

Morphometric variation in bifurcate notosetae of two *Euphrosine* species (Polychaeta, Euphrosinidae)

Ken D. VOGT & Jerry D. KUDENOV

Department of Biological Sciences
University of Alaska Anchorage
3211 Providence Drive
Anchorage, Alaska 99508 U.S.A.

ABSTRACT

Univariate and multivariate analysis of covariance based on four morphological factors reveal that prong lengths and ratios of bifurcate notosetae in two *Euphrosine* species (Polychaeta, Euphrosinidae) vary systematically along both the body axis and within setal fascicles in relation to body size. Body size was measured as a function of two covariates: total numbers of segments and body weights. Principle component analysis revealed that the majority of notosetal variation in these species is attributable to body size ; the remainder to setal position within notofascicles. Discriminant analysis correctly distinguished between the notosetae of these species in nearly all cases. These preliminary results tend to validate the traditional use of prong lengths and ratios to describe the bifurcate notosetae of euphrosinids.

RÉSUMÉ

Variation morphométrique des soies dorsales bifurquées de deux espèces d'*Euphrosine* (Polychète, Euphrosinidae)

Les analyses univariées et multivariées de la covariance basée sur quatre caractères morphologiques révèlent que la longueur des branches des soies fourchues et les proportions des soies notopodiales bifides des deux espèces d'*Euphrosine* (Polychètes, Euphrosinidae) varient selon leur position dans l'axe du corps et avec les fascicules des soies en relation avec la taille du corps. La taille du corps a été mesurée comme une fonction de deux variables liées : le nombre total de segments et le poids du corps. L'analyse en composantes principales montre que la variation majeure des soies notopodiales de ces espèces est liée à la taille du corps ; les autres variations sont liées à la position dans les faisceaux dorsaux. L'analyse discriminante permet de distinguer correctement, dans tous les cas, les soies notopodiales des espèces. Ces résultats préliminaires proposent la validation de l'utilisation traditionnelle des longueurs des branches des soies fourchues et de leur proportion pour décrire les soies notopodiales bifides des euphrosinides.

INTRODUCTION

Although the setae of polychaetous annelids are generally recognized to be highly variable morphological traits that vary over time and space (FAUCHALD, 1991), morphometric studies on setal variation have only recently

begun (GAFFNEY, 1973; GARWOOD & OLIVE, 1981; BHAUD, 1983; ORRHAGE & SUNDBERG, 1990; FAUCHALD, 1991, 1992). The need to incorporate such data into polychaete systematics is critical since: 1) simple linkages between morphology and the distinctions between biological species probably do not exist (GRASSLE & GRASSLE, 1976; LEVIN, 1984; ORRHAGE & SUNDBERG, 1990); 2) systematically significant morphological variation exists in species previously considered to be cosmopolitan or widely distributed (HARTLEY, 1984; WILLIAMS, 1984); and 3) setal morphology may be variously influenced by combinations of environmental factors as has been documented in oligochaetes (SCHROEDER, 1984; CHAPMAN & BRINKHURST, 1987, but see GAFFNEY, 1973).

There have been no quantitative studies of setae published on the Order Amphinomida, although setal variation has been noted (MOORE, 1905; HORST, 1912), and GUSTAFSON (1930) illustrated the kinds of setae present in the genera included in his study. KUDENOV (1987) suggested that bifurcate notosetae should be studied quantitatively to validate their use in the systematics of *Euphrosine*. This paper reports preliminary findings on morphometric variation of the bifurcate notosetae in two species of *Euphrosine*: *E. borealis* Örsted, 1843 and *E. bicirrata* (Moore, 1905). Work continues on morphometry of neurosetal variation and will be reported elsewhere.

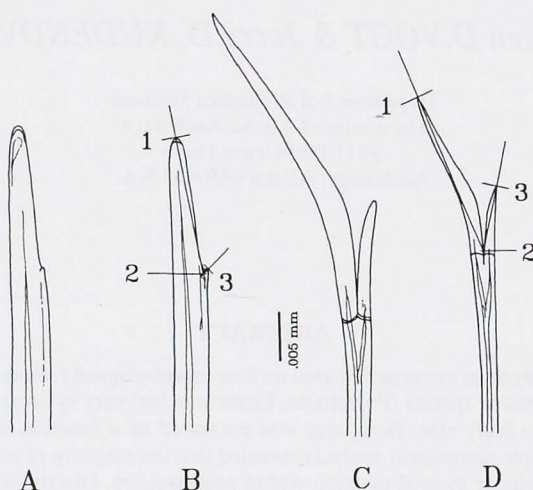


FIG. 1. — Prong lengths of bifurcate notosetae of *Euphrosine borealis* (A, B) and *E. bicirrata* (C, D), involving measurements between points 1-2 for long prongs, and points 2-3 for short prongs. A, C, superior notosetae. B, D, inferior notosetae.

MATERIALS AND METHODS

In all, 15 preserved, complete specimens (four of *E. bicirrata*; 11 of *E. borealis*) examined as part of this study were obtained on loan through the Department of Biology, Moscow State University, and the P.P. Shirshov Institute of Oceanology. Unfortunately, locality data have not yet been made available.

A two stage stratified sampling routine was used in which setae were collected from the top and bottom of notosetal fascicles of anterior (setiger 6), middle (14-18), and posterior (24-30) body regions. Setal measurements include long prong length (LP) and short prong length (SP) (Fig. 1); long to short prong ratios (LP/SP) were calculated. Abbreviations are used as subscripts for probability values given below. All setal measurements were made at 400 times magnification, and repeated nonconsecutively. Body size was estimated using two covariates: total numbers of setigers (S) and body weight (W). Body weights, recorded as wet weights of specimens stored in alcohol, were measured using an analytical balance.

Statistical analyses (Analysis of Covariance [ANCOVA]: MONTGOMERY, 1984; Multiple Analysis of Covariance [MANCOVA]: MANLY, 1990) were carried out for natural-, square root- and log_e-transformed data. Confirmation of normality was prerequisite to all parametric tests used (MONTGOMERY, 1984; MANLY, 1990). Nonparametric uni- and multivariate tests were used for *E. borealis* data that could not be normalized (CONOVER, 1980). Principal component analysis was calculated for both species (MANLY, 1990). Discriminant analysis of prong lengths and ratios for both species was constrained to a nonparametric multivariate test (K-NEAREST

NEIGHBOR: HAND, 1989) on prong lengths and ratios vs. body size and fascicle position for both species since *E. borealis* data could not be normalized. K values (1-5) were used initially for both species; those for which the fewest misidentifications occurred were selected for further analysis. Blocks for anterior, middle and posterior body regions for all statistical tests were taken to represent total numbers of segments.

Statistical software packages used for all ANCOVA and MANCOVA analyses are found in Statistical Packages for the Social Sciences, version 4.0 (SPSS Inc., 1990) and Statistical Analyses Software, version 6.06 (SAS INSTITUTE Inc., 1990). Probability limits were set at $P = 0.05$.

RESULTS

In all, 953 notosetae were measured (432 from four *E. bicirrata*; 521 from 11 *E. borealis*). In *E. bicirrata*, body segment numbers and body weights ranged from 24-31 and from 0.117-0.129 g, respectively; in *E. borealis* they ranged from 23-41 and 0.06-2.7 g. Results of statistical analyses for both species reveals the presence of continuous and verifiable variation both along the body axis and within notopodial fascicles.

The notosetae of *Euphrosine bicirrata*

Within-cell MANCOVA treatment of prong lengths vs body size and setal position within fascicles reveals the lengths of both prongs simultaneously increase from the top to bottom of a fascicle (MANCOVA: Fig. 2a-b, $P_{LP} = P_{SP} = 0.002$, Wilkes-lambda = 0.08) and along the length of the worm as body size increases (MANCOVA: Fig. 2a-b, $P_S = P_W = 0.002$, Wilkes-lambda = 0.08). Although long prongs appear to increase in length, they were found to vary independently of body size when tested alone (MANCOVA: Fig. 2c, $P_S = 0.062$, $P_W = 0.072$). In contrast, short prongs directly influence this multivariate association when examined separately. Short prongs increase in length towards posterior segments as body size increases (MANCOVA: Fig. 2d, $P_S = P_W = 0.002$, Wilkes-lambda = 0.08).

Partial cell MANCOVA treatment of prong lengths vs fascicle position (holding the influence of body size constant), reveals that both prongs increase in length from the top to bottom of fascicles (MANCOVA: $P_{LP} = P_{SP} = 0.002$, Wilkes-lambda = 0.07),

$\log_e$ -transformed ratios of prong lengths decrease from top to bottom of notofascicles (ANCOVA: $P_{LP/SP} = 0.025$) and along the length of the worm (ANCOVA: $P_{LP/SP} = 0.005$) as body-size increases. Prong lengths are positively correlated to total body segment numbers ($r = +.512$, $N = 432$), and negatively correlated to total body weight ($r = -0.531$, $N = 432$).

Principle component analysis disclosed that 91.3 % of total observed variation in this study is explained by body size while the remaining 8.7 % is attributable to setal position within notofascicles.

The notosetae of *Euphrosine borealis*

In contrast to data obtained for *E. bicirrata*, only the data for prong ratios could be normalized, and analyzed by ANCOVA; univariate analyses of prong lengths were otherwise tested nonparametrically.

Lengths of both long and short prongs, each tested separately, have statistically constant lengths and vary independently of body size (KRUSKAL-WALLIS: Fig. 3a, $P_{LP} = 0.6458$; Fig. 3b, $P_{SP} = 0.4059$). However, both long and short prongs, tested separately, increase in length from top to bottom of notofascicles (KRUSKAL-WALLIS: Fig. 4a-b, $P_{LP} < 0.0001$, $P_{SP} < 0.0001$).

$\log_e$ -transformed ratios of prong lengths, normalized through a $\log_e$ transformation, decrease from top to bottom of notofascicles (ANCOVA: $P_{LP/SP} < 0.0001$), and along the length of the worm with increasing numbers of body segments (ANCOVA: $P_{LP/SP} < 0.0001$), and are independent of total body weight (ANCOVA: $P_{LP/SP} = 0.133$). $\log_e$ -transformed ratios of prong lengths are also positively correlated to total body segment numbers ($r = +0.995$, $N = 521$) and independent of total body weight ($r = 0$, $N = 521$).

Principal component analysis revealed that 72.0 % of the total observed variation in this study is accountable by body size while the balance of 28.0 % is associated with setal positions within notofascicles.

Discriminant analysis of *Euphrosine bicirrata* and *E. borealis*

The fewest number of notosetal misidentifications for both species occurred when $K = 3$. A total of four notosetae (two from each species) were incorrectly assigned to the other species. None had any body axis or fascicle location in common, and no pattern to these errors could be discerned. In all, 99.6 % (949/953) of all

notosetae were correctly identified on the basis prong lengths of notosetae from known positions within fascicles and segments.

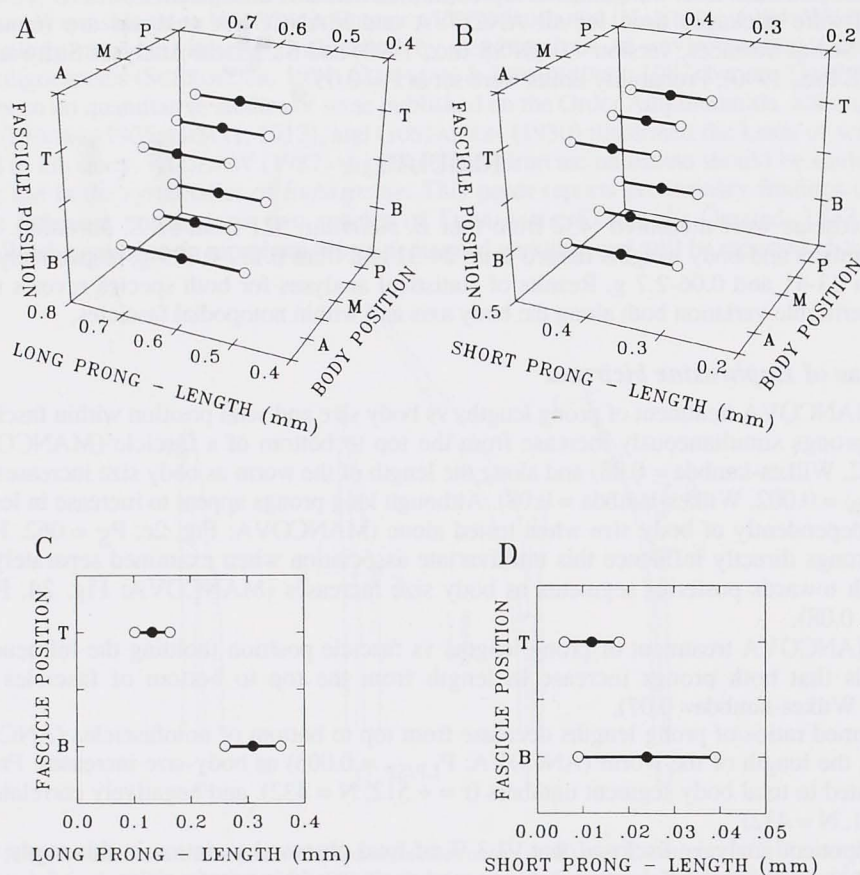


FIG. 2. — *Euphrosine bicirrata* Moore: A, mean length of long prongs from top and bottom of notofascicles along the body axis. B, mean length of short prongs from top and bottom of notofascicles along the body axis. C, mean length of long prongs in relation to position along the body axis (influence of body size removed). D, mean length of short prongs in relation to position along the body axis (influence of body size removed). A, M, P denote anterior, middle and posterior body regions; solid dots represent means; open circles ± 1 standard deviation.

Similar results were obtained for *E. borealis* prong ratios when $K = 1$ through 4, in that 99.6 % (949/953) of the notosetae in each of four tests were correctly identified; all *E. bicirrata* notosetae were correctly identified.

DISCUSSION

Bifurcate notosetae of both *Euphrosine bicirrata* and *E. borealis* vary systematically within notofascicles along the length of the body in worms of increasing body size. Few other studies are available that report similar patterns of setae variation along the body of polychaetes. The only work about which we are aware is that of FAUCHALD (1992) which found that total length of setae vary systematically along the body of preserved *Palola* species whereby: 1) all setae are initially short in anteriormost segments, becoming longest in the anterior third, and decreasing gradually in length to the end of the body; 2) setae change from top to bottom of fascicles, and that these patterns vary systematically between species; and that the 3) blades of all compound setae also vary along the body, being longest in middle body segments. Similar, but not necessarily identical, relationships also exist in the bifurcate notosetae of the two *Euphrosine* species examined in the present study. In *E. bicirrata*, prong lengths

simultaneously increase in length from top to bottom of notofascicles in segments along the length of the worm as body size increases. This relationship appears to be controlled more by the length of the short than long prong for

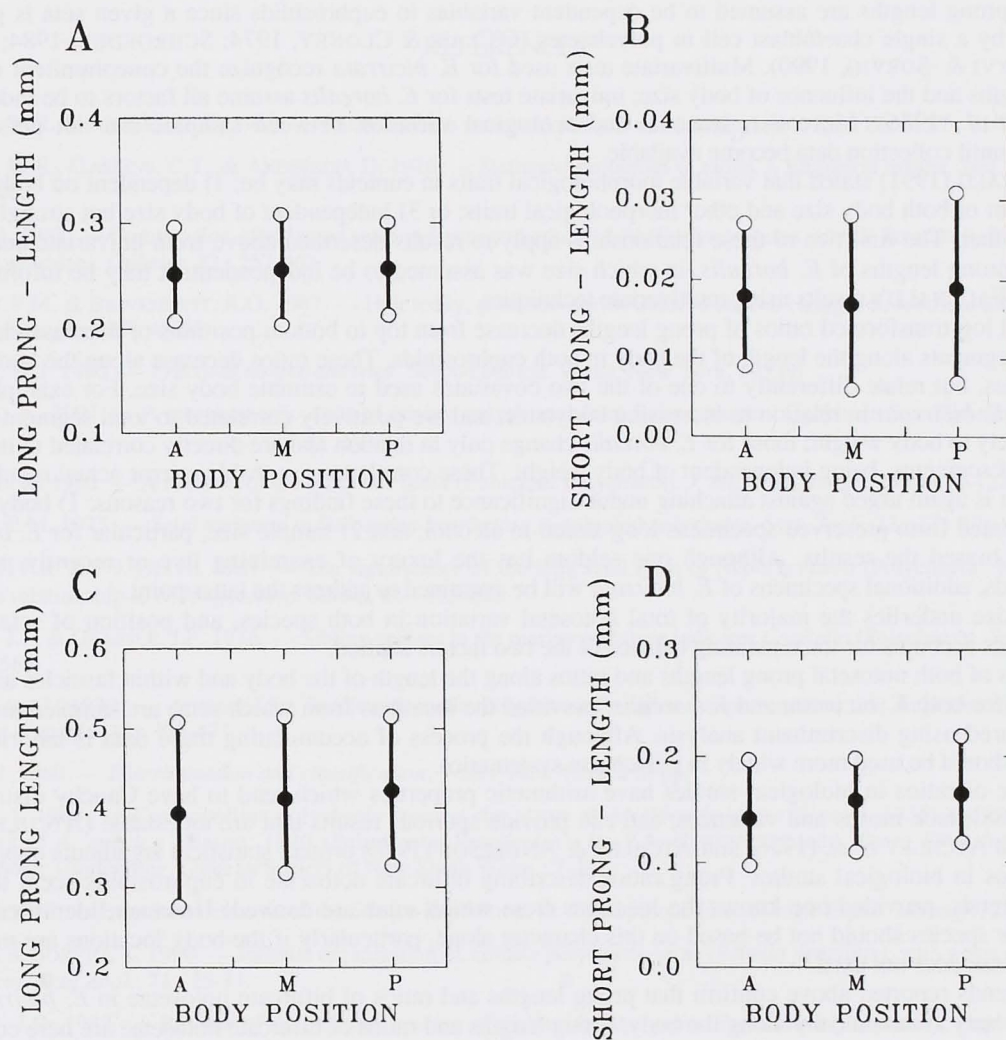


FIG. 3. — *Euphrosine borealis* Örsted: A, mean length of long prongs in relation to position along the body axis. B, mean length of short prongs in relation to position along the body axis. C, mean length of long prongs from top of notofascicles along the body axis. D, mean length of short prongs from bottom of notofascicles along the body axis. A, M, P denote anterior, middle and posterior body regions; T, B denote top and bottom region of notofascicles; solid dots represent mean values; open circles ± 1 standard deviation.

the parameters tested. Variability in the latter may be directly related to some other morphological factor not included in this study. Changes detected in prong lengths within notofascicles are not influenced by size. Prong lengths in *E. borealis* vary independently of body size, and increase from top to bottom of notofascicles.

Little if any biological significance should be attached to these findings when results for *E. bicirrata* and *E. borealis* are compared since the different statistical analyses employed make vastly disparate assumptions about the independent-dependent nature of variables examined (WILLIG *et al.*, 1986; ORRHAGE & SUNDBERG, 1990). For instance, prong lengths are assumed to be dependent variables in euphosinids since a given seta is generally produced by a single chaetoblast cell in polychaetes (O'CLAIR & CLONEY, 1974; SCHROEDER, 1984; SPECHT, 1988; KRYVI & SORVIG, 1990). Multivariate tests used for *E. bicirrata* recognize the concomitant nature of prong lengths and the influence of body size; univariate tests for *E. borealis* assume all factors to be independent (WILLIG *et al.*, 1986). Moreover, seasonal and ecological variation between samples can not presently be explained until collection data become available.

FAUCHALD (1991) stated that variable morphological traits in eunicids may be: 1) dependent on body size; 2) independent of both body size and other morphological traits; or 3) independent of body size but strongly related to one another. The first two of these relationships apply to results described above from univariate analyses of notosetal prong lengths of *E. borealis*, in which size was assumed to be independent. It may be informative to reconfirm FAUCHALD's results using multivariate techniques.

Natural log-transformed ratios of prong lengths decrease from top to bottom positions of notofascicles and in different segments along the length of the body in both euphosinids. These ratios decrease along the body axis in both species, but relate differently to one of the two covariates used to estimate body size. For example, ratios change in *E. bicirrata* in relation to increasing body size, and are positively correlated to total segment number, and inversely to body weight; those for *E. borealis* change only in relation and are directly correlated to increasing numbers of segments, being independent of body weight. These correlations probably mirror actual relationships, but caution is again urged against attaching undue significance to these findings for two reasons: 1) body weights were estimated from preserved specimens long stored in alcohol; and 2) sample size, particular for *E. bicirrata*, may have biased the results. Although one seldom has the luxury of examining live or recently preserved euphosinids, additional specimens of *E. bicirrata* will be examined to address the latter point.

Body size underlies the majority of total notosetal variation in both species, and position of setae within notofascicles accounts for the remaining balance of the two factors studied.

Patterns of both notosetal prong lengths and ratios along the length of the body and within fascicles are highly diagnostic for both *E. bicirrata* and *E. borealis*, provided the locations from which setae are sampled are known and compared using discriminant analysis. Although the process of accumulating these data is laborious, this technique should be used more widely in polychaete systematics.

The use of ratios in biological studies have arithmetic properties which tend to have Cauchy distributions (ROSS, 1988), lack means and variances, and can provide spurious results that are untestable (ATCHLEY *et al.*, 1976). Both ATCHLEY *et al.* (1976) and ATCHLEY & ANDERSON (1978) present statistical arguments opposing the use of ratios in biological studies. Prong ratios describing bifurcate notosetae in euphosinids seem to reflect expected trends, provided one knows the locations from which setae are derived! However, identifications of *Euphosine* species should not be based on this character alone, particularly if the body locations are unknown, and a database does not exist.

Setal trends reported above confirm that prong lengths and ratios of bifurcate notosetae in *E. bicirrata* and *E. borealis* vary systematically along the body. Prong lengths and ratios of bifurcate notosetae are here confirmed to be statistically valid characters that have been used traditionally in euphosinid systematics (KUDENOV, 1987), provided setal locations both within fascicles and along the body axis are known. Euphosinids should not be identified solely on the basis of bifurcate notosetal prong ratios. It is suggested that future descriptions of euphosinid taxa include prong lengths and ratios for bifurcate notosetae from the top and bottom of notosetal fascicles from anterior, middle and posterior body segments. However, prior assumptions regarding the systematic importance of prong lengths and ratios should probably not be extrapolated to other *Euphosine* species until the results of this study are corroborated in other congeners.

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REFERENCES

- ATCHLEY, W.R. & ANDERSON, D. 1978. — Ratios and the statistical analysis of biological data. *Syst. Zool.*, **27** : 71-77.
- ATCHLEY, W.R., GASKINS, C.T., & ANDERSON, D. 1976. — Statistical properties of ratios. I. Empirical results. *Syst. Zool.*, **25** : 137-148.
- BHAUD, M. 1983. — Comparision of enlarged setae in larvae and adults of *Phyllochaetopterus verrilli* Treadwell (Polychaeta : Chaetopteridae). *Ophelia*, **22** : 257-263.
- CHAPMAN, P.M. & BRINKHURST, R.O. 1987. — Hair today, gone tomorrow: induced chaetal changes in tubificid oligochaetes. *Hydrobiologia*, **155** : 45-55.
- CONOVER, W.J. 1980. — *Practical nonparametrics*. Second edition. Wiley, New York, 495 pp.
- FAUCHALD, K. 1991. — A morphometric study of eunicid polychaetes from Belize, western Caribbean Sea. *Ophelia* Suppl. **5** : 47-53.
- FAUCHALD, K. 1992. — A revision of the types of *Palola* (Eunicidae Polychaeta). *J. nat. Hist., Lond.*, **26** : 1177-1227.
- GAFFNEY, P.M. 1973. — Setal variation in *Halosydna brevisetosa*, a polynoid polychaete. *Syst. Zool.*, **22** : 171-176.
- GARWOOD, P.R. & P.J. OLIVE. 1981. — The significance of the long setal form of *Nephtys caeca* (Polychaeta : Nephtyidae) and its relationship to *N. longosetosa*. *Sarsia*, **66** : 195-202.
- GRASSLE, J.P. & GRASSLE, J.F. 1976. — Sibling species in the marine pollution indicator *Capitella* (Polychaeta). *Science*, **192** : 567-569.
- GUSTAFSON, G. 1930. — Anatomische Studien über die Polychäten-Familien Amphinomidae und Euphrosynidae. *Zool. Bidrag Uppsala*, **12** : 305-471.
- HAND, D.J. 1989. — *Discrimination and classification*. Wiley, New York, 218 pp.
- HARTLEY, J. 1984. — Cosmopolitan polychaete species: the status of *Aricidea belgicae* (Fauvel, 1936) and notes on the identity of *A. suecica* Eliason, 1920 (Polychaeta; Paraonidae). In: P.A. HUTCHINGS (ed.), *Proc. First International Polychaete Conference*. Linnean Society of New South Wales : 7-20.
- HORST, R. 1912. — Polychaeta errantia of the *Siboga*-Expedition. Pt. 1. Amphinomidae. *Siboga-Exped. Monogr.*, **24a** : 1-43.
- KRYVI, H. & SORVIG, T. 1990. — Internal organization of limbate polychaete setae (*Sabella pencillus*), with notes on bending stiffness. *Acta Zool.*, **71** : 25-31.
- KUDENOV, J.D. 1987. — Review of the primary species characters for the genus *Euphrosine* (Polychaeta: Euphrosinidae). *Bull. biol. Soc. Wash.*, **7** : 184-193.
- LEVIN, L.A. 1984. — Multiple patterns of development in *Streblospio benedicti* Webster (Spionidae) from three coasts of North America. *Biol. Bull. mar. biol. Lab, Woods Hole*, **166** : 494-508.
- MANLY, B. F. J. 1990. — *Multivariate statistical methods: A primer*. Chapman & Hall, London, 159 pp.
- MONTGOMERY, D.C. 1984. — *Design and analysis of experiments*. Wiley, New York, 538 pp.
- MOORE, J.P. 1905. — New species of polychaetes from the north Pacific, chiefly from Alaskan waters. *Proc. Acad. Nat. Sci. Phila.*, **57** : 525-554.
- O'CLAIR, R.M. & CLONEY, R.A. 1974. — Patterns of morphogenesis mediated by dynamic microvilli: chaetogenesis in *Nereis vexillosa*. *Cell Tissue Res.*, **151** : 147-157.
- ORRHAGE, L. & P. SUNDBERG. 1990. — Multivariate analysis of morphometric differentiation within the *Laonice cirrata*-group (Polychaeta, Spionidae). *Zool. Scr.*, **19** : 173-178.

- ROSS, S. 1988. — *A first course in probability*. 3rd edn MacMillan, 420 pp.
- SAS INSTITUTE INC., 1990. — *SAS/STAT user's guide*. Version 6.06, Fourth Edition. North Carolina. 1686 text + 106 pp index.
- SCHROEDER, P. 1984. — *Annelida Chaetae*. In: BEREITER-HAHN, MATOLTSY & RICHARDS (eds), *Biology of the Integument*. K.S. Springer, New York, 1 : 297-309.
- SPECHT, A. 1988. — *Chaete. Microfauna Marina*, 4 : 45-59.
- SPSS INC., 1990. — *SPSS advanced statistics student guide*. Version 4.0, Chicago, Illinois. 506 pp.
- WILLIAMS, S.J. 1984. — The status of *Terebellides stroemi* (Polychaeta; Trichobranchidae) as a cosmopolitan species, based on a worldwide morphological survey, including descriptions of new species. In: P. HUTCHINGS (ed.), *Proc. First International Polychaete Conference*. Linnean Society of New South Wales : 118-142.
- WILLIG, M.R., OWEN, R.D., COLBERT, R.L. 1986. — Assessment of morphometric variation in natural populations: The inadequacy of the univariate approach. *Syst. Zool.*, 35 : 195-203.