How does pollution affect insect diversity? A study on bark beetle entomofauna of two pine forests in Greece

Maria KALAPANIDA-KANTARTZI^{1*}, Dionissis N. MILONAS², Constantin Th. BUCHELOS² and Dimitrios N. AVTZIS¹

 ¹ National Agricultural Research Foundation, Forest Research Institute, 570 06, Vassilika, Thessaloniki, Greece
² Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, 75 Iera Odos, 11855, Athens, Attica, Greece

Received: 4 February 2009

Accepted after revision: 16 July 2009

For two successive years, the seasonal occurrence of the scolytid entomofauna was investigated in two pine forests of Northern Greece that differed with regard to the degree of anthropogenic disturbances. Bark boring insects were attracted to flight barrier traps which were baited with ethanol. In total, fifteen different bark boring insect species were collected; twelve of them were common in both sites. Repeated collection in the second year yielded the same number of species. Diversity was estimated according to the Shannon-Wiener Diversity Index (H) and Equitability (J) for the two study sites. Despite the fact that both forests are artificial, the pine forest of Chrysopigi demonstrated significantly higher values of both parameters compared to the forest of Kedrinos Hill, indicating higher diversity combined with more even distribution of individuals of the species observed. Differences in the anthropogenic disturbances, which in the case of Kedrinos Hill forest, is the polluted environment of the nearby situated city of Thessaloniki, might have affected the bark beetle's entomofauna, accounting for the unequal values.

Key words: bark beetle, Scolytinae, Shannon-Weiner Diversity Index, diversity, pine forest.

INTRODUCTION

Greece is a mountainous country with rugged terrain that demonstrates a great diversity of geomorphological formations, which, in conjunction with its geographical location and climatic environment, creates favorable conditions for the growth and proliferation of forests. Pine forests cover 879,510 hectares in Greece (Skordilis & Thanos, 1997), with *Pinus halepensis, Pinus nigra* and *Pinus brutia* being the most widely distributed pine species, covering 372,000, 282,000 and 196,000 hectares respectively. Additionally, it should be mentioned that pine forests in Greece are found in Mediterranean-type climatic conditions, where most rain falls in the cold period between October and March while during the summer months July and August rainfall is very rare (Arabatzis, 1998). Taking into account the extent of pine forests in concert with the undisputable ecological as well as economical value, it can be easily deducted that pine trees constitute a component of major significance for the ecosystems in Greece.

Bark boring beetles of the subfamily of Scolytinae are among the most economically important pests of the world's forests (Rudinsky, 1962; Wood 1982), or as it has even more vividly been stated, this group represents, "one of the most formidable groups of endophytic parasites known to mankind" (Seybold *et al.*, 2006). Despite their minute size (Wood, 1982; Pfeffer 1995; Knizek & Beaver, 2004), when population density of bark beetles exceeds a specific threshold (Raffa *et al.*, 2008) they can cause extensive damage to forests (Gregoire & Evans, 2004; Wermelin-

^{*} Corresponding author: tel.: +30 2310 461172, fax: +30 2310 461341, e-mail: mkalapan@fri.gr

ger, 2004) as it has already been demonstrated in several historical reports (Schwerdtfeger, 1973; Schwenke 1974). In order to successfully overcome host tree's defenses, bark beetles have developed several strategies, such as the use of aggregation pheromones that enables them to attack trees *en masse* (Byers, 2004), or even the mutualistic association with blue stain fungi of the *Ophiostoma* family (Kirisits, 2004).

In Greece, several different species of bark beetles have been reported over the years (Kailidis, 1985; Avtzis & Avtzis, 1999; Avtzis et al., 2006), but no study until now has tried to thoroughly investigate the composition and diversity of bark beetles communities under different environmental conditions. Here we provide an in-depth investigation of the species composition and diversity of bark beetle communities in two different pine forests of Northern Greece. Despite the fact that in both forest areas, Pinus brutia is the dominant pine tree species, the management regime and the environmental conditions differ greatly. It was thus our aim to compare the diversity indices of these pine forests, in an effort to evaluate the effect of different environmental conditions on species composition and abundance.

MATERIALS AND METHODS

In the present study, the bark beetle communities of two different pine forests of Northern Greece were studied for two successive years, 2000 and 2001 (Fig. 1A). These two pine forests, namely Kedrinos Hill (N 40° 38'51'', E 22° 58'25'', altitude 150 m. above sea level) and Chrysopigi (N 41° 08'53", E 23° 33'03", altitude 300 meters above sea level), shared some similar features: they are both the outcome of reforestation (in the mid 1960's), whereas they also demonstrate a close tree species composition, with Pinus brutia Ten. being the dominant species accompanied by small numbers of Quercus coccifera L. and Cupressus sempervirens L. The distance between these two areas is more than 100 kilometers (almost 80 km), whereas several mountains (Mavrovouni, Vertiskos and Kerdilio) and the river Strymonas together with Lake Kerkini pose physical impediments in the movement of living organisms.

In order to study the diversity of bark beetle communities, in each area, four (4) Theyson ® traps (Fig. 1B) placed in a square pattern (with a side of 100 meters) and hung on pine trees about 1.30 meters above ground, were used. These traps were baited with cotton soaked with 75% pure ethanol (EtOH), since it is widely accepted that ethanol is the prime component of host volatiles that attracts bark beetles on host trees (Klimetzek *et al.*, 1986; Shroeder, 1988; Markalas & Kalapanida, 1997; Byers, 2004).

The study period began on the 15th of March and lasted up to the 30th of November for both years. The observation and collection of individuals captured in the traps was done every 10 days, and the samples were taken to the laboratory of the Forest Research Institute where they were identified according to the taxonomic keys of Schwenke (1974), Gruenne (1977) and Pfeffer (1995).

For the estimation of diversity the index of Shannon-Weiner H' (Shannon 1948, Zar 1999), was used:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where **s**: the number of species (also mentioned as species richness), and

 \mathbf{p}_i : the relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community.

Furthermore, Equitability or Evenness J was calculated as the ratio of H' to H_{max} , with H_{max} being the theoretical maximum that maximizes diversity,

$$H_{max} = -\sum_{i=1}^{S} \frac{1}{S} \ln \frac{1}{S} = \ln S$$

RESULTS

Number and relative abundance of each species

In Kedrinos Hill *Pinus brutia* forest a total number of 3,276 and 8,079 individuals were captured in 2000 and 2001 respectively (Table 1). These individuals belong to 14 different species of Scolytinae, and as it is obvious from Table 2, two species were the most abundant ones, namely *Orthotomicus erosus* and *Hylurgus ligniperda* that accounted for 41.81% and 31.11% of the total number of individuals captured during the two year survey on Kedrinos Hill. The species, *Hylurgus micklitzi* was the third most frequently caught species with a relative abundance of 13.80% (Table 2).

The number of individuals captured in the traps of Chrysopigi pine forest was lower, with 2,598 and 2,791 individuals in 2000 and 2001 respectively (Table 1). Interestingly, 11 out of the 13 different species found in the traps of Chrysopigi forest were identical to the species collected in Kedrinos Hill forest (84.6% identical species); only *Hylastes cunicularius* and *Try*-





FIG. 1. A. Map showing the two study areas. B. Theyson® traps used for the attraction and collection of bark beetles.

Insects species	Kedrinos Hill		Chrysopigi		
	2000	2001	2000	2001	
Tomicus piniperda Linnaeus	82	781	242	284	
Carphoborus marani Pfeffer	169	27	169	236	
Crypturgus cinereus Herbst	3	0	213	664	
Crypturgus hispidulus Thomson	_*	_*	158	143	
Hylastes ater Paykull	5	3	134	115	
Hylastes augustatus Herbst	132	63	123	83	
Hylastes cunicularius Erichson	2	0	_*	_*	
Hylurgus ligniperda Fabricius	1,174	3,574	491	459	
Hylurgus micklitzi Wachtl	162	1,405	251	267	
Orthotomicus erosus Wollaston	1,396	2,136	506	386	
Pityogenes calcaratus Eichhoff	2	33	36	8	
Pityogenes trepanatus Nördlinger	9	8	50	11	
Pityophthorus micrographus Linnaeus	42	1	131	76	
Trypodedron lineatum Olivier	76	40	*	_*	
Xyleborus eurygraphus Ratzeburg	22	8	94	59	
Total	3,276	8,079	2,598	2,791	

TABLE 1. Cumulative number of species and individuals per species trapped in two pine forests of Northern Greece during two successive years

* Not recorded

TABLE 2. Shannon-Wiener Diversity Index (H) and Equitability (J) for the cumulative number of Scolytinae species and	in-
dividuals per species collected from Kedrinos Hill forest during 2000 and 2001 (p.: relative abundance of each species)	

	Species	2000	2001	Total	p _i	p _i *ln (p _i)
1	Tomicus piniperda Linnaeus	82	781	863	0.0760	0.1959
2	Carphoborus marani Pfeffer	169	27	196	0.0173	0.0701
3	Crypturgus cinereus Herbst	3	0	3	0.0003	0.0022
4	Hylastes ater Paykull	5	3	8	0.0007	0.0051
5	Hylastes augustatus Herbst	132	63	195	0.0172	0.0698
6	Hylastes cunicularius Erichson	2	0	2	0.0002	0.0015
7	Hylurgus ligniperda Fabricius	1,174	3,574	4,748	0.4181	0.3646
8	Hylurgus micklitzi Wachtl	162	1,405	1,567	0.1380	0.2733
9	Orthotomicus erosus Wollaston	1,396	2,136	3,532	0.3111	0.3632
10	Pityogenes calcaratus Eichhoff	2	33	35	0.0031	0.0178
11	Pityogenes trepanatus Nördlinger	9	8	17	0.0015	0.0097
12	Pityophthorus micrographus Linnaeus	42	1	43	0.0038	0.0211
13	Trypodedron lineatum Olivier	76	40	116	0.0102	0.0468
14	Xyleborus eurygraphus Ratzeburg	22	8	30	0.0026	0.0157
	Total	3,276	8,079	11,355		
	H I					1.4569 0.5520

		÷			• <i>′</i>		
	Species	2000	2001	Total	p _i	p _i *ln (p _i)	
1	Tomicus piniperda Linnaeus	242	284	526	0.0976	0.2271	
2	Carphoborus marani Pfeffer	169	236	405	0.0752	0.1945	
3	Crypturgus cinereus Herbst	213	664	877	0.1627	0.2955	
4	Crypturgus hispidulus Thomson	158	143	301	0.0559	0.1611	
5	Hylastes ater Paykull	134	115	249	0.0462	0.1421	
6	Hylastes augustatus Herbst	123	83	206	0.0382	0.1248	
7	Hylurgus ligniperda Fabricius	491	459	950	0.1763	0.3060	
8	Hylurgus micklitzi Wachtl	251	267	518	0.0961	0.2251	
9	Orthotomicus erosus Wollaston	506	386	892	0.1655	0.2977	
10	Pityogenes calcaratus Eichhoff	36	8	44	0.0082	0.0393	
11	Pityogenes trepanatus Nördlinger	50	11	61	0.0113	0.0507	
12	Pityophthorus micrographus Linnaeus	131	76	207	0.0384	0.1252	
13	Xyleborus eurygraphus Ratzeburg	94	59	153	0.0284	0.1011	
	Total	2,598	2,791	5,389			
	H J					2.2902 0.8929	

TABLE 3. Shannon-Wiener Diversity Index (H) and Equitability (J) for the cumulative number of Scolytinae species and individuals per species collected from Chrysopigi forest during 2000 and 2001 (p_i : relative abundance of each species)

podendron lineatum were absent from the bark beetle complex of this forest. The same species, as in Kedrinos Hill forest, namely *O. erosus* and *H. ligniperda* were the most frequent ones in both years, with a relative frequency of 17.63% and 16.55% respectively. However, this time, the third most abundant species was *Crypturgus cinereus* with 16.23% (Table 3).

Shannon-Weiner Diversity Index (H') and Equitability (J)

The Shannon-Weiner Diversity Index was calculated for each pine forest using the total number of indivi-

duals and species collected during the two-year period of the study (Tables 2, 3). This approach permitted elimination of annual variations that could bias the holistic overview of biodiversity and additionally yielded comparable results with similar studies in which the same approach was adopted (Coyle *et al.*, 2005).

Despite the fact that, the number of bark beetle species (14) as well as the total number of individuals (11,355) captured in Kedrinos Hill forest was higher in comparison with the number of species (13) and the total number of individuals (5,389) that were found in Chrysopigi forest, the Shannon-Weiner Diversity In-



FIG. 2. Bar diagrams of Shannon-Wiener Diversity Index (H) (A) and Equitability (J) (B) for the two pine forests, from the cumulative data of the traps in the two-year study period (2000 and 2001).

dex was higher for the *Pinus brutia* forest of Chrysopigi (H' = 2.2902) compared to the value calculated for the *Pinus brutia* forest of Kedrinos Hill (H' = 1.4569). The Equitability or Evenness parameter (J) demonstrated a similar pattern, with the Kedrinos Hill forest achieving a lower value (J=0.5520) compared to Chrysopigi forest (J=0.8929) (Fig. 2).

DISCUSSION

Despite the fact that both pine forests studied were mainly composed of Pinus brutia planted during 1950-1960, our analyses showed that the two forests differ regarding biodiversity of bark beetles community. The Shannon-Weiner Diversity Index (H') in concert with the Equitability Index (J) showed that the pine forest of Chrysopigi, despite the lower number of trapped individuals, demonstrated higher biodiversity (H' = 2.2902), and at the same time much more even distribution of individuals among species (J = 0.8929). On the other hand, in spite of the fact that in Kedrinos Hill forest a slightly greater number of species was observed and that the cumulative number of trapped individuals during the study period was almost twofold compared to that of Chrysopigi forest, diversity was much lower, whereas individuals were less evenly distributed among species.

The uneven distribution of individuals in the observed species of Kedrinos Hill forest becomes more evident, taking into account the fact that more than 85% of the number of individuals captured during the study period belong to the three most dominant species, namely O. erosus, H. ligniperda and H. micklitzi. The remaining eleven species observed, accounted for less than 15% of the total number of individuals caught, something that explains the low values of the indices calculated. On the other hand, the three most frequently trapped species in Chrysopigi forest, O. erosus, H. ligniperda and C. cinereus accounted for no more than 50% of the cumulative number of individuals caught in all traps during the study period, something that allowed the remaining ten species observed to contribute more evenly to biodiversity.

Biodiversity and species richness have often been considered to be sensitive indicators of anthropogenic, environment disturbances (Freedman, 1989). One of the most severe and radical disturbances, pollution, has commonly been associated with decreases in diversity of various organisms (Winner & Bewley, 1978; Newman *et al.*, 1992). Therefore, environmental degradation and pollution are often assessed with the use of diversity indices (Rapport *et al.*, 1985; Magurran, 1988). On the other hand, insect communities have long been considered to be good indicators of changes in ecosystems, since they are not only closely associated to the plants (Mattson, 1980; Rosenthal & Berenbaum, 1992; Mopper & Simberloff, 1995; Jefferies & Maron, 1997) but also play a crucial role in the operation of the ecosystem (Jefferies & Maron, 1997; Ritchie *et al.*, 1998; Reynolds & Hunter, 2001).

Taking into account previous works on biodiversity of insect communities (Oliver & Mannion, 2001; Coyle et al., 2005), in concert with the effects that environmental pollution can have (Kozlov & Zvereva, 1997; Eatouhhg Jones & Paine, 2006), it can be hypothesized that the differences of the Shannon-Weiner Diversity (H') and Equitability Index (J) between the two Pinus brutia forests of Northern Greece, are likely attributable to environmental degradation. Kedrinos Hill forest, is the sub-urban forest of Thessaloniki which, according to measurements of pollution, "is classified in the medium air pollution class (with stabilization temporal trend) based on NO2 concentrations, in the high air pollution class (with decreasing temporal trend) based on particle values and in the low air pollution class (with decreasing temporal trend) based on SO₂ levels" (Nikolaou, 2003). It can therefore be postulated that air pollution of the urban area of Thessaloniki might have affected the diversity of bark beetle communities in Kedrinos Hill contributing to the low values of Shannon-Weiner Diversity (H') and Equitability Index (J). On the other hand, Chrysopigi forest, being distant from any major source of pollution, such as overcrowded and/or industrialized cities, exhibits significantly higher values of both indices calculated. The stability of the ecosystem of Chrysopigi forest is additionally demonstrated by the almost even distribution of individuals in the species observed (J = 0.8929) compared to the less even (J = 0.5520) distribution of Kedrinos Hill.

The results presented in this study, demonstrate a difference between bark beetle diversity indices in two pine forests, despite the fact that the dominant tree species in both cases is the same (*Pinus brutia*). This difference of diversity indices was attributed to the aerial pollution of the city of Thessaloniki that lies near the Kedrinos Hill forest. However, further studies are required in order to clarify the extent as well as the exact procedure through which pollution affects the biodiversity of bark beetle communities.

ACKNOWLEDGEMENTS

We would like to thank Dr Ioannis Meliadis for providing us with the map presented in our study.

REFERENCES

- Arabatzis TI, 1998. Trees and shrubs in Greece. Volume 1. Ecological Movement of Drama – Technological Educational Institute of Drama. Drama.
- Avtzis DN, Avtzis ND, 1999. Control of the most dangerous insects of Greek Forests and Plantations. In: Proceedings-Integrated Management and Dynamics of Forest Defoliating Insects. Victoria-Canada. USDA, General Technical Report NE-277: 1-5.
- Avtzis DN, Arthofer W, Wegensteiner R, Avtzis ND, Stauffer C, 2005. The occurence of *Pityogenes chalcographus* L. (Coleoptera: Scolytidae). In: *Proceedings of the XIIth Panhellenic Forestry Conference*. Drama, Greece.
- Byers JA, 2004. Chemical ecology of bark beetles in a complex olfactory landscape. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans H, eds. *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer Academic Publishers, Dordrecht, The Netherlands: 89-134.
- Coyle DR, Booth DC, Wallace MS, 2005. Ambrosia beetle (Coleoptera:Scolytidae) species flight, and attack on living eastern cottonwood trees. *Journal of economic entomology*, 98: 2049-2057.
- Eatough Jones M, Paine TD, 2006. Detecting changes in insect herbivore communities along a pollution gradient. *Environmental pollution*, 143: 377-387.
- Freedman B, 1989. Envronmental Ecology. The impact of pollution and other stresses on ecosystem structure and function. Academic Press, New York.
- Gregoire JC, Evans HF, 2004. Damage and control of BAWBILT organisms, an overview. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans H, eds. Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis. Kluwer Academic Publishers, Dordrecht, The Netherlands: 19-37.
- Gruenne S, 1977. Handbuch zur Bestimmung der europaeischen Borkenkaefer. Verlag M. & H. Hannover.
- Jefferies RL, Maron JL, 1997. The embarrassment of riches: atmospheric deposition of nitrogen and community and ecosystems processes. *Trends in ecology and evolution*, 12: 74-78.
- Kailidis DS, 1985. Dry periods and secondary dying and bark beetle epidemics in forests of Greece. Aristotle University of Thessaloniki. Greece: 1-16. (in greek)
- Kirisits T, 2004. Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans H, eds. Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis. Kluwer Academic

Publishers, Dordrecht, The Netherlands: 185-223.

- Klimetzek D, Köhler J, Vité JP, Kohnle U, 1986. Dosage response to ethanol mediates host selection by 'secondary' bark beetles. *Naturwissenschaften*, 73: 270-272.
- Knizek M, Beaver R, 2004. Taxonomy and systematics of bark and ambrosia beetles. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans H, eds. Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis. Kluwer Academic Publishers, Dordrecht, The Netherlands: 41-54.
- Kozlov MV, Zvereva EL, 1997. Effects of pollution and urbanization on diversity of frit flies (Diptera: Chloropidae). *Acta oecologica*, 18: 13-20.
- Magurran AE, 1988. *Ecological Diversity and its Measurement*. Chapman and Hall, London.
- Markalas S, Kalapanida M, 1997. Flight pattern of some Scolytidae attracted to flight barrier traps baited with ethanol in an oak forest in Greece. *Anzeiger schädlingskde, pflanzenschutz umweltschutz*, 70: 55-57.
- Mattson WJ, 1980. Herbivory in relation to plant nitrogen content. *Annual review of ecology and systematics*, 11: 119-161.
- Mopper S, Simberloff D, 1995. Differential herbivory in an oak population: the role of plant phenology and insect performance. *Ecology*, 76: 1233-1241.
- Newman JR, Schreiber RK, Novakova E, 1992. Air pollution effects on terrestrial and aquatic animals. In: Barker JR, Tingey DT eds. *Air pollution effects on biodiversity*. Van Nostrand Reinhold. New York: 177-233.
- Nikolaou K, 2003. Air quality in European urban areas and the new EC directives. *Journal of environmental protection and ecology*, 4: 477-482.
- Oliver JB, Mannion CM, 2001. Ambrosia beetle (Coleoptera: Scolytidae) species attacking chestnut and captured in ethanol-baited traps in middle Tennessee. *Environmental entomology*, 30: 909-918.
- Pfeffer A, 1995. Zentral- und westpalaearktische Borken- und Kenkäfer. Pro Entomologia, c/o Naturhistorisches Museum Basel. Basel.
- Raffa K, Aukema BH, Bentz BJ, Carroll AL, Hicke JA, Turner MG, Romme WH, 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: The dynamics of biome-wide bark beetle eruptions. *BioScience*, 58: 501-517.
- Rapport DJ, Regier HA, Hutchinson TC, 1985. Ecosystem behavior under stress. *American naturalist*, 125: 617-640.
- Reynolds BC, Hunter MD, 2001. Responses of soil respiration, soil nutrients, and litter decomposition to inputs from canopy herbivores. *Soil biology and biochemistry*, 33: 1641-1652.
- Ritchie ME, Tilman D, Knops JMH, 1998. Herbivore effects on plant and nitrogen dynamics in oak savanna. *Eco*-

logy, 79: 165-177.

- Rosenthal GA, Berenbaum MR, 1992. *Herbivores: Their Interactions with Secondary Plant Metabolites*. Academic Press, San Diego California.
- Rudinsky JA, 1962. Ecology of Scolytidae. *Annual review of* entomology, 7: 327–348.
- Schröder LM, 1988. Attraction of the bark beetle *Tomicus piniperda* and some other bark- and wood-living beetles to the host volatiles a-pinene and ethanol. *Entomologia experimentalis et applicata*, 46: 203-210.
- Schwenke W, 1974. *Die Forstschädlinge Europas. Zweiter Band.* Verlag Paul Parey, Hamburg und Berlin.
- Schwerdtfeger F, 1973. Forest entomology. In: Smith RF, Mittler TE, Smith CN, eds. *History of Entomology*. Annual Reviews Inc. Palo Alto: 361-386.
- Seybold SJ, Huber DPW, Lee JC, Graves AD, Bohlmann J, 2006. Pine monoterpenes and pine bark beetles: A marriage of convenience for defense and chemical communication. *Phytochemistry Reviews*, 5: 143-178.
- Shannon CE, 1948. A mathematical theory of communication. *Bell systems technical journal*, 27: 379-423, 623-

656.

- Skordilis A, Thanos CA, 1997. Comparative Ecophysiology of seed germination strategies in the seven pine species naturally growing in Greece. In: Eliss RH, Black M, Mordoch AJ, Hong TD, eds. *Basic and applied aspects of seed biology*. Kluwer Academic Press, Dordrecht, The Netherlands: 623-632.
- Wermelinger B, 2004. Ecology and management of the spruce bark beetles *Ips typographus* a review of recent research. *Forest ecology and management*, 202: 67-82.
- Winner VE, Bewley JB, 1978. Contrasts between bryophyte and vascular plant synecological responses in an SO₂stressed white spruce association in central Alberta. *Oecologia*, 33: 311-325.
- Wood SL, 1982. The Bark and Ambrosia Beetles of North and Central America (Coleoptera: Scolytidae): a taxonomic monograph. *Great Basin Naturalist Memoirs* 6: 1-1359.
- Zar JH, 1999. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, NJ.