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Morphological Description and Weight-Length Relationship of the Intertidal Sipuncula *Siphonosoma australe* (Keferstein, 1865) along the Central Coast of Vietnam

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TTVN and NHT collected and prepared samples. TVG collected and prepared samples, provided key information, and helped revising manuscript. P-TH, designed and developed concept of work, carried out analyses, prepared figures, tables, and manuscript.

Key words
Anatomical description, Intertidal sipuncula, Length–weight relationship, Morphological statistical analyses, *S. australe*

ABSTRACT

The sipuncula is an important ecological and economical resource to the inter-tidal ecosystem and humans due to its environmental functions and nutritional as well as medicinal values. Thus, the current study intends to determine systematic validity and to assess the weight–length relationship of the sipuncula living along the central coast of Vietnam. Species identity is evidenced by the key features of external morphology and internal anatomy. The results show that all these sipuncula have striking similarities to the sipuncula *Siphonosoma australes* (Keferstein, 1865), which has greater than 100 fibrous tentacles around their mouth and 15-17 longitudinal bands running along the body wall. Besides, we detected significant differences body mass, body length, introvert length, BM/BL and IL/BL ratios but not trunk length and diameter, number of tentacles, and longitudinal muscular bands of those thriving at different habitats. Additionally, we examined the growth pattern for the Vietnamese sipuncula using a least-squares regression model computed for weight–length relationships. The BM – BL relationship exhibited a significantly linear regression and a negative allometric growth pattern, indicating faster length increment compared to weight. The present study has contributed important information for assessing the status and density of the *S. australe* population from the central coast of Vietnam locally and the Indo–West Pacific region generally.

INTRODUCTION

Sipuncula, non-segmented, vermiform, bilaterally symmetrical coelomate worms, was initially assigned to a protostome phylum (Cutler, 1994; Stephen and Edmonds, 1972) which is characterized according to the order of formation of the organism's mouth and anus. Over several decades, different hypotheses were proposed to place sipuncula within either Echiura, Annelida or Mollusca regarding cladistic analyses of morphological, molecular, and gene order data (Boore and Staton, 2002). Therefore, many efforts were made to resolve their evolutionary origin and phylogenetic position during the last 20 years. Currently, they are placed within the Annelida based on phylogenetic and phylogenomic studies (Dordel *et al*., 2010; Dunn *et al*., 2008; Hausdorf *et al*., 2007; Kawauchi *et al*., 2011; Mwinyi *et al*., 2009; Sperling *et al*., 2009; Statona, 2003; Struck *et al*., 2007, 2011), proteomic studies (Dordel *et al*., 2010) as well as developmental studies on the segmentation during the neurogenesis (Kristof *et al*., 2008; Wanninger *et al*., 2009) conducted by the early 2000s. Until the middle of the twenty centuries, sipuncula was officially assigned to phylum Sipunculida (Hyman, 1959). This phylum was reported to have low species diversity of 230 species described worldwide, which were

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divided into two classes, four orders, and six families (Schulze *et al*., 2005).

The sipuncula worm, also known by the vernacular names of peanut worm and star worm, is able to live in a diversity of habitats, from shallow waters to benthic zones, throughout the world's oceans including polar, equatorial, and abyssal zones (Cutler, 1994; Glynn and Enochs, 2011; Kawauchi and Giribet, 2014; Maiorova and Adrianov, 2017; Murina, 1984; Vargas and Dean, 2009). Most live in shallow waters where they can burrow in sand, mud, clay, and gravel or hide under stones, rocks, coral heads, empty seashells, and bones of dead whales. These worms were reported to be sensitive to waters with low salinity, thus they are not commonly found near estuaries (Açik, 2020; Nguyen *et al*., 2007).

Sipuncula is ecologically important to benthic ecosystems mainly based on their roles in bioerosion of coral reefs (Klein *et al*., 1991), bioturbation of sediments (Açik, 2010; Li *et al*., 2015), and adsorption of pollutants in sediments (Wu *et al*., 2020; Yan and Wang, 2002), and they are used as an indicator in environmental monitoring (Cutler, 1994; Shields and Kedra, 2009). Additionally, they also provide benefits to humans since they have been used as popular seafood and medicines in some North Mediterranean countries (Félix-Toledo *et al*., 2005; Kędra and Włodarska-Kowalczuk, 2008) and in East Asian countries such as China and Vietnam (Li *et al*., 2017; Shen *et al*., 2004).

In Vietnam, sipuncula mostly occurs in intertidal areas and island boundaries that are composed of sand and/or muddy sediments. According to previous researches, there were 20 species of sipuncula reported in the tidal areas of Mong Cai, Quang Ninh city, and Can Gio, Ho Chi Minh City (Đỗ, 1998). Later, an additional 19 species of sipunculans were reported in the intertidal areas of Nha Trang bay (Adrianov and Maiorova, 2012), Bac Viet Bay, and Con Dao islands (personal communication). Worldwide, the sipuncula *Siphonosoma australe* (Keferstein, 1865) is widely distributed in shallow and intertidal waters, preferring sandy and muddy areas, in tropical and subtropical areas. This species was originally described from Sydney, Australia, and now occurs in Indian Ocean, from Indonesia to India and Madagascar, in West Pacific, from New Zealand to South China, and Indo-Pacific region widely such as the Phillipines, Fiji Islands, Lifu, Loyalty Islands, Ambina, Zanzibar and Gulf of Manaar (Prashad, 1936). Although sipuncula are studied worldwide, those living in Vietnam, especially in the central coast of Vietnam, are not paid enough attention yet. The central coast of Vietnam is located in an intratropical area so that it has a high temperature background due to rich radiation and is characterized by a hot and humid tropical monsoon climate. This area also has large seasonal variations of rivers and tidal activities, which greatly impact the life of wild marine animals. On the other hand, together with climate change, urbanization and excess fishing acivities by humans also strongly affect growth and development of marine organisms living from along the coastline to the oceanic depth, especially the sipuncula.

Therefore, with the hope to serve management and conservation of marine resources diversity in the central coast of Vietnam, we investigate distribution of the sipuncula *Siphonosoma australe* collected from the central coasts of Vietnam including river mouth, intertidal area, and estuary in the three provinces of Quang Binh, Quang Tri, and Thua Thien Hue, respectively. Later, these samples were brought back to our laboratory for further inspecting internal anatomy and external morphology as well as measuring taxonomic features of the species, including trunk length and diameter, body length and weight, introvert length, amount of tentacles and longitudinal bands. Hence, we coducted statistical analyses to assess the differences in these taxonomic features. Since the length-length and length-weight relationships are basic biological data that can be used to compare life histories and morphological aspects of the populations inhabiting different regions (Gonçalves *et al*., 1997; Stergiou and Moutopoulos, 2001), we also conducted linear regression analyses to build a prediction for life history parameters of introvert length-body length and body length-body weight for the Vietnamese sipuncula *S. australe*. This study is important to provide information for assessing the status and density of the local sipuncula population which is used for proposing a better exploting and hunting approach to preserve and develop the regional ecosystem.

MATERIALS AND METHODS

Description of area

The study area included Gianh River in Quang Binh province (17°43'28" N; 106°26'09" E), Cua Viet beach in Quang Tri province (16°54'39" N; 107°11'28" E), and Thuan An beach in Thua Thien Hue (16°33'34" N; 107°37'10" E) (Fig. 1 and Supplementary Table SI). These areas have different tidal cycle profiles and water-level rising/falling times per day. According to Hanh and Furukawa (2007), the area in Gianh River has irregular diurnal tide with 1-2 times of rising and falling per day, while the area in Cua Viet beach has irregular semidiurnal tide but no records for water-level rising and falling per day, and the area in Thuan An beach has typical semidiurnal tide with 2 times of water-level rising and falling per day. These locations were chosen since there were no studies

carried on these areas yet. The river mouth area of Gianh River is composed of gravel, sand, and siltstone; the intertidal area of Cua Viet beach is an expanse of fine sand opening to the East Sea of Vietnam; while the estuary of Thuan An beach is a flat composed of mud and sand (Table I, Fig. 1).

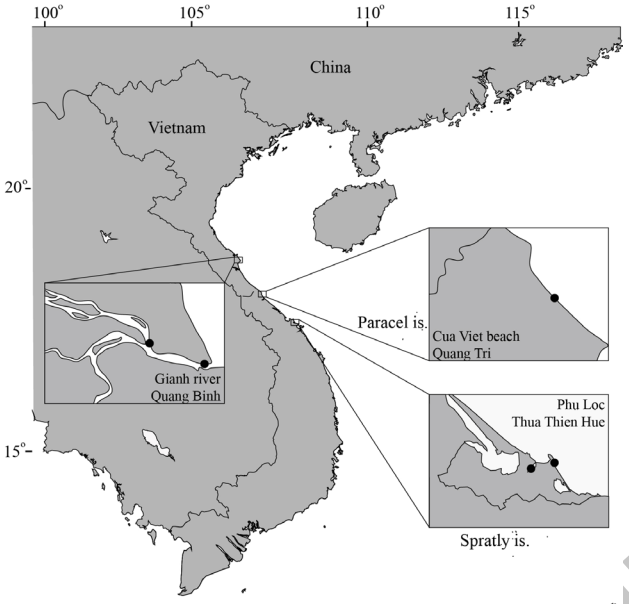

Fig. 1. Geographic map showing the sampling sites of sipuncula *Siphonosoma australe* reported in the present study. Dark gray area represents continental area and white area represents oceanic area. Black dots indicate sampling sites of the sipuncula *S. australe*.

Sampling method

A total of 151 individuals of sipuncula *Siphonosoma australe australe* (Keferstein, 1865) including 62 individuals from Quang Binh province, 32 individuals from Quang Tri province, and 62 individuals from Thua Thien Hue province, were collected early in the morning during low tide from March 2015 to June 2017 based on purposive sampling approach (Table I). To collect the samples, we determined the cave sheltering the worms and dug a hole 50-60 centimeters deep using a hand hoe. Samples from different locations were then placed in separated plastic bags filled with pure oxygen gas and brought to the laboratory of Genetics and Microorganisms, Biology Department, Hue University of Education for preservation in 70% alcohol solution (Merck, USA) for later examination.

Species identification

Morphological study was conducted using electronic scales to weight the body mass, calipers to measure the size, and sharp needles to dissect the body. The body cavity after dissection was then observed under a compound microscope (Nikon, Japan). All specimens were identified following the taxonomic keys described by Cutler (1994), Schulze *et al*. (2005), Kawauchi *et al*. (2011), and Adrianov and Maiorova (2012) including body length (mm) and mass (g), contractile introvert length (mm), number of tentacles (piece), trunk length and diameter (mm), and number of longitudinal muscle bands (piece). The body length was measured from the anterior to posterior tips of the body when the introvert was retracted within the body. The introvert was completely pulled out of the body and measured, while the trunk was measured from the posterior tip of trunk to the posterior tip of the body. The sex was determined based on microscopic observation of gonad. The ratio between length of the introvert and body was also calculated.

Statistical analyses

Since the morphological measurement data of the studied population violated assumptions of normal distribution and homogeneity variances, we applied Kruskal-Wallis test to assess any potential effects of location including province and locality on the anatomical measurement of the collected sipuncula populations. The test was conducted using kruskal.test function of rstatix package in R (R Development Core Team, 2011). The response variables are anatomical measurements including

Table I. Sampling information of the sipuncula *Siphonosoma australe* examined in the present study.

Location (Coordinates)	Sampling site ID	Number of collected samples	Sediment type	Salinity
Gianh river (17°43'28" N; 106°26'09" E)	1	32	Gravel, sand, and silt	Fresh
Quang Binh (17°45'20" N; 106°26'09" E)	2	30	Gravel, sand, and silt	Fresh
Cua Viet beach Quang Tri (16°54'39" N; 107°11'28" E)	1	32	Fine sand	Saline
Thuan An beach (16°33'34" N; 107°37'10" E)	1	32	Fine sand	Saline
Thua Thien Hue(16°36'30" N; 107°37'10" E)	2	30	Mud and sand	Fresh

trunk length (TL, cm), trunk diameter (TD, mm), body length (BL, cm), body mass (BM, g), introvert length (IL, cm), length ratio of introvert and body (R), count of tentacles (TT, pieces), and count of longitudinal bands (LB, pieces). The analyses were conducted at two levels. The first level involved comparisions of the populations from the three provinces of Quang Binh, Quang Tri, and Thua Thien Hue. The second level involved comparisions of populations from five localities that had different sediment types and salinity levels (Table I). Following the Kruskal-Wallis test, we additionally conducted pairwise comparision using pairwise wilcox test function in R with Benjamini and Hochberg correction method (Benjamini and Hocberg, 1995) to the analyses that showed significant differences in anatomical measurements between populations.

To model relationships between body mass/introvert length and body length (BM/IL – BL) of populations from provinces and localities, we used FSA package (Ogle *et al.*, 2021) implemented in R (R Development Core Team, 2011). The BM/IL–BL relationship is described by Pauly (1980) as in the following exponential regression equations:

$$BM = a_1 BL^{b_1} \quad ...(1)$$

$$IL = a_2 BL^{b_2} \quad ...(2)$$

In which BM is the body mass (g), IL is introvert length (cm), and BL is body length (cm), the parameters a_1 and a_2 are intercepts of regression line and are coefficient values relating to body form, b_1 and b_2 are the regression coefficients representing the isometric growth pattern. The parameters a and b in Equations 1 and 2 were calculated by the least-squared regression for populations from different provinces and localities. Variations in BM/IL–BL relationship of the sipuncula was tested by ANOVA using lm and anova functions implemented in R and considering geographical location with provinces and localities as fixed factor. Post-Hoc Tukey HSD test was also employed for pairwise comparisons of species weight–length measurements. The significance of the regression lines derived for the BM/IL–BL relationship was assess using F-statistic values. Allometric growth of the sipuncula is defined based on value of b_1, in which, the allometric growth occurred negatively when $b_1 < 3$ and positively when $b_1 > 3$.

RESULTS

Morphological and anatomical examination

According to the sipuncula morphological description by Cutler (1994), a morphological examination of the sipuncula from the central of Vietnam reveals a close similarity with *Siphonosoma australe australe* (Keferstein, 1865), which belongs to Siphonomatidae family, *Siphonosoma* genus (Kawauchi *et al.*, 2011). Externally, the present *S. australe* specimens are of dirty yellowish-brown color with long cylindrical and tapering body of 17.8–25.4 cm long and 9.30–9.96 mm wide (Fig. 2A). Length of the present *S. australe*'s body is nearly twice the length of the introvert ($BL_{Average}$ = 21.86 cm, $IL_{Average}$ = 9.72 cm, Table II). Mouth locates at the tip of the introvert in anterior end of the body and is surrounded by a mass of greater than 100 ciliated peripheral tentacles with yellowish oral and greenish aboral surfaces (Fig. 3A and Table II). Their body wall consists of an outer layer without cilia overlain and an inner layer composed of about 16-17 interconnected longitudinal muscular bands (Fig. 3C, D). The described *S. australe is* sexual diomorphic, but it is impossible to distinguish between males and females based on the external morphology and size of the body. In some specimens, we observed egg and sperm cells in reproductive organ which help to distinguish these individuals as female and male, respectively (Fig. 4).

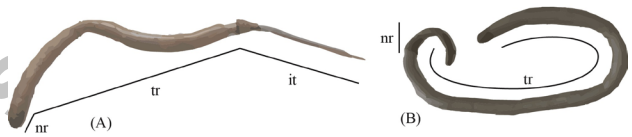

Fig. 2. A photo of a representative sipuncula *Siphonosoma australe* from Thua Thien Hue province (body length = 28.31 cm and introvert length = 10.9 cm) with introvert everted (A) and retracted (B). nr, narrower; tr, trunk and it, introvert.

Statistical analyses

We conducted Kruskal-Wallis tests to assess the differences in anatomical measurements of the sipuncula *Siphonosoma australe* from coasts of three provinces in the central Vietnam. The results indicate that there were no significant differences in length and diameter of trunk ($F_{TL}(2, 150) = 0.23$, $p = 0.89$ and $F_{TD}(2, 150) = 0.71$, $p = 0.70$, Table IIIA). However, differences in length of body and introvert were found with significant statistical support ($F_{BL}(2, 150) = 31.70$, $p = 1.31e\text{-}05$ and $F_{IL}(2, 150) = 33.22$, $p = 6.11e\text{-}08$, Table 3A). Subsequent main effect analyses showed that the average body length of the population from Thua Thien Hue was longer than those of populations from Quang Binh and Quang Tri with $BL_{Thua\ Thien\ Hue} = 24.7 \pm 6.34$, $BL_{Quang\ Binh} = 21.2 \pm 5.95$ cm, and $BL_{Quang\ Tri} = 17.8 \pm 4.99$ cm (Fig. 5C, Table II). While the average introvert length of Quang Tri population is longest with $IL_{Quang\ Tri} = 12.30 \pm 4.03$ cm, that of the Thua Thien Hue population is shorter with $IL_{Thua\ Thien\ Hue}$ = 24.7±6.34 cm, and that of the Quang Binh is shortest

Table II. Anatomical measurements of the sipuncula *Siphonosoma australe*.

Province	Locality	Population size (individual)	Trunk length (TL, cm)	Trunk diameter (TD, mm)	Body length (BL, cm)	Body mass (BM, g)	Introvert length (IL, cm)	Length ratio of introvert and body (R)	Number of tentacles (TT, pieces)	Number of longitudinal bands (BL, pieces)
Quang Binh	1	32	5.96±2.07	9.37±1.32	20.6±6.80	20.2±11.1	8.39±2.75	0.70±0.71	157±47.3	16.6±1.27
	2	30	5.27±1.84	9.73±1.85	21.8±4.92	19.4±8.93	8.36±2.77	0.41±0.22	169±48.9	16.6±1.19
	Average	62	5.63±1.97	9.55±1.60	21.2±5.95	19.8±10.00	8.38±2.76	0.56±0.54	163±48.1	16.6±1.22
Quang Tri	1	32	5.42±1.95	9.30±2.02	17.8±4.99	29.9±11.00	12.30±4.03	0.76±0.54	155±45.8	17.3±1.19
Thua Thien Hue	1	30	5.54±1.95	9.65±1.45	25.4±4.11	22.2±7.74	9.77±2.02	0.40±0.12	156±50.1	16.6±1.38
	2	32	5.66±2.06	9.72±1.45	24.0±4.50	20.6±7.19	9.74±1.88	0.43±0.14	145±41.9	16.6±1.70
	Average	62	5.60±1.99	9.96±1.44	24.7±6.34	21.3±7.44	9.75±1.93	0.41±0.13	150±46.0	16.6±1.54

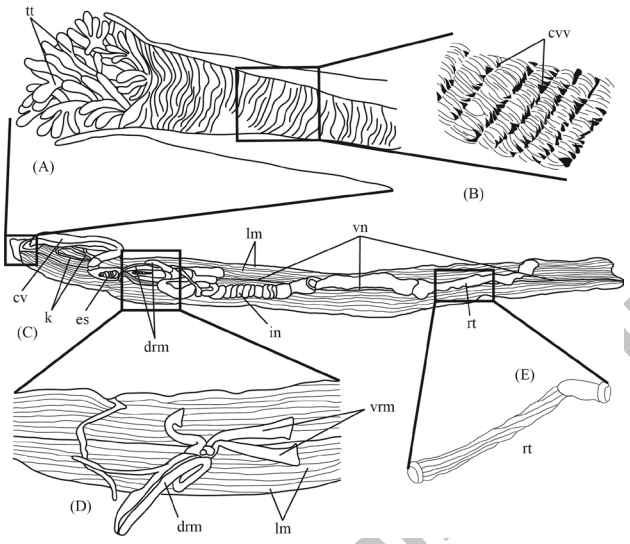

Fig. 3. Internal anatomy of the sipuncula *Siphonosoma australe* from Thua Thien Hue province (body length = 28.31 cm and introvert length = 10.9 cm). Schematic drawing of mouth (A), contractile vessel (B), coelomic cavity after dissection of the body wall (C), retractor muscles (D) and rectum (E).
cv, contractile vessel/introvert; tt, tentacles; cvv, contractile vessel villi; k, kidneys; es, esophagus; drm, dorsal retractor muscles; rt, rectum; in, intestine; lm, longitudinal muscles; vn, ventral nerve cord; vrm, ventral retractor muscles.

with $IL_{\text{Quang Binh}}$ = 8.38 ± 2.76 cm (Fig. 5E, Table II). Such significant differences in length of body and introvert resulted in significant differences in body mass of populations from the three provinces ($F_{\text{BM}}(2, 150) = 19.24$, p = 6.65e-05, Table IIIA) with $BM_{\text{Quang Tri}}$ = 29.9 ± 11.00 g, $BM_{\text{Thua Thien Hue}}$ = 21.3 ± 7.44 g, and $BM_{\text{Quang Binh}}$ = 19.8 ± 10.00 g (Fig. 5D, Table II). The analysis assessing length ratio of the introvert and body indicate that there were also significant differences between these three populations

Table III. Summary of Kruskal-Wallis test based on anatomical measurements of the sipuncula *Siphonosoma australe*.

	d.f	Kruskal wallis chi squared	p value
(A) Province			
Trunk length (TL, cm)	2	0.23	0.89
Trunk diameter (TD, mm)	2	0.71	0.70
Body length (BL, cm)	2	31.70	1.31e-05
Body mass (BM, g)	2	19.24	6.65e-05
Introvert length (IL, cm)	2	33.22	6.11e-08
Length ratio of introvert and body (R)	2	31.45	1.48e-07
Count of tentacles (TT, pieces)	2	2.36	0.31
Count of longitudinal bands (LB, pieces)	2	5.08	0.08
(B) Locality			
Trunk length (TL, cm)	4	1.21	0.88
Trunk diameter (TD, mm)	4	1.28	0.87
Body length (BL, cm)	4	33.18	1.10e-06
Body mass (BM, g)	4	19.96	5.09e-04
Introvert length (IL, cm)	4	33.27	1.05e-06
Length ratio of introvert and body (R)	4	34.53	5.79e-07
Count of tentacles (TT, pieces)	4	3.95	0.41
Count of longitudinal bands (LB, pieces)	4	4.99	0.29

d.f, degree of freedom, probability value was set at 0.05. Italics indicate statistically significant probability value.

(F_{R} (2, 150) = 31.45, p = 1.48e-07) with $R_{\text{Quang Tri}}$ = 0.76 ± 0.54, $R_{\text{Quang Binh}}$ = 0.56 ± 0.54, and $R_{\text{Thua Thien Hue}}$ = 0.41 ± 0.13. The wilcoxon signed rank test subsequently indicated that such significant difference was contributed

by the differences between Quang Binh – Quang Tri and Quang Tri – Thua Thien Hue (Table IIIA). Notably, there was no significant difference between the Quang Binh and Thua Thien Hue populations even though these two populations are far from each other, and their habitats also have different sediment type and salinity level (Supplementary Table SI). Besides this, we also could not detect significant differences in number of tentacles and longitudinal muscular bands between these three populations ($F_{TT}(2, 150) = 2.36$, $p = 0.31$ and $F_{LB}(2, 150) = 5.08$, $p = 0.08$, Table 3A) with average $TT_{Quang\ Binh} = 163 \pm 48.1$, $TT_{Quang\ Tri} = 155 \pm 45.8$, $TT_{Thua\ Thien\ Hue} = 150 \pm 46.0$ (Table II).

Further paralleled Kruskal-Wallis tests were conducted to assess the differences in anatomical measurements of the sipuncula sub-populations from different localities. As similar to the previous analyses, significant differences were detected between sub-populations in BL, IL, BM, and R ($F_{BL}(4, 150) = 33.18$, $p = 1.10e\text{-}06$; $F_{IL}(4, 150) = 33.27$, $p = 1.05e\text{-}06$; $F_{BM}(4, 150) = 19.96$, $p = 5.09e\text{-}04$; and $F_{R}(4, 150) = 34.53$, $p = 5.79e\text{-}07$, Table IIIB) but not for the TL, TD, TT and LB ($F_{TL}(4, 150) = 1.21$, $p = 0.88$; $F_{TD}(4, 150) = 1.28$, $p = 0.87$; $F_{TT}(4, 150) = 3.95$, $p = 0.41$; and $F_{LB}(4, 150) = 4.99$, $p = 0.29$, Table IIIB). Detailed pairwise comparison between sub-populations were presented in Supplementary Table SII. Such differences are considered to contribute for differences found between populations from different provinces. Notably, there were no significant differences detected between sub-populations from Thua Thien Hue ($p > 0.05$, Supplementary Table SII) in all four measurements of BL, BM, IL, and R even though these sub-populations lived in different habitats that are composed of different sediment types and different salinity levels (Table I).

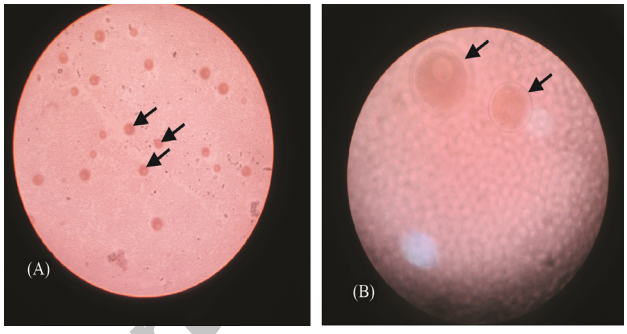

Fig. 4. Microscope images of reproductive organ of female sipuncula *Siphonosoma australe* from Thua Thien Hue province at pre-mature (A) and mature stages (B). Black arrows point eggs cells.

Table IV. Body mass/introvert length and body length relationship parameters of pooled population (A) and sub-populations categorized by province (B) and locality (C).

		a	b ± S.E	Adjusted R^2	CI (95%)	t	p value
(A) Factor							
BM – BL		40.44	0.46 ± 0.13	0.07	0.21 – 0.71	3.65	0.0004
IL – BL		89.70	0.09 ± 0.10	-0.001	0.10 – -0.28	0.91	0.37
(B) Province							
BM – BL	Quang Binh	1.56	0.88 ± 0.23	0.19	0.42 – 1.33	3.88	0.0002
	Quang Tri	42.70	0.60 ± 0.17	0.28	0.26 – 0.94	3.57	0.001
	Thua Thien Hue	24.28	0.51 ± 0.23	0.06	0.05 – 0.96	2.23	0.03
IL – BL	Quang Binh	35.97	0.17 ± 0.17	-0.0006	-0.17 – 0.51	0.99	0.33
	Quang Tri	18.09	0.42 ± 0.18	0.12	0.05 – 0.79	2.30	0.03
	Thua Thien Hue	1803.85	-0.31 ± 0.13	0.07	-0.57 – 0.05	-2.38	0.02
(C) Locality							
BM – BL	Quang Binh – 1		1.11 ± 0.25	0.38	0.60 – 1.61	4.50	9.01e -05
	Quang Binh – 2	49.07	0.37 ± 0.48	-0.01	-0.61 – 1.34	0.77	0.45
	Quang Tri	42.70	0.60 ± 0.17	0.28	0.26 – 0.94	3.57	0.001
	Thua Thien Hue – 1		1.15 ± 0.30	0.12	0.05 – 0.79	2.30	0.03
	Thua Thien Hue – 2	18.05	0.27 ± 0.19	0.03	-0.13 – 0.67	1.39	0.16
IL – BL	Quang Binh – 1	1.34	0.27 ± 0.20	0.03	-0.13 – 0.67	1.39	0.18
	Quang Binh – 2	227.03	-0.10 ± 0.35	-0.03	-0.82 – 0.62	-0.28	0.78
	Quang Tri	18.09	0.42 ± 0.18	0.12	0.05 – 0.79	2.30	0.03
	Thua Thien Hue – 1	389.22	-0.10 ± 0.21	-0.03	-0.53 – 0.33	-0.49	0.63
	Thua Thien Hue – 2	6487.84	-0.49 ± 0.17	0.20	-0.83 – 0.15	-2.94	0.006

BM, body mass; BL, body length; IL, introvert length; N, population size; a, b, regression coefficients; S.E, Standard Error; R^2, correlation coefficients; CI, confident interval (95%), range of b.

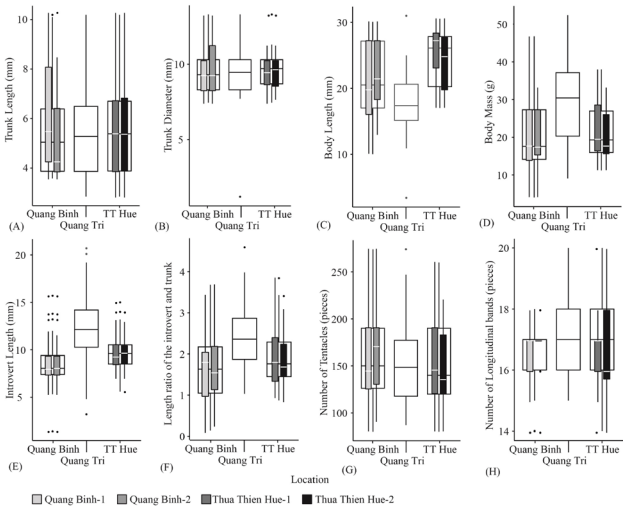

Fig. 5. The Kruskal-Wallis results assessing the differences in anatomical measurements of Trunk length (A), Trunk diameter (B), Body length (C), Body mass (D), Introvert length (E), Length ratio of the introvert and trunk (F), Count of tentacles (G) and Count of longitudinal bands (H). White colored boxes represent for measurements of the sipuncula from different provinces. Gradient black colored boxes represent the movement distance of sipuncula from different locations. The bottom and top of the boxes are the 25th, and 75th percentiles, the dashed vertical lines show the 50th percentiles, and the ends of the whiskers represent the minimum and maximum estimates of the movement distance. Outliers are represented by black dots beyond the whiskers.

Correlation between body mass/introvert length and body length

The parameters of body mass/introvert length and body length relationship were estimated for 7 sub-datasets of BM, IL, and BL corresponding to 7 sub-populations categorized by province and locality (Supplementary Table SI). The BM, IL, and BL ranges, sample sizes, estimates of a and b and the correlation coefficients from linear regression are presented in Table IV. Most of the body mass-body length (BM-BL) relationships were significantly linear with $p < 0.05$ except for those of Quang Binh– 2 and Thua Thien Hue– 2 with $p = 0.45$ and 0.16, respectively (Fig. 6 and Table IV). In contrast, most of the introvert length–body length (IL–BL) relationships were insignificant with $p > 0.05$ except for those of Quang Tri and Thua Thien Hue–2 with $p = 0.03$ and 0.006, respectively (Fig. 6 and Table IV). Later, we conducted ANOVA test to assess the difference in linear regressions of BM/IL–BL of populations from different provinces and localities. The results indicated that there were significant differences in slopes of both BM–BL and IL–BL relationships of populations from different provinces and localities with $F_{\text{BM-Province}}(2, 150) = 3.07$, $p = 0.05$; $F_{\text{IL-Province}}(2, 148) = 4.26$, $p = 0.016$; $F_{\text{BM-Locality}}(4, 146) = 4.40$, $p = 0.002$; and $F_{\text{IL-Locality}}(4, 144) = 2.94$, $p = 0.02$ (Table V). The b values of standard BM – BL regression in all collected sites exhibited lower than 3, at range of -0.83 – 1.61.

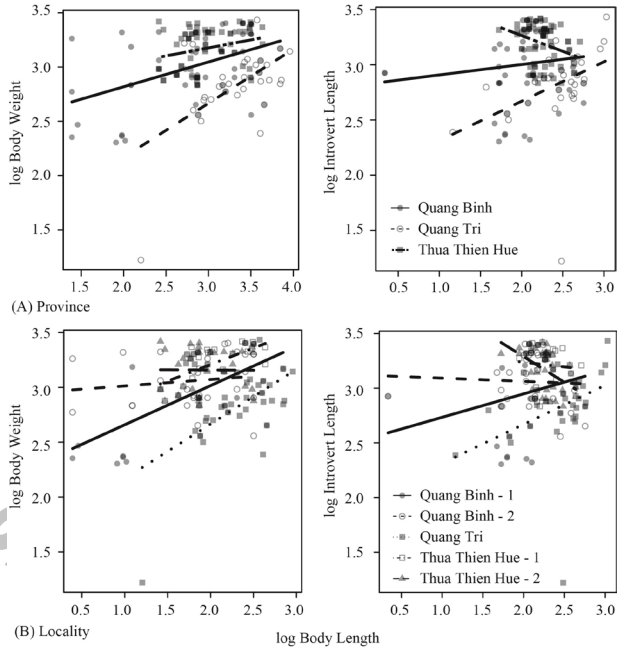

Fig. 6. Correlation between body mass/introvert length and body length of the sipuncula Siphonosoma australe from different provinces (A) and localities (B). Legend correlation lines for each sub-populations are presented inset of the graphs.

DISCUSSION

The sipuncula *Siphonosoma australe* has long been distinguished as *Phascolosoma australis* (from 1865 to 1883) and *Sipunculus australis* (from 1883 to 1907), later reclassified as *Siphonosoma australis* by 1912 and renamed as *Siphonosoma australe* by 1922 by Fischer. The key features that differentiate the *Siphonosoma* from the *Sipunculus* species are longer tentacles assembling in groups around the mouth opening; the introvert bearing distinct hooks; the dorsal and ventral retractor muscles arising at different levels; and the presence of transverse canal in the skin. The present species has all the mentioned key features and is similar to *S. rotumanun* but can be easily separated by having characteristic hooks closely associated with large papillae. The present *S. australe* is different from the Indo Pacific species of *S. cumanense* by having introvert hooks and by the origin of dorsal and

Table V. Summary of analysis of variance to compare body mass/introvert length–body length relationship of the Vietnamese *Siphonosoma australes* from different provinces (A) and localities (B).

	d.f	Sum squared	Mean squared	F	p
(A) Province					
Body mass (BM, log)	1	1.21	1.20	18.74	2.73e-5
Province	2	3.89	1.94	30.24	9.27e-12
BM X Province	2	0.39	0.20	3.07	0.05
Residuals	150	9.64	0.06		
Introvert length (IL, log)	1	0.08	0.08	1.07	0.30
Province	2	3.05	1.53	20.13	1.85e-8
IL X province	2	0.65	0.32	4.26	0.016
Residuals	148	11.22	0.08		
(B) Locality					
Body mass (BM, log)	1	1.21	1.21	20.05	1.51e-05
Locality	4	4.10	1.02	17.03	1.75e-11
BM X Locality	4	1.06	0.27	4.40	0.002
Residuals	146	8.77	0.06		
Introvert length (IL, log)	1	0.08	0.08	1.08	0.30
Locality	4	3.24	0.81	10.81	1.09e-07
IL X Locality	4	0.88	0.22	2.94	0.02
Residuals	144	10.80	0.07		

d.f, degree of freedom, probability value was set at 0.05. Italics indicate statistically significant probability value.

ventral retractor muscles at the same anterior–posterior level (Fig. 3B, D). *S. funafuti*, known from Southern Japan, Taiwan, and South China Sea, differs by lacking hooks, and *S. vastum*, also widespread in the Indo–West Pacific, has multiple clusters of caeca on the rectum that are absent in *S. australe*.

Besides, our data indicated that *S. australe* in the present study has body length double as introvert length, which is different from those thriving in Nha Trang bay having quite similar in length of the body and introvert (Adrianov and Maiorova, 2012). Even though, measuring method for body and introvert length of the *S. autsrale* is the same in both our current study and the previous work of Adrianov and Maiorova, there still remained obvious different in ratio between body length and introvert length between the populations from the central and the southern of Vietnam. The introvert can extend out or retract into the trunk or coelomic cavity to gather organic detritus particles in the waters or when feeding conditions are not suitable or danger threatens, respectively. The anterior part of introvert armed approximately 35-91 of hooks arranged in rings of scattered (Fig. 3B). These hooks have no papillae and is usually black. According to our personal observation, the larger and larger individual is the more and more rings of hooks they have. The eversion of the introvert is controlled by two pairs of dorsal and ventral retractor muscles. The ventral muscles are usually larger than the dorsal muscles. According to our results, almost *S. australe* from the central coast of Vietnam have more than 100 tentacles, while the *S. australe* found in Nha Trang bay has only 40–50 ones (Adrianov and Maiorova, 2012), which is much less than those reported in the present study. Tentacles enclosing nuchal organ are hollow and are extended via hydrostatic pressure in a similar manner as the introvert (Fig. 3A). The differences in body and introvert length as well as number of tentacles between the *S. australe* from Nha Trang and those reported in the present study can be explained by the difference of living environment or age of the specimens at the sampling tiimes, or possibly the state of relaxation of a specimen.

Their body wall consists of an outer layer without cilia overlain and an inner layer composed of about 16-17 interconnected longitudinal muscular bands, which is like those living Nha Trang bay. According to Schulze and his colleagues, the number of longitudinal muscles does not change much within species and is consider of one of critical criterion for sipuncula species identification (Schulze *et al*., 2005). These longitudinal muscular system functions as hydrostatic skeleton helps the animal move freely. Excretory organs of this species include only one pair of kidneys in elongated form with dark purple color. Digestive tract of the *S. australe* starts with esophagus located between the retractor muscles, following by the intestine which forms into 35-40 loops (personal observation) and turns anteriorly again. The downward and upward sections of the gut are coiled around each other. Rectum is fixed by wing muscles at the termination of the gut coil and ends in the anus. The rectum of the *S. australe* has no rectal caeca which is different from one of *S. astum* described by Cutler (1994). The waste product is discarded through the anterior-dorsally positioned anus which is small in size. In this species, the position of the orifice is nearly horizontal to the anus. The anus is often not visible when the introvert is retracted into the trunk. Therefore, it is important to determine the position of the anus to identify the back side of the abdomen. Spindle muscle or intestinal suspension is composed of three branches and attaches to the intestine, extends from the anus to the end of the body. Nephridia are free, about 40% of trunk length (personal observation). Nervous system consists of a dorsal cerebral ganglion or brain above the esophagus and a nerve ring around the esophagus which links the brains with the single ventral nerve cord that

runs the length of the body. Lateral nerves lead off this to innervate the muscles of the body wall.

Kruskal-Wallis siginificant differences were not found in number of tentacles and longitudinal muscular bands of populations from different provinces and localities. These results suggest that all the specimens from these provinces/localities has the same species identity and is *Siphonosoma australe*. On the other hands, we suggest that the significant differences in other measurements of body mass, body and introvert length are due to variations in age or growth rate between populations since growth rate variations are greatly influenced by local environmental and biological factors (Caddy, 1989). However, which factor and how could it affect growth rate are poorly understood, therefore it is necessary to conduct further investigation to clarify these issues.

In most marine animals, weight and length parameters are considered as distinguishable morphometric characters to identify their species. In this study, the body mass/introvert length and body length relationships were estimated (according to province and locality) for sipuncula *Siphonosoma australe* found off the central coast of Vietnam. The BM–BL relationship was significantly linear while the IL–BL relationship was not. The insignificantly linear relationship of IL–BL suggest that certain species exhibited characteristic morphological features. We considered the b values used in the BM/IL – BL as an indicator of growth for the species of *S. australe* and later used them to compare growth rate of populations from provinces or localities. When the location (province and locality) variations were considered, the b values reach a maximum of 0.88 (N= 151, adjusted R^2= 0.19) by province and 1.15 (N= 151, adjusted R^2= 0.12) by locality. Since all the computed b_1 values for all collected sipuncula were much lower than the reference test value of 3, we suggest that the allometric growth of the studied sipuncula is negative, which indicates a faster length increments rate than the weight increment. This finding is no surprised since the sipuncula has an eleongated body as a distinctive external morphology so that the rate of length increment is much higher than that of the weight increase of the sipuncula. Further, this growth patern emphasizes that all collected sipuncula are likely to be gaining less weight in all the sampling sites. Several of populations exhibited extremely low b_1 values such as Quang Binh– 2 and Thua Thien Hue– 2. Such extremely low b1 is possibly due to their feeding mechanisms and adverse climatic or seasonal effects (Ahmed *et al*., 2018; Kamaruzzaman *et al*., 2010). The central coast of Vietnam locates in an intratropical area so that it has a high-temperature background due to rich radiation and is characterized by a hot and humid tropical monsoon climate. This area also has a large seasonal variation of rivers and tidal activities, which greatly impacts the life of wild marine animals. On the other hand, together with climate change, urbanization, and excess fishing activities by humans also strongly affect qualitative, and quantitative characters of coastal, and marine sediments. Accordingly, changes in sediment characteristics negatively impact on food availability of the sipuncula and other marine animals thriving there, therefore influence their growth in terms of negative allometric growth (Ahmed *et al*., 2018; Kamaruzzaman *et al*., 2010).

CONCLUSIONS

This study firstly described external marphology and internal anatomy as well as investigate the weight–length relationships of the sipuncula in a selected areas of the central coast of Vietnam. Our morphological and anatomical data indicated that all the collected sipuncula is Siphonosoma australe which has more than 100 tentacles but only 15-17 longitudinal bands. On the other hand, our subsequent statistical analyses preveal significant differences in body mass as well as body length, introvert length and their ratio among populations and sub-populations but not in trunk length and diameter, number of tentacles, and longitudinal muscular bands. Further examinations on body mass/introvert length–body length relationships preveal significantly linear regression between BM–BL and negative allometric growth. Thus, this study would probably serve as baseline information for assessing the status and density of the *S. australe* population from the central coast of Vietnam locally and the Indo–West Pacific region in general.

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Ethical statement

No animal was harmed or disturbed during this research.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20221110081129

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

Morphological Description and Weight-Length Relationship of the Intertidal Sipuncula *Siphonosoma australe* (Keferstein, 1865) along the Central Coast of Vietnam

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Supplemental Table SI. Raw anatomy measurement of the sipuncula *Siphonosoma australe* from the central coast of Vietnam.

Province	Localities	Individual	Trunk length (mm)	Trunk diameter (mm)	Body length (mm)	Body mass (g)	Introvert length (mm)	Count of tentacles (pieces)	Count of longitudinal bands (pieces)	Length ratio of introvert and body
Quang Binh	River mouth	1	3.66	8.30	14.20	38.70	8.00	125	16	2.19
Quang Binh	River mouth	2	4.35	10.30	12.90	17.80	6.20	144	16	1.43
Quang Binh	River mouth	3	5.67	11.30	21.30	37.10	13.80	196	17	2.44
Quang Binh	River mouth	2	3.58	11.30	23.80	46.80	10.60	220	16	2.96
Quang Binh	River mouth	4	8.03	7.70	17.00	23.70	15.70	147	17	1.96
Quang Binh	River mouth	5	5.76	8.00	16.00	24.60	11.60	130	16	0.73
Quang Binh	River mouth	6	3.68	8.30	27.64	17.30	7.60	120	18	0.27
Quang Binh	River mouth	7	4.83	9.30	29.85	33.20	8.80	210	17	0.29
Quang Binh	River mouth	8	6.31	8.70	30.08	30.10	7.90	190	14	0.26
Quang Binh	River mouth	9	3.85	10.30	22.99	27.30	13.20	260	17	0.57
Quang Binh	River mouth	10	3.87	13.30	27.20	16.50	10.00	130	17	0.37
Quang Binh	River mouth	11	3.91	9.30	27.17	15.28	9.40	120	17	0.35

Table continued on next page.....

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Province	Localities	Individual	Trunk length (mm)	Trunk diameter (mm)	Body length (mm)	Body mass (g)	Introvert length (mm)	Count of tentacles (pieces)	Count of longitudinal bands (pieces)	Length ratio of introvert and body
Quang Binh	River mouth	12	8.42	9.70	24.75	19.40	7.50	130	18	0.30
Quang Binh	River mouth	13	3.68	8.30	27.64	17.30	7.60	120	18	0.27
Quang Binh	River mouth	14	4.83	9.30	29.85	33.20	8.80	210	17	0.29
Quang Binh	River mouth	15	6.31	8.70	30.08	30.10	7.90	190	14	0.26
Quang Binh	River mouth	16	8.05	10.30	27.76	20.00	8.10	140	15	0.29
Quang Binh	River mouth	17	10.19	10.70	18.65	13.80	1.40	80	17	0.08
Quang Binh	River mouth	18	8.21	8.30	17.02	17.60	5.60	120	18	0.33
Quang Binh	River mouth	19	5.47	9.70	26.11	15.90	9.60	130	16	0.37
Quang Binh	River mouth	20	4.27	7.70	18.25	17.60	8.50	130	14	0.47
Quang Binh	River mouth	21	5.26	9.70	23.19	23.48	7.40	150	17	0.32
Quang Binh	River mouth	22	6.39	10.30	19.73	11.20	8.70	100	16	0.44
Quang Binh	River mouth	23	4.27	7.70	18.25	17.60	8.50	130	14	0.47
Quang Binh	River mouth	24	6.31	8.86	24.20	8.07	5.67	178	18	0.23
Quang Binh	River mouth	25	8.05	7.44	10.52	4.02	7.65	150	17	0.73
Quang Binh	River mouth	26	10.19	8.44	10.73	7.24	6.04	124	17	0.56
Quang Binh	River mouth	27	8.21	9.01	11.80	4.30	5.32	172	18	0.45
Quang Binh	River mouth	28	5.47	10.08	10.02	6.76	5.61	247	18	0.56
Quang Binh	River mouth	29	4.27	8.88	10.19	7.53	8.16	274	16	0.80
Quang Binh	River mouth	30	10.10	9.44	10.63	7.24	6.03	123	18	0.57
Quang Binh	River mouth	31	5.26	11.30	19.10	36.60	12.00	145	17	0.63
Quang Binh	River	32	3.85	8.30	18.25	27.30	8.00	196	14	0.44
Quang Binh	River	33	3.87	9.30	23.19	16.50	6.20	220	17	0.27
Quang Binh	River	34	3.91	8.70	19.71	15.28	13.80	150	17	0.70
Quang Binh	River	35	6.39	10.30	18.26	19.40	10.60	150	17	0.58
Quang Binh	River	36	4.27	13.30	14.20	17.30	15.70	170	14	1.11
Quang Binh	River	37	6.31	10.30	12.91	33.20	11.60	178	18	0.90
Quang Binh	River	38	8.05	7.70	21.39	11.20	7.60	150	17	0.36
Quang Binh	River	39	10.19	8.86	23.80	17.60	8.80	124	17	0.37
Quang Binh	River	40	8.21	7.44	17.00	8.07	7.90	172	18	0.46
Quang Binh	River	41	3.85	8.44	16.00	4.02	13.20	124	17	0.83
Quang Binh	River	42	3.87	13.30	27.64	7.24	10.00	172	17	0.36
Quang Binh	River	43	3.91	8.30	29.85	23.70	9.40	247	16	0.31
Quang Binh	River	44	8.42	10.40	30.08	24.60	7.50	274	16	0.25
Quang Binh	River	45	3.58	11.30	22.99	17.30	7.60	145	17	0.33
Quang Binh	River	46	8.03	11.30	27.20	33.20	8.80	210	16	0.32
Quang Binh	River	47	5.76	7.70	27.17	30.10	7.90	190	17	0.29

Table continued on next page.....

Province	Localities	Individual	Trunk length (mm)	Trunk diameter (mm)	Body length (mm)	Body mass (g)	Introvert length (mm)	Count of tentacles (pieces)	Count of longitudinal bands (pieces)	Length ratio of introvert and body
Quang Binh	River	48	3.68	8.00	27.76	27.30	8.10	180	16	0.29
Quang Binh	River	49	4.83	8.30	18.65	16.50	1.40	90	18	0.08
Quang Binh	River	50	6.31	9.30	17.02	15.28	5.60	100	17	0.33
Quang Binh	River	51	3.85	8.70	26.11	19.40	9.60	150	14	0.37
Quang Binh	River	52	3.87	10.10	18.25	17.30	8.50	130	17	0.47
Quang Binh	River	53	3.91	13.30	27.17	33.20	7.40	200	17	0.27
Quang Binh	River	54	6.39	8.86	27.76	11.20	8.70	100	17	0.31
Quang Binh	River	55	3.68	7.44	18.65	17.60	8.50	172	14	0.46
Quang Binh	River	56	4.83	8.44	17.02	8.07	5.67	124	18	0.33
Quang Binh	River	57	6.31	13.30	26.11	4.02	7.65	172	17	0.29
Quang Binh	River	58	3.85	8.30	18.25	33.20	6.04	247	17	0.33
Quang Binh	River	59	3.87	10.30	23.19	30.10	5.32	274	18	0.23
Quang Binh	River	60	3.91	11.30	19.73	27.30	5.61	145	17	0.28
Quang Binh	River	61	6.39	11.30	18.25	16.50	8.16	120	17	0.45
Quang Tri	Beach	62	5.26	11.30	19.10	36.60	12.00	145	17	0.63
Quang Tri	Beach	63	6.39	11.30	18.30	23.70	14.50	87	18	0.79
Quang Tri	Beach	64	2.85	8.00	16.40	30.30	4.80	117	20	0.29
Quang Tri	Beach	65	6.80	10.30	31.00	35.60	20.70	117	18	0.67
Quang Tri	Beach	66	3.05	13.30	25.00	15.10	11.20	95	18	0.45
Quang Tri	Beach	67	2.87	9.30	10.90	37.00	3.20	174	17	0.29
Quang Tri	Beach	68	3.85	9.70	12.20	18.40	6.30	159	17	0.52
Quang Tri	Beach	69	3.87	8.30	21.00	30.70	10.30	178	18	0.49
Quang Tri	Beach	70	3.91	9.30	14.80	19.00	13.90	150	18	0.94
Quang Tri	Beach	7	8.42	8.70	17.20	25.50	8.70	124	16	0.51
Quang Tri	Beach	72	3.68	10.30	17.80	41.10	10.20	172	17	0.57
Quang Tri	Beach	73	4.83	10.70	23.20	52.40	19.20	247	18	0.83
Quang Tri	Beach	74	6.31	8.30	17.20	46.80	13.50	274	15	0.78
Quang Tri	Beach	75	8.05	9.70	14.90	20.70	11.90	145	18	0.80
Quang Tri	Beach	76	10.19	7.70	16.80	22.80	14.10	87	17	0.84
Quang Tri	Beach	77	8.21	9.70	17.60	37.20	15.80	117	20	0.90
Quang Tri	Beach	78	5.47	10.30	20.50	40.00	15.30	177	18	0.75
Quang Tri	Beach	79	4.27	8.70	13.50	16.80	11.10	206	16	0.82
Quang Tri	Beach	80	3.05	11.30	15.50	28.20	14.00	175	17	0.90
Quang Tri	Beach	81	8.70	10.30	17.80	47.40	9.70	206	17	0.54
Quang Tri	Beach	82	5.30	7.70	21.60	31.80	14.60	205	16	0.68
Quang Tri	Beach	83	7.23	9.30	20.50	35.10	14.00	93	19	0.68

Table continued on next page.....

Province	Localities	Individual	Trunk length (mm)	Trunk diameter (mm)	Body length (mm)	Body mass (g)	Introvert length (mm)	Count of tentacles (pieces)	Count of longitudinal bands (pieces)	Length ratio of introvert and body
Quang Tri	Beach	84	5.48	8.30	15.20	19.20	13.60	117	17	0.89
Quang Tri	Beach	85	5.95	8.00	24.80	16.70	20.10	118	19	0.81
Quang Tri	Beach	86	3.66	8.30	14.20	38.70	8.00	125	16	0.56
Quang Tri	Beach	87	4.35	10.30	12.90	17.80	6.20	144	16	0.48
Quang Tri	Beach	88	5.67	11.30	21.30	37.10	13.80	196	17	0.65
Quang Tri	Beach	89	3.58	11.30	23.80	46.80	10.60	220	16	0.45
Quang Tri	Beach	90	8.03	7.70	17.00	23.70	15.70	147	17	0.92
Quang Tri	Beach	91	5.76	8.00	16.00	24.60	11.60	130	16	0.73
Quang Tri	Beach	92	4.21	9.60	18.27	30.56	12.29	155	17	0.67
Quang Tri	Beach	91	4.21	1.20	3.39	9.06	12.00	150	16	3.54
Thua Thien Hue	Beach	92	2.87	8.00	21.80	16.25	11.00	140	16	0.50
Thua Thien Hue	Beach	93	3.85	10.30	22.99	27.30	13.20	260	17	0.57
Thua Thien Hue	Beach	94	3.87	13.30	27.20	16.50	10.00	130	17	0.37
Thua Thien Hue	Beach	67	3.91	9.30	27.17	15.28	9.40	120	17	0.35
Thua Thien Hue	Beach	95	8.42	9.70	24.75	19.40	15.00	130	18	0.61
Thua Thien Hue	Beach	96	3.68	8.30	27.64	17.30	7.60	120	18	0.27
Thua Thien Hue	Beach	97	4.83	9.30	29.85	33.20	8.80	210	17	0.29
Thua Thien Hue	Beach	98	6.31	8.70	30.08	30.10	7.90	190	14	0.26
Thua Thien Hue	Beach	99	8.05	10.30	27.76	20.00	8.10	140	15	0.29
Thua Thien Hue	Beach	73	10.19	10.70	18.65	13.80	14.00	80	17	0.75
Thua Thien Hue	Beach	100	8.21	8.30	17.02	17.60	9.00	120	18	0.53
Thua Thien Hue	Beach	101	5.47	9.70	26.11	15.90	9.60	130	16	0.37
Thua Thien Hue	Beach	102	4.27	7.70	18.25	17.60	8.50	130	14	0.47
Thua Thien Hue	Beach	103	5.26	9.70	23.19	23.48	7.40	150	17	0.32
Thua Thien Hue	Beach	104	6.39	10.30	19.73	11.20	8.70	100	16	0.44
Thua Thien Hue	Beach	105	2.85	8.70	17.76	11.80	8.70	100	15	0.49
Thua Thien Hue	Beach	106	6.80	11.30	20.26	14.60	8.60	120	18	0.42
Thua Thien Hue	Beach	107	3.05	8.86	30.35	32.90	8.20	210	17	0.27
Thua Thien Hue	Beach	108	8.70	7.44	28.31	19.30	10.90	150	15	0.39
Thua Thien Hue	Beach	109	5.30	8.44	28.70	32.60	10.60	220	16	0.37
Thua Thien Hue	Beach	110	7.23	9.01	26.42	17.50	7.00	120	17	0.26
Thua Thien Hue	Beach	111	5.48	10.08	27.32	26.00	10.50	180	14	0.38
Thua Thien Hue	Beach	112	5.95	8.88	30.55	29.00	9.30	190	16	0.30
Thua Thien Hue	Beach	113	3.66	11.30	28.34	21.00	8.10	150	20	0.29
Thua Thien Hue	Beach	114	4.35	11.30	27.85	30.95	9.80	200	18	0.35
Thua Thien Hue	Beach	115	5.67	8.00	27.54	19.50	9.70	150	17	0.35

Table continued on next page.....

Province	Localities	Individual	Trunk length (mm)	Trunk diameter (mm)	Body length (mm)	Body mass (g)	Introvert length (mm)	Count of tentacles (pieces)	Count of longitudinal bands (pieces)	Length ratio of introvert and body
Thua Thien Hue	Beach	116	3.58	10.30	23.62	23.20	8.70	160	18	0.37
Thua Thien Hue	Beach	117	8.03	13.30	28.98	37.90	11.90	250	17	0.41
Thua Thien Hue	Beach	118	5.76	9.30	29.00	16.10	NA	80	16	NA
Thua Thien Hue	Beach	119	4.21	9.70	24.78	38.00	13.00	260	16	0.52
Thua Thien Hue	Estuary	120	2.87	8.30	19.73	16.25	8.20	120	15	0.42
Thua Thien Hue	Estuary	121	3.85	9.70	17.76	33.20	10.90	220	18	0.61
Thua Thien Hue	Estuary	122	3.87	7.70	20.26	30.10	10.60	200	17	0.52
Thua Thien Hue	Estuary	123	3.91	9.70	27.54	20.00	7.00	140	15	0.25
Thua Thien Hue	Estuary	124	8.42	10.30	24.75	13.80	10.50	120	16	0.42
Thua Thien Hue	Estuary	125	3.68	8.30	27.64	17.60	9.30	130	17	0.34
Thua Thien Hue	Estuary	126	4.83	9.30	29.85	15.90	8.10	120	14	0.27
Thua Thien Hue	Estuary	127	6.31	8.70	30.08	17.60	9.80	140	16	0.33
Thua Thien Hue	Estuary	128	8.05	10.30	27.76	23.48	9.70	170	20	0.35
Thua Thien Hue	Estuary	129	10.19	10.70	18.65	13.80	14.00	80	18	0.75
Thua Thien Hue	Estuary	130	8.21	8.30	17.02	17.60	12.00	120	18	0.71
Thua Thien Hue	Estuary	131	5.47	9.70	26.11	15.90	9.60	130	16	0.37
Thua Thien Hue	Estuary	132	4.27	7.70	18.25	17.60	9.30	130	14	0.51
Thua Thien Hue	Estuary	133	5.26	9.70	23.19	23.48	8.10	160	17	0.35
Thua Thien Hue	Estuary	134	6.39	10.30	19.73	11.20	9.80	110	16	0.50
Thua Thien Hue	Estuary	135	2.85	8.70	17.76	11.80	9.70	110	15	0.55
Thua Thien Hue	Estuary	136	8.42	11.30	20.26	14.60	14.00	80	16	0.69
Thua Thien Hue	Estuary	137	3.68	8.00	27.54	32.90	5.60	190	20	0.20
Thua Thien Hue	Estuary	138	4.83	10.30	23.62	19.30	9.60	140	18	0.41
Thua Thien Hue	Estuary	139	6.31	13.30	28.98	32.60	8.50	210	18	0.29
Thua Thien Hue	Estuary	140	8.05	9.30	29.00	17.50	7.00	120	16	0.24
Thua Thien Hue	Estuary	141	10.19	10.08	27.32	26.00	10.50	180	14	0.38
Thua Thien Hue	Estuary	142	5.95	8.88	30.55	11.20	9.30	100	17	0.30
Thua Thien Hue	Estuary	143	3.66	11.30	28.34	11.80	8.10	100	16	0.29
Thua Thien Hue	Estuary	144	4.35	11.30	26.11	14.60	9.80	120	14	0.38
Thua Thien Hue	Estuary	145	5.67	8.00	18.25	32.90	9.70	210	17	0.53
Thua Thien Hue	Estuary	146	3.58	10.30	23.19	19.30	8.70	140	16	0.38
Thua Thien Hue	Estuary	147	8.03	13.30	19.73	32.60	11.90	220	15	0.60
Thua Thien Hue	Estuary	148	5.76	9.30	17.76	17.50	NA	90	16	NA
Thua Thien Hue	Estuary	149	4.21	9.70	24.78	26.00	13.00	200	20	0.52
Thua Thien Hue	Estuary	150	4.35	11.30	27.85	29.00	9.80	190	18	0.35
Thua Thien Hue	Estuary	151	5.67	8.00	27.54	21.00	9.70	150	18	0.35

Supplemental Table SII. Pairwise comparison of body length, body mass, introvert length, and length ratio between introvert and trunk among the sipuncula *Siphonosoma australe* populations by province and locality.

(A) By province				
	Quang Binh	**Quang Tri**		
Quang Tri	*8.84E-03*			
	1.50E-04			
	5.10E-06			
	1.90E-05			
Thua Thien Hue	*9.20E-04*	*1.30E-07*		
	0.37	*2.40E-04*		
	1.30E-04	*1.30E-04*		
	0.96	*1.90E-08*		
(B) By locality				
	Quang Binh-1	**Quang Binh-2**	**Quang Tri**	**Thua Thien Hue-1**
Quang Binh-2	0.47			
	0.76			
	0.99			
	0.32			
Quang Tri	0.13	*7.80E-03*		
	3.40E-03	*1.60E-03*		
	2.50E-04	*2.50E-04*		
	5.20E-03	*2.20E-05*		
Thua Thien Hue-1	*7.80E-03*	*9.60E-03*	*4.10E-06*	
	0.66	0.48	*9.50E-03*	
	0.13	0.16	*3.44E-03*	
	3.40	0.50	*1.80E-06*	
Thua Thien Hue-2	0.06	0.11	*5.80E-05*	0.26
	0.91	0.75	*1.60E-03*	0.66
	8.57E-03	*1.03E-02*	*2.63E-03*	0.78
	0.52	0.33	*9.40E-06*	0.56
Note:				
	Body Length			
	Body Mass			
	Introvert Length			
	Length ratio of introvert and body			