

A comparison of life-history traits in calcifying Spirorbinae polychaetes living along natural pH gradients

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Supplement 1. Site Details

The north is relatively exposed to the dominant northwestern winds, and the south is a bay-protected area. The venting area in the south is approximately 3000 m² and gases are emitted at a rate of 1.4 x 10⁶ L d⁻¹. In the north, the area is only 2000 m² and the venting rate is slightly decreased at 0.7 x 10⁶ L d⁻¹ (Hall-Spencer et al. 2008). Specific quantities of the emitted gases are comprised of the following: 90-95 % CO₂, 3-6 % N₂, 0.6-0.8 % O₂, 0.2 - 0.08 % CH₄, and 0.08-0.1 % Ar. No sulfur is present. Although neither seasonal, tidal nor diurnal variation in gas flows have been recorded, the pH does not stay static due to variable bubbling intensity, and shows quite variable values in relatively short, hourly time frames (Kroeker et al. 2011).

Site name and description:	Site GPS Coordinates:
SC: South Control	40.729467, 13.964260
S2: South Low pH	40.730075, 13.963651
S3: South Extreme Low pH	40.731148, 13.963211
NC: North Control	40.732777, 13.965218
N2: North Low pH	40.732316, 13.964464
N3: North Extreme Low pH	40.732000, 13.963716

Supplement 2. Number of spirorbids identified in each site replicate, and the ratio of specimens identified (ID'ed) at the species level to the total number of specimens found. These ratios were used to calculate the number of *Simplaria* and *P. militaris* in each replicate, as complete identification of each specimen was not possible due to lost taxonomic features.

Site	Repli- cate	Total spp.	<i>P.</i> <i>militaris</i>	<i>Simplaria</i> sp.	Other spp.	ID'ed	Ratio ID'ed	<i>Simplaria</i> sp. %
NC	<i>A</i>	146	62	48	13	123	0.84	39%
	<i>B</i>	178	68	54	12	134	0.75	40%
	<i>C</i>	161	26	32	12	59	0.37	54%
	<i>D</i>	243	78	29	5	112	0.46	26%
N2	<i>A</i>	50	12	16	0	28	0.56	57%
	<i>B</i>	80	19	33	2	54	0.68	61%
	<i>C</i>	54	25	35	1	61	1.13	57%
	<i>D</i>	94	20	54	1	75	0.8	72%
N3	<i>A-D</i>	46	5	5	0	10	0.22	50%
SC	<i>A</i>	113	4	28	3	35	0.31	80%
	<i>B</i>	71	1	25	0	25	0.35	100%
	<i>C</i>	132	0	57	2	59	0.45	97%
	<i>D</i>	180	5	78	5	83	0.46	94%
S2	<i>A</i>	75	2	39	1	42	0.56	93%
	<i>B</i>	106	9	42	3	54	0.51	78%
	<i>C</i>	234	1	99	3	103	0.44	96%
	<i>D</i>	158	0	41	1	42	0.27	98%
S3	<i>A-D</i>	47	0	13	0	13	0.28	100%

Supplement 3. Laboratory Trial Transport Details

Samples were transported from field sites by boat to the Villa Dohrn-Benthic Ecology Center and maintained inside 10 L coolers with fresh seawater from each of the collection sites. Samples were kept in seawater matching the pH level of their respective field origin; leaves were inspected to select for living spirobids, and cut in smaller portions to facilitate transport to the ENEA Laboratory in La Spezia, Italy. Transport containers were prepared with spirobids and unfiltered seawater (volume = 1300 mL; T = 21.96 ± 1.29 °C; pH: control = 8.03 ± 0.08 , low = 7.61 ± 0.26 ; S = 36; density = approx. 100 individuals *per* container) and kept in styrofoam coolers packed with ice to maintain a consistent water temperature. During the 8 h transport to ENEA, temperature and pH were recorded twice using a pH meter with integrated thermometer (SG2, Mettler-Toledo Analytical, Milan, Italy). The mean pH in the containers remained at 8.03 (control samples), or increased from 7.61 to 8.01 (low pH samples). The temperature decreased from 21.96 to 19.00 °C for 1 h in all containers. On arrival at the ENEA laboratory, containers were immediately placed in pre-conditioned temperature baths (T = 22.00 °C, S = 36). Temperature was controlled *via* two thermal baths connected to a temperature conditioner (TR 15, TECO, Naples, Italy) with heaters (V2-Them 300, São Julião do Tojal, Portugal). To enhance a homogeneous mixing of the water, and thus thermal stability of the system, submersible circulation pumps (Aquapump HJ-311, Mondial fauna, Milan, Italy) were also used. Containers were aerated with either ambient (control) air ($p\text{CO}_2 \sim 380$ μatm , for pH = 8.22), or CO₂-enriched air ($p\text{CO}_2 \sim 1000$ μatm , for pH = 7.70). CO₂ gas was slowly released into a Buchner flask to enable mixing using a CO₂ regulator (6000 CO₂, BOC, La Spezia, Italy).

Supplement 4. Taxonomy Details

The tubes and operculum of both the adults and juveniles of the *Simplaria* sp. specimens found in this study closely resemble that of *Simplaria pseudomilitaris* (Thiriot-Quévieux, 1965), a taxon first described in Villefrance sur Mer, France, and later identified in the Gulf of Naples by Harris (1968), although reported as *Spirorbis berkeleyana* (Rioja, 1942) (Knight-Jones et al. 1974), and in the fouling inside Port of Ischia, Italy (Terlizzi et al. 2000). Morphological similarities of the two are their sinistral coiled (clockwise) tube orientation, similar tube diameter (between 1.5-2 mm), latitudinal tube ridges, and 2-3 indistinct longitudinal tube ridges. The operculum also has a single opercular plate with ornamentation (protuberances, or spines, projecting from top of operculum). The operculum has been described having an elliptical cap with a partially encircling distal papillated rim that is absent on the substratum side (see also Bianchi 1981), yet this feature is not in agreement with this study's specimens, where the rim completely surrounds the distal papillated rim. This feature is, however, in better agreement with a description of *S. pseudomilitaris* from the west coast of the USA made by Beckwitt (1981), who further noted the high variation in operculum morphology in the species. The primary trait that is found in the *Simplaria* sp. specimens of this study that is not in agreement with the *S. pseudomilitaris* descriptions from the literature is the extent of 'ornamentation' on the operculum plate (e.g. Bianchi 1981; Fig. 6).

The morphology of the *Simplaria* sp. here also closely matches *Pileolaria quasimilitaris* with respect to larval and operculum morphology, a taxon first described in the Caribbean Sea (Bailey 1970). In particular, *P. quasimilitaris* has distally projecting calcareous spines on the operculum that form a complete crown. However, it still does not completely agree as there

are up to three indeterminate rows of long, slender spines observed in the operculum crown center in this study's specimens *versus* the two rows of spines originally described for *P. quasimilitaris*. Two other difference between these two species are in the tubes and chaetae: this study's specimens have latitudinal ridges and 2-3 indistinct longitudinal ridges, and no sickle chaetae on the third thoracic fascicles, *versus* the many longitudinal ridges and knobs of *P. quasimilitaris* and presence of these sickle chaetae.

The key taxonomic feature for the genus *Pileolaria* is the presence of sickle chaetae on the third thoracic fascicles (Knight-Jones et al. 1974). In the sister genus *Simplaria* erected by Knight-Jones (1984), all of the characters of the genus *Pileolaria* are found, except the sickle chaetae in the third thoracic fascicles. After examining over 40 *Simplaria* sp. specimens from this study, no sickle chaetae were found. In the original description of *P. quasimilitaris* by Bailey (1970) the chaetae of the third thoracic fascicle are defined as "hooked" chaetae. The morphology of sickle chaetae, is, however, quite variable (Knight-Jones & Fordy 1979) and "hooked" chaetae, *sensu* Bailey (1970) can be considered as sickle chaetae. Regardless, the specimens of this study also lacked hooked chaetae in the third thoracic fascicles. Therefore this relevant character, sickle chaetae absence, leads us to exclude the attribution to our specimens to any other *Pileolaria* species with spines on the operculum (e.g., as *P. semimilitaris*, Vine et al. 1972), and consider our taxon as a member of the *Simplaria* genus, and be considered or a morphotype/ecotype of *S. pseudomilitaris* having more abundant, longer, pronounced distally projecting calcareous spines covering the operculum plate, or a new species from the genus of *Simplaria*. The opercular morphology is a character quite variable in this species, which has lead also to confusion of *S. pseudomilitaris* with other species (e.g. *Spirorbis regalis* in Harris (1968)). Analysis of additional *Simplaria pseudomilitaris* material from both type locality and other areas could help to account for the possible variability in opercular morphology, however only a genetic analysis would help to determine the correct species status. Yet this is beyond the scope of this study. Therefore, the specimens of this study are designated as *Simplaria* sp.

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