Indirect biomass estimations in Collembola

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SUMMARY

We propose coefficients for regressions relating dry weight to body or tergite length in Folsomia candida, Entomobrya schoetti, Sminthurus viridis, and Hypogastrura vernalis (Collembola). Measurements were made on large batches of preserved, identified specimens. Batches were dessicated completely by critical-point drying and weighed. We compare our data with other published models and critically review the literature, finding questionable records.

KEYWORDS

Biomass estimation; Collembola; Length-weight regressions

INTRODUCTION

It has been long established that the estimation of standing crop is fundamental in studies of population and community ecology and production biology (Petersen, 1975). Many ecological measures, such as soil respiration, net production and litter turnover rates, are to be explained in terms of soil fauna activity including Collembola, which in turn requires precise measurements of its biomass (Dunger, 1968; Persson and Lohm, 1977; Petersen, 1994, 1995, 2000; Detsis, 2000).

Recently, there is increasing interest on the use of biomass when estimating biodiversity (Jordana et al., 2000), as opposed to (or complementary of) the widespread use of specimen numbers. Size differences between individuals or species may bias the use of diversity as an assessment tool for the "status" of an ecosystem. Small individuals, of correspondingly small effect on the ecosystem, are assigned an unrealistically large role by equaling them with large ones, thus violating the basic tenet of "equal differences" for the concept of diversity (Krebs, 1999). Biomass can be regarded as a homogenizer for diversity measures, once these are corrected for continuous variables.

For small soil animals, direct measurement of the biomass of samples is often difficult due to their minute size, large numbers, and triage procedures. Thus, research has been published attempting to relate easier, more reliable measurements, such as length, to biomass. Allometry has been used to relate, by least-squares regression, the biomass to a lower dimensional measure, typically body length or width, through a power function or otherwise. However, many of these papers (i.e. Huhta and Koskenniemi, 1975; Schatz, 1981; Ganihar, 1997) deal with complex groups, such as "Collembola'', which has a wide range of sizes and shapes. The goal of homogenizing counts through biomass for biodiversity studies requires good biomass estimates on a species-by-species basis.

According to Petersen (1975), the first paper dealing with specific biomass estimates for Collem-bola was that of Tanaka (1970) for some Japanese springtails, for which the coefficients for a log–log regression between body length and weight were given. Previously, Edwards (1967) had published equations based on cubic regressions for families of Collembola. Petersen's (1975) work is widely cited, containing also log–log equations similar to Tanaka's. Later, Van Straalen (1989) published parabolic curves for two species of Tomoceridae, and Teuben and Smidt (1992) added some more log–log curves.

Other information may exist as tables or unpublished data. Fjellberg (1975) referred to unpublished data on body size/weight relationship for Collembola populations on Hardangervida. Vannier (1973, 1977b, c) published individual series of data for some large Entomobridae.

The aim of this research was to add new species or populations to the set of groups of Collembola for which adequate regressions relating weight to body length are known, and to investigate whether these equations yield consistent results. We measured different lengths on individual Collembola, and performed regressions against their dry weight after grouping the animals by similar sizes. After studying the behaviour of the models, we chose one particular length for each of four species to be used as estimator for the dry weight of the individual.

MATERIALS AND METHODS

Sampling of Collembola

Four species were selected as representative of their groups. Folsomia candida was reared in the laboratory in large numbers. A very large sample of a population of Hypogastrura vernalis was collected from the surface of a swimming pool in Labiano (Navarra) in 2000. Entomobrya schoetti and Sminthurus viridis were collected in large numbers from an alfalfa (lucerne) field in Navarra where they had become a pest, in 1997.

Preparation and measurements

The animals were preserved in 70% ethanol. The length of a straight line between the anal papillae and the front margin of the first thoracic segment (in all species but E. schoetti); the length of the fourth abdominal segment (in E. schoetti); the total length excluding antennae (in S. viridis); and the length of the straight line between the posterior margin of the head and the base of the antennae, or the end of the labrum in S. viridis, were measured, placing the specimens on their side under a stereomicroscope, by a pre-tabulated eyepiece micrometer. Animals were grouped by size classes so as to obtain up to 10 different, homogeneous classes, which were then subject, batch by batch, to dessication by critical point drying with liquid carbon dioxide. Shape and size were thus subsequently fully retained. In order to avoid rehydratation, dried specimens were kept at all times in dessicators with silicagel. Size batches, each containing 30–300 specimens of one species, were weighed repeatedly with a one-microgram microbalance (10 µm scale for E. schoetti).

Data analysis

The ratio between head length and body length, or between head length and body length excluding head, was established by least-squares regressions. Results of this analysis were used to determine which length to use for the regressions between length and weight. We chose the measure having the largest intrinsic variation (i.e. largest coefficient of variation) to separate between classes. Precision factors for the measurements were obtained by dividing the unit length by the range for each measurement.

The regressions were calculated assuming a power function of the type $W = \alpha L^b$, where L is a linear length (mm) and W the weight (micrograms). We used the average length of the body excluding the head (or the abdominal segment IV in E. schoetti), measured as described above, plotted against the last weighing before rehydratation would take place for the batch. Although other, slightly better fitting functions (mostly polynomial) were tested through the use of mathematical software (Tablecurve v. 2.12), for the sake of homogeneity and comparability we chose to stick to this most commonly used allometric function.

In order to find agreement between our data and published coefficients, we plotted our functions together with the published functions. In case they described the relationship

between length and fresh weight, the function was corrected using published fresh weight to dry weight ratios for the corresponding family. However, in many cases comparisons were not possible, as the lengths used for the regressions were measured differently among studies and there could not be set a homogeneous “unit length” for all species. Where this was possible, a “unit type” (i.e. the weight of an animal measuring one unit in length) was also used for comparison among species and groups. A quick quality check was performed on the coefficients to discard obvious errors by calculating the minimal density of a unit sphere according to the regression coefficients. No correction was included for “swelling factor’’, as they have been found to be poorly documented.

RESULTS

A total of 4061 specimens were measured. The achieved precision for the length measurements was 16 µm for the measurements in H. vernalis (precision 1.1% for body length) and E. schoetti (precision 3.1%), and 24µm for S. viridis (precision 1.3%) and F. candida (precision 1.7% for length of abdominal IV).

We chose the measure that maximized the measure range for the whole group (i.e. that of maximal CV) in order to set up as many classes as feasible for the weighing (Table 1). Therefore, we used the length of the body without head except for E. schoetti, where we used the abdominal segment IV, for subsequent analyses.

All species fit well within a power function, with different coefficients for each species (Fig. 1). All our data fit within the published biomass ranges for their corresponding groups of Collembola. However, taking into account that for a given length we must obtain a weight slightly higher than that obtained from regressions where total length was used, it is surprising that our curves fall just about or slightly below the corresponding published weights (Fig. 1). This affects both the regression curve and the actual data. Comparisons of E. schoetti with published data were not possible since we were unable to find regression data of head length vs. weight for Entomobrya.

DISCUSSION

Our results are in range with published data and regressions (Fig. 1 and Table 2), with some exceptions. This is somewhat surprising, since we were not using total lengths but a fraction (i.e. body without head, or just the length of abdominal segment IV for E. schoetti) whereas other authors used total length, or length without head for Entomobryidae (where we measured just one segment). The calculated dry weight per unit length was expected to be higher for our animals, accounting for the weight of the remaining part of the body. As indicated in Table 2, the drying methods varied among the studies, and no two studies used the same drying procedure before weighing; Edwards (1967) and Van Straalen (1989) reported their equations for fresh weight although a correction factor is given in Table 2 for comparison. It should be noted, however, that the water content in Collembola is highly variable and depends on the degree of starvation, ambient conditions, transportation conditions and catching procedures (Vannier, 1976, 1977a; Thibaud and Vannier, 1978; Vannier and Verhoef, 1978; Petersen and Luxton, 1982).

Differences in body weight estimations from linear measurements, all things being equal, should be related solely with the material density or the aspect factor (i.e. the degree to which the shape differs from a regular, equal shape). Thus, more slender bodies should correlate to smaller volumes for unit length, and more globular ones to greater volumes (Fig. 2). Shapes for a given species should be similar; therefore, only density is left to account for differences. Our drying procedure used (critical point drying by phase change of liquid CO_2, a procedure commonly used in electron microscopy to ensure complete removal of water without any volume or shape loss), together with our use of preserved specimens, has rendered dry materials which are somewhat "drier", or lighter, than that obtained by other drying procedures such as freeze drying drying in an oven. Vannier (1976) and Thibaud and Vannier (1978) have reported fat contents of Collembola tissue and, although they were comparatively low, they may affect dry weight measurements. Teuben and Smidt (1992) reported as much as 15% loss of dry matter after storage in preservative fluids for 3 weeks.

Other discrepancies may stem from typographic errors on published data. Some of the coefficients published in Teuben and Smidt (1992) are mistaken. If true, a 1 mm long Hypogastrura would have a dry weight of 5.5×10^6 µg: its lowest possible specific gravity, were it perfectly spherical, would be over one thousand, that is, almost fifty times heavier than the heaviest element (iridium). Likewise, the coefficients for Isotoma notabilis and Orchesella cincta in this paper cannot be used as they are. It seems also that Teuben and Smidt did indeed obtain correct estimates for the coefficients, as results in the same paper based on these coefficients appear to be reasonable and do not reflect these errors; however, a number of papers may have used the published equations for biomass calculations (e.g. Detsis, 2000). We have not found a correction for these estimates, although it is likely that it might exist in the literature. As regards to the parabolic equation in Van Straalen (1989), the third term should be read "$a_2(\log Y)^2$" (Van Straalen, pers. comm.)

The range of differences in weight per unit length of Collembola found in the reported literature (see Fig. 2 for Arthropleona) suggest that the estimation of biomass from length measurements varies with the procedure used for the calculation of the regression curves. Thus, we conclude that precise figures for the dry weight of Collembola are yet to be published, much as they are needed for respiration or biodiversity studies. Our data, based on a large number of measurements, added figures for a few common species; more such studies are necessary for a more complete analysis of the structure and function of Collembola communities.

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Table 1. Mean lengths and range of lengths (in µm) of head, body, abdominal segment IV or body without head of the specimens

Species	N	Measurement	Mean	Range	CV	Precision (%)	Classes
H. vernalis	635	Head	131.7	82–197	0.188	14.3	8
		Body excl. head	797.1	393–1229	0.231	2.0	
F. candida	750	Head	140.9	73–340	0.459	9.1	6
		Body excl. head	685.6	242–1673	0.563	1.7	
E. schoetti	741	Head	193.0	82–377	0.384	5.6	10
		Abdominal IV	303.7	98–623	0.481	3.1	
S. viridis	1935	Head	493.5	244–1292	0.460	2.3	8
		Body excl. head	700.6	268–2195	0.593	1.3	
		Body total	836.5	341–2731	0.578	1.0	

N=number of specimens; CV=coefficient of variation. “Classes” is the number of size classes into which specimens were grouped for weighing.

Table 2. Published equations and coefficients for the relationship between body length and body weight

	Code	A	B		C		D			E		F	
	Author	Edwards (1967)	Tanaka (1970)		Petersen (1975)		Van Straalen (1989)			Teuben and Smidt (1992)		Caballero et al. (this paper)	
	Formula	$W=(bL)^3$	$W=aL^b$		$W=aL^b$		$\log W=a+b\log L+c(\log L)^2$			$W=a(20L)^b$		$W=aL^b$	
	Drying mode	FW	OD 80 °C 2 h		VO 60 °C 100 mm Hg 24 h		FW			FD > 16 h		CPD CO_2	
	Coefficients	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*c*	*a*	*b*	*a*	*b*
1.	*Hypogastrura* cf. *manubrialis*		9.772	2.550									
2.	*Hypogastrura* spp.									1.000[a]	4.412[a]		
3.	*Hypogastrura vernalis*											*4.217*	*3.087*
4.	Hypogastruridae	2.810											
5.	*Neanura* sp.		10.715	2.240									
6.	Onychiuridae	2.220								0.645	0.546		
7.	*Onychiurus armatus* adults				5.598	2.769							
8.	*Onychiurus armatus* juveniles				5.572	2.532							
9.	*Onychiurus furcifer* adults				6.152	3.126							
10.	*Onychiurus furcifer* juveniles				6.792	2.725							
11.	*Onychiurus* spp.		4.266	2.750									
12.	*Tullbergia krausbaueri*				3.192	2.502							
13.	*Folsomia candida*		6.457	2.990								*6.371*	*2.565*
14.	*Folsomia hasegawai*		5.129	2.550									
15.	*Folsomia quadrioculata* adults				5.623	2.799							
16.	*Folsomia quadrioculata* juveniles				4.592	2.439							
17.	*Isotoma notabilis* adults				8.472	3.223				1.000[a]	3.417[a]		
18.	*Isotoma notabilis* juveniles				4.732	2.183							
19.	*Isotoma trispinata*		5.623	3.280									
20.	Isotomidae	3.060											
21.	*Isotomiella minor* adults				6.252	2.881							
22.	*Isotomiella minor* juveniles				4.592	2.439							
23.	*Entomobrya schoetti*[b]											*115.540*	*2.419*
24.	*Entomobryidae*	2.460	*11.749*	*2.520*									
25.	*Lepidocyrtus cyaneus*									0.025	2.060		
26.	*Lepidocyrtus lanuginosus*				*21.777*	*2.960*				0.026	2.088		
27.	*Lepidocyrtus lignorum*				*14.256*	*2.708*							
28.	*Orchesella cincta*						1.358	3.242	−0.624	1.000[a]	2.705[a]		
29.	*Tomocerus flavescens*				9.204	2.744							
30.	*Tomocerus minor*						1.532	3.219	−0.546	0.009	2.435		
31.	*Dicyrtoma minuta* adults				*39.628*	*3.796*							
32.	*Dicyrtoma minuta* juveniles				*31.696*	*2.280*							
33.	Sminthuridae	3.800											
34.	*Sminthurus aureus*				*190.546*	*3.627*							
35.	*Sminturus viridis*											*66.428*	*3.048*

Author and taxon codes refer to Fig. 3. FW: fresh weight; OD: oven dried; VO: vacuum-oven dried; FD: freeze dried; CPD: critical point drying. Figures in *italics* apply to length of body excluding head.

[a]Unrealistic data.

[b]Length of Abd. IV only.

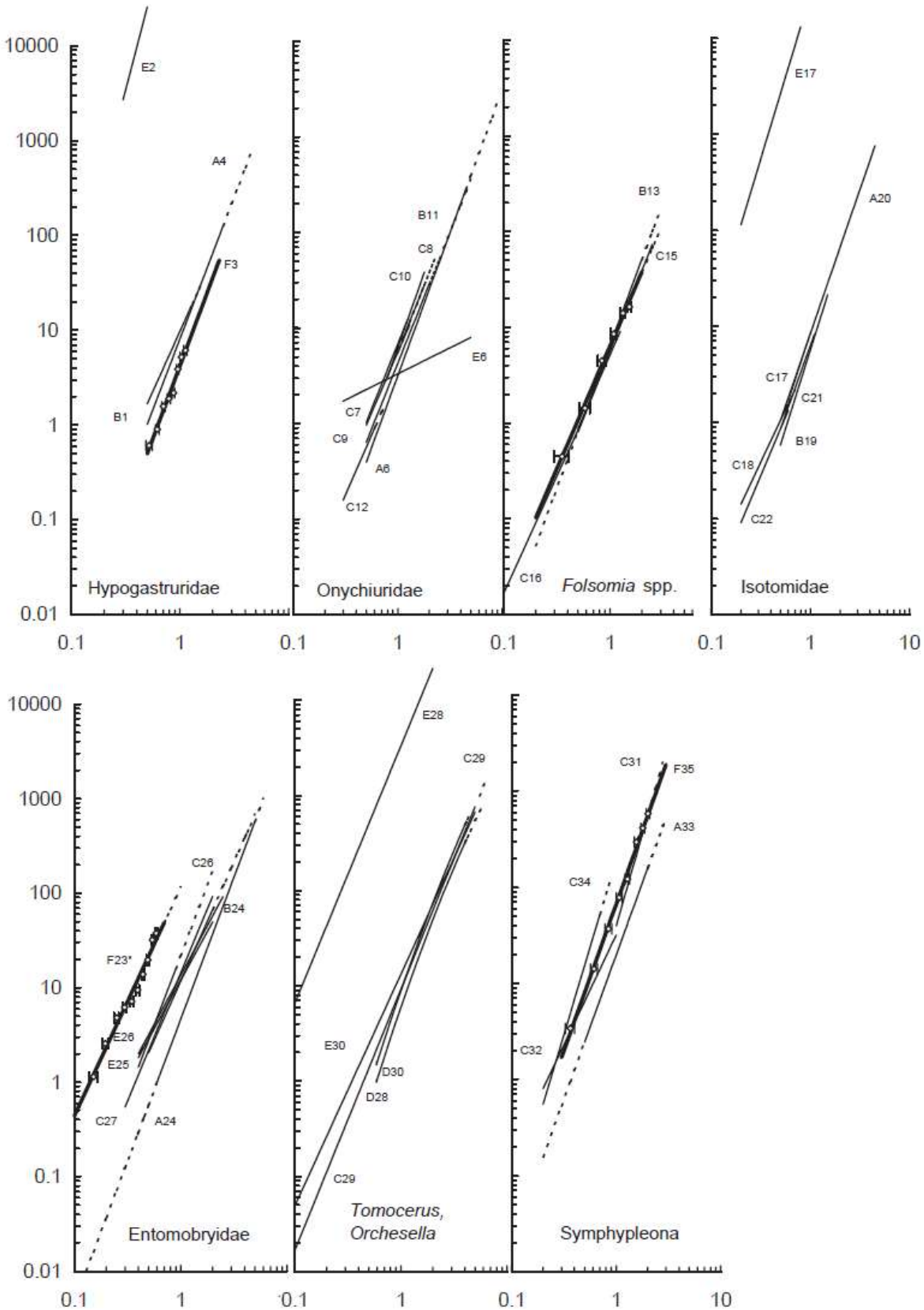

Figure 1. Dry weight-length regression lines from literature (thin) and this work (thick, with error bars). Solid lines indicate the range for which the regression was defined; dotted lines indicate the size range for the taxon. Codes indicate author (A–F, from Edwards, 1972 to Caballero et al.) and taxon (number) as seen in Table 2. F23 (E. schoetti, this work) based on length of abdominal segment IV only.

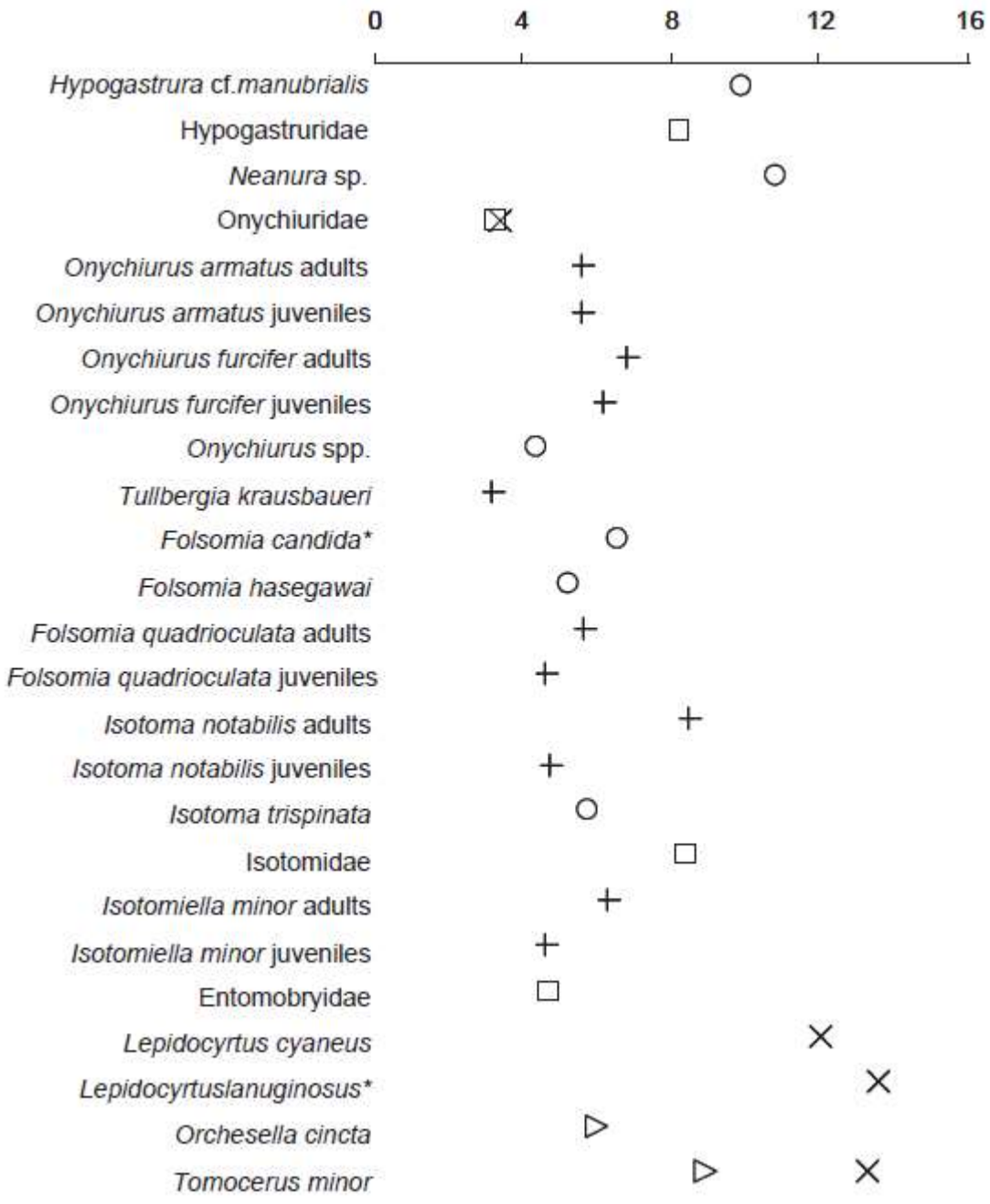

Figure 2. Weight (in micrograms) of specimens of Arthropleona measuring 1 mm (total body length) calculated according to Edwards (1967, squares); Tanaka (1970, circles); Petersen (1975, crosses); Van Straalen (1989, triangles); Teuben and Smidt (1992, X symbol).