# Within-host adaptive speciation of commensal yoyo clams leads to ecological exclusion, not co-existence (#97354)



First submission

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# Within-host adaptive speciation of commensal yoyo clams leads to ecological exclusion, not co-existence

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Symbionts dominate planetary diversity and three primary symbiont diversification processes have been proposed: co-speciation with hosts, speciation by host-switching, and within-host speciation. The latter mechanism is prevalent among members of an extraordinary marine symbiosis in the Indian River Lagoon, Florida, composed of a host mantis shrimp, *Lysiosquilla scabricauda*, and seven host-specific commensal vasconiellline "yoyo" clams (Galeommatoidea) that collectively occupy two distinct niches: burrow-wall-attached, and host-attached/ectocommensal. This within-host symbiont radiation provides a natural experiment to test how symbiont coexistence patterns are regulated in a common ancestral habitat. The competitive exclusion principle predicts that sister taxa produced by adaptive speciation (with distinct morphologies and within-burrow niches) are most likely to coexist whereas the neutral theory predicts no difference among adaptive and non-adaptive sister taxa co-occurrence. To test these predictions, we engaged in 1) field-censusing commensal species assemblages; 2) trophic niche analyses; 3) laboratory behavioral observations. Although predicted by both models, the field census found no mixed-niche commensal assemblages: multi-species burrows were exclusively composed of burrow-wall commensals. Their co-occurrence matched random assembly process expectations, but presence of the single ectocommensal species had a highly significant negative effect on recruitment of all burrow-wall commensal species ($P < 0.001$), including on its burrow-wall commensal sister species ($P < 0.001$). Our stable isotope data indicated that commensals are suspension feeders and that co-occurring burrow-wall commensals may exhibit trophic niche differentiation. The artificial burrow behavioral experiment yielded no evidence of spatial segregation among burrow-wall commensals, and it was terminated by a sudden breakdown of the host-commensal relationship resulting in a mass mortality of all commensals unattached to the host. This study system appears to contain two distinct, superimposed patterns of commensal distribution: 1) all burrow-wall

commensal species; 2) the ectocommensal species. Burrow-wall commensals (the plesiomorphic condition) broadly adhere to neutral theory expectations of species assembly but the adaptive evolution of ectocommensalism has apparently led to ecological exclusion rather than coexistence, an inverse outcome of theoretical expectations. The ecological factors regulating the observed burrow-wall/ectocommensal exclusion are currently obscure but potentially include differential recruitment to host burrows and/or differential survival in “mixed” burrow assemblages, the latter potentially due to changes in host predatory behavior. Resampling host burrows during commensal recruitment peak periods and tracking burrow-wall commensal survival in host burrows with and without added ectocommensals could resolve this outstanding issue.

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## Abstract

Symbionts dominate planetary diversity and three primary symbiont diversification processes have been proposed: co-speciation with hosts, speciation by host-switching, and within-host speciation. The latter mechanism is prevalent among members of an extraordinary marine symbiosis in the Indian River Lagoon, Florida, composed of a host mantis shrimp, *Lysiosquilla scabricauda*, and seven host-specific commensal vasconiellinе “yoyo” clams (Galeommatoidea) that collectively occupy two distinct niches: burrow-wall-attached, and host-attached/ectocommensal.

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This study system appears to contain two distinct, superimposed patterns of commensal distribution: 1) all burrow-wall commensal species; 2) the ectocommensal species. Burrow-wall commensals (the plesiomorphic condition) broadly adhere to neutral theory expectations of species assembly but the adaptive evolution of ectocommensalism has apparently led to ecological exclusion rather than coexistence, an inverse outcome of theoretical expectations. The ecological factors regulating the observed burrow-wall/ectocommensal exclusion are currently obscure but potentially include differential recruitment to host burrows and/or differential survival in “mixed” burrow assemblages, the latter potentially due to changes in host predatory behavior. Resampling host burrows during commensal recruitment peak periods and tracking burrow-wall commensal survival in host burrows with and without added ectocommensals could resolve this outstanding issue.

## Introduction

A striking feature of life on Earth is its high degree of ecological nestedness, a condition famously satirized by Swift (1733): “So, naturalists observe, a flea hath smaller fleas that on him prey, and these have smaller still to bite 'em; and so proceed ad infinitum”. An important but often-overlooked consequence of this feature is that most species are in fact symbionts (parasites/commensals/mutualists) whose habitats consist of other (host) species (Windsor, 1998; Poulin & Morand, 2004; Moran, 2006).

Symbiont diversification processes have therefore played outsized roles in generating our planet's fundamental biodiversity and two main generative evolutionary mechanisms have been proposed: co-speciation with hosts, and speciation by host-switching (*e.g.*, Ricklefs, Fallon & Bermingham, 2004). The former mechanism is host-driven – host lineage speciation events lock-stepping symbiont lineage speciation – and it is thought to be highly prevalent in Nature, *e.g.*, co-speciation of bacterial endosymbionts with insect hosts alone may form the bulk of all speciation events (Larsen et al., 2017; Hernández-Hernández et al., 2021). The latter mechanism is symbiont-driven – colonization of new hosts providing new ecological portals for symbiont speciation – and it has been proposed as a major driver of speciation in both terrestrial (Coyne & Orr, 2004; Matsubayashi, Ohshima & Nosil, 2010) and marine (Duffy, 1996; Goto et al., 2012; Hurt et al., 2013; Fritts-Penniman et al., 2020; Rodriguez & Krug, 2022) biotas.

A third diversification mechanism, within-host speciation, has received less attention and it involves the evolution of sister species that retain the same ancestral host. Co-existence of sister taxa on their host might *a priori* be expected to approximate neutral theory (Hubbel, 2001) expectations of species assembly because of their joint persistence within a shared, highly specialized ancestral habitat. However, competitive exclusion principle-based perspectives (Grinnell, 1904; Hardin, 1960; Chesson, 2003) have dominated species diversity studies over the past century (Simha, Pardo-De La Hoz & Carley, 2022). This is also apparent for within-host speciation case histories where niche differentiation or allopatry is implicitly expected, *e.g.*, within-host phytophagus insect speciation studies emphasize cases of either adaptive speciation, *e.g.*, specialization for discrete host tissues (Cook et al., 2002; Joy & Crespi, 2007; Althoff, 2014), or discrete host life history stages (Zhang et al., 2015), or non-adaptive allopatric speciation occurring in exclusive subsets of a host range (Imada, Kawakita & Kato, 2011). In contrast, there have been few studies of sympatric, ecologically non-differentiated sister species that share the same host.

Almost all evolutionary radiations have the potential to produce new members through either adaptive or non-adaptive speciation processes (Czekanski-Moir & Rundell, 2019; Matsubayashi & Yamaguchi, 2020). In principle, a within-host symbiont radiation that contained sympatric sister species pairs respectively generated by adaptive and by non-adaptive speciation processes could represent an ideal natural experiment to test how symbiont coexistence patterns are regulated in a common ancestral habitat. The competitive exclusion principle (Grinnell, 1904; Hardin, 1960; Chesson, 2003) predicts that sister taxa produced by adaptive

speciation and occupying distinct host niches are most likely to coexist on individual hosts. In contrast, the neutral theory (Hubbel, 2001) predicts that sister taxa produced by non-adaptive speciation and having similar host niches are equally likely to coexist with each other as with their ecologically differentiated co-symbionts.

A single-host marine symbiont assemblage documented in the Indian River Lagoon (IRL) on the East coast of Florida exhibits many of the model attributes outlined above. The host, *Lysiosquilla scabricauda* (Lamarck, 1818), is a benthic ambush predator "spearing" mantis shrimp (Caldwell & Dingle, 1976; deVries, Murphy & Patek, 2012) that lives in large burrows up to 10m in length within sandy substrates (Christy and Salmon, 1991) and is widely distributed in the Western Atlantic from the southeastern USA to southern Brazil (Tavares, 2002; Reaka *et al., 2009*). In the IRL, *L. scabricauda* hosts 7 species of commensal galeommatoidean bivalves currently placed in two vasconiellinе genera – *Divariscintilla* (6 species) and *Parabornia* (1 species) – that appear to be host-specific, *i.e.*, are known to occur only within *L. scabricauda* burrows (Mikkelsen & Bieler 1989,1992; Goto, Harrison & Ó Foighil, 2018). The 6 species of *Divariscintilla* [*D. yoyo* Mikkelsen & Bieler, 1989*, D. troglodytes* Mikkelsen & Bieler, 1989, *D. octotentaculata* Mikkelsen & Bieler, 1992, *D. luteocrinit*a Mikkelsen & Bieler, 1992, *D. cordiformis* Mikkelsen & Bieler, 1992, and a new, undescribed species *D.* aff. *yoyo* (Goto *et al.*, 2018)] are currently known only from the IRL and nearby Floridian locations (Mikkelsen & Bieler 1992; Mikkelsen, Mikkelsen & Karlen, 1995). All 6 attach to the smooth, hard-packed host burrow walls (Figure 1) via a long, thin posterior foot extension that secretes anchoring byssus threads, and contraction/relaxation of this "hanging foot" structure produces characteristic yoyo-like movements (Mikkelsen & Bieler 1989; Mikkelsen & Bieler 1992; Goto *et al*., 2018 Supplementary Movie 1) – hence the informal "yoyo clams" moniker (Mikkelsen & Bieler 1989). In contrast, the *Parabonia* species, *P. squillina* Boss, 1965, is an ectocommensal, attaching directly to the host (Figure 1), specifically to the lateral portion of its pleonal sternite (Goto *et al*., 2018). Its known range extends from Panama to Florida (Boss, 1965; Moore & Boss, 1966; Abbott, 1974) and it has one very similar ectocommensal congener, *P. palliopillata* Simone, 2001, recorded from Southern Brazilian *L. scabricauda* host populations (Simone, 2001; Goto et al., 2018).

A vasconiellinе molecular phylogenetic analysis (Goto *et al., 2018*) illuminated the evolutionary relationships among 6/7 of the IRL *L. scabricauda* commensals (the

rarest species, *D. cordiformis*, was unavailable for genotyping). One species, *D. troglodytes*, was phylogenetically distinct and placed topologically among Pacific Ocean burrow-wall lysiosqillid commensals, implying that its presence in *L. scabricauda* burrows involved an ancestral host-switching event coupled with inter-ocean basin migration. The remaining 5 *L. scabricauda* commensals formed a host-specific clade, a result consistent with within-host speciation, but not necessarily in sympatry as initial differentiation may have occurred in allopatry (Rundell & Price, 2009), *i.e.*, in discrete subsets of the host's extensive Western Atlantic range (Goto *et al., 2018*). The host-specific clade contained two well-supported clade tip sister relationships. One involved a cryptic sister species pair of burrow-wall commensals – *D. yoyo* and *D.* aff. *yoyo* – that are apparent products of non-adaptive speciation. They are effectively identical in external appearance and in within-burrow habitat but can be distinguished morphologically by details of their (mantle-covered) anterior shell margins, in addition to their gene sequences (Goto *et al.*, 2018). The other comprised *D. octotentaculata*, a burrow-wall commensal, and *P. squillina*, the ectocommensal, two species that differ not only in within-burrow habitat but also in many aspects of their morphologies. Goto *et al.* (2018) concluded that *P. squillina* was a product of adaptive speciation and ecological character displacement (Grant and Grant, 2006) from a burrow-wall commensal common ancestor with *D. octotentaculata*. This evolutionary process involved an ecological shift to an ectocommensal niche along with a suite of associated morphological changes: loss of specialized "hanging foot" structures, loss of hypertrophied mantle tissue enveloping the shell, loss of prominent sensory tentacles, as well as gain of specialized mantle margin papillae.

Mikkelsen & Bieler's (1992) focus was primarily taxonomic, but they also commented on the relative frequency of burrow-wall commensals recovered from individual IRL *L. scabricauda* burrows. Most burrows with these commensals contained *D. octotentaculata*, usually in combination with one or more of 4 congeners: *D. yoyo, D. troglodytes, D. luteocrinita,* and *D. cordiformis*. They concluded that "no ecological niche separation between the five sympatric species was recognized, leaving interesting questions for future research". Mikkelsen & Bieler (1992) did not provide data on the frequency of the ectocommensal *P. squillina* in IRL host burrows but anecdotally noted that it "has not been collected in burrows containing *Divariscintilla* species".

These preliminary ecological observations (Mikkelsen & Bieler, 1992) are broadly consistent with neutral theory (Hubbel, 2001) expectations for IRL commensal vasconiellline species. Our aim in this study was to revisit this issue in light of the new evolutionary relationships data among the commensals, including the sister species pairs produced by adaptive (*D. octotentaculata* and *P. squillina*) and non-adaptive (*D. yoyo* and *D.* aff. *yoyo*) within-host speciation (Goto *et al., 2018*). We employed a diversity of approaches including 1) censusing commensal species assemblages in host burrows; 2) testing for dietary differentiation *via* isotope composition analyses; 3) laboratory behavioral observations of artificial burrow commensal assemblages with, and without, hosts. Although our results are the inverse of competitive exclusion expectations, they are also not fully consistent with neutral theory predictions, and they imply that the adaptive evolution of ectocommensalism may have disrupted ancestral coexistence modalities among members of this host-specific commensal community.

## Materials & Methods

*Sampling sites*

From June 14th to July 25th, 2017, the first author (after obtaining a Florida state collecting permit) performed extensive low tide field sampling of *Lysiosquilla scabricauda* burrows at 5 adjacent shallow water sandflat study sites within the IRL's Ft. Pierce Inlet (Figure 2). The three sites (1, 4 & 5) on the northern margin of the Inlet (Figure 2) collectively contain the type localities of 5 commensal clam species: *Divariscintilla octotentaculata, D. luteocrinita, D. troglodytes, D. yoyo,* and *D. cordiformis* (Mikkelsen & Bieler 1989, Mikkelsen & Bieler 1992).

*Host burrow identification and host capture*

At the beginning of each field session, all visible host burrows within the targeted study site were flagged for sampling. *Lysiosquilla scabricauda* burrows were identified by their characteristic openings: irregularly square, about 1 $cm^2$ in area, and covered by a sand cap that was differentially textured than the surrounding sand flat. Presence of a host burrow was confirmed by lightly touching the burrow cap and observing it give way, a disturbance that occasionally caused a resident stomatopod to appear briefly at the burrow mouth prior to withdrawing from sight. *L. scabricauda* specimens were collected manually using a bait-and-capture technique (Goto *et al.*, 2018); see here a video summary. A bait fish was placed directly over a submerged burrow opening and held for a 3-minute trial period to elicit an attack by a resident stomatopod. If no host response occurred during that interval, a new host burrow was

then attempted. The raptorial appendages of lysiosquillid stomatopods are barbed, razor sharp, and designed to impale soft-bodied prey (deVries *et al.,* 2012). Thus, thick fishing gloves were worn for protection and once a resident stomatopod impaled the bait or otherwise presented at the mouth of the burrow, its raptorial appendages were sequentially grasped by hand (Figure 3) and held firmly as it attempted to pull itself downward into its burrow. As the restrained stomatopod tired, it was slowly pulled upward out of the burrow.

*Collecting burrow-wall commensal clams*

Once a host *L. scabricauda* had been collected, its burrow was then sampled for yoyo clam burrow wall commensals using a stainless-steel bait pump ("yabby pump"). As emphasized by Mikkelsen and Bieler (1989, 1992), this method effectively samples only its own length (0.5-1.0m) of the vertical parts of the stomatopod's U-shaped burrow, leaving the deeper horizontal section unsampled. The contents of single pulls of the yabby pump were expelled into a 2 mm sieve and this process was repeated until three pulls failed to return any observable clams, or until the vertical arm of the host burrow collapsed from the repeated suctioning. Regardless of species, yoyo clams were readily recognized by their characteristic off-white, mucoid appearance against the mesh and the residual sediment particles retained in the sieve. Individual clams were carefully picked up using a feather weight forceps and placed into 50 ml tubes of seawater. Any ectocommensals detected on the stomatopod host were similarly detached from the base of the host pleopods and placed in seawater tubes. Back in the laboratory, all live commensal clams sampled from individual host burrows were maintained together in burrow-specific, labelled finger bowls containing filtered sea water with slight aeration. All clams were then identified to species using a dissecting microscope and their mantle lengths measured. Species counts and the number of individuals per species were recorded for each burrow sample.

*Statistical analyses of co-occurrence data*

Several statistical tests were performed on the commensal species frequency data. These included over-dispersion tests for the two most frequent species (*P. squillina* and *D. octotentaculata*) using the "overdispersion.test" function in R 4.3.1, to determine if the observed individual distributions in host burrows were more clustered than expected by chance.

In addition, we conducted several simulations tests of the co-occurrence patterns of the different commensal species to determine if they fall within the expectation of a random larval settling

process. IRL *Divariscintilla* species have “mixed” larval development in which early developmental stages are ctenidially-brooded, then released into the water column as early, straight-hinged “D” veligers to undergo an obligate period of planktotrophic larval development and dispersal, thereby greatly reducing the likelihood of resettlement in parental burrows (Mikkelsen & Bieler 1989; Mikkelsen & Bieler 1992). The details of *Parabornia squillina*’s early development are currently unknown but its prodissoconch structure is consistent with it also having an obligate planktototrophic larval dispersal phase (Supplementary Figure 1). [Similarly, presence of large numbers of small (<100 µm) brooded “ova” in the ctenidia of its Brazilian congener, *P. palliopapillata*, (Simone, 2001; Figure 13 therein) is indicative of planktotrophic larval development in Galeommatoidea (Ó Foighil, 1988)].

We therefore assumed that each IRL commensal clam was independently recruited to its host burrow from a planktonic pool of metamorphosing veliger larvae. We were particularly interested in testing for settlement/survival effects among the ectocommensal *P. squillina* and the burrow-wall commensal *Divariscintilla* species. The competitive exclusion principle (*Grinnell, 1904; Hardin, 1960; Chesson, 2003*) predicts that IRL *Divariscintilla* species (which all share the same burrow-wall niche) will co-occupy burrows host less frequently with each other that with the niche-differentiated ectocommensal *P. squillina*. In contrast, the neutral theory of species assembly (*Hubbel, 2001*) predicts that each member of the IRL commensal vasconielline community will occupy host burrows irrespective of the presence or absence of any other member, and that the observed co-occurrence pattern will therefore match random larval settling expectations.

Expected co-occurrence distributions of different species pairs were generated by randomly allocating clams to burrows based on the observed proportions of each species across all burrows. For example, a total of 73 *D. octotentaculata* and 20 *P. squillina* were sampled from all burrows, meaning that 78% [73/(73+20)] of that combined species pair were *D. octotentaculata* and 22% were *P. squillina*. In the simulation, the two species were then randomly allocated to all burrows based on this probability, keeping the total clam count in each burrow equal to the observed value (*i.e.*, if a burrow had five clam individuals, then simulation was done five times for that burrow). This process was repeated 1000 times. After the simulation, the numbers of burrows where the two species randomly co-occurred were counted and summarized in a histogram. The actual observed number of co-occurred burrows was also plotted on the histogram to compare with the theoretical distribution. P-values were calculated by the

272 percentile of the actual observed value in the simulated distribution. This comparison was
273 performed between *P. squillina* and all wall-commensal species (treated as the same type); *P.*
274 *squillina* and *D. octotentaculata*; *P. squillina* and *D. luteocrinita*; and *D. octotentaculata* and *D.*
275 *octotentaculata*. The other wall commensal species had low occurrences therefore they were
276 not compared with *P. squillina* individually.
277
278 *Stable isotope analyses*
279 An organism's stable isotope composition is shaped by, and indicate of, its diet/trophic niche
280 (Layman et al. 2007). To test if the IRL *L. scabricauda* vasconiellinе commensal species differ in
281 their trophic niches, 18 burrow-wall commensals (11 *D. octotentaculata*, 4 *D. luteocrinita,* 2 *D.*
282 *yoyo* and 1 *D. troglodytes*) and 20 *P. squillina* ectocommensals, together with samples of within-
283 burrow potential basal trophic resources — tissue from 19 *L. scabricauda* specimens,
284 suspended particulate organic matter from 35 burrows, and deposited organic matter from 34
285 burrows — were collected (Figure 4) to measure their respective isotopic niche widths.
286
287 The clams were housed separately in petri dishes of filtered sea water for 12 hours to allow for
288 their gut contents to empty, after which their soft tissues were separated from their shells prior
289 to further processing. Host stomatopod specimens were euthanized in an ice-water slurry. To
290 sample burrow water particulate organic matter (POM) the flow of ambient water into burrows
291 was first blocked with a cylindrical barrier (bucket with the bottom removed) enclosing the
292 burrow opening. For each burrow, 1 liter of burrow water was field-collected with a large syringe
293 (with a 1 mm filter attachment) and filtered in the laboratory onto 4.7 cm diameter Whatman
294 GF/F glass microfiber filters using a six-manifold filtration system. Deposited organic matter was
295 sampled by collecting the oxygenated layer of burrow wall sediment with a shallow teaspoon.
296 All commensal tissue specimens and potential basal resource samples were lyophilized using a
297 Labconco Freeze Dry System prior to further processing. This involved grinding the commensal
298 samples, and the POM samples (first removed from the filter paper with a spatula), with
299 disposable mortar and pestles, grinding the right *merus* of each host specimen for 4 minutes in
300 a ball-mill grinder, and grinding each sediment sample for 4 minutes in a bead-mill grinder.
301 Approximately 2.5 mg of each ground host and commensal species sample was weighed into
302 individual 5 x 9 mm pressed tin capsules. Approximately 5 - 7 mg of each POM and 35 - 40 mg
303 of each sediment sample were weighed in 10.5 x 9 pressed, light-weight silver capsules and
304 acidified with reagent grade HCL to remove any undetected shell fragments. All samples were
305 analyzed at the Center for Applied Isotope Studies at the University of Georgia for carbon and

nitrogen isotopic signatures. The isotopic niche width of each species was quantified as Standard Ellipse Areas ($SEA_B$) which estimate mean population-level isotopic niche spaces while accounting for variation in population size, in the R package SIAR (Jackson et al. 2011).

*Artificial burrow construction & observations*

Two observable, artificial stomatopod burrows were constructed at the Smithsonian Marine Station at Fort Pierce. Each was positioned within a 110 L flow-through glass aquarium tank that was partitioned with a sheet of PVC to form a 9 cm wide cross-section of sediment observable through the front facing wall of the tank. The PVC barrier was 10 cm shorter than the tank, allowing flow to pass over it, and it was kept in place by a series of 3.8 cm diameter PVC tubes (Supplementary Figure 2). To construct artificial stomatopod burrows, a 3.8 cm diameter electrical conduit tube was first planed in half and then taped to form a U shape. The inner surfaces of the tube were lightly coated with silicone rubber sealant, packed with dry sand, and allowed to set overnight. The unattached sand was then removed, and the sand-coated tube halves were individually placed into separate aquarium tanks with the cut edge held in place against the tank walls with additional dry sand and the tube openings flush with the sand surface (Figure 5). The aquaria were then filled with aerated sea water and allowed to settle for 24 hours.

A host *Lysiosquilla scabricauda* was introduced to one of the aquaria, was acclimated for a week, and offered shrimp and small fish as food. It entered the artificial burrow on the first day and remained in the burrow for the duration of the experiment (Figure 5). During the acclimation period, the stomatopod used loose sand in the tank to form a cap for the artificial burrow and consistently maintained this cap by collecting excess sand in the burrow with its maxillipeds. The other aquarium was kept host-free.

An experimental community of four species (*D. octotentaculata*, *D. luteocrinita*, *P. squillina*, and *D. troglodytes*) were introduced, in proportions approximate to their natural IRL frequencies, to both tanks. The host-free aquarium housed 47 clams (34 *D. octotentaculata*, 7 *D. luteocrinita*, 5 *P. squillina*, and 1 *D. troglodytes*). The host-containing aquarium housed 46 clams (33 *D. octotentaculata*, 7 *D. luteocrinita*, 5 *P. squillina*, and 1 *D. troglodytes*). Clams were introduced in small cohorts of conspecifics placed between the burrow openings and observed for 15 minutes. Those that did not enter the burrow within 15 minutes (*i.e.*, remained on the sand surface or climbed the aquarium glass) were manually transferred into the burrow after this

observation period. A mix of cultured unicellular green and brown algae was added to the tanks once a day and each tank was observed for patterns of spatial use, grouping behavior, and mortality. To simulate the assumed light conditions of natural stomatopod burrows, the exposed side of the artificial burrow was covered with thick black plastic bags level with the burrow entrances when observations were not taking place.

## Results

*Commensal occurrence and distribution*

A total of 86 host burrows were sampled and 29 of these (33.7%) yielded ≥1 commensal vasconielline clam(s), collectively totaling 112 specimens from 6/7 of the known IRL vasconielline species (Goto *et al.*, 2018) and ranging in frequency from 1-21 commensals per burrow (Supplementary Table 1). The burrow-wall commensal *Divariscintilla octotentaculata* was numerically dominant with 73 individuals sampled from 18 burrows (Figure 6) distributed among 4/5 sampling sites (Table 1). It's ectocommensal sister species, *Parabornia squillina*, was the next most numerous with 20 individuals recovered from 8 host burrows (Figure 6), also distributed among 4 sampling sites (Table 1). Respective numbers for *D. luteocrinita* and *D. troglodytes* were 13 and 3 individuals from 9 and 2 host burrows (Figure 6) among 3 and 2 sites (Table 1). The products of non-adaptive within-host speciation, sister species *D. yoyo* and *D.* aff. *yoyo*, were the least numerous commensals recovered: respectively 2 and 1 and both from single burrows (Figure 6, Table 1). Sampled individuals of the 4 most common species ranged two-fold in mantle length (Supplementary Table 2), likely representing different age classes. No specimens of the rarest IRL commensal vasconielline (Mikkelsen and Bieler, 1992), *D. cordiformis*, were recovered.

Of the 29 burrows with commensals, 22 (75%) were monospecific (either *Divariscintilla octotentaculata*, *D. luteocrinita*, or *Parabornia squillina*), 4 had 2-species assemblages, and 3 had 3-species assemblages (Figure 7). Burrows with multispecies assemblages (N=7) shared two characteristics: they were exclusively comprised of burrow-wall commensals, and all contained *D. octotentaculata* individuals. The ectocommensal *P. squillina* was not recovered from any host burrow that also yielded burrow-wall commensals (*Divariscintilla* spp.).

The over-dispersion tests of *P. squillina* and *D. octotentaculata* rejected the null hypothesis, meaning that for each species, the observed frequency distribution was significantly ($P < 0.001$) more clustered than that expected by chance alone. For the comparative recruitment simulation

tests (Figure 8), when the 5 burrow-wall commensal species were collectively treated as one group in comparison to the ectocommensal *P. squillina*, the null hypothesis of random assembly was strongly rejected ($P < 0.001$). *P. squillina* was never observed to co-occur with burrow-wall commensals in the field, whereas in the simulated random recruitment scenarios there were always at least four burrows where the two groups co-occurred. Similar results were found for individual ectocommensal/burrow-wall commensal simulation tests: *P. squillina* and *D. octotentaculata*, as well as *P. squillina* and *D. luteocrinita* (Figure 8). In stark contrast, when evaluating co-occurrence among the burrow-wall commensals *D. octotentaculata* and *D. luteocrinita* (Figure 8), the observed field value fell well within the simulated random recruitment distribution ($P = 0.405$), indicating these two burrow-wall commensal species likely co-recruited to IRL host burrows following a random process.

*Stable isotope analyses of dietary niche*

Individuals of the 6 IRL commensal species sampled, together with samples of their potential basal resources (host tissue, burrow-water POM, and burrow-wall sediment), were analyzed to determine their respective carbon and nitrogen isotopic signatures (Supplementary Table 3). Only 3 of the 6 commensals — *Divarscintilla octotentaculata, Parabornia squillina* and *D. luteocrinita* — were recovered in sufficient numbers to generate isotopic analysis Bayesian-estimated Standard Ellipse Areas ($SEA_B$) plots, and their respective SEAc values were 0.184, 1.128, and 0.255. The corresponding SEAc values for host tissue, burrow-water POM, and burrow-wall sediment samples were respectively 0.826, 3.354, and 7.336.

*Divariscintilla octotentaculata's* inferred isotopic niche space (its Bayesian-estimated ellipse area) was distinct from that of *D. luteocrinita* (Figure 9), a fellow burrow-wall commensal, but it overlapped substantially with that of *Parabornia squillina* (Figure 9), its ectocommensal IRL sister species. All three commensal species did not overlap in isotopic niche space with any of their three potential basal resources but they placed closest to burrow-water POM, and furthest away from the host *Lysiosquilla scabricauda* (Figure 9).

*Artificial burrow observations*

Individuals of all 4 commensal species (*Divarscintilla octotentaculata, Parabornia squillina*, *D. luteocrinita* and *D. troglodytes*) introduced into aquaria containing artificial host burrows (with and without a mantis shrimp host) preferred firmer surfaces to the loosely packed aquarium surface sand. Most clams that encountered the edge of an artificial burrow opening crawled

down that burrow and most that encountered the aquarium glass wall crawled up that surface (prior to being manually relocated into the artificial host burrow).

In all 3 observations of the host-free aquarium (Figure 10A-C), commensal clam species were partially intermixed throughout the artificial burrow. The most numerous species, *Divarscintilla octotentaculata*, exhibited the clearest spatial aggregation with most individuals dominating the left 1/3$^{rd}$ of the burrow, leaving the rest of the burrow occupied primarily by a mixture of *Parabornia squillina* and *D. luteocrinita* (Figure 10A-C). Most individuals, regardless of species, were located within the horizontal segment of the artificial burrow, primarily attached to the lateral and upper burrow walls. Commensals were typically sedentary during observation periods, though there were changes in individual positioning between observations and 2 *D. octotentaculata* specimens left the burrow and attached to the aquarium walls.

In the host-containing aquarium, the initial disturbance associated with commensal introduction led the host mantis shrimp to attempt to cover up the light-exposed glass with a sand-mucus mixture. Following this, it rested within the burrow and the commensal clams gradually positioned themselves around it. By the first observation period, 15 hrs post-introduction (Figure 10D), most commensals had formed a mixed species assemblage in the horizontal segment of the artificial burrow where the host primarily rested (although 3 *Divarscintilla octotentaculata* individuals had exited the burrow and were attached to the aquarium walls). Most burrow-wall commensals attached to the upper burrow wall where many engaged in characteristic “yo-yo” behavior in response to being touched by the host. Within-burrow positioning of the 5 ectocommensal *Parabornia squillina* individuals varied from observation to observation (Figure 10D-F). Although none immediately moved onto the host, at +15 hrs (Figure 9D) 3 had attached to the base of the host pleopods and 2 were attached to the upper burrow wall. At +27 hrs (Figure 10E) 2 *P. squillina* individuals remained attached to the host and 3 to the burrow wall (2 upper, 1 lower), and at +63 hrs (Figure 10F) 1 individual remained attached to the host.

Commensal survivorship in the experimental artificial burrows decreased with time and two quantitatively and qualitatively distinct patterns of commensal mortality were evident during the observation period (Figure 10A-F). One pattern was independent of host presence: in 5/6 burrow observations (Figure 10A-E), a “background” rate of mortality, ranging from 1-8 individuals per time increment, was characterized by the presence of dead clam bodies lying on the bottom of the

burrows. In contrast, a greatly elevated mortality rate (34/35 commensals) was detected in the +63 hrs observation of the host-occupied burrow (Figure 10F), characterized by the absence of observable dead clam bodies or clam tissue/shell fragments within or outside the burrow. The sole survivor was a single individual of the ectocommensal *Parabornia squillina* attached to the base of the host pleopods (Figure 10F).

## Discussion

Our study investigated the regulation of IRL *Lysiosquilla scabricauda* commensal species coexistence using three complementary approaches and it uncovered a complex mix of congruence and incongruence with both neutral model (Hubbel, 2001) and competitive exclusion principle (Grinnell, 1904; Hardin, 1960; Chesson, 2003) expectations.

Some of this complexity was evident in the field census results for individual IRL host burrows for which competitive exclusion principle expectations are of co-occurrence of commensals occupying distinct host niches (burrow-wall commensals and ectocommensals) and neutral theory expectations are of a mix of commensals with and without distinct host niches. Mixed-niche commensal assemblages (predicted by both models) were absent ($P < 0.001$), and all observed cases of multi-species co-occurrence were exclusively composed of burrow-wall commensals, two of whom (*Divariscintilla octotentaculata* and *D. luteocrinita*) met random co-recruitment expectations (Figures 7 and 8). We were particularly interested in the coexistence dynamics of two constituent sister species pairs alternatively generated by adaptive and by non-adaptive speciation. A reciprocal, robustly negative recruitment effect was apparent for the adaptive sister species pair: burrow-wall commensal *D. octotentaculata* on ectocommensal *Parabornia squillina*, and *vice versa*, ($P \leq 0.001$); a result explicitly incompatible with competitive exclusion principle expectations. Unfortunately, the rarity of the non-adaptive sister species pair [*Divariscintilla yoyo* (N=2) and *D.* aff. *yoyo* (N=1)] precluded meaningful statistical analyses of their census data.

Most host burrows sampled (66%) lacked detectable commensal clams (Figure 7) implying that host individuals and resources may not be limiting factors for commensals. However, some of the sampled burrows without commensals could also be 1) occupied but undetected because of incomplete sampling; 2) empty because aspects of commensal life history, *e.g.*, mating behavior (Mikkelsen & Bieler 1992), promote within-species clustering, (as implied by the over dispersion

test results for *Divariscintilla octotentaculata* and *Parabornia squillina*); or 3) subsets of host burrows may be otherwise inhospitable for commensal species recruitment/survival. We know that incomplete sampling was an issue for both burrow-wall commensals and ectocommensals. As noted by Mikkelsen and Bieler (1989, 1992), yabby pumps (used to sample burrow-wall commensals) are ineffective in sampling the deeper, horizontal sections of *Lysiosquilla scabricauda* burrows. In addition, burrows in this stomatopod genus are typically occupied by a resident male-female monogamous pair (Christy and Salmon, 1991) and our host bait-and-capture method (Figure 3), used to sample ectocommensals, was effective only in capturing one resident host/burrow, most likely the resident male (Ahyong, Caldwell and Erdmann, 2017).

Nevertheless, it is important to note that key aspects of our field census results are consistent with Mikkelsen & Bieler's (1992) sampling of these same populations ≥25 years earlier, despite major IRL ecological changes (involving extensive eutrophication, algal blooms and seagrass habitat loss) in the interim (Morris et al., 2022). These include the numerical dominance of *Divariscintilla octotentaculata* [recorded from 88% of host burrows containing burrow-wall commensals by Mikkelsen & Bieler (1992) *versus* 85% in our study], its co-occurrence with other burrow-wall commensals (80% *versus* 38%, respectively), the absence of co-occurring burrow-wall commensals and ectocommensals (0% *versus* 0%, respectively) and the prevalence of commensal-free host burrows ("most" *versus* 66%, respectively). Further comparisons of the same metric among the two studies indicate that *D. leucocrinita* may have increased in occurrence [22.8% of host burrows containing burrow-wall commensals recorded by Mikkelsen & Bieler (1992) *versus* 42.8% in our study], but that the remaining burrow-wall commensals appear to have declined: *D. yoyo* + *D.* aff. *yoyo* [57% *versus* 9.5%, respectively; note that Mikkelsen and Bieler (1992) were unaware of *D.* aff. *yoyo*'s existence], *D. troglodytes* (54% *versus* 9.5%, respectively) and *D. cordiformes* (5.7% *versus* 0%, respectively). The collective >80% decrease of the non-adaptive sister species pair *D. yoyo* + *D.* aff. *yoyo* implies that their relative rarity may be a recent development.

Regarding trophic niche differentiation, neutral theory allows co-existence irrespective of trophic niche overlap, whereas competitive exclusion principle expectations are that co-existing commensals will occupy distinct trophic niches. Combined stable isotope/field census data were available for only 3 commensal species and the results were mixed. The only multispecies

combination observed among the 3 — co-occurring burrow-wall commensals *Divarscintilla octotentaculata* and *D. luteocrinita* (Figure 7, 6/7 multispecies assemblages) — exhibited qualitative separation in their isotopic niches (Figure 9), thereby conforming with competitive exclusion principle expectations. In contrast, the other possible known heterogeneous trophic niche combination — burrow-wall commensal *D. luteocrinita* and ectocommensal *Parabornia squillina* (Figure 9) — was not detected in any IRL host burrow (Figure 7).

A consumer's stable isotope composition is shaped by that of the species it consumes in a broadly predictable manner: empirical studies have shown that in consumer tissues, the ratio of $^{15}N$ to $^{14}N$ is generally 2.5-5 greater, and the ratio of $^{13}C$ to $^{12}C$ is generally similar or as much as 1 greater, than that of their diets (Bearhop et al., 2004). Applying this general expectation to our commensal species stable isotope data (Figure 9) yields a pronounced mismatch with host tissue $SEA_B$, implying that host tissues/wastes/food scraps are not significant trophic resources for the three commensals, including the ectocommensal *Parabornia squillina*. Of the two remaining putative commensal trophic resources tested (burrow-wall deposited organic material and burrow-water POM) the placement of burrow-water POM SEAB (Figure 9) is most consistent with it being the commensal's primary trophic resource, a conclusion in agreement with Mikkelsen and Bieler's (1989) description of the IRL commensal species as "filter-feeders". The qualitative isotopic niche separation shown by *Divariscintilla luteocrinita* from the other two commensal species (Figure 9) could stem from a variety of factors including qualitative differences in 1) the subset of burrow-water POM material being assimilated; 2) how their respective microbiomes process ingested material; 3) accession of another basal resource (*e.g.*, dissolved organic matter) that was not sampled in our study.

Our artificial burrow behavioral experiment was designed to test if a competitive exclusion principle expectation — the evolution of symbiont specialization for discrete within-host niches (*Cook et al., 2002; Joy & Crespi, 2007; Althoff, 2014*) — applied to other members of the IRL *Lysiosquilla scabricauda* commensal community in addition to the adaptive sister species pair of the burrow-wall commensal *Divariscintilla octotentaculata* and the ectocommensal *Parabornia squillina* (Goto *et al.*, 2018). *Lysiosquilla scabricauda* burrows are large enough to potentially facilitate fine-scale spatial partitioning among co-occurring burrow-wall commensals that might be undetectable by yabby pump sampling. Our results (Figure 10) yielded no

544 evidence of spatial segregation among burrow-wall commensals in the
545 presence of a resident host: all three species clustered around the host's
546 primary resting location. However, this experiment did yield two unexpected
547 new behavioral insights, although we cannot rule out the possibility that they are
548 both experiment-induced artifacts.
549
550 One surprise concerned a hitherto unknown behavioral flexibility of the
551 ectocommensal *Parabornia squillina.* In the control artificial burrow, lacking a
552 host, 5/5 individuals attached to the burrow wall (Figure 10A-C). In the
553 treatment artificial burrow, containing a host, only 3/5 assumed the
554 ectocommensal condition and at least one of these subsequently moved off the
555 host and attached to the burrow wall during the observation period (Figure 10D-
556 F). This was somewhat surprising because Goto *et al.* (2018; Supplementary
557 Movie 2) found that individuals detached from hosts rapidly reattach and, to our
558 knowledge, extensive yabby pump sampling of IRL *Lysiosquilla scabricauda*
559 burrows (*Mikkelsen & Bieler 1989; Mikkelsen & Bieler 1992; Goto et al., 2018; this study)* have
560 not recovered non-host-attached *P. squillina* individuals. It remains to be
561 determined to what degree *P. squillina* clams alternate between ectocommensal
562 and burrow-wall attachments in the wild.
563
564 The most surprising result of the artificial burrow behavioral experiment was the
565 sudden breakdown of the commensal relationship resulting in a mass mortality of
566 34/35 commensals, apparently due to targeted predation by the host (Figure109E
567 &F). The only survivor was a host-attached specimen of *Parabornia squillina*
568 and all others, including at least 3 non-host attached *P. squillina* and an
569 aquarium wall-attached specimen of *Divariscintilla octotentaculata* (Figure 10F),
570 were apparently consumed by the host. We cannot of course rule out the
571 possibility that this sudden switch in host behavior was an artifact triggered by
572 stressful artificial culture conditions and it is unclear if *Lysiosquilla scabricauda*
573 also targets non-host attached commensals in the wild, and if so, under what
574 conditions?
575
576 Galeommatoidea is a highly speciose superfamily (Bouchet et al., 2002;
577 Paulay, 2003) and the vast majority of commensal members occur in soft-bottom habitats

in association with larger, bioturbating macroinvertebrate hosts (collectively from diverse phyla) that provide a within-sediment depth refuge from predation (Li et al., 2012). Within this group, *Lysiosquilla scabricauda*'s commensals are exceptional in four aspects of their evolutionary ecology: species richness, predominant evolutionary origin mechanism, host trophic ecology, and potential for host predation. We currently know of 8 host-specific commensals (Simone, 2001; Goto et al., 2018), more than any other single galeommatoidean host to-date, and this is likely an underestimate because only a tiny sliver of the host's range – the IRL - has been studied in detail. Goto et al's (2018) phylogeny of 6/7 IRL commensals was consistent with a 5:1 ratio of within-host to host-switching speciation events, a much higher ratio than that documented in other galeommatoidean clades (Goto et al. 2012, Li et al. 2016). Mantis shrimp are one of the few predators to host galeommatoidean commensals (Yamamoto & Habe 1961, Morton 1980, Goto et al. 2012) and are the only known galeommatoidean hosts that engage in active, visual predation (Cronin et al., 2022). To our knowledge, *L. scabricauda*'s mass killing of 34/35 commensals, albeit in captivity (Figure 10 E&F), is the first report of galeommatoidean commensals being actively preyed upon by their host.

*Synthesis*

Collective consideration of the IRL field census, stable isotope, and captive behavioral data yields a heterogenous vista of symbiont coexistence and of symbiont exclusion. It may therefore be useful to view this study system as being composed of two distinct, superimposed patterns of commensal distribution: 1) all burrow-wall commensal species; 2) the ectocommensal species.

In this framing, the 6 burrow-wall commensals broadly adhere to neutral theory (*Hubbel, 2001*) expectations of species assembly in that they co-occur seamlessly in space and time, at least in the sections of IRL host burrows reached by yabby pump sampling (Mikkelsen & Bieler, 1992; this study). This is consistent with numerous studies that have found little evidence for competitive exclusion in marine benthic communities (Stanley, 2008; Shinen and Navarette, 2014; Klompmaker and Finnegan, 2018). Such studies often emphasize the role of high rates of marine predation and disturbance in minimizing competition (Klompmaker and Finnegan, 2018) but another, possibly more apt, model for IRL burrow-wall commensal coexistence might be Laird and Schamp's (2006) finding that coexistence of ≥3 competitors is possible if the competition is non-hierarchical. That important detail remains to be determined but at least one

burrow-wall commensal (*Divariscintilla luteocrinita*) showed evidence of trophic differentiation (Figure 9), and 4/6 appear to have fluctuated in relative frequency between 1992 and 2017 (Mikkelsen & Bieler, 1992; this study). Goto *et al*'s (2018) vasconielline phylogeny established that the burrow-wall niche and its associated "hanging-foot" morphology is plesiomorphic among IRL commensals, implying that within-burrow coexistence may also be the ancestral condition. If so, it has proven to be remarkably stable and has survived the repeated addition of new burrow-wall commensals, mainly through within-host (ostensibly non-adaptive) speciation, but also through host switching (Goto *et al*., 2018).

In contrast, *Parabornia squillina*'s apparent inability to coexist with other *Lysiosquilla scabricauda* commensals in IRL host burrows, despite its unique ectocommensal niche, is incongruent with both competitive exclusion principle and neutral theory expectations. Goto *et al*'s (2018) vasconielline phylogeny shows that the ectocommensal niche is 1) a derived condition among IRL commensals; 2) a product of within-host adaptive speciation. In this case, within-host adaptive speciation involving clear ecological character displacement (Goto *et al*., 2018) has apparently led to the introduction of strict ecological exclusion (and a truncation of realized niches) to a commensal community hitherto characterized by comprehensive co-existence: an inverse outcome of theoretical expectations!

The ecological factors regulating the observed IRL burrow-wall commensal/ectocommensal exclusion are currently obscure but potentially include differential recruitment to individual IRL host burrows and/or differential survival in "mixed-niche" burrow assemblages. Our field census data unfortunately could not distinguish among those possibilities because they did not include newly recruited juvenile commensals: based on prodissonconch sizes, they metamorphose out of the plankton at 350-390 µm in length (*Mikkelsen & Bieler 1989,1992*; Supplemental Figure 1) and our smallest recovered specimen was 2.5 mm in mantle length. Resampling IRL host burrows during commensal recruitment peak periods using a sufficiently fine mesh sieve, together with microscopic examination of sediment and host samples, could address this deficiency. Replication of the adult exclusion pattern by juveniles, or detection of juvenile-specific "mixed-niche" burrow assemblages, would respectively support differential recruitment, or differential survival, exclusion mechanisms.

Current evidence for either potential exclusion mechanism is fragmentary at best. Regarding differential recruitment, the challenge is to explain the lack of *Parabornia squillina* recruitment to

host burrows supporting multi-species burrow-wall commensal assemblages, and/or *vice versa*. Commensal galeommatoideans typically display positive chemotaxes to their respective hosts (Morton, 1962; Gage, 1968, 1979; Ockelmann and Muus, 1978), as apparently does *P. squillina* (Goto *et al.,* 2018). Preventing "mixed-niche" IRL recruitment of the 7 IRL commensal species to the same exclusive host might require counteracting among-commensal negative chemotaxes/behaviors (burrow wall commensal species vs ectocommensal species or *vice-versa*). Our laboratory behavior experiments (Figure 10), show no clear evidence for such.

A variety of potential drivers, competitive and/or predatory, might contribute to differential burrow-wall commensal vs ectocommensal survival in "mixed" burrow assemblages. Note that the formal concept of competitive exclusion (Hardin, 1960) turns out to be inapplicable to this study system because it requires the species that cannot coexist — burrow-wall commensals and the ectocommensal (not subsets of the burrow-wall commensals as initially hypothesized) — to have identical niches, and this is clearly not the case (Figure 1). As discussed above, evidence that a trophic competition driver is influential in regulating this system is mixed at best for the 3 commensal species with characterized trophic niches, with the sole member of the 3 to show trophic differentiation, *Divariscintilla leuteocrinita* (Figure 9), co-occurring only with other burrow-wall commensals (Figure 7).

A context-specific change in *Lysiosquilla scabricauda* predatory behavior is another potential driver of differential commensal survival *i.e.*, selective host exclusion. Our artificial burrow behavioral experiment was unexpectedly terminated by the host-induced mass mortality of all non-host-attached commensals (Figure 10). That host behavioral change might have been triggered by starvation because captive mantis shrimp refused to feed on offered prey fishes (a response readily seen in the field). However, a host-starvation trigger does not explain the absence of ectocommensals in IRL burrows containing burrow-wall commensals (Figure 7). An alternative trigger might be that the act of ectocommensal attachment itself induces a change in host predatory behavior leading to the eradication of co-occurring burrow wall commensals. This may seem far-fetched, but it is fully congruent with the observed field distribution data (Figure 7) and mantis shrimp are behaviorally complex organisms with extraordinary visual systems (Theon et al., 2014, 2017; Franklin et al., 2017; Patel and Cronin, 2020). It could also be tested experimentally by tracking burrow-wall commensal survival in host

burrows with (treatment) and without (control) added ectocommensals.

## Conclusions

This study aimed to investigate how *Lysiosquilla scabricauda*'s extraordinary IRL galeommatoidean commensal community (*Mikkelsen & Bieler 1989; Mikkelsen & Bieler 1992; Goto et al., 2018*), incorporating sympatric sister species pairs generated by adaptive and by non-adaptive speciation processes, is regulated. Although the unexpected rarity of the non-adaptive species pair did not allow us to fully address this goal, our results confirmed the presence of a trenchant ecological exclusion in this commensal community that violates both competitive exclusion principle and neutral theory expectations. This intriguing ecological puzzle is potentially resolvable through additional field sampling of commensal recruitment and additional host-commensal behavioral experiments. However, a fuller understanding of this commensal communities' evolutionary ecology will also require its study outside of the narrow confines of the IRL. It would be particularly interesting to investigate Southern Brazilian *L. scabricauda* commensal populations to establish if its ectocommensal, *Parabornia palliopillata*, also exhibits an ecological exclusion from regional burrow-wall commensals.

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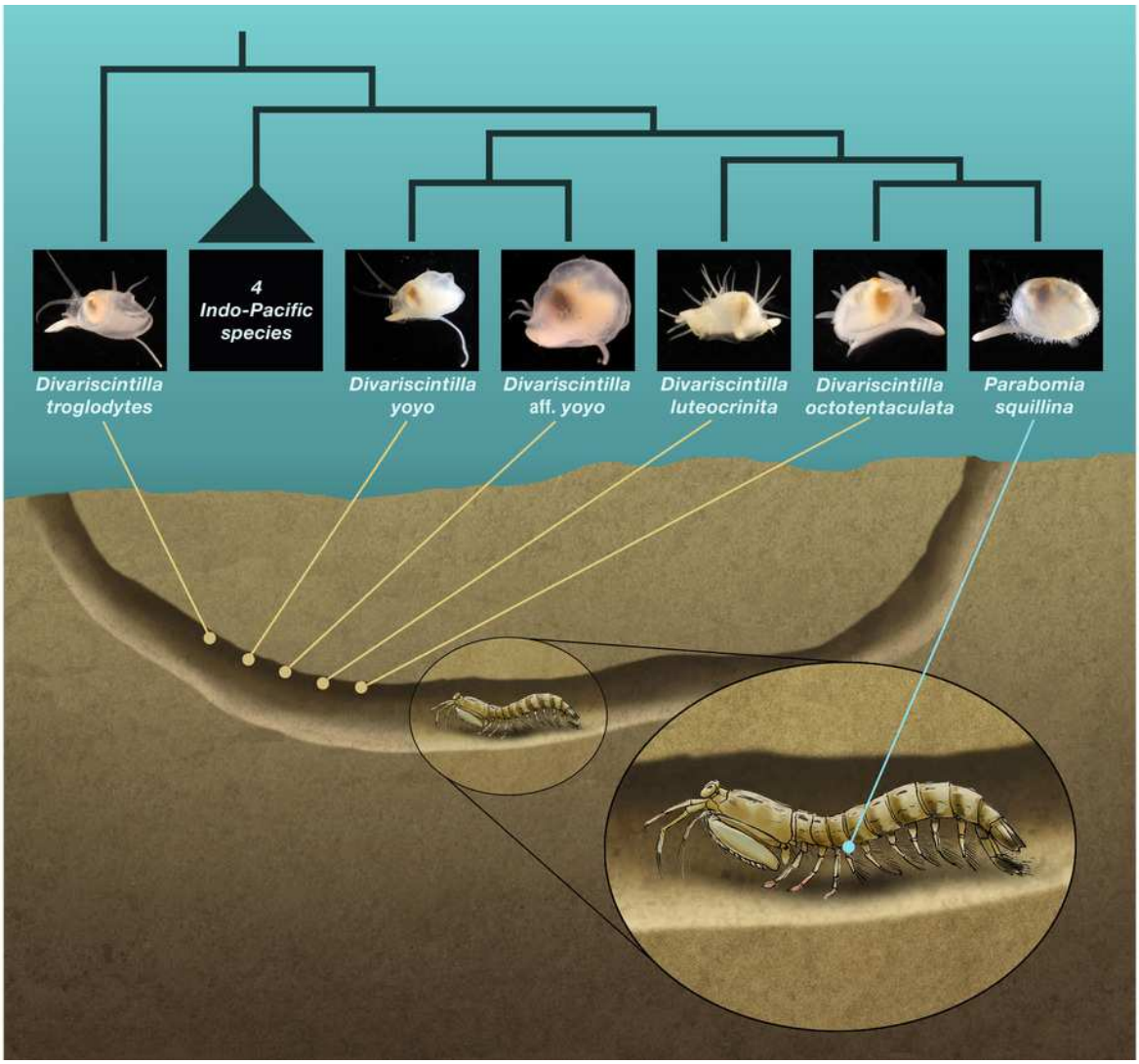
# Figure 1

Schematic section of a composite Indian River Lagoon *Lysiosquilla scabricauda* burrow.

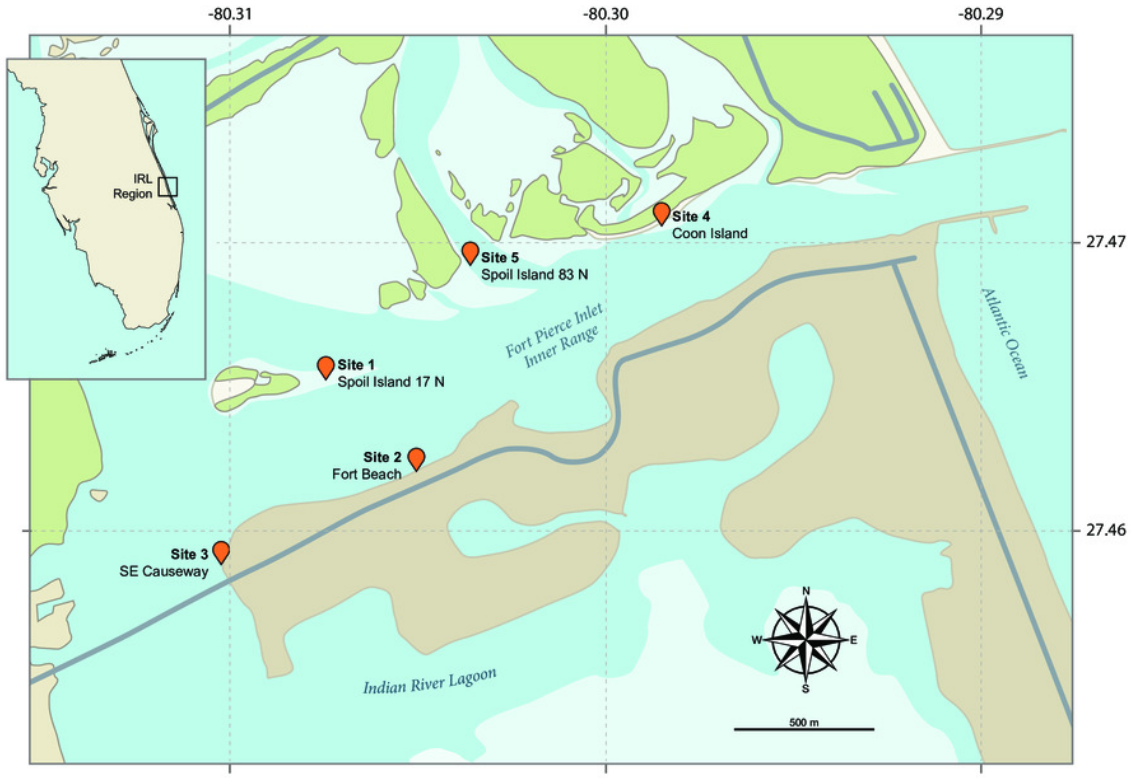
This shows the relative positioning (by yabby pump field sampling) of ~~the~~ 5 burrow-wall commensal species (*Divariscintilla* spp.), and the single ectocommensal (*Parabornia squillina*) species, collected in this study. Also shown, in outline, are the inferred phylogenetic relationships of the 6 IRL commensals (Goto et al., 2018).



# Figure 2

Maps of the field study sites.

The small inset map (top left) shows the position of the Indian River Lagoon (IRL) study area on the East coast of Florida. The main map illustrates the 5 intertidal study field sites flanking the IRL's Fort Pierce Inlet.



# Figure 3

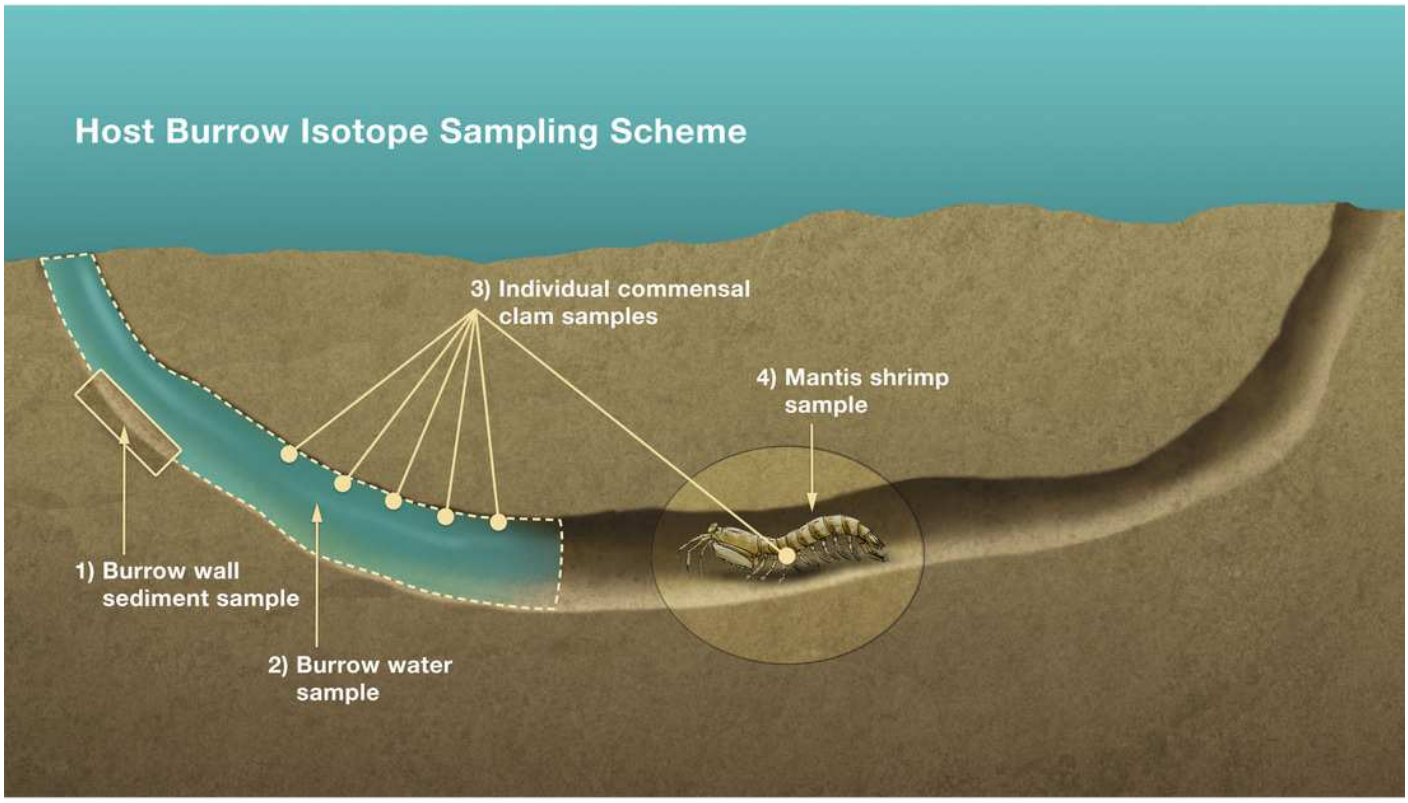
Field photograph showing capture of a host *Lysiosquilla scabricauda* specimen.

The first author is shown firmly grasping the host specimen's two raptorial appendages prior to carefully lifting it out of its flagged burrow opening. See here (insert link: https://figshare.com/articles/media/Mantis_Shrimp_Capture_Technique_m4v/24847938) a video recording of an entire host capture sequence.

# Figure 4

Sampling scheme for Stable Isotope Analyses.

Schematic diagram of a composite Indian River Lagoon mantis shrimp *Lysiosquilla scabricauda* host burrow showing the 4 primary burrow components sampled for isotope analyses: individual commensal clams (3) and their potential basal trophic resources [deposited organic matter (1), suspended particulate organic matter (2), and host tissue (4)].



# Figure 5

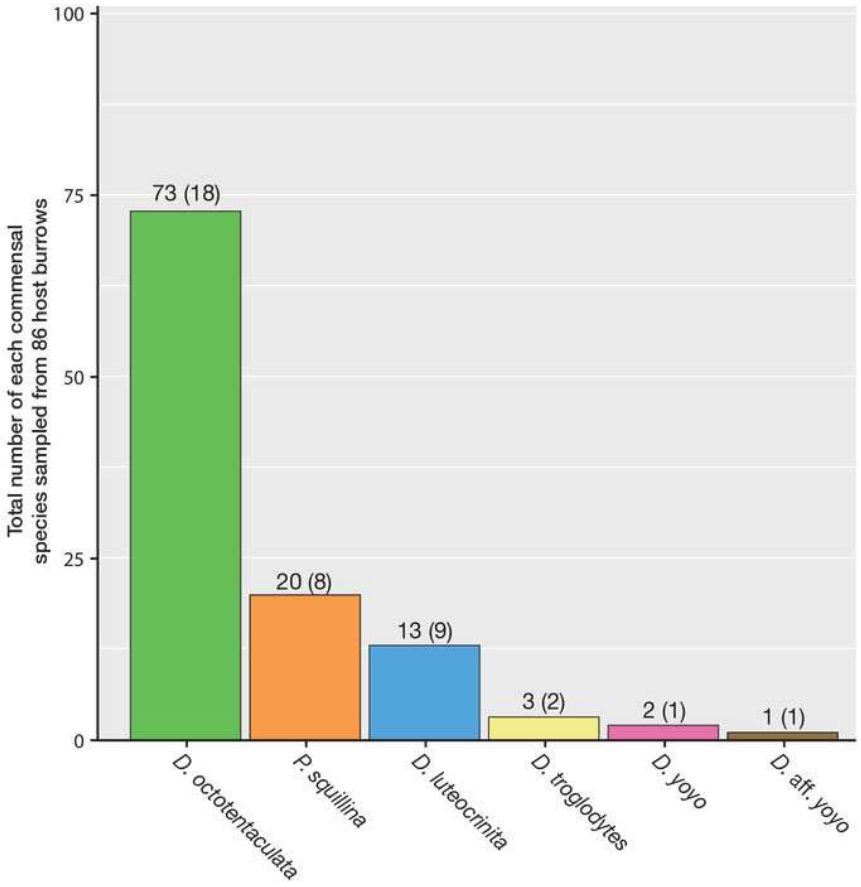
Aquarium artificial host burrow for viewing commensal/host interactions in the laboratory.

Note the host *Lysiosquilla scabricauda* (arrow) within the sand-coated PVC artificial burrow structure. This photo was taken prior to the addition of commensals.

# Figure 6

Summary frequencies of each commensal clam species recovered in the field census of Indian River Lagoon host burrows.

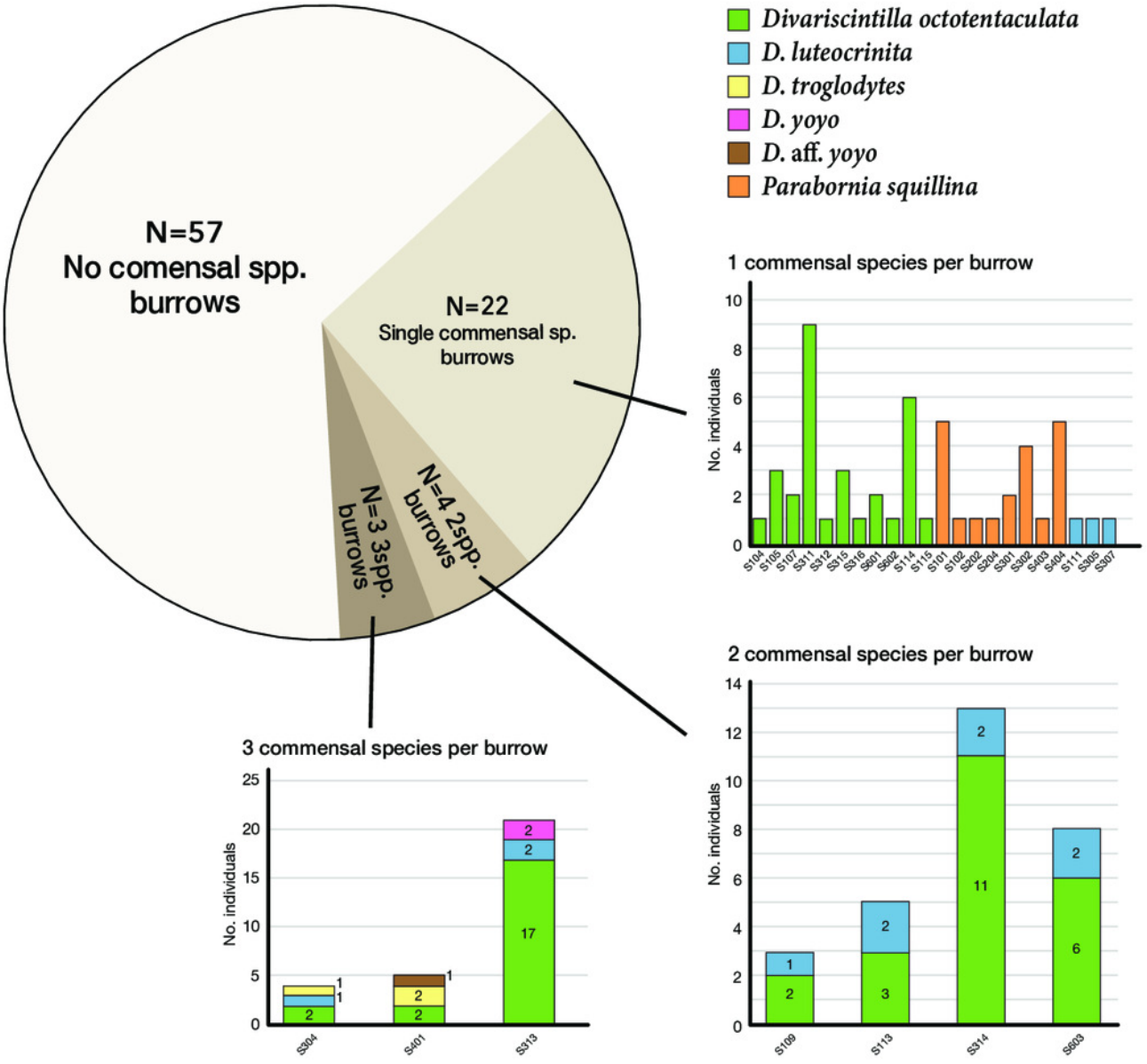
The total number of commensal clams recovered, and burrows occupied (in parentheses), for each of the 6 commensal species (5 *Divariscintilla* spp. and 1 *Parabornia* sp.) sampled from 86 IRL host *Lysiosquilla scabricauda* burrows.


Total number of each commensal species sampled from 86 host burrows
100
75
50
25
0
73 (18)
20 (8)
13 (9)
3 (2)
2 (1)
1 (1)
D. octotentaculata
P. squillina
D. luteocrinita
D. troglodytes
D. yoyo
D. aff. yoyo

# Figure 7

Graphical summary of Indian River Lagoon commensal clam co-occurrence

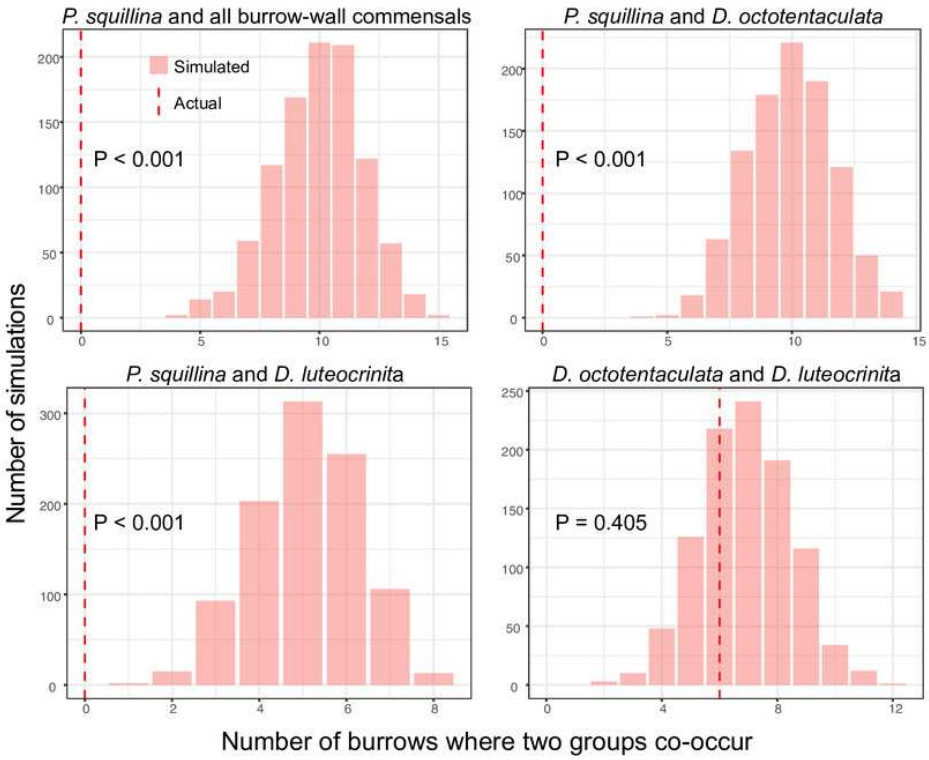
Census data from 29 commensal-occupied host burrows (out of a total of 86 burrows sampled) grouped by their level of commensal species diversity: mono-, bi-, and tri-specific. See Supplementary Table 1 for site locations of individual burrow IDs (bar graph x axes notation) yielding ≥1 commensal clam(s).


Divariscintilla octotentaculata
D. luteocrinita
D. troglodytes
D. yoyo
D. aff. yoyo
Parabornia squillina
N=57
No comensal spp. burrows
N=22
Single commensal sp. burrows
N=4 2spp. burrows
N=3 3spp. burrows
1 commensal species per burrow
No. individuals
S104 S105 S107 S311 S312 S315 S316 S601 S602 S114 S115 S101 S102 S202 S204 S301 S302 S403 S404 S111 S305 S307
2 commensal species per burrow
No. individuals
S109 S113 S314 S603
3 commensal species per burrow
No. individuals
S304 S401 S313

# Figure 8

Simulated random recruitment expectations for co-occurrence of commensal species in Indian River Lagoon host burrows

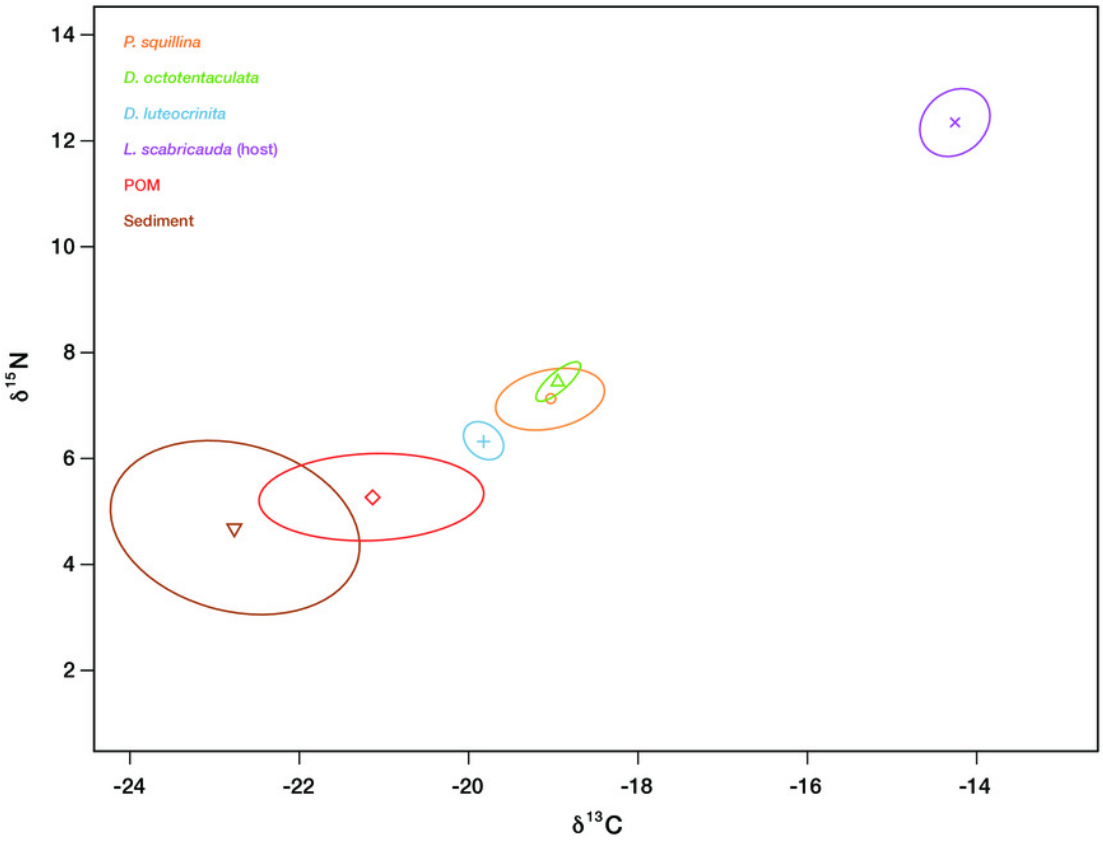
Comparison of the actual observed (dashed red lines) co-occurrence of 4 commensal vasconielline species combinations in IRL host burrows to their simulated co-occurrence distributions (histograms) expected under random larval recruitment and post-larval survival dynamics.


P. squillina and all burrow-wall commensals
Simulated
Actual
P < 0.001
P. squillina and D. octotentaculata
P < 0.001
P. squillina and D. luteocrinita
P < 0.001
D. octotentaculata and D. luteocrinita
P = 0.405
Number of simulations
Number of burrows where two groups co-occur

# Figure 9

Inferred isotopic niche widths of commensal species and potential basal resources.

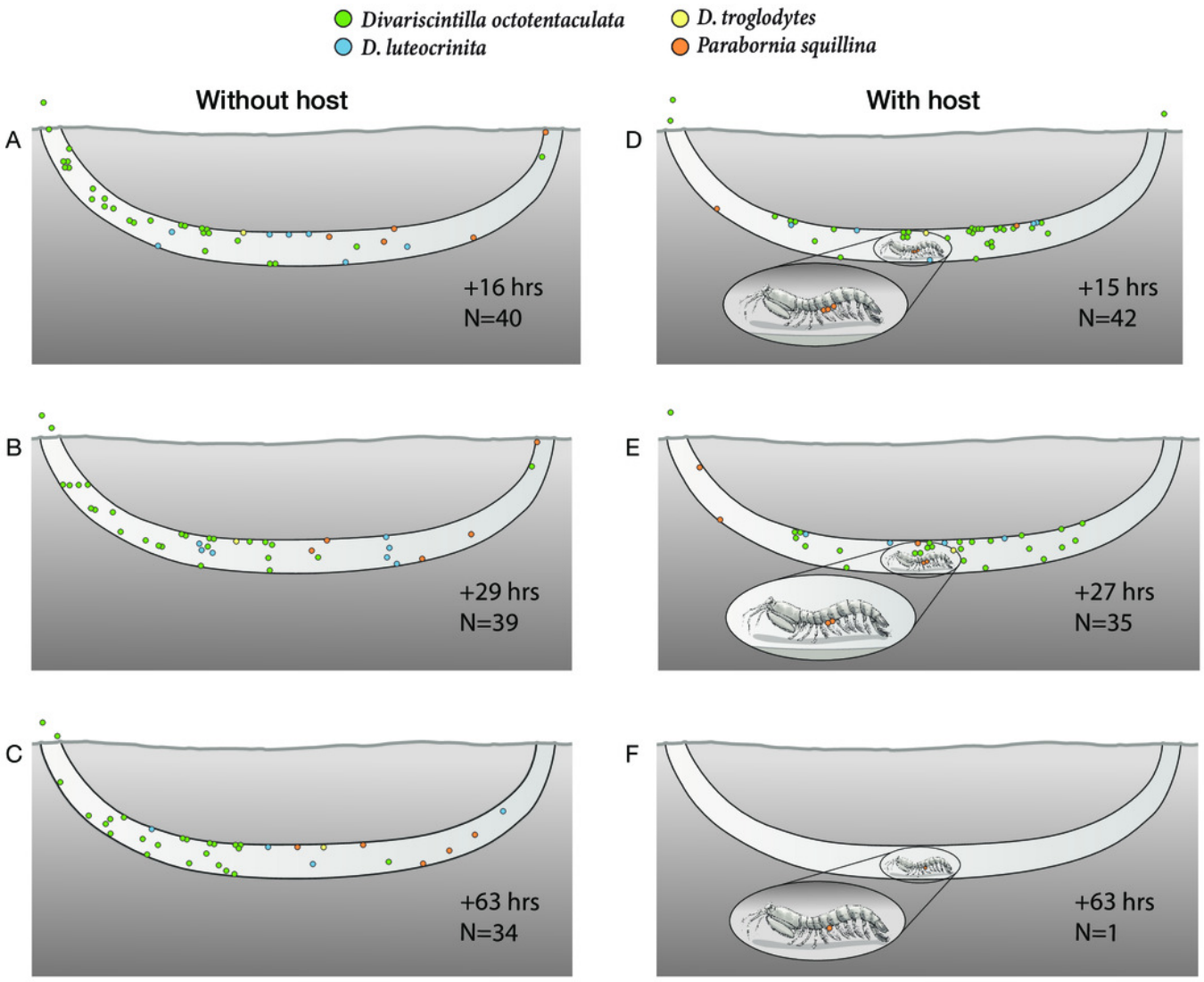
Bayesian-estimated Standard Ellipse Areas ($SEA_B$) of 3 IRL commensal clam species - *Divariscintilla octotentaculata* (N=10; 1 sample failed), *D. luteocrinita* (N=4) and *Parabornia squillina* (N=20) - together with that of their potential basal resources: suspended particulate organic matter (POM, N=33; 2 samples failed), deposited organic matter (sediment; N=33; 1 sample failed), and mantis shrimp (*Lysiosquilla scabricauda*; N=19). Three other IRL commensals (*D. troglodytes, D. yoyo,* and *D.* aff. *yoyo*) were not sampled in large enough quantities to produce $SEA_B$ plots for this analysis. X axis units, expressed as $\delta^{15}N$, are the ratios of $^{15}N$ to $^{14}N$ obtained from the labelled samples, whereas Y axis units, expressed as $\delta^{13}C$, are the corresponding ratios of $^{13}C$ to $^{12}C$. See Supplemental Table 3 for individual specimen isotopic data values and sampling details.


P. squillina
D. octotentaculata
D. luteocrinita
L. scabricauda (host)
POM
Sediment
14
12
10
8
6
4
2
δ15N
-24
-22
-20
-18
-16
-14
δ13C

# Figure 10

Artificial burrow laboratory observations of commensal clam behavior and survival with and without a host

A series of time-specific observations of the spatial positioning and survival of 4 Indian River Lagoon commensal clam species within experimental artificial burrows without (left), and with (right), a resident *Lysiosquilla scabricauda* mantis shrimp host. At the beginning of the experiment (Time 0), 47 clams were introduced to the host-free burrow (34 *Divariscintilla octotentaculata*, 7 *D. luteocrinita*, 5 *Parabornia squillina*, and 1 *D. troglodytes*), and 46 clams were introduced to the host-occupied burrow (33 *D. octotentaculata*, 7 *D. luteocrinita*, 5 *P. squillina*, and 1 *D. troglodytes*). N denotes the number of surviving clams observed for each treatment at the accompanying time point.


Divariscintilla octotentaculata
D. luteocrinita
D. troglodytes
Parabornia squillina
Without host
With host
A
+16 hrs
N=40
B
+29 hrs
N=39
C
+63 hrs
N=34
D
+15 hrs
N=42
E
+27 hrs
N=35
F
+63 hrs
N=1

## Table 1(on next page)

Summary table showing commensal species recovery from each of the 5 Indian River Lagoon sampling sites

For each of the 5 IRL sampling sites (see Figure 2), the respective numbers of commensal clams recovered (“Count”), and of*Lysiosquilla scabricauda*host burrows occupied (“Burrows”), are shown for all 6 commensal clam species sampled in this study: *Divariscintilla octotentaculata* (O), *Parabornia squillina* (S), *D. luteocrinita* (L), *D. troglodytes* (T), *D. yoyo* (Y), and *D.* aff. *yoyo* (AY). The two rightmost columns (“All”) collectively display the combined totals of all species of commensal clams collected at each site.

| | O | | S | | L | | T | | Y | | AY | | All | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Count | Burrows | Count | Burrows | Count | Burrows | Count | Burrows | Count | Burrows | Count | Burrows | Count | Burrows |
| Spoil Is. 17 N | 18 | 7 | 6 | 2 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 28 | 10 |
| Ft. Beach | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| SE Causeway | 44 | 7 | 6 | 2 | 7 | 5 | 1 | 1 | 2 | 1 | 0 | 0 | 60 | 11 |
| Coon Is. | 2 | 1 | 6 | 2 | 0 | 0 | 2 | 1 | 0 | 0 | 1 | 1 | 11 | 3 |
| Spoil Is. 83 N | 9 | 3 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 3 |