

Chapter 5

The Bacteria *Endozoicomonas*: Community Dynamics, Diversity, Genomes, and Potential Impacts on Corals



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Abstract *Endozoicomonas*, a recently identified bacterial genus, is commonly detected in corals, sponges, and other reef invertebrates. These bacteria have attracted attention, as there are many indications that they have important roles in coral health. This chapter includes introduction to *Endozoicomonas* and is divided into four main sections – (1) introducing history and type strain of *Endozoicomonas*; (2) ecological distribution; (3) abundance, diversity, and phylogeny; and (4) genomes – followed by a general discussion and future research directions in the end. In the first section, the taxonomy and classification of the genus *Endozoicomonas* are clarified and discussed. Important characteristics of all cultivable *Endozoicomonas* strains isolated from marine invertebrates are also described. The studies of *Endozoicomonas* in different hosts, locations, and time are described in the second section. The third section will discuss the variation of *Endozoicomonas* in abundance, phylogeny, and

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diversity. Finally, the genomes of *Endozoicomonas* and possible interactions between these bacteria and reef invertebrates, especially coral holobiont, are elucidated that paves the way for the future study of *Endozoicomonas*.

Keywords *Endozoicomonas* · Bacteria · Reef invertebrate · Coral

5.1 Introduction to *Endozoicomonas*

5.1.1 History of *Endozoicomonas* and *Spongiobacter*, Family Hahellaceae

The genus *Endozoicomonas*, under the family *Hahellaceae*, the order *Oceanospirillales*, and the class *Gammaproteobacteria*, was widely detected in various marine environments and abundant in various marine invertebrates. However, the taxonomic name of this genus is confusing, due to the use of alternate terminology in some reports, that is, *Spongiobacter* (genus) and *Endozoicimonaceae* or *Endozoicomonaceae* (family), transition errors, or provisional usage.

The genus name *Endozoicomonas* was first proposed by Kurahashi and Yokota in 2007 [1]. Prior to that, sequences similar to *Endozoicomonas* were annotated or referred to as *Spongiobacter*, the other “genus” name proposed by Nishijima (2005) in an unpublished conference paper, in which she introduced a nickel-tolerant bacterial isolate from a marine sponge (information unavailable). Unfortunately, the “genus” name *Spongiobacter* (should be *Candidatus Spongiobacter*) was never formally registered as a taxon, there was no description of characteristics, and no culture of this bacterium was deposited in an authorized collection center or institute, all of which are essential for publication of a new bacterial species. Furthermore, the genera *Endozoicomonas* and *Spongiobacter* are phylogenetically closely related; consequently, their 16S rRNA gene sequences are always entangled in phylogenetic analyses (Fig. 5.1) [2, 3], indicating that presumably they are the same genus (i.e., *Endozoicomonas*). Nonetheless, some sequences, with great similarity to *Endozoicomonas*, were recently annotated as *Spongiobacter* and deposited in public databases.

Regarding the family name, both *Endozoicomonaceae* and *Endozoicimonaceae* were used in recent reports [4, 5]. However, neither of those family names has been accepted as an official name of the taxonomic rank, resulting in confusion during data mining. For example, informal family names are included in the bacterial database of Greengenes (greengenes.lbl.gov), but they are absent in Silva (www.arb-silva.de) and RDP (rdp.cme.msu.edu). Furthermore, the same sequences assigned to *Endozoicomonaceae* in Greengenes were assigned to *Hahellaceae* in RDP [6]. Apparently, taxonomic issues of *Endozoicomonas* remain unsettled, but it should be clarified as soon as possible, to minimize further confusion. Due to a paucity of evidence to distinguish those informal taxonomic names, sequences designated *Spongiobacter*, *Endozoicomonaceae*, or *Endozoicimonaceae* are all referred as the genus *Endozoicomonas* in this chapter.

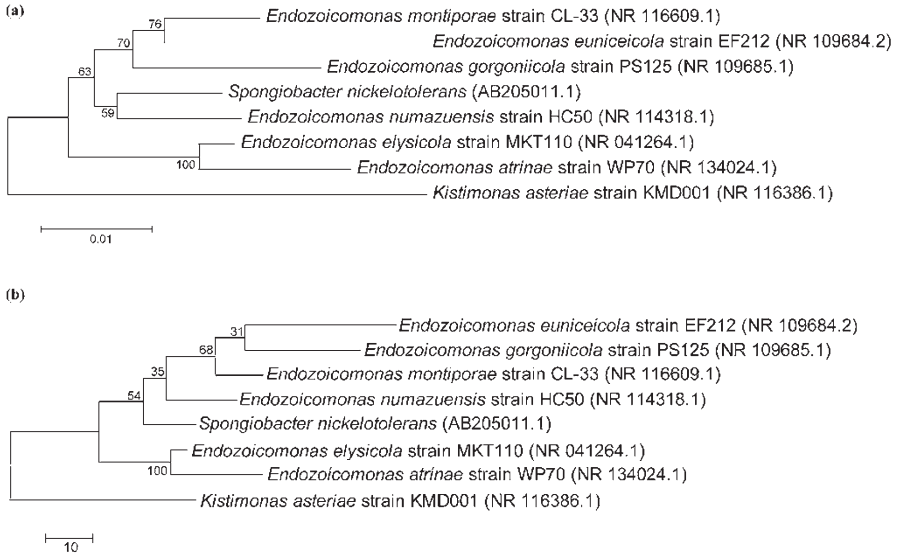


Fig. 5.1 Phylogenetic trees of partial 16S rRNA gene sequences (1397 sites) were constructed with 1000 bootstrap replicates using (a) neighbor-joining method based on Kimura 2P + G model and (b) maximum parsimony method. Bootstrap values are shown on nodes. *Kistimonas asteriae* was used as an outgroup

5.1.2 Cultivable Species of *Endozoicomonas*

Eight cultivable species in this genus have been isolated and published, including *E. elysicola* [1], *E. montiporae* [7], *E. numazuensis* [8], *E. euniceicola*, *E. gorgoniicola* [9], *E. atrinae* [10], *E. arenosclerae* [11], and *E. cretensis* [12]. With the exception of *E. cretensis* that was isolated from fish, all other species were originally isolated from marine invertebrates: three from corals, two from sponges, and one each from sea slugs and pen shells (Table 5.1). Some unique features of each species are described below.

E. elysicola MKT110^T was the first *Endozoicomonas* isolated from the sea slug *Elysia ornate* at depth of 15 m near the coast of Izu-Miyake Island, Japan. The bacterium was Gram-negative, rod-shaped, approximately 0.4–0.6 μm in diameter, and 1.8–2.2 μm long, with a single polar flagellum, pili, and releasable vesicles. The colony on marine agar (Difco Laboratories, Detroit, MI, USA) was 4–5 mm in diameter, circular, convex, and beige with intact edges. The optimal temperature range for aerobic growth conditions on agar plates was 25–30 $^{\circ}\text{C}$ in the presence of seawater-like salt [1].

E. montiporae CL-33^T was the first cultivable coral-associated *Endozoicomonas* from the reef-building coral *Montipora aequituberculata*, subclass Hexacorallia, collected at a depth of 10–15 m in tropical Taiwan [7]. Cultivation conditions were more fastidious and stringent than other species. For example, a sugar supply (e.g., glucose) is necessary for culture. Moreover, the bacterium grows relatively slower

Table 5.1 Differential phenotypic and genotypic characteristics of type strains of *Endozoicomonas* species

Characteristic	<i>E. ephysicola</i>	<i>E. montiporae</i>	<i>E. gorgonicicola</i>	<i>E. eumicicicola</i>	<i>E. numazuensis</i>	<i>E. arenosclerae</i>	<i>E. attrinae</i>
Strain	DSM22380	LMG24815	CECT8353	DSM26535	DSM25634	CBAS572	JCM19190
Isolation source	Sea slug	Hexacoral	Octocoral	Octocoral	Sponge	Sponge	Pen shell
Motility	+	+	+	+	-	+	-
Cell length (µm)	0.4-0.6	0.5-0.7	0.4-0.9	0.6-0.9	3.0-10.0	N.D.	1.2-3.6
Cell diameter (µm)	1.8-2.2	1.0-3.0	1.7-2.5	1.7-2.6	0.4-0.8	0.5-1.0	0.7-1.0
Growth temp. (°C)							
Range	4-37	15-35	15-30	15-30	15-37	12-35	15-37
Optimum	25-30	25	22-30	22-30	25	20-30	30
Growth NaCl (% w/v)							
Range	>0	1-3	1-4	1-4	1-5	2-5	1-4
Optimum	>0	2-3	2-3	2-3	2	3	2
pH optimum	N.D.	8	8	8	7.5-8.0	N.D.	7
Aerobic/anaerobic	Aerobic	Aerobic	Both	Both	Both	Aerobic	Aerobic
Nitrate reduction	+	+	-	-	+	N.D.	+
Major fatty acids	C ₁₆ : ₁ ω7c	C ₁₆ : ₁ ω6c and/or C ₁₆ : ₁ ω7c	C ₁₆ : ₁ ω6c and/or C ₁₆ : ₁ ω7c	C ₁₆ : ₁ ω6c and/or C ₁₆ : ₁ ω7c	C ₁₈ : ₁ ω7c	N.D.	C ₁₆ : ₁ ω6c and/or C ₁₆ : ₁ ω7c
	C ₁₆ : ₀	C ₁₈ : ₁ ω6c and/or C ₁₈ : ₁ ω7c	C ₁₈ : ₁ ω6c and/or C ₁₈ : ₁ ω7c	C ₁₆ : ₀	C ₁₆ : ₁ ω7c	N.D.	C ₁₈ : ₁ ω6c and/or C ₁₈ : ₁ ω7c
	C ₁₄ : ₀	C ₁₆ : ₀	C ₁₆ : ₀	C ₁₈ : ₁ ω6c and/or C ₁₈ : ₁ ω7c	C ₁₆ : ₀	N.D.	C ₁₆ : ₀
Quinones	Q-9	Q-9, Q-8	Q-9, Q-8	Q-9, Q-8	Q-9, Q-8, MK-9	N.D.	Q-9
G + C content	50.4	50.0	47.5	48.6	48.7	47.6	50.5
References	[1]	[7]	[9]	[9]	[8]	[11]	[10]

than other isolates (e.g., *E. elysicola* and *E. numazuensis*) [13]. Colonies growing on marine agar (Difco Laboratories) were 1–2 mm in diameter (smaller than *E. elysicola*). Optimal aerobic growth conditions on agar plates were 25 °C at pH 8.0 and 2–3% NaCl [7].

E. euniceicola and *E. gorgoniicola* [9] were isolated from the octocorals, *Eunicea fusca* and *Plexaura* sp. collected in Florida, USA, and Bimini, Bahamas, respectively. Unlike *E. montiporae* and *E. elysicola*, colonies on agar plates are white and creamy white for *E. euniceicola* and *E. gorgoniicola*, respectively. Moreover, they are facultative anaerobic but lack the ability to reduce nitrates to nitrites.

E. numazuensis [8] and *E. arenosclerae* [11] were both isolated from sponges, the purple sponge *Haliclona* sp. at Numazu in Japan, and the sponge *Arenosclera brasiliensis* in Rio de Janeiro, Brazil, respectively. *E. numazuensis* is longer (3–10 µm) and non-motile, facultative anaerobic, whereas *E. arenosclerae* is motile and aerobic. Although their phenotype profiles differ, they share >99% in sequence identity of the 16S rRNA gene (i.e., 1427 informative sites) [11].

E. atrinae [10] was isolated from the gut of the comb pen shell *Atrina pectinate* in the southern sea of Yeosu in Korea. Similar to *E. elysicola*, this is the other *Endozoicomonas* isolated from mollusks. This bacterium is non-motile, like *E. numazuensis*. Furthermore, it has the smallest colony size on agar plates, 0.6–1.1 µm, and the highest DNA GC content (50.5) of all seven *Endozoicomonas* species.

Although these seven *Endozoicomonas* species were all isolated from marine invertebrates, there were many differences in their distribution, phenotypic characteristics, and metabolic potentials. Therefore, we inferred that diversity is a characteristic of this genus.

5.2 Distribution of *Endozoicomonas*

5.2.1 Host Variation

Culture-independent bacterial community studies have provided valuable ecological information regarding distribution of *Endozoicomonas*. Using 16S rRNA gene sequencing, several surveys of *Endozoicomonas*-related bacteria have been reported from various marine invertebrates, including hexacorals [4, 14, 15], octocorals [16, 17], sea anemones [18], hydras [19], sponges [20–22], polychaetas [23], ascidians [5], sea slugs [1], oysters [3, 24], and bivalves [2]. These bacteria were associated with marine animals and were also detected in some environmental niches, including sediment [25] and seawater [26], although relative abundance of *Endozoicomonas* was much lower in environments than in marine animals [16].

5.2.2 *Spatial Discovery*

Regarding their geographic distribution, *Endozoicomonas*-related bacteria were widely detected in various regions, including South Africa (Mayotte); Asia (Japan and Taiwan); North, Middle, and South America (Florida, Caribbean, Belize, and Brazil); Europe (Mediterranean, Rockall Banks, and Norway); Red Sea; and Great Barrier Reef [1, 2, 7, 15–17, 19, 21, 22, 27–30]. Their geographic habitats were also variable, from intertidal zones [7, 31] to ocean locations at depths exceeding 700 m [6, 17, 26].

5.2.3 *Temporal Distribution*

Endozoicomonas species associated with corals have been discovered in various seasons and climate zones. For example, in the tropic zone, *Endozoicomonas* were regularly identified in a time-series survey of the coral *Isopora*, collected from the southern coast of Taiwan [32]. In the temperate zone, *Endozoicomonas* was also detected in summer and winter or summer and autumn in various gorgonian octocoral species, *Paramuricea clavata* and *Eunicella verrucosa*, respectively [27, 33]. In the Great Barrier Reef, *Endozoicomonas* were dominant in all seasons in the coral *Acropora muricata* of inshore reefs [34].

Regardless of spatial and temporal factors, *Endozoicomonas* were detected not only in various regions with wide longitudes and latitudes but also from intertidal areas to deep oceans and in various marine invertebrates and corals. Therefore, *Endozoicomonas* is a common resident bacterial group associated with marine invertebrates around the world, particularly invertebrates in coral reefs.

5.3 **Abundance, Phylogeny, and Diversity of *Endozoicomonas* in Corals**

5.3.1 *Abundance*

Although absolute abundance of *Endozoicomonas* in their hosts has not been well characterized, there is evidence of changes in relative abundance of the bacteria, often in association with specific factors. For example, relative abundance of *Endozoicomonas* was correlated with their habitats [35]. Similarly, bacterial abundance in fungid corals (*Ctenactis echinata*) was only dominant in sheltered sides of the offshore (i.e., open rocky substrates and clear water habitats) but less abundant in the nearshore (characterized by loose substrates and turbid water) [36], suggesting that these bacteria had environmental preferences that matched those of coral species in the central Red Sea. There was also habitat specificity of *Endozoicomonas*

in a dominant reef-building coral (*Acropora millepora*) in the Great Barrier Reef [34], although the association was opposite to the other study [36], as there was higher abundance of *Endozoicomonas* on the midshore reef than the offshore reef [34]. There are many potential explanations for these apparently contradictory results, including differences in *Endozoicomonas* species, coral hosts, and environmental conditions. In addition, in some studies, *Endozoicomonas* in octocorals had stable abundance in different seasons [27, 33]. Although these studies have provided preliminary insights, much more work is needed to characterize changes in abundance of *Endozoicomonas* in corals.

There are some reports on the effects of stress and environmental factors in abundance of *Endozoicomonas* of corals. For example, relative abundance of *Endozoicomonas* was considerably decreased in response to abiotic stresses, e.g., temperature increases [37], ocean acidification [38], or anthropogenic impacts (viz., sedimentation and sewage) [39]. Furthermore, bleaching of *Acropora* corals in the Great Barrier Reef caused *Endozoicomonas* to dynamically disappear, although it largely recovered during the coral's resurgence over the summer of 2001 to 2002 [40]. Furthermore, loss of *Endozoicomonas* from the surface mucus layer was also a characteristic of lesions in *Pocillopora* in Belize [15]. Similarly, Vezzulli et al. (2013) reported that *Endozoicomonas* were a predominant group on healthy Mediterranean gorgonians but declined greatly when the host was compromised [41]. In addition, *Endozoicomonas* had higher relative abundance in new mucus of healthy coral *Porites astreoides* but was less abundant in aged mucus and disturbed coral [42]. These results strongly support that *Endozoicomonas* is highly associated with coral health (alternatively, host health). Furthermore, we inferred that *Endozoicomonas* may have important roles in marine invertebrates or their holobionts, although it is noteworthy that these bacteria have also been implicated as a potential cause of disease in fish [12].

5.3.2 Phylogeny

Phylogenetic analysis of *Endozoicomonas* may provide clues regarding relationships between these bacteria and their hosts or habitats. For example, some specific *Endozoicomonas* species were present due to adaptation to the environment or host [17]. In that study, *Endozoicomonas* populations in the coral *Madrepora oculata* were grouped together in a phylogenetic tree of 16S rRNA gene that differed from *Endozoicomonas* detected in other species of octocorals and sponges from other places. Similarly, other studies provided evidence that *Endozoicomonas* was host specific [29, 30, 43], whereas it was even proposed that the relationship between the *Endozoicomonas* species and their gorgonian host *Eunicella cavolini* was an ancient evolutionary association, as two *Endozoicomonas* populations, both collected from gorgonians albeit from different locations in the Mediterranean and Caribbean, were closely related in a phylogenetic analysis [16]. The same team reported a similar pattern in another study with two hexacorals, *Porites damicornis* and *Acropora*

spp., from the Red Sea and the Great Barrier Reef [31]. However, in the latter study, some *Endozoicomonas* sequences were mixed with the *Endozoicomonas* from different host species (e.g., *Stylophora pistillata* with *Goniastrea edwardsi* or *Pocillopora damicornis*) in a monophyletic branch of the phylogenetic tree with good bootstrapping values (93 and 78, respectively).

Endozoicomonas species in the hexacoral *Seriatopora hystrix* also clustered with *Endozoicomonas*, not only from other corals but also other marine invertebrates, e.g., sea slugs, sea cucumber, and sea anemones [35]. Besides, *Endozoicomonas* was only detected in three *Acropora* species at Magnetic Island, but not in the same coral species on Orpheus Island, <80 km away [28]. Both studies concluded that location or habitat effects were more important than species effects on bacterial community in host. Hence, host specificity of *Endozoicomonas* is still a complex and unresolved question.

5.3.3 Diversity

Detailed studies of diversity and absolute abundance of *Endozoicomonas* are lacking and cannot be easily done using current data sets. One reason is that the approaches used to conduct community surveys varied among studies. Therefore, there is an urgent need to use only a standardized method. For example, sequencing the same regions or the full length of 16S rRNA gene will facilitate comparative analysis. If possible, the development of universal or common primers to detect the *Endozoicomonas* community should be even more sensitive and helpful to characterize the diversity of these bacteria. Furthermore, measuring absolute abundance of *Endozoicomonas* should be based on the same method of normalization (e.g., equivalent numbers of host cells). Additionally, similar molecular or bioinformatic methods should be used to minimize bias among studies.

5.4 Genomes of *Endozoicomonas*

Several studies proposed or emphasized potential functions of *Endozoicomonas* or their interactive relationships with marine invertebrates, for example, an intimate relationship with the coral host, as *Endozoicomonas* cells were present in coral cells [2, 31]. In addition, *Endozoicomonas* may have a role in sulfur cycling [44], DMSP degradation [45], and production of antimicrobial compounds in its coral host [22]. However, there is no direct evidence that these actually occur.

Genomic approaches are useful to identify potential metabolic and other functions of *Endozoicomonas*. Three cultivable strains of *Endozoicomonas*, *E. elysicola*, *E. montiporae*, and *E. numazuensis*, were sequenced [46]. All three genomes were estimated to exceed 5 Mbp (Table 5.2), with potential capacity for the Embden-Meyerhof-Parnas (EMP) glycolytic pathway, tricarboxylic acid cycle, in addition to

Table 5.2 Genome information from *Endozoicomonas*-type strains

Characteristic	<i>E. elysicola</i>	<i>E. elysicola</i>	<i>E. numazuensis</i>	<i>E. montiporae</i>	<i>E. montiporae</i>
Strain	DSM 22380	DSM 22380	DSM 25634	LMG 24815	CL-33 ^T
Host	Sea slug, <i>Elysia ornata</i>	Sea slug, <i>Elysia ornata</i>	Sponge, cf. <i>Haliclona</i> spp.	Hexacoral, <i>Montipora aequituberculata</i>	Hexacoral, <i>Montipora aequituberculata</i>
Genome (Mbp)	5.55	5.61	6.34	5.6	5.43
Gene	4669	4693	5405	5113	5033
Protein	4515	4532	5129	4837	4761
16S rRNA	N/A	6	5	4	7
23S rRNA	N/A	6	2	4	7
5S rRNA	6	8	3	8	8
tRNA	78	85	90	104	109
GC content (%)	46.7	46.8	47.1	48.5	48.37
Pseudogene	72	55	175	155	198
References	[47]	[46]	[46]	[46]	[13]

genes for the conversion and assimilation of nitrate. Ding and co-workers [13] provided a high-quality, nearly completed genome (e.g., 99.8%) of *E. montiporae* and detailed characterizations, including comparative analysis of the three species. All of these had the capacity to synthesize all proteinogenic amino acids and most cofactors, prosthetic groups, and electron carriers required for growth, except vitamin B12 [13]. The researcher provided evidence-based inferences and speculation regarding how *E. montiporae* interacted with its host. Based on a physiological experiment, all three bacteria had the genes to degrade testosterone, implicating this male sex hormone as a potential “animal sign” for attracting *Endozoicomonas* [13]. Unique genes detected in *E. montiporae* for N-deglycosylation enzyme might be able to partially dissociate glycoproteins inside the coral mucus (without harming the host) and thereby enable bacteria to penetrate through the mucus layer to reach specific ephrin receptors on the coral cell membrane; an interaction of these receptors and ephrin ligands of *E. montiporae* enables the bacterium to enter the coral cell by endocytosis [13]. Moreover, a secreted protein of *E. montiporae* might modulate trafficking inside the host’s cell and prevent attacks by the lysosome inside the host cells. More interestingly, various type III secretion effectors (e.g., T3SS: involved in survival inside hosts, regulating metabolism and increasing the fitness of the host) were detected in the *E. montiporae* genome; perhaps they are able to interact with hosts or provide certain responses when the coral host under stress (e.g., thermal-induced mitochondrial dysfunction). Finally, based on several striking features, including unusual high-repeat sequences, mobile elements, pseudogenes, and several eukaryotic genes detected in the genome, the authors inferred that *E. montiporae* was involved in genomic erosion and gene exchange and could be a facultative endosymbiont [13].

5.5 Future Directions

Although there are many reports of *Endozoicomonas*, their ecology and evolution remain unclear. Due to variations in regions of 16S rRNA gene that have been sequenced, and inconsistencies among reports, it is hard to clarify how abiotic or biotic factors affect distribution, composition, relative abundance, or phylogeny of *Endozoicomonas* communities. Nevertheless, some hypotheses can still be constructed. For example, occurrences of *Endozoicomonas* populations in marine invertebrates are not randomly distributed, and various marine invertebrates may provide distinct niches for certain *Endozoicomonas* spp. or strains. In addition, we suggest that some *Endozoicomonas* sp. have specific roles, including being endosymbiotic with marine invertebrates, pathogenic for fish, or free-living in seawater or sediment [12, 13, 23, 25, 31].

To demonstrate or comprehend biotic (e.g., host species) and abiotic effects on communities of *Endozoicomonas* and propose potential relationships of *Endozoicomonas* community with habitats or host species specificity and temporal variations, it is necessary to conduct large-scale surveys of *Endozoicomonas* community in various environments, habitats, or hosts and monitor the temporal transition of the *Endozoicomonas* community in long-term studies.

In addition, detailed molecular and physiological experiments, based on genomic information, should be conducted to clarify physiological functions or ecological roles of *Endozoicomonas*. These experiments should not only be done on cultivable-type strains in vitro but also be examined in vivo, i.e., inside host cells. These studies will provide insights into coral microbiology, as well as a big impetus to conduct additional studies of *Endozoicomonas* and interactions with their hosts.

5.6 Summary

- (1) *Endozoicomonas* bacteria are highly diverse and widely dispersed across various hosts, geographies, and times.
- (2) The relative abundance of *Endozoicomonas* is affected by habitat, environmental stress, or health of their host, suggesting *Endozoicomonas* spp. may have habitat or host specificity.
- (3) Based on genomic results, *E. montiporae* may be a facultative symbiont.
- (4) We hypothesize that occurrences of *Endozoicomonas* populations in marine invertebrates are not randomly distributed. Various marine invertebrates may serve as distinct niches for certain *Endozoicomonas* spp., whereas specific *Endozoicomonas* sp. has separate ecological roles.
- (5) Standardization of the strategy and methods to characterize *Endozoicomonas* is essential for effective comparative analyses.

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