MORPHOMETRIC AND ELECTROPHORETIC COMPARISON BETWEEN THE PACIFIC RUBBER BOA (*CHARINA BOTTAE BOTTAE*) AND THE SOUTHERN RUBBER BOA (*CHARINA BOTTAE UMBRATICA*)

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SIGNATURE PAGE

THESIS: MORPHOMETRIC AND ELECTROPHORETIC COMPARISON BETWEEN THE PACIFIC RUBBER BOA (*CHARINA BOTTAE BOTTAE*) AND THE SOUTHERN RUBBER BOA (*CHARINA BOTTAE UMBRATICA*)

AUTHOR: Cathy Marie Weisman

DATE SUBMITTED: Summer 88

Department of Biological Sciences

Dr. Glenn R. Stewart
Thesis Committee Chairman,
Biological Sciences

Dr. Stephen S. Bryant
Biological Sciences

Dr. Curtis Clark
Biological Sciences

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INTRODUCTION

The rubber boa snake, *Charina bottae* (Blainville), is one of the two North American members of the family Boidae. It is more secretive, thus less well known, than the rosy boa (*Lichanura trivirgata*). The rubber boa is a relatively small snake, ranging from 180-800 mm. It is sometimes called the "two-headed" snake because the short blunt tail resembles the head. The head is not distinct from the neck and has large frontal, supraocular, and parietal scales. The snakes are uniform in color, ranging from chocolate brown to camel tan. Dorsal scale counts range from 32-53, ventrals 182-231, and subcaudals 24-43.

Populations of the rubber boa are found from southern British Columbia to southern California and eastward into southern Utah at altitudes from sea level to 3100 meters (Stebbins, 1985). Over the past several years, the validity of subspecies of *Charina bottae* has been repeatedly reevaluated. The criteria for the division into subspecies have been variation in the numbers of scale counts and head plate configuration (see below).

Before 1890, the rubber boa had four different generic names. Stejneger (1890) revised the taxonomy, providing a synonymy, which retained the genus *Charina* and dividing it into three species. Van Denburgh (1920) described *Charina bottae utahensis*, the Rocky Mountain Rubber Boa, using only the number of dorsal scale rows to distinguish it from the Pacific Rubber Boa (*C. b. bottae*). Stejneger and Barbour (1923) revised the 1920 findings and synonymized all *Charina* into one species. A third subspecies, the Southern Rubber Boa (*C.b.umbratica*), was described in 1943 by Klauber. He based the diagnosis of *umbratica* on the number of ventral scales and the posterior margin of the frontal scale, which is straight in *umbratica* as contrasted with curved or pointed in *bottae* and *utahensis*. Nussbaum and Hoyer (1974) compared *bottae* with *utahensis* and synonymized them. They also suggested that *umbratica* was not a valid subspecies and variations in scale counts and head plate morphology were clinal. In the same year, Erwin proposed that *umbratica* be elevated to full species status due to its distinctiveness. Stewart (1977) agreed with Nussbaum and Hoyer regarding the status of *utahensis*, but suggested the retention of *umbratica* as a subspecies based on its morphology.

The purpose of this study was to examine differences between *C.b. bottae* and *C. b. umbratica* using both morphometric and electrophoretic analyses. Although morphological data have yielded inconclusive results in the past, for this study, I repeated measurements on a sample of specimens and compared them by multivariate analyses. In addition, I used biochemical analysis of enzymes by electrophoresis. Comparative protein analysis has been a useful technique to study systematics and genetics of other reptiles (Lawson and Dessaur 1979, Matthew 1975).

MATERIALS AND METHODS

Twenty one snakes from fourteen different localities were collected for electrophoretic analysis. These included six *Charina bottae umbratica* and three specimens from the Mt. Piños-Tehachapi Mountains area which seem to represent an intermediate population. Morphological data were also taken from these 21 specimens (appendices 1 and 2). The small sample size used for the electrophoretic study was due to the threatened status of the Southern Rubber Boa (*C. b. umbratica*) and the limitation of collecting permits. Morphological data were taken from an additional 193 live and museum specimens (appendices 1 and 2). These data from live specimens were recorded in the field by Gary Keasler. Museum specimen data were recorded by Glen Stewart and the author.

Electrophoretic analysis

Specimens were anesthetized using a 0.005 ml dose of Sodium Pentobarbitol injected 0.5 cm posterior to the heart. Tissue sampling was initiated when the snakes no longer had the ability to regain their balance after being placed on their backs. Sampling procedures were undertaken in a systematic fashion, beginning with the blood, which was collected in small capillary tubes after puncturing of the posterior vena cava or atria of the heart. Blood samples were centrifuged to separate the cells from the plasma.

Multiple tissue samples of about 5 mg each were then taken from the heart, liver, stomach, kidney, and skeletal muscle. The samples were individually wrapped in foil, labeled, and placed on a block of dry ice until the entire procedure was finished. The complete sample series from each specimen was stored in an individually labeled box and placed in the deep freeze at -80 C. Long-term cold storage does not appear to affect the electrophoretic patterns of tissue samples (Lawson and Dessauer, 1979).

All tissue samples, except for the blood, were homogenized in a glass mortar and pestle with 2 ml of grinding solution (5.0 g sucrose, 50 ml 0.1 m TBEDTA buffer, 1 flake bromphenol blue to color), and 100 ul of each sample was loaded into each pocket of a polyacrylamide vertical slab gel. Gels were run for three hours at 300 volts in a Tris Borate EDTA buffer, pH 8.9. Standards were not used during the gel runs because the primary goal was to look for electrophoretic variation. Samples from several individuals were run side by side and compared together to compensate for variations in mobility of the proteins between gel runs. Gel recipes from Harris (1976) were used except for LDH (Bryant, pers. comm.). Gels were stained for the following enzymes: lactate dehydrogenase (skeletal muscle, liver, heart, kidney), malate dehydrogenase (liver), 6-phosphogluconate dehydrogenase (red blood cells), glucose-6-phosphate dehydrogenase (liver), peptidase (method A; liver, heart), esterase (method B; (liver), and octanol dehydrogenase (liver). Since no electrophoretic analyses had been done on the genus *Charina*, Lawson and Dessaur's (1979) biochemical study of *Thamnophis* served as a guideline for the choice of enzymes. The enzymes which exibited the highest degree of polymophism in the *Thamnophis* complex were chosen. To establish effective techniques, a number of gels were run using *Thamnophis* tissues before the *Charina* tissues were run. Relative enzyme mobilities were compared by measuring the distance each enzyme traveled down the gel matrix. The frequencies of alleles were calculated and used to determine genetic distance (Hartl, 1980).

Morphometric analysis

Dorsal and subcaudal scale counts and snout-vent and tail length measurements were taken according to procedures outlined by Stebbins (1985). Ventral scales were counted by the method of Dowling (1951). In addition, drawings of the supraoccular, frontal and parietal head scales were made from live and preserved specimens. Head scale drawings were numerically coded by determining whether the supraocular was pointed (0) or blunt (1), the posterior margin of the frontal was convex (0), pointed (1), or straight (2), and whether the parietal was undivided (1) or divided (2-5). The sex of each individual and the locality and date of capture also were recorded.

Comparisons of dorsal and ventral scale counts of 134 snakes were made by analyses of variance (ANOVA) using the Bonferroni probability distribution for multiple samples. Both tests were performed with STATA version 1.5 (Computing Resource Center) on an AST 286 microcomputer.

Preliminary examination of dorsal and ventral scale counts and the numerically coded data for the parietal and frontal scales was performed by a principle component analysis (PCA) and detrended reciprocal averaging (DRA). The PCA and DRA were made with Biostat II (Pimental and Smith) on an AST 286 microcomputer.

RESULTS

Morphometric analyses

The supraocular scale shape was coded, but not included in the original analysis since the shape of the supraocular is dependent on the shape of the frontal scale. Principal component analysis and detrended reciprocal averaging showed that ventral and dorsal scale counts accounted for the major amount of information which I found to be biologically significant to this study. The dorsal and ventral scale count data strongly loaded on one axis, frontal on another, and parietal on a third.

A bivariate scatter plot of ventral scale count and the coded frontal scale margin (Figure 1) shows that for the characters examined populations from the Mt. Piños-Tehachapi Mountains region are mophologically intermediate between *C. b. bottae* and *C. b. umbratica*. All three character states (convex, pointed, and flat) for the posterior margin of the frontal scale are found in the Mt.

Piños-Tehachapi specimens. All except one of the *umbratica* specimens have a flat posterior margin of the frontal scale. Both the convex and pointed frontal scale margin are found in *bottae*. The presence of divided versus undivided parietals was fairly evenly distributed throughout both subspecies and had no apparent taxonomic value.

Ventral scale counts were also compared by the Bonferroni multiple comparison test (Fig. 2 and Table 1). There is an overlap in the ventral scale counts, with *umbratica* at the lower end of the range (180 to 200 ventrals; mean=189.1) and *bottae* at the upper end of the range (184 to 230 ventrals; mean=210). The ventral scale counts of the Mt. Piños-Tehachapi specimens fall within the middle of the ranges of the counts of the two subspecies (187-204; mean= 196.8). All of the means were significantly different with the exception of *utahensis* and the Mt. Piños-Tehachapi populations.

Midbody dorsal scale row counts were compared by the Bonferroni multiple comparison test (Fig. 3 and Table 2). Almost complete overlap is seen between the dorsal counts of *umbratica* (35-42; mean=38.55) and the Mt. Piños-Tehachapi populations (35-42; mean=39.39). The dorsal scale row counts of *bottae* were at the upper end of the range (40-52; mean=45.58) while the counts for *utahensis*(41-43; mean=41.60) are intermediate between *bottae* and the Mt. Piños-Tehachapi specimens. When the means of the midbody dorsal scale row counts were compared (Table 4), significant differences were found between all of the groups with the exception of *umbratica* and the Mt. Piños-Tehachapi populations.

Electrophoretic analyses

Banding patterns (Table 3) for homozygous and heterozygous snakes were similar to those of *Thamnophis* (Lawson and Dessaur 1979). However, *Charina* displays more evidence of homozygosity throughout the populations.

6-phosphogluconate dehydrogenase (6-PGD) exhibited a single band for homozygotes and a triple band for heterozygotes. The heterozygous condition was only found in the Mt. Piños-Tehachapi specimens. In skeletal muscle and liver, lactate dehydrogenase (LDH) did not produce the expected five band pattern characteristic of this enzyme. However, different banding patterns between *bottae* and *umbratica* were evident. What appears to be a heterozygous pattern is seen in the Mt. Piños-Tehachapi specimens. The banding patterns of both the octanol dehydrogenase (ODH) and malate dehydrogenase (MDH) enzymes show *bottae* and *umbratica* each to be different and homozygous, while the

Mt. Piños-Tehachapi individuals exhibit the same banding as *bottae*. Peptidase (PEP) displayed the highest overall activity in kidney tissue. The three different banding patterns which resulted were found in representatives of both subspecies. Only patterns "a" and "c" appeared in the intermediate snakes. The bands from both the Esterase (EST) and Glucose 6-Phosphate Dehydrogenase (G-6 PGD) indicate a homozygous condition for both enzymes in all subspecies/populations.

The genetic distances (*bottae* versus *umbratica*,.372; *bottae* versus the Mt. Piños-Tehachapi population, .388; *umbratica* versus the Mt. Piños-Tehachapi population, .358) indicate separation between the groups at the species level (Hartl, 1980). The values are skewed toward the higher end of the range (subspecies, D=0.02 to 0.2; species, D= 0.10 to 2.0) since the enzymes were chosen for their high degree of polymorphism in *Thamnophis* (Lawson and Dessaur, 1979).

DISCUSSION

Previous studies of the rubber boa have involved only the comparison of morphological characteristics (Nussbaum and Hoyer, 1974). Morphological analyses have provided guidelines that investigators may use to classify subspecies, (Van Denburgh 1920; Klauber 1943). Due to a large amount of overlap in size, scale counts and individual variation of head plate configuration, however, morphological data have yielded inconclusive results. The controversy over the validity of subspecies has centered around differences in scale counts and head plate morphology (Nussbaum and Hoyer, 1979; Stewart,1977). Nussbaum and Hoyer (1979) felt the differences observed may be a result of geographic variation in a north-south cline. In contrast, Erwin (1974) suggested that *umbratica* be considered a separate species. Thus, it is clear that the division of the rubber boa into subspecies cannot be based entirely on these characteristics.

In the present study, the comparisons of both midbody dorsal scale row and ventral scale counts (Figures 2 and 3) show a large overlap of the ranges of counts between the groups. This analysis shows that the most taxonomically significant variable is the shape of the posterior margin of the frontal scale. In fact, this one character can be used to separate the two subspecies into distinct groups (Fig 1). Previous studies concentrated on the number of head scales, not the shape (Nussbaum and Hoyer, 1979). This analysis also provides evidence that the populations of the Mt. Piños-Tehachapi Mountain region are intermediates in some sense, but they clearly do not represent a population of F_1 hybrids. While one specimen may display a scale count of *C. b. bottae*, the same individual may have a frontal scale with a flat posterior edge (typical of *C. b. umbratica*). However, the majority of these intermediates do resemble *C.b.bottae* more than they resemble *C.b. umbratica* in head plate morphology and color. The dorsal and ventral scale counts are intermediate between the two. The existence of these intermediate scale counts supports clinal variation as the reason for the differences. The intermediate head plate morphology suggests the Mt. Piños-Tehachapi population is a result of secondary contact between *bottae* and *umbratica*.

Of the nine enzymes analyzed by electrophoresis, 6-PGD, MDH, ODH, and LDH yielded differnces between *C. b bottae* and *C. b. umbratica*. Banding patterns of the intermediates showed heterozygous genotypes in the LDH and the 6-PGD enzymes. The existence of the heterozygous genotype in the intermediate populations suggests secondary contact between *bottae* and *umbratica* (perhaps during the last glaciation) and implies the northern and southern populations are divergent. Results of the analysis of the MDH and ODH enzymes indicate that the intermediates are more closely related to *C. b. bottae* than to *C. b. umbratica*. The results of the remaining gels show a homozygous condition throughout the species. Although the genetic distances between the groups indicates that they each be declared separate species, this might be misleading due to the following: (1. enzymes were chosen due to their high degree of polymorphism, 2. only seven enzymes were analyzed, 3. a limited number of specimens from each population were sacrificed for the analysis).

This study has shown that both morphological and enzymatic differences do exist between *C. b. bottae* and *C. b. umbratica*. To clarify the evolutionary relationship between the two subspecies, research in the future should concentrate on the Mt. Piños-Tehachapi populations. The rubber boa populations at the zone of intergradation between *bottae* and *utahensis* should be examined as well. Further avenues of research could include additional enzymatic analyses by electrophoresis, mitochondrial DNA restriction site analyses, and a comprehensive field study. At present, a conservative approach should be taken regarding the taxonomy of *C. b. umbratica* and it should be retained as a distinct subspecies of the rubber boa.

LITERATURE CITED

Blainville, H. D. de. 1835. Description de quelques especes de reptiles de la Californie. Nouv. Ann. Mus. d'Hist. Nat. (Paris) 4:233-296.

Dowling, H.G. 1951. A proposed standard system for counting ventrals in snakes. Brit. J. of Herp. Vol. I, No. 5 Nov.

Erwin, D.B. 1964. Taxonomic status of the southern rubber boa, *Charina bottae umbratica*. Copeia 1964:996-997.

Harris, H., and D.A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. Academic Press, New York and London.

Hartl, D. L. 1980. Principles of population genetics. Sinauer Associates, Inc., Sunderland, Massachuetts.

Klauber, L.M. 1943. The subspecies of the rubber boa, *Charina*. Trans. San Diego Natur. Hist. 10:83-90.

Lawson, R. and H.C. Dessaur. 1979. Biochemical genetics and systematics of garter snakes of the *Thamnophis elegans-couchi-ordinoides* complex. Occas. papers of Mus. of Zoology. 56:1-24.

Matthews, T.C. 1975. Biochemical polymorphism in populations of the Argentine toad, *Bufo arenarum*. Copeia 1975:454-456

Nussbaum, R.A. and R.F. Hoyer. 1974. Geographic variation and the validity of subspecies in the rubber boa, *Charina bottae*(Blainville). Northwest Sci. 48:219-229.

Stebbins, R.C. 1985. A field guide to western reptiles and amphibians, 2nd edition, revised. Houghton Mifflin Co., Boston.pg. xvi + 336 pp.

Stejneger, L. 1890. On the snakes of the genus *Charina*. Proc. U.S. Nat. Mus. 13:177-182.

_______, and T. Barbour. 1923. A checklist of north american amphibians and reptiles, 2nd ed. Harvard Univ. Press, Cambridge, MA. 125 pp.

Stewart, G.R. 1977. *Charina*. *C. bottae*. Catalog Amer. Amphib. Rept. 205.1-205.2.

Van Denburgh, J. 1920. Description of a new subspecies of boa (*Charina bottae utahensis*) from Utah. Proc. Calif. Acad. Sci. 4th ser. 10:31-32.

Table 1. Comparison of the means of ventral scale counts by Bonferroni.
(u = *umbratica*, i = Mt. Pinos/Tehachapi specimens, b = *bottae*, y = *utahensis*).

	u	i	b
i	$\alpha \approx 0.001$		
b	$\alpha \approx 0.0005$	$\alpha \approx 0.0005$	
y	$\alpha \approx 0.001$	$\alpha \approx 1.000$	$\alpha \approx 0.018$

Table 2. Comparison of the means of midbody dorsal scale counts by Bonferroni (u = *umbratica*, i = Mt. Pinos/Tehachapi specimens, b = *bottae*, y = *utahensis*).

	u	i	b
i	$\alpha \approx 0.270$		
b	$\alpha \approx 0.0005$	$\alpha \approx 0.0005$	
y	$\alpha \approx 0.0005$	$\alpha \approx 0.021$	$\alpha \approx 0.0005$

Table 3. Relative mobilities of enzymes.

ENZYMES	PEP	MDH	6-PGD	ODH	LDH
bottae					
1	1.01	1.009	1.01	1.009	1.01
2	.90/1.08	1.00	1.00	.99	1.01
4	1.00	1.00	1.00	1.00	1.00
7	1.00	.99	1.00	.99	.99
8	.89/1.05	1.00	1.01	.98	1.00
9	.93	.98	1.01	.98	1.00
10	.91	.98	1.00	.97	1.006
11	.95/1.06	.99	.98	.98	1.01
12	1.04	.98	1.00	.99	1.00
18	.89/1.05	.99	.96	.99	1.006
20	.91	.98	1.00	1.00	1.006
21	.94	1.009	1.00	1.00	1.00
umbratica					
3	1.05	1.17	1.27	1.37	1.11
5	.94/1.05	1.16	1.29	1.36	1.10
6	1.06	1.18	1.25	1.38	1.12
13	.89	1.17	1.29	1.38	1.12
15	.91	1.17	1.29	1.39	1.12
16	.97/1.10	1.16	1.27	1.38	1.11

ENZYMES	PEP	MDH	6-PGD	ODH	LDH
Mt.Pinos-Tehachapi population					
14	.97	1.009	.94/1.08/1.22	1.03	1.07
17	1.06	.99	.96/1.10/1.20	1.04	1.06
19	1.09	.98	1.01/1.20	1.03	1.06

Fig. 1. Bivariate scatter plot of ventral scale counts and frontal scale shapes (0=convex, 1=pointed, 2=straight).

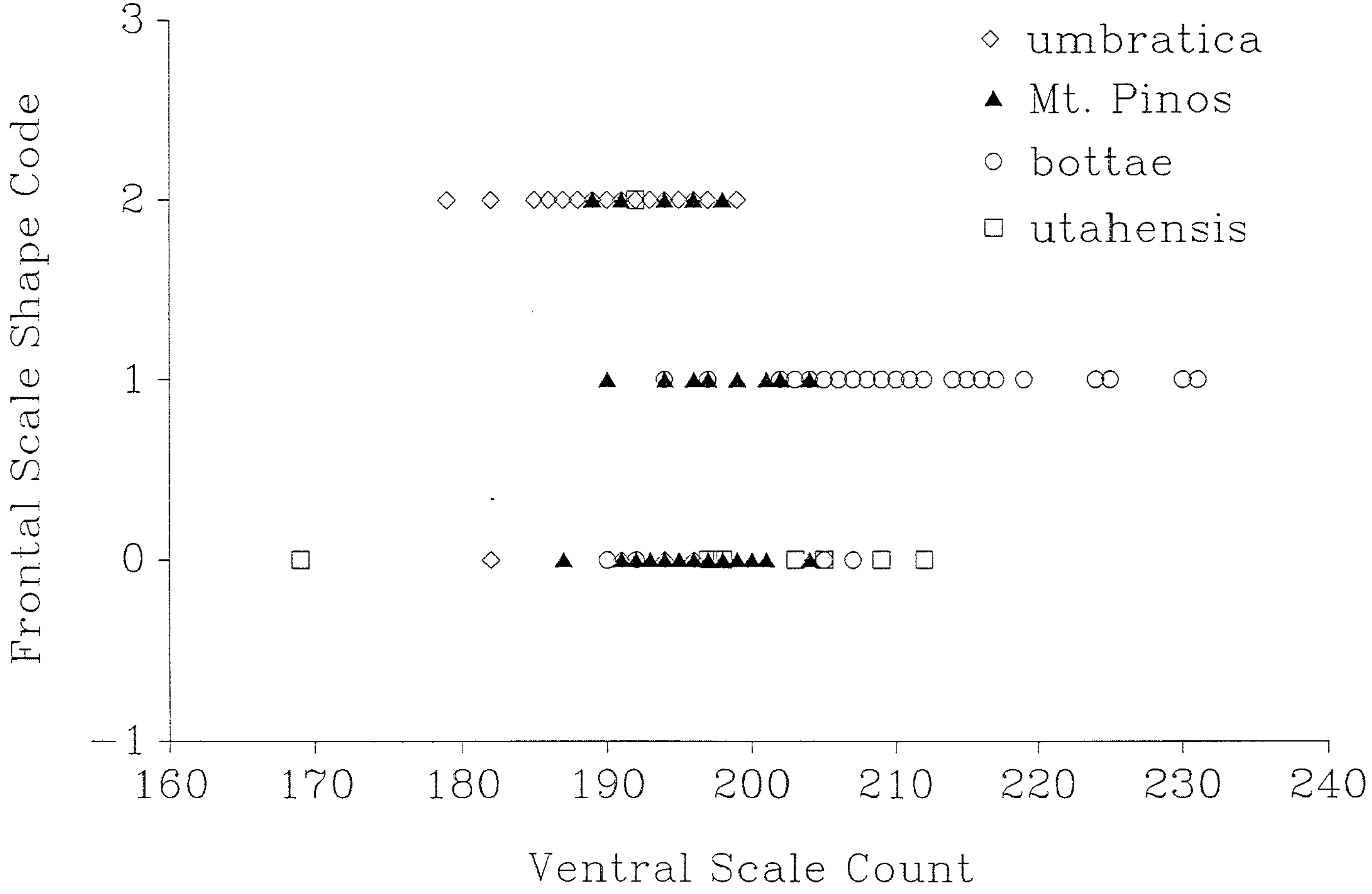
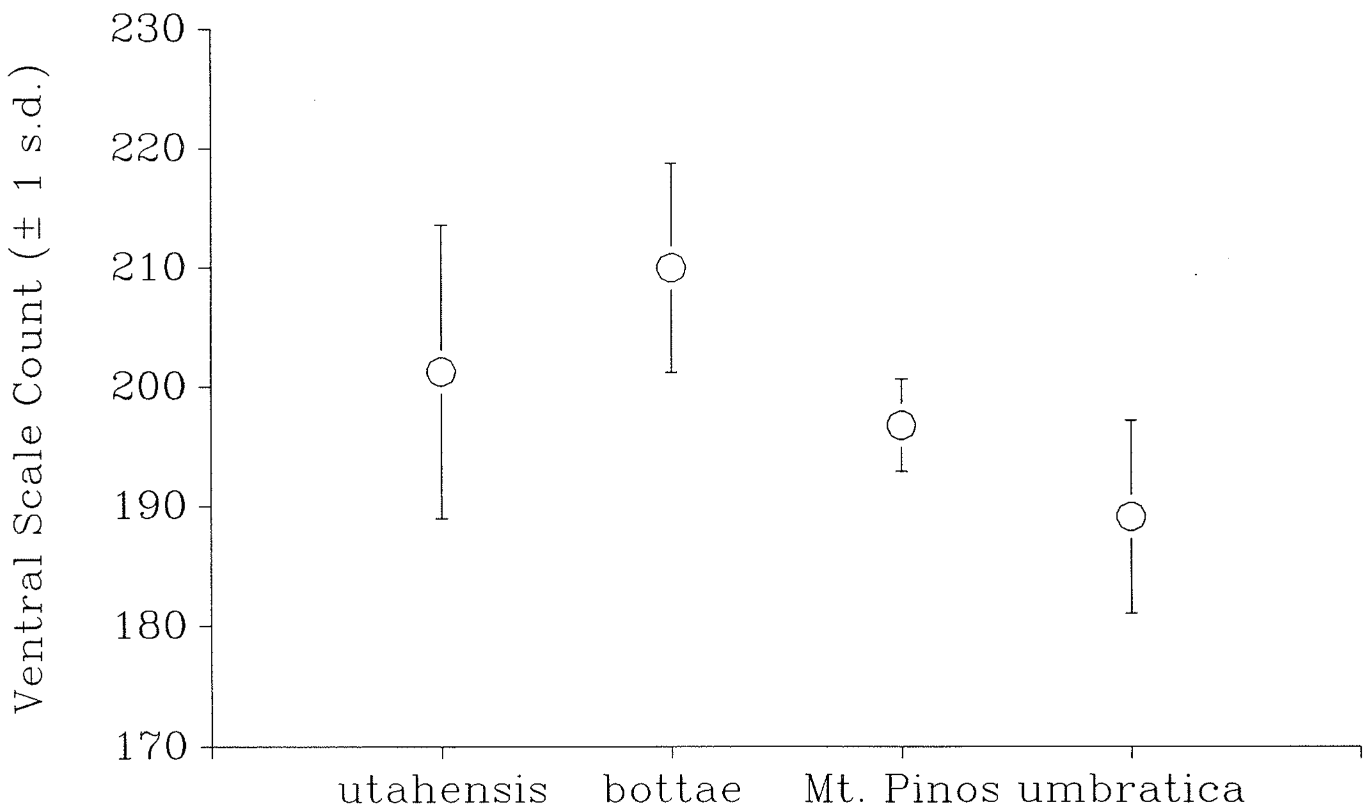
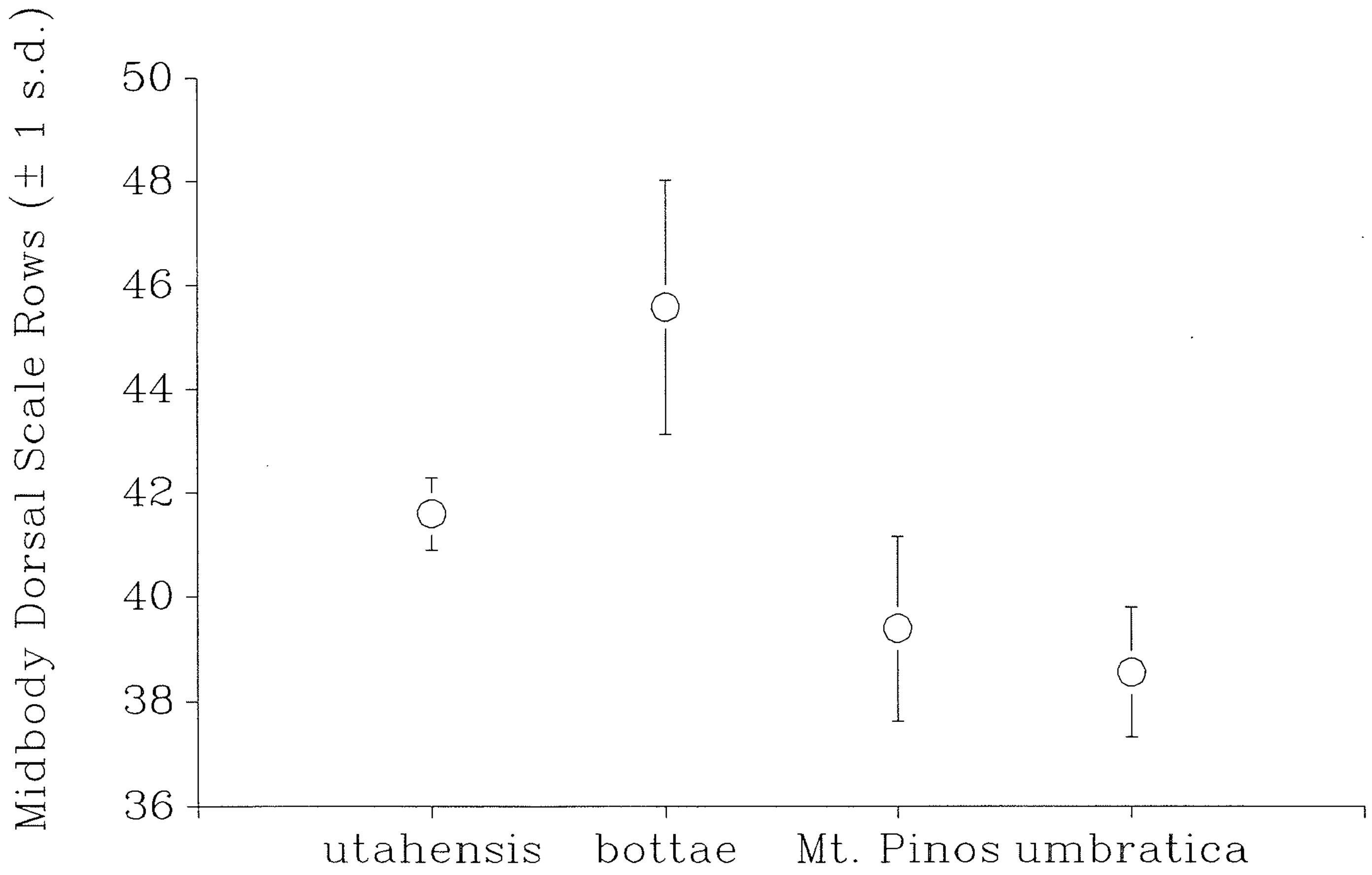

umbratica
Mt. Pinos
bottae
utahensis
Frontal Scale Shape Code
3
2
1
0
−1
160
170
180
190
200
210
220
230
240
Ventral Scale Count

Fig. 2. Means of ventral scale counts.



Ventral Scale Count (± 1 s.d.)
230
220
210
200
190
180
170
utahensis
bottae
Mt. Pinos
umbratica

Fig. 3. Means of midbody dorsal scale counts.



Midbody Dorsal Scale Rows (± 1 s.d.)
50
48
46
44
42
40
38
36
utahensis
bottae
Mt. Pinos
umbratica

Appendix 1. List of specimens examined.

(e = specimens from which tissue samples were taken for electrophoresis; l = live specimens)

Code	Number	Locality
e	1 (CP02227)	California. Kern Co.: Tehachapi Mts.
l	2	California. Kern Co.: Tehachapi Mts.
l	3	California. Kern Co.: Tehachapi Mts.
l	4	California. Kern Co.: Tehachapi Mts.
	5 (MUZ12168)	Nevada. Lauder Co.
	6 (MUZ12169)	Nevada. Lauder Co.
	7 (MUZ39173)	Nevada. Nye Co.
	8 (MUZ57740)	Nevada. Eureka Co.: Snow Cyn
	9 (MUZ18459)	Nevada. Humbolt Co.: 14 mi. N. Paradise Valley
	10 (MUZ67609)	Nevada. Lauder Co.: Big Creek
	11 (MUZ12167)	Nevada. Lauder Co.: Kingston Creek
	12 (MUZ16178)	Nevada. Nye Co.: Summit Creek
	13 (MUZ39174)	Nevada. Nye Co.: E. side Toiyabe range
	14 (MUZ42082)	Nevada. Nye Co.: Wisconsin Creek
	15 (CAS 38421)	Utah. Wasatch Co.: Wasatch Mts.
	16 (CAS771	California. Kern Co.: Alta Sierra
l	17	California. Alpine Co.
l	18	California. Alpine Co.
l	19	California. Riverside Co.: Devils Slide Trail, Humber Park

e	20 (CP02230)	California. Ventura Co.: Mt. Piños
l	21	California. Ventura Co.: Mt. Piños
l	22	California. Ventura Co.: Mt. Piños
l	23	California. Ventura Co.: Mt. Piños
l	24	California. Ventura Co.: Mt. Piños
l	25	California. Ventura Co.: Mt. Piños
l	26	California. Ventura Co.: Mt. Piños
l	27	California. Ventura Co.: Mt. Piños
l	28	California. Ventura Co.: Mt. Piños
l	29	California. Ventura Co.: Mt. Piños
l	30	California. Ventura Co.: Mt. Piños
l	31	California. Ventura Co.: Mt. Piños
	32 (CP01354)	California. Mono Co.: Lee Vining
	33 (CP01355)	California. Mono Co.: Lee Vining
	34 (CAS50165)	California. Mariposa Co.: Yosemite Ntl. Park
	35 (CAS7751)	California. Mono Co.: Mono Lake
	36 (CAS81561)	California. Mono Co.: Mono Lake
	37 (CAS81560)	California. Mono Co.: Mono Lake
	38 (CAS5316)	California. El Dorado Co.
	39 (CAS39644)	California. El Dorado Co.
	40 (CAS8869)	California. Tulare Co.: Sequoia Natl. Park
	41 (CAS4372)	California. El Dorado Co.
	42 (CAS4373)	California. Tuolumne Co.
	43 (CAS7646)	California. Tuolumne Co.
	44 (CAS6673)	California. Tuolumne Co.

45 (CAS6674)	California. El Dorado Co
46 (CAS8870)	California. Tulare Co.: Sequoia Ntl. Pk
47 (CAS55383)	California. Mariposa Co.: Yosemite Ntl. Park
48 (CAS81563)	California. Mono Co.: Mono Lake
49 (CAS8922)	California. Tulare Co.: Sequoia Ntl. Park
50 (MVZ77999)	California. El Dorado Co.
51 (MVZ13807)	California. Mariposa Co.: Yosemite Ntl. Park
52 (MVZ46817)	California. Tuolumne Co.
53 (MVZ8178)	California. Mariposa Co.: Yosemite Ntl. Park
54 (MVZ66403)	California. Alameda Co.: Berkeley
55 (MVZ6994)	California. Tulare Co.
56 (MVZ43607)	California. Alameda Co.: Berkeley
57 (MVZ3607)	California. Alameda Co.: Berkeley
58 (MVZ4306)	California. Alameda Co.: Berkeley
59 (MVZ7199)	California. Alameda Co.: Berkeley
60 (MVZ13109)	California. Alameda Co.: Berkeley
61 (MVZ771118)	California. Kern Co.: Alta Sierra
62 (MVZ42846)	California. Mono Co.
63 (MVZ396)	California. Tuolumne Co.
64 (MVZ18632)	California. Tuolumne Co.
65 (MVZ4112)	California. Tuolumne Co.
66 (MVZ3778)	California. Tuolumne Co.
67 (MVZ9254)	California. Tuolumne Co.
68 (MVZ22432)	California. Tuolumne Co.
69 (MVZ77072)	California. Calaveras Co.

	70 (MVZ7067)	California. Calaveras Co.
	71 (MVZ7068)	California. Calaveras Co.
	72 (MVZ7066)	California. Calaveras Co.
	73 (MVZ77074)	California. Calaveras Co.
	74 (MVZ43604)	California. Alameda Co.
	75 (MVZ77069)	California. Alameda Co.
	76 (MVZ6332)	California. Calaveras Co.
	77 (MVZ6331)	California. Fresno Co.
	78 (MVZ77073)	California. Calaveras Co.
	79 (MVZ24394)	California. Calaveras Co.
	80 (MVZ38312)	California. Fresno Co.
	81 (MVZ8171)	California. El Dorado Co.
	82 (MVZ77070)	California. Calaveras Co.
	83 (MVZ7071)	California. Tulare Co.: Sequoia Natl. Pk
	84 (MVZ21888)	California. Tuolumne Co.
	85 (MVZ50197)	California. Tuolumne Co.
	86 (MVZ40670)	California. Tuolumne Co.
	87 (MVZ68222)	California. Tuolumne Co.
	88 (MVZ58313)	California. Tuolumne Co.
l	89	California. Ventura Co.: Mt. Piños
l	90	California. Ventura Co.: Mt. Piños
l	91	California. Ventura Co.: Mt. Piños
l	92	California. Ventura Co.: Mt. Piños
l	93	California. Ventura Co.: Mt. Piños
l	94	California. Ventura Co.: Mt. Piños

l	95	California. Ventura Co.: Mt. Piños
l	96	California. Ventura Co.: Mt. Piños
l	97	California. Ventura Co.: Mt. Piños
l	98	California. Ventura Co.: Mt. Piños
l	99	California. Ventura Co.: Mt. Piños
l	100	California. Ventura Co.: Mt. Piños
l	101	California. Ventura Co.: Mt. Piños
l	102	California. San Bernardino Co.: Running Springs
	103 (SBCM1357)	California. San Bernardino Co.: Running Springs
	104 (LACM20269)	California. San Bernardino Co.: Camp O-Ongo
	105 (LACM2141)	California. Orange Co.: (pet?)
	106 (LACM27660)	California. San Bernardino Co.: Jenks Lake
	107 (LACM20268)	California. San Bernardino Co.: Camp O-Ongo
	108 (LACM20264)	California. San Bernardino Co.: Camp-O-Ongo
	109 (LACM20265)	California. San Bernardino Co.: Camp-O-Ongo
	110 (LACM20270)	California. San Bernardino Co.: Camp-O-Ongo
	111 (LACM20266)	California. San Bernardino Co.: Camp-O-Ongo
	112 (LACM20267)	California. San Bernardino Co.: Camp-O-Ongo
	113 (SDCM36011)	California. San Bernardino Co.: Camp-O-Ongo
	114 (SDCM40725)	California. Riverside Co.: Marion Mts.
	115 (SDCM36549)	California. San Bernardino Co.: 8 mi. E. Barton Flats
	116 (LACM19332)	California. Los Angeles Co.: Bouquet Cyn
	117 (LACM101269)	California. San Bernardino Co.: 4 mi. E. Sky Forest
	118 (LACM101270)	California. San Bernardino Co.: E. of Sky Forest
l	119	California. San Bernardino Co.: San Bernardino Mts.

	120 (CP01163)	California. San Bernardino Co.: Running Springs
	121 (CP01164)	California. San Bernardino Co.: Camp Helendale
e	122 (CP02218)	California. San Bernardino Co.: Green Valley Road
l	123	California. San Bernardino Co.: Camp Helendale
l	124	California. San Bernardino Co.: Camp Helendale
l	125	California. San Bernardino Co.: Running Springs heliport
e	126 (CP02226)	California. San Bernardino Co.: Running Springs heliport
e	127	California. San Bernardino Co.: Running Springs heliport
l	128	California. San Bernardino Co.: Running Springs heliport
e	129 (CP02216)	California. San Bernardino Co.: Big Bear Dam
l	130	California. San Bernardino Co.: Camp O-Ongo
e	131 (CP02219)	California. San Bernardino Co.: Twin Peaks
l	132	California. San Bernardino Co.: Burnt Mill Road, Arrowhead
l	133	California. Ventura Co.: Mt Piños
l	134	California. San Bernardino Co.: Running Springs heliport
l	135	California. San Bernardino Co.: Running Springs heliport
l	136	California. San Bernardino Co.: Running Springs heliport
l	137	California. San Bernardino Co.: E. of Camp O-Ongo
l	138	California. San Bernardino Co.: Running Springs heliport
l	139	California. San Bernardino Co.: Running Springs heliport
l	140	California. San Bernardino Co.: Running Springs heliport
e	141 (CP02228)	California. Riverside Co.: Humber Park, Fern Valley
e	142 (CP02229)	California. Riverside Co.: Humber Park, Fern Valley
l	143	California. San Bernardino Co.: Barton Flats
l	144	California. San Bernardino Co.: Barton Flats

l	145	California. San Bernardino Co.: Big Bear City
l	146	California. San Bernardino Co.: Arrowhead
l	147	California. San Bernardino Co.: Idyllwild
l	148	California. San Bernardino Co.: Idyllwild
l	149	California. San Bernardino Co.: Idyllwild
l	150	California. San Bernardino Co.: Running Springs dump
l	151	California. San Bernardino Co.: Running Springs dump
l	152	California. San Bernardino Co.: Daley Cyn. Road, Blue Jay
l	153	California. San Bernardino Co.: Daley Cyn. Road, Blue Jay
l	154	California. San Bernardino Co.: Daley Cyn. Road, Blue Jay
l	155	California. San Bernardino Co.: Sheep's Creek
	156 (CP02170)	California. San Bernardino Co.: Santa's Village Lake Arrowhead
l	157	California. San Bernardino Co.: Lake Arrowhead
l	158	California. San Bernardino Co.: Lake Arrowhead
l	159	California. San Bernardino Co.: Lake Arrowhead
l	160	California. Ventura Co.: Mt. Piños
l	161	California. Ventura Co.: Mt. Piños
l	162	California. Ventura Co.: Mt. Piños
l	163	California. Ventura Co.: Mt. Piños
l	164	California. Ventura Co.: Mt. Piños
l	165	California. Ventura Co.: Mt. Piños
l	166	California. Ventura Co.: Mt. Piños
l	167	California. Ventura Co.: Mt. Piños
l	168	California. Ventura Co.: Mt. Piños
l	169	California. Ventura Co.: Mt. Piños

l	170	California. Ventura Co.: Mt. Piños
l	171	California. Ventura Co.: Mt. Piños
l	172	California. Ventura Co.: Mt. Piños
l	173	California. Ventura Co.: Mt. Piños
l	174	California. Ventura Co.: Mt. Piños
l	175	California. Ventura Co.: Mt. Piños
l	176	California. Ventura Co.: Mt. Piños
l	177	California. Ventura Co.: Mt. Piños
l	178	California. Kern Co.: Mt. Abel
l	179	California. Ventura Co.: Mt. Piños
l	180	California. Ventura Co.: Mt. Piños
l	181	California. Ventura Co.: Mt. Piños
l	182	California. Ventura Co.: Mt. Piños
l	183	California. Ventura Co.: Mt. Piños
l	184	California. Kern Co.: Mt. Abel
l	185	California. Kern Co.: Mt. Abel
l	186	California. Kern Co.: Camp Earl, Tehachapi Mts.
l	187	California. Kern Co.: Camp Earl, Tehachapi Mts.
l	188	California. Kern Co.: Camp Earl, Tehachapi Mts.
	189 (MVZ6331)	California. Fresno Co.: Huntington Lake
	190 (MVZ26994)	California. Tulare Co.: 10 mi. E. Badger Pass
	191 (MVZ43604)	California. Tulare Co.
	192 (MVZ16605)	California. Tulare Co.
	193 (MVZ43607)	California. Tulare Co.
	194 (MVZ13109)	California. Tulare Co.

	195 (MVZ43606)	California. Tulare Co.
	196 (MVZ46817)	California. Tuolumne Co.
	197 (MVZ13807)	California. Mariposa Co.: Yosemite Ntl. Park
	198 (MVZ77999)	California. El Dorado Co.: Lake Tahoe
	199 (CP02224)	California. Tuolumne Co.: Strawberry
	200 (CP01388)	California. Mono Co.: Lee Vining
	201 (CP01389)	California. Mono Co.: Lee Vining
	202 (CP01390)	California. Mono Co.: Lee Vining
e	203 (CP02222)	California. Tulare Co.
e	204 (CP02221)	California. Shasta Co.: Rock Creek
e	205 (CP02231)	California. Mariposa Co.: Yosemite Natl. Park
e	206 (CP02224)	California. El Dorado Co.: Strawberry
e	207 (CP02225)	Oregon. Benton Co.: Corvallis
e	208 (CP02223)	Oregon. Benton Co.: Corvallis
e	209 (CP02220)	Oregon. Benton Co.: Corvallis
e	210 (CP02217)	Oregon. Benton Co.: Corvallis
e	211 (CP02215)	Oregon. Benton Co.: Corvallis
e	212 (CP02232)	California. Ventura Co.: Alamo Mt.
e	213 (CP02233)	California. Plumas Co.
e	214 (CP02234)	California. Santa Cruz Co.

Appendix 2. Morphological data.

specimen number	dorsal scales	ventral scales		snount/vent length	tail length/sex	parietal	supraocular shape	post. margin of frontal
1	42	199		395	52f	d	pt	semicirc
2	42	195		352	57m	d/3	pt	semicirc/2
3	40	204		425	51f	d/3	pt	triang/ 3/4 div
4	42	193		467	54f	d/3	pt	semicirc
5(12168)	41	198	35		m	u	pt	semicirc
6(12169)	42	209	35		f	u	pt	semicirc
7(39173)	41	169	35		f	u	pt	semicirc
8(57740)								
9(18459)	41	197	38			u	pt	semicirc
10(67609)	42	203	36			d/3	pt	semicirc
11(12167)	41	205	37			d/3	pt	semicirc
12(16178)	43	205	36			u	pt	semicirc
13(39174)	42	210	37		f			
14(42082)	41	205	35		f	u	pt	semicirc/90%div
15	42	212	34		f	u	pt	semicirc
16(77118)	42	192	34		f	d/2	pt	semicirc
17	42	203	32	210	26f	d/3	sq	triang
18	41	205	33	287	40m	u	sq	semicirc
19	38	189		12.75 in	2 in m	d/3	sq	sq

20	39	195	33		f	u	sq	sq
21	42	193	32	350	45f	d/2	sq	semicirc
22	37	187	32	161	19m	d/2	sq	semicirc
23	39	191	35	293	46m	u	sq	semicirc
24	39	196	36	305	45m	d/2	sq	sq w/pt
25	39	191	31	337	43f	u	sq	flat
26	41	199	34	440	57f	u	pt	triang
27	41	190	34	279	39f	d/2	pt	triang
28	41	202	33	267	m	u	pt	triang
29	40	194	32	270	35m	d/2	pt	flat/wider
30	42	196	29	343	42m	u	pt	triang
31	39	198	31	332	51f	u	pt	flat
32	45	219	36	596	67f	u		
33	45	219	43	660	77f	u		
34(cas50165)	45	204	36	358	57m	d/3		
35cas7751)	46	211	34	546	64f	u		
36(cas81561)	46	216	39	610	63f	d/2		
37(cas81560)	45	209	34	366	51m	u		
38(cas5316)	46	210	39	397	63	u		
39(cas39644)	45	214	34	570	60	d		
40(cas8869)	52	212	34	540	60	d		
41(cas4372)	44	202	33	261	39	d		
42(cas4373)	41	538	47	217	41			
43(cas7646)	46	210	33	373	52	d/2		
44(cas6673)	41	210	36	299	41	d/2		

45(cas6674)		225		214	26	u
46(cas8870)				182	20	d/2
47(cas55383)	47	217	41	231	29	d
48(cas81563)	44	231	39	234	32	u
49(cas8922)	50	225	37	258	37	u
50(mu277999)	48	215	36	662	68	u
51(mu213807)	48	212	39	569	68	d/3
52(mu246817)	48	204	39	569	66	u
53(mu28178)	46	203	33	434	56	1 u
54(mu266403)	46	206	32	380	55	d/3
55(mu26994)	45	215	38	286	39	u
56(mu243607)	47	197	34	524	61	d/2
57(mu243607)	44	199	30	569	61	d/2
58(mu24306)	46	207	34	597	58	d/2
59(mu27199)	45	207	39	369	56	d/2
60(mu213109)	46	208	32	409	56	d/2
61(mu2771118)	42	194	35	450	52	d/2
62(mu242846)	44	230	38	350	50	u
63(mu211396)	44	203	34	308	42	u
64(mu218632)	43	221	34	334	40	u
65(mu264112)				188	22	d/2
66(mu233778)				174	20	d/3
67(mu29254)	46	202	31	417	53	d/2
68(mu22432)	47	210	34	500	60	d/2
69(mu277072)	46	217	32	638	57	u

70(mu277067)	42	217	33	425	45	u		
71(mu277068)	46	211	35	426	56	d/2		
72(mu277066)				203	27	u		
73(mu277074)				196	28	u		
74(mu43604)				214	26	d/2		
75(mu2(77069)				240	30	d/2		
76(mu277069)				200	27	u		
77(mu26331)				177	23	u		
78(mu277073)	45	208	41	288	42	d/3		
79(mu224394)	49	210	37	496	60	d/2		
80(mu238312)	48	219	35	484	56	u		
81(mu28171)	47	216	35	440	60	d/2		
82(mu277070)	47	203	27	562	48	u		
83(mu277071)	45	214	34	438	57	u		
84(mu221888)	51	224	34	490	52	u		
85(mu250197)	45	203	31	392	50	d/2		
86(mu240670)	47	205	34	527	62	u		
87(mu268222)	48	214	31	410	47	d/2		
88(mu258313)				233	27	d/3		
89	40	201	32	395	46	d/2	blunt	semicirc
90	41	196	37	404	50f	d/2	pt	semicirc
91	37	196	33	226	28f	u	pt	semicirc
92	38	198	34	225	28f	u	pt	semicirc
93	38	201	32	343	52m	u	pt	triang
94	39	194	37	235	32m	d/2	pt	triang

95	39	195	30	372	50m	d/2	pt	semicirc
96	36	199	35	224	28f	u	pt	semicirc
97	38	194	34	258	36m	d/2	blunt	triang
98	38	197	31	242	31m	d/3	pt	semicirc
99	38	197	34	202	226f	d/2	pt	triang
100	40	204	35	321	51m	d/2	sq	semicirc
101	39	201	31	227	29m	u	pt	triang
102	38	188		325	39f	u	sq	rect/flat
103(1357)	38	187			f	d/2	sq/blunt	flat
104(20269)	40	188		317	44f	d/2	sq	flat
105(2141)	40	190		297	54m	u	sq	semicirc
106(27660)	40	179		348	49f	u	sq	long/flat
107(20268)	38	192		206	29f	u	sq/blunt	long/flat
108(20264)	39	185		267	40m	u	blunt/rect	long/flat
109(20265)	38	185		326	48f	d/2	blunt/rect	lonf/flat
110(20270)	40	186		251	42m	d/2	blunt	flat
111(20266)	37	188		325	47f	d/2	rect/circ edg	flat/pt in mid
112(20267)	39	192		330	51f	d/2	blunt	flat
113(36011)	39	186		243	37m	u	sq/blunt	rect/flat
114(40725)a	39	188		312	33f	d/4	sq pt in mid	flat
115(36549)a	40	179		417	38f	d/5	rect	flat div/3
116(19332)a	46	207		479	56f	d/3	blunt	semicirc
117(1098)	37	189		330	46f	u	blunt/rect	long/flat
118(1099)	36	186		345	47f	d/2	blunt/rect	long/flat
119	36	182		287	37f	u	blunt/rect	long/flat

	120	40	191		345	39f	u	blunt	long/flat
	121	36	189		271	36m	u	blunt/rect	long/flat
	122	36	192	30	175	21f	d/2	blunt/sq	long/flat
	123	41	197	35	411	37f	u	sq/blunt	long/flat
	124	41	195	24	386	20f	d/2	blunt/sq	long/flat
	125	38	192	34	286	48m	u	blunt/sq	long/flat
126@		40	192	30	390	45f	1/2 div	pt/sq	long/flat 7/8 div
	127	39	190	31	325	55m	u	blunt/sq	long/flat
	128	40	192	32	319	54m	u	sq/blunt	long.flat
	129	39	191	25	435	44f	d/2	rect/blunt	flat
	130	38	190	29	230	22f	d/3	sq w/pt	long/flat
	131	38	192	34	377	56f	d/2	rect/pt	deep/semicirc
	132	39	194	33	308	38f	d/3	sq	long/flat
133@		42	200	36	339	50f	u	rect/blunt	semicirc
134@		39	142	35	367	51f	u	rect/slant	long/flat
135@		39	196	34	237	30f	u/pt	sq/blunt	long/flat
	136	38	194	31	373	48f	u	rect/blunt	long/flat
137@		38	190	33	223	34m	u	rect/pt	long/flat
	138	38	193	34	303	41f	u	sq/blunt	long/flat
139@		39	196	34	309	48m	d/3	rect/pt	deep/semicirc div/2
	140	39	193	31	282	32f	d/2	sq/blunt	long/flat
141@		41	199	36	441	54f	u	rect/pt	semicirc
142@		35	196	34	394	49f	d/3	rect/pt	long/semicirc
143@		37	196	35	282	32f	d/4	rect/pt	long pt in mid
144@		39	193	33	374	50f	u	rect/pt	long/flat

145a		38	185	24	411	41f	d/2	rect pt in mid	deep long/flat
	146	37	196	31	189	24f	d/2	rect/blunt	long/flat
	147	38	195	26	362	40m	u	rect/pt	long/flat
	148	38	190	33	285	49m	u	rect/blunt	long/flat
	149	39	199	35	406	55f	u	rect/blunt	long/flat
	150	39	191	35	221	36f	d/3	rect/pt	long/circ div/2
	151	39	185	32	304	37m	u	rect/blunt	long/flat
	152	38	194	31	337	52m	u	rect/blunt	long/semicirc
	153	38	192	34	311	46m	d/2	rect/blunt	long/flat
	154	37	191	32	375	49f	u	rect/blunt	long/flat
	155	41	194	35	329	51m	u	sq/blunt	long/semicirc
	156	39	191	33	337	48m	u	blunt/rect	long/flat div/3
	157	40	182	33	317	52m	d/2	rect/pt	long/semicirc
	158	39	187	29	342	48f	d/2	sq/blunt	long/pt in mid
	159	40	190	30	187	22f	d/2	sq/blunt	long/flat
	160	39	195	33		f			semicirc
	161	42	195	32		f			semicirc
	162	37	189	32					almost st
	163	41	204	33		m			triang
	164	39	194	35		m			triang
	165	39	197	36		m			triang
	166	39	194	31		f			almost st/deep
	167	41	201	34		f			semicirc
	168	41	194	34		f			semicirc
	169	40	196	32		m			triang

170	39	198	31	f		triang
171	40	201	32	f		triang
172	41	196	37	f		triang
173	37	196	33	f		triang
174	38	199	34	f		triang
175	38	201	32	m		triang
176	39	194	37	m		triang
177	39	195	30	m		triang
178	36	199	35	f		semicirc
179	38	194	34	m		triang
180	38	197	31	m		triang
181	38	197	34	f		triang
182	40	204	35	m		triang
183	39	201	31	m		triang
184	42	198	29	m		triang
185	36	199	35	f		semicirc
186	42	199		f		triang
187	42	198		m		triang
188	40	204		f		triang
189	42	193	32	f		triang
189(MVZ6331)				u	pt	triang
190(MVZ26994)				u	pt	triang
191(MVZ43604)				d/2	pt	triang
192(MVZ16605)				d/2	pt	triang
193(MVZ13109)				d/2	pt	triang

194(MVZ43606)						d/2	pt	triang
195(MVZ46817)						u	pt	triang
196(MVZ13807)						d/3	pt	triang
197(MVZ77999)						u	pt	triang
198(MVZ66403)						d/2	pt	triang
199(CP02224)			521		f			
200(CP01388)	45	214	267	58	f			
201(CP01389)	43	204	250	33	f			
202(CP01390)	46	211	336	49	m			
203(CP02222)			500	67	f			
204(CP02221)			260	32	f			
205(CP02231)			453	58	f			
206(CP02224)			521	65	f			
207(CP02225			258	40	m			
208(CP02223)			210	28	m			
209(CP02220)			230	31	f			
210(CP02217)			400	68	m			
211(CP02215)			556	64	f			
212(CP02232)	41	189	310	62		u	sq	semicirc
213(CP02233)	43	210	263	43	m	d/2	pt	semicirc
214(CP02234)	42	200	284	31	f	u	pt	semicirc