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Identification of deep water lantern sharks (Chondrichthyes: Etmopteridae) using morphometric data and multivariate analysis

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Taxonomic distinction to species level of deep water sharks is complex and often impossible to achieve during fisheries-related studies. The species of the genus Etmopterus *are particularly difficult to identify, so they often appear without species assignation as* Etmopetrus *sp. or spp. in studies, even those focusing on elasmobranchs. During this work, the morphometric traits of two species of* Etmopterus, E. spinax *and* E. pusillus *were studied using 27 different morphological measurements, relatively easy to obtain even in the field. These measurements were processed with multivariate analysis in order to find out the most important ones likely to separate the two species. Sexual dimorphism was also assessed using the same techniques, and it was found that it does not occur in these species. The two* Etmopterus *species presented in this study share the same habitats in the overlapping ranges of distribution and are caught together on the outer shelves and slopes of the north-eastern Atlantic.*

Keywords: *Etmopterus*, lantern shark, morphology, multivariate analysis, Squalidae, taxonomy

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INTRODUCTION

The correct taxonomic identification of species provides a critical baseline that supports the rest of the biological research (Last, 2007). Elasmobranchs in general have suffered major taxonomic constraints that have led to identification problems when by-catch and fisheries data are analysed, usually solved by grouping the data in higher (genus or family) taxonomic levels (e.g. Girard *et al.*, 2000; Zeeberg *et al.*, 2006).

The identification of the deep water elasmobranchs is even more problematic because of their morphological similarities and the lack of studies on these groups. Among these, thelantern sharks (genus *Etmopterus*), are particularly problematic because the small size of most species and lack of commercial interest, and even specific elasmobranch studies where most taxa were identified to species level have failed to identify lantern sharks to species level (e.g. McKinnell & Seki, 1998). In the FAO official fisheries data (FAO, 2007) there are only two categories to list lantern sharks (*Etmopterus* spp. and *Etmopterus spinax*) indicating that identification problems occur in this group.

Elasmobranch fish are vulnerable to exploitation, due to their slow growth rates, late maturity and low fecundities (Cortés, 2000), with the potential for overexploitation even at low levels of fishing mortality (Stevens *et al.*, 2000). Commercially exploited deep water shark species are amongst elasmobranchs with the highest risks of extinction (Fowler *et al.*, 2005), and implementing fisheries management or species conservation programmes is impossible when identification is still an issue.

Two species of lantern sharks of the genus *Etmopterus* are common in southern Portuguese waters: the velvet belly lantern shark, *Etmopterus spinax* (Linnaeus, 1758) and the smooth lantern shark, *Etmopterus pusillus* (Lowe, 1839). A third species, *Etmopterus princeps* Collett, 1904, has been recorded in these waters but it has not been recorded in any recent studies that have surveyed the area (e.g. Borges *et al.*, 2001; Erzini *et al.*, 2001; Monteiro *et al.*, 2001; Coelho *et al.*, 2005).

Both these species occur in deep waters, on or near the bottoms of the outer continental shelves and slopes and have the same habitats in their overlapping ranges of distribution; *E. spinax* has been recorded between 70 and 2000 m depth (mostly between 200 and 500 m) and *E. pusillus* between 274 and 1000 m depth (Compagno *et al.*, 2005). *Etmopterus spinax* is restricted to the eastern Atlantic Ocean, including the Mediterranean Sea, whereas *E. pusillus* has a worldwide distribution, having been recorded on both sides of the Atlantic, the western and south-east Pacific, and the western Indian Oceans (Compagno *et al.*, 2005).

Even though both species of lantern shark presented in this study are valid, the morphological differences between them are not very evident and there is a need to determine which morphometric measurements are most useful for researchers to quickly distinguish between them. Even though these species can be separated by studying their exterior marks, there is a need for alternative methods for separating them, especially when identification cannot be done on board the research vessel. The use of multivariate analysis allows the processing of an entire set of morphometric measurements. Furthermore, when each morphometric measurement is contributing very little to the overall differences, multivariate analysis allows for those small differences to be added and analysed as a whole (Clarke & Warwick, 2001). The objectives of the present study were to: (1) determine if the two *Etmopterus* species could be separated using multivariate analysis based on morphological features; (2) determine the most important morphometric measurements for separating the two species; and (3) determine if sexual dimorphism occurs in each species.

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MATERIALS AND METHODS

For this work, a total of 104 specimens were sampled, corresponding to 69 *Etmopterus spinax* and 35 *Etmopterus pusillus*. Both species had samples from both sexes and covered a wide range of lengths (Table 1). The samples were collected between February and June 2003 by commercial fishing vessels using deep water trawls and longlines and operating off the southern Portuguese coast.

Once in the laboratory, specimens were sexed and measured for a total of 27 different morphometric characteristics. These measurements covered all the general body areas of these sharks (head, trunk and tail), including general length measurements, body heights and widths and measurements on each of the fins (Table 2). Most measurements were taken with a digital calliper with 0.01 mm precision, except the largest body measurements that were taken with a 1 mm precision. All measurements throughout this study refer to millimetres and names and abbreviations were defined according to Compagno (2001). All measurements were expressed in % of total length (TL).

For the paired structures, such as the pectoral and pelvic fins, both the left and the right side structures were measured and compared with analysis of covariance (ANCOVA), using TL as the covariate. Once it was determined that no differences occurred between the two sides, only the left side structure was used for the multivariate analysis.

Multivariate analysis was carried out with the PRIMER 6 software (Clarke & Gorley, 2006). A matrix of the morphometric data as percentages of TL was created, and the variables species (*E. spinax* or *E. pusillus*) and sex (male or female) added as factors to test. This matrix was then used to build a similarity matrix, using Euclidean distances. Non-metric multidimensional scaling (MDS) of distances in this similarity matrix was used to visually evaluate differences between the two species and between sexes in each species. Analysis of similarities (ANOSIM) statistical tests were used to assess if the differences observed in the MDS plots were significant, both between species and between sexes. This statistic tests the null hypothesis that no differences occur between groups. In the cases where differences were found, a SIMPER (similarities of percentages) analysis was carried out, in order to determine the percentage of contribution of each morphological measurement to the overall differences. This analysis was carried out until the cumulative differences were higher than 50%.

In order to create a model capable of correctly separating these two species, a discriminant analysis was carried out with the STATISTICA 6 software (Statsoft, 2004). All the morphometric measurements (as percentages of TL) were used and the model was constructed using a backward stepwise approach, where all variables are included in the initial model and variables that contribute least to the prediction of group membership are sequentially removed. Thus, only the most important variables (those that contribute the most to the discrimination of the groups) are kept (Statsoft, 2004).

The coefficients of the discriminant analysis were calculated by determining optimal variable combinations in different functions (canonical analysis). Given that in this case there were only two species (groups), only one canonical function was calculated, given by:

$$Group = a + b_1x_1 + b_2x_2 + \ldots + b_mx_m$$

where a is the constant and b_1 through to b_m are the canonical coefficients of the morphometric variables x_1 through to x_m. The correct identification to the species level of an *Etmopterus* specimen can be obtained by applying this formula.

Table 1. Size (N) and total length (TL, in mm) range of the sample used in this study, for each sex and each species.

Sex	*Etmopterus spinax*			*Etmopterus pusillus*		
	N	Min TL	Max TL	N	Min TL	Max TL
Males	27	143	335	24	223	430
Females	42	120	365	11	231	455
Total	69	120	365	35	223	455

Min, minimum; Max, maximum.

RESULTS

The complete morphometric characterization of the two *Etmopterus* species is presented in Table 3. Some measurements such as pre caudal-fin length (PCL), fork length (FL) or dorsal caudal-fin margin (CDM) were fairly different

Table 2. Morphometric measurements used in this study, with the corresponding abbreviations (Compagno, 2001). All measurements were taken with a precision of 0.01 mm except for measurements marked with an asterisk (*), that were taken with 1 mm precision.

Place of measurement	Abbreviation	Name
Body length	TL*	Total length
	FL*	Fork length
	PCL*	Pre caudal-fin length
	GIR*	Girth
Body height	HDH	Head height
	TRH	Trunk height
	ABH	Abdomen height
	TAH	Tail height
	CPH	Caudal-fin peduncle height
Body width	HDW	Head width
	TRW	Trunk width
	ABW	Abdomen width
	TAW	Tail width
	CPW	Caudal-fin peduncle width
Head	HDL	Head length
	POB	Pre-orbital length
	POR	Pre-oral length
	EYL	Eye length
	ING	Inter-gill length
Caudal fin	CDM	Dorsal caudal-fin margin
	CPV	Pre-ventral caudal-fin margin
	CFL	Caudal-fin fork length
Dorsal fins	D1A	1st dorsal-fin anterior margin
	D2A	2nd dorsal-fin anterior margin
	IDS	Inter-dorsal space
Pectoral fin	P1A	Pectoral-fin anterior margin
Pelvic fin	P2A	Pelvic-fin anterior margin

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Table 3. Morphometric characterization of males and females of *Etmopterus spinax* and *E. pusillus*. All values are presented as percentage of total length (TL), except TL that is given in mm. The mean values and ranges (in parentheses) are given.

Morphometric	*Etmopterus pusillus*		*Etmopterus spinax*	
	Female	Male	Female	Male
TL	365 (231–455)	345 (223–430)	268 (120–365)	239 (143–335)
FL	89.0 (87.7–89.9)	89.4 (88–90.9)	86.4 (83.6–88.6)	86.2 (85.3–87.7)
PCL	80.7 (79.7–82.4)	81.1 (79.5–83.3)	77.2 (72.1–80.1)	76.9 (73.6–78.7)
GIR	32.2 (29.2–36.5)	31.8 (27.5–36.5)	34.7 (29.1–43.0)	33.4 (29.0–39.1)
HDL	23.7 (21.6–25.2)	23.6 (21.5–25.5)	22.5 (20.7–24.7)	22.9 (20.9–25.6)
POB	7.0 (5.7–7.9)	7.1 (6.5–8.3)	7.1 (5.1–8.6)	7.3 (6.2–8.6)
POR	9.9 (9.2–10.7)	9.6 (8.4–11.4)	10.2 (8.5–11.9)	10.3 (9.0–11.7)
EYL	3.6 (3.0–4.1)	3.7 (2.8–4.2)	4.3 (3.3–5.5)	4.1 (3.1–5.2)
ING	5.3 (4.2–6.2)	5.5 (4.6–7.1)	4.9 (3.2–5.9)	5.2 (4.2–7.2)
CDM	18.8 (17.4–19.6)	18.7 (16.0–21.2)	22.5 (20.2–25.9)	22.6 (19.4–24)
CPV	9.8 (8.8–10.8)	9.4 (8.4–10.7)	10.4 (8.9–14.3)	10.3 (8.6–11.7)
CFL	10.4 (9.6–11.4)	10.0 (8.5–11.8)	11.4 (10.4–13.6)	11.5 (10.3–13.7)
D1A	5.5 (5.1–5.8)	6.0 (5.0–9.1)	6.5 (4.7–8.8)	6.3 (5.1–7.6)
D2A	6.8 (5.9–7.3)	7.0 (5.7–9.5)	8.5 (6.2–11.2)	8.5 (7.1–11.2)
IDS	24.2 (22.2–26.5)	24.3 (21.8–26.8)	23.0 (21.0–25.9)	22.8 (16.3–25.9)
P1A	8.4 (6.8–9.9)	8.1 (6.4–9.2)	8.4 (6.5–10.1)	8.6 (6.5–10.2)
P2A	6.3 (5.3–7.6)	6.1 (5.2–7.2)	6.4 (4.9–8.3)	6.6 (5.1–8.6)
HDH	8.2 (7.0–9.4)	8.4 (6.6–10.2)	8.0 (6.9–9.6)	7.4 (6.1–9.1)
TRH	11.0 (8.8–12.2)	10.5 (8.2–12.2)	11.4 (7.1–14.8)	10.4 (6.7–13)
ABH	11.2 (8.5–13.5)	10.1 (7.2–12.3)	13.0 (6.5–17.4)	11.9 (9.0–14.6)
TAH	5.1 (4.2–6.0)	5.1 (4.3–5.8)	6.5 (4.6–9.2)	5.7 (4.8–6.6)
CPH	2.1 (1.7–2.5)	2.1 (1.9–2.5)	2.7 (2.2–3.3)	2.6 (2.2–2.9)
HDW	9.6 (8.6–10.6)	9.0 (7.9–11.1)	10.0 (8.2–11.9)	9.4 (8.0–12.6)
TRW	10.9 (9.5–13.3)	10.2 (8.6–13.5)	11.1 (6.6–14.6)	10.2 (8.5–12.4)
ABW	10.2 (8.2–11.6)	8.9 (6.9–11.3)	11.6 (6.9–15.3)	10.6 (8.3–13.5)
TAW	4.2 (3.2–5.2)	4.5 (3.4–5.7)	5.0 (3.5–6.9)	4.4 (3.6–5.7)
CPW	1.8 (1.6–2.4)	1.8 (1.4–2.4)	1.9 (1.1–2.4)	1.8 (1.4–2.7)

For definition of abbreviations see Table 2.

between species, with a very slight overlapping of the ranges whilst others, such as the pectoral-fin anterior margin (P1A) or the pelvic-fin anterior margin (P2A) were very similar, with the ranges of values mostly overlapped. For some measurements such as abdomen width (ABW), there were large differences between males and females, probably due to the fact that the ABW in females increases proportionally more than in males once maturity is achieved.

In both species, no differences were detected for the measurements taken for the paired structures, namely the left and right side P1A (ANCOVA $_{E.\ spinax}$: $F = 1.01$; $P = 0.32$; ANCOVA $_{E.\ pusillus}$: $F = 0.08$; $P = 0.78$) and the left and right side P2A (ANCOVA $_{E.\ spinax}$: $F = 1.36$; $P = 0.25$; ANCOVA $_{E.\ pusillus}$: $F = 0.64$; $P = 0.43$). Therefore, for the remaining multivariate analysis only the left side structures were considered.

In both *Etmopterus* species, no sexual dimorphism was detected (Figure 1). Statistically, the visual analyses made with the MDS were corroborated by ANOSIM tests, with no differences detected between sexes: ANOSIM $_{E.\ spinax}$: $R = 0.055$; $P = 0.078$ and ANOSIM $_{E.\ pusillus}$: $R = 0.057$, $P = 0.220$.

Given that no sexual dimorphism was detected, the comparison between the two species was made using the sexes combined. The multivariate visualization of the data with a MDS plot produced a clear separation between the species (Figure 2). Statistically, these differences were significant (ANOSIM: $R = 0.491$, $P < 0.01$).

The SIMPER analysis evidenced the morphometrics that contributed most to the species separation (Table 4). A >50% cumulative difference was achieved when 11 morphometric traits were considered: the measurement that individually contributed more for the species separation was the FL, and accounted for 5.94% of the differences, followed by the CDM with 5.84% and PCL with 5.64%.

With the backward stepwise discriminant analysis a model using only 6 variables (morphometrics) was created that adequately explains the variability between the two species (Wilks' Lambda = 0.077; approximately $F = 178.69$; $P < 0.001$). Given that in this case only two groups (species) were being separated, only one canonical function was needed to separate the data. With these canonical coefficients, an equation for identifying the species was created and defined as:

$$Group = -25.51 + 0.52PCL - 0.44CDM + 0.71HDH - 0.75ABH - 2.89CPH + 0.44TRW$$

The centroids of this model were respectively −2.176 for *Etmopterus spinax* and 5.362 for *Etmopterus pusillus* when applied to the original data (Figure 3). The best cutting point is half way between these values (1.593) and in this way it is possible to determine the category (species) of a new observation (specimen) depending on the value obtained. For values greater than 1.593 the specimen is probably *E. pusillus* and if it is lower it is probably *E. spinax*. When a *post hoc* prediction was run on all specimens observed

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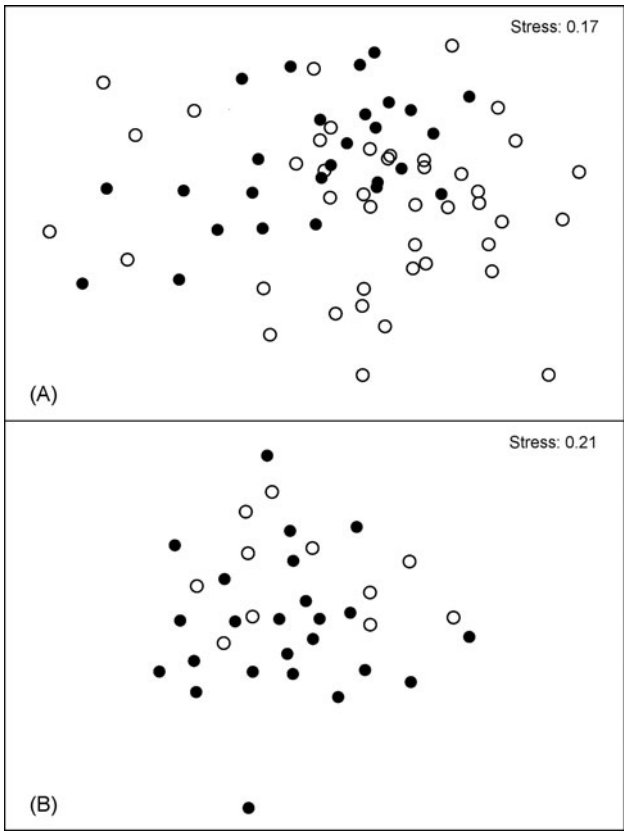

Fig. 1. Non-metric multidimensional scaling of the morphometric differences between males (black dots) and females (white dots) of (A) *Etmopterus spinax* and (B) *Etmopterus pusillus*. The stress value refers to the error that is created when multidimensional data are plotted in two dimensions.

during this study, 100% accuracy was obtained between the model estimated and the actual observed species.

DISCUSSION

This work showed both the importance and difficulty of separating some morphologically similar deep water shark species, in this case from the *Etmopterus* genus. Difficulties in separating elasmobranch catches to species level are common in fisheries and discards studies, and many authors have had to analyse the data considering a higher taxonomic level such as genus or even family. Examples of such cases are the deep water squaloid fishery in northern France where commercialized squaloid sharks are all identified under the same common name (Girard *et al.*, 2000) and the analysis of by-catch from pelagic trawlers in western Africa (Zeeberg *et al.*, 2006). In Portugal, the official fisheries statistics have most of the elasmobranch catch listed under a general 'unidentified sharks and rays' group (DGPA, 2006).

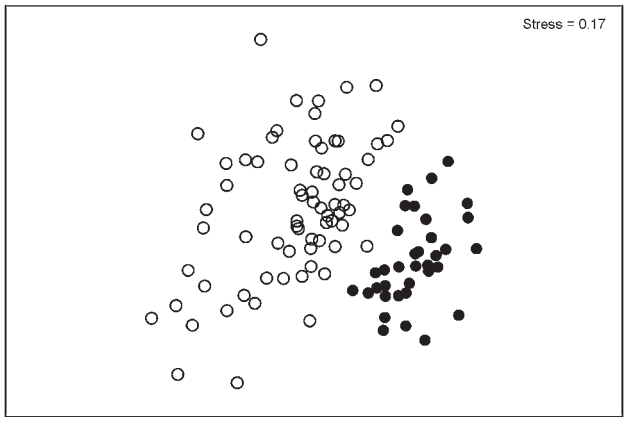

Fig. 2. Non-metric multidimensional scaling of the morphometric differences between the two *Etmopterus* species. White dots refer to *E. spinax* and black dots refer to *E. pusillus*. The stress value refers to the error that is created when multidimensional data are plotted in two dimensions.

The particular case of the genus *Etmopterus* seems to be even more problematic and there are some studies, where most elasmobranch catches have been identified to species level except for the genus *Etmopterus* where specimens were only identified to genus level. Examples of such cases are the studies by Anderson & Clark (2003) on the by-catch of the orange roughy, *Hoplostethus atlanticus*, in New Zealand or the work of McKinnell & Seki (1998) on shark by-catch in a Japanese squid fishery.

However, even though these species are morphologically very similar, they have different ecological life cycles and in order to have accurate fisheries management and conservation programmes, there is a need to correctly discriminate them to species level. In fact, deep water elasmobranch fish are amongst the most vulnerable marine organisms (Fowler *et al.*, 2005) and there is an urgent need for the implementation of efficient management and conservation programmes in the short term that will require fisheries and by-catch data specified to species level.

Even though lantern sharks are mainly discarded species, part of the catch may sometimes be landed and should therefore be recorded by the countries' fisheries statistics. The FAO fisheries statistics (FAO, 2007) have two categories to list lantern sharks: the general 'lantern sharks' group that refers to *Etmopterus* spp. and the 'velvet belly (ETX)' group that refers specifically to *Etmopterus spinax*. Up until 1994 no recordings appeared for either of these groups, meaning that before this date these species were being identified only to family level. Starting in 1995, some catches started to be recorded but most of the problems remained. In the case of Europe, from 1999 to 2005, the *Etmopterus* spp. group recorded 871 tonnes while the *E. spinax* group only recorded 75 tonnes, and this evidences clear limitations of these data sets at two levels: (1) most *E. spinax* are not being correctly identified to species level and therefore are being placed in the general *Etmopterus* spp. group; and (2) the relatively low biomass of these groups indicates that most data are not even being identified to genus level and are probably being placed in the Squalidae family group.

In this work the two *Etmopterus* species studied were well separated by multivariate analysis. Each morphometric measurement contributed relatively little for the overall differences, but when all these small differences were considered together it was possible to separate the species.

The discriminant analysis carried out is typically used to determine which variables discriminate between two or more naturally occurring groups (Statsoft, 2004). In this study, it was possible to create a model that could effectively separate between the two species, in this case using a backward stepwise approach that used only 6 of the 27 possible morphometric measurements. The *post hoc* tests, used to compare

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Table 4. Morphometric measurements that contributed most to the differences found between the two species. Only the morphometrics up to a cumulative difference of >50% are indicated.

Measurement	Average values (% TL)		Differences (%)	
	Etmopterus spinax	*Etmopterus pusillus*	Contribution	Cumulative
FL	86.30	89.26	5.94	5.94
CDM	22.25	18.73	5.84	11.78
PCL	77.07	80.97	5.64	17.43
TRW	10.75	10.44	5.22	22.65
CFL	11.43	10.13	4.52	27.17
D2A	8.51	6.93	4.51	31.68
HDW	9.75	9.23	4.20	35.89
EYL	4.23	3.63	3.86	39.75
HDL	22.69	23.63	3.80	43.55
IDS	22.89	24.28	3.79	47.34
CPH	2.65	2.11	3.79	51.13

For definition of abbreviations see Table 2.

what was observed to what was being predicted by the model showed an accuracy of 100%, meaning that the model created accurately identified all the specimens used for this study. Four morphometric measurements were common both to the SIMPER and to the discriminant analysis and two of them relate the proportion between the specimen length and the caudal-fin length. This fact is important since these measurements are relatively easy to obtain: even in photographed specimens, these measurements can be estimated with relative ease and eventually allow for a posterior identification or confirmation of the species.

Some measurements, such as the abdomen width were clearly not adequate for separating these species. For these particular measurements, there is large intra-specific variability that is mostly related with sex and maturity stage and not so much with species characteristics. Once maturity is achieved, the abdomen width of females increases proportionally more than that of males due to the presence of large oocytes or embryos, and this intra-specific variability prevents these measurements from reflecting specific species characteristics.

No sexual dimorphism was detected in these species. Sexual dimorphism in terms of maximum sizes of Squalidae sharks is common with females usually attaining larger sizes than males and heavier weights for the same size (e.g. Ford, 1921; Wetherbee, 1996; Girard & Du Buit, 1999; Coelho & Erzini, 2005; McLaughlin & Morrissey, 2005). However, the results presented here evidence that even though females might reach larger sizes than males, the body proportions are maintained and therefore no sexual dimorphism is observed, once the effect of growth is removed.

The two *Etmopterus* species studied have overlapped distributions, both in terms of depth and geographical areas, so most fisheries and by-catch studies carried out in the outer shelves and slopes of the eastern Atlantic Ocean are likely to catch both. With this work, a relatively easy way to separate both species based on morphometric traits that can inclusively be applied after the biological sampling procedures, using detailed photographs of the specimens, was demonstrated.

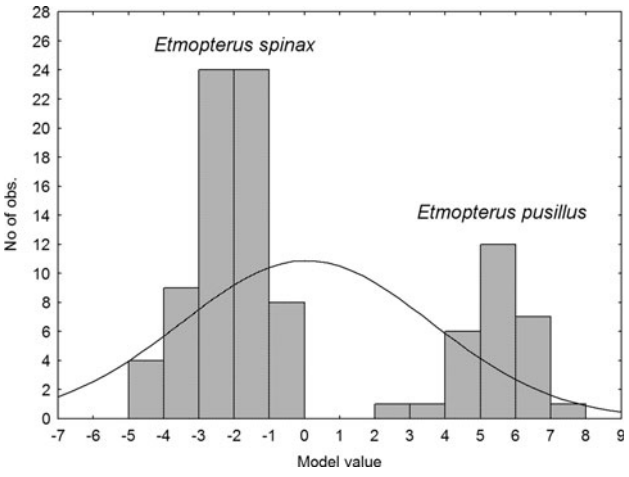

Fig. 3. Histogram of the values obtained when applying the model to the specimens used in this study. The expected normal curve, if only 1 group occurred, is also given.

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