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Observations on the geographical distribution and morphometrics of *Enoploides stewarti* NICHOLAS, 1993 (Nematoda; Thoracostomopsidae)

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Abstract

Two populations of *Enoploides stewarti* NICHOLAS, 1993 have recently been found in freshwater, one in Lake Alexandrina and the other a new population in streams in Macquarie Island in the sub-antarctic. In the latter population the adults are distinctly larger, but otherwise morphologically indistinguishable. The statistical relationship between body length, indicative of size, and the growth of taxonomically significant characters, has been studied in adults and juveniles of both populations. The lengths of pharynx, tail, spicules, labial and cephalic setae, mandible and onchium, head width, and the position of the vulva and male supplement, were measured. It is concluded that the two populations belong to the same species with a very discontinuous distribution. The value of DE MAN's indices is discussed.
Key-words: *Enoploides,* Nematoda, taxonomy, morphometrics, Macquarie Is.

Introduction

Enoploides stewarti NICHOLAS, 1993, from Lake Alexandrina in South Australia, is one of only two species described from freshwater in an otherwise marine genus with over 40 species. Recently another population of *Enoploides* with larger adults has been found in freshwater streams in sub-antarctic Macquarie Island (MARCHANT & LILLYWHITE, 1994). The two populations differ in their measurements, but are otherwise indistinguishable morphologically. The other freshwater species, *E. fluviatilis* MICOLETZKY, 1923 from the Volga River, can be distinguished by several morphological characters (NICHOLAS, 1993). Taxonomic descriptions of new species of nematodes invariably include measurements with the implied or explicit inference that these measurements are of significance in distinguishing between closely related species. The Macquarie Island and Lake Alexandrina populations differ in measurements of characters taxonomically important in Thoracostomopsidae, to which *Enoploides* belongs. The question is, are the different measurements entirely explicable by differences in size? With this question in mind, the mathematical relationships between such measurements and body size have been investigated in adults and juveniles of both populations, taking body length as the most general indicator of size.

Allometric growth has been studied in other nematodes, most thoroughly by GERAERT (1968, 1978, 1979a, b, 1983) with reference to terrestrial Tylenchida, Dorylaimida and Rhabditida, but not in Enoplina, which is primarily marine and includes the Thoracostomopsidae. DE MAN's ratios, originally introduced to taxonomic descriptions to compensate for differences in size, are mathematically unsound when used for this purpose (GERAERT, 1968, and ROGGEN & ASSELBERG, 1971). However, FORTUNER (1990) has cogently advocated the alternative use of DE MAN's ratios in taxonomic descriptions, as indicators of shape or position. Fortuner recommends re-naming DE MAN's ratios DE MAN's indices to avoid misinterpretation of their significance. We have also investigated the relationship between these indices and growth in length in both populations, referring to the ratios as DE MAN's indices.

Materials and methods

The specimens from Lake Alexandrina were collected in sandy cores at the waters edge of Lake Alexandrina and fixed in 5% formalin as described by NICHOLAS, BIRD & STEWART (1992). Specimens from Macquarie Island were collected by kick sampling, as described by MARCHANT & LILLYWHITE (1994) and fixed in 4% formalin. They came from Stoney, Lusitania, Finch, Jessie Nicol, Sawyer and Nuggets Creeks and Red River. In the laboratory, specimens were transferred to 5% aqueous glycerol and slowly dehydrated to absolute glycerol. Specimens were then mounted on slides in anhydrous glycerol on microscope slides with cover slips supported by glass beads (Ballatini), selected under the microscope to match the width of the worms, and the cover slips ringed with Glyceel (Gurr).

Measurements were taken from *camera lucida* drawings. The structures measured are shown in Fig. 1. All measurements are reported in μm, those of adults from Macquarie Island and juveniles from both Macquarie Island and Lake Alexandrina are new, but those of adults from Lake Alexandrina have previously been published in Nicholas (1993). DE MAN's indices are: **a** = body length divided by greatest body width, **b** = length divided by length of pharynx, **c** = length divided by tail length,

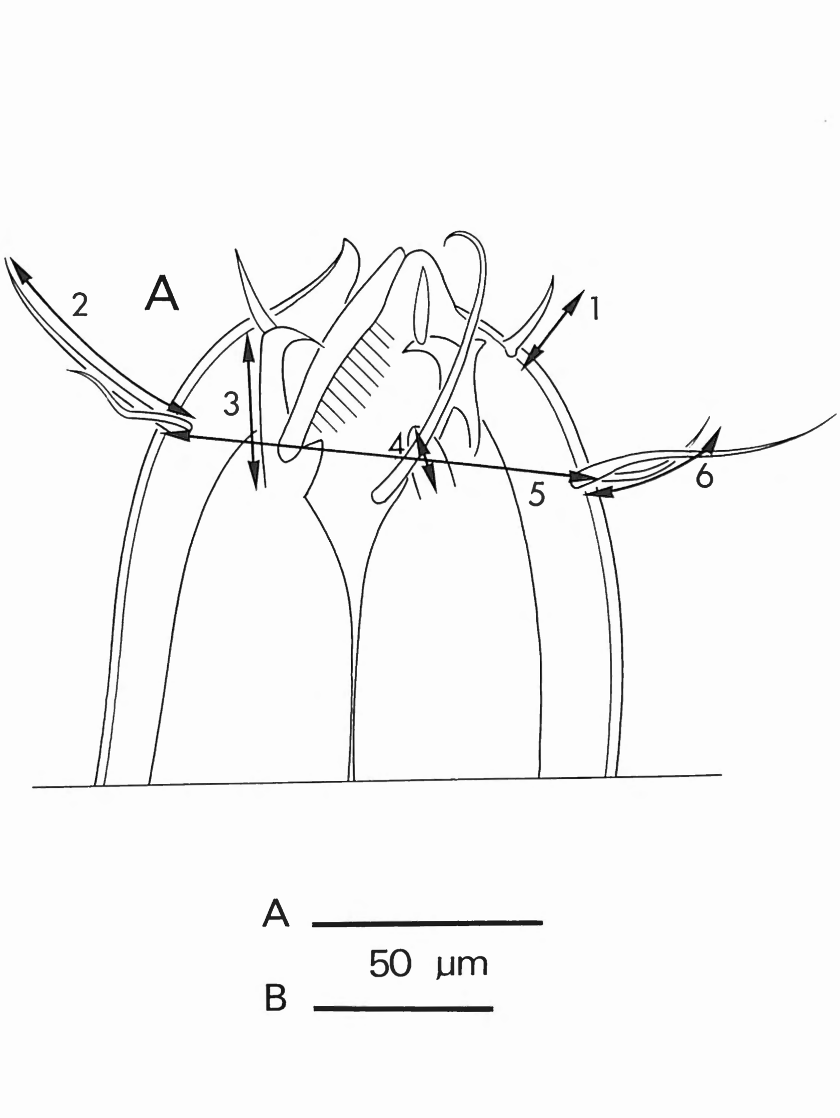


Fig. 1. – Distances measured on *cameria lucida* drawings of the pharynx (A) and anal (B) regions of *Enoploides stewarti.* 1. Inner labial seta; 2. Outer labial seta; 3. Mandible height; 4. Onchium length; 5. Head width; 6. Cephalic seta; 7. Anus to Supplement distance; 8. Spicule arc length; 9. Width at Anus; 10. Tail length.

c' = tail length divided by width at anus. For statistical analysis, the measurements were regressed against body length and the residuals calculated using the Macintosh program StatView SE+Graphics. Significant ($p<0.05$) regression equations are shown in the figures. Multivariate analysis of the residuals was carried out using the Macintosh program SuperAnova.

Results

The measurements upon which the mathematical analyses are based are set out in Table 1. Females are larger than males at both localities, and adults from Macquarie Island are larger than adults from Lake Alexandrina. When taxonomically interesting measurements of Macquarie Island adults are compared, there is significant sexual dimorphism in body length, pharyngeal length, and tail length, but not in the langths of inner labial, outer labial, and cephalic setae, head width, mandible height, or onchium length (Table 2). For DE MAN's indices, only the index **c'** is significantly different (Table 2). The significance of sexual dimorphism in Lake Alexandrina was not tested because there were only three adults of each sex.

The statistical significance of differences in the same characters and indices between the adults of both sexes from the two localities has also been compared (Table 3). Measurements of all the characters listed were significantly different, except for the inner labial setae, but of DE MAN's indices, only **c'** was significantly different. The arc length of the spicules, and distance from anus to supplementary organ differed significantly in males from the two localities but the position of the vulva (DE MAN's V%) in females did not (Table 4). However,

Table 1.
Mean and standard errors of measurements of males, females and juveniles of *Enoploides stewarti* from Macquarie Island and Lake Alexandrina, South Australia. The data from the longest and shortest individuals are included in the columns Max and Min

	Body Length				Spicule Arc Length			
Macquarie Island	Mean	S.E.	Max	Min	Mean	S.E.	Max	Min
Males (n=8)	5280	146	6063	4679	143	2.8	168	133
Females (n=9)	7009	383	8388	6433				
Juveniles (n=10)	4023	252	5110	2426				
L. Alexandrina								
Males (n=3)	2375	357	3080	1930	112	4.5	121	107
Females (n=3)	2612	71	2750	2510				
Juveniles (n=11)	1794	116	2638	1350				

	Pharynx Length				Tail Length			
Macquarie Island	Mean	S.E.	Max	Min	Mean	S.E.	Max	Min
Males (n=8)	936	38	1061	908	138	1.8	148	131
Females (n=9)	1136	58	1401	1108	180	5.4	187	173
Juveniles (n=10)	806	36	945	648	141	6.1	152	110
L. Alexandrina								
Males (n=3)	527	53	632	491	105	14.4	134	93
Females (n=3)	591	15	621	572	139	3.2	145	134
Juveniles (n=11)	448	19	579	389	123	3.6	127	116

	Head Width				Width at Anus			
Macquarie Island	Mean	S.E.	Max	Min	Mean	S.E.	Max	Min
Males (n=8)	59.1	1.5	66	54	66.5	1.7	73	59
Females (n=9)	69.0	4.4	8.5	47	76.1	2.6	92	64
Juveniles (n=10)	48.3	2.9	73	38	56.1	3.0	70	42
L. Alexandrina								
Males (n=3)	35.0	0.0	35	35	48.0	3.1	52	42
Females (n=3)	45.0	2.1	49	42	50.7	1.9	53	47
Juveniles (n=11)	25.9	1.4	33	22	39.2	1.1	43	34

	Inner Labial Setae				Outer Labial Setae			
Macquarie Island	Mean	S.E.	Max	Min	Mean	S.E.	Max	Min
Males (n=8)	12.8	0.8	17	10	42.9	1.6	50	38
Females (n=9)	13.6	1.1	20	11	46.0	2.0	58	34
Juveniles (n=10)	10.2	0.3	12	8	34.0	1.7	36	26
L. Alexandrina								
Males (n=3)	11.3	2.0	15	8	29.3	1.2	31	27
Females (n=3)	10.3	0.9	12	10	34.7	2.3	37	30
Juveniles (n=11)	9.4	0.3	10	8	26.1	0.9	28	26

	Cephalic Setae				Mandible Height			
Macquarie Island	Mean	S.E.	Max	Min	Mean	S.E.	Max	Min
Males (n=8)	23.5	1.5	21	18	14.3	1.0	18	10
Females (n=9)	26.3	1.7	31	22	17.3	0.8	20	13
Juveniles (n=10)	18	1.5	31	13	12.6	0.8	16	7
L. Alexandrina								
Males (n=3)	15.3	0.9	17	14	10.3	0.9	10	9
Females (n=3)	18.3	0.7	19	17	13.7	0.3	14	13
Juveniles (n=11)	13.8	0.6	17	12	9.4	0.3	10	8

Table 1 Cont.	Anus to Supplement Distance				V %			
Macquarie Island	Mean	S.E.	Max	Min	Mean	S.E.	Max	Min
Males (n=8)	153	6.5	169	140				
Females (n=9)					55.2	1.0	59.4	50.2
Juveniles (n=10)								
L. Alexandrina								
Males (n=3)	98.7	11.7	118	78				
Females (n=3)					56.0	0.1	56.1	56.0
Juveniles (n=11)								

	Onchium Length				DE MAN's Index **a**			
Macquarie Island	Mean	S.E.	Max	Min	Mean	S.E.	Max	Min
Males (n=8)	9.9	0.5	12	8	60.1	2.4	71	56
Females (n=9)	11.4	0.8	15	12	60.0	6.2	89	36
Juveniles (n=10)	8.1	0.6	8	7	52.8	3.5	55	36
L. Alexandrina								
Males (n=3)	7.0	0.0	7	7	37.4	6.2	49	28
Females (n=3)	6.3	0.3	7	6	26.0	2.1	30	23
Juveniles (n=11)	6.6	0.2	8	6	28.9	1.1	33	26

	DE MAN's Index **b**				DE MAN's Index **c**			
Macquarie Island	Mean	S.E.	Max	Min	Mean	S.E.	Max	Min
Males (n=8)	5.7	0.2	6.8	5.2	38.4	1.3	44.3	33.4
Females (n=9)	6.2	0.2	7.8	4.7	40.2	1.7	44.9	37.2
Juveniles (n=10)	5.0	0.1	5.4	3.7	28.4	1.5	33.6	22.1
L. Alexandrina								
Males (n=3)	4.5	0.3	4.9	3.9	22.5	0.9	23.0	21.0
Females (n=3)	4.4	0.2	4.8	4.3	19.0	1.2	21.0	17.0
Juveniles (n=11)	4.0	0.1	4.6	3.5	14.6	0.8	20.8	11.6

	DE MAN's Index **c'**			
Macquarie Island	Mean	S.E.	Max	Min
Males (n=8)	2.1	0.5	2.2	1.8
Females (n=9)	2.5	0.1	3.1	2.0
Juveniles (n=10)	2.6	0.1	3.1	2.4
L. Alexandrina				
Males (n=3)	2.2	0.3	2.7	1.8
Females (n=3)	2.8	0.1	3.0	2.5
Juveniles (n=11)	3.2	0.1	3.9	2.8

Table 2.
The significance of differences in measurements and indices between males and females from Macquarie Island; unpaired two-tailed t-tests with 15 degrees of freedom

Length	t	p	Index	t	p
Body	−6.901	0.0001	DE MAN's Index **a**	0.046	0.963
Pharynx	−3.318	0.0047	DE MAN's Index **b**	−1.452	0.167
Tail	−7.002	0.0001	DE MAN's Index **c**	−0.286	0.779
Inner labial setae	−0.587	0.566	DE MAN's Index **c'**	−2.708	0.0162
Outer labial setae	−0.937	0.364			
Cephalic setae	−1.997	0.064			
Head width	−2.006	0.063			
Onchium	−1.012	0.3267			
Mandible	−0.979	0.343			

Table 3.
The significance of differences between adults of both sexes from Macquarie Island and Lake Alexandrina; unpaired two-tailed t-tests with 21 degrees of freedom

Length	t	p	Index	t	p
Body	6.909	0.0001	DE MAN's Index **a**	0.352	0.729
Pharynx	6.466	0.0001	DE MAN's Index **b**	−1.014	0.322
Tail	3.206	0.0042	DE MAN's Index **c**	−0.006	0.995
Inner labial setae	1.826	0.0821	DE MAN's Index **c'**	−3.103	0.005
Outer labial setae	4.135	0.0005			
Cephalic setae	4.173	0.0001			
Head width	4.482	0.0002			
Onchium	4.317	0.0003			
Mandible	2.794	0.0109			

Table 4.
The significance of differences between the genitalia of adults from Macquarie Island and Lake Alexandrina; unpaired two-tailed t-tests

Structure	df	t	p
Spicule arc length	9	5.301	0.0005
Distance of supplementary organ from anus	9	2.696	0.0245
Vulva from head end, %	10	−0.506	.6241

Table 5.
Coefficient of variation of accessory sexual organs in Macquarie Island adults

Males	COV %	Females	COV %
Spicule arc length	6.3	Vulva position/Length %	5.6
Supplement to anus	6.6		

there was considerable variation in all three measurements within each population (Table 5 and Fig. 2).
The question is to what extent can the significant differences between the two populations be accounted for by differences in size? Are the characters not significantly different less dependent on body size? Juveniles from both localities have also been measured increasing the size range. The dependence of measurements on total body length are shown in Figs 2 and 3, and where appropriate the linear regression of the measurement on body length calculated.
In Macquarie Island adults, spicule length and anus to supplement distance increase with increasing body length, but the relative position of the vulva does not (Fig. 2). All three measurements show similar large variability (Table 2). Too few adults from Lake Alexandrina were measured to make similar comparisons of variability worthwhile. In contrast the linear regressions of pharyngeal length on body length for both sexes from both localities give a close fit (Fig. 2).
When juveniles and adults from both localities are combined, all the characters measured increase with increasing body length, but not in direct proportion, so that when body length doubles, the three sets of cephalic setae increase in length about 45%, and head width about 75%. The rate of increase of lengths of setae falls off with increasing body length. Pharyngeal length in contrast, increases linearly with body length, increasing about 65% for a doubling in body length. Tail length increases more slowly, about 36% for twice body length and fits a straight line much less closely. Mandibles and onchium increase relatively little with body length.
Linear regression of DE MAN's indices **a**, **b**, and **c** fit a straight line fairly closely (Fig. 4), but do not increase as rapidly as body length, for a doubling in length they increase by 56, 40 and 65% respectively. In contrast,

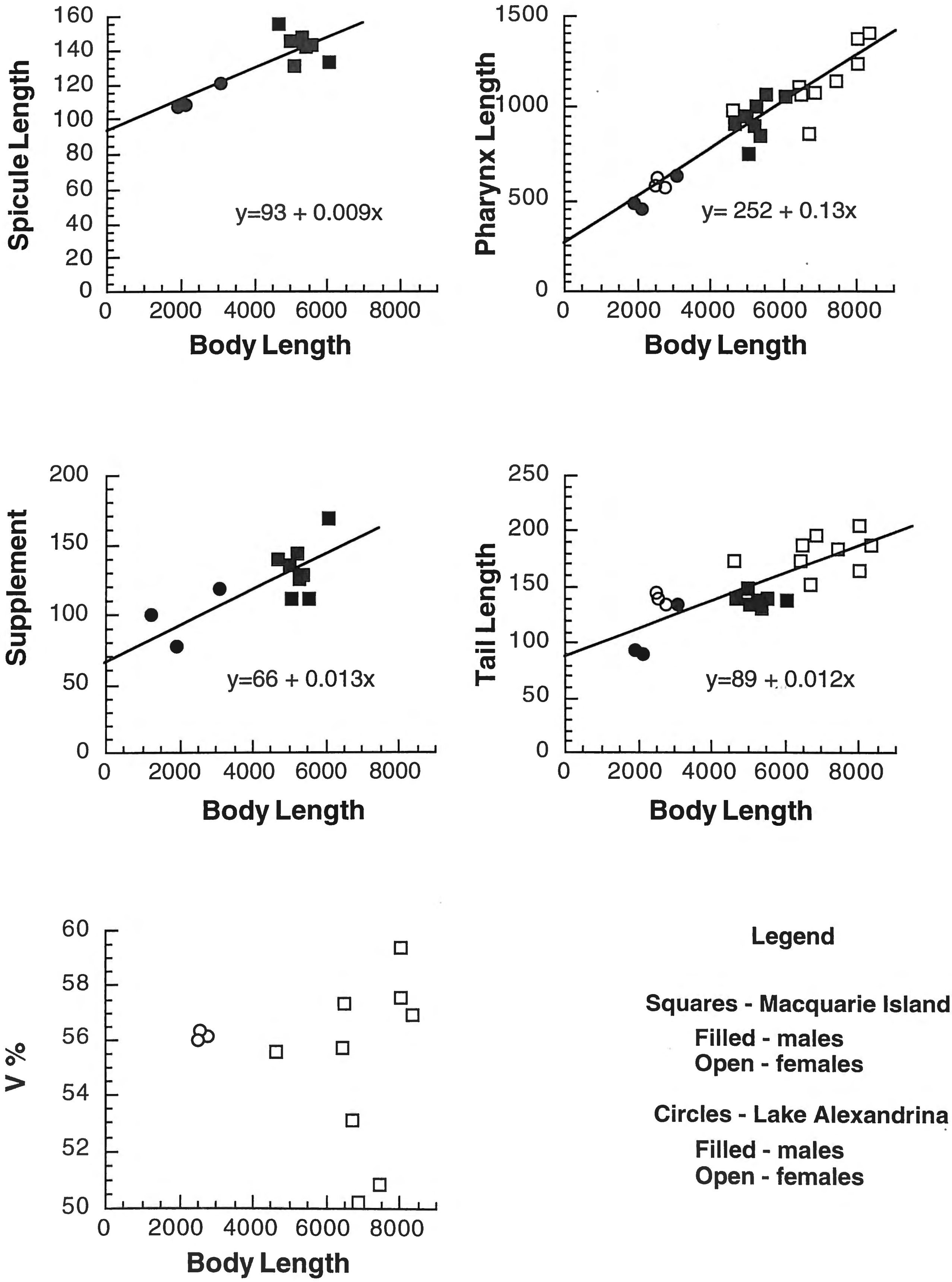
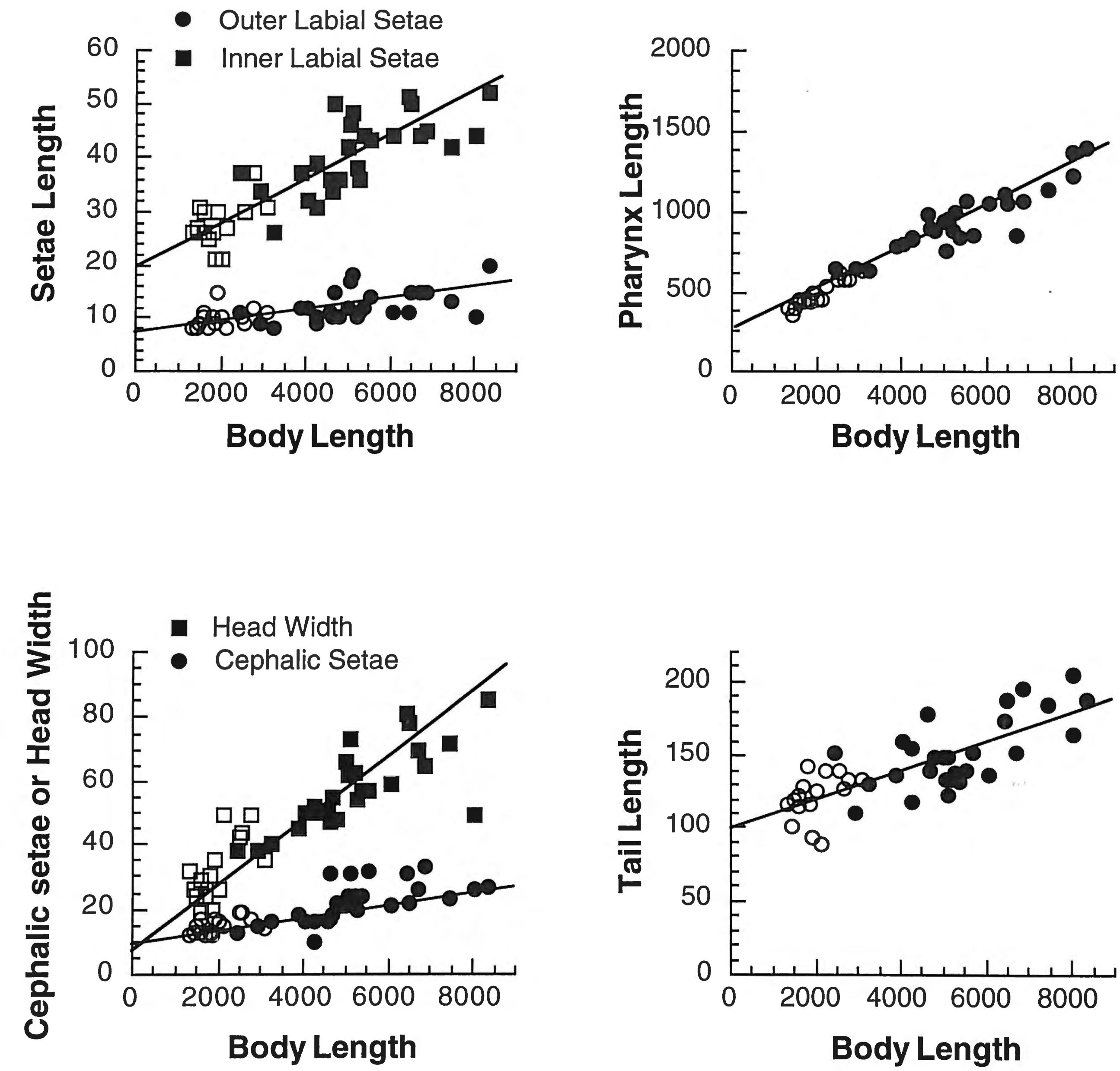


Fig. 2. – Comparisons between measurements of adults of both sexes from Macquarie Island and Lake Alexandrina, South Australia.



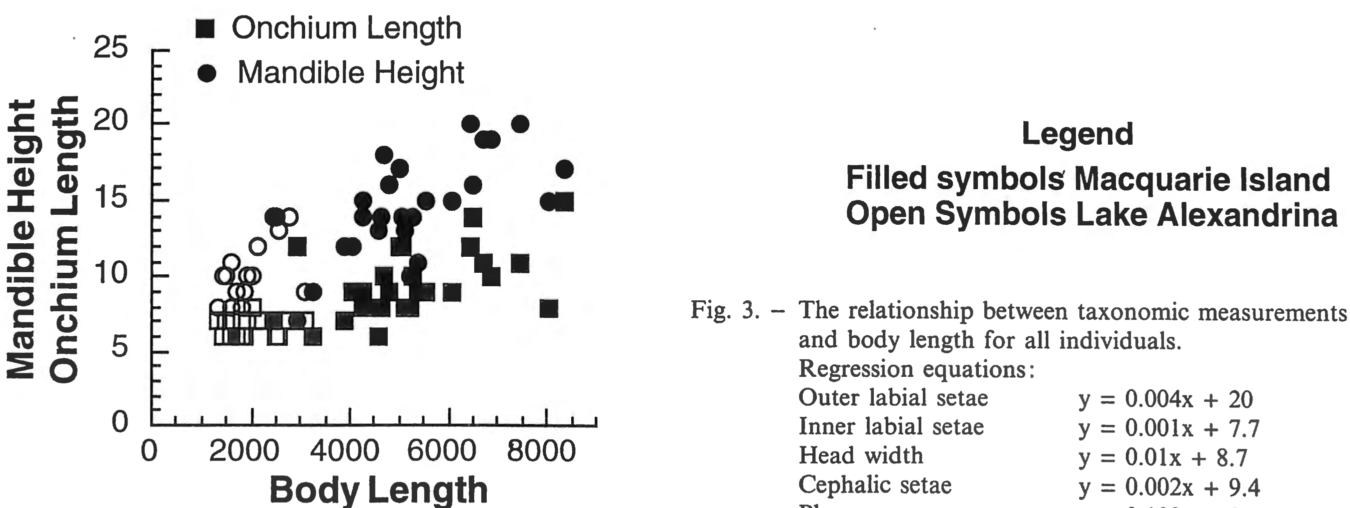


Fig. 3. – The relationship between taxonomic measurements and body length for all individuals.
Regression equations:

Outer labial setae	y = 0.004x + 20
Inner labial setae	y = 0.001x + 7.7
Head width	y = 0.01x + 8.7
Cephalic setae	y = 0.002x + 9.4
Pharynx	y = 0.132x + 235
Tail	y = 0.01x + 100

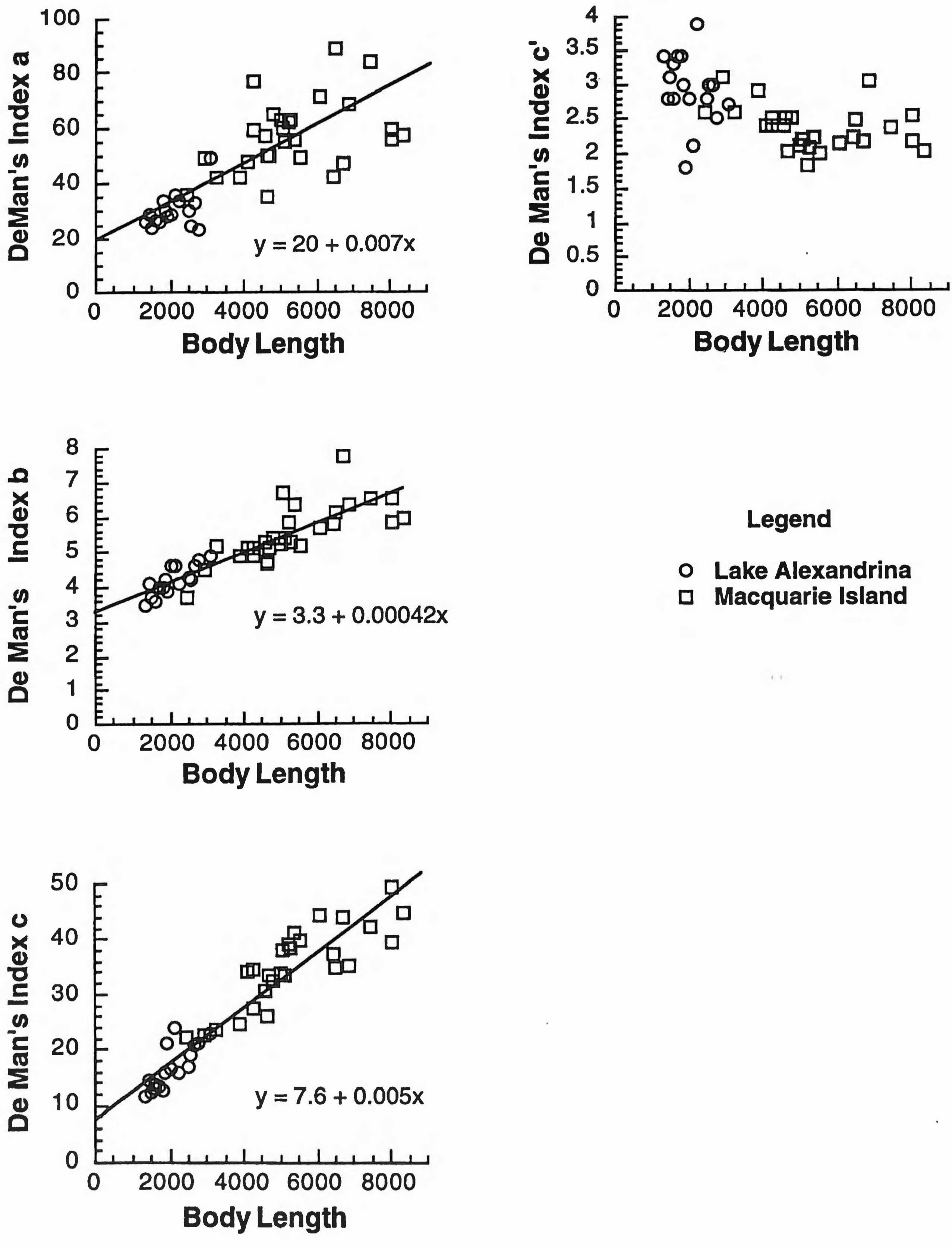


Fig. 4. – The dependence of DE MAN's indices on body length; adults and juveniles combined.

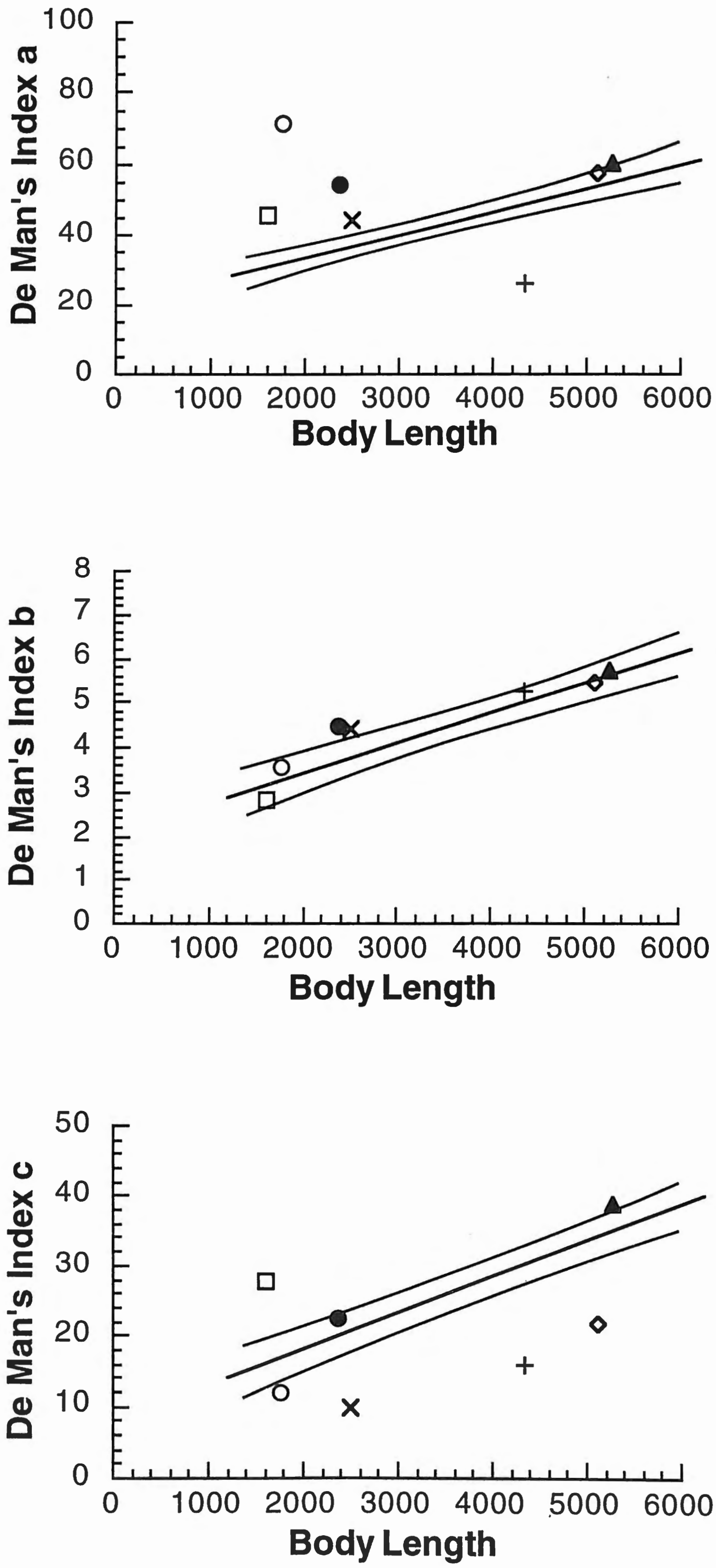


Fig. 5. – The value of DE MAN's indices for male holotypes taken from the literature, together with the regression lines and their 95% confidence limits from the samples of *Enoploides stewarti* from Macquarie Island and Lake Alexandrina, South Australia.

c' does not increase, and is the only property measured in which nematodes from Lake Alexandrina differ from those from Macquarie Island independently from body length (Fig. 4).

A multivariate analysis of variance was carried out on the residuals calculated when the following measurements were regressed against body length: onchium length, mandible length, head width, outer labial setae length, inner labial setae length, tail length, pharynx length, and cephalic setae length. The effect of using residuals was to remove the size difference between the two populations. There was no difference between the populations, based on these measurements (ROY's Greatest Root = 0.009; $p > 0.9$).

Discussion

Differences in temperature have been shown in the laboratory to cause differences in adult size, higher temperatures reducing size, i.e. Bergmann's rule (GYSELS, 1964, SOHLENIUS, 1968). A decrease in size at higher temperatures may be associated with increased and earlier egg production, but may also be an indirect effect of temperature on food supply. A colder climate in Macquarie Island may account for the larger adult size of that population. Differences in temperature affected body length and morphometrics, including DE MAN's indices, in laboratory populations of *Ditylenchus* (EVANS & FISCHER, 1970).

The relationship between length and other body dimensions has frequently been studied, mostly in plant pathogenic Tylenchida and Dorylaimida. Often interest has been in organs such as the pharyngeal bulbs, glands or stylet which are not represented in Thoracostomopsidae. Inherent variability and the effects of environmental factors on the important taxonomic characters in Thoracostomopsidae have not been investigated.

GERAERT (1978) compared the growth of the pharynx and body width and body length in many Tylenchida and found that pharyngeal growth does not keep pace with body length while body width changes with maturity. The relative growth of the pharynx in plant and animal parasites is affected by changes in the life cycle and food and is not relevant here, but the relationship of pharyngeal length to body length during the post embryonic development of a variety of free living nematodes is pertinent (GERAERT, 1979a, b, 1983). The relationship is best fitted by a second degree polynomial (parabola) and is relatively little affected by environmental factors. GERAERT (1979b, 1983) also studied the relationship between the growth of the tail and position of the vulva with body length in a variety of free living and plant pathogenic nematodes. Tail length was very variable within and between species. The position of the vulva varied very little in a species. In contrast with GERAERT's findings, a linear equation fitted the increase in pharyngeal length with increasing body length of adults and juveniles of both populations of *Enoploides*. A linear equation fitted tail length on body length for both populations less well, with points more widely scattered about the regression line. Vulva position was not constant and showed no relationship with length. Spicules and supplement position increased with size but the nature of the relationship was not evident.

The dimensions of the characters particularly appropriate to Thoracostomopsidae, i.e. head width, length of the setae (inner labial, outer labial and cephalic), mandible and onchium increased steadily with size, though not linearly, without any discontinuity between the two populations. DE MAN's indices fit linear relationships with body length quite well, without discontinuity between the populations; a wider scatter of index **a** shown by adult females is a consequence of variations in egg production. The simplest explanation is that the two populations belong to a single species differing in adult size for environmental reasons, probably primarily temperature.

To compare differences in DE MAN's indices between the two populations of *E. stewarti* with that between closely related species, and test FORTUNER's (1990) contention that the indices retain their usefulness in taxonomy, we have compared the indices in several related species. We have used published data on *E. cirrhatus, E. alexandriae, E. dispirilis,* and *E. fluviatilis,* which together with *E. stewarti,* form a discrete sub-group in the genus, distinguishable by their shorter spicules (NICHOLAS, 1993). Figure 5 presents the data for male holotypes plotted against body length, with the regression and confidence intervals calculated for *E. stewarti.* Specific differences are evident between related species with indices **a** and **c** but not **b**.

Acknowledgments

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