Chapter 15
Molecular Phylogenetic Analysis Confirms the Species Status of *Electra verticillata* (Ellis and Solander, 1786)

Species Status of *Electra verticillata*

Elena A. Nikulina, Hans De Blauwe, and Oscar Reverter-Gil

Abstract *Electra verticillata* was original described by Ellis and Solander ((1786) The natural history of many curious and uncommon zoophytes collected from various parts of the globe by the late John Ellis, systematically arranged and described by the late D. Solander, London), and since then the species status of this bryozoan has been in dispute. Many bryozoologists considered *E. verticillata* as one variety of colony morphology of *Electra pilosa* (Linnaeus 1767). To test the species status of *E. verticillata,* we analysed DNA sequences from material from the Bay of Douarnenez (near Morgat, France), together with sequences from *E. pilosa, E. posidoniae, E. scuticifera, E. indica*, and *Electra omanensis*. Phylogenetic analyses based on fragments of the 18S, 16S and 12S ribosomal RNA genes confirmed the status of *E. verticillata* as a separate species. We also examined the morphology of specimens of *E. pilosa* and *E. verticillata* in various institutions as well as in own collections. This study revealed morphological and ecological differences between these two species and clarified the geographical distribution of *E. verticillata*.

Keywords Taxonomy • Biodiversity • DNA • *Electra pilosa* • Ecology • Distribution

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Introduction

Flustra verticillata was originally described by Ellis and Solander (1786) from the Mediterranean Sea. In the same publication, the authors also mentioned two other species: *Flustra pilosa* (Linnaeus, 1767) and the new species *Flustra dentata*, both from the Atlantic Ocean. Several authors subsequently cited these species (e.g., Gmelin 1789; Bosc 1802; Lamarck 1816). Lamouroux (1816, 1821) erected the genus *Electra* for *F. verticillata*, the history of which is thus significant for members of this genus. Many species of *Electra* were described to be globally distributed, and differences in morphology can reflect geographical variation, but can also represent cryptic species (Nikulina et al. 2007; Nikulina 2008a). Some species of *Electra* were synonymised with other species after their description or, conversely, divided into several varieties (e.g., Farre 1837; Smitt 1867; Norman 1894; Borg 1931), some of which were subsequently accorded specific rank (Powell 1968; Gautier 1954; Nikulina 2008a, b).

Electra and other electrids exhibit a high degree of morphological plasticity, as well as high ecological tolerance. This combination may partially explain the existence of numerous morphological types (Norman 1894; Borg 1931). Since the middle of the nineteenth century, bryozoologists have tended to synonymize *E. verticillata*, *E. pilosa*, and *E. dentata*, or to view them as varieties or subspecies of *E. pilosa* (e.g., Farre 1837; Smitt 1867; Fischer 1870; Norman 1894; Hayward and Ryland 1998). Nevertheless, some zoologists doubted the synonymy of *E. pilosa* and *E. verticillata* (e.g., Bobin and Prenant 1960; Gautier 1962; Cook 1968; d'Hondt and Goyffon 2002). Bobin and Prenant (1960) studied *E. verticillata* from the Bay of Douarnenez and showed that it is similar to yet distinct from *E. pilosa*. Cook (1968) stated that the characters used by Bobin and Prenant (1960) were not representative, but came to the same conclusion. In contrast, investigating enzymatic polymorphism in *E. pilosa* and *E. verticillata* from the Bay of Biscay, d'Hondt and Goyffon (2002) found that the zymograms of the two were identical for the main enzymatic systems. Hence, the taxonomic status of *E. verticillata* and of other varieties or species of *Electra* remained unclear. The status of *E. verticillata* as a valid species is especially important, as it is the type species for *Electra*.

Recent studies employing DNA sequences have resolved similar taxonomic questions (Ryland et al. 2009), including those concerning electrids (Nikulina et al. 2007; Nikulina 2008a). A study of the geographic population structure of the putatively cosmopolitan species *Electra pilosa* and *Einhornia crustulenta* (Pallas, 1766) (formerly *Electra crustulenta*) revealed several morphologically similar species with restricted distributions, including *Electra scuticifera* Nikulina 2008b; *Einhornia korobokkura* (Nikulina, 2006); *Einhornia moskvikvendi* (Nikulina 2008a); and *Electra oligopora* Gordon, 2009, and confirmed the specific status of some varieties, e.g., *Einhornia arctica* (Borg, 1931), but failed to support other varieties (*typica* and *baltica*) as distinct species (Nikulina 2008a).

To test the species status of *E. verticillata*, we sequenced ribosomal RNA genes. Fragments of the mitochondrial 16S and 12S RNA gene were aligned and analysed

with homologous sequences from *E. pilosa*. As the mitochondrial genome represents only a maternal perspective of evolution (Degnan 1993; Palumbi and Baker 1994), we validated our phylogeny based on mitochondrial data by adding a nuclear marker, part of the 18S rRNA gene, which has been broadly used in phylogenetic inference (Hillis and Dixon 1991; Grechko 2002; Halanych and Janosik 2006). Our study included four other species similar and closely related to *E. pilosa*: *Electra posidoniae* Gautier, 1954; *Electra scuticifera* Nikulina 2008b; *Electra indica* Menon and Nair, 1975; and *Electra omanensis* Nikulina et al. (this volume). The more distantly related *E. crustulenta* and *E. korobokkura* were used as outgroup taxa. Although phylogenetic relationships within electrids remain unknown, the genus *Einhornia* Nikulina 2007 was assumed to be most likely the sister taxon to our ingroup (Nikulina et al. 2007). Morphology was studied by using scanning electron microscopy (SEM) to reveal differences between *E. verticillata* and *E. pilosa*. The distribution of *E. verticillata* was revised using data from the literature, museum specimens, and our own material.

Material and Methods

Sampling

Colonies of *E. verticillata* were collected from the Atlantic coast of France (Morgat and St-Jean-de-Luz), Spain (Ria de Coruña), and Portugal (Praia de Falésia). A fragment of a colony in the Bay of Douarnenez, near Morgat (48°14′N 4°29′W) (Fig. 15.1) was preserved in 98% ethanol for molecular genetic analyses. The colony occurred in a hole eroded in the rock beneath a steep cliff bordering a sandy beach. The hole was situated among rocks at the edge of the beach, was partly filled with sand, and was probably only exposed to the air during extreme low tides. The zooids covered a slender, ramifying seaweed, with the colony forming a nearly complete sphere about 30 cm in diameter. Water entered and left the hole through a gully. The base of the colony extended into the sand, forming a mat of stolons about 2 cm thick and filled with sand grains.

Table 15.1 lists the samples included in the study. For the morphological and morphometric analyses, colonies of *E. pilosa* were collected from the Atlantic coast of France (Bay of Arcachon), The Netherlands (Eastern Scheldt estuary), and Ireland (Galway Bay).

Other Material Examined

For revision of the geographic distribution of *E. verticillata*, material in various institutions was also examined: Muséum National d'Histoire Naturelle, Paris

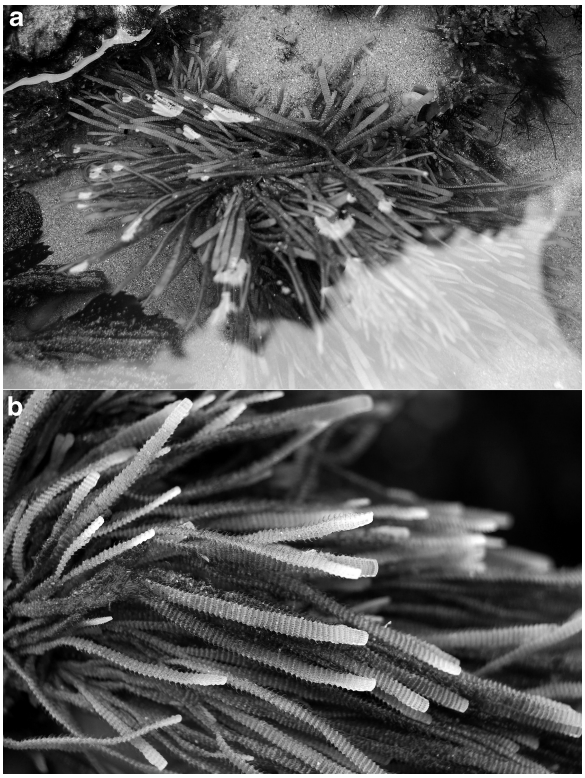

Fig. 15.1 *Electra verticillata* in situ, Bay of Douarnenez, Morgat, France; we used this sample in the molecular phylogenetic study

(MNHN); Natural History Museum, London (NHMUK); Museo Nacional de Ciencias Naturales, Madrid (MNCN); Manchester Museum (MM).

Molecular Techniques

Total DNA was extracted from ethanol-preserved colonies (about 10–20 zooids) using the Qiagen DNEasy Tissue Extraction Kit. Part of the 18S gene was amplified using primers we designed, U-18-4F (AGGAGTGGAGCCTGCGGCTTAA-TTTGACTC-3) and U-18-4R (AGGTTCACCTACGGAAACCTTGTTACGAC-3). Part of the 16S gene was amplified using the universal metazoan primers 16Sar and 16Sbr (Palumbi et al. 1991) or primers we designed, 16SF4 (CTCGGCAAAGAAGGGCTCCGCCTGTTTATCAAAAACAT-3) and 16SLr (TTCTCTTTTTCTGTTCCTTTCGTAAT-3). We designed 12S primers based on protostome sequences in the EMBL Nucleotide Sequence Database: 12SF-I

Table 15.1 Specimens included in the molecular phylogenetic study, gene fragments with GenBank accession numbers, and sample locality information

Species	12S	16S	18S	Locality
E. verticillata	FR754521	FR754524	FR754534	NW Atlantic, Bay of Douarnenez, Morgat
E. pilosa (1)	FR754511	AJ971066*	FR754527	NW Atlantic, North Sea, Helgoland
E. pilosa (2)	FR754512	AJ971065*	AM075768*	NW Atlantic, North Sea, Helgoland
E. pilosa (3)	FR754513	AJ971067*	FR754530	NW Atlantic, North Sea, Helgoland
E. posidoniae (1)	–	AJ971084*	AM75770*	Mediterranean, Adriatic Sea, Pab
E. posidoniae (2)	FR754514	AJ971085*	AM75771*	Mediterranean, Mallorca
E. scuticifera (1)	–	AM886854*	AM886854*	IW Pacific, Tasman Sea, Maori Bay
E. scuticifera (2)	FR754515	AJ971086*	FR754533	IW Pacific, Tasman Sea, West Coast
E. omanensis	FR754510	FR754522	FR754525	Indian Ocean, Arabian Sea, Oman coast
E. indica	–	FR754523	–	Indian ocean, Arabian Sea, Kerala Bay
E. crustulenta	–	AJ853844*	AM92413*	Western Baltic Sea, Lolland
E. korobokkura	FR754516	AJ853947*	AM158087*	NW Pacific coast, Hokkaido, Akkeshi Bay

An asterisk indicates a sequence from GenBank

(GGAAAAAATTGTGCCAGCADCCGCGGTTA-3) and 12SRLen (CACTTTCA-AGTACGCCTACTGTGTTACGAC-3).

Amplifications were performed in 25 μl of PCR mixture (20 mM Tris–HCl, 10 mM (NH4)2SO4, 10 mM KCl, 2 mM MgSO4, 0.1% Triton X-100, pH 8.8) containing 0.5 units Taq polymerase (New England Biolabs), 200 mM dNTPs, 0.5 mM primers, and 1 μl template DNA. Cycling parameters were as follows: 94°C for 2 min; 35 cycles of 94°C for 30 s, 45°C (54°C for 18S) for 30 s, and 72°C for 40 s; and 7 min at 72°C. PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) and sequenced directly in both directions using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI3100 automatic sequencer.

Phylogenetic Analyses

Sequences were assembled and edited using SeqMan and EditSeq software (DNASTAR Lasergene). ClustalX (Thompson et al. 1997) was used with default settings to perform automatic sequence alignments. Phylogenetic analyses were undertaken using PAUP* v4.0b10 (Swofford 2000) and MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). Phylogenetic trees were obtained using the maximum parsimony (MP), maximum likelihood (ML), neighbour-joining (NJ), and Bayesian (BA) methods. Nodal support was estimated by bootstrapping (Felsenstein 1985), with analyses of 1,000 pseudoreplicates by full heuristic searches. MP analyses were conducted by heuristic searches with the tree-bisection-reconnection

branch-swapping algorithm (Swofford and Olsen 1990). A first tree was obtained by random addition of sequences, 200 replicates were then generated, and 10 trees were kept for a search for the most parsimonious trees. Gaps were treated as missing data. ModelTest v.3.6 (Posada and Crandall 1998) was used to find the model of DNA substitution that best fit the data (Posada and Buckley 2004). The parameters of this best-fit model selected with the Akaike information criterion (Posada and Buckley 2004) were subsequently used for ML and NJ analyses in PAUP* and BA analyses in MrBayes. For ML analyses, we used a full heuristic search, with 100 random-addition replicates and search parameters as described for the MP analysis. Bayesian inference of phylogeny was conducted using MrBayes running four Metropolis-coupled Markov-Chain Monte-Carlo chains (MCMC) simultaneously for 10,000,000 generations, sampling every 100th generation. Each Markov chain was started from a random tree. The MCMC output was analysed with TRACER v1.3 (Rambaut and Drummond 2004); 10% of the samples were discarded as burn-in; 90,000 samples were used to estimate parameters, parameter variance, and the posterior probabilities of particular nodes to construct a majority rule consensus tree.

Morphological Study

Parts of colonies *of E. verticillata* and *E. pilosa* were dried, coated with Pd-Pt, and photographed with a SciScan scanning electron microscope. The following zooidal characters were measured from SEM images: zooid length and width, opesia length, and opesia inclination. Two specimens of *E. verticillata* (Bay of Douarnenez and Praia de Falésia) and three colonies of *E. pilosa* (Eastern Scheldt Estuary, Arcachon Bay, and Galway Bay) were measured. Zooid length and width, and opesia length, were measured on 20 zooids, and opesia inclination on 10 zooids, for each colony studied. All statistical calculations and tests to evaluate the significance of morphological differences were conducted with PAST software (Hammer et al. 2001). We prepared the permutation *t*-test with 10,000 permutations to test the equality of means, and the Mann–Whitney *U* test to test the equality of medians.

Results

Phylogenetic Analysis

18S rRNA gene. The alignment of ten 18S sequences was 510 bp long, with 18 variable and seven parsimony-informative sites. No variability was found within any of the species, and therefore the data set consisted of six unique sequences,

corresponding to six species: *E. verticillata, E. omanensis*, *E. scuticifera* (1, 2), *E. posidoniae* (1, 2), *E. pilosa* (1–3), and the outgroup taxon *E. korobokkura*. An MP analysis using equally weighted characters yielded a single tree 32 steps long, with a consistency index (CI) of 1 and retention index (RI) of 1. ML, NJ, and BA analyses were conducted using the F81 model of nucleotide substitution (Felsenstein 1981) with a gamma shape parameter of 0.0062 and estimated nucleotide frequencies of A = 0.19080, C = 0.2771, and G = 0.2955. The ML and NJ analyses resulted in single trees. A BA majority rule consensus tree was created. All trees were completely resolved and had identical topologies, and included the highly supported (88–100%) clade (*E. verticillata* (*E. omanensis, E. scuticifera*)). *Electra posidoniae* and *E. pilosa* formed a sister clade with lower nodal support (76–95%). Figure 15.2a shows the Bayesian phylogram, with nodal support values from all four methods.

16S rRNA gene. The alignment of 11 sequences was 447 bp long, with 190 variable and 49 parsimony-informative sites. The data set consisted of 11 unique sequences obtained from seven species: *E. verticillata, E. omanensis*, *E. indica*, *E. scuticifera* (1, 2), *E. posidoniae* (1, 2), *E. pilosa* (1–3), and the outgroup taxon *E. korobokkura*. An equally weighted MP analysis yielded a single tree 331 steps long, with the CI = 0.8218 and RI = 0.8343. The tree topology was stable to Goloboff's weighting, and the best-fit tree was obtained with k = 0 (Goloboff fit = −112.8333). The HKY85 model of nucleotide substitution (Hasegawa-Kishino-Yano 1985) with a gamma shape parameter of 0.2648, a transition/transversion ratio of 2.0386, and assumed nucleotide frequencies of A = 0.3654, C = 0.1431, G = 0.1693 was used in the ML, NJ, and BA analyses. All trees from the NJ, ML, and MP analyses were identical in topology and consisted of two sister clades: the highly supported (98–100%) clade (*E. posidoniae, E. pilosa*), and the less well supported (77–94%) clade (*E. scuticifera* (*E. verticillata* (*E. omanensis, E. indica*))). A Bayesian majority rule consensus tree was created. Figure 15.2b shows the Bayesian phylogram, with nodal support values from all four methods.

12S rRNA gene. The alignment of eight sequences was 575 bp long, with 329 variable and 187 parsimony-informative sites. The data set consisted of six unique sequences obtained from *E. verticillata, E. omanensis*, *E. scuticifera* (2), *E. posidoniae* (2), *E. pilosa* (1–3), and the outgroup taxon *E. korobokkura*. An equally weighted MP analysis yielded a single tree 581 steps long, with the CI = 0.8468 and RI = 0.6888. The tree topology was stable to Goloboff's weighting, the best-fit tree was obtained with k = 0 (Goloboff fit = −143.5). The general time-reversible model of nucleotide substitution (Rodriguez et al. 1990) with estimated base frequencies of A = 0.3577, C = 0.1687, G = 0.1886, a specified substitution rate matrix, and a gamma shape parameter of 0.4938 was used in the ML analysis. A similar model was applied to construct NJ and BA trees. All methods yielded an identical tree topology, consisting of two sister clades with high nodal support (85–100%): (*E. posidoniae, E. pilosa*) and (*E. omanensis* (*E. scuticifera* (*E. verticillata*))). Figure 15.2c shows the Bayesian phylogram, with nodal support values from all four methods.

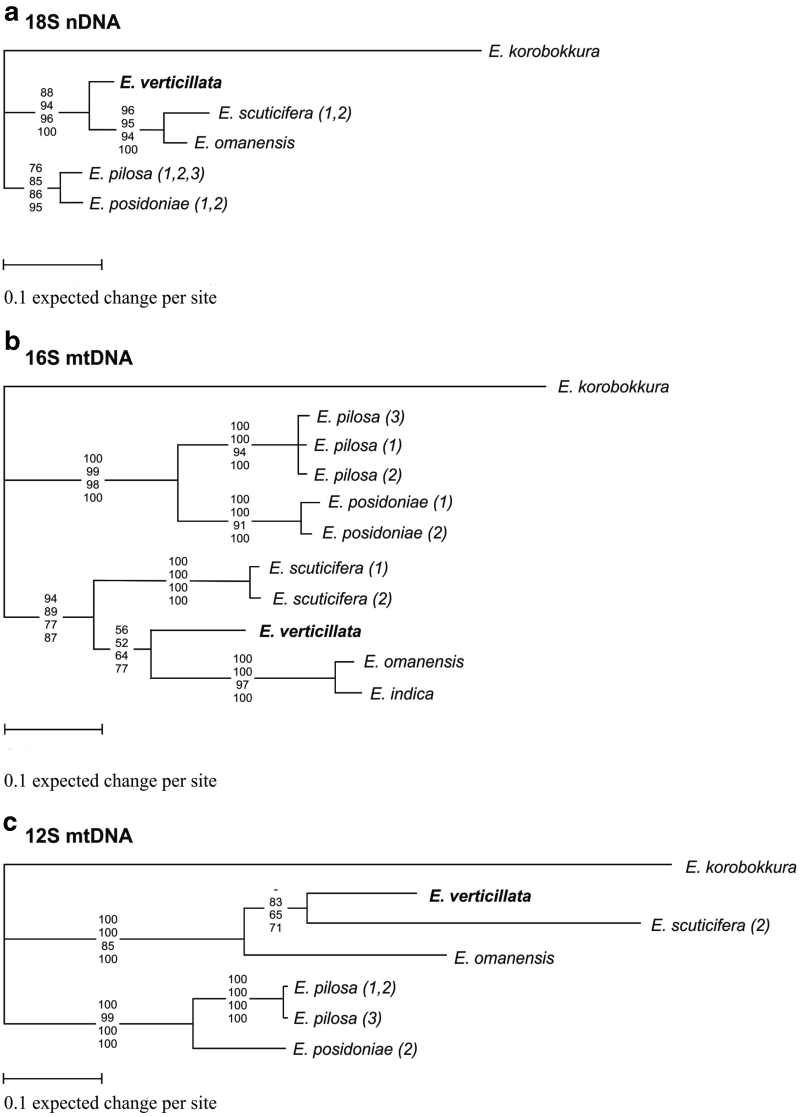

Fig. 15.2 Phylograms constructed from 18S (**a**), 16S (**b**), and 12S (**c**) data. The topology depicts the strict (**a**, **b**) or majority rule (**c**; MP analysis provided 70% bootstrap support for the [*E. verticillata, E. omanensis*] clade) consensus between the MP, NJ, ML and Bayesian trees. The four-number columns at nodes show bootstrap support values and posterior probabilities from the MP, NJ, ML, and BA analyses, respectively (*top* to *bottom*). Branch lengths were calculated with the Bayesian approach

Morphology of Electra verticillata

The colony of *E. verticillata* forms erect tufts (Fig. 15.1). The base of the colony consists of linear stolons, highly branched and matted, forming a more or less dense mat up to 2 cm thick. The stolons are made up primarily of kenozooids and more or less degraded autozooids. The kenozooids are sometimes very large, without visible differentiation, with simple ectocyst, non-calcified and devoid of pores. An underdeveloped opesia, operculum, pores, or spines may be present. The stolons attach to solid substrates (shells, seaweed, etc.) by means of young buds, each of which can develop into an autozooid. Numerous erect branches grow from the encrusting base; these are cylindrical to ribbon-like and consist of autozooids arranged in regular verticils, each composed of five autozooids in encrusting branches and up to 15 zooids in free tufts (Fig. 15.3a, b). The tufts are bilaminar (Fig. 15.3c). Branching may occur by simple separation of the autozooidal series into two branches. Most frequently, new branches arise from a lateral bud (Fig. 15.3d); this gives rise to two autozooids jointed back to back, which in turn give rise to four autozooids in the next generation.

Autozooids have the shape of an obliquely truncate cone, with the truncation corresponding to the opesia (Fig. 15.3e). The opesia is rounded rectangular, slightly elongate, and occupies about half the zooidal length. The edge of the opesia bears five (rarely six) spines, largely chitinous: one proximal, pointed, curved above the opesia, rarely exceeding the length of the autozooid, frequently more prominent in the zooids in the laterals of the verticil; a shorter pair at the proximolateral corners of the opesia; and a distolateral pair, poorly developed, on the lateral walls level with the hinges of the operculum. The rim around the opesia distal to this last pair of spines projects slightly distally. The marginal sclerite of the operculum is narrow, light brown, the bulge at the hinge bending sharply toward the midline and often decorated with various extensions. The cryptocyst is thin and transparent, well developed proximally, tapering laterally, sometimes surrounding the opesia distally. The gymnocyst is smooth, translucent, with numerous rounded pseudopores; pseudopores are absent in the most proximal area. Communication is via pore chambers or multiporous plates.

When the polypide is retracted, the long oesophagus forms a curve that prolongs the cardiac loop and represents the most proximal part of the polypide, because the cardia is relatively short. There are 9–13 (usually 11 or 12) short tentacles that do not exceed the proximal edge of the opesia when the polypide is retracted.

The ancestrula is smaller and flatter than autozooids, with a rounded base; its gymnocyst is uniformly perforated by 50–60 pores. It bears the five spines seen in astogenetically mature zooids, plus an additional two thin spines, one on each side lateral to the main spine (ancestrulae of *E. pilosa* invariably have five spines). The ancestrula buds two or three daughter autozooids, and subsequent growth tends to form linear branching series appressed to one another laterally and connecting by lateral septula; the autozooids are lined up in transverse rows starting from the second generation. This growth can lead to a very regular whorled arrangement of

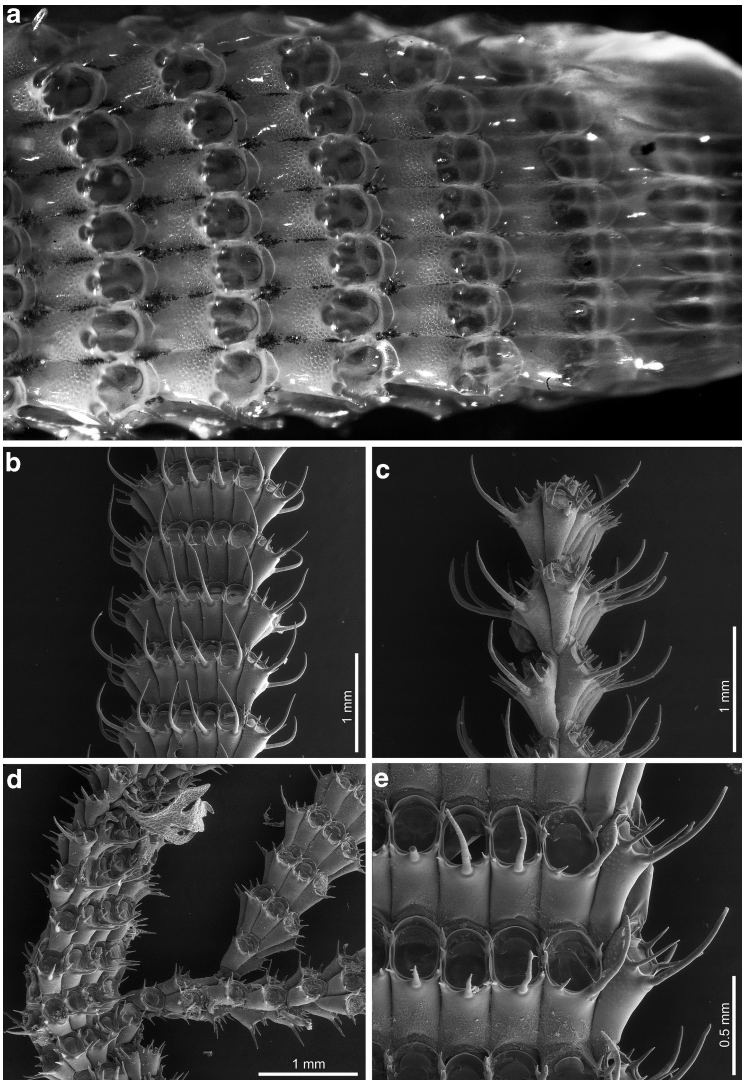

Fig. 15.3 *Electra verticillata*. (**a**) Colony from Bay of Douarnenez, Morgat, France; light microscopy. (**b**–**e**) Details of the branch structure of *E. verticillata*; SEM images of bleached samples from Praia de Falésia, Portugal (**b**, **c**); Arcachon Bay, France (**d**); and Bay of Douarnenez, France (**e**) (From the collection of Hans De Blauwe)

tufts, parts of which encrust algae (Fig. 15.4b), with bilaminar branches arising from the crusts (Fig. 15.3). For comparison, the morphology of *E. pilosa* is shown in Fig. 15.4c–f.

Table 15.2 lists measurements of some zooidal characters for *E. verticillata* and *E. pilosa*. The statistical analyses indicated statistically significant differences

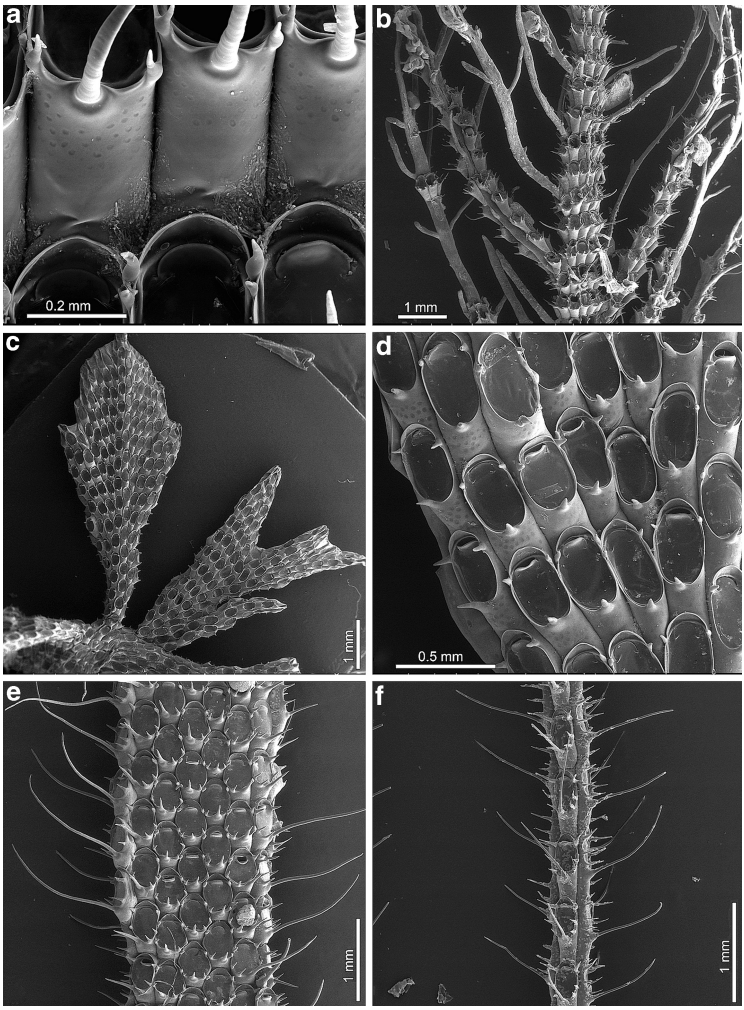

Fig. 15.4 *Electra verticillata* (**a**, **b**) and *E. pilosa* (**c**–**f**). (**a**) Pseudopores on the gymnocyst of *E. verticillata* (Bay of Douarnenez, Morgat, France). (**b**) Colony of *E. verticillata* overgrowing an alga (Bay of Douarnenez, St-Jean-de-Luz, France). (**c**–**e**) Erect, bifoliate branches in *E. pilosa* incrusting an algal frond, demonstrating some variation in zooidal arrangement; specimens are from the Eastern Scheldt Estuary, The Netherlands (**c**, **d**) and Galway Bay, Ireland (**e**, donated by Marco Faasse). (**f**) *Electra pilosa* encrusting a hydroid stem (Arcachon Bay, France) (From the collection of Hans De Blauwe)

between *E. verticillata* and *E. pilosa* in three characters: zooid length (greater in *E. verticilata*), opesia length (lesser in *E. verticillata*), and opesia inclination (greater in *E. verticillata*) (Table 15.3), all at a significance level of $p < 0.001$. The assumption of similarity was not rejected for zooid width, as the significance level was $p > 0.05$.

Table 15.2 Measurements for zooids of *E. verticillata* and *E. pilosa*. Two colonies of *E. verticillata* (Bay of Douarnenez, France; Praia de Falésia, Portugal) and three colonies of *E. pilosa* (Bay of Arcachon, France; Eastern Scheldt estuary, The Netherlands; Galway Bay, Ireland) were used for measurements

	E. verticillata	*E. pilosa*
Zooid length (mm)		
N	40	60
Range	0.52–0.68	0.47–0.66
Mean	0.61	0.57
Median	0.63	0.57
Standard error	0.009	0.010
Standard deviation	0.041	0.045
Zooid width (mm)		
N	40	60
Range	0.24–0.30	0.20–0.29
Mean	0.27	0.25
Median	0.27	0.25
Standard error	0.004	0.007
Standard deviation	0.017	0.029
Opesia length (mm)		
N	40	60
Range	0.27–0.34	0.37–0.47
Mean	0.32	0.42
Median	0.32	0.42
Standard error	0.004	0.006
Standard deviation	0.017	0.028
Opesia inclination (°)		
N	20	30
Range	42–70	30–45
Mean	57	39
Median	59	40
Standard error	3.0	1.6
Standard deviation	9.3	6.1

Discussion

Phylogenetic Analyses

Phylogenetic analyses of mitochondrial and nuclear ribosomal genes demonstrated not only that *E. verticillata* is a species distinct from *E. pilosa*, but also that these two species are not directly related. *Electra verticillata* belongs to a Pacific group of *pilosa*-like species (*E. scuticifera, E. indica,* and *E. omanensis*) with high nodal support (77–100%, mean 93%), although the relationship among these species remain unresolved, as the three genes provided different results regarding relationships (Fig. 15.2).

Table 15.3 P-values in the permutation *t*-test and Mann–Whitney *U* test

	Zooid length	Zooid width	Opesia length	Opesia inclination
Permutation *t*-test	<0.0001	0.08	<0.0001	<0.0001
Mann–Whitney *U* test	$2.8 \cdot 10^{-5}$	0.06	$1.8 \cdot 10^{-16}$	$6.3 \cdot 10^{-9}$

Morphological Differences Between E. verticillata *and* E. pilosa

Electra verticillata and *E. pilosa* are discriminated by zooidal characters and colony morphology. Zooids of *E. verticillata* have a more conical shape due to the steep incline of the opesia to the frontal plane; the zooids are significantly longer and opesiae are significantly shorter than in *E. pilosa* (Tables 15.2 and 15.3). The ratio of opesium to zooid length is about 0.5 in *E. verticillata* and 0.7 in *E. pilosa*. The most conspicuous differences in colony morphology are the very regular verticillate zooid arrangement in erect branches of *E. verticillata* and a well-developed stolonial system, compared to quincuncial zooid arrangement and absence of stolons in *E. pilosa*.

Electra pilosa encrusts various substrata, sometimes giving rise to two-layered branches (Fig. 15.4c–f) that are sporadically ribbon-like. De Blauwe (2009) illustrates an encrusting (his Fig. 159) and a folious colony part (his Fig. 158) of *E. pilosa*. The arrangement of autozooids is normally quincuncial, but may change with the nature of the substratum, and in contrast to *E. verticillata* only sporadically appears somewhat verticillate. The possibility for the colony to produce erect parts, where some zooids are arranged in more or less transversal rows, can lead to confusion with *E. verticillata*.

Bobin and Prenant (1960) and Prenant and Bobin (1966) first described stolons in *E. verticillata*. Although Marcus (1926) experimentally induced stolon production in *E. pilosa*, they are not normally present in this species. The structure of stolons and their function in propagation of the colony are similar between these two species and other electrids such as *Einhornia arctica* (Borg, 1931) and *E. korobokkura* (Nikulina 1999, 2006), in which stolons are, however, very rare.

Ecological Notes

Both *E. verticillata* and *E. pilosa* produce cyphonautes larvae, though the larvae of *E. verticillata* are not well known (Bobin and Prenant 1960). In the southern North Sea, *E. pilosa* is the first species to colonise new substrates such as wrecks and foundations of offshore wind turbines (De Blauwe unpublished). Bobin and Prenant (1960) suggested that in *E. verticillata*, colony tufts are annual and are regenerated from the perennial stolonal system every year; colonies are therefore less likely to originate from ancestrulae. The long-lived larvae of *E. pilosa* and their potential to settle on almost any substrate makes this species a successful coloniser, whereas

Fig. 15.5 Distribution of *E. verticillata*, reconstructed from data in the literature data and material in collections (see Supplements 15.1 and 15.2)

E. verticillata seems to be more selective with regard to substrate choice (rocks, sand, and algae are needed), but the stolonial system is a good survival strategy once a colony is established in a suitable habitat.

Electra verticillata can adhere to stones, shell fragments, and especially algae, particularly *Gracilaria gracilis* (Stackhouse) M. Steentoft, L.M. Irvine and W.F. Farnham 1995, on which the most luxuriant colonies develop; we have never seen *E. verticillata* encrusting hydroids, as Hayward and Ryland (1998) described.

Bobin and Prenant (1960) provided detailed information on the ecology of *E. verticillata* at two stations in the Bay of Douarnenez, and described the habitat of the species and its commonly used substrates. The ecological conditions and morphology of the colony we found in the Bay of Douarnenez (see Material and Methods) near these previous two stations were identical to those Bobin and Prenant (1960) described and seem to be typical for this species.

Geographical Distribution of E. verticillata

Analysis of material in museum collections, data from the literature, and some unpublished sources presented in Supplements 15.1 and 15.2 allowed us to map the geographic range of *E. verticillata* (Fig. 15.5). The species occurs along the Atlantic coast of the Iberian Peninsula, the Atlantic coast of North Africa, and in the Western Mediterranean. Cook (1968, 1985) reported *E. verticillata* at various stations along the West coast of Africa, reaching South Africa, but a detailed study of the original material will be necessary to confirm Cook's records. *Electra verticillata* has not been found north of Brittany, France (Bobin and Prenant 1960) and is thus absent from the North Sea. Populations in situ are probably absent between Hendaye near the French-Spanish border and Brittany, as suitable habitat is rare along this coast. Bobin and Prenant (1960) searched unsuccessfully for *E. verticillata* in the bay and channels of Arcachon, but found neither colonies nor ancestrulae on *G. gracilis,* the most common algal substrate for *E. verticillata*. Along this coast, colony fragments are beached regularly, probably originating from populations along the north coast of Spain.

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Supplements

Suppl. 15.1 Atlantic records of *E. verticillata* from North to South

Station	Date	Source	Checked or identified by
France, Roscoff	No data	MNHN-12147, MNHN-12162	Bobin, ORG
France, Brittany"Finistère"	1826	Bobin and Prenant (1960), pl I. Fig. 7	P and B
France, Rade de Brest	No data	Guérin-Gavinet (1911)	No data
France, Bay of Douarnenez	No data	Bobin and Prenant (1960)	P and B
France, Brittany, Morgat	29/03/2010	This paper	HDB
France, Island Re	No data	de Beauchamps (1923)	P and B
France, Dept. Gironde, Arcachon and SW France	No data	Fischer (1870)	Fischer
France, Arcachon	03/08/2001	HDB (unpublished)	HDB
France Basque coast, Hendaye	No data	Bobin and Prenant (1960)	P and B
France Basque coast, Northeast of Hendaye	No data	d'Hondt and Goyffon (2002)	d'Hondt and Goyffon
France Basque coast, St-Jean-de-Luz	12/08/2001	HDB (unpublished)	HDB
France Basque coast, Bidart	11/1935	D'Hondt (1987)	D'Hondt
France Basque coast, Biarritz	04/09/1909	NHMUK-1882.7.7.10	ORG
		MNHN-5974	ORG
France Basque coast, Biarritz	No data	Station Biologique d'Arcachon (in Bobin and Prenant 1960)	P and B
Spain, Santander	No data	Barroso (1912)	Álvarez
		MMC 3/M/60	
		MMC 3/M/61	
Spain, Asturias, Gijón	1923	MNCN-25.03/24	Álvarez
Spain, Galicia: several localities in the North and South	No data	Reverter-Gil and Fernández-Pulpeiro (2001)	ORG
Portugal, not specified	No data	NHMUK-1897.5.1.486	ORG
Portugal: Oporto	No data	NHMUK-1897.5.1.485	ORG
Portugal: Matosinhos, Figueira da Foz, Nazaré	No data	ORG (unpublished)	Javier Souto
Portugal: Albufeira, Praia da Falésia	03/2004	HDB (unpublished)	HDB
Morocco: Mohammedia (Fedhala)	No data	Canu and Bassler (1925)	P and B
Morocco: Medina (Mogador)	No data	Canu and Bassler (1925)	P and B
Canary Islands	No data	Arístegui-Ruiz (1984)	Arístegui-Ruiz
Mauritania: Cabo Blanco	No data	O'Dea and Okamura (2000)	O'Dea and Okamura
Gabon, Pointe Noir		Cook (1968, 1985)	Cook
Lagos		Cook (1968, 1985)	Cook

MMC Museo Marítimo del Cantábrico, *NHMUK* Natural History Museum, London, *MNHN* Muséum National d'Histoire Naturelle, Paris, *MNCN* Museo Nacional de Ciencias Naturales, Madrid, *P and B* Prenant and/or Bobin, *HDB* Hans De Blauwe, *ORG* Oscar Reverter-Gil

Suppl. 15.2 Mediterranean records of *E. verticillata* from west to east

Station	Date	Source	Checked or identified by
Spain, Andalusia: La Atunara	No data	López de la Cuadra and García-Gómez (1988)	ORG by photographs
		López de la Cuadra (1991)	
Spain, Castellón	No data	Rioja lo Bianco (1920)	Rioja lo Bianco
		Barroso (1921)	Barroso
		Zabala (1986)	Zabala
Spain, Catalonia: Sitges	05/1962	NHMUK-1965.8.18.16	ORG
		NHMUK-1975.7.18.53	ORG
Spain, Malaga	1862	Zoological museum of CAU, Kiel	No data
France, Camargue	1966, 1980, 1983	Harmelin	Harmelin
France, Nice (Nizza)	No data	Carus (1893) (Risso)	No data
Islas Baleares, Mahón	No data	Zabala (1986)	Zabala
Italy, Mare della Toscana	No data	Carus (1893)	No data
Sicily	No data	Gautier (1958)	Gautier

MMC Museo Marítimo del Cantábrico, *NHMUK* Natural History Museum, London, *MNHN* Muséum National d'Histoire Naturelle, Paris, *MNCN* Museo Nacional de Ciencias Naturales, Madrid, *P and B* Prenant and/or Bobin, *HDB* Hans De Blauwe, *ORG* Oscar Reverter-Gil

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