



ORIGINAL ARTICLE

Discrete genetic boundaries of three *Streblospio* (Spionidae, Annelida) species and the status of *S. shrubsolii*

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Abstract

Spionid polychaetes are a dominant component of benthic assemblages in marine and estuarine habitats and are important in assessing ecological function and integrity. Ecological and biogeographic studies rely upon the ability to distinguish between morphologically similar species, as well as knowledge of evolutionary relationships between species. Unfortunately, differentiating between congeneric species of spionids can be challenging thereby potentially creating mistakes in scientific investigations. For example *Streblospio* contains three recognized species (*S. benedicti*, *S. shrubsolii*, *S. gynobranchiata*) with few distinguishing morphological characters. Herein, we use ~500 bases of the mitochondrial cytochrome c oxidase subunit I gene (COI) to investigate the status of *S. shrubsolii* relative to other *Streblospio*. COI has previously been used to distinguish North American *Streblospio benedicti* and *S. gynobranchiata*. Results indicate all three *Streblospio* species are reciprocally monophyletic with *S. shrubsolii* basal to a well-supported *S. benedicti*/*S. gynobranchiata* clade.

Key words: *Annelida*, *COI*, *mtDNA*, *phylogeny*, *poecilogony*, *Polychaeta*

Introduction

The polychaete family Spionidae includes ca. 1000 described species in 34 genera (Blake 1996; Sigvaldadóttir et al. 1997). These worms are widely distributed in marine and estuarine habitats and are found from intertidal zones to deep canyons (Foster 1971). Spionids are found in calcareous substrates, rock crevices, and a wide diversity of sedimentary habitats throughout the world's oceans (Blake 1996). Because of physiological and reproductive characteristics of Spionidae (e.g. short generation times, regenerative and asexual reproductive capabilities, and a diversity of sexual reproductive and developmental modes), they are often characterized as density dominants in benthic assemblages (Blake & Arnofsky 1999). Numerous spionid species are classified as euryhaline opportunists and indicative of a variety of stressed environments, making them useful indicators of environmental alterations (Boesch 1977; Pearson & Rosenberg 1978; Dauer et al. 1992; Weisberg et al. 1997; Ritter & Montagna

1999). As such, proper identification can be critical to accurately assessing biodiversity or changes in ecosystems.

In addition to their use in environmental survey and monitoring studies, *Streblospio* species are also noteworthy for their developmental diversity. A range of developmental modes associated with brooding occur in *Streblospio* including (1) release of planktotrophic larvae, (2) release of lecithotrophic larvae, (3) direct development and (4) poecilogony (Levin 1984; Levin et al. 1991; Levin & Bridges 1995; Schulze et al. 2000).

Currently, three species are recognized in *Streblospio*: *S. benedicti* Webster, 1879, *S. shrubsolii* Buchanan, 1890 and *S. gynobranchiata* (Rice & Levin 1998). All individuals within *Streblospio* possess a single pair of branchiae on the first setiger, a dorsal hood on setiger two, and notosetae only as capillaries (Fauchoald 1977; Rice & Levin 1998; Dauer et al. 2003).

Streblospio benedicti described from the northeast region of North America (Webster 1879)¹ is reported

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Table I. Taxa included in this study, collection localities, and GenBank accession numbers.

Species	Sample #	Sample/haplotype collection locality	Genbank accession number	
<i>Streblospio benedicti</i>	Bened_Laf_River_1	Lafayette River, Norfolk, VA	EU151697	
	Bened_Laf_River_2	Lafayette River, Norfolk, VA	EU151698	
	Bened_Piver_River_1	Bogue Sound, Beaufort, NC	EU151699	
	Bened_Piver_River_3	Bogue Sound, Beaufort, NC	EU151700	
	Bened_Piver_River_6	Bogue Sound, Beaufort, NC	EU151701	
	Bened_Tar_River_2	Tar Landing, Atlantic Beach, NC	EU151702	
	Bened_Ware_River_1	Ware River, Gloucester County, VA	EU151703	
	Bened_Ware_River_4	Ware River, Gloucester County, VA	EU151704	
	GB1*	Galveston Bay, TX	AF138926	
	GB2*	Galveston Bay, TX	AF138927	
	GB3*	Galveston Bay, TX	AF138928	
	GB4*	Galveston Bay, TX	AF138929	
	GB5*	Galveston Bay, TX	AF138930	
	BS2*	Bogue Sound, NC	AF138934	
	BS3*	Bogue Sound, NC	AF138935	
	BS4*	Bogue Sound, NC	AF138936	
	BS5*	Bogue Sound, NC	AF138937	
	BS6*	Bogue Sound, NC	AF138938	
	BS7*	Bogue Sound, NC	AF138939	
	BSL2*	Bogue Sound, NC	AF138940	
	NO1CH1BS1BSL1*	Norfolk, VA, Charleston, SC, Bogue Sound, NC	AF138941	
	CH2*	Charleston, SC	AF138942	
	CH3*	Charleston, SC	AF138943	
	CH4*	Charleston, SC	AF138944	
	DB2CH5*	Daytona Beach, FL, Charleston, SC	AF138945	
	DB3*	Daytona Beach, FL	AF138946	
	DB4*	Daytona Beach, FL	AF138947	
	SF1*	San Francisco, CA	AF138948	
	SF2*	San Francisco, CA	AF138949	
	TS1*	Tijuana Slough, CA	AF138950	
	TS2DB1*	Tijuana Slough, CA, Daytona Beach, FL	AF138951	
	TS3*	Tijuana Slough, CA	AF138952	
	TS4*	Tijuana Slough, CA	AF138953	
	TS5*	Tijuana Slough, CA	AF138954	
	<i>Streblospio gynobranchiata</i>	AR3*	Alafia River, FL	AF138906
		AR7*	Alafia River, FL	AF138907
		AR4*	Alafia River, FL	AF138908
		AR5*	Alafia River, FL	AF138909
		AR6*	Alafia River, FL	AF138910
		AR1HR1*	Alafia River, FL, Hillsborough River, FL	AF138911
		HR3*	Hillsborough River, FL	AF138913
HR4*		Hillsborough River, FL	AF138914	
VB1*		Vero Beach, FL	AF138915	
VB2*		Vero Beach, FL	AF138916	
VB3*		Vero Beach, FL	AF138917	
VB4*		Vero Beach, FL	AF138918	
MB1GB6*		Mobile Bay, AL, Galveston Bay, TX	AF138919	
MB2*		Mobile Bay, AL	AF138920	
MB3*		Mobile Bay, AL	AF138921	
MB4*		Mobile Bay, AL	AF138922	
MB5*		Mobile Bay, AL	AF138923	
MB6*		Mobile Bay, AL	AF138924	
MB7*		Mobile Bay, AL	AF138925	
GB7*		Galveston Bay, TX	AF138931	
GB8*	Galveston Bay, TX	AF138932		
GB9*	Galveston Bay, TX	AF138933		
<i>Streblospio shrubsolii</i>	shrub_1	Tagus River, Portugal	EU151707	
	shrub_2	Tagus River, Portugal	EU151713	
	shrub_4	Tagus River, Portugal	EU151717	
	shrub_5	Tagus River, Portugal	EU151705	
	shrub_6	Tagus River, Portugal	EU151718	
	shrub_7	Tagus River, Portugal	EU151706	
	shrub_8	Tagus River, Portugal	EU151719	
	shrub_11	Tagus River, Portugal	EU151708	

Table I (Continued)

Species	Sample #	Sample/haplotype collection locality	Genbank accession number
	shrub_12	Tagus River, Portugal	EU151720
	shrub_13	Tagus River, Portugal	EU151709
	shrub_14	Tagus River, Portugal	EU151710
	shrub_16	Tagus River, Portugal	EU151711
	shrub_17	Tagus River, Portugal	EU151712
	shrub_20	Tagus River, Portugal	EU151714
	shrub_21	Tagus River, Portugal	EU151715
	shrub_22	Tagus River, Portugal	EU151716
<i>Marenzelleria viridis</i>	Mv_1**	Eastern North Sea/Baltic Sea	DQ309273
	Mv_2**	Baltic Sea	DQ309274
<i>Marenzelleria arctia</i>	Ma_1**	Baltic Sea	DQ309271
	Ma_2**	Baltic Sea	DQ309272
<i>Marenzelleria neglecta</i>	Mn_1**	Baltic Sea	DQ309263
	Mn_2**	Baltic Sea	DQ309264
	Mn_3**	eastern North Sea	DQ309265
	Mn_4**	eastern North Sea	DQ309266
	Mn_5**	Baltic Sea	DQ309267
	Mn_6**	Baltic Sea	DQ309268
	Mn_7**	Baltic Sea	DQ309269
	Mn_8**	Baltic Sea	DQ309270
<i>Prionospio steenstrupi</i>	Pr_s1*	–	AF138955
<i>Prionospio</i> sp.	KJO-2005***	Monterey Bay, CA	DQ209266
<i>Scololepis squamata</i>	Sc_s1*	–	AF138956

*Schulze et al. (2000); **Bastrop and Blank (2006); ***Osborn et al. (2007).

from estuarine and coastal waters of the Atlantic coast of North America, the Gulf of Mexico and the Caribbean (Levin 1984; Foster 1971). Occurrences of *S. benedicti* from the Pacific coast of North America and from the North Atlantic coast of Europe are generally considered to represent introductions (Carlton 1979; Fonseca-Genevois & Cazaux 1987; Schulze et al. 2000).

Streblospio shrubsolii was originally described from the northern coast of England (Buchanan 1890) and is recorded from the Mediterranean, the Atlantic coast of Europe, and the Baltic Sea (see Dauer et al. 2003). The species was later placed as a junior synonym of *S. benedicti* based solely upon morphological considerations (Foster 1971; Light 1978). Cazaux (1985) reported direct development in *S. shrubsolii*, but this is not necessarily a compelling distinction due to the known occurrence of poecilogony in *S. benedicti* (Levin 1984). However, Fonseca-Genevois & Cazaux (1987) argued the two taxa should be regarded as distinct species because of differences in the number of hooded hooks and the segment where these hooks first appear, but specimens intermediate in these setal characteristics have been reported (Garcia-Abberas & Rollo 2004). *Streblospio benedicti* and *S. shrubsolii* have the same functional ciliary groups on their palps (Dauer et al. 2003), but they differ in the distribution of prostomial papillae and the shape of an interbranchial tentacle (Dauer et al. 2003).

The third species, *Streblospio gynobranchiata*, was originally described from the Gulf of Mexico and southern Florida and is morphologically indistinct from *S. benedicti* except in reproductive females (Rice & Levin 1998; Schulze et al. 2000). Recent reports of *S. gynobranchiata* in the eastern Mediterranean Sea have been attributed to an introduction, most likely through ballast water release (Çinar et al. 2005, 2006).

The goal of this study was to assess the taxonomic status of *S. shrubsolii* as it relates to the other recognized *Streblospio* species. Building on previous work that distinguished North American *S. benedicti* and *S. gynobranchiata* (Schulze et al. 2000), we use ~500 bases of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene to molecularly characterize these three morphologically similar species.

Materials and methods

Table I lists taxa included in this study, collection localities, and GenBank accession numbers. All novel samples included in this investigation were preserved in 95% non-dantaured EtOH and morphologically identified by HKM and/or DMD.

DNA was extracted using the DNeasy tissue kit (Qiagen) according to the manufacturer's protocols. Approximately 500 bases of the cytochrome c oxidase subunit I (COI) gene were amplified using the Folmer et al. (1994) primers and the Schulze et al. (2000) protocol. PCR products were purified

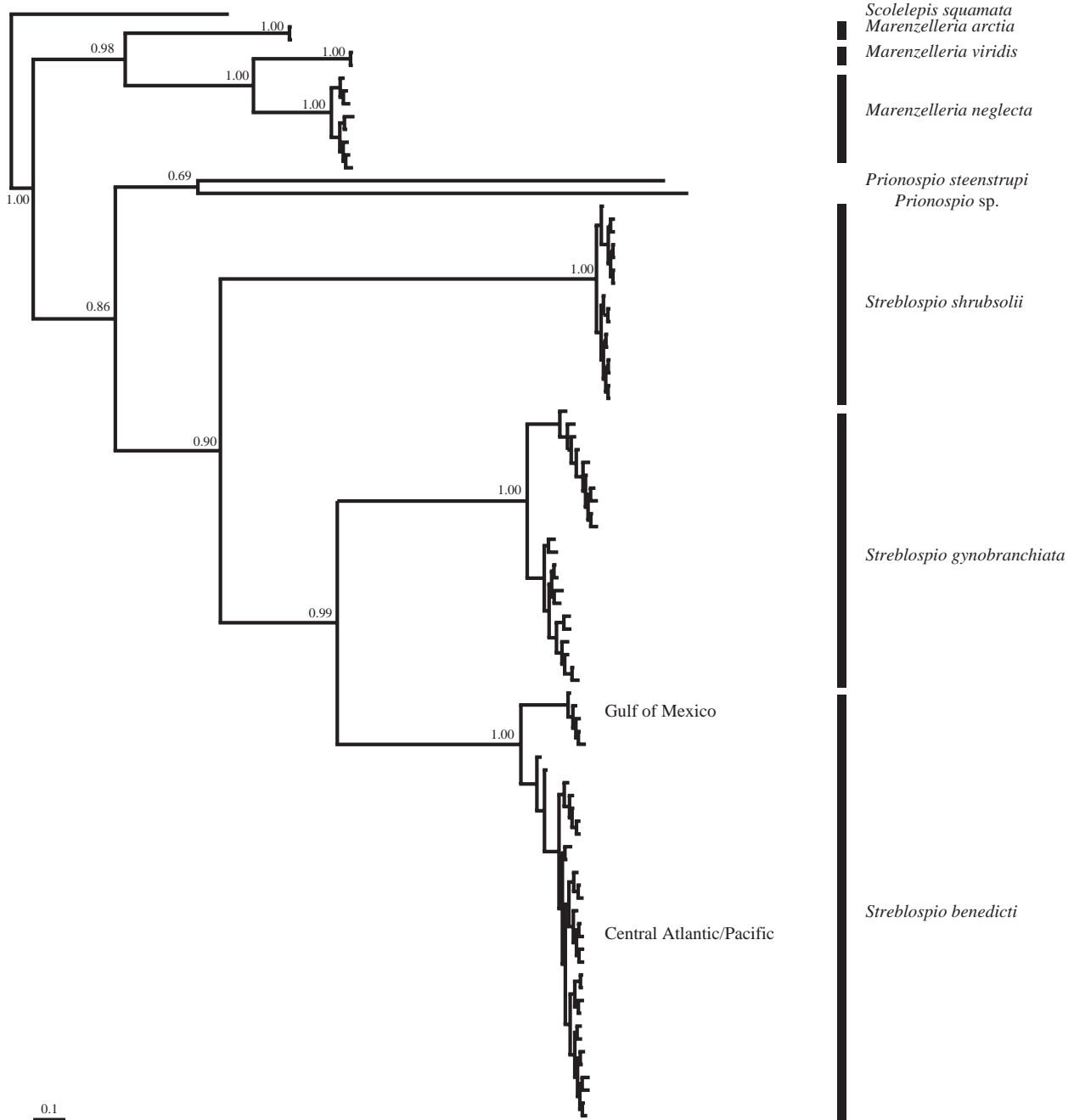


Figure 1. Bayesian inference consensus topology. Posterior probabilities for major clades are shown next to relevant node. Details of the analysis are given in the text. Within *Streblospio benedicti*, geographic origins for species are noted (Gulf of Mexico or Central Atlantic/Pacific).

with the Qiaquick PCR purification kit (Qiagen). Sequences were generated using Genome Lab™ Quick Start Mix (Beckman Coulter) and bidirectionally sequenced on a Beckman CEQ 8000 Genetic Analysis System (Beckman Coulter). Herein, we sequenced 8 individuals of *Streblospio benedicti* and 16 of *S. shrubsolii*. We also included 29 haplotype sequences of *S. benedicti* and 22 of *S. gynobranchiata* from Schulze et al. (2000). Note that the four Vero Beach, FL haplotypes from Schulze

et al. (2000) were morphologically identified as *S. benedicti* but were molecularly characterized as *S. gynobranchiata*. Thus, they are designated as *S. gynobranchiata* in this investigation (as in GenBank). Additional data included as outgroups in this study were chosen based on available sequences for other spionid taxa and included members of the genera *Marenzelleria*, *Prionospio*, and *Scolelepis* (see Table I).

All sequences were aligned using ClustalX (Thompson et al. 1997) and proofread using

MacClade (Maddison & Maddison 2000). Aligned data are available at TREEBASE (www.treebase.org). Uncorrected (p) values of sequence divergence both within and between groups were calculated in MEGA 3.1 (Kumar et al. 2004). Bayesian analysis was performed using MrBayes 3.1 (Huelsenbeck & Ronquist 2001) employing the GTR+I+G model as suggested by MrModeltest 2.2 (Nylander 2004). Two sets of four unlinked chains, three heated and one cold, were run for 3×10^6 generations being sampled every 1000 generations. Based on the convergence of likelihood scores, the first 300 trees (10%) were discarded as *burn-in*. The outgroup *Scolecipis squamata* was used to root the resultant topology.

Results

The final dataset consisted of 499 base pairs of mtDNA COI gene for 72 ingroup and 14 outgroup operational taxonomic units (OTUs). Within *Streblospio*, 163 (33%) of the COI characters were variable and 151 (30%) were parsimony informative. Table II gives maximum and average within group and between group uncorrected pairwise differences (p). Within *Streblospio* species, the uncorrected (p) value ranged from 0.44 to 2.60%. Between *Streblospio* species, the average uncorrected (p) value calculated was 19.68% (ranging from 15.51 to 22.50%). This value is comparable to that found between the three *Marenzelleria* species (also in the family Spionidae; mean 14.69%) included as outgroups in this investigation.

Figure 1 shows the Bayesian inference consensus tree with posterior probabilities (PP) >0.90 indicated on the relevant branches. *Streblospio* is monophyletic with nodal support (PP) of 0.90. Likewise *S. benedicti*, *S. gynobranchiata*, and *S. shrubsolii* were all found to be reciprocally monophyletic (PP = 1.00 for all). As discussed in Schulze et al. (2000), *S. benedicti* forms two clades, one from the Central Atlantic/Pacific and one from the Gulf of Mexico. All novel *S. benedicti* sequences reported here are found in the

Central Atlantic/Pacific clade. Noteworthy, the three *Marenzelleria* form a monophyletic clade (PP = 0.98).

Discussion

Mitochondrial COI sequence data confirm the status of *Streblospio shrubsolii* as a valid species that is evolutionarily distinct from *S. benedicti* and *S. gynobranchiata* despite relatively minor morphological differences. All three taxa are clearly delineated as monophyletic clades with large interclade distances (Table II, Figure 1). Thus, our results support the validity of *S. shrubsolii* and are consistent with previous morphological (Fonseca-Genevois & Cazaux 1987; Dauer et al. 2003), ecological (Sardá & Martin 1993) and reproductive (Cazaux 1985) investigations. Previous studies synonymizing *S. shrubsolii* with *S. benedicti* (Foster 1971; Light 1978) appear to be incorrect as sequence divergences of *S. shrubsolii* and congeners are comparable to other well-established species.

Interestingly, the European *S. shrubsolii* is basal to (PP = 0.99) the two North American *Streblospio* species. The sister relationship between *S. benedicti* and *S. gynobranchiata* is not surprising given the close association reported for many marine species along the Atlantic and Gulf coasts of North America (Avisé 1992, 1994). However, the uncorrected genetic distances between these two *Streblospio* species are considerably greater (roughly 3–10 times greater) than other Atlantic/Gulf of Mexico sister relationships (reviewed in Avisé 1992). Presumably the greater genetic divergence represents either an early speciation event or much faster rates of mtDNA substitution than seen in other marine and coastal species of the area. Unfortunately, spionid fossils that could be used to calibrate a molecular clock are lacking. Thus we are not able to estimate timing of lineage-splitting events and/or rates of mtDNA evolution in this investigation.

Streblospio gynobranchiata, *S. benedicti*, and *S. shrubsolii* are routinely found in temperate near shore marine and estuarine shallow water sediments, and

Table II. Average uncorrected (p) distances between species. Intraspecific distances shown on diagonal when available.

	<i>Marenzelleria viridis</i>	<i>Marenzelleria arctia</i>	<i>Marenzelleria neglecta</i>	<i>Scolecipis squamata</i>	<i>Prionospio steenstrupi</i>	<i>Prionospio</i> sp.	<i>Streblospio shrubsolii</i>	<i>Streblospio benedicti</i>	<i>Streblospio gynobranchiata</i>
<i>M. viridis</i>	0.00%								
<i>M. arctia</i>	17.40%	0.00%							
<i>M. neglecta</i>	10.56%	16.10%	1.01%						
<i>S. squamata</i>	19.60%	19.38%	17.56%	–					
<i>P. steenstrupi</i>	25.99%	25.55%	24.66%	22.69%	–				
<i>Prionospio</i> sp.	24.23%	24.45%	24.85%	24.23%	28.19%	–			
<i>S. shrubsolii</i>	24.33%	22.63%	24.24%	24.51%	27.78%	26.28%	0.44%		
<i>S. benedicti</i>	21.76%	21.04%	21.06%	20.76%	23.71%	24.83%	21.04%	1.95%	
<i>S. gynobranchiata</i>	19.61%	19.35%	18.59%	19.85%	24.95%	24.62%	22.50%	15.51%	2.60%

can occur in disturbed or stressed habitats making them useful indicators of environmental change (Boesch 1977; Dauer et al. 1992; Weisberg et al. 1997; Rice & Levin 1998; Ritter & Montagna 1999; Dauer et al. 2003). Environmental assessments that use species diversity or species tolerance metrics will necessarily be dependent upon accurate species determinations. Utilization of molecular tools to identify closely related species, such as the members of the genus *Streblospio*, will be extremely useful in such studies so that these stressed habitats are correctly analysed.

In general, reproduction and development of spionid polychaetes is among the most extensively investigated among annelids (see Blake & Arnofsky 1999), and *Streblospio* is no exception (e.g. Levin 1984; Levin & Bridges 1994, 1995; Rice & Levin 1998; Blake & Arnofsky 1999). Development among spionids ranges from planktotrophic and lecithotrophic larvae to brooded young and viviparity (Blake & Arnofsky 1999) and development mode has implications for dispersal, connectivity, and ultimately speciation. Despite slight morphological differences in the three species placed within the *Streblospio*, there are considerable differences in their reproductive biology which may hold clues for how barriers to gene flow arose in these taxa. The loss of planktotrophy, and/or gain of lecithotrophy or brooding, is recognized as being an easier evolutionary change(s) than the gain of planktotrophy (Strathmann 1993). Because the planktotrophic larvae of North American taxa are fairly typical necktochaete larvae, they are not likely to be secondarily derived. The ancestral *Streblospio* likely had some form of planktonic development even though the data presented herein found that the brooding *S. shrubsolii* is the basal lineage. Given this line of reasoning, *S. gynobranchiata* likely has the least derived larval biology of the three taxa.

Here we provide molecular data, COI barcodes, that allow for accurate determination of the three recognized species of *Streblospio*. However, as the described distribution of these species is expanding with new investigations (e.g. recent reports of *Streblospio* sp. and *S. gynobranchiata* from Brazil by Lana et al. 2006 and V. Radashevsky pers. comm.) and as unintended introductions into new biogeographic regions continue, studies such as ours will enable evaluations of the evolutionary origins within the genus as well as putative negative impacts of introductions.

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Note

1 Webster's original description of *Streblospio benedicti* should be cited in this work as Webster (1879). The type locality for *S. benedicti* is Great Egg Harbor, NJ USA. The confusion in the literature arises due to the fact that the 1879b paper was reprinted in 1880 with different pagination and a different volume number. The following references

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