

Metal concentrations of insects associated with the South African Ni hyperaccumulator *Berkheya coddii* (Asteraceae)

ROBERT S. BOYD¹, MICHEAL A. DAVIS², MICHAEL A. WALL³ and KEVIN BALKWILL⁴

¹Department of Biological Sciences, Auburn University, Auburn, ²Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, ³Entomology Department, San Diego Natural History Museum, San Diego, USA; ⁴C.E. Moss Herbarium, School of Animal and Plant Sciences, University of the Witwatersrand, South Africa

Abstract The high levels of some metals in metal hyperaccumulator plants may be transferred to insect associates. We surveyed insects collected from the South African Ni hyperaccumulator *Berkheya coddii* to document whole-body metal concentrations (Co, Cr, Cu, Mg, Mn, Ni, Pb, Zn). We also documented the concentrations of these metals in leaves, stems and inflorescences, finding extremely elevated levels of Ni (4 700–16 000 µg/g) and high values (5–34 µg/g) for Co, Cr, and Pb. Of 26 insect morphotypes collected from *B. coddii*, seven heteropterans, one coleopteran, and one orthopteran contained relatively high concentrations of Ni (> 500 µg/g). The large number of high-Ni heteropterans adds to discoveries of others (from California USA and New Caledonia) and suggests that members of this insect order may be particularly Ni tolerant. Nymphs of the orthopteran (*Stenoscepa*) contained 3 500 µg Ni/g, the greatest Ni concentration yet reported for an insect. We also found two beetles with elevated levels of Mg (> 2 800 µg/g), one beetle with elevated Cu (> 70 µg/g) and one heteropteran with an elevated level of Mn (> 200 µg/g). Our results show that insects feeding on a Ni hyperaccumulator can mobilize Ni into food webs, although we found no evidence of Ni biomagnification in either herbivore or carnivore insect taxa. We also conclude that some insects associated with hyperaccumulators can contain Ni levels that are high enough to be toxic to vertebrates.

Key words *Berkheya coddii*, biomagnification, food webs, heavy metals, hyperaccumulation, South Africa

DOI 10.1111/j.1744-7917.2006.00071.x

Introduction

Metals are an important group of pollutants because they often are toxic to organisms in relatively small quantities. Much research has documented the biological effects of metals in metal-contaminated systems or in laboratory situations that mimic them (e.g. Heikens *et al.*, 2001). However, high levels of metals occur naturally in some

ecosystems. Ultramafic rocks (also called serpentine rocks) may contain relatively large amounts of Co, Cr, Fe, Mg, Mn and Ni, and these elements may be relatively abundant in soils derived from these rocks (Brooks, 1987). As a consequence, plant species from ultramafic sites often possess adaptations that allow them to tolerate this unusual soil chemistry (Brooks, 1987) and specialized floras have often evolved (Brooks, 1987; Kruckeberg, 1984).

Although they are relatively well studied botanically (Brooks, 1998), ultramafic ecosystems are relatively under-studied in some important ecological contexts. In particular, plant-animal interactions on ultramafic sites have rarely been studied. Isolated studies have been published (e.g. Proctor & Whitten, 1971; Shapiro, 1981), but

Correspondence: Robert S. Boyd, Department of Biological Sciences, 101 Life Sciences Building, Auburn University, Auburn, Alabama 36849-5407, USA. Tel: 334 844 1626; fax: 334 844 1626; e-mail: boydrob@auburn.edu

generally little is known. Kruckeberg (1984) reported a general lack of information on the fauna of North American ultramafic sites.

Ultramafic sites may contain chemically unique plant species. These plants, termed hyperaccumulators by Brooks *et al.* (1977), are unusual in that they contain large amounts of elements not normally found in abundance in plants. Nickel hyperaccumulators are defined by Reeves and Baker (2000) as species containing $>1\,000\ \mu\text{g Ni/g}$ dry weight in the aboveground parts of at least one specimen collected from the wild. For comparison, “normal” plants growing on “normal” (non-metalliferous) soils might have leaf Ni concentrations of $1\text{--}10\ \mu\text{g/g}$ dry weight (Reeves & Baker, 2000). At the time of their review, Reeves and Baker (2000) estimated that 418 hyperaccumulator taxa had been documented, with the majority of these (76%) hyperaccumulating Ni. The remaining taxa hyperaccumulated other metals or metalloids, such as Cd, Co, Cu, Mn, Pb, Se or Zn (Reeves & Baker, 2000). Hyperaccumulators are globally widespread, having been reported from every continent except Antarctica, but most hyperaccumulator taxa are restricted to ultramafic soils (Proctor, 1999).

Because plants form the foundation of most terrestrial food webs, the elements in plants are a major source of elements for organisms in higher trophic levels. Therefore, hyperaccumulator plants provide an opportunity for metals to enter food webs in relatively large quantities. Movement of metals into animals in metal-polluted areas has received much attention (e.g. Heikens *et al.*, 2001), but study of metal movements in ecosystems with naturally elevated metal concentrations has rarely been attempted. Davison *et al.* (1999) compared the metal concentrations of insects from an ultramafic site in Scotland to insects from two non-ultramafic sites, finding elevated levels of Mn, Mg and/or Cr in some insect orders from the ultramafic site (Ni was not included in their study). Peterson *et al.* (2003) contrasted metal concentrations of invertebrates collected from sites in Portugal with and without a Ni hyperaccumulator species. They found increased Ni concentrations (but not Co and Cr) in those collected from the Ni hyperaccumulator sites, indicating that Ni was being transferred to other trophic levels and implicating the Ni hyperaccumulator in that process.

Commonly used metal analysis techniques require a relatively large sample mass compared to the masses of many individual insects, so that individual insects are often combined into samples representing relatively large taxonomic groups (e.g. orders). Both Davison *et al.* (1999) and Peterson *et al.* (2003) combined arthropod morphotypes into large taxonomic categories (orders in the former case and spiders and grasshoppers in the latter case) and thus

were unable to study metal concentration at finer taxonomic levels. A few studies have attempted to identify invertebrate species with elevated metal concentrations that may be feeding upon Ni hyperaccumulator plant species (Wall, 1999; Mesjasz-Przybylowicz & Przybylowicz, 2001). These efforts have uncovered some high-Ni insect species. Schwartz and Wall (2001) described a plant bug from California ultramafics, *Melanotrichus boydi* Schwartz and Wall (Heteroptera: Miridae), containing $770\ \mu\text{g Ni/g}$. Mesjasz-Przybylowicz and Przybylowicz (2001) reported a high-Ni beetle from South African ultramafics, *Chrysolina pardalina* (Fabricius) (Coleoptera: Chrysomelidae), that contained a maximum of $2\,600\ \mu\text{g Ni/g}$. Boyd (2005, personal communication) reported a high-Ni bug, *Utana viridipuncta* (Heteroptera: Pentatomidae), that fed on a Ni hyperaccumulator in New Caledonia. This heteropteran contained $2\,600\ \mu\text{g Ni/g}$ in nymphs and $750\ \mu\text{g Ni/g}$ in adults.

These examples show that some insects feeding on hyperaccumulators may have elevated whole-body metal concentrations. This may have consequences for the applied uses of metal hyperaccumulating plants. Hyperaccumulators have been studied for potential use in phytoextraction, the removal of metals from soils using plants (Reeves, 2003). These uses include cleanup of metal-polluted sites (Pilon-Smits, 2004) as well as using metal hyperaccumulators to mine metals from high-metal sites, which has been termed phytomining (Anderson *et al.*, 1999). There is some concern that herbivores feeding on hyperaccumulators may mobilize metals into ecosystems (Whiting *et al.*, 2004) and affect local food webs. Study of food webs that involve hyperaccumulators in their native environments is needed to illustrate the pathways through which metals can move between plants and higher trophic levels.

This research reports the results of surveys of the insect fauna associated with a South African Ni hyperaccumulator species, *Berkheya coddii* (Asteraceae). Our research had two main objectives. First, we wanted to compare results when data were analyzed at both broad (order) and fine (morphotype) taxonomic levels (a morphotype is a collection of specimens that appear to comprise a single species, based on their appearance in the field). We examined metal concentrations among orders and then among morphotypes to illustrate the value of sorting taxa to the finest taxonomic scale possible. Second, we examined morphotype-level data to discover additional high-metal (especially high-Ni) insects. Identification of these high-metal insects helps us understand the role of insects in metal mobilization within ultramafic ecosystems. It is also a necessary first step that permits future studies on the ecology and ecophysiology of these unusual organisms.

Materials and methods

Plant species

Berkheya coddii Roessler is a summer-active perennial herb that reaches 1.5 m in height, forming new stems from rootstocks each spring and dying back to the rootstocks during late summer (Anderson *et al.*, 1997). Endemic to ultramafic soils of Mpumalanga Province, South Africa (Anderson *et al.*, 1997), it can form relatively large stands with high biomass (Augustyniak *et al.*, 2002). Robinson *et al.* (1997) reported that *Berkheya coddii* can produce annually as much as 22 t/ha of aboveground biomass in experimental plantings. This species is also notable for its high levels of foliar Ni. Morrey *et al.* (1991) reported a mean of 12 000 µg/g, but Anderson *et al.* (1997) found up to 37 000 µg/g and Mesjasz-Przybylowicz & Przybylowicz (2001) reported as much as 77 500 µg/g. The combination of exceptional biomass and high Ni levels have made this species attractive as a potential Ni phytoextractor (Robinson *et al.*, 2003).

Study sites

Insects were collected from several stands of *B. coddii* located on ultramafic soils located about 30 km ENE of Badplaas, Mpumalanga Province, South Africa. In this area, *B. coddii* grows only on ultramafic soils and often in dense stands where it is the dominant biomass. The stands sampled for insects included those from which plant samples were collected for analysis (see below), but insects also were collected from other sites that contained *B. coddii* in the area.

Plant sampling

Plant biomass was collected from three of the populations used as sources of insects in this study. The three sites were: Doyershoek, where plants were located along a firebreak on a steep hillside; Groenvaly, which had plants at the foot of an ultramafic hill; and Groenvaly Mine, an abandoned mine site about 1 km from the afore-mentioned ultramafic hill. In each population, several plants were collected and subsamples made from leaf, stem and inflorescence material. Subsamples were dried at 60 °C for several days, ground, and then combined into a single sample to represent each plant part from each of the three sample populations. Plant material was not rinsed with water prior to grinding and analysis to avoid leaching metals from the tissues.

Insect sampling

Collections were made at the season of peak flowering of

B. coddii, from January 10 to February 5, 2000. Insects associated with *B. coddii* were visually located on plants and captured. Only individuals that had been observed to physically contact a *B. coddii* plant were collected. Observations of insect activity were made if possible, along with notes on which plant organ(s) the insects were found. We were particularly interested in noting which morphotypes consumed *B. coddii* tissues.

We also collected morphotypes of herbivorous insects from plant species other than *B. coddii*. The metal concentrations of these “reference morphotypes” were compared against those of morphotypes collected from *B. coddii* and thus provided a benchmark to help us identify exceptional metal concentrations in insects from *B. coddii*. These four reference morphotypes were given names that indicated both their provenance and their family. The first reference morphotype (called *Acacia* Curc) comprised two samples of a small weevil (Coleoptera: Curculionidae) collected from *Acacia* sp. growing on ultramafic soil at the Groenvaly site. The second and third reference morphotypes were grasshoppers (Orthoptera: Acrididae) collected from plants other than *B. coddii* growing on ultramafics at the Groenvaly site. These morphotypes were named Ultramafic Acri 1 and Ultramafic Acri 2 and each consisted of two samples. The fourth reference morphotype comprised three samples of a grasshopper (Orthoptera: Acrididae) that was collected from a nearby non-ultramafic grassland area. This morphotype was named Non-Ultramafic Acri 1.

Metal concentration analyses

Plant material was analyzed by dry-ashing and analysis of the ash dissolved in concentrated acids (Boyd & Davis, 2001). Plant samples were dry-ashed at 485 °C, further oxidized in 1 mol/L HNO₃, and the residues were re-dissolved in 1 mol/L HCl. For all metals except Ni (Co, Cr, Cu, Mg, Mn, Pb, Zn), the solutions were analyzed using an inductively coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000). Nickel concentrations were determined using an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251).

Insect specimens were sorted according to morphotype and representatives of each morphotype were pinned and labeled for later identification to the lowest taxonomic level that could be readily attained. Upon returning from the field, samples were air-dried for at least 72 h at 67 °C and weighed. Individuals of the same morphotype weighing less than 50 mg were combined in order to create samples of at least that mass for analysis. Many morphotypes could not be analyzed for Ni concentration because we had only a few specimens with insufficient mass for analysis.

Insect samples were analyzed for metal concentrations

in a manner similar to the plant samples except that they were wet-digested (Wall & Boyd, 2002). As with plant samples, insect samples were not rinsed with water prior to analysis to avoid leaching metals from the insect bodies. Samples were digested in borosilicate glass test tubes using 3–5 mL of concentrated HNO_3 at 110°C for 6–8 h, after which time most of the liquid had evaporated. Residue was redissolved in 3–5 mL of 1 mol/L HCl at 110°C for 2–4 h. The solutions were then diluted with distilled water to a volume of 25 mL. Solutions were analyzed for all metals except Ni using an inductively coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000). Nickel concentrations were determined using an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251).

Statistical analyses

Plant metal concentrations were analyzed using one-way analysis of variance (ANOVA) to determine if metal concentrations varied between plant organs (leaf, stem, inflorescences). Fisher's Protected Least Significant Difference (PLSD) test was used for post-hoc means separations at $P = 0.05$ (Abacus Concepts, 1998). We analyzed metal concentration data from insects in two ways. First, we analyzed data from morphotypes without regard to their classification into orders. We used one-way ANOVA on data from reference morphotypes to determine if they varied significantly from one another, using Fisher's PLSD test for pairwise means comparisons. Then one-way ANOVA was used to examine metal concentrations of all morphotypes (both reference morphotypes and morphotypes collected from *B. coddii*) with Fisher's PLSD test for pairwise means comparisons. We were particularly interested in identifying morphotypes from *B. coddii* that contained significantly more metal than all four

of the reference morphotypes, and identifying morphotypes collected from *B. coddii* that contained greater metal levels than the other morphotypes collected from *B. coddii*. Second, we calculated the mean concentration of each metal for each morphotype belonging to the four best-represented orders collected from *B. coddii* (Heteroptera, Coleoptera, Orthoptera and Hymenoptera). We then used one-way ANOVA to test whether orders varied in metal concentration, comparing means using Fisher's PLSD test.

Results

Plant sampling

Organs of *B. coddii* differed in metal concentrations for most of the metals examined (Table 1). Nickel concentrations were extremely high in all organs sampled. Leaves contained the largest amounts of Ni (up to a maximum of 19 000 $\mu\text{g/g}$), followed by inflorescences (to 11 600 $\mu\text{g/g}$) and then stems with a maximum of 5 300 $\mu\text{g/g}$ (Table 1). Mean Ni levels were at least 470-fold above the upper limit of the normal range of Ni concentration in plant tissues (Table 1). Three other metals, Co, Cr and Pb, reached concentrations in *B. coddii* that exceeded the normal range for plants (Table 1), although to a much lesser extent than for Ni. Concentrations of these metals ranged from 1.8- to 17-fold that of the upper limit of the normal range in plants. Lead had a similar pattern of accumulation to that of Ni, with maximum values in leaves and minimum values in stems, although for Pb the greatest concentration measured (a value of 39 $\mu\text{g/g}$ in a leaf sample from the Groenvaly Mine site) was much less than any values for Ni. Three metals (Co, Cr, Zn) reached greatest concentrations in leaves, but stems and inflorescences did not differ signifi-

Table 1 Mean metal concentrations (in $\mu\text{g/g}$, dry mass) of samples of plant organs collected from three populations of *B. coddii*.

Metal	Plant organ						Normal range		
	Leaf		Stem		Inflorescence				
Co	34.00 ^a	(3.40)	5.50 ^b	(0.69)	12.00 ^b	(0.26)	0.03	—	2.00
Cr	20.00 ^a	(1.40)	6.70 ^b	(0.47)	8.20 ^b	(0.50)	0.20	—	5.00
Cu	10.00 ^b	(0.91)	7.70 ^c	(0.70)	13.00 ^a	(0.00)	5.00	—	25.00
Mg	5 900.00	(890.00)	4 400.00	(1 100.00)	3 800.00	(290.00)	1 000.00	—	6 000.00
Mn	99.00	(54.00)	18.00	(7.30)	44.00	(13.00)	20.00	—	400.00
Ni	16 000.00 ^a	(2 100.00)	4 700.00 ^c	(360.00)	9 600.00 ^b	(1 100.00)	1.00	—	10.00
Pb	33.00 ^a	(3.30)	8.90 ^c	(1.70)	18.00 ^b	(2.40)	0.10	—	5.00
Zn	71.00 ^a	(2.20)	39.00 ^b	(3.10)	48.00 ^b	(3.90)	20.00	—	400.00

SE in parentheses, $n = 3$ for all values. Means for a metal that differ significantly among plant organs (Fisher's PLSD test, $P = 0.05$) are denoted by differing superscripts. Normal ranges of metal concentrations in terrestrial plants, as reported by Reeves and Baker (2000), are provided as a benchmark against which values from *B. coddii* can be compared.

cantly from one another in concentration (Table 1). In each case, however, the trend matched that of Ni and Pb in that mean values for inflorescences were greater than mean values for stems. The pattern for Cu differed from these other metals, in that greatest values were found for inflorescences, intermediate values in leaves, and lowest values in stems. Two metals (Mg and Mn) did not differ significantly in concentration among plant organs (Table 1).

Insect sampling

A total of 26 morphotypes were collected from *B. coddii* in sufficient mass for Ni analysis, along with the four reference morphotypes collected from species other than *B. coddii*. Many morphotypes collected from *B. coddii* were identified to genus or species (Table 2), although identifications were difficult in other cases. For example, nymphs of a pyrgomorph grasshopper were tentatively identified to the genus *Stenoscepa* but adults are required for definitive identification. In other cases we were only able to identify morphotypes to family (Table 2). These morphotypes were assigned a short name derived from an abbreviation of their family name (e.g., the unidentified mantid morphotype was called Mant 1). Major orders of insects represented were Coleoptera (10 morphotypes), Heteroptera (6 morphotypes), Orthoptera (4 morphotypes), and Hymenoptera (3 morphotypes). Species/morphotypes were fairly evenly divided between those collected only from vegetative portions of the plant (10: Table 2), and those collected only from inflorescences (11). Several of the heteropterans and one coleopteran were collected from both stems and inflorescences (Table 2). Feeding by 13 morphotypes was observed, but two of these were not herbivores. The ant (*Polyrhachis* sp.) was tending aphids, and Mant 1 was hunting floral visitors on *B. coddii* inflorescences.

Metal concentrations varied significantly among morphotypes for most metals. For each metal, we first explored variation among the reference samples to determine if significant differences occurred. We next analyzed data from all morphotypes, both reference and *B. coddii* samples, to identify which (if any) morphotypes from *B. coddii* had unusually large concentrations of a metal when compared to both reference morphotypes and other morphotypes from *B. coddii*.

Nickel

Extreme variation was observed for Ni, for which mean concentrations ranged from a low of 3.3 µg/g to a high of 3 500 µg/g. Concentrations of Ni were least in the reference sample from the non-ultramafic area (Non-ultramafic Acri 1:

Table 3) and increased by ten-fold or more for those collected on ultramafic substrates from plant species other than *B. coddii*. However, ANOVA of these data did not reveal statistically significant variation in Ni concentration ($F_{3,6} = 1.0$, $P = 0.45$) because of high variation among sample values.

The ANOVA of Ni concentrations for all morphotypes showed highly significant variation in Ni concentration ($F_{29,67} = 13$, $P < 0.0001$). Fisher's PLSD test revealed that six morphotypes had greater Ni concentrations than all four of the reference taxa (Fig. 1). Four of these high-Ni taxa were heteropterans, for which mean Ni concentration ranged from 630 µg/g for *Spilostethus* to 1 800 µg/g for *Veterna*. The value for *Veterna* was the second greatest mean Ni concentration, and was significantly greater than all but three other morphotypes (*Stenoscepa*, *Agonoscelis* and *Boerias*: Fig. 1). Two other heteropterans had mean Ni concentrations above 500 µg/g and thus are worthy of note: *Anoplocnemis* at 510 µg/g and *Sphaerocoris* at 530 µg/g, but these values were not significantly greater than those for all four of the reference taxa.

Besides the heteropterans, two other taxa had extremely elevated Ni concentrations. Curc 1 had a Ni concentration of 1 080 µg/g, which was significantly greater than that of all but six morphotypes and greater than that of all other beetles collected from *B. coddii*. However, the greatest Ni concentration was documented for the orthopteran *Stenoscepa*, a grasshopper species that was observed consuming leaves of *B. coddii* in the field (Table 2). The mean Ni concentration of *Stenoscepa* was 3 500 µg/g, a value significantly greater than those of all other morphotypes examined. For this insect, Ni values of individual samples ranged between 3 050–4 100 µg Ni/g.

Lead

Lead concentrations ranged from 0 µg/g to a maximum of 76 µg/g. Values for the reference samples varied significantly for lead ($F_{3,5} = 11$, $P = 0.012$), which was low in the non-ultramafic sample and for two of the three morphotypes collected from ultramafic plants other than *B. coddii* (Table 3). However, *Acacia* Curc had a very large amount of Pb (76 µg/g), more than ten-fold that of the other reference morphotypes.

The ANOVA of Pb values for all morphotypes showed significant variation ($F_{29,66} = 4.9$, $P < 0.0001$). Several morphotypes collected from *B. coddii* had relatively great concentrations of Pb (> 40 µg/g), including two heteropterans, three coleopterans, two hymenopterans, and one lepidopteran (Fig. 2). However, none of these morphotypes contained significantly more Pb than all others (Fisher's PLSD test, $P > 0.05$).

Table 2 Insect taxa (morphotypes) collected from *B. coddii* and analyzed for whole-body metal concentrations, noting their typical location on plants and observations regarding their activities.

Insect taxon	Location on plant	Observed activity	<i>n</i>
Heteroptera			
Pentatomidae			
<i>Veterna Sanguineirostris</i> (Thb.) ³	Stem, receptacle	—	4
<i>Boerias ventralis</i> (Dallas) ³	Stem, receptacle	—	4
<i>Agonoscelis erosa</i> (Westwood) ³	Stem, receptacle	Feeding	4
Coreidae			
<i>Anoplocnemis curvipes</i> (Fabricius) ³	Stem, leaf	Feeding	4
Lygaeidae			
<i>Spilostethus rivularis</i> (Germar) ³	Inflorescence	Feeding	4
Scutelleridae			
<i>Sphaerocoris testudogriseus</i> (DeGeer) ³	Inflorescence/infructescence	Feeding	4
Coleoptera			
Chrysomelidae			
<i>Demotina</i> sp. ¹	Inflorescence	—	3
Undetermined (subfamily Alleculinae) ¹	Inflorescence	—	3
<i>Chrysolina clathrata</i> (Clark) ¹	Leaf	Feeding	4
<i>Cryptocephalus</i> sp. ¹	Leaf	—	2
<i>Monolepta</i> sp. ¹	—	—	3
Meloidae			
<i>Hycleus lunatus</i> (Pallas)	Inflorescence	Feeding	4
Curculionidae			
Undetermined ³	Stem, leaf	Feeding	3
Scarabaeidae			
<i>Cyrtothyrea marginalis</i> (Swartz) ³	Inflorescence	Resting	4
<i>Anisorrhina umbonata</i> (Gory & Percheron) ³	Stem, inflorescence	Resting	3
<i>Leucocelis haemorrhoidalis</i> (Fabricius) ³	Inflorescence	—	4
Orthoptera			
Pyrgomorphidae			
<i>Stenoscepa</i> sp. ³	Leaf	Feeding	4
Undetermined ³	Leaf, stem	—	2
Tettigoniidae			
Undetermined ³	Growing stem tip	—	2
Acrididae			
Undetermined ³	Leaf, stem	—	2
Mantodea			
Mantidae			
Undetermined ³	Inflorescence	Hunting floral visitors	3
Hymenoptera			
<i>Pachyhalictus</i> sp. ³	Inflorescence	Foraging: floral rewards	3
Apidae			
<i>Apis mellifera</i> (Linnaeus) ³	Inflorescence	Foraging: floral rewards	3
Formicidae			
<i>Polyrhachis</i> sp. ³	Stem, leaf	Tending aphids	4
Homoptera			
Aphididae			
Undetermined ³	Stem, young leaf	Feeding	4
Lepidoptera			
Pieridae			
<i>Belenois aurota</i> (Fabricius) ³	Inflorescence	Foraging: nectar	3

Superscripts for each taxon denote location of voucher specimens: 1=Australian Museum; 2=Museo el Instituto di Zoologia Sistemática, Torino; 3=San Diego Natural History Museum. The number of samples of each taxon analyzed for metal concentration is shown as *n*.

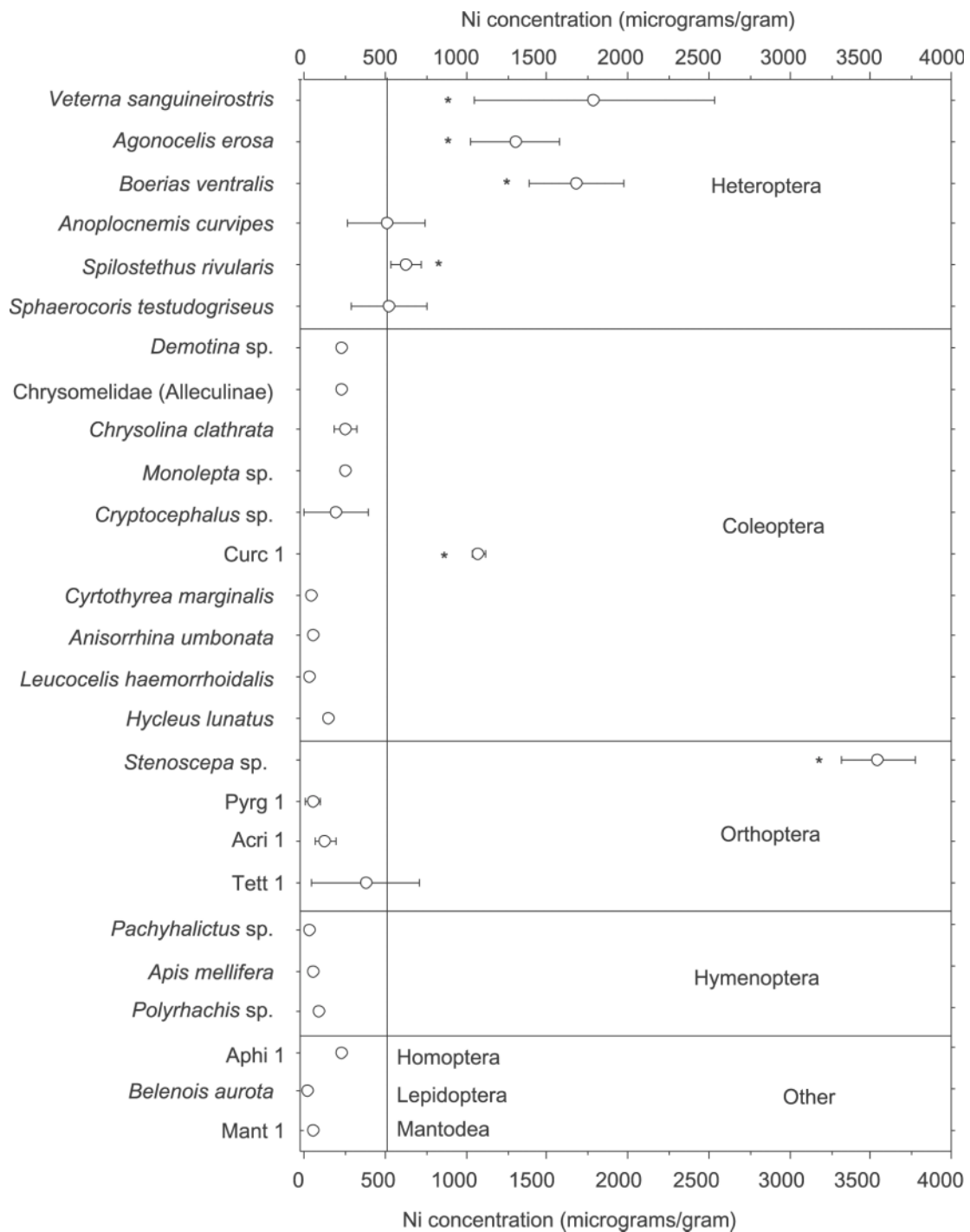


Fig. 1 Mean Ni concentrations (in $\mu\text{g/g}$ dry mass, bars showing SE) in morphotypes collected from *B. coddii*. Lack of error bars for a mean indicates that the bars are too small to be shown at the scale of the graph. Stars indicate morphotypes with significantly greater Ni concentrations than in all four reference morphotypes collected from non-hyperaccumulator species (means for reference morphotypes in Table 3). The line at 500 $\mu\text{g/g}$ indicates a general threshold for unusually high-Ni insects.

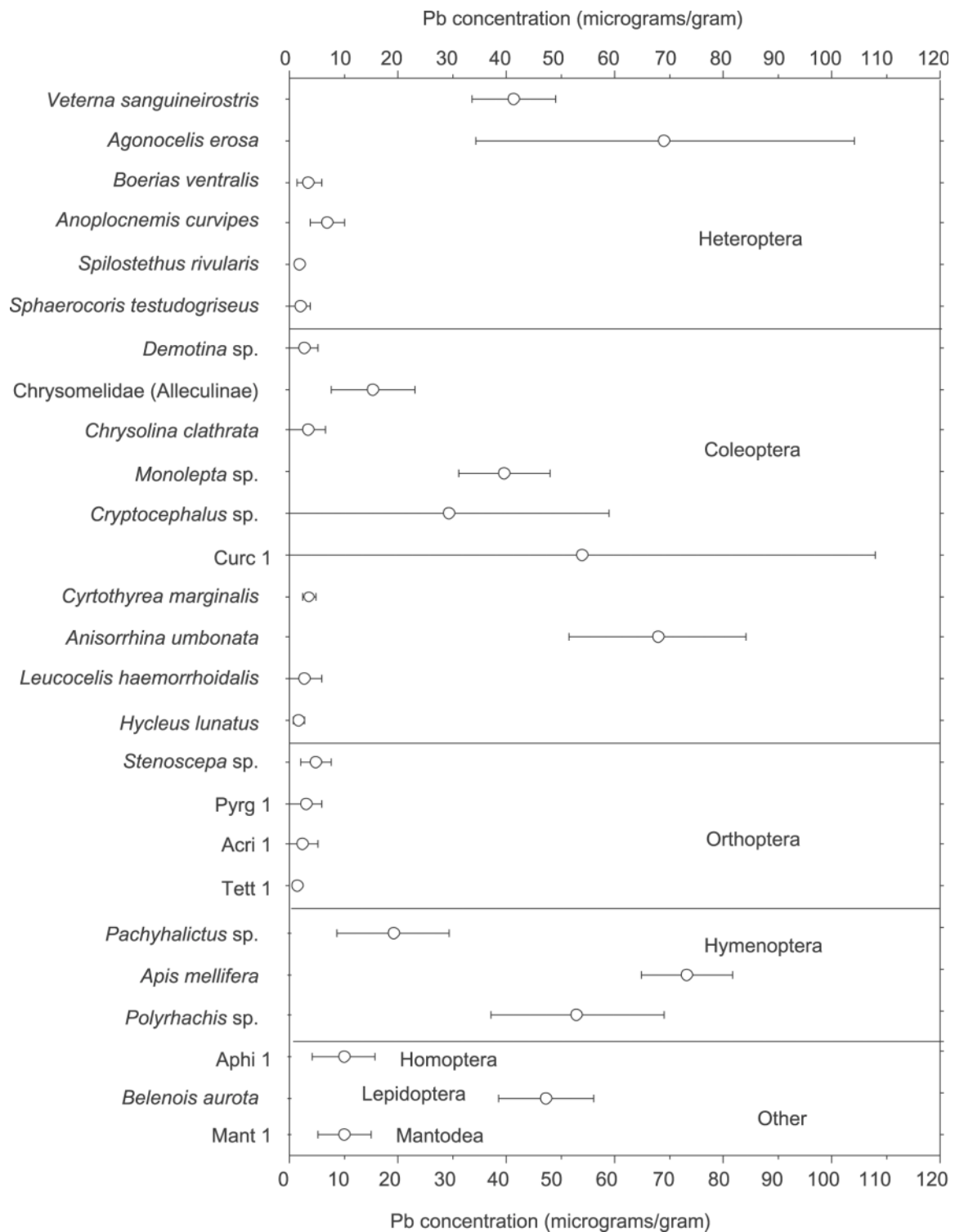


Fig. 2 Mean Pb concentrations (in $\mu\text{g/g}$ dry mass, bars showing SE) in morphotypes collected from *B. coddii*. Lack of error bars for a mean indicates that the bars are too small to be shown at the scale of the graph.

Copper

Mean concentrations of Cu varied from 15–110 $\mu\text{g/g}$ (Fig. 3). However, Cu concentrations were similar among the four reference morphotypes (ANOVA: $F_{3,5} = 0.18$, $P = 0.91$), the means of which ranged from 53–62 $\mu\text{g/g}$ (Table 3).

The ANOVA for all morphotypes showed significant variation ($F_{29,66} = 3.8$, $P < 0.0001$), and Fisher's PLSD test revealed one morphotype (Curc 1) with a mean value (110 $\mu\text{g/g}$) greater than for all four reference taxa (Fig. 3). Several other taxa with elevated Cu values were also found, so that this curculionid was not significantly different from five other taxa (*Monolepta*, the chrysomelid from the subfamily Alleculinae, *Chrysolina*, *Sphaerocoris* and *Anoplocnemis*; Fisher's PLSD test, $P > 0.05$; Fig. 3).

Zinc

Mean Zn concentrations varied from 79–1100 $\mu\text{g/g}$ (Fig. 4). The reference taxa differed in Zn concentration ($F_{3,5} = 5.5$, $P = 0.048$), with *Acacia* Curc having significantly more Zn (1100 $\mu\text{g/g}$) than the other three reference taxa, which ranged from a mean of 260 $\mu\text{g/g}$ for Ultramafic Acrid 1 to 380 $\mu\text{g/g}$ for Non-ultramafic Acrid 1 (Table 3). However, ANOVA of all morphotypes revealed only a marginally significant influence of morphotype on Zn concentration ($F_{29,66} = 1.5$, $P = 0.088$). Thus we conclude that, overall, the morphotypes did not vary significantly in concentration of this metal.

Magnesium

Mean Mg concentrations ranged greatly, from a low of 870 $\mu\text{g/g}$ to a high of 3000 $\mu\text{g/g}$. Values for Mg did not differ among the reference taxa ($F_{3,5} = 1.5$, $P = 0.32$),

ranging from a mean of 940 $\mu\text{g/g}$ for Non-ultramafic Acrid 1 to 1900 $\mu\text{g/g}$ for Ultramafic Acrid 1 (Table 3).

The ANOVA of all morphotypes revealed a significant effect of morphotype on Mg concentration ($F_{29,66} = 8.1$, $P < 0.0001$). Comparisons with the reference morphotypes identified two taxa with values greater than those for all four reference taxa (Fisher's PLSD test, $P < 0.05$): the chrysomelid from the Alleculinae, with a mean of 2900 $\mu\text{g/g}$, and *Hycleus*, with a mean of 3000 $\mu\text{g/g}$ (Fig. 5). Values for Mg of these same two taxa also were greater than for all other taxa collected from *B. coddii* (Fisher's PLSD test, $P < 0.05$).

Manganese

Values for Mn varied considerably, from 0–200 $\mu\text{g/g}$ (Fig. 6). Concentrations of Mn in the reference taxa differed significantly from each other ($F_{3,5} = 6.1$, $P = 0.040$), with *Acacia* Curc having more Mn than both Non-ultramafic Acrid 1 and Ultramafic Acrid 2, with Ultramafic Acrid 1 containing an intermediate value (Table 3).

Results of the ANOVA for all morphotypes revealed significant variation ($F_{29,66} = 9.6$, $P < 0.0001$), but no morphotype contained more Mn than all others (Fisher's PLSD test, $P > 0.05$). The heteropteran *Spilostethus* contained significantly more Mn than all four reference taxa (Fig. 6) and three other taxa collected from *B. coddii* (*Veterna*, *Boeris* and *Apis mellifera*) contained amounts of Mn that were statistically similar to *Spilostethus* (Fisher's PLSD test, $P > 0.05$).

Cobalt

Most morphotypes contained undetectable levels of Co. One of the reference taxa (Ultramafic Acrid 1) contained significantly more Co than the other three reference taxa

Table 3 Mean metal concentrations (in $\mu\text{g/g}$ dry mass, SE in parentheses) of the four insect taxa collected from other plant species to form a basis of comparison for those collected from *B. coddii*.

Metal	Non-ultramafic Acrid 1	Ultramafic Acrid 1	Ultramafic Acrid 2	<i>Acacia</i> Curc
Co	0.0 ^b (0.0)	6.9 ^a (2.1)	0.0 ^b (0.0)	0.0 ^b (0.0)
Cr	0.0 ^b (0.0)	9.3 ^a (0.3)	0.0 ^b (0.0)	0.0 ^b (0.0)
Cu	63.0 (9.7)	62.0 (19.0)	53.0 (12.0)	56.0 (3.7)
Mg	940.0 (88.0)	1900.0 (330.0)	1400.0 (190.0)	1700.0 (580.0)
Mn	18.0 ^b (2.8)	59.0 ^{a,b} (14.0)	21.0 ^b (6.7)	120.0 ^a (41.0)
Ni	3.3 (2.4)	50.0 (15.0)	53.0 (32.0)	33.0 (33.0)
Pb	3.8 ^b (3.8)	6.9 ^b (2.1)	3.3 ^b (3.3)	76.0 ^a (24.0)
Zn	380.0 ^b (140.0)	260.0 ^b (26.0)	330.0 ^b (42.0)	1100.0 ^a (350.0)

Means for a metal that differ significantly among these four taxa (Fisher's PLSD test, $P = 0.05$) are denoted by differing superscripts. Sample sizes: $n = 2$ for Non-ultramafic Acrid 1 (except for Ni value, for which $n = 3$), $n = 2$ for Ultramafic Acrid 2, $n = 3$ for Ultramafic Acrid 1, and $n = 2$ for *Acacia* Curc.

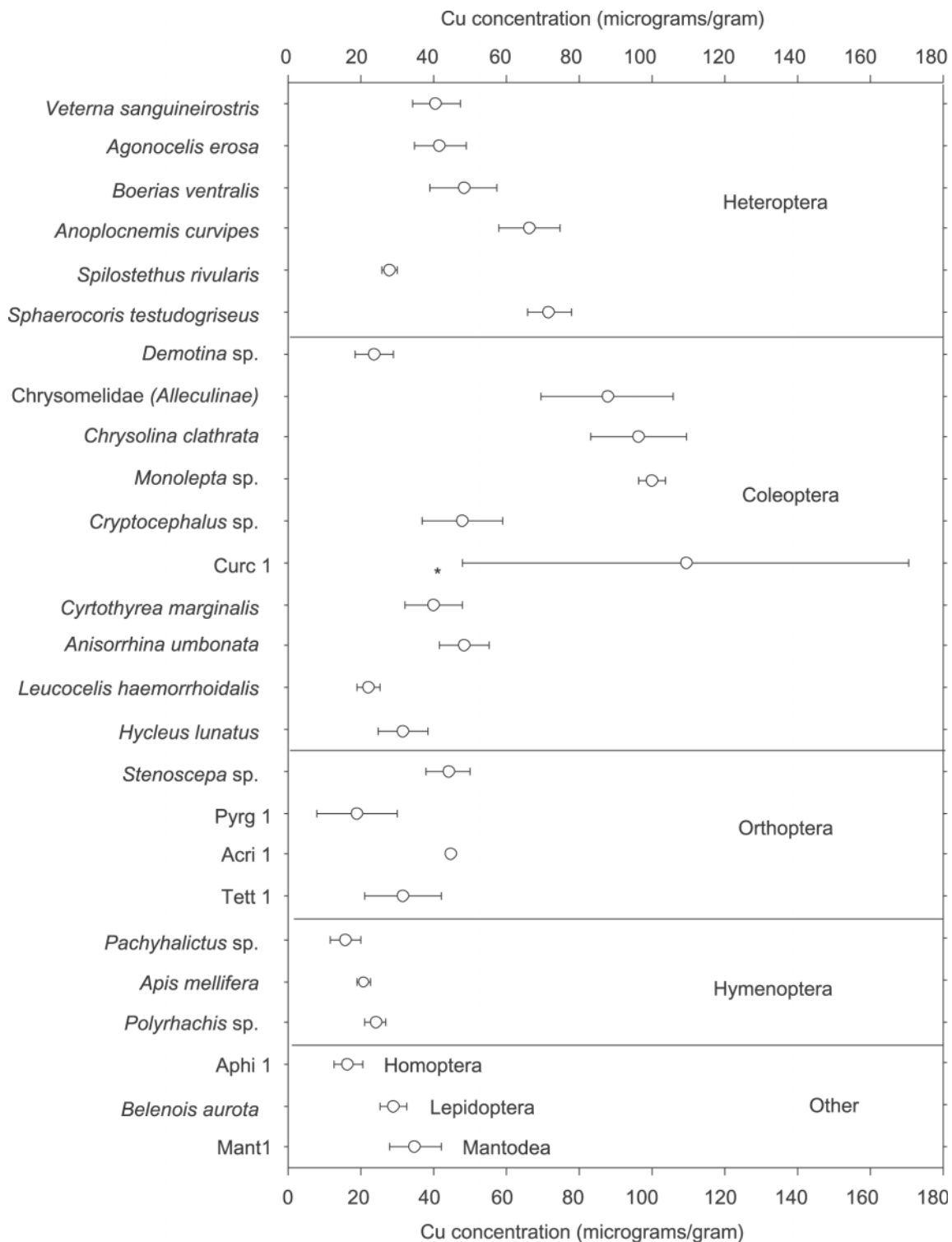


Fig. 3 Mean Cu concentrations (in $\mu\text{g/g}$ dry mass, bars showing SE) in morphotypes collected from *B. coddii*. Lack of error bars for a mean indicates that the bars are too small to be shown at the scale of the graph. A star indicates a morphotype with significantly greater Cu concentration than in all four reference morphotypes collected from non-hyperaccumulator species (means for reference morphotypes in Table 3).

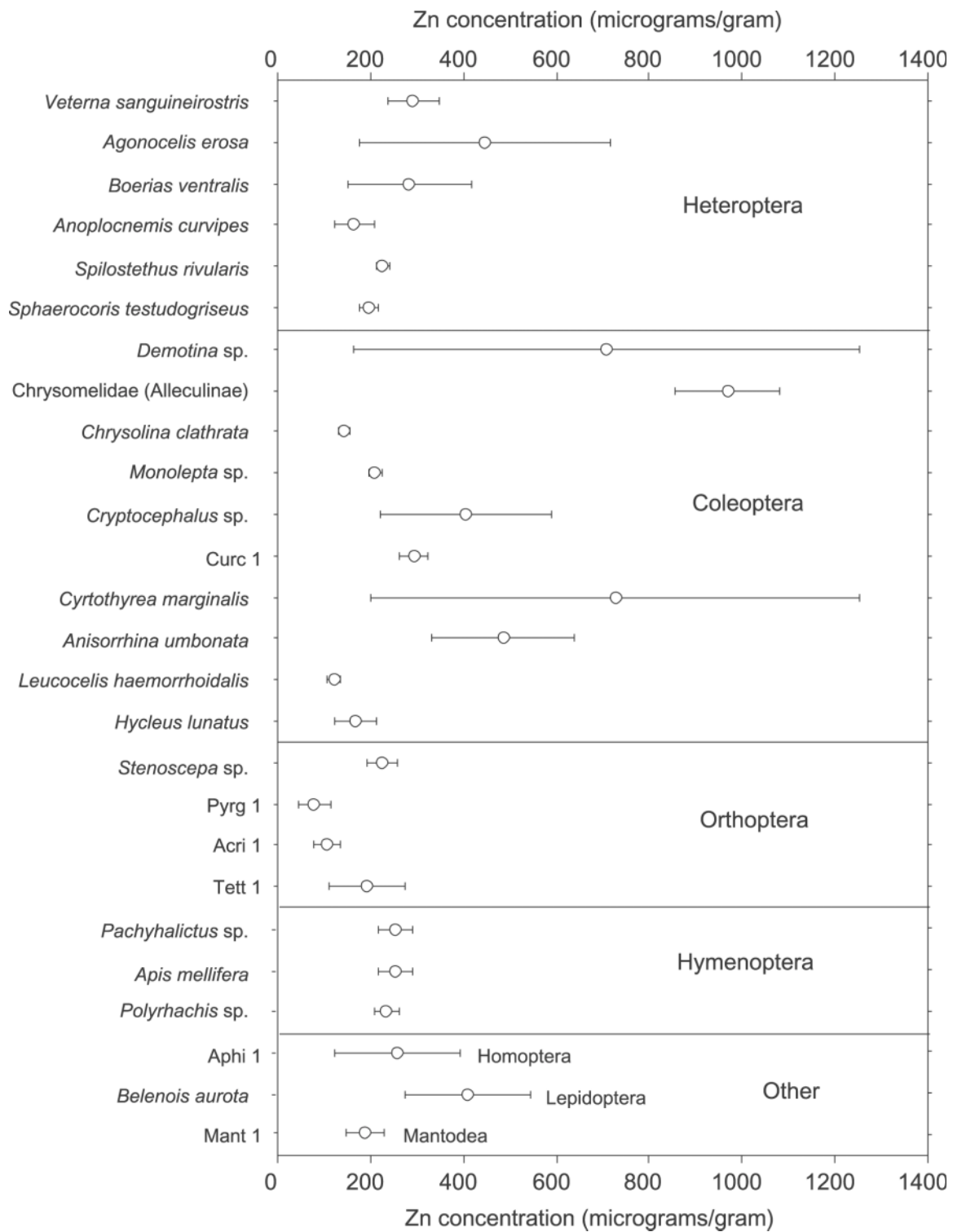


Fig. 4 Mean Zn concentrations (in $\mu\text{g/g}$ dry mass, bars showing SE) in morphotypes collected from *B. coddii*. Lack of error bars for a mean indicates that the bars are too small to be shown at the scale of the graph.

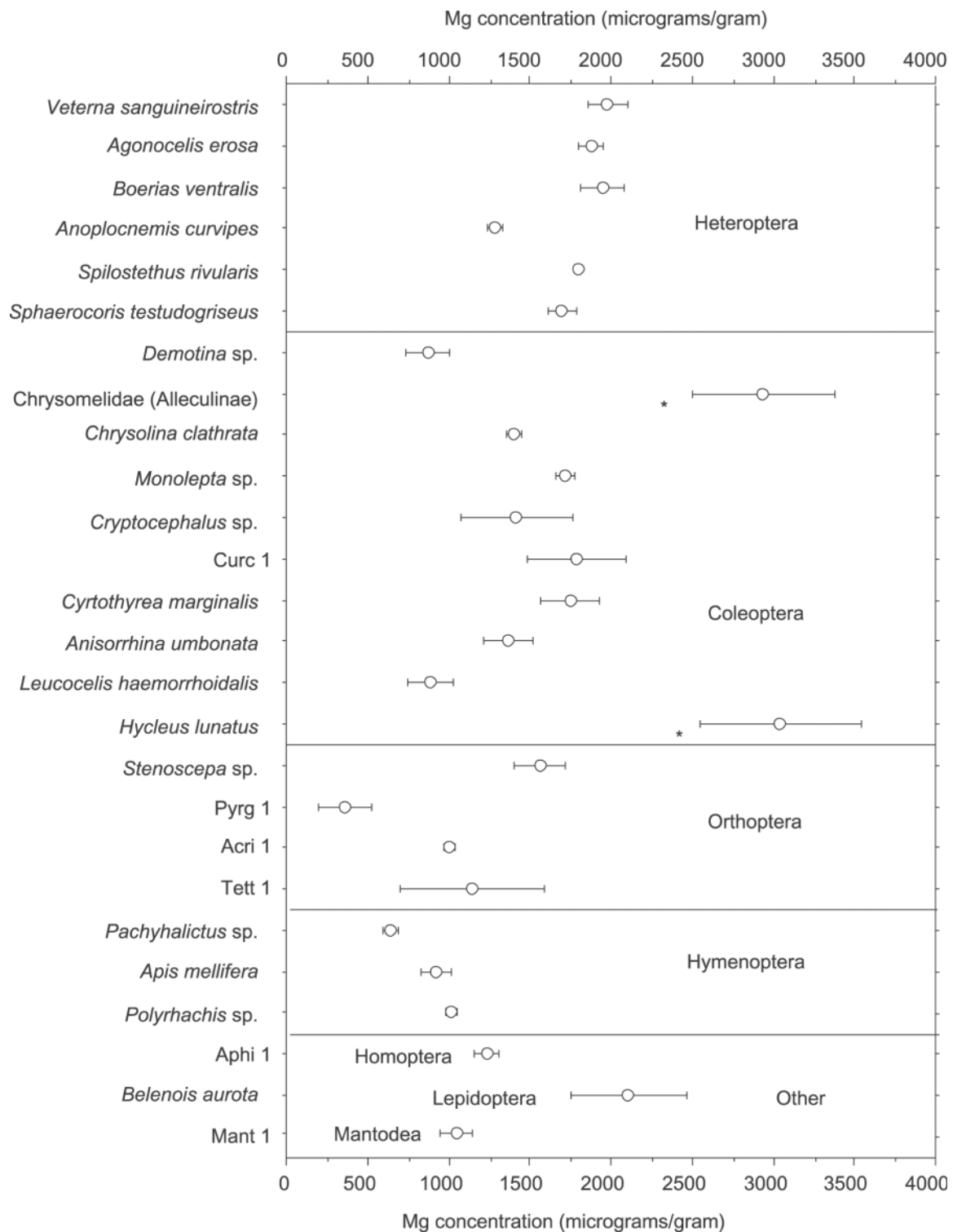


Fig. 5 Mean Mg concentrations (in $\mu\text{g/g}$ dry mass, bars showing SE) in morphotypes collected from *B. coddii*. Lack of error bars for a mean indicates that the bars are too small to be shown at the scale of the graph. Stars indicate morphotypes with significantly greater Mg concentrations than in all four reference morphotypes collected from non-hyperaccumulator species (means for reference morphotypes in Table 3).

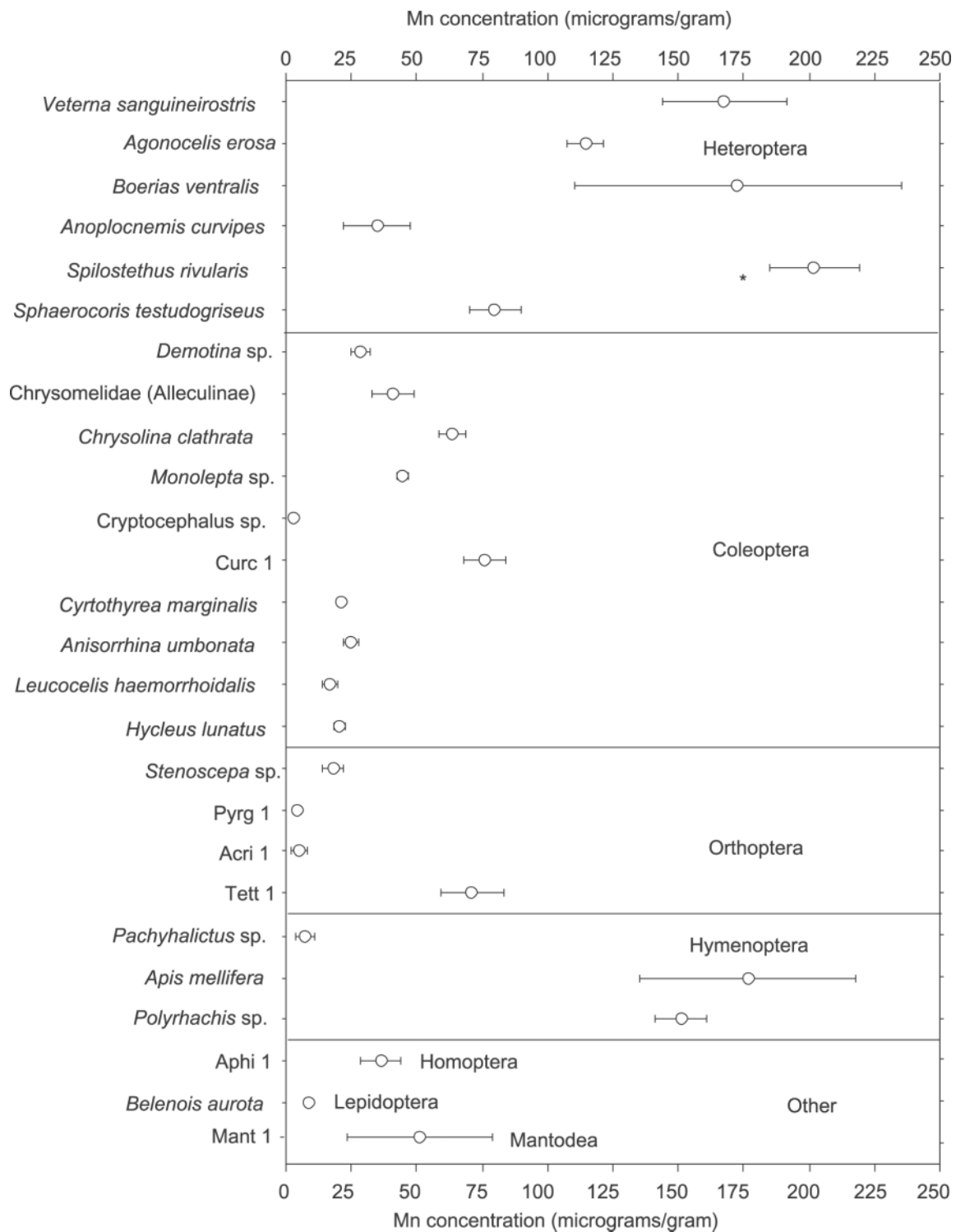


Fig. 6 Mean Mn concentrations (in µg/g dry mass, bars showing SE) in morphotypes collected from *B. coddii*. Lack of error bars for a mean indicates that the bars are too small to be shown at the scale of the graph. A star indicates a morphotype with significantly greater Mn concentration than in all four reference morphotypes collected from non-hyperaccumulator species (means for reference morphotypes in Table 3).

($F_{3,5} = 14.0$, $P = 0.007$ 0; Table 3). Only three other morphotypes contained detectable Co levels, and ANOVA of all morphotypes revealed significant variation ($F_{29,66} = 2.6$, $P = 0.000$ 7). Fisher's PLSD test showed that *Stenoscepa* (4.8 ± 2.8 $\mu\text{g/g}$; mean \pm SE), *Chrysolina* (2.6 ± 1.5 $\mu\text{g/g}$; mean \pm SE), and Ultramafic Acri 1 (6.9 ± 2.1 $\mu\text{g/g}$; mean \pm SE) contained statistically equivalent amounts of Co and more than all other taxa ($P < 0.05$). Only the ant *Polyrhachis* showed a value of Co other than 0 $\mu\text{g/g}$ (2.0 ± 2.0 $\mu\text{g/g}$; mean \pm SE), but Fisher's PLSD test showed this value did not differ significantly from 0 $\mu\text{g/g}$ ($P > 0.05$).

Chromium

As for Co, Cr was undetectable for most samples. The reference taxon Ultramafic Acri 1 contained significantly more Cr than the other three reference taxa ($F_{3,5} = 1$ 200, $P < 0.000$ 1; Table 3). Five other morphotypes contained at least some Cr, and ANOVA of all morphotypes revealed significant variation among morphotypes ($F_{29,66} = 2.4$, $P = 0.001$ 8). Two taxa, Aphi 1 and Ultramafic Acri 1, had statistically similar Cr concentrations (Aphi 1 with 9.8 ± 5.7 $\mu\text{g/g}$; Ultramafic Acri 1 with 9.3 ± 0.30 $\mu\text{g/g}$; Fisher's PLSD test, $P > 0.05$) and more than all other taxa (Fisher's PLSD test, $P < 0.05$). The remaining four morphotypes with detectable levels of Cr: *Hycleus* with 0.85 ± 0.85 $\mu\text{g/g}$ (mean \pm SE); Curc 1 with 3.8 ± 3.8 $\mu\text{g/g}$; Mant 1 with 0.83 ± 0.83 $\mu\text{g/g}$; and *Cyrtothyrea* with 1.7 ± 1.7 $\mu\text{g/g}$, did not differ significantly from 0 $\mu\text{g/g}$ (Fisher's PLSD test, $P > 0.05$).

Order-level analysis

Few differences were found when mean metal concentrations were analyzed for differences between orders (Table 4). A significant effect of order was found for Mg

and Mn levels. Levels of Mg were greatest in Heteroptera and Coleoptera, and levels of Mn were highest in Heteroptera and Hymenoptera (Table 4). Further analysis of the Ni data showed how sensitive this order-level analysis was to morphotype outliers. Despite the initial non-significant result for Ni ($F_{3,19} = 2.4$, $P = 0.10$), reanalysis of the Ni data when the values for the extremely high-Ni orthopteran *Stenoscepa* were excluded gave a highly significant result ($F_{3,18} = 7.7$, $P = 0.001$ 7). The reanalysis showed that the Heteroptera contained significantly more Ni than the other orders (Fisher's PLSD test, $P < 0.05$).

Discussion

Tissues of *B. coddii* contained elevated levels of four metals relative to the normal ranges in plant tissues (Table 1). Values for Ni were most extreme: the mean for leaves was 1 600-fold the upper boundary of the normal range of Ni in plant tissues, as reported by Reeves and Baker (2000). Concentrations of Pb were up to 6-fold that of normal (Table 1), whereas concentrations of Cr were 5-fold greater than normal values reported by Reeves and Baker (2000). Cobalt levels also were elevated, up to 4-fold the upper boundary of the normal range (Table 1). Concentrations of the other metals (Zn, Cu, Mg and Mn) all fell within the normal ranges for plant tissues (Table 1).

It is difficult to identify exceptionally elevated levels of metals in insects, as the literature is not as well characterized as that for plants. Our comparison of insect morphotypes collected from *B. coddii* against the four reference morphotypes revealed several from *B. coddii* with unusually elevated levels of some metals. The greatest number of insects contained elevated Ni levels: a total of seven heteropterans, one coleopteran, and one orthopteran contained mean whole-body Ni concentrations > 500 $\mu\text{g/g}$. We

Table 4 Mean metal concentrations (in $\mu\text{g/g}$ dry mass, SE in parentheses) of morphotypes from the four insect orders that were represented by at least three morphotypes in the dataset.

Metal	Heteroptera	Coleoptera	Orthoptera	Hymenoptera
Co	0.00 (0.00)	0.26 (0.26)	1.20 (1.20)	0.67 (0.67)
Cr	0.00 (0.00)	0.64 (0.40)	0.00 (0.00)	0.00 (0.00)
Cu	50.00 (6.80)	61.00 (11.00)	35.00 (6.00)	20.00 (2.30)
Mg	1 800.00 ^a (110.00)	1 700.00 ^a (230.00)	1 000.00 ^{a,b} (250.00)	860.00 ^b (110.00)
Mn	130.00 ^a (26.00)	34.00 ^b (7.20)	25.00 ^b (16.00)	110.00 ^a (53.00)
Ni	1 100.00 (240.00)	250.00 (96.00)	830.00 (680.00)	66.00 (17.00)
Pb	21.00 (12.00)	22.00 (7.70)	2.80 (1.00)	48.00 (16.00)
Zn	270.00 (41.00)	420.00 (93.00)	150.00 (35.00)	250.00 (6.70)

Means for a metal that differ significantly among the four orders (Fisher's PLSD test, $P = 0.05$) are denoted by differing superscripts. Numbers of morphotypes for each order were: 6 for Heteroptera, 10 for Coleoptera, 4 for Orthoptera and 3 for Hymenoptera.

also found two beetles with elevated levels of Mg, one high in Cu, and one heteropteran with elevated levels of Mn, relative to the reference taxa. No cases of exceptionally elevated concentrations of the other metals (Co, Cr, Pb or Zn) relative to reference taxa were encountered.

We also did not find any single morphotype that contained significantly more of a metal than all other morphotypes examined, except for the extremely high-Ni grasshopper *Stenoscepa* (Fig. 1). The 3 500 $\mu\text{g/g}$ in this insect was significantly greater than all other insects sampled, including the other high-Ni insects. To our knowledge, *Stenoscepa* has the greatest whole-body Ni concentration reported from any animal. Discovery of high-Ni insects is a first step toward characterizing other aspects of these species, including how they can withstand their high-Ni diets (Przybylowicz *et al.*, 2003). Whereas the ecophysiology of metals in insects from metal polluted sites has received some investigation (e.g. Hopkin, 1989), nothing is known about the ecophysiology of insects from ultramafic sites other than the pioneering studies described by Mesjasz-Przybylowicz *et al.* (2004). Our discovery of these additional high-Ni insects provides the opportunity for more ecophysiological studies.

There are many pathways by which metals can enrich the bodies of insects. These include consuming high-metal plant material (such as *B. coddii*), consuming low-metal plant material and concentrating metals from the gut contents, consuming soil enriched in metals (especially as subterranean larvae) and through dust and soil that adheres to the exoskeleton. Our work did not identify these pathways for the morphotypes we examined, but we hope our study will stimulate more detailed explorations. We also did not determine how much metal was in either insect tissues or in gut contents. It is likely that insects consuming *B. coddii* leaves (such as *Stenoscepa*) will have high whole-body Ni concentrations in part due to the high Ni level of their gut contents. Investigations of tissue-level metal concentrations of these insects, such as those performed by Mesjasz-Przybylowicz *et al.* (2004), would help resolve this question.

Our study shows both the challenges and the value of studying insect metal levels at the relatively fine taxonomic level of morphotype. The challenges include obtaining adequate biomass for analysis, high variability of data and the difficulty of obtaining insect identifications. The value is that a larger taxonomic scale can obscure the existence of exceptionally high-metal morphotypes. For example, we showed that insect orders did not significantly vary in Ni concentration but that result was primarily due to the extremely high level of Ni in *Stenoscepa*. When that morphotype was excluded from the analysis, order had a highly significant influence with the Heteroptera contain-

ing higher Ni levels than all other orders. Some earlier studies (Davison *et al.*, 1999; Peterson *et al.*, 2003) have presented results based upon analysis of samples representing composites of members of large taxa (orders or above). While this approach has value, it can fail to detect fine-scale ecologically significant variation. For example, Peterson *et al.* (2003) reported high Ni levels (1 000 $\mu\text{g Ni/g}$) for samples of "Other Invertebrates" collected from sites containing a Ni hyperaccumulator plant. It seems likely that these samples contain one or more morphotypes that contain exceptional levels of Ni, possibly because they feed on the Ni hyperaccumulator, but their approach did not allow them to identify these morphotypes.

Our results show an extraordinary concentration of high-Ni insects associated with *B. coddii*. Earlier work by Mesjasz-Przybylowicz and Przybylowicz (2001) reported five beetle species feeding on *B. coddii*. One of these species, *Chrysolina pardalina* Fabricius, contained as much as 2 650 $\mu\text{g Ni/g}$ (Mesjasz-Przybylowicz & Przybylowicz, 2001). Including that *Chrysolina* species, to date 10 high-Ni ($\geq 500 \mu\text{g/g}$) insect species are reported to be associated with this single Ni hyperaccumulator plant species. Although there are few other surveys of insect species associated with hyperaccumulators, those that exist have found fewer high-Ni taxa. For example, Boyd (2005, personal communication) reported only one high-Ni insect species from an initial survey of Ni hyperaccumulating plants at an ultramafic site in New Caledonia. Similarly, Wall and Boyd (2002) reported only one high-Ni insect from a more extensive survey of 33 arthropod species associated with the Ni hyperaccumulator *Streptanthus polygaloides* Gray (Brassicaceae) from an ultramafic site in California, USA.

Several factors may explain the large number of high-Ni insects associated with *B. coddii*. First, *B. coddii* has a relatively elevated Ni level even among Ni hyperaccumulators. The highest Ni value reported for *B. coddii*, 77 500 $\mu\text{g/g}$ (Mesjasz-Przybylowicz & Przybylowicz, 2001), is one of the greatest leaf Ni concentrations ever reported. A review by Reeves and Baker (2000) found only a few taxa with maximum Ni values exceeding 31 000 $\mu\text{g/g}$: the highest value they reported (60 170 $\mu\text{g/g}$) was from a *Phyllanthus* species in Cuba. Thus, insects feeding on *B. coddii* must deal with exceptionally large amounts of Ni. Second, *B. coddii* forms dense stands of fairly large plants, thereby representing a significant resource for herbivorous insect species and providing multiple niches. For example, among the heteropterans, *Anoplocnemis* was found on stems and leaves whereas *Spilostethus* and *Sphaerocoris* were found on inflorescences (Table 2). Similarly, within the coleopterans, the chrysomelid species *Chrysolina clathrata* was observed feeding on leaves, whereas the

meloid beetle *Hycleus lanatus* fed on inflorescences (Table 2).

One of our striking results was the relatively large number of heteropteran species with elevated body Ni concentrations. We thus add to a growing list of heteropteran species with high body Ni levels ($\geq 500 \mu\text{g/g}$) found on Ni hyperaccumulator plants. Studies of arthropods associated with the Ni hyperaccumulator *Streptanthus polygaloides* from California, USA have found two heteropterans that feed on the plant and contain elevated body Ni levels. One, *Melanotrichus boydi* Schwartz & Wall (Miridae), apparently specializes on the plant and contains $770 \mu\text{g Ni/g}$ dry mass (Schwartz & Wall, 2001). The other, *Coquillettia insignis* Uhler (Miridae), has a more broad host range but those collected from *S. polygaloides* contained $500 \mu\text{g Ni/g}$ (Boyd *et al.*, 2004). A survey in New Caledonia (Boyd, 2005, personal communication) discovered a relatively high-Ni heteropteran, *Utana viridipuncta* (Pentatomidae), with $2\,600 \mu\text{g Ni/g}$ in nymphs and $750 \mu\text{g Ni/g}$ in adults. Furthermore, surveys in Portugal of a Ni hyperaccumulator (Peterson *et al.*, 2003) documented high Ni levels in composite samples of invertebrates that included large-bodied heteropterans (Pollard AJ, 2003, personal communication). These results suggest heteropterans may be particularly important herbivores of hyperaccumulators.

There are at least two reasons why many high-Ni insects are heteropterans. First, heteropterans may be more Ni tolerant than other insect groups, although we know of no available test of this hypothesis. Second, heteropterans may be better able to feed on Ni hyperaccumulators due to their piercing-sucking mouthparts, which allow them to feed by cellular disruption. Boyd and Martens (1998) suggested that one way by which herbivores circumvent metal-based defenses is to selectively feed on low-Ni tissues. *Berkheya coddii* stores much of its leaf Ni in cuticle (Robinson *et al.*, 2003) and in epidermal cells (Mesjasz-Przybyłowicz *et al.*, 1996; Robinson *et al.*, 2003). Heteropterans may be able to avoid the greatest concentrations of Ni by penetrating beyond epidermal cells to feed on deeper (lower Ni) cells. Jhee *et al.* (2005) reported that the mirid heteropteran *Lygus lineolaris* (Palisot de Beauvois) was able to feed without apparent harm on *S. polygaloides*, perhaps using this same mechanism. On *B. coddii*, we found two high-Ni heteropteran species present in inflorescences (Table 2), where Ni concentrations were generally less than in leaves (Table 1). Feeding in this location may have allowed them access to relatively low-Ni tissues.

Insects have important roles in terrestrial food webs. Through these webs, the elevated whole-body Ni concentration of some insect species may have consequences for other organisms in ultramafic sites. For example, Boyd and Martens (1998) suggested that high-metal insect herbi-

vores might be protected against their predators by sequestered metal. An initial test of this hypothesis (Boyd & Wall, 2001) found that survival of crab spiders (*Misumena vatia* Clerck: Thomisidae) was decreased when fed insect prey that contained $800 \mu\text{g Ni/g}$. Our discovery that *Stenoscepa* grasshopper nymphs feeding upon *B. coddii* contain 4-fold that concentration suggests that further tests of the predator defense hypothesis be conducted using *Stenoscepa*, as it is likely that such a high Ni concentration will have toxic (or even repellent) effects against some predators.

A large amount of Ni in insects may cause toxicity to vertebrate predators. Outridge and Scheuhammer (1993) suggest that $> 500 \mu\text{g Ni/g}$ in the diet of mammals can cause reduced growth and survival, whereas young birds may be negatively affected by $300\text{--}800 \mu\text{g Ni/g}$. In our study the mean Ni concentrations of three heteropterans were above $1\,200 \mu\text{g/g}$, and the grasshopper *Stenoscepa* had $3\,500 \mu\text{g/g}$ (Fig. 1). If sufficient quantities were ingested, these species could be toxic to vertebrates. However, since insect-eating birds and mammals generally consume a wide variety of prey, it is uncertain if under natural conditions these vertebrates would receive a harmful Ni dose. It is also possible that high Ni insects might be distasteful to predators and thus be rejected as prey or avoided, but this hypothesis has not been tested.

Our results indicate that Ni is mobilized into some insect species associated with *B. coddii*. In addition to mobilization, it is also important to assess biomagnification of metals in food webs (van Straalen & Ernst, 1991). Our study shows that Ni is not biomagnified at the herbivore level, as Ni concentration in plant parts (Table 1) was far greater than that in insect bodies (Fig. 1). Our study also included two carnivores, the ant *Polyrhachis* and a mantid (Mant 1). Both of these carnivores contained relatively low amounts of Ni (Fig. 1), thus providing no evidence of Ni biomagnification at that trophic level. These results are in general agreement with other studies that have concluded that Ni is not biomagnified in terrestrial food webs (e.g. Outridge & Scheuhammer, 1993; Barceloux, 1999), including food webs involving Ni hyperaccumulators (Boyd & Wall, 2001; Peterson *et al.*, 2003).

Acknowledgments

We wish to thank Auburn University and the University of the Witwatersrand for providing travel funds for this research. We are grateful to Sappi Limited for access to study sites and permission to sample insects and harvest *B. coddii*. Aid with elemental analyses was graciously provided by J. Odom of Auburn University. We thank Eugene Marais, Mauro Daccordi and Chris Reid for identification

of insect specimens and two anonymous reviewers for helpful comments on the original manuscript.

References

- Abacus Concepts (1998) *StatView*. SAS Institute Inc., Cary, USA.
- Anderson, T.R., Howes, A.W., Slatter, K. and Dutton, M.F. (1997) Studies on the nickel hyperaccumulator, *Berkheya coddii*. *The Ecology of Ultramafic and Metalliferous Areas* (eds. T. Jaffré, R.D. Reeves and T. Becquer), pp. 261–266. ORSTOM, Noumea, New Caledonia.
- Anderson, C.W.N., Brooks, R.R., Chiarucci, A., LaCoste, C.J., Leblanc, M., Robinson, B.H., Simcock, R. and Stewart, R.B. (1999) Phytomining for nickel, thallium and gold. *Journal of Geochemical Exploration*, 67, 407–415.
- Augustyniak, M., Mesjasz-Przybylowicz, J., Miroslaw, N., Dybowska, M., Przybylowicz, W. and Migula, P. (2002) Food relations between *Chrysolina pardalina* and *Berkheya coddii*, a nickel hyperaccumulator from South African ultramafic outcrops. *Fresenius Environmental Bulletin*, 11, 85–90.
- Barceloux, D.G. (1999) Nickel. *Clinical Toxicology*, 37, 239–258.
- Boyd, R.S. and Davis, M.A. (2001) Metal tolerance and accumulation ability of the Ni hyperaccumulator *Streptanthus polygaloides* Gray (Brassicaceae). *International Journal of Phytoremediation*, 3, 353–367.
- Boyd, R.S. and Martens, S.N. (1998) The significance of metal hyperaccumulation for biotic interactions. *Chemoecology*, 8, 1–7.
- Boyd, R.S. and Wall, M.A. (2001) Responses of generalist predators fed high-Ni *Melanotrichus boydi* (Heteroptera: Miridae): Elemental defense against the third trophic level. *American Midland Naturalist*, 146, 186–198.
- Boyd, R.S., Wall, M.A. and Davis, M.A. (2004) The ant-mimetic plant bug, *Coquillettia insignis* (Heteroptera: Miridae) feeds on the Ni hyperaccumulator plant, *Streptanthus polygaloides* (Brassicaceae). *Ultramafic Rocks: Their Soils, Vegetation and Fauna* (eds. R.S. Boyd, A.J.M. Baker and J. Proctor), pp. 227–231. Science Reviews 2000 Ltd, St Albans, UK.
- Brooks, R.R. (1987) *Serpentine and its Vegetation: A Multi-disciplinary Approach*. Dioscorides Press, Portland, USA.
- Brooks, R.R., Lee, J., Reeves, R.D. and Jaffré, T. (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of Geochemical Exploration*, 7, 49–57.
- Brooks, R.R. (1998) Geobotany and hyperaccumulators. *Plants that Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Mineral Exploration and Phytomining* (ed. R.R. Brooks), pp. 55–94. CAB International, Wallingford, UK.
- Davison, G., Lambie, C.L., James, W.M., Skene, M.E. and Skene, K.R. (1999) Metal content in insects associated with ultramafic and non-ultramafic sites in the Scottish Highlands. *Ecological Entomology*, 24, 396–401.
- Heikens, A., Peijnenburg, W.J.G.M. and Hendricks, A.J. (2001) Bioaccumulation of heavy metals in terrestrial invertebrates. *Environmental Pollution*, 113, 385–393.
- Hopkin, S.P. (1989) *Ecophysiology of Metals in Terrestrial Invertebrates*. Elsevier, New York, USA.
- Jhee, E.M., Boyd, R.S. and Eubanks, M.D. (2005) Nickel hyperaccumulation as an elemental defense of *Streptanthus polygaloides* (Brassicaceae): influence of herbivore feeding mode. *New Phytologist*, 168, 331–344.
- Kruckeberg, A.R. (1984) *California Serpentine: Flora, Vegetation, Geology, Soils, and Management Problems*. University of California Press, Berkeley, USA.
- Mesjasz-Przybylowicz, J., Balkwill, K., Przybylowicz, W.J., Annegarn, H.J. and Rama, D.B.K. (1996) Similarity of nickel distribution in leaf tissue of two distantly related hyperaccumulating species. *The Biodiversity of African Plants* (eds. L.J.G. van der Maesen, X.M. van der Burgt and J.M. van Medenbach de Rooy), pp. 331–335. Kluwer Academic, Boston, USA.
- Mesjasz-Przybylowicz, J. and Przybylowicz, W.J. (2001) Phytophagous insects associated with the Ni-hyperaccumulating plant *Berkheya coddii* (Asteraceae) in Mpumalanga, South Africa. *South African Journal of Science*, 97, 596–598.
- Mesjasz-Przybylowicz, J., Migula, P., Nakonieczny, M., Przybylowicz, W., Augustyniak, M., Tarnawska, M. and Glowacka, E. (2004) Ecophysiology of *Chrysolina pardalina* Fabricius (Chrysomelidae), a herbivore of the South African Ni hyperaccumulator *Berkheya coddii* (Asteraceae). *Ultramafic Rocks: Their Soils, Vegetation and Fauna* (eds. R.S. Boyd, A.J.M. Baker and J. Proctor), pp. 233–241. Science Reviews 2000 Ltd, St Albans, UK.
- Morrey, D.R., Balkwill, K., Balkwill, M.-J. and Williamson, S. (1991) A review of some studies of the serpentine flora of southern Africa. *The Vegetation of Ultramafic (Serpentine) Soils*. (eds. A.J.M. Baker, J. Proctor and R.D. Reeves), pp. 147–157. Intercept, Andover, UK.
- Outridge, P.M. and Sheuhamer, A.M. (1993) Bioaccumulation and toxicology of nickel: implications for wild animals and birds. *Environmental Review*, 1, 172–197.
- Peterson, L.R., Trivett, V., Baker, A.J.M., Aguiar, C. and Pollard, A.J. (2003) Spread of metals through an invertebrate food chain as influenced by a plant that hyperaccumulates nickel. *Chemoecology*, 13, 103–108.
- Pilon-Smits, E. (2004) Phytoremediation. *Annual Review of Plant Biology*, 56, 15–39.
- Proctor, J. (1999) Toxins, nutrient shortage and droughts: the serpentine challenge. *Trends in Ecology and Evolution*, 14, 334–335.
- Proctor, J. and Whitten, K. (1971) A population of valley pocket

- gopher on a serpentine soil. *American Midland Naturalist*, 85, 517–521.
- Przybylowicz, W.J., Mesjasz-Przybylowicz, J., Migula, P., Glowacka, E., Nakonieczny, M. and Augustyniak, M. (2003) Functional analysis of metals distribution in organs of the beetle *Chrysolina pardalina* exposed to excess of nickel by Micro-PIXE. *Nuclear Instruments and Methods in Physics Research B*, 210, 343–348.
- Reeves, R.D. (2003) Tropical hyperaccumulators of metals and their potential for phytoextraction. *Plant and Soil*, 249, 57–65.
- Reeves, R.D. and Baker, A.J.M. (2000) Metal-accumulating plants. *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment* (eds. I. Raskin and B.D. Ensley), pp. 193–228. John Wiley & Sons, New York, USA.
- Robinson, B.H., Brooks, R.R., Howes, A.W., Kirkman, J.H. and Gregg, P.E.H. (1997) The potential of the high biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. *Journal of Geochemical Exploration*, 60, 115–126.
- Robinson, B.H., Lombi, E., Zhao, F.J. and McGrath, S.P. (2003) Uptake and distribution of nickel and other metals in the hyperaccumulator *Berkheya coddii*. *New Phytologist*, 158, 279–285.
- Schwartz, M.D. and Wall, M.A. (2001) *Melanotrichus boydi*, a new species of plant bug (Heteroptera: Miridae: Orthotylini) restricted to the nickel hyperaccumulator *Streptanthus polygaloides* (Brassicaceae). *Pan-Pacific Entomologist*, 77, 39–44.
- Shapiro, A.M. (1981) Egg-mimics of *Streptanthus* (Cruciferae) deter oviposition by *Pieris sisymbrii* (Lepidoptera: Pieridae). *Oecologia*, 48, 142–143.
- van Straalen, N.M. and Ernst, W.H.O. (1991) Metal biomagnification may endanger species in critical pathways. *Oikos*, 62, 255–256.
- Wall, M.A. 1999. Nickel accumulation in serpentine arthropods with emphasis on *Melanotrichus boydi* (Heteroptera: Miridae). MS thesis, Auburn University, USA.
- Wall, M.A. and Boyd, R.S. (2002) Nickel accumulation in serpentine arthropods from the Red Hills, California. *Pan-Pacific Entomologist*, 78, 168–176.
- Whiting, S.N., Reeves, R.D., Richards, D., Johnson, M.S., Cooke, J.A., Malaisse, F., Paton, A., Smith, J.A.C., Angle, J.S., Chaney, R.L., Ginocchio, R., Jaffré, T., Johns, R., McIntyre, T., Purves, G.W., Salt, D.E., Schat, H., Zhao, F.J. and Baker, A.J.M. (2004) Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation. *Restoration Ecology*, 12, 106–116.

Accepted January 11, 2006