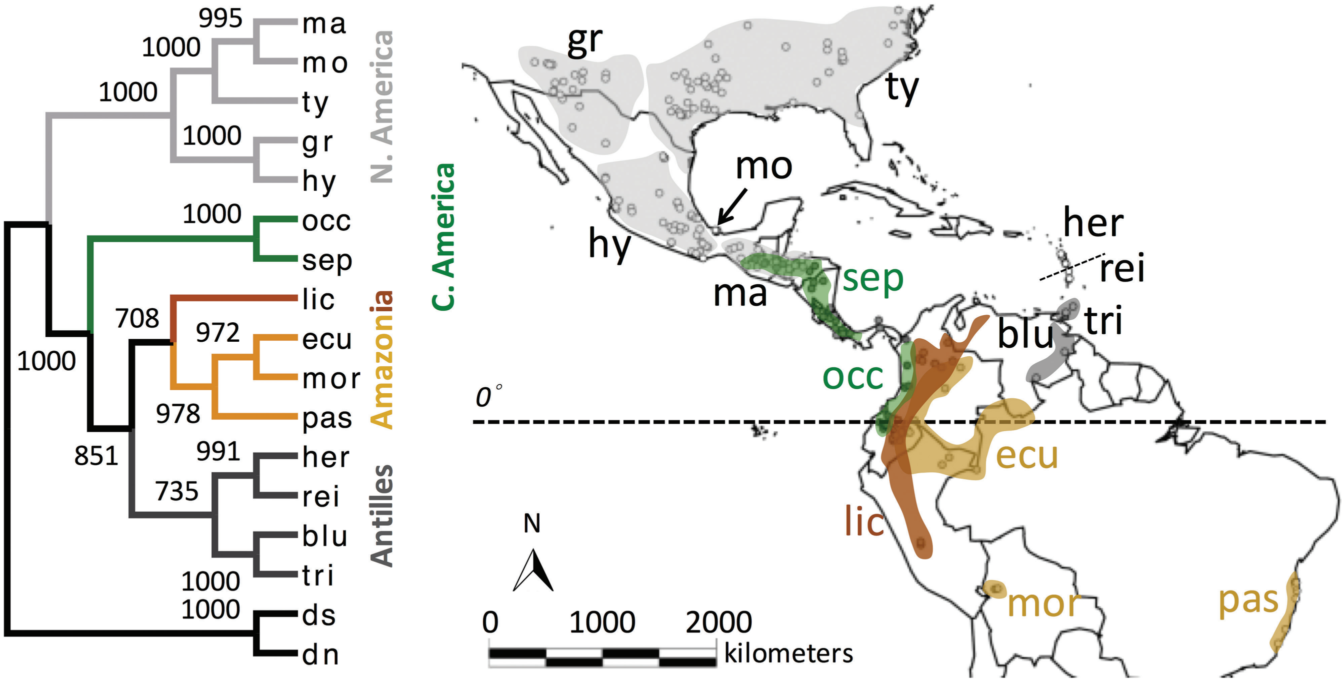


Figure 35— The *Dynastes* beetle species tree reconstructed using 43,205 unlinked SNPs via SVDQuartets. Geographic clades are identified by differently colored branches and highlighted geographic areas. Numbers on the branches indicate bootstrap supports from 1000 bootstrap replicates. The species abbreviations are defined in Figs. 30, 31 and the appendix.

is evolutionarily independent of the other taxa in its sister lineage. The geographic area separating *D. paschoali* from the nearest Amazonian Giant Hercules beetle, *D. ecuatorianus*, is savanna (Cerrado), which is uninhabitable for Hercules beetles. *D. paschoali* has therefore been evolving along its own distinct path and has been and will remain in a geographically isolated region far from all other congeneric species. It can be recognized as a distinct biological species.

4. Parapatric taxa with absolute divergences based on phenotypic and molecular evidence.

Dynastes ecuatorianus versus *D. lichyi* and *D. occidentalis* versus *D. lichyi*: These taxa belong to distantly related lineages, and their evolutionary independence from other Giant Hercules species is supported by both molecular and phenotypic data (Huang and Knowles, 2016). The predicted geographic ranges between *D. lichyi* and *D. ecuatorianus* and between *D. lichyi* and *D. occidentalis* widely overlap both currently and historically (Huang and Knowles, 2016). These pairs of taxa can be found in geographic proximity (e.g. Huang, 2012). Their relationships fit the definition of completely divergent biological species well (Mayr, 1942, 1963), where reproductive isolation must have been established to help maintain the differences between these taxa in geographic proximity.

THE SPECIATION CONTINUUM AND SPECIES DELIMITATION IN HERCULES BEETLES

Speciation can be a continuous process, and divergence between evolutionary lineages can occur and accumulate at different rates along different possible axes (e.g. phenotypic and genetic; de Queiroz, 2007). Studies that use different types of data to determine species delimitation may come to different conclusions, but even studies that use the same data may produce different species designations due to the application of different species concepts (de Queiroz, 2007). The study of the system of *Dynastes* beetles is no exception.

If species are defined as evolutionarily independent lineages, then recent divergences supported only by molecular data can be used as yardsticks to define species-level divergence (e.g. those highlighted in blue in Fig. 36). If only (potential) reproductive isolation is used as evidence for designating different species, however, then divergence between sympatric/parapatric species should be used as a yardstick to define species-level divergence (e.g. those highlighted in red in Fig. 36). Defining species in the system of Hercules beetles is highly uncertain, i.e., there is a wide range of species designation gray zone (Fig. 36, also see discussion in de Queiroz, 2007). I acknowledge that my

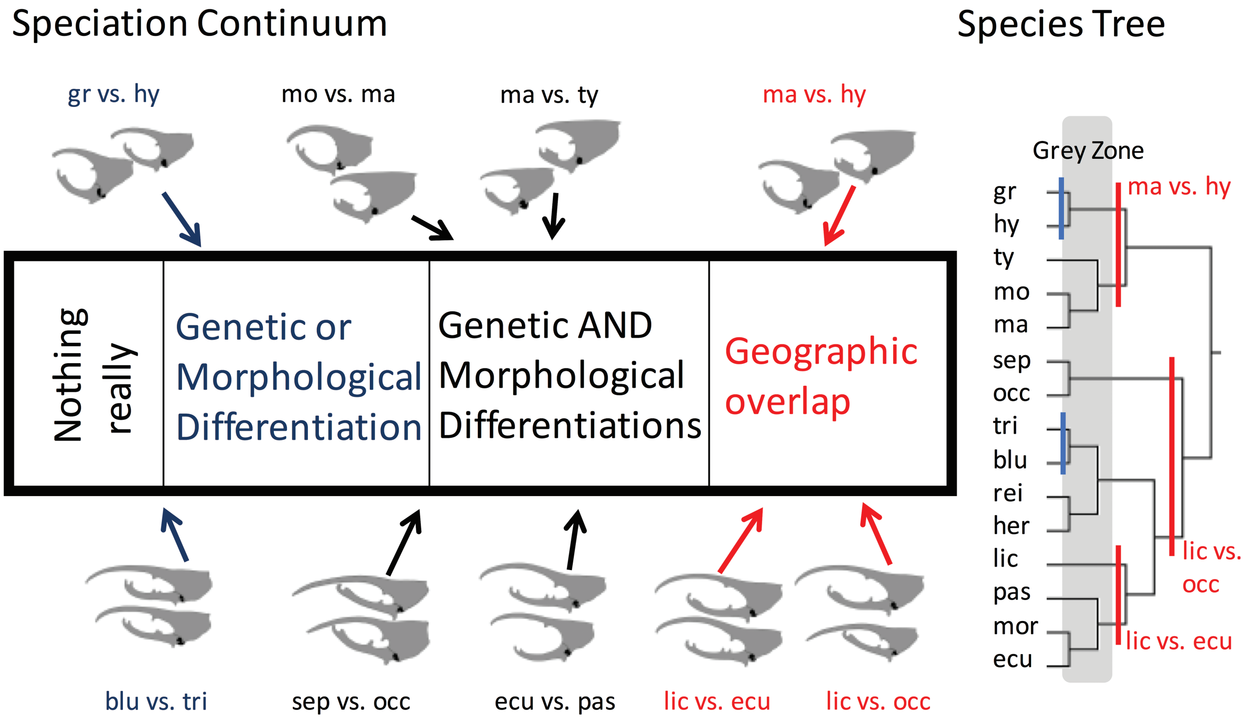


Figure 36— The *Dynastes* beetle species tree and speciation continuum represented by molecular and morphological differentiations and the predicted geographic distribution. Red colors highlight example splits in the species tree that coexist in sympatry/parapatry between taxa from different lineages predicted as possible–the most stringent way of defining biological species. Blue colors indicate sister taxa whose evolutionary independence is supported using either molecular or morphological data–the least stringent way of delimiting species as evolutionarily independent lineages. Uncertainty between the two extreme views of species designations is high (de Queiroz, 2007). The species abbreviations are defined in Figs. 30, 31 and the appendix.

taxonomic treatments of the various Hercules beetle taxa may differ from those of other taxonomists using the same data presented here, and I welcome critiques and discussions in future taxonomic studies. I would like, however, to emphasize that taxonomic treatments can have a profound effect on other biological fields, such as conservation. My treatment, by recognizing all evolutionarily unique entities as species, will likely help the study of conservation by directing efforts of protection toward each genetically/phenotypically unique group without the danger of ignoring any of them.

ACKNOWLEGEMENTS

As a student trained in the era of molecular phylogenetics, I have never been fully confident that I could complete a taxonomic monograph. Due to the constant encouragement from Dr. L. Lacey Knowles, especially about the use of non-traditional methods of species description and diagnosis, I have finally discovered how to use the quantitative methods with which I am comfortable and to incorporate evolutionary perspectives that should be meaningful in this study. I would also like to especially thank the encouragement and help from Dr. Brett C. Ratcliffe from the University of Nebraska and Mr. Jonathan Lai, an amateur entomologist and beetle breeder, who supported my pursuit of the systematics of *Dynastes* beetles.

This revisionary monograph would not have been possible without the help for obtaining fresh wild individuals from Diego Alvarado-Serrano, Marco Bolaños, Fortuné Chalumeau, Wei-Yun Chen, Francis Deknuydt, Patrick Demez, Michael Ivie, Cliff Keil, Javier Lamber, Elisa Levy, Edwin Levy, Hervé Magnin, Rajindra Mahabir, Franklin Neira, Diego Peña, J. Mark Rowland, Felix Stumpe, William Wallin, and Guy Van-Laere and from the Ministerio del Ambiente of Ecuador, Pontificia Universidad Católica del Ecuador, Parc national de la Guadeloupe, Yanayacu Biological Station, and Parque Nacional Yasuní. Additionally, Hsin-Ping Ko, Jonathan Lai, and Ping Feng Tsai kindly shared captive-bred individuals of some species that augmented the molecular data set used in this study. Tristan MacKnight significantly helped this manuscript by providing important insights and comments from the perspective of a taxonomist. Dr. William Blackhall helped with English edits. Completion of this revision was aided by a Graduate Student

Curatorial Assistantship from the Museum of Zoology and by financial support from the Ammerman Endowment to the Insect Division of the University of Michigan, and NSF DEB-15-01462.

LITERATURE CITED

Amadon, D. 1966. The superspecies concept. Systematic Zoology 15: 245–249.

Amadon, D. 1968. Further remarks on the superspecies concept. Systematic Zoology 17: 345–346.

Arrow, G. J. 1937. Systematic notes on beetles of the subfamily Dynastinae with descriptions of a few species in the British Museum collection. Transactions of the Royal Entomological Society of London 86: 35–58.

Bonhomme, V., A. Picq, C. Gaucherel, and J. Claude. 2014. Momocs: outline analysis using R. Journal of Statistical Software 56: 1–24.

Bryant, D. and V. Moulton. 2004. Neighbor-Net: an agglomerative method for the construction of phylogenetic networks. Molecular Biology and Evolution 21: 255–265.

Chalumeau, F. and W. Reid. 2002. Aperçus sur le complexe *hercules* et statut du *Dynastes alcides* (Coleoptera, Dynastidae). Nouvelle Revue d'Entomologie 19: 83–91.

Chifman, J. and L. Kubatko. 2014. Quartet inference from SNP data under the coalescent model. Bioinformatics 30: 3317–3324.

Dixon, P. 2003. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science 12: 927–930.

Dutrillaux, D. and A. M. Dutrillaux. 2013. A south American origin of the genus *Dynastes* (Coleoptera: Scarabaeidae: Dynastinae) demonstrated by chromosomal analyses. Cytogenetic and Genome Research 141: 37–42.

Eaton, D. A. 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics 30: 1844–1849.

Endrödi, S. 1947. Über die Gattung *Dynastes* Kirby. Folia Entomologica Hungarica 2: 54–59.

Fiduccia, C. M. and R. M. Mattheyses. 1982. A linear time heuristics for improving network partitions. Proceedings of the 19th Design Automation Conference 175–181.

Gompert, Z., L. K. Lucas, C. C. Nice, J. A. Fordyce, M. L. Forister, and C. A. Buerkle. 2012. Genomic regions with a history of divergent selection affect fitness of hybrids between two butterfly species. Evolution 66: 2167–2181.

Hardy, M. 2003. Description of a new species of *Dynastes* Kirby (Coleoptera Scarabaeidae Dynastinae) from North and Central America. Besoiro 9: 3–7.

Hijmans, R. J., L. Guarino and E. Rojas. 2002. DIVA-GIS, version 2. A geographic information system for the analysis of biodiversity data. Manual.

Hinton, E. and M. Jarman. 1973. Physiological colour change in the elytra of the Hercules beetle, *Dynastes hercules*. Journal of Insect physiology 19: 533–549.

Huang, J-P. 2012. Ecuador—Land of the Giant Hercules beetles. Scarabs, 71: 1–13.

Huang, J-P. 2016a. The Great American Biotic Interchange and diversification history in *Dynastes* beetles (Scarabaeidae; Dynastinae). Zoological Journal of the Linnean Society 178: 88–96.

Huang, J-P. 2016b. Parapatric genetic introgression and phenotypic assimilation: testing conditions for introgression between Hercules beetles (*Dynastes*, Dynastinae). Molecular Ecology 25: 5513–5526.

Huang, J-P. and L. L. Knowles. 2016. The species versus subspecies conundrum: quantitative delimitation from integrating multiple data types within a single Bayesian approach in Hercules beetles. Systematic Biology 65: 685–699.

Huson, D. H. and D. Bryant. 2006. Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23: 254–267.

Hwang, S-M-R. 2011. The Dynastini of the World. Nature and Ecology, Seoul, Korea.

Jarman, M. and E. Hinton. 1974. Some defense mechanisms of the Hercules beetle, *Dynastes hercules*. Journal of Entomology 1: 71–80.

Kubatko, L. and J. H. Degnan. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescent. Systematic Biology 56: 17–24.

Kuhl, F. P. and C. R. Giardina. 1982. Elliptic Fourier features of a closed contour. Computer Graphics and Image Processing 18: 236–258.

Lai, J. and H-P. Ko. 2008. For the Love of Rhinoceros and Stag Beetles (2nd Ed.). Morning Star, Taipei, Taiwan.

Maddison, W. P. 1997. Gene trees in species trees. Systematic Biology 46: 523–536.

Marquez, E. J. and L. L. Knowles. 2007. Correlated evolution of multivariate traits: detecting co-divergence across multiple dimensions. Journal of Evolutionary Biology 20: 2334–2348.

Mayr, E. 1942. Systematics and the Origin of Species, from the Viewpoint of a Zoologists. Harvard University Press, Cambridge, MA.

Mayr, E. 1963. Animal Species and Evolution. Harvard University Press, Cambridge, MA.

Morón, M. A. 2009. El género *Dynastes* MacLeay, 1819 en la zona de transición Mexicana (Coleoptera: Melolonthidae: Dynastinae). Boletín Socieda Entomológica Aragonesa 45: 23–38.

Nagai, S. 2002. Two new subspecies of *Dynastes hercules* (Linnaeus, 1758) (Scarabaeidae, Dynastinae). Gekkan-Mushi 381: 2–4.

Nagai, S. 2005. Two new subspecies of the genus *Dynastes* Kirby (Coleoptera, Scarabaeidae) from Mexico and Venezuela. Gekkan-Mushi 418: 31–35.

Noor, M. A. 1999. Reinforcement and other consequences of sympatry. Heredity 83: 503–508.

Nosil P., L. J. Harmonand, O. Seehausen. Ecological explanations for (incomplete) speciation. Trends in Ecology and Evolution 24: 145–156.

Pickrell, J. K. and J. K. Pritchard. 2012. Inference of population splits and mixtures from genome-wide allele frequency data. PLoS Genetics 8: e1002967.

de Queiroz, K. 2007. Species concepts and species delimitation. Systematic Biology 56: 879–886.

Ratcliffe, B. C., R. D. Cave and E. B. Cano. 2013. The Dynastine Scarab Beetles of Mexico, Guatemala, and Belize. Bulletin of the University of Nebraska Museum, Lincoln, NE, Volume 27.

Ratcliffe, B. C. and R. D. Cave. 2015. The Dynastine Scarab Beetles of the West Indies. Bulletin of the University of Nebraska Museum, Volume 28, Lincoln, Ne.

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Rowland, J. M. and K. B. Miller. 2012. Phylogeny and systematics of the giant rhinoceros beetles (Scarabaeidae: Dynastini). Insecta Mundi 0263: 1–15.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313.

Silvester-Bradley, P. C. 1954. The superspecies. Systematic Zoology 3: 145–146.

Smith, B. T., C. C. Ribas, B. M. Whitney, B. E. Hernández Baños and J. Klicka. 2013. Identifying biases at different spatial and temporal scales of diversification: a case study in the Neotropical parrotlet genus *Forpus*. Molecular Ecology 22: 483–494.

Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods) 4.0 Beta. Sinauer Associates, Inc., Sunderland, MA.

Venable, W. N. and B. D. Riply. 2002. Modern Applied Statistics with S (4th Ed). Springer, New York.

APPENDIX

The x-y coordinates of the outline data and the new molecular data used in the phylogenetic and species-tree reconstructions can be retrieved from the dryad data repository: doi:10.5061/dryad.p4f5v

R scripts for processing and analyzing the data can be obtained upon request.

MATERIAL EXAMINED

Morphological Data. The material listed below are specimens physically examined or with digitized images examined by the author. Published records, although included on the maps, are not listed among the individuals. Label data were not copied verbatim, although all provided locality information was included (Museum labels and the corresponding specimen information are available in the figures).

Dynastes bleuzeni (Silvestre and Dechambre, 1995)

VENEZUELA: *Bolivar* [1 captive-bred male] (UMMZ); *Bolivar, Cerro Sarisariñama* [1 male] (UMMZ); *Bolivar, La Escalera* [1 male image retrieved from Lai and Ko, 2008] (Dr. Pascoal Grossi, personal collection); *Bolivar* [1 male image retrieved from Hwang, 2011] (personal collection).

Dynastes ecuatorianus (Ohaus, 1913)

COLOMBIA: *Unknown origin* [3 males] (UMMZ); **ECUADOR:** *Napo, Misahualli* [6 males] (UMMZ); *Orellana, Loreto* [1 male] (UMMZ); **PERU:** *Loreto, Iquitos* [3 males] (UMMZ).

Dynastes granti Horn, 1870

USA: *Arizona, Gila County* [*Payson*: 2 males; *Star Valley*: 3 males; *Pine*: 3 males] (UMMZ).

Dynastes hercules (Linnaeus, 1758)

GUADELOUPE: *captive-bred sample* [originally from Guadeloupe 1 male] (UMMZ); *Guadeloupe* [1 male image retrieved from Lai and Ko, 2008] (Jason Chen, personal collection); **UNKNOWN:** *Guadeloupe* or *Dominica* [1 male type-specimen image retrieved from Hwang, 2011] (Linnean Society of London).

Dynastes hyllus Chevrolat, 1843

MEXICO: *Chiapas* [1 male] (UMMZ); *Sinaloa, Potrerillos* [1 male] (UMMZ); *Puebla* [2 males] (UMMZ); *Puebla* [2 male images retrieved from Lai and Ko, 2008] (Dr. Paschoal Grossi, personal collection and Jason Chen, personal collection).

Dynastes lichyi (Lachaume, 1985)

COLOMBIA: *Santander, La Bellela* [3 males] (UMMZ); **ECUADOR:** *Napo, Consanga* [5 males] (UMMZ); *Pichincha, El Reventador* [2 males] (UMMZ); *Pastaza, Santa Clara* [2 males] (UMMZ); *Napo, San Pablo* [2 males] (UMMZ); **PERU:** *Selva Central* [1 male] (UMMZ); *Junin, Satipo* [1 male] (UMMZ); **VENEZUELA:** *Rancho Grande* [1 male type-specimen image retrieved from Hwang, 2011] (Muséum national d'Histoire naturelle, Paris).

Dynastes maya Hardy, 2003

HONDURAS: *Cortez, El Cusuco* [2 males] (UMMZ); *Captive-bred sample* [1 male image originating from Honduras retrieved from BeKuwa magazine] (personal collection); **GUATEMALA:** *Alta Verapaz, San Cristobal* (originally denoted as Alta Veracruz) [1 male image retrieved from Lai and Ko, 2008] (Dr. Paschoal Grossi, personal collection); **UNKNOWN:** [1 male image retrieved from the online guide to Neotropical Scarab beetles hosted by the University of Nebraska State Museum] (The Smithsonian Scarab collection at the University of Nebraska State Museum).

Dynastes morishimai (Nagai, 2002)

BOLIVIA: *a captive-bred individual* [1 male originally from La Paz] (UMMZ); *La Paz, Near Tipuani* [1 male holotype image retrieved from Hwang, 2011] (Entomological Laboratory, Ehime University, Matsuyama).

Dynastes moroni (Nagai, 2005)

MEXICO: *Veracruz, Sierra de Los Tuxtlas, Volcan San Martin* [1 male] (UMMZ); *Captive-bred sample* [1 male image originating from the state of Tuxtlas retrieved from BeKuwa magazine] (personal collection); *Santiago Tuxla, Cerro El Vigia* [1 male image retrieved from Lai and Ko, 2008] (Dr. Miguel Morón, personal collection); **UNKNOWN:** [1 captive-bred male image retrieved from Lai and Ko, 2008] (personal collection).

Dynastes occidentalis (Lachaume, 1985)

ECUADOR: *Esmeraldas, Chuchuvi* [3 males] (UMMZ); *Pichincha, Los Bancos* [3 males] (UMMZ); **COLOMBIA:** *Cali* [1 male type-specimen image retrieved from Hwang, 2011] (Muséum national d'Histoire naturelle, Paris).

Dynastes paschoali (Grossi and Arnaud, 1993)

BRAZIL: *Espirito Santo, Anchieta* [1 male holotype image retrieved from Lai and Ko, 2008] (E. J. Grossi, personal collection); *Unknown localities* [2 male paratype-specimen images retrieved from Lai and Ko, 2008] (Museu Nacional do Rio de Janeiro and Museu de Zoologia de São Paulo).

Dynastes reidi (Chalumeau, 1977)

SAINT LUCIA: *Soufriere* [1 male] (UMMZ); **MARTINIQUE:** *Morne Bleue* [1 male image retrieved from Lai and Ko, 2008] (Dr. Paschoal Grossi, personal collection).

Dynastes septentrionalis (Lachaume, 1985)

COSTA RICA: *Near Chiripo* [3 males] (UMMZ); **HONDURAS:** *Cortez, El Cusuco* [1 male] (UMMZ); **PANAMA:** *Cerro Azul* [2 males] (UMMZ); *Chiriqui* [2 male images retrieved from Lai and Ko, 2008] (Jason Chen, personal collection); **GUATEMALA:** *Sierra de Coral, Finca La Firma* [1 male] (UMMZ); *Alta Verapaz, Quixal* [1 male type-specimen image retrieved from Hwang, 2011] (Muséum national d'Histoire naturelle, Paris).

Dynastes trinidadensis (Chalumeau and Reid, 1995)

TRINIDAD: *Morne Bleu* [3 males, one UMMZ collection, one retrieved from Lai and Ko 2008 and another from the color plate from Chalumeau and Reid 1995] (UMMZ; Dr. Paschoal Grossi, personal collection; Institut de Recherches Entomologiques de la Caraïbe).

Dynastes tityus (Linnaeus, 1763)

USA: *North Carolina, Franklin County* [1 male] (UMMZ); *Tennessee, Montgomery* [1 male] (UMMZ); *Tennessee, Rock Island* [1 male] (UMMZ); *Georgia, Suburb of Atlanta* [1 male] (UMMZ); **UNKNOWN:** *historical collections* [4 males] (UMMZ).

Molecular Data. The material listed below refers to UMMZ specimens that have DNA sequence data used in the section of this study on phylogenetic reconstruction. Label data were copied verbatim (same as in Figs. 32, 33). Abbreviations of species names are the same as those used in Figs. 34, 35.

<u>Outgroups:</u>

***Dynastes neptunus* (dn):** Consanga, Napo, Ecuador (**CSGDn1**); Los Bancos, Pichincha, Ecuador (**LBDn1**)

***Dynastes satanas* (ds):** a captive-bred sample originally from La Paz, Bolivia (**DS01**).

<u>Hercules beetles:</u>

***Dynastes bleuzeni* (blu):** a captive-bred sample originating from Bolivar, Venezuela (**DhbV1Br**); Cerro Sarisariñama, Bolivar, Venezuela (**DhbV2**).

***Dynastes ecuatorianus* (ecu):** Colombia (**DheCO2** and **DheCO6**); Iquitos, Loreto, Peru (**DheP8**); Yasuni Station, Ecuador (**YSNE1** and **YSNE2**).

***Dynastes granti* (gr):** Pine, Arizona, USA (**DgAZ5**); Payson, Arizona, USA (**DGRP1**); Reserve, New Mexico, USA (**DGRR1** and **DGRR2**).

***Dynastes hercules* (her):** Dominica (**DhhD1**, **DhhD2**, and **DhhD3**); Saint-Claude, Guadeloupe (**DhhG2**).

***Dynastes hyllus* (hy):** a captive-bred individual originating from Mexico (**Dhy3Br**); El Palmito, Sinaloa, Mexico (**Dhy6**); Poterillos, Sinaloa, Mexico (**Dhy8**); Puebla, Mexico (**Dmi2**).

***Dynastes lichyi* (lic):** La Bellela, Santander, Colombia (**DhlCO1** and **DhlCO2**); Selva Central, Peru (**DhlP2**); Satipo, Junin, Peru (**DhlP7**); El Reventador, Ecuador (**ERVL1** and **ERL2**).

***Dynastes maya* (ma):** a captive-bred sample originating from El Cusuco, Cortez, Honduras (**Dma7Br**); Honduras (**Dma2** and **Dma5**).

***Dynastes morishimai* (mor):** captive-bred sample from Bolivia (**Dhm2Br**).

***Dynastes moroni* (mo):** Volcan San Martin, Sierra de Los Tuxtlas, Veracruz, Mexico (**Dhym2**, **Dhym3**, **Dhym5**, and **Dhym10**).

***Dynastes occidentalis* (occ):** Chuchuvi, Esmeraldas, Ecuador (**CCVO1**); Los Bancos, Pichincha, Ecuador (**DhoE1** and **DhoE3**).

***Dynastes paschoali* (pas):** captive-bred individuals from Bahia, Brazil (**DhpB1Br** and **DhpB2Br**).

***Dynastes reidi* (rei):** Martinique (**DhrM1**); Soufriere, Saint Lucia (**DhrSL5**).

***Dynastes septentrionalis* (sep):** Finca La Firmeza, Sierra de Coral, Guatemala (**DhsG1** and **DhsG2**); Santa Barbara, Honduras (**DhsH3**); Cerro Azul, Panama (**DhsP1**).

***Dynastes trinidadensis* (tri):** Morne Bleu, Trinidad (**DhtT8** and **DhtT9**).

***Dynastes tityus* (ty):** Fort White, Florida, USA (**DtFL2**); Montgomery, Tennessee, USA (**DtTN1**).