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A New Host and Range Record for the Gall Crab *Fungicola fagei* as a Symbiont of the Mushroom Coral *Lobactis scutaria* in Hawai'i[1]

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Abstract: The coral crab *Fungicola fagei* (Decapoda: Brachyura: Cryptochiridae) is recorded for the first time from the Hawaiian Islands, where it was discovered in a previously unknown association with the solitary, free-living mushroom coral *Lobactis scutaria* (Anthozoa: Scleractinia: Fungiidae). The associated crab species was discovered off Hilo on the island of Hawai'i, where it appeared to be relatively common. It could have been previously overlooked because of its small size (max. ca. 1 cm long) and its hidden life style inside the host coral. Species identification is based on the morphology of the carapace and use of the cytochrome oxidase subunit I (COI) barcode gene as molecular marker. *Fungicola fagei* is known from other localities in the Indo-West Pacific region, where it is only hosted by mushroom coral species of the genera *Podabacia* and *Sandalolitha*. The record of *F. fagei* from the Hawaiian Islands represents a northeastward extension of its known geographical distribution range, 6,000 km away from its nearest locality in Vanuatu, despite the availability of host species as eastward as the Cook Islands and French Polynesia. Because these other host species do not occur in the Hawaiian Islands, *F. fagei*'s occurrence here is only possible because of its association with *L. scutaria*, uniquely recorded from Hawai'i.

Keywords: Brachyura, Cryptochiridae, Scleractinia, Fungiidae, COI, host switch, Indo-West Pacific

Lobactis scutaria (Lamarck, 1801) is a solitary, free-living scleractinian coral of the family Fungiidae that shows a wide distribution range in the Indo-West Pacific region, from the Red Sea to the Hawaiian Islands, and French Polynesia (Hoeksema 1989, Gittenberger et al. 2011). It is the largest and most common mushroom coral in the Hawaiian archipelago, where it was traditionally known as *Fungia scutaria* (Edmondson 1946, Maragos 1977, Hoover 1999, Fenner 2005). This species has been used in coral reef research in Kāne'ohe Bay and Waikīkī Reef (O'ahu Island) or in laboratory experiments with specimens taken from those localities, which has resulted in numerous publications on its ecology and physiology over a long period of time (e.g., Edmondson 1929, Stephens 1962, Bosch 1967, Yamazato 1970, Pearse 1971, Jokiel and Cowdin 1976, Krupp 1982, 1983, 1984, 1985, Chadwick 1988, Romano 1988, Krupp et al. 1993, 2006, Jokiel and Bigger 1994, Schwarz et al. 1999, Weis et al. 2001, Hagedorn et al. 2006, Concepcion et al. 2010, Schnitzler et al. 2012). Additional references can be found in the work by Coles et al. (2002).

Despite all the research on *Lobactis scutaria* in the Hawaiian Islands, limited attention has been given to its associated fauna. For example, Bosch (1965) and Bell (1985) reported on

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epitoniid snails and their egg capsules found on the undersurface of several *L. scutaria* corals in Kāneʻohe Bay, Oʻahu, which probably feeds on its host's tissue and can be considered parasitic. They were described as *Epitonium ulu* Pilsbry, 1921, with Hilo at Hawaiʻi as type locality (Pilsbry 1921, Edmondson 1946). Now, this gastropod is classified as *Epifungium ulu* and considered widespread in the Indo-West Pacific region, where it has been recorded from 11 mushroom coral species (Gittenberger and Gittenberger 2005, Gittenberger and Hoeksema 2013).

Furthermore, Maragos (1977) mentioned that small xanthid crabs find shelter underneath mushroom corals in the Hawaiian Islands. Finally, van der Meij, Fransen, et al. (2015) reported on empty galls of cryptochirids found in museum specimens of *L. scutaria* from Hawaiʻi, which were assigned to *Fungicola* sp. In an earlier review paper, Hoeksema et al. (2012) reported on two gall crab species, *Fungicola fagei* (Fize & Serène, 1956) and *F. utinomi* (Fize & Serène, 1956), in association with *L. scutaria*, but they did not give specific locality data or references. In a successive, more elaborate study on cryptochirids associated with mushroom corals, van der Meij, Fransen, et al. (2015) mentioned that *F. fagei* crabs were known to dwell only in *Podabacia* and *Sandalolitha* corals and that *F. utinomi* crabs were hosted by *Ctenactis*, *Danafungia*, *Fungia fungites* (Linnaeus, 1758), *Halomitra*, *Lithophyllon*, and *Sandalolitha* corals, whereas *F. syzygia* van der Meij, 2015, was predominantly associated with *Cycloseris* and *Pleuractis*. Hence, there was no certainty about which gall crab species lived in association with *Lobactis* in the Hawaiian Islands, and no occurrence records of this association are known from elsewhere.

During exploratory dives at the reef off Leleiwi Beach, Hilo, Hawaiʻi, in July 2016, J.E.G.-H. came across numerous mushroom corals of *Lobactis scutaria* and of another solitary, free-living mushroom coral species, *Pleuractis granulosa* (Klunzinger, 1879). Individuals of *L. scutaria* were common, and many of them contained visible injuries (Figure 1). Closer inspection revealed that these injuries were gall crab dwellings and that most of them contained living crabs. This finding offered the opportunity to investigate the identity of the gall crab species associated with *L. scutaria*, which so far has remained unknown.

MATERIALS AND METHODS

Mushroom corals off Leleiwi Beach, Hilo, island of Hawaiʻi (19° 44′ 05.6″ N, 155° 01′ 00.1″ W), were observed and photographed in situ at ca. 10–15 m depth (4 July 2016). Individuals containing galls of cryptochirids were investigated more closely. A total of six crabs (two females and four males) was extracted from the corals with the help of forceps and stored in 96% ethanol. The crabs were brought to the laboratory for identification by morphological examination and molecular analyses based on the cytochrome oxidase subunit I (COI) barcoding gene as marker. Close-up photographs of the crabs were made with a stereo microscope (Carl Zeiss Discovery V20) equipped with a camera (Axiocam Mrc5) for stacking photography.

Genomic DNA was extracted using the Macherey-Nagel NucleoMag 96 Tissue Kit and the KingFisher Flex (Thermo Scientific). One leg of a gall crab was used for the DNA extraction and was lysed overnight in Lysis buffer T1 and Proteinase K at 56°C. A polymerase chain reaction (PCR) was carried out by using a M13-LepFol primer mix (M13F-LepFolF mix and M13R-LepFolR mix) with an amplification product between the primers of 658 base pairs (bp). M13F-LepFolF primer mix was a mix of M13F-LepF1 and M13F-LCO1490, and M13R-LepFolR primer mix was a mix of M13R-LepR1 and M13R-HCO2198 (Folmer et al. 1994, Herbert et al. 2004). A PCR amplification of the mitochondrial COI was performed in a volume of 25 µl containing 2 µl DNA template, 2.5 µl of 10x PCR Coralload buffer (Qiagen), 0.5 µl of 10 µM of each primer, 0.5 µl of 2.5 mM dNTP, and 0.25 µl of 5 U/µl Taq DNA polymerase (Qiagen) under standard PCR conditions (initial denaturation at 94°C for 3 min, 40 cycles of: denaturation at 94°C for 15 sec, annealing at 50°C for 30 sec, elongation at 72°C for 40 sec, and a final elongation step

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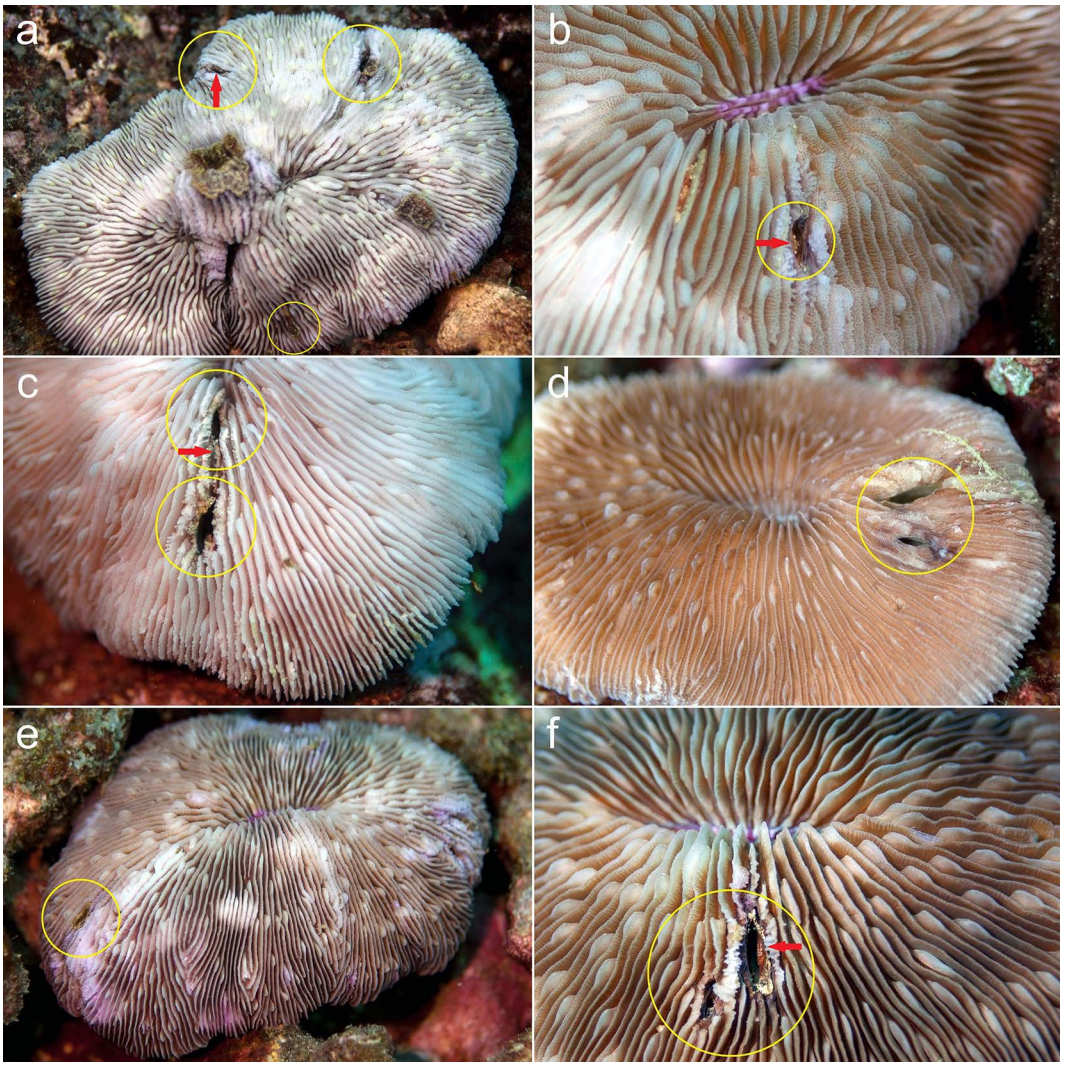


FIGURE 1. Gall grab (*Fungicola fagei*) dwellings in mushroom corals (*Lobactis scutaria*), Hilo, Hawai'i (marked by yellow circles). Crabs are marked by red arrows. (*a*) One coral containing two female crabs [at the top (BPBM-S17057, RMNH.CRUS.D.57237)] and at least one male (bottom). (*b*) Host coral with one male crab, clearly visible (RMNH. CRUS.D.57238). (*c*) One host coral with two dwellings from which one male crab was extracted (RMNH.CRUS.D.57239). (*d*) One host coral with two pits, no visible crabs. (*e*) One coral with one crab pit, crab not visible. (*f*) One coral with two crabs, one of which is clearly visible.

at 72°C for 5 min). PCR products were visualized by agarose gel electrophoresis (2% agarose E-Gel, Invitrogen), and suitable amplicons were sequenced by Baseclear Leiden using the M13 primers (forward and reverse sequencing). Four out of six sequences were successful. The raw sequences were edited by using Geneious software (version 8.1.8). For the purpose of molecular identification, a Neighbor-Joining tree was constructed to show the phylogenetic affinities of the crabs from Hawai'i, along with all three *Fungicola*

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species recognized so far (van der Meij, Fransen, et al. 2015). Data are derived from GenBank (NCBI) based on specimens from Southeast Asia and the West Pacific (van der Meij 2015*a*). Because *Fungicola fagei* and *F. syzygia* together form a sister group of *F. utinomi* (van der Meij 2015*a*), the latter was selected as outgroup.

The six specimens have been deposited in the collections of the Bernice B. Bishop Museum in Honolulu (BPBM) and Naturalis Biodiversity Center (previously known as Rijksmuseum van Natuurlijke Historie) in Leiden (RMNH). The following catalog numbers were assigned: BPBM-S17057 (one female and one male), RMNH.CRUS.D.57237 (one female), RMNH.CRUS.D.57238 (one male), RMNH.CRUS.D.57239 (one male), RMNH.CRUS.D.57240 (one male). The latter four had sequences that were deposited in Genbank (NCBI) under accession numbers MF973150–MF973153.

RESULTS

Based on the association with mushroom corals (Figure 1), the crabs could be identified at family level as belonging to the Cryptochiridae (Decapoda: Brachyura). The elongated pits were wedged in between the host's septa, with the crabs themselves in perpendicular position (Figure 1*f*). These dwellings were similar to those made by *Fungicola* crabs in corals of *Cycloseris* and *Pleuractis* (van der Meij, Fransen, et al. 2015: fig. 1). Most crabs lived as a pair consisting of a large female and a small male (Figure 1*a*, *c*, *d*, *f*), whereas others lived alone (Figure 1*b*, *e*). The females were about 8–11 mm long and the males 3–4 mm, a ratio of ca. 3:1 (Figure 2). The upper surface of the female carapace shows a keyhole-shaped sculpture (Figure 2*a*, *b*), which is also seen in *Fungicola fagei* (van der Meij 2015*a:* figs. 1*A*, 4*A*) but not in its congeners.

In the COI-based phylogenetic tree, all four crabs from Hawai'i clustered with specimens of *F. fagei* found in corals of the fungiid genera *Podabacia* and *Sandalolitha*, which were collected from Indonesia, Malaysia, and New Caledonia (Figure 3). Hence, results of the molecular analysis are consistent with those of the morphological examination, confirming the crab's identity as *F. fagei.*

DISCUSSION

Gall crabs belonging to the genus *Fungicola* were previously unknown from the Hawaiian Islands, except for the listing by Hoeksema et al. (2012). Until now, only four gall crab species (family Cryptochiridae) were previously recognized from the Hawaiian Islands (Kropp 1989, 1990, Castro 2011): (1) *Hapalocarcinus marsupialis* Stimpson, 1859, associated with corals of the scleractinian family Pocilloporidae; (2) *Opecarcinus crescentus* (Edmondson, 1925), known from corals of the genus *Pavona* (Agariciidae); (3) *Pelycomaia minuta* (Edmondson, 1933), found in corals of the scleractinian genera *Cyphastrea* (family Merulinidae) and *Leptastrea* (family incertae sedis); and (4) *Utinomiella dimorpha* (Henderson, 1906) in association with pocilloporid corals. Hence, *F. fagei* is the fifth cryptochirid species recorded from the Hawaiian Islands.

The new record is also an addition to the known associated fauna of *L. scutaria*. Besides the parasitic snail *Epifungium ulu*, *L. scutaria* is known to host various other invertebrates. It has been observed to host three barnacle species, an excavating mytilid bivalve, a coralliophiliid snail, and a hydroid (Gittenberger and Gittenberger 2011, Hoeksema et al. 2012, Montano et al. 2015).

Until now, the easternmost record of *F. fagei* is from New Caledonia and Vanuatu (Figure 4), where it was reported from the corals *Podabacia motuporensis* Veron, 1990, and *Sandalolitha robusta* (Quelch, 1886), respectively (van der Meij 2015*a*; van der Meij, Fransen, et al. 2015). Hawai'i is about 6,000 km away from the nearest locality of *F. fagei* at Vanuatu (Figure 4). Other *F. fagei* host records concern *Podabacia crustacea* (Pallas, 1766) from eastern Indonesia and northeastern Borneo (Malaysia); *Podabacia sinai* Veron, 2000, from Palau; and *Sandalolitha dentata* Quelch, 1884, also from northeastern Borneo. Some of the coral species themselves have more eastward distribution localities, such as *P. crustacea* in French Polynesia (Hoeksema 1989); *S. dentata* in the Mar-

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FIGURE 2. Microphotographs of two females and two males of *Fungicola fagei* from Hilo, Hawai'i. Scale bars: 1 mm. (*a*) Female, BPBM-S17057. (*b*) Female, RMNH.CRUS.D.57237. (*c*) Male, BPBM-S17057. (*d*) Male, RMNH.CRUS.D.57239.

shall Islands, Caroline Islands, and French Polynesia (Hoeksema 1989, Hoeksema and Benzoni 2013); and *S. robusta* in the Cook Islands (Hoeksema 1989). *Fungicola fagei* may occur at these localities, in which case it has been overlooked, as previously in Hawai'i. Other easternmost records for *Fungicola* concern *F. syzygia* from French Polynesia (van der Meij, Fransen, et al. 2015) and *F. utinomi* from Guam and Palau (Kropp 1990, Paulay et al. 2003).

The new record presented here for *F. fagei* is not only unique because of the eastward extension of its known distribution range, but

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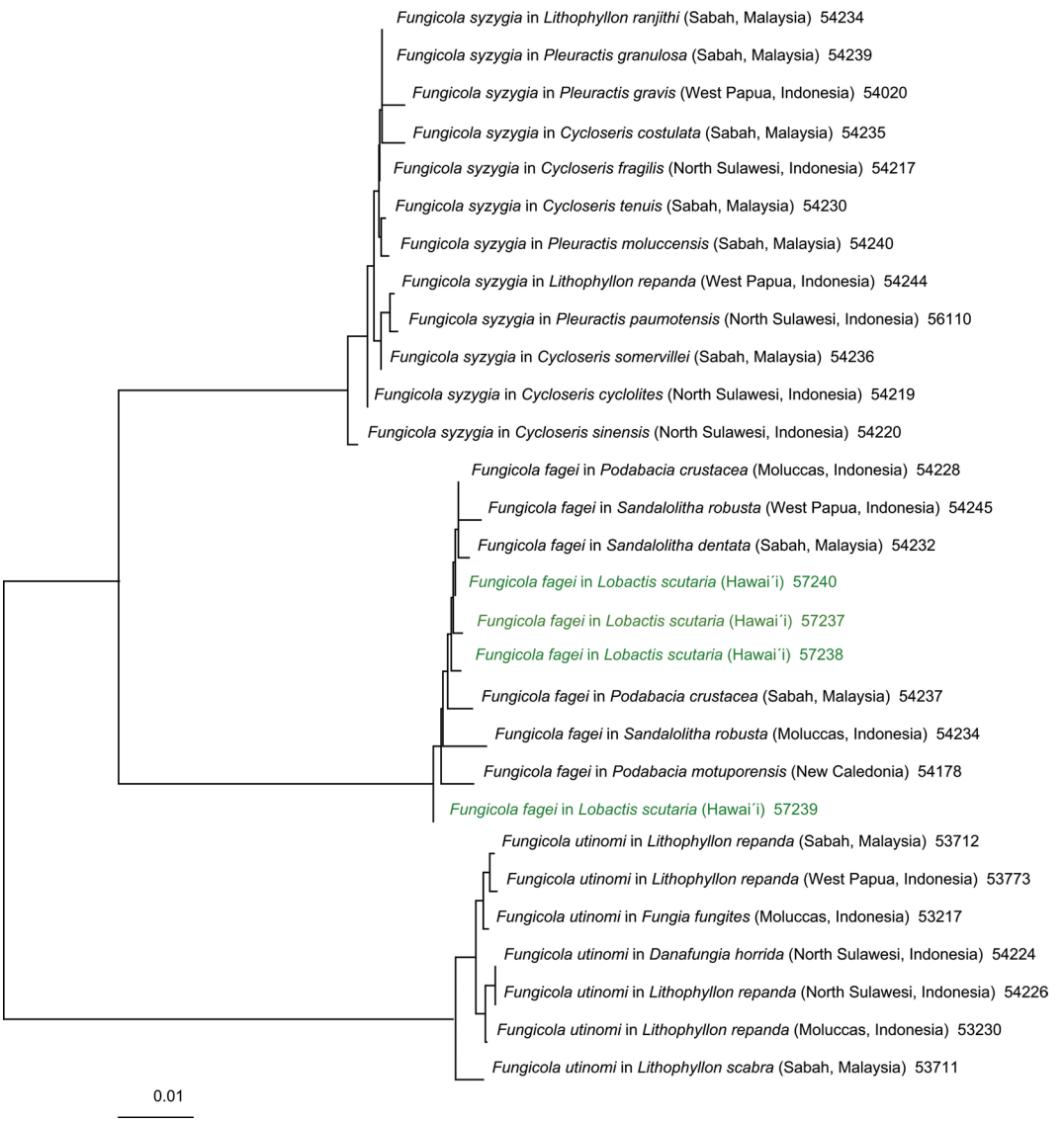


FIGURE 3. Neighbor-Joining tree showing the phylogenetic affinities (COI) of *Fungicola fagei* crabs from Hawai'i (green lettering) with *Fungicola* specimens from Southeast Asia and the West Pacific represented in GenBank (black lettering). Numbers refer to collection catalog entries (RMNH.Crus.D).

also because *L. scutaria* is documented as a newly recorded host coral (Figure 1). Extensive surveys of the mushroom coral fauna have been performed in various localities in eastern Indonesia and eastern Malaysia, where *L. scutaria* is most common on offshore reefs: the Spermonde archipelago, South Sulawesi (Hoeksema 2012*a*,*b*); Ternate, Moluccas (Gittenberger et al. 2015); and Kudat and Semporna, Sabah (Waheed and Hoeksema 2013, Waheed et al. 2015). Corals at the same localities were also searched for cryptochirids, but this did not result in host records for *L. scutaria* (van der Meij and Hoeksema 2013;

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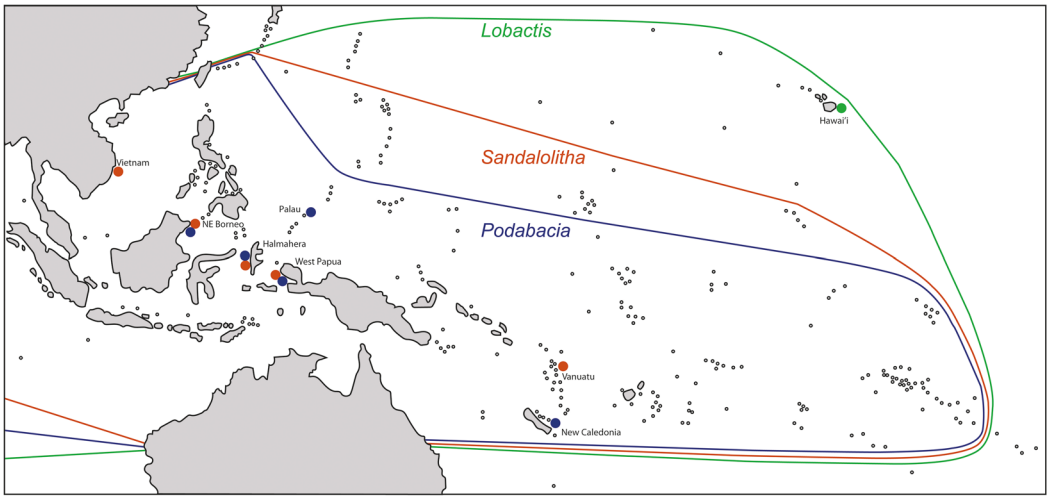


FIGURE 4. Distribution map of *Fungicola fagei* (host and locality records from van der Meij 2015*a;* van der Meij, Fransen, et al. 2015; this study) and the known ranges of its host coral genera in the West and central Pacific (Hoeksema 1989, Hoeksema and Dai 1991, Hoeksema and Benzoni 2013). (Green dots, *Lobactis;* red dots, *Sandalolitha;* dark blue dots, *Podabacia*.)

van der Meij, Fransen, et al. 2015). Unfortunately, we have no occurrence data on *F. fagei* from localities between Palau/Vanuatu and Hawai'i. Nevertheless, the Hawaiian crabs appear closely related to those from remote localities (Figure 3), which indicates that they may have been introduced relatively recently.

It is important to note that the population of *F. fagei* would not have been able to establish and maintain itself in Hawai'i without a host switch to *L. scutaria* from *Podabacia/Sandalolitha* spp., which are absent in the Hawaiian Islands. The reverse is unlikely because then *L. scutaria* would also have been found as a host in other investigated areas. Because *Lobactis* is not directly related to *Podabacia/Sandalolitha*, and elsewhere it is unknown to serve as a host for *F. fagei*, it is also not likely that this association originated in a shared ancestral host taxon. A host switch is therefore more probable, but it is unclear when and where this could have happened, either in the Hawaiian Islands or in more westward localities. Host switches have also been reported from other crustacean taxa, such as pea crabs (Becker and Türkey 2017), xanthid crabs (García-Hernández et al. 2016), and palaemonid shrimps (Brinkmann and Fransen 2016, Horká et al. 2016, Hoeksema and Fransen 2017).

Because *F. fagei* appears to be common in Hawai'i, it is likely that this association also occurs at other islands throughout the Hawaiian archipelago. The absence of field records despite a long research history concerning its host off O'ahu is probably due to a lack of focus on coral-associated fauna. Cryptochirid crabs are small and live concealed inside their pits, and therefore they are usually not easily noticed (Hoeksema 2017), except perhaps when they appear in extremely high densities (Hoeksema and van der Meij 2013). Recent research targeting coral gall crabs resulted in the discovery of a number of new species (Badaro et al. 2012, Zayasu et al. 2013, van der Meij 2014*a*, 2015*b*, 2017, van der Meij, Berumen, and Pauley 2015) and new host records (van der Meij 2012, 2014*b*, Hoeksema et al. 2017). Likewise, recent surveys on mushroom coral hosts have also led to new discoveries of associated fauna, including some undescribed species, such as a benthic ctenophore (Hoeksema et al. 2013, Alamaru et al. 2016), a serpulid worm (Hoeksema and ten Hove 2014), a hydroid (Montano

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et al. 2015), and cryptobenthic fishes (Bos and Hoeksema 2015, 2017). The discovery reported here will undoubtedly be succeeded by new observation records of *F. fagei* and perhaps of other coral-associated fauna within the Hawaiian archipelago. Because of its common occurrence in Hawai'i, the abundance of the association between *F. fagei* and *L. scutaria* could become the subject of in-depth ecological studies.

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