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## Towards a standardisation of morphological measurements in the phylum Kinorhyncha

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### ABSTRACT

Taxonomic studies of the phylum Kinorhyncha follow a standardised structure, which is extremely useful in many aspects, such as making it easier to read and compare the different species descriptions. Nevertheless, the morphological measurements methods, essential for formal description of species, may differ according to the authors. In the present contribution, we propose a standardised method of taking and representing the measurements, with the aim of obtaining comparable and repeatable morphometric results. Additionally, we propose an online repository to make the measurements accessible to all researchers in the same format, facilitating future comparisons and studies. Finally, a glossary that compiles and defines all the measurements that may be included in Kinorhyncha descriptions is presented.

### 1. Introduction

Morphometrics have revealed to be useful for different aspects in meiofauna research, including species discrimination (Tchesunov et al., 2015; Karanovic et al., 2016; Michaloudi et al., 2018; Kim et al., 2021), sexual dimorphism (Da Silva et al., 2009), detection of evolutionary patterns (Cepeda et al., 2019b; Cerca et al., 2019), ecological adaptations (Bell et al., 1987; Cepeda et al., 2020a; Grzelak et al., 2020), and functional diversity (Sroczyńska et al., 2021). In Kinorhyncha, specifically, measurements of selected body traits have been traditionally included in all formal descriptions of new species since the very

beginning (Greeff 1869; Zelinka 1928; Blake 1930). However, it was not until Higgins (1967) when the most relevant morphological traits that would allow characterising the Kinorhyncha species were defined and established.

At this point, the total trunk length, the maximum sternal width, the standard sternal width, and the length of the spines were compiled in a standardised table for the first time (Higgins 1967). The total trunk length was taken on the midline from the anterior margin of segment 1 to the posterior margin of segment 11, excluding spines but including sternal and tergal extensions of segment 11, whereas the maximum and standard sternal widths were measured at the anterior margin of the

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widest pair of sternal plates, and the sternal plates of segment 10, respectively (Higgins 1967; 1983).

Measurements of the dorsal, lateral and terminal series of spines were calculated as arithmetic means per segment, although individual values of spines on each segment were also provided in the text (Higgins 1967; 1968; 1969a). In addition, relative proportions of spine lengths compared to the total trunk length (especially those of the terminal series) were also included (Higgins 1967; 1968; 1969a; 1969b).

Higgins frequently incorporated length values of each body segment as part of the text in the taxonomic descriptions (Higgins 1967; 1968; 1969a; 1977a; 1977b). The way to capture a segment length was first defined by Zelinka (1928), from the anterior margin of the pachycyclus to the end of the primary pectinate fringe, but the position in which to take the measure was also not specified. In addition, Kinorhyncha segments are not perfect, geometric cylinders, and they overlap each other quite variably, which increases the need to standardise how to take these measurements to minimise errors.

Under this framework, we consider it necessary to revise and standardise the way to measure certain Kinorhyncha morphological traits. This standardisation will allow minimising the measurement error linked to the person who takes the measurement. The main objective of the present paper is to reach reproducibility for future Kinorhyncha researchers regarding: (i) the current measurements included in Kinorhyncha descriptions, (ii) new measurements that can be useful for species characterisation, and (iii) standardizing the way certain measurements are taken (i.e. segment lengths) to find the lowest data variability between observers.

## 2. Material and methods

### 2.1. Compilation of information about Kinorhyncha measurements

A bibliographic search of taxonomic studies (descriptions of new taxa, redescriptions, et cetera) related to Kinorhyncha since the discovery of the phylum has been carried out. The keywords ‘Kinorhyncha’, ‘kinorhynch’ and ‘mud dragon’, followed by ‘measurement’, ‘measure’, ‘new species’ and ‘description’ were used in Google Scholar and the Web of Science™ for this survey. For each search, the title, material and methods, species description and morphometric tables of corresponding

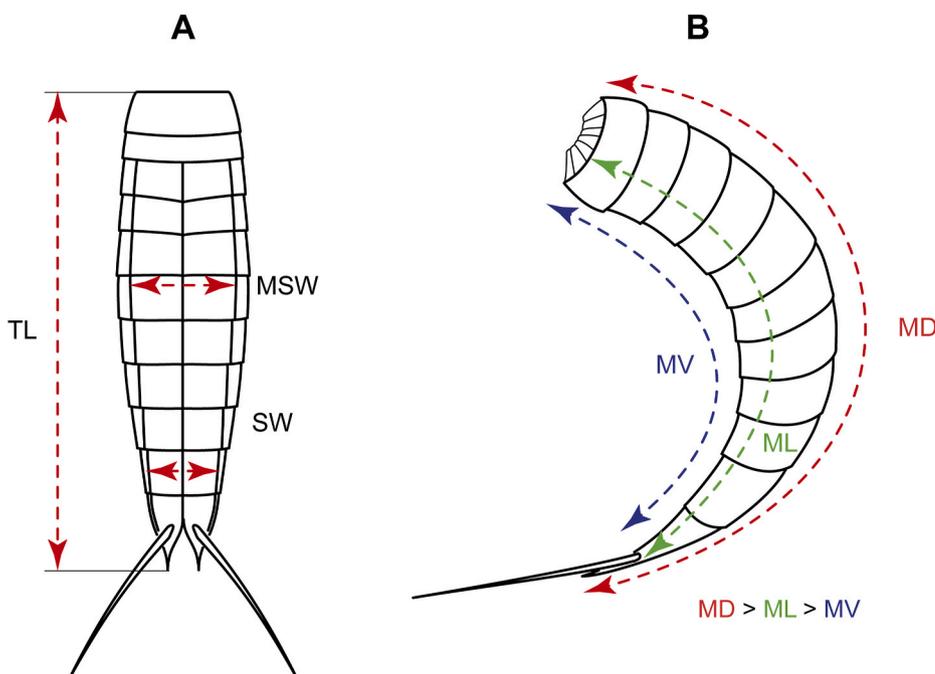
publications were screened to search for measurement definitions and details. The oldest publication that specified how to take a measurement was retained. All relevant papers were carefully consulted to extract all the information on Kinorhyncha measurements.

Not all the information about the measurement uptake methods was included in these publications (e.g. preferred anatomical position to take trunk and segment lengths). Because of that, experts on the phylum, as well as attendees of the VI Scalidophora International Workshop (15–19th August, 2022; Helgoland, Germany) were consulted to compile this missing information.

### 2.2. Morphometric study

To accomplish the objective (iii) of the present study (see Introduction), two species of Kinorhyncha were selected as representatives of the two most diverse families: Echinoderidae and Pycnophyidae. A total of 30 specimens (15 males and 15 females) of each species were chosen from the material stored at the Complutense University of Madrid (UCM) Meiofauna Laboratory. Specimens of *Echinoderes cantabricus* Pardos et al., 1998 were originally sampled in Galicia (north-western Spain, Atlantic Ocean), whereas those of *Setaphyes dentatus* (Reinhard 1881) are from Galicia, Huelva (south-western Spain, Atlantic Ocean) and Tarragona (north-eastern Spain, Mediterranean Sea).

Specific body segments and regions were selected and measured by three independent observers (authors A. González-Casarrubios, D. Cepeda and N. Sánchez of the present contribution). The length of segments 1, 5, 7 and 9 were measured in middorsal, midlateral and ventromedial regions (Figs. 1; 4). These segments were chosen for several reasons: i) the length of the segments in *E. cantabricus* and *S. dentatus* increases progressively towards the posterior trunk, showing no noticeable differences between adjacent segments (see Table 3 in Pardos et al., 1998 and Supplementary Table I in González-Casarrubios et al., In press); ii) only three segments do not follow this trend, showing larger dimensions (segments 1 and 10), or being smaller and hard to measure properly (segment 11) (see section 4.2.1. *Segment length*); iii) segments 2 and 3 tend to overlap each other more than the remaining ones, which makes discrimination of the anterior edge of the pachycyclus and the posterior end of the primary pectinate fringe difficult. In addition, measuring only even or odd segments provides enough



**Fig. 1.** Schematic line art representation of a standardised echinoderid kinorhynch morphology. A: Ventral view showing the most common trunk measurements; B: lateral view showing the three possible lines to take the morphological measurements (note how the total trunk length may vary depending on the selected line due to the natural curved shape of the trunk). Abbreviations: MD, middorsal; ML, midlateral; MSW, maximum sternal width; MV, midventral; SW, standard sternal width; TL, total trunk length.

**Table 1**

Coefficients of variation (CV, %) and average length ( $\mu\text{m}$ ) of *Echinoderes cantabricus* and *Setaphyes dentatus* based on 90 measurements (the same 30 specimens measured by three independent observers). Bold numbers indicate the lowest value among the three regions.

	<i>Echinoderes cantabricus</i>				<i>Setaphyes dentatus</i>			
	Segment 1	Segment 5	Segment 7	Segment 9	Segment 1	Segment 5	Segment 7	Segment 9
<b>CV</b>								
Dorsal	8.5	7.3	<b>6.6</b>	5.4	<b>6.6</b>	9	7.3	7.6
Lateral	<b>6.9</b>	<b>5.8</b>	7	<b>4.2</b>	6.7	<b>6.9</b>	<b>6.9</b>	<b>5.9</b>
Ventral	11	8.1	6.9	7	7.2	7.9	7	8.4
<b>Average</b>								
Dorsal	37	48	54	55	95	72	75	78
Lateral	37	40	46	51	81	68	71	74
Ventral	40	36	43	50	94	69	72	72

information on segment length variability throughout the whole body.

The coefficients of variation of these three regions, which represent the ratio of the standard deviation ( $\sigma$ ) to the mean ( $\mu$ ) as  $\frac{\sigma}{\mu}$ , were compared to determine which one had the lowest variability. Measurements were taken in all cases from the most anterior region of the pachycyclus to the most distal part of the pectinate fringe (see Fig. 2 in Herranz et al., 2012). The measurements of the three observers were combined to calculate the coefficients of variation, giving a total of 90 measurements per segment and position. Finally, in order to avoid the introduction of a factor producing undesirable variability, the left side of the specimens were preferably measured whenever possible (see section 4.2.1. *Segment length*).

Specimens were measured using the Olympus® CellD software in an Olympus® BX51-P microscope with differential interference contrast optics equipped with an Olympus® DP-23 camera. Plots and coefficients of variation were performed in R v.1.1.453 (R Core Team 2022) using the ‘stats’ basic package.

### 3. Results

Concerning the bibliographic search, a total of 140 published sources were checked to extract any information related to morphological measurements, including research articles published in peer-reviewed journals, books or chapter books, and doctoral thesis.

Regarding the morphometric analysis, the coefficients of variation of *Echinoderes cantabricus* were lower in lateral position for segments 1, 5 and 9 and in dorsal position for segment 7 (Table 1). Similarly, those of *Setaphyes dentatus* were lower in lateral position for segments 5, 7 and 9, and in dorsal position for segment 1 (Table 1).

### 4. Discussion

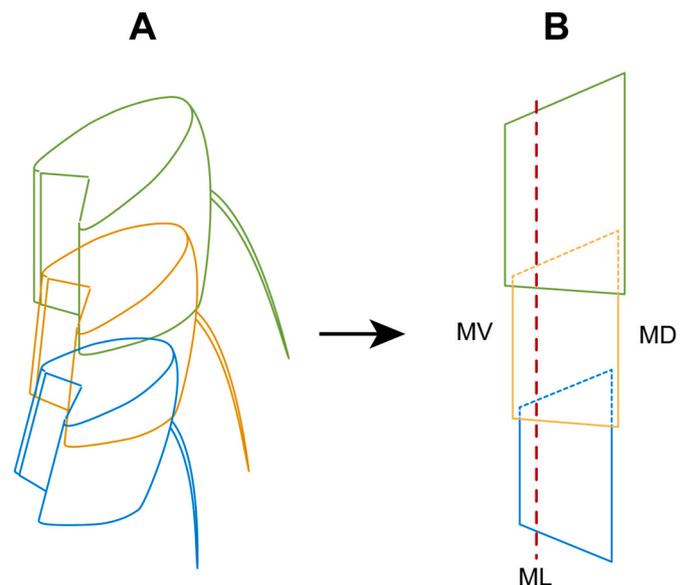
#### 4.1. Biological shape of kinorhynchs and its implication in measurements

Kinorhyncha segments are cylindrical. In fact, the cuticle of the first two segments forms a ring in some taxa (Neuhauser 2013). This is true regarding the segment cuticular composition, since they are single, closed cuticular pieces, but they do not have constant length according to our observations. The dorsal body line of kinorhynchs is longer than the ventral one (Fig. 2), and the overlapping degree of segments is usually greater on the tergal plates due to the contraction of the longitudinal muscles on this side. This is especially evident in Echinorhagata, whose body architecture can be biologically explained by the curvy shape that species show in their natural environment (Fig. 2A - B). As a result of this shape, the length of a segment varies depending on the region at which it is measured, decreasing from the middorsal towards the midventral region (Fig. 1B). These differences are greater in the mid-body segments (where animals show the highest degree of

curvature), especially in Echinoderidae.

Kinorhynch specimens for light microscopy examinations must preferably be mounted in dorsoventral position (Sørensen & Pardos 2020). This mounting process modifies the natural body shape, making it straight rather than curved. Also, it roughly equalises the trunk length in dorsal and ventral position by further overlapping the tergal plates of the segments. However, the way to measure segment length is less sensitive to the forced dorsoventral position of the specimen (although it is somewhat sensitive, see section 4.2.1. *Segment length*). This happens because the anterior and posterior ends of the measurement (i.e. anterior margin of pachycyclus and posterior one of primary pectinate fringe) belong to the same cuticular plate, which has low flexibility.

These differences are crucial because they indicate that not only the way in which the segment is measured matters, but also the anatomical position where the measurement is taken (Table 1, Fig. 1B). Therefore, to compare morphometric data, measurements should be taken in the same region of the segment, whether they were taken by the same or different observers.



**Fig. 2.** Schematic representation of adjacent and isolated segments in the Echinoderidae. A: Segment nesting due to the natural biological shape of Echinoderidae. B: Schematic representation of the overlap of the tergal plates. Abbreviations: MD, middorsal; ML, midlateral; MV, midventral; colours distinguish adjacent segments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

## 4.2. Standardisation of measurements

### 4.2.1. Segment length

We suggest that the segment length must be taken in the midlateral region. This way of measuring is supported by the morphometric study, as the coefficients of variation for both species were generally lower in this region (Table 1).

Another important issue to get accurate measurements is that the initial and final measurement points must be clearly visible. These points are the anterior margin of the pachycyclus and the posterior edge of the primary pectinate fringe (see Fig. 2 in Herranz et al., 2012). The anterior margin of the pachycyclus is relatively easy to observe in dorsal, lateral and ventral positions in species with a well-developed, sclerotised cuticle, but the same does not apply to the end of the primary pectinate fringe. This feature is sometimes difficult to see, especially in old or deteriorated preparations, or in specimens with poorly sclerotised cuticle. In these cases, the easiest position to undoubtedly detect the end of the primary pectinate fringe is the midlateral region.

An exception to this rule is segment 11. This segment is highly variable in morphology among kinorhynch groups, which makes it difficult to take its length in the midlateral region. We suggest that this segment should be measured in the middorsal region, including the end of the tergal extensions (if present).

### 4.2.2. Other measures

The remaining structures traditionally measured in kinorhynchs (total trunk length, sternal widths, cuticular appendage lengths, etcetera) are already defined sufficiently in the literature and hence standardisation is not required. We suggest that all traditional and new morphological measurements should be taken as they appear in the Glossary of the present contribution.

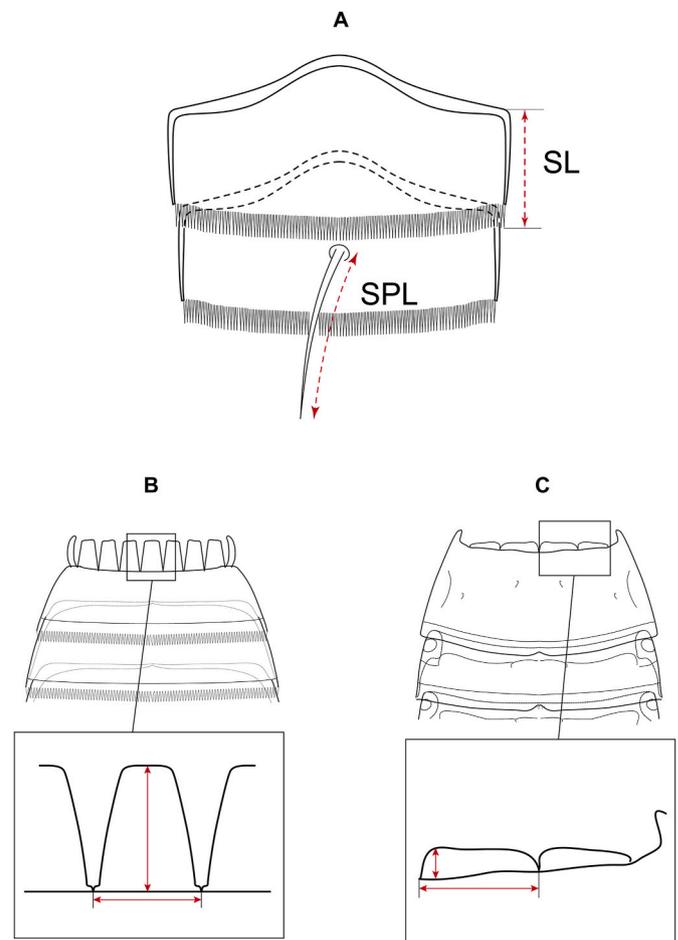
### 4.2.3. New proposed measurements

In addition to the measurements traditionally used (total trunk length, sternal widths, segment lengths, cuticular appendage lengths and placid measurements), we suggest that the following measures be taken:

Cumulative length, defined as the cumulative sum of the segment lengths. The total trunk length depends on the length of the animal, but also on fixatives, position and mounting of the specimens (see section 4.1. Biological shape of kinorhynchs and its implication in measurements). Thus, in a more flattened preparation, the total trunk length will be greater than in one in which the animal has not completely lost its biological bulging shape. We suggest that the total trunk length should continue to be taken, because it gives a good approximation to the length of a living specimen, but that a further addition of the length of the segments (measured as indicated in section 4.2.1. Segment length) should be included. This cumulative length is a fixed length, invariable for every specimen and does not depend on the contraction state of the specimen. Cumulative length can be especially valuable in some instances, for example, species in the *Echinoderes coulli* complex in which segments 11 and 10 can be withdrawn into segment 9 (Kennedy et al., 2022). In such a case the cumulative length can be calculated, and would be more valuable than the trunk length of contracted specimens.

Midsternal plate, defined as the proportions of the midsternal cuticular plate. The proportions of this structure, one of three sternal plates of segment 1 in the family Pycnophyidae, can vary greatly. Among the representatives of the family, we commonly find midsternal plates rather rectangular, but some species have markedly narrowed shapes in the anterior region (Erlenmeyer-flask-like). To reflect these variable proportions numerically, we suggest that the length and anterior and posterior widths of the midsternal plate should be measured.

Length of tergal extensions, and corresponding proportion to the total trunk length (expressed as a percentage). Tergal extensions of segment 11 can vary in both shape and length between species of Echinoderidae. This character is considered diagnostic in some



**Fig. 3.** Schematic representation of measurements in Echinoderidae and Pycnophyidae. A: two consecutive trunk segments of a generalised Echinoderidae, showing the way to measure the length of a segment as well as the way to take a spine length; B: Representation of generalised anterior body region of a species of Echinoderidae with detail of the neck placid to show the recommended way to take the placid measurements (length and width); C: Same representation with a species of Pycnophyidae. Abbreviations: SL, segment length; SPL, spine length.

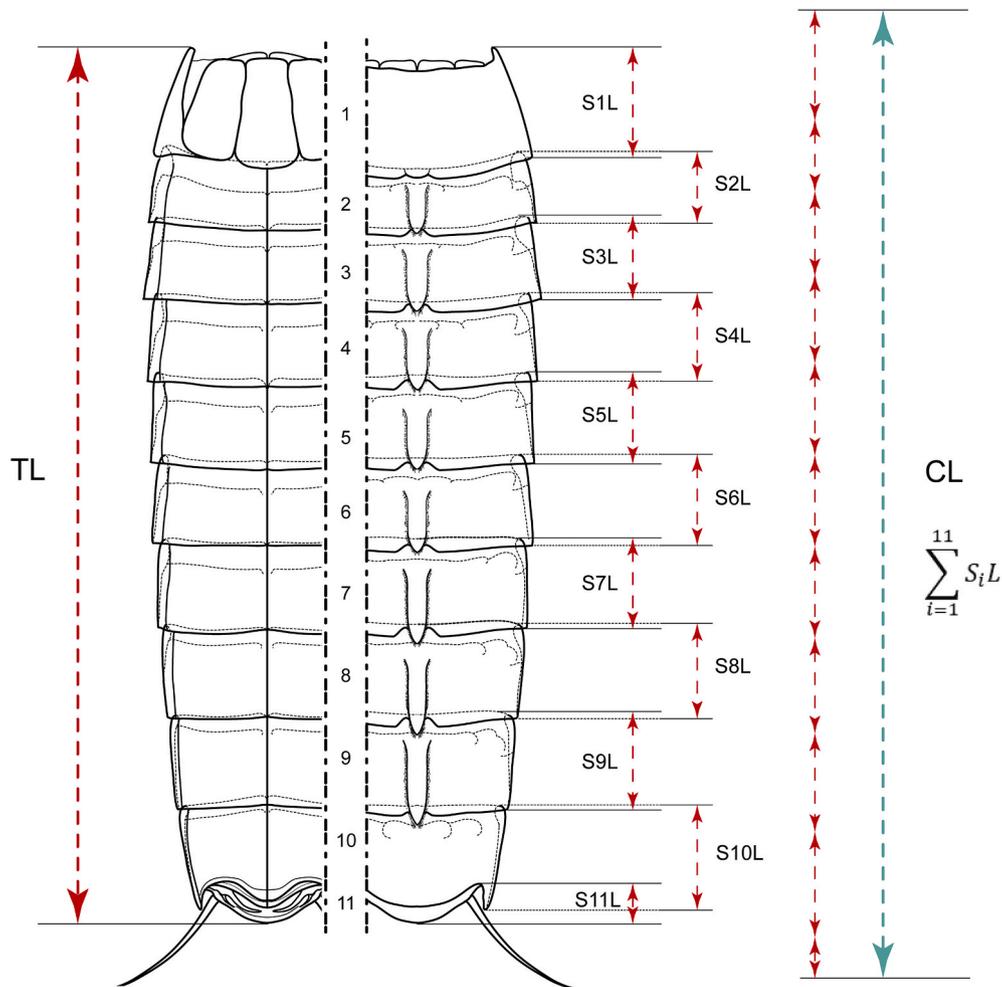
*Echinoderes* Claparède, 1863, including the *Echinoderes spinifurca*-group (Landers & Sørensen 2018; Sørensen et al., 2018). Due to the growing importance of this character in the taxonomy of some Echinoderidae, we propose to take its measure from the base of the extension (i.e. posterior edge of segment 11) to the tip.

## 4.3. Methodology

### 4.3.1. Presentation and analyses of the measurements

According to the Abbe's resolution limit (Abbe 1873), lateral resolution is limited to 200 nm and axial resolution is limited to 500 nm in an ideal light microscope due to the refraction of the light. Thus, measurements in light microscopy usually cannot be more accurate than 1 µm. For this reason, we suggest that all measures are rounded to the nearest unit, unless they are percentages, ratios, or other mathematical operations.

Furthermore, we suggest that the measurement database (see section 4.3.5. Measurement database) should only include values without decimals, and that all morphometric analyses should be carried out with the data presented in this way.



**Fig. 4.** Schematic representation of the total trunk length and cumulative length. Abbreviations: CL, cumulative length; SL, segment length, including the number of the segment; TL, total trunk length; formula:  $\sum_{i=1}^{11} S_i L = S_1 L + S_2 L + \dots + S_{11} L$

#### 4.3.2. Traits to be measured

All morphological measurements included in the Glossary of the present paper may be included in any future taxonomic description of kinorhynchs. New measurements (in addition to those included in the present contribution) can be proposed in future contributions if accurate instructions regarding the procedure are stipulated.

However, the morphological characters with more relevance and which should therefore be included in any taxonomic description of a kinorhynch species (if present) are the following:

1. Cumulative length
2. Total trunk length
3. Maximum sternal width
4. Standard sternal width
5. Segment lengths
6. Spine lengths
7. Tube lengths

#### 4.3.3. Number and type of specimens

Taxonomic descriptions based on a low number of specimens sometimes occur. In these cases, we recommend measuring all available specimens. On the other hand, sometimes a large number of specimens are found. In this case, in order to find a balance between a high time-consuming task and descriptions covering the real spectra of a species variability in terms of measurements, we suggest measuring 30 specimens per species if possible. If this task involves too much time due to

the specific characteristics of the species, a minimum number of 10 specimens must always be measured.

In addition, whenever possible, we recommend measuring the same number of males and females.

#### 4.3.4. Measurement tables

Measurement tables are added in every new description of a kinorhynch since Higgins (1967). However, each author makes the tables in their own way to some extent. This is not a problem itself, because the tables are not necessarily suitable for morphometric analysis (see section 4.3.5. *Measurement database*). Nevertheless, tables arranged differently may disturb readability. Therefore, we suggest a consensus way of presenting the information within tables depending on the number of measured specimens.

We suggest that holotype measurements should be detailed in a separate column as recommended by the ICZN (1999, Recommendation 73C.1). Male and female values should be separated if sexual dimorphism is detected. Also, we consider it important to include ranges, mean values, standard deviations, and number of measured specimens (specifying the number of specimens of each sex if they are not separated), each in a different column. An example of such a table can be found in Sánchez et al. (2019, 2022).

#### 4.3.5. Measurement database

Tables are an excellent way of presenting scientific research data in an organised way. However, excessively long tables may disturb the

readability of a paper, especially if the summarised data are not the objective or the aim results of the research. That is why in the measurement tables, only the ranges and not the individual data of each specimen are proposed. The problem is that these individual data, which are extremely useful for other types of analyses, such as morphometric ones, are unavailable to the scientific community.

We suggest using a common spreadsheet model to include the measurements in the same format by all researchers (see [Appendix A. Supplementary data](#)). In this spreadsheet, only the most used measures for allometric and morphometric analysis will be included. We also suggest that this sheet should be used each time a species is described and uploaded to the Kinorhyncha Measurement Database (González-Casarrubios & Yamasaki, 2022) (<https://sites.google.com/a/meiobenthos.com/laboratory/database/kinorhyncha-measurement-database>). If possible, we also suggest that the file be added as additional material to the taxonomic publication.

In this way, all authors will have access to the individual data in the same format, and it will be much easier to edit and create a dataset for any kind of morphometric analysis. When used, columns should not be deleted, even if the described species does not have some of the characters used in the spreadsheet (these columns will appear as blank spaces). For an efficient search in the database, we recommend naming the additional file as “*Genus\_species\_measurements*” and saving it in.xls/.xlsx format.

## Glossary

This glossary includes a list of the measurements that are currently provided in Kinorhyncha descriptions and the original references. Also, new proposed measurements in the present paper are included.

- Cumulative length. Cumulative sum of the segment lengths (defined in the present contribution, [Fig. 4](#)).
- Length and width of sieve plate (nephridiopore). These two measurements determine the dimensions of the enlarged sieve plate (i.e. nephridiopore) that characterizes the *Echinoderes coulli*-group. Length of this structure, taken from most anterior to most posterior edges, was first introduced by [Lundbye et al. \(2011\)](#), whereas width (taken from the left to the right of the structure) was more recently measured for the first time ([Kennedy et al., 2022](#)). Sometimes, proportion of the sieve plate length to length of corresponding segment (segment 9) is mentioned in the description of the species ([Kennedy et al., 2022](#)).
- Length, maximum and minimum widths of the midsternal plate. These three measurements give an idea of the dimensions and shape of the midsternal cuticular plate in the family Pycnophyidae. The length is measured from the anterior margin to the posterior end of the plate, the maximum width at the most posterior margin of the midsternal plate, and the minimum width at the narrowest area of the midsternal plate. These measurements are defined in the present contribution.
- Length of spines. Spines are measured from base to tip. This term includes the measurement of the dorsal, lateral and terminal series of spines ([Higgins 1967, Fig. 3A](#)). In the case of the lateral terminal spines and the midterminal spines, proportions to the total trunk length are expressed in percentages. In *Echinoderes*, proportion of female lateral terminal accessory spines to lateral terminal spines is frequently included and expressed as a percentage.
- Length of tubes. Tubes are measured from base to tip. This term includes the measurement of the dorsal and lateral series of tubes ([Higgins 1967](#)).
- Maximum sternal width. Measured at the anterior margin of the widest pair of sternal plates ([Higgins 1967, Fig. 1A](#)). Also, proportion to the total trunk length is measured and expressed in percentage.
- Placid height and width. These two measurements give an idea of the dimensions of the neck placids. Placid height is measured from the

base to the top of both dorsal and ventral placids, and placid width is measured at the basal edge of both dorsal and ventral placids ([Fig. 3B - C](#)).

- Segment length. Measured from the anterior margin of the pachycyclus to the posterior end of the primary pectinate fringe ([Zelinka 1928; Fig. 2 in Herranz et al., 2012](#)). After the present contribution, segment lengths should be measured at the midlateral position (for segments 1–10) and the middorsal position (for segment 11).
- Standard sternal width. Measured at the anterior margin of the tenth sternal plates ([Higgins 1967, Fig. 1A](#)). Also, proportion to the total trunk length is measured and expressed in percentage.
- Tergal extension length. This measurement is newly proposed for *Echinoderes* (especially for the *E. spinifurca*-group) and gives an idea of the tergal extension dimensions. The length is captured from the base of the extension (i.e. posterior edge of segment 11) to the tip. Also, proportion to the total trunk length should be calculated and expressed in percentage.
- Total trunk length. Measured on the midline, from the anterior margin of segment 1 to the posterior margin of segment 11, including sternal and tergal extensions if present ([Higgins 1967, Figs. 1A; 4](#)).

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcz.2022.11.015>.

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