



Biological systematics of *Zaitzevia thermae* (Hatch)
by Mark Mitchell Hooten

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Entomology
Montana State University
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Abstract:

Zaitzevia thermae (Hatch) is a riffle beetle restricted to a small warm spring in the Bozeman, Montana, area. It is of interest to systematists and ecologists due to its limited range and thermophilic habits. Additionally, *Z. thermae* is found literally next to its sister species, *Zaitzevia parvula* (Horn), a cold water inhabitant found commonly in foothill streams. The basic problem in understanding the two species lay in their cryptic nature with respect to each other.

A systematic analysis of *Z. thermae* was undertaken to determine its taxonomic identity. Analysis of *Z. thermae*'s morphology, ecology, and genetic identity was pursued.

Results revealed morphological and isozyme markers that clearly identify *thermae* and suggest that it is reproductively isolated from regional populations of *Z. parvula*. Investigations into the thermal ecology of the two species indicates that they have different preferences.

It is concluded that *thermae* is a distinct taxonomic unit at the species level.

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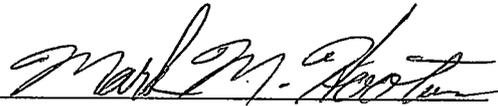
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09 April 1991

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TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES	viii
NOMENCLATURE	ix
ABSTRACT	x
INTRODUCTION	1
TAXONOMIC HISTORY	6
Introduction	6
Synonymy of <u>Zaitzevia thermae</u> (Hatch)	6
MORPHOLOGICAL ANALYSIS	8
Introduction	8
Methods and Materials	9
Standard Analysis	9
Morphometric Analysis	11
Larval Instar Analysis and Morphology	12
Results	13
Standard Analysis	13
Pronotum	13
Wings	15
Metendosternite	16
Genitalia	16
Morphometric analysis	21
Larval Instar Analysis	22
Larval Morphology	23
ECOLOGY	25
Introduction	25
Respiration	26
Avoidance	27
Dispersal	30
Development and Thermal Ecology	31
Methods and Materials	33
Observational	33
Experimental	33

TABLE OF CONTENTS - Continued

	Page
ECOLOGY - <u>Continued</u>	
Results	36
Observational	36
Experimental	37
ELECTROPHORETIC ANALYSIS	41
Introduction	41
Methods and Materials	43
Results	45
CONCLUSION	53
Summary	64
REFERENCES CITED	66
APPENDICES	75
Appendix A - Conservation Management of <u>Zaitzevia</u> <u>thermae</u> (Hatch)	76
Appendix B - Materials Studied of <u>Zaitzevia</u> Champion	81

LIST OF TABLES

Table	Page
1. Summary of ANOVA on size (volume) of <u>Zaitzevia</u> spp. ...	21
2. Summary of LSD on size (volume) of <u>Zaitzevia</u> spp.	22
3. Summary of ANOVA on pronotal proportional size	22
4. Summary of LSD on pronotal proportional size	23
5. Summary of ANOVA on thermal gradient analysis	39
6. Summary of LSD on thermal gradient analysis	40
7. Tray and gel buffers used in electrophoresis with corresponding loci examined	44
8. Allelic frequencies at all observed loci in electrophoresis	46
9. Genetic similarity and distance values for populations examined	48
10. Mean heterozygosity values for populations examined ..	49

LIST OF FIGURES

Figure	Page
1. Map of localities from which <u>Zaitzevia</u> spp. were collected for this study	10
2. Habitus photograph of <u>Zaitzevia thermae</u> (Hatch), BCWS, and <u>Zaitzevia parvula</u> (Horn), NS	14
3. Wing of <u>Zaitzevia thermae</u> , BCWS	15
4. Wing of <u>Zaitzevia parvula</u> , BC	17
5. Wing of <u>Zaitzevia parvula</u> , NS	18
6. Wing of <u>Zaitzevia parvula</u> , SFS	19
7. Wing of <u>Zaitzevia</u> sp., BRWS	19
8. Metendosternites of <u>Zaitzevia</u> spp.	20
9. Histogram of larval head widths of <u>Zaitzevia thermae</u> , BCWS	24
10. Photograph of thermal gradient bar	34
11. Histograms of thermal gradient trials	38
12. WPGMA tree of <u>Zaitzevia</u> spp.	51
13. Distance Wagner tree of <u>Zaitzevia</u> spp.	52

NOMENCLATURE

List of Textual Acronyms *

1. ANOVA - Analysis of Variance
2. BC - Bridger Creek
3. BCWS - Bridger Canyon Warm Spring
4. BFCDC - Bozeman Fish Cultural Development Center
5. BRWS - Brooks Warm Spring
6. LPPC - Little Prickly Pear Creek
7. LSD - Least Significant Difference
8. MR - Musselshell River
9. MSU - Montana State University
10. MTEC - Montana State University Entomology Collection
11. NS - Nimrod Spring
12. OTC - Otter Creek
13. PWS - Potosi Warm Spring
14. SFS - Snowflake Spring
15. UPGMA - Unweighted Pair-group Method with Arithmetic Averaging
16. USFWS - United States Fish and Wildlife Service
17. WFS - West Fork Spring
18. WPGMA - Weighted Pair-group Method with Arithmetic Averaging
19. WSC - Warm Spring Creek

* Acronyms used as citations are found in REFERENCES CITED.

ABSTRACT

Zaitzevia thermae (Hatch) is a riffle beetle restricted to a small warm spring in the Bozeman, Montana, area. It is of interest to systematists and ecologists due to its limited range and thermophilic habits. Additionally, Z. thermae is found literally next to its sister species, Zaitzevia parvula (Horn), a cold water inhabitant found commonly in foothill streams. The basic problem in understanding the two species lay in their cryptic nature with respect to each other.

A systematic analysis of Z. thermae was undertaken to determine its taxonomic identity. Analysis of Z. thermae's morphology, ecology, and genetic identity was pursued.

Results revealed morphological and isozyme markers that clearly identify Z. thermae and suggest that it is reproductively isolated from regional populations of Z. parvula. Investigations into the thermal ecology of the two species indicates that they have different preferences.

It is concluded that Z. thermae is a distinct taxonomic unit at the species level.

INTRODUCTION

Zaitzevia thermae (Hatch), (Elmidae Coleoptera), is restricted to Bridger Canyon Warm Spring (BCWS), a warm spring of 21°C average temperature, in Gallatin County, Montana (MBMG 1981). It is unusual, though not unique, among elmids because of its restricted range and thermophilic habits. The other North American member of the genus is the sister-species, Z. parvula (Horn), which is widespread and common throughout Western North America. This makes Z. thermae a unique organism for systematic investigation. A brief summary of our recent understanding of, and interaction with Z. thermae, will elucidate the current importance of such an investigation.

Zaitzevia thermae is a microphytophage (Brown 1987, Chapman and Demory 1990), found mostly on the lithic substrate of Bridger Canyon Warm Spring (BCWS). Like other elmids, however, Z. thermae is commonly found amid aquatic vegetation, and may derive some of its oxygen supply from aquatic plants (Thorpe and Crisp 1949, Brown 1987). The species' members are flightless, and their life cycle is restricted entirely to the warm spring environment.

Bridger Canyon Warm Spring is located on United States Fish and Wildlife Service (USFWS) land emptying directly into Bridger Creek (BC), a typical foothill stream, at the

base of Drinking Horse Mountain (MBMG 1981). The elevation of BCWS is approximately 1518 m. and the universal transverse mercator measurement is 502.10 east by 5061.33 north (USGS 1987). The spring's source is in the Madison limestone group (MBMG 1981) and the topographic surface of the spring occupies an area of approximately 35 square meters.

Bridger Canyon Warm Spring is one of several naturally occurring warm springs in Montana whose waters originate in the Madison limestone geologic group (MBMG 1981). These springs are of approximately $\geq 10,000$ years of age, many of which are of Pleistocene age or older (Dr. John Montagne, personal communication, Mitchell 1974). Most of these springs have similar water chemistry and average temperatures (16°C to 30°C). Flow rates may vary dramatically from spring to spring (300 to 273,000 liters/minute). In comparison, BCWS exhibits relatively low flow (300 to 1000 l/min.). (See MBMG (1981) for details on water chemistry, temperature and flow).

Less than five square meters of BCWS is habitable by Z. thermae due to anthropogenic alterations in the spring environment. The Bozeman Fish Cultural Development Center (BFCDC) is located approximately one-quarter of a mile south of BCWS and utilizes water from the spring in hatchery operations. Since its construction in 1890, the BFCDC has utilized water from BCWS for fish culture.

In the early years (approximately 1890 to 1920) water was piped directly from the spring to the BFCDC with little modification to the spring. Though the exact date is not known, an open-topped spring house was constructed in this early period, raising the level of the spring. However, shortly after Emma M. Braton reportedly committed suicide in the spring house on March 22, 1918, a cover was constructed to avoid subsequent incidents (Gallatin County Hall of Records, BFCDC records).

The first cover(s) on the spring house were made of wood. A wooden cover probably allowed light to penetrate the joints between boards, thus allowing algal and planktonic growth inside the spring house (BFCDC records). Thus, the population of beetles was likely able to persist inside the spring box as long as light entered. Evidence of this is revealed in a collection of Z. thermae by D.J. Pletsch, 6 March, 1938, "from impounded water" (Montana Entomology Collection (MTEC), Montana State University).

The history of the spring box becomes obscured in BFCDC records after 1938. Local residents recall a metal cover being installed in the early 1950's (Ina Denton and Melvin Osborn, personal communication). The structure was reroofed with corrugated iron in the early 1970's (Carlie Smith, BFCDC Director, personal communication). This structure persists to the present time. These metal covers completely exclude light from the spring box, thus eliminating it as

suitable long-term habitat for Z. thermae.

The USFWS lists Z. thermae as a Category 2 candidate species (USDI 1989). A Category 2 candidate is a species under consideration for protection under the Federal Endangered Species Act (Category 1 Species). Category 2 species are given Category 1 status or Category 3 status (species of special concern) only after a clear understanding of their biological and population status is established. An understanding of the taxonomic, biological, and ecological status of Z. thermae is therefore critical to making sound management decisions designed for preserving the species as well as perpetuating historically important human activities in the area (i.e. BFCDC operations).

However, the taxonomic status of Z. thermae has been uncertain due to the morphological similarities it shares with Z. parvula. In addition, the two species occur literally side-by-side (Z. parvula is abundant in BC). Thus, uncertainties of the taxonomic status of Z. thermae have demanded an in-depth approach to the biological systematics of the species.

Since its description, Z. thermae (Hatch 1938) has received little attention in the taxonomic and ecological literature. Various authors have dealt haphazardly with its taxonomy, speculating over the validity of the species without systematic study (Hinton 1939a; Sanderson 1954; Hatch 1965; Brown 1972). In the latest review of North

American Elmidae, Brown (1972) treats Z. thermae as a possible ecological variant of the sister-species Z. parvula.

In testing the hypothesis that Z. thermae is a valid species, I will also examine the null hypothesis that Z. thermae is not a biological species. This will be undertaken with a multifaceted systematic approach including: 1) the taxonomic history of Z. thermae; 2) a morphological comparison of the skeletal morphology of Z. thermae and Z. parvula adults and larvae; 3) a descriptive and experimental examination of the ecology of Z. thermae, with particular reference to thermal ecology; and 4) an isozyme electrophoretic analyses for comparison of identified allelic frequencies in Z. thermae and Z. parvula populations.

TAXONOMIC HISTORY

Introduction

The first records of Z. thermae were collected by C.J.D. Brown of Montana State University from Bridger Canyon Warm Spring on 6 December, 1936 (Hatch 1938). Holotype and paratype specimens were housed in the Hatch collection, and are now in the collections at the National Museum of Natural History, Washington, D.C. and Oregon State University, respectively.

Subsequent records are widely separated, chronologically. Since its description, the species was collected by Pletsch, 6 March 1938 (MTEC), and not again until 16 January, 1987, by M.A. Ivie and D.L. Gustafson. Since that time, Z. thermae has been observed in situ repeatedly by D.L. Gustafson, M.A. Ivie, and M.M. Hooten (also see Appendix B).

Despite the lack of specimens of Z. thermae, various authors have synonymized Z. thermae with or split it from its sister-species Z. parvula, without apparent justification. The taxonomic history of Z. thermae can best be summarized as follows.

Zaitzevia thermae (Hatch)

Macronychus thermae Hatch 1938 : 18.

Zaitzevia thermae: Sanderson 1954 : 11. Brown 1972 : 21.

Zaitzevia parvulus (not Horn) : Hinton 1939 : 181 (in part).

Zaitzevia parvula (not Horn): Hatch 1965 : 11 (in part).

Hinton (1936) moved Macronychus parvulus Horn (type locality, California) to Zaitzevia following Champion's (1923) generic description. Hatch (1938) described Z. thermae from Bridger Canyon Warm Springs, near Bozeman, Montana, in Macronychus, apparently missing Hinton's work.

Hinton (1939a) synonymized M. thermae Hatch with Z. parvulus, but Sanderson (1954) resurrected it under the new combination Z. thermae. Hatch (1965) missed or chose not to follow Sanderson's view and listed Z. thermae as a synonym of Z. parvula.

Brown (1972) discussed Z. thermae as a possible ecological variant of Z. parvula, but recognized it as a valid species.

MORPHOLOGICAL ANALYSIS

Introduction

Identification of sympatric sister-species is often difficult on morphological grounds due to lack of divergence (Wiley 1981) and an inexactness of ecological scale involved in capture techniques (Allen and Star 1982, Wiens 1989). This may lead to errors in the recognition of sister-species (Howard 1983, Crego et. al. 1990).

Hinton (1939a) synonymized Z. thermae with Z. parvula on the basis of gross morphological similarities. However, even a cursory comparison showed a subtle, but constant difference in habitus. Therefore, a comparative and detailed investigation of skeletal morphology of both species was undertaken in order to test Hinton's hypothesis.

In addition to adult morphology, larval morphology was pursued in the same vein. Separation of congeneric larvae at the species level has proven to be a difficult task in the Elmidae and has met with little or no success (Brown 1972, Brown and White 1978). In order to make direct comparisons between instars, a larval instar analysis of Z. thermae preceded the morphological comparisons of sister-taxa larvae.

Methods and Materials

Standard Analysis

Methods similar to those of Hinton (1939b), Doyen (1966), and Brown (1981) were employed in a sclerite-by-sclerite comparison of Z. thermae and Z. parvula. Fresh specimens were soaked in lactic acid for at least 24 hours prior to dissection in order to clear soft tissues. Sclerites were then removed, put in glycerine mounts and examined under dissecting and compound microscopes.

Dorsal, ventral, and lateral views were compared and a series of working drawings were made using a drawing tube on Wild dissecting and compound microscopes. Drawings, as well as side-by-side comparisons of sclerites served to identify structural differences between the species.

Specimens dissected included approximately fifty Z. thermae from BCWS, and seventy Z. parvula from West Fork Spring (WFS), Snowflake Spring (SFS), and Bridger Creek (BC). In addition, wing and metendosternite morphology were examined in Z. parvula from Nimrod Spring (NS), Potosi Warm Spring (PWS), and Brooks Warm Spring (BRWS). Specimens from each locality have been deposited as vouchers in the MTEC (Appendix B). (See Figure 1 for a map of localities of materials utilized in this and all subsequent analyses).

Since it was not the purpose of this study to redescribe the sister-taxa but to elucidate potential morphological differences, discussion and illustrations are

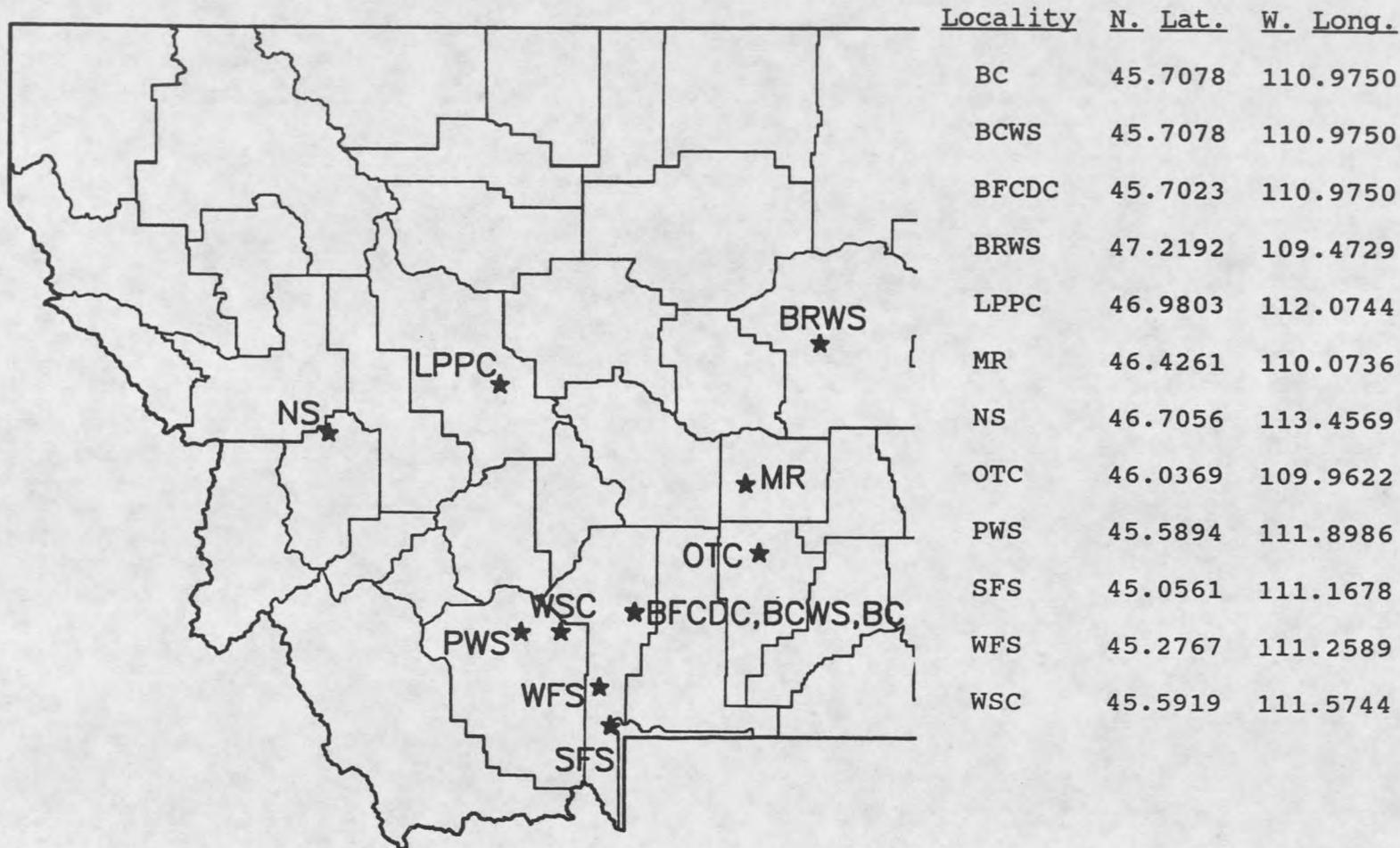


Figure 1. Map of Western Montana showing localities of collection of *Zaitzevia* spp. See list of textual acronyms (page ix) and text for further explanation.

limited to divergent morphological characters, with additional mention of genitalia.

Morphometric Analysis

In addition to standard morphological techniques, morphometric analyses were used to measure potential differences in overall size and proportion between populations. Six mixed-sex populations of Zaitzevia spp. were used for these analyses, in order to cover the breadth of temperature regimes that Zaitzevia occupies. Two cold stenothermic populations (WFS and SFS), two cold eurythermic populations (BC and Musselshell River (MR)), and two warm stenothermic populations (BCWS and BRWS) were represented. Twenty-five specimens were measured from WFS, SFS, BC, and BCWS, eleven from MR, and seventeen from BRWS. All specimens represent point-specific localities, yet most are from variable dates of collection (Appendix B). This was done purposefully to avoid bias of potential seasonal effects.

Measurements included pronotal length and width, as well as elytral length. Pronotal length was measured from the posterior edge of the pronotum at the anterior-most point adjacent to the scutellum, to the apex of the anterior pronotal curve. Pronotal width was measured at the widest point. Elytral length was measured from the median pronotal juncture to the posterior tip of the elytra. Specimens were measured with an ocular micrometer under 40X magnification

with 10X oculars on a Nikon dissecting microscope and calibrated with a Minitool device. Measurements were accurate to within $\pm 1/2$ of an empirical unit (1 unit=0.0194 mm). All specimens examined were dry when measured.

All measurements were added to achieve a gross measure of individual size (referred to herein as total size), and pronotal length was divided by pronotal width to determine relative proportional size. ANOVA models were employed to analyze the data on MSUSTAT (Lund 1988). Mean population total size measurements were analyzed using a fixed model one-way ANOVA. Multiple comparisons of means were analyzed utilizing LSD procedures following the ANOVA. Proportional values were transformed by $2\arcsin(\text{square root} [\text{proportion}/2])$ and analyzed under the same assumptions as above. Values under this transformation are positively correlated with increasing proportion. Null hypotheses of no significant total size and proportion differences between populations were tested.

Larval Instar Analysis and Morphology

Morphological examination of larvae was necessarily preceded by an analysis of larval instars so that larvae of the same developmental stage could be examined. One hundred sixty-two and four hundred fifty-two larvae of Z. thermae collected by D.L. Gustafson on June 20 and November 1, 1987, respectively, were examined for head capsule diameter. Head

capsule measurement techniques for the determination of instars in Coleoptera larvae were reviewed by Bartell and Roberts (1974) and Crowson (1981). A multimodal distribution of head width measurements is hypothetically indicative of distinct developmental stages (Crowson 1981).

Larvae were removed from alcohol and blotted on Kimex paper to remove excess liquid. Head capsules were removed under a Wild dissecting scope and placed in rows in a thin, uncovered, glycerine slide mount. An ocular micrometer was placed in the Wild dissecting scope with 20X oculars and calibrated using a Minitool device. Head capsules were measured under 50X across the occipital bulge and widths were recorded on an empirical scale from 7 to 22. Translation of empirical widths is from 0.128 to 0.403 mm (0.0183 mm per empirical unit). Histograms of measurement data were constructed to indicate larval instar classes of Z. thermae.

Results

Standard Analysis

Analyses of the skeletal features revealed three anatomical markers distinguishing Z. thermae and Z. parvula. These include differential curvature of the pronota, and variations in the form of the wings and metendosternites. Individual features are discussed below.

Pronotum. The pronotum of Z. thermae is strongly

downcurved at the lateral margins. When viewed from above, the tooth of the anterior angles appears acutely produced from the anterior pronotal margin (Figure 2). Lateral rotation of the pronotum reveals this illusion, showing the tooth to be, in truth, sinuously produced.

The pronotum of Z. parvula is less strongly downcurved at the lateral margins. When viewed from above, the tooth of the anterior angles appears smoothly produced from the anterior pronotal margin (Figure 2).

These differences appear to be consistent, even in relatively large Z. thermae and small Z. parvula, respectively.

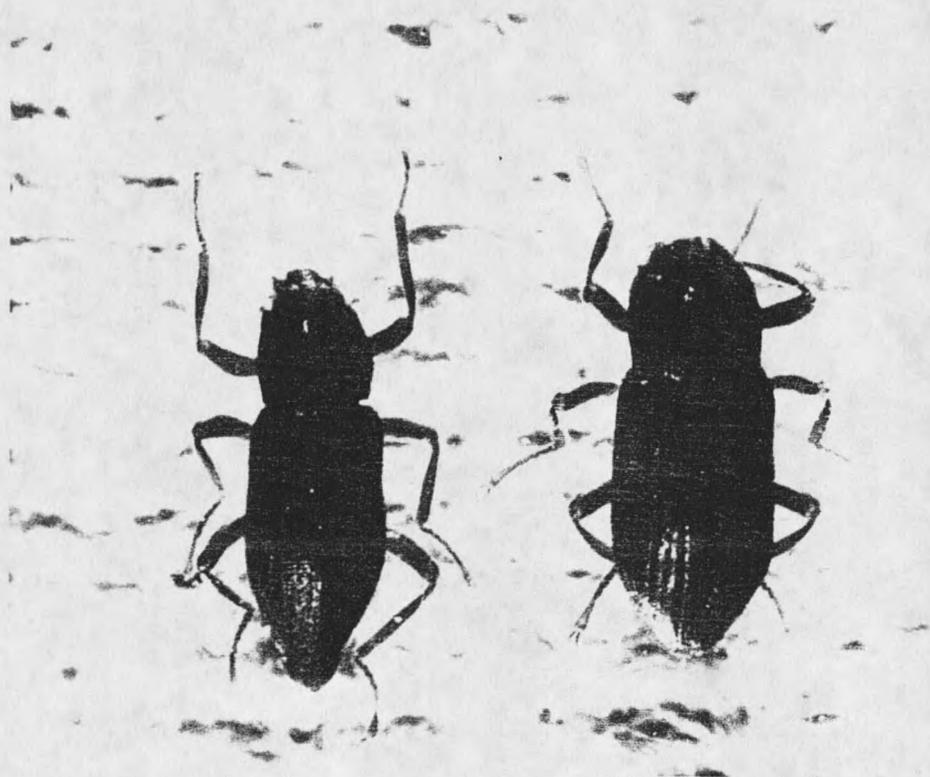
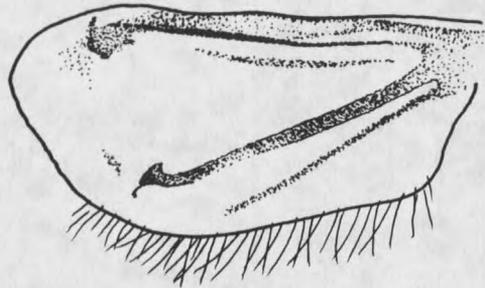


Figure 2. Habitus of Zaitzevia thermae and Z. parvula, NS. Note the slender form of Z. thermae. See text for further explanation.

Wings. Hind wings in Z. thermae are vestigial (Segal 1933) showing reductions in relative length, venation, and setation (Figure 3).



1 mm.

Figure 3. Wing of Z. thermae . See text for further explanation.

The distal membranous region is greatly shortened, with the loss of some thickened areas (flecks) found in the region in Z. parvula (Figures 4 through 7). Wings in Z. thermae are $\leq 2/3$ the length of the elytra. Those in Z. parvula are consistently $> 2/3$ the length of the elytra, with NS and SFS populations exhibiting the relative extremes of development (Figures 5 and 6, respectively). Zaitzevia parvula, PWS, BC, WFS, and BRWS exhibited intermediate wing development.

Venation in the wing of Z. thermae is also reduced, particularly in the radial region (sensu Crowson 1955). In all Z. parvula populations examined, complete wing venation appeared to be conserved in all size classes (Figures 4

through 7).

Wing surfaces of all Zaitzevia are microtrichiate, a feature omitted from illustration for the sake of clarity. Marginal setation follows the posterior edge of the wings from the proximal side of the anal angle proceeding distally. In Z. thermae, setation extends to the cubital region, thus not extending to the most distal tip of the wing (Figure 3) as it does in Z. parvula (Figures 4 through 7).

Metendosternite. Although typically used as a higher taxonomic character (Crowson 1938, 1955), the metendosternite proved to be a key character differentiating the sister taxa. The metendosternite in Z. thermae is reduced to a simple T-shaped structure, completely lacking lobes on the apophyseal arms (Figure 8). All Z. parvula examined showed marked lobation on the anterioventral side of the apophyseal arms (Figure 8). The size of the lobes appears to vary somewhat with wing size, as one may expect (Doyen 1966), yet Z. parvula exhibits apophyseal lobes across the range of variation.

Genitalia. Examination of the genitalia of both sexes revealed no significant differences between the taxa. In fact, it would appear that genitalia are mechanically compatible, possessing no "lock and key" mechanism (Shapiro and Porter 1989). Undoubtedly, this finding leaves open the

