

Biology of a “babysitting” symbiosis in brittle stars: analysis of the interactions between *Ophiomastix venosa* and *Ophiocoma scolopendrina*

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Abstract. “Babysitting” symbioses between brittle star species involve juveniles of one species and adults of another. During this phenomenon, reported from many localities in the Indo-Pacific Ocean, juveniles are attached to the disk or lie in the bursa of the adults. The symbiosis between members of *Ophiomastix venosa* and their host, *Ophiocoma scolopendrina*, was investigated on the Great Barrier Reef of Toliara (Madagascar) during a 14-month period. The population of *O. scolopendrina* only occurs on rocky spurs that frequently emerge at low tide, while the population of *O. venosa* lies in adjacent surge channels that are always immersed. Only juveniles of *O. venosa* associated with adults of *O. scolopendrina* may occur on the rocky spurs. Analyses conducted on the populations of the two species showed that (1) the symbiosis is facultative and that symbiotic juveniles migrate into the channels when they reach a disc diameter of 6 mm, and (2) recruitment in the channels occurs in April. Host choice experiments, Y-tube experiments, and experiments assessing the resistance of the ophiuroids to air-drying were conducted in the laboratory. The experiments clearly demonstrated that (1) symbiotic juveniles of *O. venosa* specifically recognize adults of *O. scolopendrina*, while free juveniles of the same size do not, and (2) juveniles of *O. venosa* would not survive air-drying conditions similar to those observed on the spurs at low tides if they were not in symbiosis with adults of *O. scolopendrina*.

Additional key words: symbiosis, commensalisms, echinoderm, ophiuroid

“Babysitting” symbiosis in brittle stars concerns juveniles of one species and adults of another species. During this phenomenon, reported from many localities in the Indo-Pacific Ocean, juveniles are attached to the disk or lie in the bursa of the adults. “Babysitting” symbiosis between the subtidal populations of *Ophiomastix annulosa* and the intertidal populations of *Ophiocoma scolopendrina* (LAMARCK 1816) was first studied by Hendler et al. (1999) at Sesoko Island (Okinawa, Japan). These authors found that juveniles of *O. annulosa* were usually in the bursae of the hosts while a few others were attached to their disk. They suggested that members of *Ophiomastix* species in general would be found to be associated with members of *Ophiocoma* species, and that juveniles of *O. annulosa* may always be symbiotic with adults of *O. scolopendrina* before they move into the

subtidal. According to Hendler et al. (1999), symbiotic ophiuroids must avoid desiccation, overheating, and isolation. It would seem advantageous for these juveniles to take refuge within large, abundant, widespread, mobile, calcified animals that occupied moist, sheltered crevices. Their results, however, do not allow them to find selective advantages that would explain why it is advantageous for subtidal symbiotic ophiuroids to recruit to the intertidal.

A similar inter-ophiuroid symbiosis was observed on the barrier reef of Toliara (Madagascar) between juveniles of *Ophiomastix venosa* PETERS 1851 and adults of *O. scolopendrina*. Investigations made on the reproductive cycles of both ophiuroids indicated that members of *O. scolopendrina* reproduce continuously and that they have a planktotrophic larva, while individuals of *O. venosa* breed once a year in the austral summer, mainly in January–February (Fourgon 2006). Members of *O. venosa* have a lecithotrophic larva that metamorphoses spontaneously after 2 d of development in cultures (Fourgon et al.

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2005). Yet, analyses of the natural abundance of carbon and nitrogen stable isotopes were performed to investigate the feeding habits of the two ophiuroids and their symbiotic juveniles (Fourgon et al., 2006). The results suggest that symbiotic juveniles steal neuston from their host, *O. scolopendrina*.

The present article aims to explain why juveniles of the subtidal species, *O. venosa*, are found in association with adults of the intertidal species, *O. scolopendrina* and it explores the reasons why this symbiosis is specific. It presents the results of a 14-month survey conducted on the populations of the two species. It also further investigates this symbiosis by laboratory experiments that were conducted to study (1) the host choice shown by ophiuroid symbionts, (2) the degree of recognition of host odors by ophiuroid symbionts, and (3) the tolerance of ophiuroid symbionts to air-drying conditions similar to those they face in the intertidal.

Methods

In situ observations

Members of *Ophiomastix venosa* and *Ophiocoma scolopendrina* occur in the boulder tract of the barrier reef of Toliara (Madagascar) (Fig. 1; see Clausade et al. 1971 for a detailed description of the Toliara barrier reef). There, rocky spurs alternate with surge channels, in parallel to each other and perpendicular to the external reef slope. Spurs and surge channels continue ≤ 20 m deep into the subtidal. At the intertidal level, spurs are ~ 100 m long and 10 m wide. They consist of rocks of dead corals emerging at low tides. Surge channels are permanently immersed (channel width: ~ 30 m; water height at low tide: ~ 50 cm) and their bottom consists of sand with living and dead corals.

Ophiuroids were collected at low tide by hand. At first, transects were performed at five different sites along the barrier reef in February 2000 (Fig. 1A–E). Each of the transects consisted of four 1-m^2 quadrats haphazardly placed on the spurs and four others in the channels (40 quadrats in total). All dead coral rocks were removed and analyzed to ensure that all ophiuroids were counted. The occurrence of the inter-ophiuroid symbiosis was recorded and the prevalence (i.e., the percentage of hosts associated with at least one symbiont individual) was calculated. The prevalences were statistically equal in transects A, B, D, and E, but significantly higher in transect C (Mann–Whitney *U* Test, $p < 0.05$). The latter was thus selected as the study site for the inter-ophiuroid symbiosis. The investigated area comprises a channel

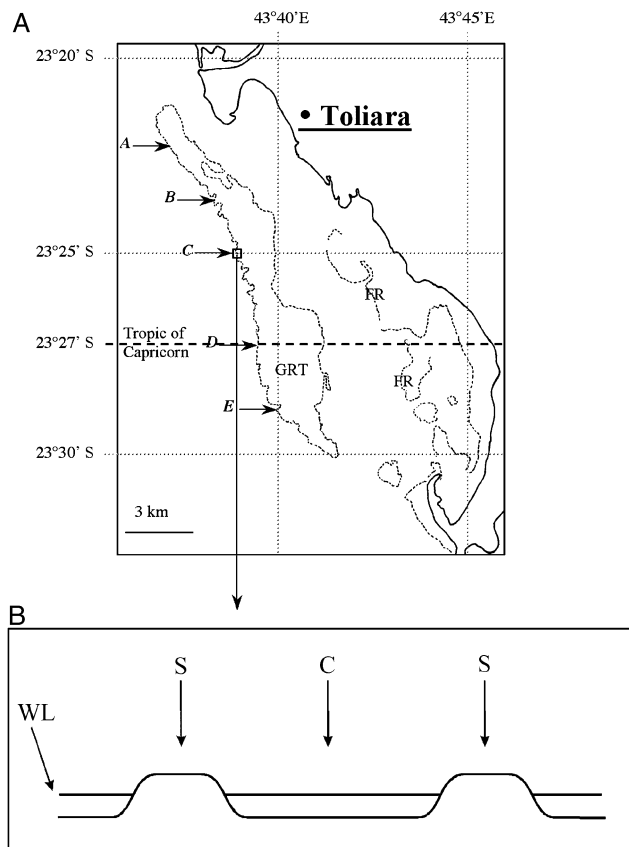


Fig. 1. A. Map of the Great Barrier Reef of Toliara with the location of transect sites (A–E). B. Schematic transverse section of study site C. c, channel; s, spur; fr, fringing reef; grt, Great Barrier Reef of Toliara; wl, water level at low tide.

and the southern adjacent spur. This site was then investigated monthly from March 2000 to April 2001 at low tide: each month, eight 1-m^2 quadrats were placed on the spur and eight others in the channel; all the ophiuroid species, as well as the occurrence of symbiotic juveniles of *O. venosa* on adults of *O. scolopendrina* or other species were recorded. Disc diameters (DDs) of symbionts and hosts were measured in the field using a calliper and the number of symbionts per host was noted. Yet, each month, the DD of the individuals that were found in one (for *O. scolopendrina*) to up to ten quadrats (for *O. venosa*) was measured (>180 individuals of both species were measured each month). Yet, because juveniles of *O. venosa* were also found in the host's bursae, and because it was difficult to dissect individuals in the field, a minimum of 180 individuals of *O. scolopendrina* was dissected monthly in the laboratory, and the bursal infestation was recorded.

To determine recruitment events in the population of *O. venosa*, monthly disk-size frequency

distributions were generated by pooling individuals in size classes of 2 mm. Size-frequency data were first tested against a normal distribution (Kolmogorov–Smirnov test); any deviation from normality would indicate possible multimodality. When multimodality was suspected, separation of the observed polymodal length–frequency distributions into component normal distributions (subgroups) was achieved by C.A.MAN program (Böhning et al. 1992; freely available at www.medizin.fu-berlin.de/sozmed/caman.html). This program estimates finite mixtures of distributions using the maximum-likelihood method. Information such as the number of population subgroups, their mean values, and relative sizes expressed as weights, was extracted from the length–frequency data. The information generated by C.A.MAN was used as initial values in the MIX program (Macdonald & Pitcher 1979). MIX analyzes histograms as mixtures of statistical distributions by finding a set of overlapping component distributions that gives the best fit to the histogram. The optimal criterion used by MIX is maximum likelihood.

Behavioral approach

Two types of experiments were performed to analyze the possible attraction exerted by adults of *O. scolopendrina* on juveniles of *O. venosa*. Individuals used during these experiments were collected outside the study site mentioned above. Experiments were conducted with filtered seawater at ambient temperature ($24 \pm 1^\circ\text{C}$). Tested ophiuroids were taken in the field 2 d before the experiments and acclimatized in 50-L tanks. In both experiments, two groups of juveniles of *O. venosa* were tested: free juveniles collected in the surge channel and symbiotic juveniles collected from adults of *O. scolopendrina* from the rocky spur. Symbiotic juveniles were separated from the host and kept in aquaria 2 d before being tested. Both types of juveniles were of the same size (DD: 5–9 mm).

Host-choice experiment. The goal of this experiment was to assess the ability of one juvenile of *O. venosa* to choose between one adult of *O. scolopendrina* and one adult of *O. venosa* when the three individuals were placed in a confined area. The experimental system consisted of an aquarium ($30 \times 30 \times 10$ cm) filled with 4 L of filtered seawater aerated with fine air bubbling. During a trial, one adult of *O. scolopendrina* and one adult of *O. venosa* (DD of both 1.6–2.0 cm) were placed at the opposite ends of the aquarium. Between them, either a free juvenile of *O. venosa* or a symbiotic juvenile was introduced at the center of the aquarium. Thirty trials

were conducted using 120 ophiuroids (30 ophiuroids for each of the four types). Each trial lasted 15 h, at the end of which the position of the juvenile in relation to the co-occurring adults was recorded. Juveniles tested could be found (1) alone without touching any of the two adults, (2) in loose contact with any part of any of the two adults, or (3) firmly attached to any part of any of the two adults.

Y-tube experiments. This assessed the ability of juveniles of *O. venosa* to recognize water flows conditioned by either *O. scolopendrina* or *O. venosa*. The experimental system used was a Y-tube system adapted from Davenport (1950). It consisted in a Y-shaped glass tube, 3 cm in diameter, whose branches each measured 10 cm long. The paired branches were connected to two separate aquaria ($17 \times 17 \times 10$ cm; aquaria A and B; Fig. 2) while the unpaired branch was closed by a perforated plug, crossed by a flexible pipe provided with a tap. Each aquarium was in turn connected to another aquarium ($17 \times 17 \times 10$ cm; Fig. 2C,D) filled with either filtered seawater or

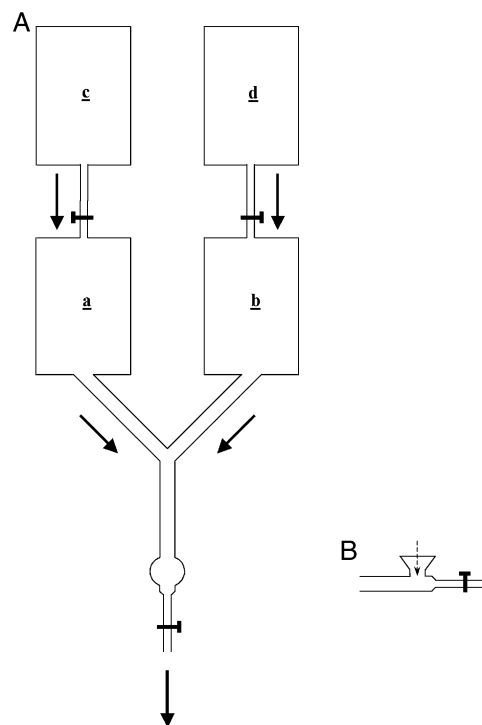


Fig. 2. A. Schematic drawing of the Y-tube system used during the experiment. B. Lateral view of the starting point of the unpaired branch of the Y-tube, showing where juveniles were introduced into the system. Arrows indicate the direction of water flow. Aquaria A and B contain water coming from aquaria C and D, respectively. One of the last aquaria included adults of either *Ophiomastix venosa* or *Ophiocoma scolopendrina*, depending on the test.

filtered seawater conditioned by one of the tested ophiuroid species (water conditioning consisted of placing 25 adults of the tested ophiuroid species in 4 L of seawater for 20 min). Water flowed from the upper aquarium to the lower one, then to the paired branches, and was evacuated through the flexible pipe. Water flow ($50\text{--}100\text{ mL min}^{-1}$) was regulated using a tap and monitored by adding drops of fluorescein (50 mg L^{-1}).

Four tests were performed that included 30 trials each: either adults of *O. scolopendrina* or *O. venosa* were placed in aquarium C and tested against seawater of aquarium D, and either free or symbiotic juveniles of *O. venosa* were tested. A control consisted of opposing only seawater and recording the behavior of juveniles. In total, 180 juveniles were used. Each juvenile was placed 3 cm deep in the unpaired branch of the Y-tube system and two types of behavior were recorded (VandenSpiegel et al. 1998; Eeckhaut et al. 2000): odor-mediated ophiuroid motion and odor-mediated ophiuroid orientation. The odor-mediated ophiuroid motion was investigated by comparing the number of times individuals started moving under stimulation with the number of times they started moving under "no stimulation" (when only seawater filled the two aquaria). The statistical significance was assessed with a Yates-corrected chi-square test (Zar 1996). The odor-mediated ophiuroid orientation was investigated by comparing the number of times ophiuroids tested entered the paired branch carrying ophiuroid odor with the frequency "50%." The statistical significance was assessed with a chi-square goodness-of-fit test (Zar 1996). When ophiuroids did not move after 15 min, the trial was considered nul. Between each assay, the Y-tube system was washed with detergent and thoroughly rinsed with seawater. After 15 assays, the system was turned over (the right branch became the left, and vice versa) to avoid any other parameter that could have influenced the choice of the animals.

Resistance to air drying

Temperatures below the rocks of the spurs and in the water of the channels were recorded many times during the survey. The highest temperatures observed occurred in summer (25 February) and reached 39°C under rocks and 35°C in the channels. The following experiment was conducted to study the tolerance exhibited by adults of *O. scolopendrina* and *O. venosa*, and free and symbiotic juveniles of *O. venosa*, to air drying that occurs at low tide on the spurs. Thirty individuals of each of the four categories of ophiuroids were placed separately in dry plastic containers

($12 \times 12 \times 12\text{ cm}$) (the symbiotic juveniles were tested in association with their host) and maintained in a ventilated oven (WTB Binder) kept at 39°C for 20 min, 1, 2, 3, 4, or 5 h (in all, 180 individuals for each of the four categories were used). To minimize variations in air-drying tolerance that would be due to ophiuroid age, adults with a disk $16\text{--}19\text{ mm}$ in diameter, and juveniles with a DD of $6\text{--}9\text{ mm}$, were used in the experiment. After each assay, individuals were transferred overnight to 50-L tanks and the number of survivors was counted. We used the Gompertz function to represent the survivorship curves of ophiuroids (Ebert 1999). In addition, adults were weighed before and after assays to quantify the body weight lost due to water evaporation.

Results

In situ observations

Individuals of *Ophiocoma scolopendrina* were abundant on the spur and few in the channel: pooled over all the sampling periods, a mean of 124 and 0.5 m^{-2} individuals, respectively. The highest density on the spur was observed in April and the lowest in February (155 and 102 individuals m^{-2} , respectively) (Table 1). The DD of *O. scolopendrina* varied $0.1\text{--}2.2\text{ cm}$, the average size of the population observed monthly varying $1.10\text{--}1.28\text{ cm}$. At all periods, small size classes (DD $< 0.8\text{ mm}$) were present on the spurs (data not shown).

In contrast, the population of *Ophiomastix venosa* was much denser in the channel: we observed 0.2 and 13 individuals m^{-2} on average (all periods pooled), respectively. All spur-inhabiting members of *O. venosa* were symbiotic juveniles (Fig. 3). Their DD varied $0.1\text{--}1\text{ cm}$ (Table 2) while individuals of *O. venosa* (all free) observed in the channels varied $0.1\text{--}2.4\text{ cm}$ (Fig. 4). The average prevalence of the infestation over the whole survey period was 2.2%, varying 0.4–11% in monthly samplings (Table 2). In most cases, there was one juvenile per host, rarely two or three. The DD of the hosts varied $0.5\text{--}2\text{ cm}$, but most had a large size (mean DD = 1.53 mm) (Table 2). The disk size of the symbionts was correlated to the DD of the hosts (Spearman's rank correlation, $n = 305$, $r = 0.47$, $p < 0.001$).

Most of the symbiotic juveniles were located on the host aboral side (50%); the others were on the oral side (30%), on the disk margin (19%), or in the bur-sae (1%). Individuals on the aboral side were firmly attached to the disk with their arms located in the host interradii that often extend up to the host mouth. Individuals on the oral side had their mouth

Table 1. Density and size of the spur population of *Ophiocoma scolopendrina* over the 14-month period.

Month	Density (ind m ⁻²)		Disk size (mm)	
	Mean (SD)	Minimum–Maximum	Mean (SD)	Minimum–Maximum
March	138 (25)	96–182	1.14 (0.5)	0.3–2.1
April	155 (25)	129–211	1.16 (0.4)	0.2–2.2
May	129 (42)	47–186	1.17 (0.4)	0.3–2.2
June	127 (42)	74–190	1.28 (0.3)	0.2–2.1
July	135 (25)	96–172	1.27 (0.3)	0.2–1.9
August	136 (45)	75–200	1.25 (0.3)	0.2–1.8
September	112 (56)	28–215	1.22 (0.4)	0.1–2
October	108 (36)	41–154	1.18 (0.3)	0.2–2.1
November	104 (29)	36–130	1.27 (0.3)	0.2–1.9
December	105 (63)	20–218	1.12 (0.3)	0.1–1.9
January	125 (33)	76–167	1.10 (0.3)	0.2–1.9
February	102 (25)	72–137	1.15 (0.3)	0.3–1.9
March	117 (18)	90–141	1.25 (0.3)	0.3–1.9
April	141 (44)	94–226	1.28 (0.3)	0.2–2

facing that of the host; those on the disc margin extended their arms to the oral and aboral sides of the host disk. Symbionts in the bursae were generally smaller (mean disc size = 0.30 mm) than those located externally on the hosts (mean disc size = 0.57 mm) ($p < 0.05$; student *t*-test). Intrabursal juveniles appeared harmless, as their hosts showed no sign of wound or disease.

External infestation occurred at any time of the year. It was higher at the beginning of the survey than at the end (11% vs. 0.5%, Table 2). Intrabursal infestation was very low, observed March–August in the first year of the survey, and in February of the second year, and zero in other months (Table 2).

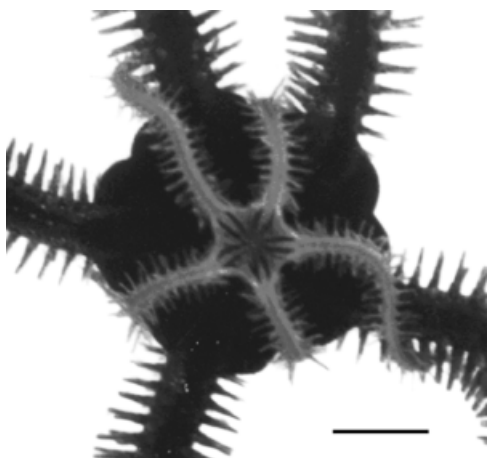


Fig. 3. Macrophotography showing a symbiotic juvenile of *Ophiomastix venosa* attached on the disk of an adult of *Ophiocoma scolopendrina*. Scale bar, 5 mm.

When internal infestation occurred, prevalence varied 0.5–2.4% over sampling periods; there was always only one intrabursal juvenile per infested ophiuroid.

The size-frequency distributions in the channel population of *O. venosa* from March 2000 to February 2001 are illustrated in Fig. 4. Small size classes ($DD \leq 10$ mm, similar to symbiotic juveniles) were present at all periods. They represented 15–50% of the samples. Even very small individuals ($DD < 4$ mm) were present at most sampling periods, except in November and December 2000, and February 2001. When present, they were rather scarce, representing $< 1.4\%$ of the samples, except from March to May 2000, where they accounted for 2.7–5.1%. In April 2000, individuals 0.6–0.8 mm DD reached their highest abundance, representing $> 25\%$ of the sampling, while the other size classes did not reach 15%. A C.A.MAN analysis suggested that one or two cohorts explain the size–frequency distributions best; two cohorts from April to October 2000, one in March 2000 and November 2000–February 2001 (Fig. 4). According to this analysis, recruits appear in the channels in April 2000 and this cohort of juveniles would totally mix with older individuals by November 2000.

Figure 5 gives a comparison of the size-frequency distributions of all symbiotic juveniles (intrabursal and external) and that of free individuals of the same size ($DD < 10$ mm). The distribution of symbiotic juveniles is normal with a maximal number of individuals of DD 4–6 mm. The distribution of free individuals is exponential with a maximal number of individuals of DD 6–8 mm.

Table 2. Infestation of adults of *Ophiocoma scolopendrina* by juveniles of *Ophiomastix venosa*. The prevalence (%) is the portion of host population infested; the number in brackets indicate the prevalence of intrabursal infestations. "Juv/Host" refers to the minimum and maximum number of symbiotic juveniles associated with one host. Host and symbiont sizes express the DD of the ophiuroids. SD, standard deviation.

Month	Infestation		Host size (cm)		Symbiont size (cm)	
	Prevalence	Juv/Host	Mean	Minimum–Maximum	Mean (SD)	Minimum–Maximum
March	11 (2.4)	1–3	1.58	0.5–2.1	0.60 (0.20)	0.2–1
April	8 (0.6)	1–2	1.61	1.2–1.9	0.68 (0.16)	0.3–1
May	8 (1.1)	1–3	1.68	1.3–2	0.62 (0.14)	0.3–0.8
June	4 (0.5)	1–3	1.44	0.9–1.7	0.55 (0.16)	0.3–0.7
July	3 (0.5)	1	1.58	1.2–1.8	0.76 (0.18)	0.3–1
August	3 (0.5)	1	1.64	1.5–1.8	0.54 (0.24)	0.2–0.8
September	3 (0)	1–2	1.65	1.3–1.9	0.46 (0.19)	0.2–0.7
October	3 (0)	1–2	1.60	1.6	0.40 (0.10)	0.4
November	2 (0)	1	1.47	0.9–1.9	0.57 (0.16)	0.3–0.8
December	1 (0)	1	1.55	1.4–1.7	0.60 (0.14)	0.5–0.7
January	1 (0)	1	1.33	1.1–1.5	0.48 (0.10)	0.4–0.6
February	1 (0.5)	1	1.53	1.5–1.6	0.73 (0.15)	0.6–0.9
March	0.4 (0)	1	1.10	1.1	0.40 (0.10)	0.4
April	0.5 (0)	1	1.70	1.7	0.70 (0.10)	0.7

Three other ophiuroids were observed on the spurs: *Ophioplocus imbricatus*, *Ophiocoma erinaceus*, and *Ophiolepis cincta* (Table 3). All three were observed with *O. venosa* and seven other species in the channels: *Ophiothrix tricuspida*, *Ophiocoma brevipes*, *Ophiarachnella gorgonia*, *Ophiocoma valenciae*, *Ophionereis porrecta*, *Ophiolepis superba*, and *Ophiomyxa australis*. Juveniles of *O. venosa* were never observed to be associated with these species, except for *O. brevipes*, for which 58% of the individuals carried juveniles of *O. venosa*. Furthermore, juveniles of *O. scolopendrina* were sometimes found on the disk of conspecific adults. They were not attached as strongly as juveniles of *O. venosa* and they could be removed easily from their hosts. The prevalence of this intraspecific association was low, 0.016–0.069%.

Behavioral approach

Host choice experiments. The results of these experiments are shown in Table 4. They indicate a clear difference in the behavior of symbiotic and free juveniles of *O. venosa*. Twenty-two out of the 30 symbiotic juveniles were found to be firmly attached to adults of *O. scolopendrina* at the end of the experiment while only two out of the 30 free juveniles were doing so ($p < 0.001$). In contrast, free juveniles appeared to be more attracted by conspecific adults, as 15 out of 30 were found in loose contact with adults of *O. venosa* at the end of the experiments

($p = 0.019$). Very few free juveniles and symbiotic juveniles were found to be in loose contact with adults of *O. venosa*.

Y-tube experiments. When the aquaria were filled with fresh filtered seawater (control experiments), both free and symbiotic individuals of *O. venosa* made no choice between aquaria A and B (Table 5, I and IV).

When exposed to water conditioned with adults of *O. scolopendrina*, symbiotic juveniles were, most of the time, stimulated because only one specimen remained motionless (Table 5, II). Furthermore, these juveniles preferred host-conditioned seawater to regular seawater because 24 out of 29 juveniles that responded to the stimulus chose the conditioned aquaria (Table 5, II). In contrast, free-living juveniles exposed to the same experimental conditions (Table 5, V) did not significantly move toward seawater conditioned by members of *O. scolopendrina* (Test 1) and those that moved did not significantly discriminate between that and regular seawater (Test 2).

When exposed to water conditioned with adults of *O. venosa* (Table 5, III), symbiotic juveniles were not significantly attracted nor did they show any preference between conspecific-conditioned seawater and regular seawater. Similarly, free juveniles were not significantly attracted by a chemical cue from adult conspecifics, and those that moved did not show any preference between the two water-flow treatments (Table 5, VI).

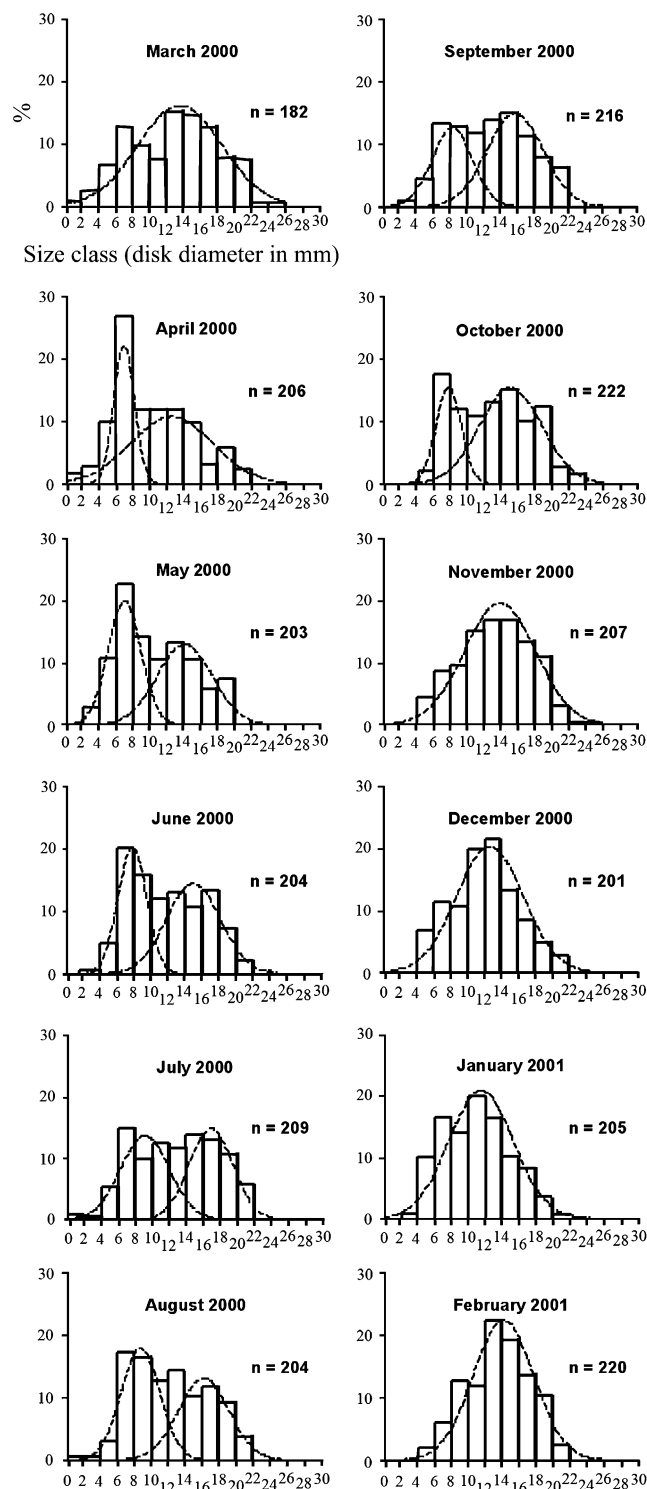


Fig. 4. Size–frequency distributions (%) of the channel population of *Ophiomastix venosa* during the 14-month survey period. n, number of individuals.

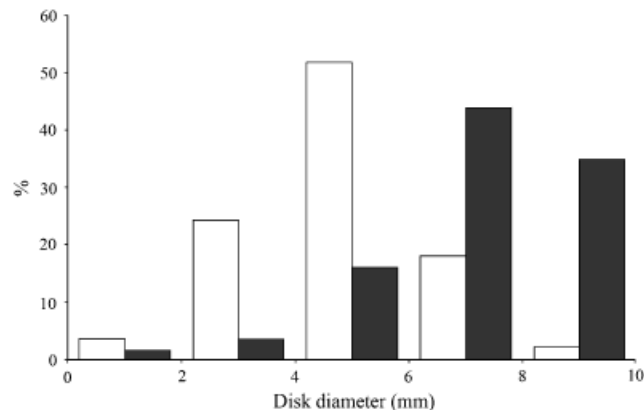


Fig. 5. Size–frequency distributions of the symbiotic juveniles (in white; intrabursal and external; n = 306) and of free individuals of the same size (in black; disc diameter < 10 mm; n = 891) observed during the 14-month survey period.

Resistance to air drying

When individuals of *O. scolopendrina* were exposed to air at low tide, they sheltered under dead corals or in rock crevices, and curled their arms above their disk. A similar posture was observed in specimens exposed to air-drying in the experimental system. Most (97%) survived ≤ 3 h while 25% survived to 4 h (Fig. 6). Individuals of *O. venosa* did not curl their arms when air-dried, and they were much less resistant to hot air exposure, most dying after 2 h (Fig. 6).

Free-living and symbiotic juveniles of *O. venosa* reacted differently to the treatment (Fig. 6). All 30

Table 3. Ophiuroid species observed on the spurs and in the channels of the Great Barrier Reef of Toliara (Madagascar). N, number of individuals observed during the 14-month survey period; D, mean density expressed in individuals m^{-2} .

Species	Spurs		Channels	
	N	D	N	D
<i>Ophiocoma scolopendrina</i>	13,934	124.41	110	0.48
<i>Ophiomastix venosa</i>	21	0.19	2968	13.02
<i>Ophioplocus imbricatus</i>	1253	11.19	1107	4.86
<i>Ophiocoma erinaceus</i>	86	0.77	410	1.80
<i>Ophiolepis cincta</i>	1	0.01	126	0.55
<i>Ophiothrix tricuspida</i>	0	0	104	0.46
<i>Ophiocoma brevipes</i>	0	0	91	0.40
<i>Ophiarachnella gorgonia</i>	0	0	26	0.11
<i>Ophiocoma valenciae</i>	0	0	7	0.03
<i>Ophionereis porrecta</i>	0	0	5	0.02
<i>Ophiolepis superba</i>	0	0	2	0.01
<i>Ophiomyxa australis</i>	0	0	1	0.004

Table 4. Host-choice experiment. Numbers of juveniles of *Ophiomastix venosa* that were observed free, in loose contact, or firmly attached to adults of *Ophiocoma scolopendrina* or *Ophiomastix venosa*. Thirty free juveniles (collected in channels) and 30 symbiotic juveniles (collected with their hosts on spurs) were used (all of similar size: DD 5–9 mm).

State at the end of the test	Free juvenile	Symbiotic juvenile
Observed free	8	3
Loose contact with <i>O. scolopendrina</i>	15	3
Firmly attached to <i>O. scolopendrina</i>	2	22
Loose contact with <i>O. venosa</i>	3	2
Firmly attached to <i>O. venosa</i>	2	0

free juveniles died after 1 h of air-drying. In contrast, most of the symbiotic juveniles attached to adults of *O. scolopendrina* were still alive after 3 h (Fig. 6). Even after 4 h, >25% of symbiotic juveniles were still alive, their survivorship curve being very similar to that of their host. Comparison of body weight loss between adults of the two species shows that during 2 h of air drying, individuals of *O. venosa* lost significantly more water than *O. scolopendrina* ($p < 0.001$). The adults of the two species died after 30% loss of body weight (Fig. 7).

Discussion

Is the association between juveniles of *Ophiomastix venosa* and adults of *Ophiocoma scolopendrina*

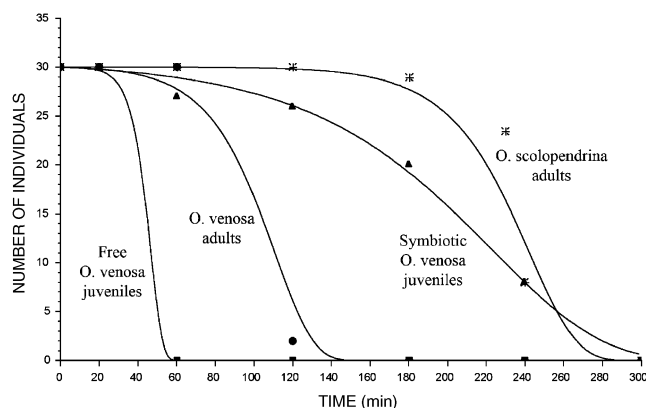


Fig. 6. Survival curves of ophiuroids when exposed to air-drying (40°C). The curves illustrate the survival of 30 individuals of free juveniles of *Ophiomastix venosa*, symbiotic juveniles of *O. venosa*, and adults of *O. venosa* and *Ophiocoma scolopendrina* during air-drying.

facultative or obligatory? The answer is facultative. Symbiotic juveniles observed in association with adults of *O. scolopendrina* on the spurs of the reef have a 1–10 mm DD, the size of small individuals observed in the channels. Yet, in the channels, small individuals of *O. venosa*, <6 mm DD, represent a non-negligible 21% of the population, pooled over all samples. It is thus very probable that these small individuals of *O. venosa* never entered in symbiosis with adults of *O. scolopendrina*.

If this really is the case, why does a fraction of juveniles of *O. venosa* enter in association with adults of *O. scolopendrina* on the spurs while others do not?

Table 5. Y-tube experiment. The results illustrate the behavior of symbiotic juveniles and free juveniles when placed in a Y-tube and exposed to various water-mediated chemical cues. Aquarium A contains the chemical cue; aquarium B, regular seawater. Thirty assays were performed for each of the 6 tests (180 juveniles total). In the results, “nul”, “A,” and “B” indicate the number of times the tested ophiuroids remain motionless, run into the aquarium A, and run into the aquarium B, respectively. Test 1 investigated the odor-mediated ophiuroid motion by comparing the number of times individuals started moving under a species stimulation with the number of times they started moving when only seawater filled the two aquaria; Test 2 investigated the odor-mediated ophiuroid orientation by comparing the number of time tested juveniles entered the paired branch carrying ophiuroid odor with a frequency 50% (see Methods).

Type of juvenile	Aquarium A	Aquarium B	Results			Statistics	
			Nul	A	B	Test 1	Test 2
Symbiotic juvenile							
(I)	Seawater	Seawater	20	5	5	—	$p = 1$
(II)	<i>Ophiocoma scolopendrina</i>	Seawater	1	24	5	$p < 0.001$	$p < 0.001$
(III)	<i>Ophiomastix venosa</i>	Seawater	16	8	6	$p = 0.43$	$p = 0.59$
Free juvenile							
(IV)	Seawater	Seawater	14	8	8	—	$p = 1$
(V)	<i>O. scolopendrina</i>	Seawater	8	13	9	$p = 0.18$	$p = 0.39$
(VI)	<i>O. venosa</i>	Seawater	6	13	11	$p = 0.055$	$p = 0.68$

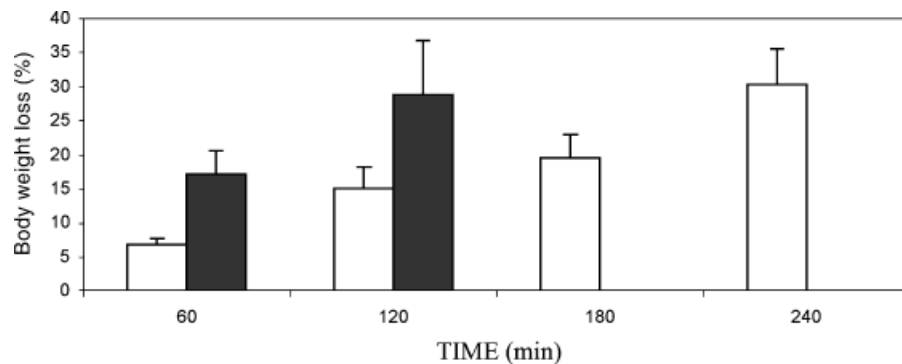


Fig. 7. Resistance to air-drying. Bars indicate the body-weight loss in adults of *Ophiocoma scolopendrina* (white) and *O. venosa* (black) after air-drying (40°C). Loss is expressed in % of the initial body weight. The initial average body weights were 6.7 ± 0.3 g for *O. scolopendrina* and 7.3 ± 0.3 g for *Ophiomastix venosa*.

It is known that *O. venosa* has a lecithotrophic larva that metamorphoses without the help of any metamorphic inducer, 2 d after fertilization (Fourgon et al. 2005). In 6 d, the metamorphosis is complete and it gives birth to star-shaped juveniles (Fourgon et al. 2005). The gonadic cycles in *O. venosa* and spawning experiments both indicate that this species breeds once a year in the austral autumn, mainly in January–February (Fourgon 2006). Lecithotrophic larvae of *O. venosa* are thus scattered on and around the reef during these months. The larvae that metamorphose out of the channels and spurs certainly die, as no members of *O. venosa* are found outside of these biotopes. Those that metamorphose in the channels are in adequate biotopes to grow close to their conspecifics. A last portion of larvae would metamorphose on the spurs where environmental conditions are such that, if they do not find an appropriate way to adapt to air-drying at low tides, they would die. The adaptation that juveniles of *O. venosa* have developed is the symbiosis with adults of *O. scolopendrina*. The latter species is one of the most abundant macroinvertebrates that live on the spurs; they resist air-drying by curling up their arms, which allows them to remain wet during low tides.

The experiment on air-drying resistance shows that the main advantage gained by symbiotic juveniles of *O. venosa* is protection: without the symbiosis, no juveniles of *O. venosa* are able to survive air-drying conditions similar to that occurring on the spurs at low tide. Another advantage gained by symbiotic juveniles of *O. venosa* is that they access a new energy-rich food source, the neuston carried by the host's arms (Fourgon et al. in press).

To ensure the symbiosis with adults of *O. scolopendrina*, juveniles of *O. venosa* have the ability to recognize their host. Our results demonstrate that host recognition is, at least partially, mediated by host odor. It is well known that echinoderm hosts attract various types of symbionts thanks to stimuli

mediated by water currents; for example, symbiotic crabs (Eckhaut et al. 2000) and shrimps (Vanden Spiegel et al. 1998) are chemically attracted by crinoid hosts. Odor-mediated host recognition was also observed in (1) polychaetes living either on asteroids or holothuroids (Davenport 1950, 1953a, b; Davenport & Hickock 1951; Dimock & Davenport 1971), (2) bivalves (Gage 1966), gastropods (Vañtiligon et al. 2004), shrimp (Ache & Davenport 1972), and crabs (Gray et al. 1968) associated with echinoids, and (3) fish living with holothuroids (Van Meter & Ache 1974). Such odor-mediated host recognition was not known in any symbiosis involving ophiuroids as hosts.

Juveniles of *O. venosa* on the spurs grow on adults of *O. scolopendrina*. When they reach a DD close to 6 mm, most of them leave the spurs and migrate into the channels. The symbiotic association probably lasts 2–3 months, because *O. venosa* reproduces mainly in January–February (Fourgon 2006) and we observed a very large recruitment of individuals measuring 6–8 mm DD in the channels in April. The reason for this emigration is probably related to the huge size reached by symbiotic juveniles and the high territoriality that characterizes *O. scolopendrina*.

To answer whether this facultative symbiosis is useful for *O. venosa* at the population level, we estimated the number of symbiotic juveniles compared with similarly sized individuals in the channels. A spur has an estimated surface of ~ 1000 m² and the channel surface is $\sim 3 \times$ larger. There are ~ 500 spurs and ~ 500 channels on the Great Reef of Toliara, which is 20 km long. The prevalence of the infestation by symbiotic juveniles varies 0.4–11% and the host density is 124 individuals m⁻². Small individuals of *O. venosa* (DD < 0.8 mm) occurring in the channels have a density ranging 2–5.4 individuals m⁻². The total number of symbiotic juveniles on the Great Reef of Toliara during the period of the survey can be estimated at 248,000 during the lowest

infestations and $\leq 6,820,000$ during the highest infestations. In comparison, small individuals of *O. venosa* in the channels are estimated at 3–8.1 million. This facultative symbiosis ensures the survival of at least $\sim 10\%$ of the juveniles of *O. venosa*.

We found that the symbiosis is facultative for juveniles of *O. venosa*, while Hendler et al. (1999) suggested that the symbiosis between juveniles of *O. annulosa* and adults of *O. scolopendrina* is obligatory. However, as they mentioned in their discussion, they did not investigate juveniles of *O. annulosa* on sites other than the intertidal, and thus certainly missed free juveniles. The symbiosis studied here also differs from Hendler's et al. (1999) description in the lowest number of juveniles found in the bursa; our results lead us to suggest that bursal infestation does not occur systematically when a juvenile of *O. venosa* comes in contact with an adult of *O. scolopendrina*: the number of intrabursal juveniles was very low and they were not smaller than some of the juveniles located on the host surface. The mean sizes of symbiotic juveniles of *O. annulosa* mentioned by Hendler et al. (1999) were lower than those we observed, and it might be possible that symbiotic juveniles of this species entered the bursae of their hosts more easily. Yet, Hendler et al. (1999) considered the babysitting symbiosis that occurs in brittle stars as a brood parasitism. Even if a portion of the host's food is consumed by the symbionts, it never appears to be detrimental for the hosts and we would consider this symbiosis more as a brood commensalism.

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