

**AN ASSESSMENT OF THE POLYTYPIC STATUS OF THE NAMIB
DARKLING BEETLES *ONYMACRIS UNGUICULARIS* AND
*ONYMACRIS RUGATIPENNIS***

by

Rachel Pollard

A Senior Honors Project Presented to the

Honors College

East Carolina University

In Partial Fulfillment of the

Requirements for

Graduation with Honors

by

Rachel Pollard

Greenville, NC

May 2014

Approved by:

Faculty Mentor:



Trip Lamb, Ph.D.

**AN ASSESSMENT OF THE POLYTYPIC STATUS OF THE NAMIB
DARKLING BEETLES *ONYMACRIS UNGUICULARIS* AND
*ONYMACRIS RUGATIPENNIS***

by

Rachel Pollard

A Senior Honors Project Presented to the

Honors College

East Carolina University

In Partial Fulfillment of the

Requirements for

Graduation with Honors

by

Rachel Pollard

Greenville, NC

May 2014

Approved by:

Faculty Mentor:

Trip Lamb, Ph.D.

I hereby declare I am the sole author of this thesis. It is the result of my own work and is not the outcome of work done in collaboration, nor has any of it been submitted elsewhere for another degree.

Signed: Rachel M. Pollard

Date: 5/10/14

Rachel M. Pollard

**An assessment of the polytypic status of the Namib darkling beetles *Onymacris*
unguicularis and *Onymacris rugatipennis***

Rachel M. Pollard

Department of Biology, East Carolina University, Greenville N.C. 27858

ABSTRACT - The southern African beetle genus *Onymacris* (family Tenebrionidae) comprises 14 species, six of which are polytypic. Despite longstanding research on the physiological and behavioral adaptations of these desert beetles, little is known about their evolutionary relationships, particularly regarding the validity of currently recognized subspecies. In this study, I examined the polytypic status of the species *Onymacris unguicularis* and *O. rugatipennis*, each composed of two subspecies. The first, *Onymacris unguicularis*, is restricted to vegetationless dunes and is renowned for an unusual drinking behavior called fog basking. Northern populations—isolated from southern populations by approximately 300 km of duneless land—compose the subspecies *O. u. schulzeae*, which exhibits only minor morphological differences from the southern subspecies, *O. u. unguicularis*. The second species, *Onymacris rugatipennis*, also contains two subspecies—*O. r. rugatipennis* and *O. r. albotessellata*—which are distinguished by the latter's white wax bloom on the dorsum. Their ranges are contiguous and slightly overlapping, with *O. r. rugatipennis* occurring primarily along riverbanks and *O. r. albotessellata* occurring on dune bases. To assess the validity of the subspecies of *O. unguicularis*, I analyzed morphological variation in 35 specimens of *O. u. schulzeae* and 95 of *O. u. unguicularis* as well as mitochondrial DNA (mtDNA) sequence variation in five *O. u. schulzeae* and ten *O. u. unguicularis*. For *O. rugatipennis*, I examined only mitochondrial DNA sequence variation, generating sequence data for 11 *O. r. rugatipennis* and 12 *O. r. albotessellata*. Phylogenetic analysis of the mtDNA data revealed reciprocal monophyly between *O. u. unguicularis* and

O. u. schulzeae, a pattern complementary to their morphological variation. On the basis of congruent phenotypic diversity, geographic delimitation, and genetic variation, I support the recognition of *O. u. unguicularis* and *O. u. schulzeae* as valid taxa. Conversely, the limited genetic divergence, absence of phylogeographic structuring, and number of shared haplotypes between *O. r. rugatipennis* and *O. r. albotessellata* suggest this species would be better regarded as a monotypic taxon.

Acknowledgments

I thank Ruth Müller of the Ditsong National Museum of Natural History, Pretoria, South Africa for arranging specimen loan and shipment to Dr. Lamb. Dr. Thomas Fink kindly assisted with digital imaging and measurements. I would especially like to thank Dr. Trip Lamb for his time, knowledge, guidance, and support. This project was funded in part by two East Carolina University Undergraduate Research and Creative Activity Awards and a George T. Barthalmus Undergraduate Research Grant.

Table of Contents

Introduction	9
Brief History of Subspecific Taxonomy	9
Definitions of Subspecies	11
Subspecific Delimitation in Invertebrates	11
Subspecies in <i>Onymacris</i>	11
Materials and Methods	18
Molecular Phylogenetic Analysis	18
Morphometrics	19
Results and Discussion	22
Future Studies	26
Broader Implications	26
Literature Cited	28
Appendices	30

List of Tables

Table 1. Pronotal, prosternal, and elytral ratio means and ranges.	22
Table 2. Evaluation of Braby et al.'s (2012) criteria for <i>Onymacris unguicularis</i> and <i>Onymacris rugatipennis</i>	26

List of Figures

Figure 1. Range maps for (A) <i>Onymacris unguicularis</i> and (B) <i>Onymacris rugatipennis</i> depicting subspecific geographic distributions and collecting localities	15
Figure 2. Dorsal aspect of <i>Onymacris unguicularis unguicularis</i>	16
Figure 3. Dorsal aspect of <i>Onymacris unguicularis schulzeae</i>	16
Figure 4. Dorsal aspect of <i>Onymacris rugatipennis rugatipennis</i>	16
Figure 5. Dorsal aspect of <i>Onymacris rugatipennis albotessellata</i> .	16
Figure 6. Pronotum of <i>Onymacris unguicularis unguicularis</i>	20
Figure 7. Pronotal ratio calculations	20
Figure 8. Prosternum of <i>Onymacris unguicularis unguicularis</i>	20
Figure 9. Prosternal ratio calculations	20
Figure 10. Lateral aspect of the prosternal process of <i>Onymacris unguicularis schulzeae</i>	20
Figure 11. Lateral aspect of the prosternal process of <i>Onymacris unguicularis unguicularis</i>	20
Figure 12. Elytra of <i>Onymacris unguicularis unguicularis</i> , depicting measurements used for elytral ratio calculations	21
Figure 13. Elytra of <i>Onymacris unguicularis schulzeae</i> , depicting measurements used for elytral ratio calculations	21
Figure 14. Maximum likelihood tree for populations of <i>O. unguicularis</i>	23
Figure 15. Maximum likelihood tree for populations of <i>O. rugatipennis</i>	24

Introduction

Beetles of genus *Onymacris* (family Tenebrionidae) are dominant ecological entities in the sand seas and other dune systems of the Namib Desert in southern Africa. Six of the 14 named species of *Onymacris* are polytypic, containing two to four subspecies. Although behavioral and physiological adaptations of these flightless, diurnal beetles have been studied extensively (e.g., Hamilton and Seely 1976; Naidu 2008; De Villiers and Hanrahan 1991; Cooper 1982; Nørgaard and Dacke 2010) little is known about their evolutionary relationships, particularly regarding the validity of the currently recognized subspecies. I examined the polytypic status of two species: *Onymacris unguicularis* and *Onymacris rugatipennis*.

Brief History of Subspecific Taxonomy

The concept of subspecies first arose in the late 19th century as a way to formally recognize intraspecific geographic and morphological variation (Braby et al. 2012). The Swedish ornithologist Carl Sundevall is credited as the first to use trinomial nomenclature, assigning a third name to poorly delimited species of birds, which he described as geographic varieties, in 1840. Sundevall's use of trinomial nomenclature was followed closely by Herman Schlegel (1844), who is considered the first to routinely use trinomials (Winker 2010). One of the major proponents of trinomial nomenclature was the American ornithologist Elliott Coues, who greatly aided its usage and popularization. He argued that the Linnaean system was a hindrance to our understanding of evolution and speciation.

It [the Linnaean system] answered, when a thing was either square or else it was round—when species were held for fixed facts as separate creations; but now that we know a thing may be neither square nor round, but something between, it is lamentably defective. Not many years hence, we trust, naturalists will have discarded it for some better method of notion; and then

the wonder will be that we advanced so far with such a stumbling-block in the way. (Coues 1871)

Coues stressed that because all differentiation begins gradually and imperceptibly, intraspecific variation is visible evidence of divergence and naming divergent forms is therefore necessary. The concept of trinomial nomenclature quickly became established among ornithologists as well as entomologists in Europe and soon became a standard in the International Code of Zoological Nomenclature (ICZN), as it remains today (Mallet 2013). As a result of the trinomial revolution, taxonomists began directing a great deal of effort toward the characterization and discovery of new subspecies in the late 1800s and early 1900s. Over this time frame, trinomials were often grossly misused by taxonomists to recognize subtle intraspecific morphological differences. In extreme cases, species have been assigned up to 150 subspecies (Lamb and Avise 1992).

However, not all naturalists agreed with the widely accepted practice of official subspecific delimitation. Wilson and Brown (1953), two ant systematists, vehemently opposed the use of trinomials, which they considered a subjective and useless practice that merely appeases our “unceasing search for novelties.” In fact, they stated that “the subspecies concept is the most critical and disorderly area of modern systematic theory,” deeming its function both “illusory and superfluous” and predicting its imminent disappearance. Wilson and Brown’s (1953) major issues with subspecific delimitation were: (1) the lack of concordance between genetically independent characters and geography; (2) the recurrence of specific characters in more than one geographic location and thus the existence of polytopic races; (3) microgeographic races, or distinct local populations (common in snail species), that show extensive character variation and would therefore accumulate a long list of trinomials over time; and (4) the arbitrary lower limit of the subspecies, which they claimed would never be satisfactory due to borderline cases. They suggested that taxonomists

redirect their efforts by examining geographical variation of genetically independent characters before attempting to create racial groupings, and further recommended that the description of intraspecific variation be limited to locality citations or brief statements of variant ranges.

Definitions of Subspecies

Despite its strong opposition—and contrary to Wilson and Brown’s expectations—the subspecies concept has extended into the present day. Recognition of polytypic species has resulted in a huge reduction in the number of species-level taxa, from more than 20,000 in the 1920s to approximately 9,000 in the 1980s, with a fair amount of “overlumping” occurring and many valid allopatric species being designated as subspecies (Winker 2010). Although the battle over nomenclature is over, the ICZN does not provide a clear definition of what constitutes a subspecies or, in other words, which cases justify the application of trinomialism (Winker 2010). In a broad sense, trinomial labels function as descriptions of the intraspecific variation that is representative of evolutionary and developmental responses to geographically heterogeneous biotic and abiotic phenomena. The debate over the subspecies concept is ultimately the result of dissatisfaction with this broad definition and a lack of a better, more detailed one (Winker 2010). Concern over this issue is longstanding; Darwin (1859), noted

no clear line of demarcation has as yet been drawn between species and subspecies—that is, the forms which in the opinions of some naturalists come very near to, but do not quite arrive at, the rank of species: or, again, between sub-species and well-marked varieties, or between lesser varieties and individual differences.

Different definitions have since evolved in an attempt to provide a clear line of demarcation and resolve the subspecies debate. Hartert (1903) defined subspecies as “the

geographically separated forms of one and the same type, which taken together make up a species. Therefore, not just a small number of differences, but differences combined with geographic separation, permit us to determine a form as a subspecies.” Mayr (1942, 1963) described a subspecies as an “aggregation of phenotypically similar populations of a species inhabiting a geographical subdivision within the overall range and differing from other such subdivisions of the species.”

Despite the multitude of reasonable definitions, taxonomists have not strictly abided by any one, and the criteria used to describe subspecies have continued to be inconsistent and somewhat subjective. The development of genetic tools has further complicated our definition of subspecies; now both phenotypic variation and phylogeographic patterns, as demonstrated by neutral genetic variation in markers such as microsatellites and mitochondrial DNA (mtDNA), can be taken into consideration when delimiting subspecific groups (James 2010). Avise (2004) supported using a phylogenetic approach to designate subspecies, recommending subspecies be defined as

groups of actually or potentially interbreeding populations (normally mostly allopatric) that are genealogically highly distinctive from, but reproductively compatible with, other such groups. Importantly, the empirical evidence for genealogical distinction must come, in principle, from concordant genetic partitions across multiple, independent, genetically based molecular (or phenotypic; Wilson and Brown 1953) traits.

Subspecific Delimitation in Invertebrates

Taxonomic problems in mammals, birds, and plants have been studied extensively, but the importance of subspecific delimitation in invertebrates has been largely neglected. Braby et al. (2012) argued that true subspecies represent valid, important evolutionary units, and the recognition of subspecies is important because it gives insight into intraspecific

variation, adaptation, and speciation. However, it is vital to use a clear, well-defined set of criteria for subspecific delimitation. The major issue involves setting the lower limit of what constitutes a subspecies in order to avoid arbitrary assignment of trinomials to forms that vary only slightly in phenotypic characteristics such as coloration, pattern, or size as recommended by Ernst Hartert in 1891 (Winker 2010).

Braby et al. (2012) recently provided an update of the subspecies concept, recommending subspecific delimitation be restricted to extant groups of “evolving populations representing partially isolated lineages of a species that are allopatric, phenotypically distinct, have at least one fixed diagnosable character state, and that these character differences are correlated with evolutionary independence according to population genetic structure.” Although limiting their definition to allopatric populations, they acknowledged that the subspecies concept could also be applied to “broadly sympatric lineages diverging in ecological space,” noting that subspecies status may be appropriate if differentiated sympatric lineages are phenotypically distinct with at least one fixed diagnosable character state (Braby et al. 2012). Molecular phylogenetic analysis is helpful in the reassessment of subspecific taxa. If genetic variation exhibits discordance with phenotypic or geographic variation in traditionally defined subspecies, morphological differences can be interpreted as local adaptation or clinal variation, and subspecific designation is therefore not valid (Lamb et al. 2013). Alternatively, if genetic variation corroborates phenotypic variation in subspecies, retention of the trinomial designations or elevation to full species should be considered.

Subspecies in Onymacris

One of the more intensely studied species of *Onymacris*, *O. unguicularis* has been the subject of much behavioral and physiological research (De Villiers and Hanrahan 1991; Cooper 1982; Nørgaard and Dacke 2010). The species is renowned for a unique behavior

called fog-basking; these otherwise diurnal beetles climb to the top of sand dunes at night, tilt head-down towards incoming fog, and use their bodies to collect drinking water (Hamilton and Seely 1976; Naidu 2008). Despite its interesting behavioral adaptations, *O. unguicularis* has been poorly studied from an evolutionary standpoint, though it is currently recognized as a polytypic species. Northern populations, which compose the subspecies *O. u. schulzeae*, are separated from the southern subspecies, *O. u. unguicularis*, by approximately 300 km of duneless land (Fig. 1A). The two subspecies, characterized by slight morphological differences in the shapes of the pronotum, prosternal process, and elytra, were described by Penrith (1984), following Schulze's (1964) earlier notations of differences in larval morphology (Figs 2-3). Whether or to what degree genetic variation is congruent with morphological and geographic delimitation in *O. unguicularis* is unknown.

Schulze (1964) described an additional species of *Onymacris*—*O. albotessellata*—splitting it from *Onymacris rugatipennis* on the basis of differences in larval morphology, the larva of *O. rugatipennis* having a rounder apex and shorter setae (Osberg et al. 1986). Penrith (1984) later argued that from the standpoint of adult morphology, she could provide no justification for separating the two taxa beyond the level of subspecies. Their ranges are contiguous and slightly overlapping, with *O. r. albotessellata* occurring primarily on the vegetated dune bases of the south central Namib dune system and *O. r. rugatipennis* along the sandy riverbanks of the Kuiseb and Swakop rivers (Fig. 1B). Adults of *O. r. albotessellata* are distinguished from *O. r. rugatipennis* by the presence of a white wax bloom that protects the integument and by dense aggregations of microgranules in the elytral sculpturing (Penrith 1975). The bloom, which is composed of wax filaments that are extruded through pore openings to coat the insect's body, is thought to affect water diffusion resistance and reflection of solar radiation, providing protection from desiccation in the hot, dry interior of the Namib (Hauffe and Seely 1995). Osberg et al. (1986) conducted laboratory

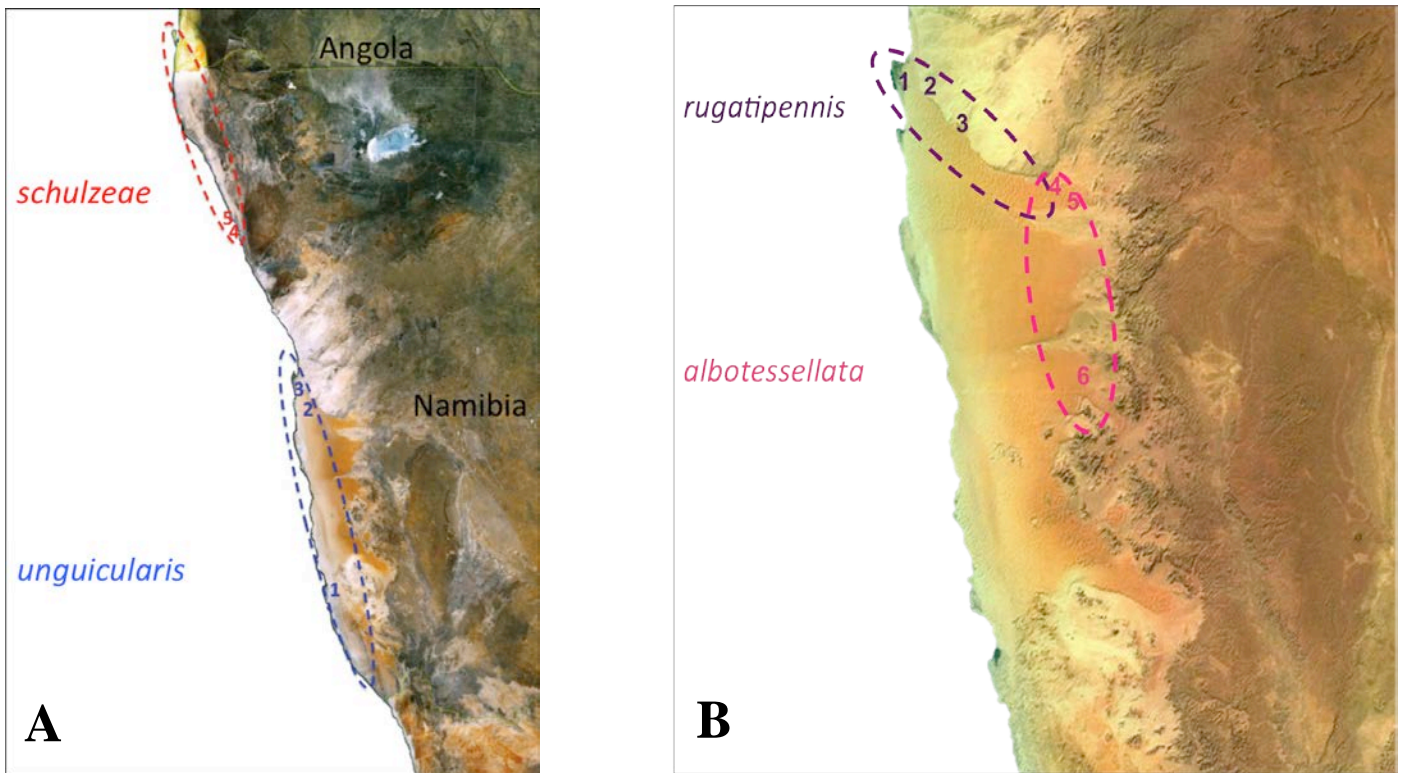


Figure 1. Range maps for (A) *Onymacris unguicularis* and (B) *Onymacris rugatipennis* depicting subspecific geographic distributions and collecting localities. Ovals represent the approximate ranges of each subspecies. Collecting localities for *O. unguicularis*, listed from south to north, are: **1** Luderitz **2** Gobabeb **3** Walvis Bay, and **4, 5** Torra Bay. Collecting localities for *O. rugatipennis* are: **1** Walvis Bay **2** Rooibank **3** Gobabeb **4, 5** Rostock Farm, and **6** Hwy 707.

breeding experiments demonstrating that *O. r. albotessellata* and *O. r. rugatipennis* are fully interfertile. However, the apparent non-existence of intermediates in the zone of overlap indicated that introgression between the two interfertile types does not occur in the field. Thus, they recommended *O. r. rugatipennis* and *O. r. albotessellata* should be considered separate species.

Alternatively, behavioral studies and allozyme analysis (Hauffe et al. 1993) indicated that introgression between the two subspecies does, in fact, occur and that there is limited genetic divergence among populations across the species range. Hauffe et al. (1993) found



Figures 2-3. Dorsal aspects of *Onymacris unguicularis unguicularis* (2) and *O. u. schulzeae* (3).



Figures 4-5. Dorsal aspects of *Onymacris rugatipennis rugatipennis* (4) and *O. r. albotessellata* (5).

that the morphotypes share similar precopulatory and copulatory timing and behaviors as well as daily activity patterns, and they provided evidence of intermediate forms in as much as the density of elytral microgranules varies continuously in *O. rugatipennis*, gradually increasing from west to east along the Kuiseb River. Additionally, the lack of genetic divergence revealed by their allozyme data indicated continuous gene flow or recent divergence between waxy and non-waxy forms. More recently, Hauffe and Seely (1995) applied a multidisciplinary approach to help solve the taxonomic problem in *O. rugatipennis*, considering morphological, physiological, behavioral, and genetic studies of adult individuals. They determined that the two forms of *O. rugatipennis* should be considered a single species, stating that they are probably not dissimilar enough to warrant even subspecific status (Hauffe and Seely 1995).

I will employ Braby et al.'s (2012) criteria to examine the validity of the subspecies in these two species of *Onymacris*, relying heavily on the use of molecular phylogenetic analysis. If the subspecies satisfy each criterion—allopatry, the presence of at least one fixed diagnosable character state, and the correspondence of character differences with evolutionary independence—I will consider them valid taxa. Alternatively, if they fail to meet these criteria overall, I will suggest that their subspecific status be reconsidered.

Materials and Methods

Molecular Phylogenetic Analysis

I obtained genetic data for 15 specimens of *Onymacris unguicularis*: five *O. u. schulzeae*, representing two populations, and ten *O. u. unguicularis*, representing three populations. Specimens were collected by T. Lamb in Namibia and preserved in 100% ethanol prior to DNA extraction with DNeasy Blood & Tissue Kits (Qiagen). I amplified two mitochondrial genes—cytochrome oxidase I (COI) and cytochrome oxidase II (COII)—using the following PCR conditions: initial denaturation at 94°C for 1 minute, annealing at 48 °C for 45 seconds, and extension at 72 °C for 1 minute (repeated five times), followed by denaturation at 94 °C for 45 seconds, annealing at 50 °C for 45 seconds, and extension at 72°C for 1 minute (repeated 45 times). Amplification products were cleaned using exoSAP-IT (USB Corp.) and sequenced on an Applied Biosystems 3130 capillary sequencer, yielding 1547 nucleotides for COI and 680 nucleotides for COII. Sequences were edited using Sequencher 4.9 (GeneCodes, Ann Arbor, MI) and Geneious R7 7.1.4 (Biomatters, Auckland, New Zealand) and aligned using ClustalX ver. 2.0. I calculated uncorrected pairwise genetic distance values within and among populations as well as between the two subspecies and produced a phylogenetic tree using maximum likelihood (ML) analysis, executed in RAxMLHPC and involving 1,000 random sequence addition replicates (RAS). Bootstrap support values, calculated using the same search parameters with 1,000 replicates, were applied to the best tree recovered in the RAS search. Two additional species of *Onymacris*, *Onymacris laeviceps* and *O. plana*, shown to be sister taxa to *O. unguicularis* (Lamb and Bond 2013), served as outgroups.

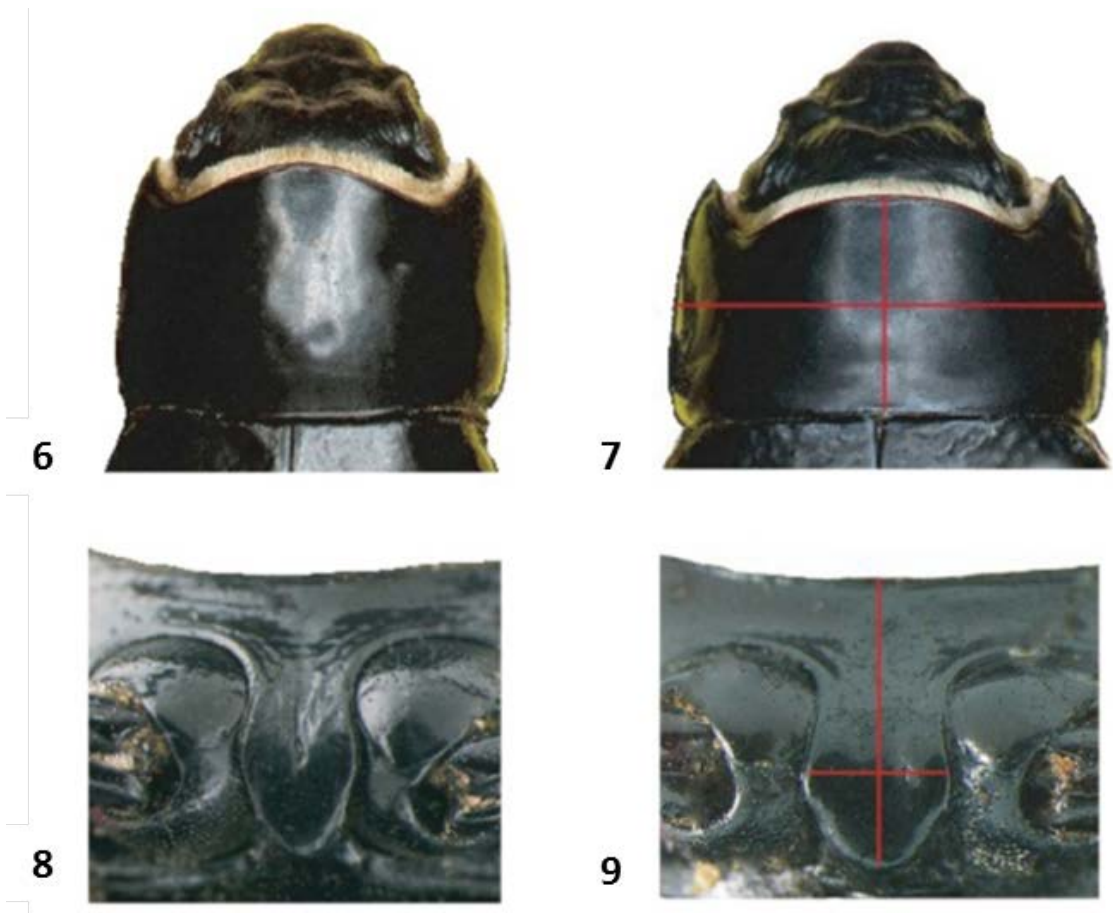
I generated COII sequence data for 23 *O. rugatipennis* individuals (collected by J.E. Bond and T. Lamb) using the methods described above, yielding 708 nucleotides. Specimens consisted of 11 *O. r. rugatipennis*, representing three populations, and 12 *O. r. albotessellata*,

representing three populations. Phylogenetic analytical procedures were as above, with the sister taxon, *O. boschimana*, serving as the outgroup.

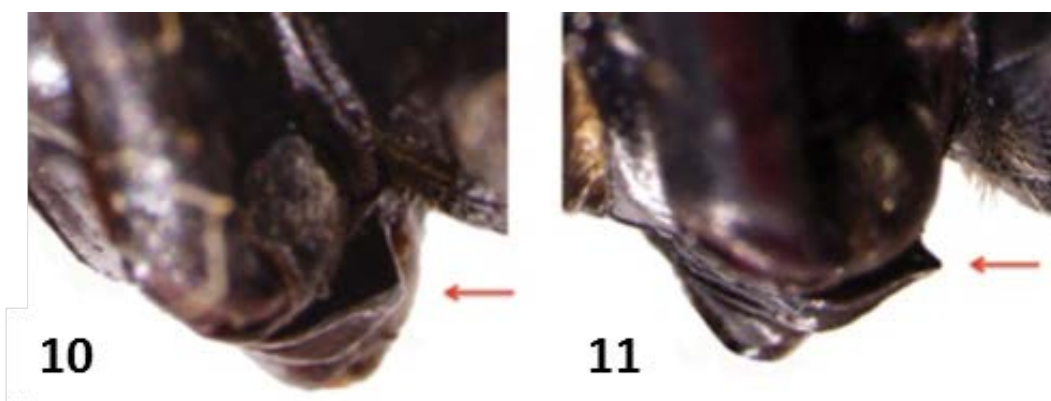
Morphometrics

I also conducted morphometric analysis to verify Penrith's (1984) diagnostic morphological characters of *O. u. unguicularis* and *O. u. schulzeae*, which involved shape differences of the pronotum and prosternal process (Figs. 6-9). Specifically, she noted that the pronotum is more strongly transverse in *O. u. schulzeae*, and its prosternal process is typically larger, with a blunt apex that is not clearly visible in lateral aspect (Fig. 10). In contrast, the prosternal process in *O. u. unguicularis* can be seen in lateral aspect, its apex often appearing as a tooth-like projection (Fig. 11). I examined pinned specimens of *O. unguicularis* on loan from the Ditsong National Museum of Natural History (formerly Transvaal Museum) in Pretoria, South Africa. The museum specimens included 30 *O. u. schulzeae* representing four populations and 93 *O. u. unguicularis* representing 11 populations. I also included two *O. u. unguicularis* and five *O. u. schulzeae* from T. Lamb's collection. I photographed the ventral and dorsal aspects of each beetle using an Olympus BX41 Laboratory Microscope with the assistance of Dr. Thomas Fink.

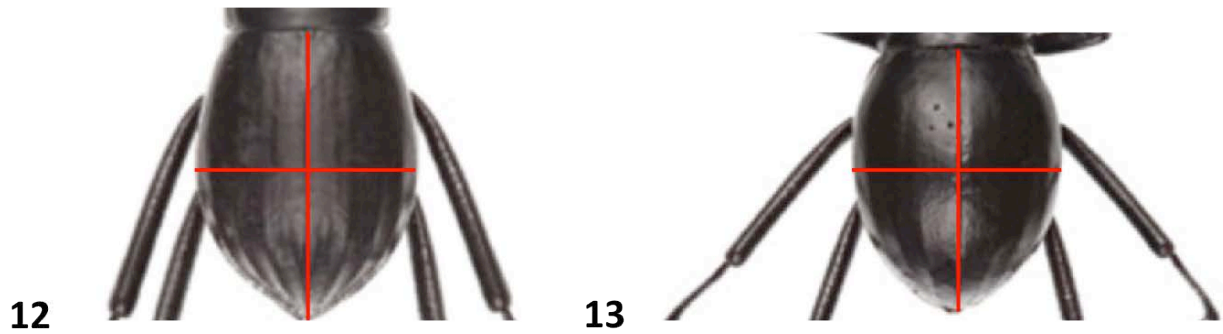
Penrith (1984) used the ratio of pronotal length to pronotal width (PL/PW) to quantify morphological differences between subspecies (Figs. 6-7). I repeated these measurements using Image J software and also quantified differences in the prosternal process by generating length/width ratios (Figs. 8-9). Additionally, Penrith (1984) identified potential differences in elytral shape, suggesting that it was "less elongate, broader, and more abruptly tapered posteriorly" in *O. u. schulzeae*. Therefore, I also measured the elytral length and elytral width (at the midpoint of elytral length) of each specimen and calculated elytral ratios (EL/EW) to assess putative differences in dorsal shape (Figs. 12-13). Although sexual dimorphism may potentially contribute to elytral shape variation, I chose to include both males and females in



Figures 6-9. Pronotum (6-7) and prosternum (8-9) of *Onymacris unguicularis unguicularis* (6,8) and *O. u. schulzeae*, illustrating measurements used for pronotal (7) and prosternal (9) ratio calculations.



Figures 10-11. Lateral aspects of the prosternal process, depicting a blunt apex (10) in *O. u. schulzeae* and a tooth-like apex (11) in *O. u. unguicularis*.



Figures 12-13. Elytra of *Onymacris unguicularis unguicularis* (12) and *O. u. schulzeae* (13), depicting measurements used for elytral ratio calculations.

the morphometric analysis. Sexual dimorphism is evident in *Onymacris unguicularis*: males have longer legs and, uniquely within the genus, possess setose brushes on the anterior femora (Penrith 1975). However, only one species of *Onymacris*, *O. plana*, exhibits significant sexual dimorphism in elytral shape; in all others there is substantial overlap, with female elytra being only slightly broader than male elytra (Lamb et al. 2013).

Results and Discussion

Genetic divergence was negligible within and among populations for each subspecies of *O. unguicularis*; however, genetic divergence was substantial between subspecies (3.2% for COII and 3.0% for COI). Observed divergence levels were complemented by my results from the likelihood analysis: no haplotypes were shared between subspecies, which were identified as two well-supported lineages in the ML tree (Fig. 14). Additionally, morphological analyses revealed significant subspecific differences in the elytral, prosternal, and pronotal shapes (all $p < 0.0001$), with minimal overlap in the prosternal and pronotal data but broad overlap in the elytral ratios (Table 1). I also noted the position of the greatest elytral width in each specimen relative to the midpoint, finding that elytral width is widest anterior to the midpoint in both subspecies but closer to the pronotal suture in *Onymacris unguicularis schulzeae*.

Table 1. Pronotal, prosternal, and elytral ratio means and ranges.

Character	Subspecies	N	Mean	Range
pronotum	<i>O. u. unguicularis</i>	95	1.66 ± 0.08	1.47–1.83
	<i>O. u. schulzeae</i>	35	1.97 ± 0.13	1.73–2.35
prosternum	<i>O. u. unguicularis</i>	94	2.22 ± 0.17	1.86–2.71
	<i>O. u. schulzeae</i>	33	2.01 ± 0.14	1.65–2.34
elytra	<i>O. u. unguicularis</i>	95	1.44 ± 0.08	1.25–1.61
	<i>O. u. schulzeae</i>	34	1.35 ± 0.07	1.24–1.47

Alternatively, genetic distance values between subspecies of *O. rugatipennis* were negligible (0.397% for COII) throughout its geographic range. Maximum likelihood analysis

revealed that the two groups are not reciprocally monophyletic; rather, haplotypes placement in the ML tree showed no structure or pattern with respect to geography or subspecific assignment (Fig. 15).

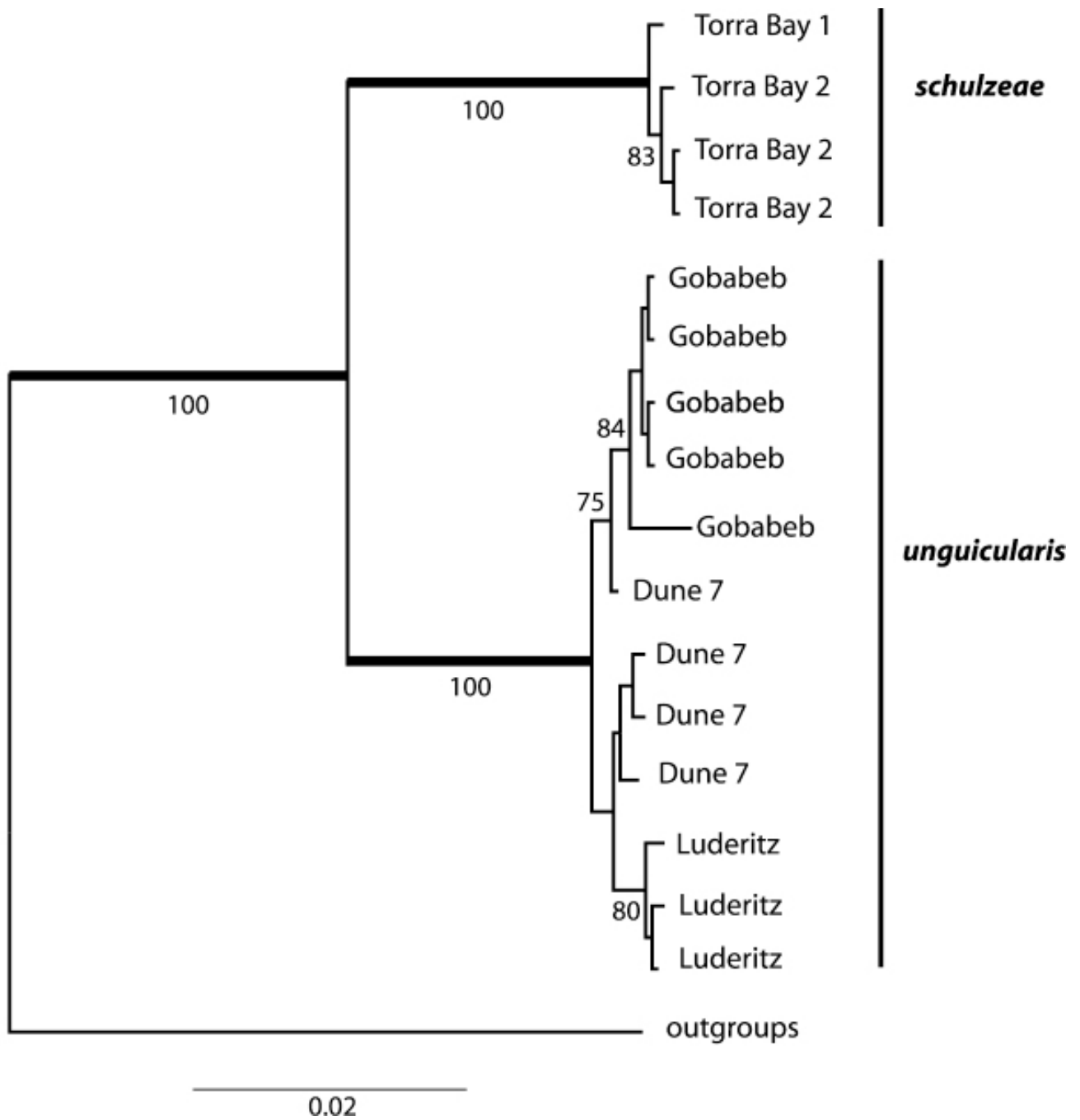


Figure 14. Maximum likelihood tree for populations of *O. unguicularis*.

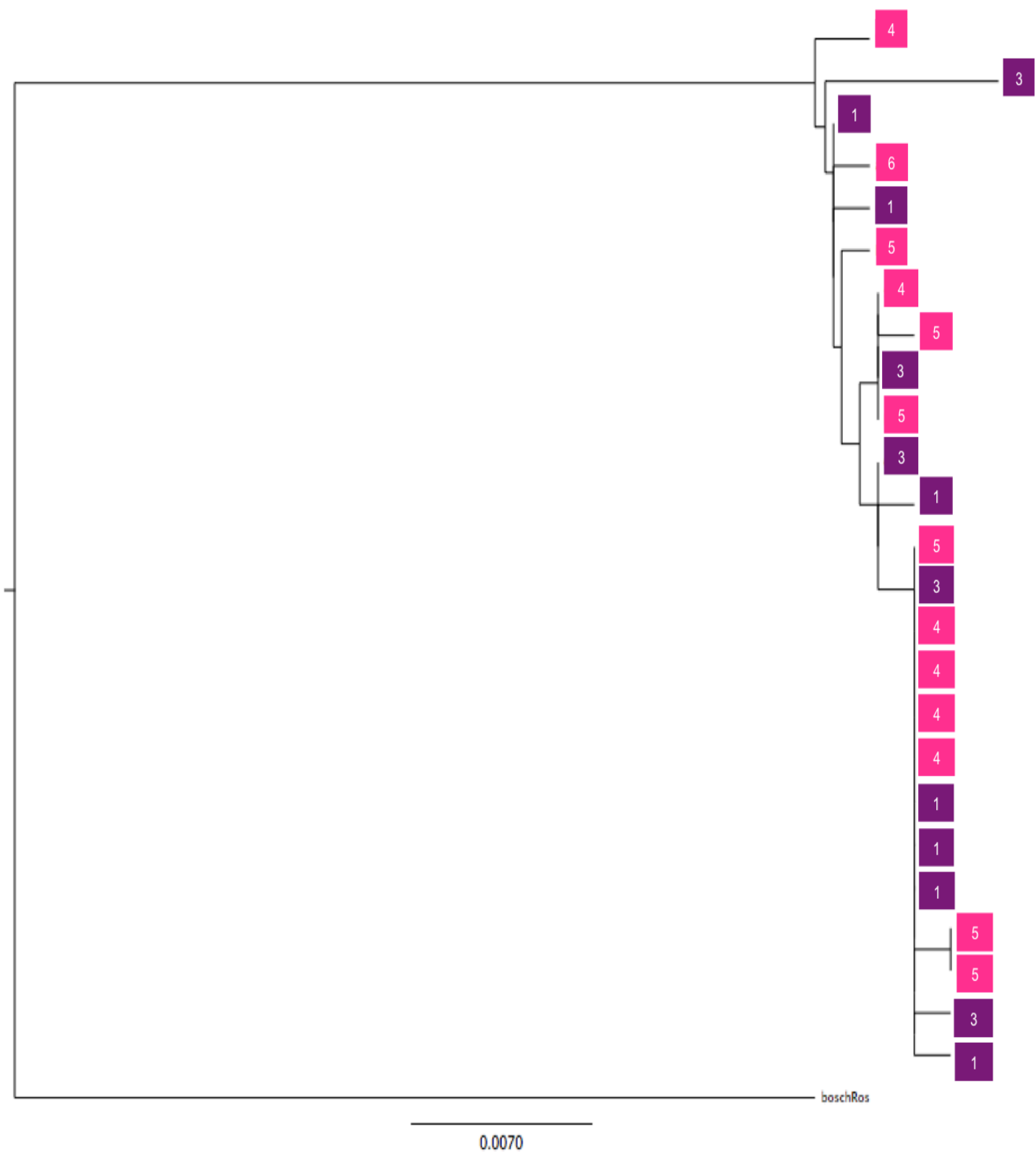


Figure 15. Maximum likelihood tree for populations of *O. rugatipennis*. Purple squares represent haplotypes of *O. r. rugatipennis* and pink squares represent haplotypes of *O. r. albotessellata*. Numbers represent collecting localities listed in Fig. 1B.

To reiterate, I employed Braby et al.'s (2012) criteria for subspecific delimitation to evaluate the polytypic status of *Onymacris unguicularis* and *O. rugatipennis* (Table 3), which recommended subspecies be defined as “partially isolated lineages of a species that are allopatric, phenotypically distinct, have at least one fixed diagnosable character state, and that these character differences are correlated with evolutionary independence according to population genetic structure.” For *Onymacris unguicularis*, I found support for each criterion: (1) The two subspecies are allopatric, confined to dune fields that are separated by ~300 km of unsuitable habitat; (2) *O. u. unguicularis* and *O. u. schulzeae* are phenotypically distinct; they are distinguished by quantitative differences in pronotal, prosternal, and elytral shape—as demonstrated by significantly different ratios. Subspecies also exhibit distinguishing patterns in larval variation, with the ninth abdominal tergum shorter and broader in northern populations (Schulze 1964); (3) Phenotypic differences correspond with genetic variation. The phylogeographic profile is perfectly congruent with the north-south partition in phenotypic variation, and reciprocal monophyly observed between northern and southern haplotypes in the phylogenetic tree—with associated levels of genetic divergence—indicate evolutionary independence. Therefore, I support Penrith's (1984) taxonomic interpretation that *Onymacris u. unguicularis* and *O. u. schulzeae* are valid subspecific taxa.

Alternatively, I found support for only one of Braby et al.'s (2012) criteria for *Onymacris rugatipennis*: (1) The two subspecies are not allopatric but parapatric, occupying contiguous, slightly overlapping ranges; (2) *O. r. rugatipennis* and *O. r. albotessellata* are phenotypically distinct; they are distinguished by differences in larval morphology and the presence of the waxy bloom in *O. r. albotessellata*. However, the adult forms are otherwise indistinguishable. (3) Molecular phylogenetic analysis revealed limited genetic divergence and no indication of evolutionary independence. The ML analysis did not resolve reciprocally monophyletic groups: rather the subspecies share two haplotypes, including a common haplotype found in

five of the six populations, which are distributed across the ML tree with no phylogeographic structure or subspecific patterning. On the basis of limited genetic divergence, parapatry, and a lack of evolutionary independence, I do not support *O. r. rugatipennis* and *O. r. albotessellata* as phylogenetically distinct lineages and therefore concur with Hauffe and Seely (1995) that a polytypic *O. rugatipennis* should be abandoned.

Table 3. Evaluation of Braby et al.'s criteria for *Onymacris unguicularis* and *Onymacris rugatipennis*

Criterion	<i>O. unguicularis</i>	<i>O. rugatipennis</i>
1	Met	Not met
2	Met	Met
3	Met	Not met

Future Studies

Although *Onymacris u. unguicularis* and *O. u. schulzeae* display reciprocal monophyly, evolutionary independence, and phenotypic distinctiveness, I would be reluctant to argue their elevation to full species because I analyzed only mtDNA sequences. Monophyly of alleles arises more quickly in mtDNA than nuclear loci because the genetically effective population size of organellar DNAs is approximately four times smaller than that of nuclear loci and thus genetic drift is faster (Palumbi et al. 2001). In mtDNA, this reduced genetically effective population size is due to uniparental inheritance and haploidy. Elevation of *O. u. unguicularis* and *O. u. schulzeae* to separate species should require demonstration of genetic divergence using nuclear genes; therefore, the next step of this study would be to analyze a series of appropriate nuclear loci.

Broader Implications

This study has broader implications due to its relevance to biodiversity and conservation. By some estimates (Maderspacher 2008), one out of every four animal species is a beetle! As

a result of their diversity and species-richness, many beetle taxa have not been well studied from an evolutionary perspective. Subspecific delimitation and confirmation thus have important applications in conservation and may be considered with respect to protective legislation in certain countries. Subspecies are often the focus of conservation efforts supported by substantial funding because they represent distinct evolutionary lineages with important genetic diversity. It is necessary to be able to recognize and confirm subspecies in order to ensure appropriate protective measures are taken to conserve them and their environments, thereby maintaining biodiversity.

Literature Cited

- Braby, M., Eastwood, R. & Murray, N. 2012 The subspecies concept in butterflies: has its application in taxonomy and conservation biology outlived its usefulness? *Biological Journal of the Linnean Society*. 106, 699-716.
- Cooper, P. D. 1982 Water balance and osmoregulation in a free-ranging tenebrionid beetle, *Onymacris unguicularis*, of the Namib Desert. *J. Insect Physiol.* . 28, 737-742.
- Coues, E. 1871 Review: Progress of American Ornithology. On the Mammals and Winter Birds of East Florida, with an Examination of Certain Assumed Specific Characters in Birds, and a Sketch of the Bird-Faunae of Eastern North America. by J. A. Allen. *The American Naturalist*. 5, 364-373.
- Hamilton, W. & Seely, M. 1976 Fog basking by the Namib Desert beetle, *Onymacris unguicularis*. *Nature*. 262, 284-285.
- Hauffe, H. C., Searle, J. B. & Seely, M. K. 1994 The taxonomic status of two morphotypes of the tenebrionid *Onymacris rugatipennis*: indications from behavioural and genetical studies. *Journal of Zoology* . 233, 193-201.
- James, F. 2010 Avian Subspecies: Introduction. *Ornithological Monographs* . 67, 1-5.
- Lamb, T. & Bond, J. 2013 A multilocus perspective on phylogenetic relationships in the Namib darkling beetle genus *Onymacris* (Tenebrionidae). *Molecular Phylogenetics and Evolution* . 66, 757-765.
- Lamb, T., Pollard, R. & Bond, J. 2013 Genetic variation corroborates subspecific delimitation in the Namib fog-basking beetle, *Onymacris unguicularis* (Haag) (Tenebrionidae, Coleoptera). *ZooKeys* . 353, 47-60.
- Maderspacher, F. 2008 Genomics: An Inordinate Fondness for Beetles. *Current Biology*. 18, R466-R468.

- Mallet, J. 2013 Subspecies, Semispecies, Superspecies. In *Encyclopedia of Biodiversity*, vol. 7, pp. 45-48. Waltham, Massachusetts: Academic Press.
- Naidu, S. 2008 Why does the Namib Desert tenebrionid *Onymacris unguicularis* (Coleoptera: Tenebrionidae) fog-bask?. *Eur. J. Entomol.* . 105, 829-838.
- Norgaard, T. & Dacke, M. 2010 Fog-basking behaviour and water collection efficiency in Namib Desert Darkling beetles. *Frontiers in Zoology* . 7, 23.
- Osberg, D. C. & Hanrahan, S. A. 1986 Laboratory studies on hybridization among Namib Desert Tenebrionids (Coleoptera: Tenebrionidae). *Madoqua* . 14, 345-354.
- Palumbi, S. R., Cipriano, F. & Hare, M. P. 2001 Predicting Nuclear Gene Coalescence from Mitochondrial Data: The Three-Times Rule. *Evolution* . 55, 859-868.
- Schulze, L. 1964 The Tenebrionidae of southern Africa. XXXIX. A revised key to the larvae of *Onymacris* Allard (Coleoptera: Adesmiini). *Scientific Papers of the Namib Desert Research Station No 23: 1-7* .
- Wilson, E. O. & Brown, W. L. 1953 The Subspecies Concept and Its Taxonomic Application. *Systematic Zoology* . 2, 97-111.
- Winker, K. 2010 Subspecies Represent Geographically Partitioned Variation, A Gold Mine of Evolutionary Biology, and a Challenge for Conservation. In *Ornithological Monographs*, vol. 67, pp. 6-23. Washington: University of California Press.

Appendices

Appendix 1. Collecting localities and vouchers for *Onymacris* specimens.

Subspecies	Dataset	N	Locality (locality number)	Vouchers
<i>unguicularis</i>	morphometrics	10	Anigab	
		9	Blauberg	
		10	Bogenfels	
		1	Chaneis	
		12	Gobabeb	
		8	Grillental	
		9	Hottentot Bay	
		5	Luderitz	
		10	Spencer Bay	
		10	Swakopmund	
		11	Walvis Bay	
<i>schulzeae</i>	genetics	4	Dune 7, near Walvis Bay (3)	TL022-025
		5	Gobabeb (2)	TL026-030
		3	20 km E Luderitz (1) near Foz du Cunene, 8 km from coast,	TL031-033
<i>schulzeae</i>	morphometrics	3	Angola	
		4	Lacrau, 13 km N Fos du Cunene, Angola	
		11	Kaokoveld, between Koichab-Unjab rivers	
		12	Unjab River, 8 km from mouth	
		5	near Torra Bay	
<i>schulzeae</i>	genetics	3	near Torra Bay (1)	TL034-36
		1	near Torra Bay (2)	TL037
<i>rugatipennis</i>	genetics	7	Walvis Bay (1)	JEB 67, 69-75
		1	Rooibank (2)	G1
		4	Gobabeb (3)	G2-G5
<i>albotessellata</i>	genetics	6	Rostock Farm (4)	N19-24 91-92, 94, 95, 97
		6	Rostock Farm (5)	97
		1	dunes 69km S Hy 407 on Rd 707 (6)	707

Appendix 2. Aligned COI sequences for *Onymacris unguicularis*. Localities for haplotype numbers are listed in Appendix 1.

TL026 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL027 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL022 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL023 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL024 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL025 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL031 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL032 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL033 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL037 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL036 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT
 TL034 AATAAATGATTATTTTCAACTAACCACAAAGACATTGGAACACTTTACTTTATTTTGGT

TL026 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL027 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL022 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL023 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL024 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL025 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL031 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL032 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL033 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL037 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL036 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 TL034 GCATGAGCAGGAATAGTGGGAACATCTCTAAGTTTATTAATTCGAACAGAATTAGGAAAC
 ***** ** *****

TL026 CCCGGATCCCTAATTTGGAGACGATCAAACTATAACGTTATTGTAACCGCCACGCATTT
 TL027 CCCGGATCCCTAATTTGGAGACGATCAAACTATAACGTTATTGTAACCGCCACGCATTT
 TL022 CCCGGATCCCTAATTTGGAGACGATCAAACTATAACGTTATTGTAACCGCCACGCATTT
 TL023 CCCGGATCCCTAATTTGGAGACGATCAAACTATAACGTTATTGTAACCGCCACGCATTT
 TL024 CCCGGATCCCTAATTTGGAGACGATCAAACTATAACGTTATTGTAACCGCCACGCATTT
 TL025 CCCGGATCCCTAATTTGGAGACGATCAAACTATAACGTTATTGTAACCGCCACGCATTT
 TL031 CCCGGATCCCTAATTTGGAGACGATCAAACTATAACGTTATTGTAACCGCCACGCATTT
 TL032 CCCGGATCCCTAATTTGGAGACGATCAAACTATAACGTTATTGTAACCGCCACGCATTT
 TL033 CCCGGATCCCTAATTTGGAGACGATCAAACTATAACGTTATTGTAACCGCCACGCATTT
 TL037 CCCGGATCCCTAATTTGGAGATGATCAAACTATAACGTTATTGTAACCGCTCACGCATTT
 TL036 CCCGGATCCCTAATTTGGAGATGATCAAACTATAACGTTATTGTAACCGCTCACGCATTT
 TL034 CCCGGATCCCTAATTTGGAGATGATCAAACTATAACGTTATTGTAACCGCTCACGCATTT

TL026 ATTATAATTTTCTTTATAGTTATACCTATTATAATCGGGGGATTGGAAATTGATTAGTC
 TL027 ATTATAATTTTCTTTATAGTTATACCTATTATAATCGGGGGATTGGAAATTGATTAGTC
 TL022 ATTATAATTTTCTTTATAGTTATACCTATTATAATCGGGGGATTGGAAATTGATTAGTC
 TL023 ATTATAATTTTCTTTATAGTTATACCTATTATAATCGGGGGATTGGAAATTGATTAGTC
 TL024 ATTATAATTTTCTTTATAGTTATACCTATTATAATCGGGGGATTGGAAATTGATTAGTC
 TL025 ATTATAATTTTCTTTATAGTTATACCTATTATAATCGGGGGATTGGAAATTGATTAGTC
 TL031 ATTATAATTTTCTTTATAGTTATACCTATTATAATCGGGGGATTGGAAATTGATTAGTC
 TL032 ATTATAATTTTCTTTATAGTTATACCTATTATAATCGGGGGATTGGAAATTGATTAGTC
 TL033 ATTATAATTTTCTTTATAGTTATACCTATTATAATCGGGGGATTGGAAATTGATTAGTC
 TL037 ATTATAATTTTCTTTATAGTTATACCGATTATAATTTGGGGGATTGGAAATTGATTAGTT
 TL036 ATTATAATTTTCTTTATAGTTATACCGATTATAATTTGGGGGATTGGAAATTGATTAGTT
 TL034 ATTATAATTTTCTTTATAGTTATACCGATTATAATTTGGGGGATTGGAAATTGATTAGTT

TL026 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGG
 TL027 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGA
 TL022 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGG
 TL023 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGG
 TL024 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGG
 TL025 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGA
 TL031 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGG
 TL032 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGG
 TL033 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGG
 TL037 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGA
 TL036 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGA
 TL034 CCCCTAATATTAGGTGCACCCGATATAGCATTTCACCGTATAAATAATATAAGATTCTGA

TL026 CTCTTACCCCATCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCAGGA
 TL027 CTCTTACCCCATCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCAGGA
 TL022 CTCTTACCCCATCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCAGGA

TL023 CTCTTACCCCATCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCAGGA
TL024 CTCTTACCCCATCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCAGGA
TL025 CTCTTACCCCATCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCAGGA
TL031 CTCTTACCCCATCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCAGGA
TL032 CTCTTACCCCATCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCAGGA
TL033 CTCTTACCCCATCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCAGGA
TL037 CTCTTACCTCCCTCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCCGGA
TL036 CTCTTACCTCCCTCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCCGGA
TL034 CTCTTACCTCCCTCATTAACTATTATTAATAAGAAGAATCGTAGAAAGAGGGGCCGGA
***** ** *****

TL026 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCTCATGGTGGGTCTCCGTT
TL027 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL022 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL023 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL024 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL025 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL031 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL032 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL033 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL037 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL036 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
TL034 ACTGGATGAACAGTCTACCCCTTATCATCAAATATTGCCATGGTGGGTCTCCGTT
***** *****

TL026 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL027 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL022 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL023 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL024 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL025 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL031 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL032 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL033 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL037 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL036 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
TL034 GATTTAGCTATTTTGTCTTTCATTTAGCAGGAATCTCATCAATTCTTGGAGCAGTAAAC
***** *****

TL026 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL027 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL022 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL023 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL024 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL025 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL031 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL032 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL033 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL037 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL036 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
TL034 TTCATTACTACAGTTATTAATATACGACCCAGGGTATAACATTTGACCGAATACCTTTA
***** *****

TL026 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTACTCCTGTCTCTCCAGTATTAGCG
TL027 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTGTCTCTCCAGTATTAGCG
TL022 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTGTCTCTCCAGTATTAGCG
TL023 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTGTCTCTCCAGTATTAGCG
TL024 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTGTCTCTCCAGTATTAGCG
TL025 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTGTCTCTCCAGTATTAGCG
TL031 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTACTCCTGTCTCTCCAGTATTAGCG
TL032 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTGTCTCTCCAGTATTAGCG
TL033 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTGTCTCTCCAGTATTAGCG
TL037 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTACTCCTATCTCTCCAGTATTAGCA
TL036 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTACTCCTATCTCTCCAGTATTAGCA
TL034 TTTGTTTGAGCTGTCGTAATTACAGCAGTTCTCCTACTCCTATCTCTCCAGTATTAGCA
***** *****

TL026 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCG
TL027 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCG
TL022 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCA
TL023 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCA
TL024 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCA
TL025 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCA
TL031 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCA
TL032 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCA
TL033 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCA
TL037 GGAGCTATTACCATATTATTAACAGACGAAACATTAACACTTCATTCTTTGACCCAGCA

TL036 GGAGCTATTACCATATTATTAACCGACCGAAACATTAACACCTCATTTTTTTGACCCAGCA
TL034 GGAGCTATTACCATATTATTAACCGACCGAAACATTAACACCTCATTTTTTTGACCCAGCA

TL026 GGGGGTGGAGATCCCATCCTATACCAACACTTATTTTGATTTTTTTGGACACCCCGAAGTA
TL027 GGGGGTGGAGATCCCATCCTATACCAACACTTATTTTGATTTTTTTGGACACCCCGAAGTA
TL022 GGGGGTGGAGATCCCATCCTATACCAACACTTATTTTGATTTTTTTGGACACCCCGAAGTA
TL023 GGGGGTGGAGATCCCATCCTATACCAACACTTATTTTGATTTTTTTGGACACCCCGAAGTA
TL024 GGGGGTGGAGATCCCATCCTATACCAACACTTATTTTGATTTTTTTGGACACCCCGAAGTA
TL025 GGGGGTGGAGATCCCATCCTATACCAACACTTATTTTGATTTTTTTGGACACCCCGAAGTA
TL031 GGAGGTGGAGATCCCATCCTATACCAACACTTATTTTGATTTTTTTGGACACCCCGAAGTA
TL032 GGAGGTGGAGATCCCATCCTATACCAACACTTATTTTGATTTTTTTGGACACCCCGAAGTA
TL033 GGAGGTGGAGATCCCATCCTATACCAACACTTATTTTGATTTTTTTGGACACCCCGAAGTA
TL037 GGGGCGGAGATCCCATCCTCTACCAACACTTGTTTTGATTTTTTTGGCCACCCCGAAGTA
TL036 GGGGGTGGAGATCCCATCCTCTACCAACACTTGTTTTGATTTTTTTGGCCACCCCGAAGTA
TL034 GGGGGTGGAGATCCCATCCTCTACCAACACTTGTTTTGATTTTTTTGGCCACCCCGAAGTA
**

TL026 TATATTCCTAATTTTACCTGGATTTCGGAATAATTTCTCATATTATTAGTCAAGAAAGAGGA
TL027 TATATTCCTAATTTTACCTGGATTTCGGAATAATTTCTCATATTATTAGTCAAGAAAGAGGA
TL022 TATATTCCTAATTTTACCTGGATTTCGGAATAATTTCTCATATTATTAGTCAAGAAAGAGGA
TL023 TATATTCCTAATTTTACCTGGATTTCGGAATAATTTCTCATATTATTAGTCAAGAAAGAGGA
TL024 TATATTCCTAATTTTACCTGGATTTCGGAATAATTTCTCATATTATTAGTCAAGAAAGAGGA
TL025 TATATTCCTAATTTTACCTGGATTTCGGAATAATTTCTCATATTATTAGTCAAGAAAGAGGA
TL031 TATATTCCTAATTTTACCTGGATTTCGGAATAATTTCTCATATTATTAGTCAAGAAAGAGGG
TL032 TATATTCCTAATTTTACCTGGATTTCGGAATAATTTCTCATATTATTAGTCAAGAAAGAGGG
TL033 TATATTCCTAATTTTACCTGGATTTCGGAATAATTTCTCATATTATTAGTCAAGAAAGAGGG
TL037 TATATTTAATTTTACCCGGATTTCGGAATAATTTCTCATATCATTAGTCAAGAAAGAGGA
TL036 TATATTTAATTTTACCCGGATTTCGGAATAATTTCTCATATCATTAGTCAAGAAAGAGGA
TL034 TATATTTAATTTTACCCGGATTTCGGAATAATTTCTCATATCATTAGTCAAGAAAGAGGA

TL026 AAAAAGGAAGCTTTTGGAACTAGGAATAATTTACGCCATAATAGCAATCGGATTACTC
TL027 AAAAAGGAAGCTTTTGGAACTAGGAATAATTTACGCCATAATAGCAATCGGATTACTC
TL022 AAAAAGGAAGCTTTTGGAACTAGGAATAATTTACGCCATAATAGCAATCGGATTACTT
TL023 AAAAAGGAAGCTTTTGGAACTAGGAATAATTTACGCCATAATAGCAATCGGATTACTT
TL024 AAAAAGGAAGCTTTTGGAACTAGGAATAATTTACGCCATAATAGCAATCGGATTACTT
TL025 AAAAAGGAAGCTTTTGGAACTAGGAATAATTTACGCCATAATAGCAATCGGATTACTC
TL031 AAAAAGGAAGCTTTTGGAACTAGGAATAATTTACGCCATAATAGCAATCGGATTACTT
TL032 AAAAAGGAAGCTTTTGGAACTAGGAATAATTTACGCCATAATAGCAATCGGATTACTT
TL033 AAAAAGGAAGCTTTTGGAACTAGGAATAATTTACGCCATAATAGCAATCGGATTACTT
TL037 AAAAAGGAAGCTTTTGGAACTGGGGATAATTTACGCCATAATAGCAATCGGATTACTT
TL036 AAAAAGGAAGCTTTTGGAACTGGGGATAATTTACGCCATAATAGCAATCGGATTACTT
TL034 AAAAAGGAAGCTTTTGGAACTGGGGATAATTTACGCCATAATAGCAATCGGATTACTT

TL026 GGGTTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL027 GGGTTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL022 GGGTTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL023 GGGTTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL024 GGGTTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL025 GGGTTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL031 GGGTTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL032 GGGTTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL033 GGGTTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL037 GGATTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL036 GGATTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
TL034 GGATTCGTTGTATGAGCACACCACATATTTACAGTTGGAATAGATGTAGATACACGAGCA
**

TL026 TATTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGGTGG
TL027 TATTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGGTGG
TL022 TATTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGGTGG
TL023 TATTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGGTGG
TL024 TATTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGGTGG
TL025 TATTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGGTGG
TL031 TATTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGGTGG
TL032 TATTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGGTGG
TL033 TATTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGGTGG
TL037 TACTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGATGA
TL036 TACTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGATGA
TL034 TACTTTACATCAGCAACTATAATTATTGCTGTTCCAACAGGAATTAATAATTTTTAGATGA
**

TL026 CTAGCAACTCTCCAGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGG
TL027 CTAGCAACTCTCCAGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGG
TL022 CTAGCAACTCTCCAGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGG

TL023 CTAGCAACTCTCCACGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGG
TL024 CTAGCAACTCTCCACGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGG
TL025 CTAGCAACTCTCCACGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGG
TL031 CTAGCAACTCTCCACGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGG
TL032 CTAGCAACTCTCCACGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGG
TL033 CTAGCAACTCTCCACGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGG
TL037 CTAGCAACTCTCCACGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGT
TL036 CTAGCAACTCTCCACGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGT
TL034 CTAGCAACTCTCCACGGAACACAATTAATTAACAGGCCATCAATACTTTGAGCCCTAGGT

TL026 TTCGTGTTTTTATTCACGGTTGGGGGACTAACTGGAGTAATTTTAGCAAATTCCTCTATC
TL027 TTCGTGTTTTTATTCACGGTTGGGGGACTAACTGGAGTAATTTTAGCAAATTCCTCTATC
TL022 TTCGTGTTTTTATTCACGGTTGGGGGACTAACTGGAGTAATTTTAGCAAATTCCTCTATC
TL023 TTCGTGTTTTTATTCACGGTTGGGGGACTAACTGGAGTAATTTTAGCAAATTCCTCTATC
TL024 TTCGTGTTTTTATTCACGGTTGGGGGACTAACTGGAGTAATTTTAGCAAATTCCTCTATC
TL025 TTCGTGTTTTTATTCACGGTTGGGGGACTAACTGGAGTAATTTTAGCAAATTCCTCTATC
TL031 TTCGTGTTTTTATTCACGGTTGGGGGACTAACTGGAGTAATTTTAGCAAATTCCTCTATC
TL032 TTCGTGTTTTTATTCACGGTTGGGGGACTAACTGGAGTAATTTTAGCAAATTCCTCTATC
TL033 TTCGTGTTTTTATTCACGGTTGGGGGACTAACTGGAGTAATTTTAGCAAATTCCTCTATC
TL037 TTCGTGTTTTTATTCACGGTTCGGAGGATTAACAGGAGTAATTTTAGCAAATTCCTCAATC
TL036 TTCGTGTTTTTATTCACGGTTCGGAGGATTAACAGGAGTAATTTTAGCAAATTCCTCAATC
TL034 TTCGTGTTTTTATTCACGGTTCGGAGGATTAACAGGAGTAATTTTAGCAAATTCCTCAATC
***** ** **

TL026 GACATTATACTTCATGATACTTACTATGTTGTGGCCCACTTCCATTACGTTCTTTCCATG
TL027 GACATTATACTTCATGATACTTACTATGTTGTGGCCCACTTCCATTACGTTCTTTCCATG
TL022 GACATTATACTTCATGATACTTACTATGTTGTGGCCCACTTCCATTACGTTCTTTCCATG
TL023 GACATTATACTTCATGATACTTACTATGTTGTGGCCCACTTCCATTATGTTCTTTCCATG
TL024 GACATTATACTTCATGATACTTACTATGTTGTGGCCCACTTCCATTACGTTCTTTCCATG
TL025 GACATTATACTTCATGATACTTACTATGTTGTGGCCCACTTCCATTACGTTCTTTCCATG
TL031 GACATTATACTTCATGATACTTACTATGTTGTGGCCCACTTCCATTACGTTCTTTCCATG
TL032 GACATTATACTTCATGATACTTACTATGTTGTGGCCCACTTCCATTACGTTCTTTCCATG
TL033 GACATTATACTTCATGATACTTACTATGTTGTGGCCCACTTCCATTACGTTCTTTCCATG
TL037 GACATTATACTTCATGATACTTACTATGTTGTAGCCCACTTCCATTACGTTCTTTCCATA
TL036 GACATTATACTTCATGATACTTACTATGTTGTAGCCCACTTCCATTACGTTCTTTCCATA
TL034 GACATTATACTTCATGATACTTACTATGTTGTAGCCCACTTCCATTACGTTCTTTCCATA

TL026 GGGGCAGTATTTGCCATTTTCGGGAGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL027 GGGGCAGTATTTGCCATTTTCGGGAGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL022 GGGGCAGTATTTGCCATTTTCGGGAGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL023 GGGGCAGTATTTGCCATTTTCGGGAGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL024 GGGGCAGTATTTGCCATTTTCGGGAGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL025 GGGGCAGTATTTGCCATTTTCGGGAGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL031 GGGGCAGTATTTGCCATTTTCGGGGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL032 GGGGCAGTATTTGCCATTTTCGGGGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL033 GGGGCAGTATTTGCCATTTTCGGGGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL037 GGGGCAGTATTTGCCATTTTCGGGGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL036 GGGGCAGTATTTGCCATTTTCGGGGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA
TL034 GGGGCAGTATTTGCCATTTTCGGGGGACTAGTTCACTGATTCCCATTATTTACAGGTTTA

TL026 TCCTTAAATATAAAAATATGTAATAATTCATTTATTACCATATTCATTGGGGTAAACCTA
TL027 TCCTTAAATATAAAAATATGTAATAATTCATTTATTACCATATTCATTGGGGTAAACCTA
TL022 TCCTTAAATATAAAAATATGTAATAATTCATTTATTACCATATTCATTGGGGTAAACCTA
TL023 TCCTTAAATATAAAAATATGTAATAATTCATTTATTACCATATTCATTGGGGTAAACCTA
TL024 TCCTTAAATATAAAAATATGTAATAATTCATTTATTACCATATTCATTGGGGTAAACCTA
TL025 TCCTTAAATATAAAAATATGTAATAATTCATTTATTACCATATTCATTGGGGTAAACCTA
TL031 TCCTTAAATATAAAAATATGTAATAATTCATTTATTACCATATTCATTGGGGTAAACCTA
TL032 TCCTTAAATATAAAAATATGTAATAATTCATTTATTACCATATTCATTGGGGTAAACCTA
TL033 TCCTTAAATATAAAAATATGTAATAATTCATTTATTACCATATTCATTGGGGTAAACCTA
TL037 TCCTTGAACATAAAAATATGTAATAATCCAATTTATTACTATATTCATTGGGGTAAACCTA
TL036 TCCTTGAACATAAAAATATGTAATAATCCAATTTATTACTATATTCATTGGGGTAAACCTA
TL034 TCCTTGAACATAAAAATATGTAATAATCCAATTTATTACTATATTCATTGGGGTAAACCTA

TL026 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL027 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL022 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL023 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL024 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL025 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL031 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL032 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL033 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL037 ACATTTTTTCCCAACATTTCTTGGATTAAGAGGAATACCTCGACGATATTCTGACTAC

TL036 ACATTCTTTCCCAACATTTTCTTGATTAAGAGGAATACCTCGACGATATTCTGACTAC
TL034 ACATTCTTTCCCAACATTTTCTTGATTAAGAGGAATACCTCGACGATATTCTGACTAC

TL026 CCAGATGCCTACATAATATGAAATATTGTTTCATCAGTGGATCAATAATTTTCGTTAATT
TL027 CCAGATGCCTACATAATATGAAATATTGTTTCATCAGTGGATCAATAATTTTCGTTAATT
TL022 CCAGATGCCTACATAATATGAAATATTGTTTCATCAGTGGATCAATAATTTTCGTTAATT
TL023 CCAGATGCCTACATAATATGAAATATTGTTTCATCAGTGGATCAATAATTTTCGTTAATT
TL024 CCAGATGCCTACATAATATGAAATATTGTTTCATCAGTGGATCAATAATTTTCGTTAATT
TL025 CCAGATGCCTACATAATATGAAATATTGTTTCATCAGTGGATCAATAATTTTCGTTAATT
TL031 CCAGATGCCTACATAATATGAAATATTGTTTCATCAGTGGATCAATAATTTTCGTTAATT
TL032 CCAGATGCCTACATAATATGAAATATTGTTTCATCAGTGGATCAATAATTTTCGTTAATT
TL033 CCAGATGCCTACATAATATGAAATATTGTTTCATCAGTGGATCAATAATTTTCGTTAATT
TL037 CCGGATGCCTACATAATATGAAATATTGTTTCATCTATTGGATCAATAATTTTCGTTAATT
TL036 CCGGATGCCTACATAATATGAAATATTGTTTCATCTATTGGATCAATAATTTTCGTTAATT
TL034 CCGGATGCCTACATAATATGAAATATTGTTTCATCTATTGGATCAATAATTTTCGTTAATT
** * ***** * *****

TL026 AGAGTTTTATATTTAATTTTATCGTGTGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL027 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL022 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL023 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL024 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL025 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL031 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL032 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL033 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL037 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL036 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT
TL034 AGAGTTTTATATTTAATTTTATCGTATGAGAATCATTTTCATCTTCGCGAAAACTGTT

TL026 TTCCCCCTTAGAATAACCTCATCAATTGAATGACTACAATTAACACCTCCCGCAGAACAC
TL027 TTCCCCCTTAGAATAACCTCATCAATTGAATGACTACAATTAACACCTCCCGCAGAACAC
TL022 TTCCCCCTTAGAATAACCTCATCAATTGAATGACTACAATTAACACCTCCCGCAGAACAC
TL023 TTCCCCCTTAGAATAACCTCATCAATTGAATGACTACAATTAACACCTCCCGCAGAACAC
TL024 TTCCCCCTTAGAATAACCTCATCAATTGAATGACTACAATTAACACCTCCCGCAGAACAC
TL025 TTCCCCCTTAGAATAACCTCATCAATTGAATGACTACAATTAACACCTCCCGCAGAACAC
TL031 TTCCCCCTTAGAATAACCTCATCAATTGAATGACTACAATTAACACCTCCCGCAGAACAC
TL032 TTCCCCCTTAGAATAACCTCATCAATTGAATGACTACAATTAACACCT-----
TL033 TTCCCCCTTAGAATAACCTCATCAATTGAATGACTACAATTAAC-----
TL037 TTCCCCCTTAGAATAACCTCATCAATTGAGTGACTACAATTAAC-----
TL036 TTCCCCCTTAGAATAACCTCATCAATTGAGTGACTACAATTAAC-----
TL034 TTCCCCCTTAGAATAACCTCATCAATTGAGTGACTACAATTAAC-----

TL026 AGATATTCTGAATTACCAATACTATCTTCTAA
TL027 AGATATTCTGAATTACCAATACTATCTTCTAA
TL022 AGATATTCTGAATTACCAATACTATCTTCTAA
TL023 AGATATTCTGAATT-----
TL024 AGATATTCTGAA-----
TL025 AGATATTCTGAATTACCAATACTATCTTCTAA
TL031 AGATATTCTGAATTACCAATACTATCTTCTAA
TL032 -----
TL033 -----
TL037 -----
TL036 -----
TL034 -----

Appendix 3. Aligned COII sequences for *Onymacris unguicularis*. Localities for haplotype numbers are listed in Appendix 1.

TL026 ATAGCCACATGAAAAACCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAAATTA
TL027 ATAGCCACATGAAAAACCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAAATTA
TL022 ATAGCCACATGAAAAACCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAAATTA
TL025 ATAGCCACATGAAAAACCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAAATTA
TL031 ATAGCCACATGAAAAACCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAAATTA
TL037 ATAGCCACATGAAAAACCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAAATTA
TL034 ATAGCCACATGAAAAACCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAAATTA

TL026 TCATTCTTTACGACCATACCCTCTTAATTTTAGTAATTATTACTATTTTAGTTGGGCAA
TL027 TCATTCTTTACGACCATACCCTCTTAATTTTAGTAATTATTACTATTTTAGTTGGGCAA
TL022 TCATTCTTTACGACCATACCCTCTTAATTTTAGTAATTATTACTATTTTAGTTGGGCAA
TL025 TCATTCTTTACGACCATACCCTCTTAATTTTAGTAATTATTACTATTTTAGTTGGGCAA

TL031 TCATTCTTTCACGACCATACCCTCTTAATTTTAGTAATTACTATTTTAGTTGGGCAA
TL037 TCATTCTTTCATGACCATACCCTCTTAATTTTAGTAATTACTATTTTAGTTGGGCAA
TL034 TCATTCTTTCATGACCATACCCTCTTAATTTTAGTAATTACTATTTTAGTTGGGCAA

TL026 ATACTAACAGGGCTATTTTAAATAAATACACCACCGATACCTTTTAGAGGGGACAAC
TL027 ATACTAACAGGGCTATTTTAAATAAATACACCACCGATACCTTTTAGAGGGGACAAC
TL022 ATACTAACAGGGCTATTTTAAATAAATACACCACCGATACCTTTTAGAGGGGACAAC
TL025 ATACTAACAGGGCTATTTTAAATAAATACACCACCGATACCTTTTAGAGGGGACAAC
TL031 ATACTAACAGGGCTATTTTAAATAAATACACCACCGATACCTTTTAGAGGGGACAAC
TL037 ATACTAACAGGACTATTCTTCAACAAATACACCACCGATACCTTTTAGAGGGGACAAT
TL034 ATACTAACAGGACTATTCTTCAACAAATACACCACCGATACCTTTTAGAGGGGACAAT

TL026 ATTGAAATCATCTGAACAATCCTTCCCGAATCACCTTAATCTTCATTGCACTACCATCC
TL027 ATTGAAATCATCTGAACAATCCTTCCCGAATCACCTTAATCTTCATTGCACTACCATCC
TL022 ATTGAAATCATCTGAACAATCCTTCCCGAATCACCTTAATCTTCATTGCACTACCATCC
TL025 ATTGAAATCATCTGAACAATCCTTCCCGAATCACCTTAATCTTCATTGCACTACCATCC
TL031 ATTGAAATCATCTGAACAATCCTTCCCGAATCACCTTAATCTTCATTGCACTACCATCC
TL037 ATTGAAATCATCTGAACAATCCTTCCCGAATCACCTTAATCTTCATTGCACTACCATCC
TL034 ATTGAAATCATCTGAACAATCCTTCCCGAATCACCTTAATCTTCATTGCACTACCATCC

TL026 CTTCAACTAATTTATATCCTAGACGAAACTAATAACCCATTAATTTCAATTAAGCCATT
TL027 CTTCAACTAATTTATATCCTAGACGAAACTAATAACCCATTAATTTCAATTAAGCCATT
TL022 CTTCAACTAATTTATATCCTAGACGAAACTAATAACCCATTAATTTCAATTAAGCCATT
TL025 CTTCAACTAATTTATATCCTAGACGAAACTAATAACCCATTAATTTCAATTAAGCCATT
TL031 CTTCAACTAATTTATATCCTAGACGAAACTAATAACCCATTAATTTCAATTAAGCCATT
TL037 CTTCAACTAATTTATATCCTAGACGAAACTAATAACCCATTAATTTCAATTAAGCCATT
TL034 CTTCAACTAATTTATATCCTAGACGAAACTAATAACCCATTAATTTCAATTAAGCCATT

TL026 GGACATCAATGATACTGGTCATATGAATATTCAGATTTCAAAAACATTGAATTCGATTCT
TL027 GGACATCAATGATACTGGTCATATGAATATTCAGATTTCAAAAACATTGAATTCGATTCT
TL022 GGACATCAATGATACTGGTCATATGAATATTCAGATTTCAAAAACATTGAATTCGATTCT
TL025 GGACATCAATGATACTGGTCATATGAATATTCAGATTTCAAAAACATTGAATTCGATTCT
TL031 GGACATCAATGATACTGGTCATATGAATATTCAGATTTCAAAAACATTGAATTCGATTCT
TL037 GGACATCAATGATACTGGTCATATGAATATTCAGATTTCAAAAACATTGAATTCGATTCT
TL034 GGACATCAATGATACTGGTCATATGAATATTCAGATTTCAAAAACATTGAATTCGATTCT

TL026 TATATGATTCCTCAAACAGAAATAAATCAATTCACCTCCGGCTATTAGACGTTGACAAT
TL027 TATATGATTCCTCAAACAGAAATAAATCAATTCACCTCCGGCTATTAGACGTTGACAAT
TL022 TATATGATTCCTCAAACAGAAATAAATCAATTCACCTCCGGCTATTAGACGTTGACAAT
TL025 TATATGATTCCTCAAACAGAAATAAATCAATTCACCTCCGGCTATTAGACGTTGACAAT
TL031 TATATGATTCCTCAAACAGAAATAAATCAATTCACCTCCGGCTATTAGACGTTGACAAT
TL037 TATATGATTCCTCAAACAGAAATAAATCAATTCACCTCCGGCTATTAGACGTTGACAAT
TL034 TATATGATTCCTCAAACAGAAATAAATCAATTCACCTCCGGCTATTAGACGTTGACAAT

TL026 CGAATAGTAGTCCCCTATCTTTCACAAATTCGCATACTTATCTCAGCAGCAGACGTTATT
TL027 CGAATAGTAGTCCCCTATCTTTCACAAATTCGCATACTTATCTCAGCAGCAGACGTTATT
TL022 CGAATAGTAGTCCCCTATCTTTCACAAATTCGCATACTTATCTCAGCAGCAGACGTTATT
TL025 CGAATAGTAGTCCCCTATCTTTCACAAATTCGCATACTTATCTCAGCAGCAGACGTTATT
TL031 CGAATAGTAGTCCCCTATCTTTCACAAATTCGCATACTTATCTCAGCAGCAGACGTTATT
TL037 CGAATAGTAGTCCCCTATCTTTCACAAATTCGCATACTTATCTCAGCAGCAGACGTTATT
TL034 CGAATAGTAGTCCCCTATCTTTCACAAATTCGCATACTTATCTCAGCAGCAGACGTTATT

TL026 CACTCTTGAACGTTCATCTCTAGGAGTTAAAGTAGATGCAACACCAGGACGATTAAT
TL027 CACTCTTGAACGTTCATCTCTAGGAGTTAAAGTAGATGCAACACCAGGACGATTAAT
TL022 CACTCTTGAACGTTCATCTCTAGGAGTTAAAGTAGATGCAACACCAGGACGATTAAT
TL025 CACTCTTGAACGTTCATCTCTAGGAGTTAAAGTAGATGCAACACCAGGACGATTAAT
TL031 CACTCTTGAACGTTCATCTCTAGGAGTTAAAGTAGATGCAACACCAGGACGATTAAT
TL037 CACTCTTGAACGTTCATCTCTAGGAGTTAAAGTAGATGCAACACCAGGACGATTAAT
TL034 CACTCTTGAACGTTCATCTCTAGGAGTTAAAGTAGATGCAACACCAGGACGATTAAT

TL026 CAAGTTAGATTCATTGCAACCCGACCAGGATTAATATATGGACAATGTTCTGAAATTTGT
TL027 CAAGTTAGATTCATTGCAACCCGACCAGGATTAATATATGGACAATGTTCTGAAATTTGT
TL022 CAAGTTAGATTCATTGCAACCCGACCAGGATTAATATATGGACAATGTTCTGAAATTTGT
TL025 CAAGTTAGATTCATTGCAACCCGACCAGGATTAATATATGGACAATGTTCTGAAATTTGT
TL031 CAAGTTAGATTCATTGCAACCCGACCAGGATTAATATATGGACAATGTTCTGAAATTTGT
TL037 CAAGTTAGATTCATTGCAACCCGACCAGGATTAATATATGGACAATGTTCTGAAATTTGT
TL034 CAAGTTAGATTCATTGCAACCCGACCAGGATTAATATATGGACAATGTTCTGAAATTTGT

TL026 GGAGCAAATCATAGATTCATGCCAATCGTAGTAGAAAGAATCTCCCCTAATTACTTCATT
 TL027 GGAGCAAATCATAGATTCATGCCAATCGTAGTAGAAAGAATCTCCCCTAATTACTTCATT
 TL022 GGAGCAAATCATAGATTCATGCCAATCGTAGTAGAAAGAATCTCCCCTAATTACTTCATT
 TL025 GGAGCAAATCATAGATTCATGCCAATCGTAGTAGAAAGAATCTCCCCTAATTACTTCATT
 TL031 GGAGCAAATCATAGATTCATGCCAATCGTAGTAGAAAGAATCTCCCCTAATTACTTCATT
 TL037 GGAGCAAATCATAGATTCATGCCAATCGTAGTAGAAAGAATCTCCCCTAATTACTTCATT
 TL034 GGAGCAAATCATAGATTCATGCCAATCGTAGTAGAAAGAATCTCCCCTAATTACTTCATT

TL026 AAATGAATTTCTAAAATA
 TL027 AAATGAATTTCTAAAATA
 TL022 AAATGAATTTCTAAAATA
 TL025 AAATGAATTTCTAAAATA
 TL031 AAATGAATTTCTAAAATA
 TL037 AAATGAATTTCTAAGATA
 TL034 AAATGAATTTCTAAGATA

Appendix 4. Aligned COII sequences for *Onymacris rugatipennis*. Localities for haplotype numbers are listed in Appendix 1.

67 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 69 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 71 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 72 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 73 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 74 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 75 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 90 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 91 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 92 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 94 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 95 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 97 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 G1 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 G2 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 G3 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 G4 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 G5 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 N19 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 N20 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 N21 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 N22 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 N23 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 N24 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA
 albo707 AGAATAGCTACATGAAAAACCCCTTCTTCTTCAAGATAGAGTATCACCATTAATAGAACAA

67 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 69 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 71 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 72 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 73 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 74 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 75 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 90 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 91 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 92 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 94 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 95 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 97 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 G1 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 G2 CTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATCCTAGTTGGA
 G3 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 G4 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 G5 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 N19 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 N20 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 N21 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 N22 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 N23 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA
 N24 TTATCATTCCTTTCATGATCATACTCTCCTAATTTTAGTAATTATTACTATTCTAGTTGGA

94 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
95 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
97 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
G1 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
G2 TGTGGAGCAAATCACAGATTTATACCAATCGTAGTAGAAAAGAATTTCTCCTAATTACTTC
G3 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
G4 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
G5 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
N19 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
N20 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
N21 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
N22 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
N23 TGTGGAGCAAATCACAGATTTATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
N24 TGTGGAGCAAATCACAGATTCATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC
albo707 TGTGGGGCAAATCACAGATTTATACCAATTGTAGTAGAAAAGAATTTCTCCTAATTACTTC

67 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
69 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
71 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
72 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
73 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
74 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
75 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
90 ATTAAATGAATTTCTAAAATAGTTGAAATTTTCATTAGATGGCTGAAAG
91 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
92 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
94 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
95 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
97 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
G1 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
G2 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
G3 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
G4 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
G5 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
N19 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
N20 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
N21 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
N22 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
N23 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
N24 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
albo707 ATTAAATGAATTTCTAAAATAATTGAAATTTTCATTAGATGGCTGAAAG
