Host relations and DNA reveal a cryptic gall crab species (Crustacea: Decapoda: Cryptochiridae) associated with mushroom corals (Scleractinia: Fungiidae)

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Key words: cryptic species, Fungicola, host specificity, Indo-West Pacific region, Tropical Eastern Pacific region, Scleractinia

Abstract

Mushroom corals of the Indo-West Pacific Fungiidae (Scleractinia) provide habitats for a rich associated fauna, including three species of gall crabs (Cryptochiridae). During the course of the present study gall crabs were sampled from many different fungiid hosts. Based on this ‘reversed’ approach - by studying coral symbionts from a host perspective - a previously unnoticed host specificity pattern was detected. The sampling of gall crab fauna per host coral combined with molecular analyses of H3 nDNA, 16S and COI mtDNA revealed a cryptic gall crab species closely related to Fungicola fagei. This new species, described hereafter as Fungicola syzygia sp. nov., is predominantly associated with the mushroom coral genera Cycloseris and Pleuractis, whereas its sibling species F. fagei is only known to be associated with the host genera Podabacia and Sandalolitha. Based on morphology F. syzygia sp. nov. is difficult to distinguish from F. fagei, but there are subtle differences in carapace shape, the lateral carapace margins, the border between the orbital angles and the merus of the third maxilliped, as well as in the carapace length/width ratio. The type material of F. utinomi and F. fagei is figured for comparison.

Contents

Introduction ........................................................................................................................................ 39
Material and methods ..................................................................................................................... 41
Nha Trang collections .................................................................................................................. 41
Molecular analyses ...................................................................................................................... 41
Results .......................................................................................................................................... 43
Molecular analyses ...................................................................................................................... 43
Host specificity ............................................................................................................................. 44
Discussion ..................................................................................................................................... 44
Acknowledgements ..................................................................................................................... 44
References ..................................................................................................................................... 45
Appendix ....................................................................................................................................... 49

Introduction

The mushroom coral family Fungiidae occurs in the Indo-West Pacific and tropical Eastern Pacific regions with a distribution ranging from the Red Sea and eastern Africa to the west coast of Central America (Hoeksema, 1989). Several species have been recorded in association with these funguids (Hoeksema et al., 2012). Most of the associated fauna consists of crustaceans and molluscs, but fishes have also been recorded to live in symbiosis with funguids (Bos, 2012; Hoeksema et al., 2012). Such heterospecific associations between a host and an associated organism can range from brief facultative encounters to lifelong obligate partnerships (Castro, 1988). Studies on the associated fauna of stony corals are often studied from a symbiont perspective. A ‘reversed approach’ aims to study associated fauna from the perspective of the host, by collecting specimens from as many host coral species as possible. This reversed approach has previously been applied to several endolithic and epibiotic mollusc taxa obligately associated with funguids, resulting in the discovery of several cryptic, adaptive radiations (Hoeksema and Kleemann, 2001; Kleemann and Hoeksema, 2002; Gittenberger and Gittenberger, 2005, 2010).

The reversed approach in the examination of a complete inventory of associated fauna was also used in the present study on fungiid associated gall crabs (Cryptochiridae). Gall crabs are obligate symbionts of scleractinians and reside in dwellings in their host coral. They occur worldwide but are most diverse in the Indo-West Pacific region (Fize and Serène, 1957; Kropp, 1990). Three gall crab species, Fungicola fagei (Fize and Serène, 1956), F. utinomi (Fize and Serène, 1956) and Dacryomaia sp., have so far been recorded from 32 mushroom corals (Fize and Serène, 1957; Takeda and Tamura, 1979; Kropp, 1990; Hoeksema et al., 2012; van der Meij and Hoeksema, 2013). The reversed approach for studying gall crab associations already yielded new coral hosts (Hoeksema et al., 2012; van der Meij and Hoeksema, 2013; van der Meij, 2014). Based on host relations and molecular analyses, a cryptic species

References

Castro, C.O. (1988). Heterospecific associations in symbiosis with funguids (Decapoda, Crypto-
Fig. 1. Types in the Nha Trang collections; A) dorsal view and B) ventral view of the female holotype of *Troglocarcinus fagei* Fize and Serène, 1956 (E.38.444), carapace 4.5 × 4.0 mm; C) dorsal view, D) marsupium, E) pereiopod and F) ventral view of the female holotype of *T. utinomi* Fize and Serène, 1956 (E.37.277), carapace 5.0 × 4.0 mm; G) dorsal view, H) pereiopods and I) ventral view of the male allotype of *T. utinomi* Fize and Serène, 1956 (E.37.766), carapace 4.0 × 3.0 mm. Photos by SET van der Meij and BT Reijnen.
closely related to *F. fagei* was discovered (van der Meij and Hoeksema, 2013) and described herein as *Fungicola syzygia* sp. nov. It is the third species assigned to the genus, and the fourth species recorded from mushroom corals.

**Material and methods**

Material was collected during fieldwork in Raja Ampat (Papua, Indonesia, Nov.-Dec. 2007), Bunaken National Marine Park (N Sulawesi, Indonesia, Dec. 2008), Ternate (Halmahera, Indonesia, Oct.-Nov. 2009), Semporna (E Sabah, Malaysia, Nov.-Dec. 2010), Lembeh Isl. (N Sulawesi, Indonesia, Jan.-Feb. 2012), and Kudat (N Sabah, Malaysia, Sep. 2012). A few additional samples were available from New Caledonia (2012), Payar Isl. (Malaysia, 2013), Loyalty Isl. (New Caledonia, 2014) and the Maldives (2014). Gall crabs were collected with their host coral and taken to the field station. After being photographed with a digital SLR camera with a 50 mm macro-lens, the crabs were preserved in 80% ethanol. The gall crabs are deposited in the collections of Naturalis Biodiversity Research, collection coded as ZRC). The mushroom coral identifications are based on revisions of the Fungiidae (Hoeksema, 1989; Gittenberger et al., 2011), all identifications were (confirmed) by Dr. Bert W. Hoeksema. Species authorities for the Fungiidae are provided in the list of material examined (Appendix).

Drawings were made with a stereo microscope with camera lucida. The chelipeds were drawn with the outer surface of the manus parallel to the plane of the paper, which somewhat distorts the other segments. Carapace lengths and widths were measured using an eyepiece micrometre. Abbreviations used: ovigerous, ovig.; MXP, maxillipeds; P, pereiopod; PLP, pleopod; CL, carapace length at midpoint; CW, carapace width at widest point. Carapace measurements are given as CL × CW.

**Nha Trang collections**

Fize and Serène (1956, 1957) described 16 new gall crab species in their monograph on the gall crabs of Vietnam and deposited the types in the museum of the Institute of Oceanography in Nha Trang (Vietnam). The Nha Trang collections were examined in October 2012 to search for the type material. Various specimens in the museum got displaced and subsequently lost during Vietnam’s turbulent history, however, seven out of the 16 Fize and Serène’s holotypes were located, including the types of *Troglocarcinus fagei* and *T. utinomi* (Fig. 1A-F). The allotype of *T. utinomi* was also located (Fig. 1G-I), but the vial that should have contained the allotype of *T. fagei* was empty, and this allotype is therefore considered lost. The list of material examined (see Appendix) includes all the *Fungicola* specimens that were located in the Nha Trang museum. Collection numbers were mentioned on labels on the outside of the collection jars and on labels inside the vials. In case of discrepancy, the information on the labels inside the vial was accepted as most likely the ‘correct’ information for the specimen(s) concerned. The locality data, ‘Rte’ (recolte) on the Vietnamese labels can be retrieved from Fize and Serène (1957).

Fize and Serène deposited duplicates of *Fungicola* specimens collected in Vietnam in the Muséum national d’Histoire naturelle (MNHN) in Paris, France. Additional specimens from Fize and Serène were unexpectedly encountered in the collections of the Natural History Museum (BMNH) in London, the United Kingdom. The details of this material are also included in the list of material examined.

**Molecular analyses**

For the molecular analyses specimens of *Fungicola fagei* and *F. utinomi* were included from many different host corals. *Utinomiella dimorpha* (Henderson, 1906) and *Pseudocryptochirus viridis* Hiro, 1938, associated with the coral genera *Pocillopora* and *Turbinaria*, respectively, were selected as outgroups because they belong to more distant clades within the monophyletic Cryptochiridae (van der Meij and Reijnen, 2014; van der Meij and Schubart, 2014).

Analyses of sequences from the mitochondrial cytochrome c oxidase subunit I gene (COI, partially, Folmer *et al.* (1994) and 16S mtDNA (16L2 and 16H10, Schubart (2009)) were used to infer phylogenetic relationships between the examined taxa. In addition, Histone H3 (H3) was used (Colgan *et al.*, 2000). The reverse primer was optimized for gall crabs (H3_R_SET: 5’-GCCGACMAGGTARGCCTCKG-3’).

DNA was isolated from the fifth pereiopod, using the QIAGEN DNeasy Kit according to the manufacturer’s protocol for animal tissue. Maceration took place
Fig. 2. Phylogeny reconstruction of the genus *Fungicola* based on a concatenated dataset of 16S and COI mtDNA and H3 nDNA of 50 taxa (including outgroups). Topology derived from Bayesian inference 50% majority rule, significance values are posterior probabilities. Numbers refers to collection numbers (RMNH.Crus.D, see material examined in the Appendix).
Sequences were aligned using ClustalW on the Guiding outgroups. Are included in the three marker dataset (n=50, edited in Sequencer 4.10.1. A total of 1520 base pairs were obtained were included in the analysis (n=40).

**Results**

**Molecular analyses**

The analysis in MrBayes resulted in a tree with high posterior probabilities. Based on the results of this analysis and host specificity data a cryptic species of *Fungicola* was revealed, closely related to *Fungicola fagei* (Fig. 2). This new species is described as *F. syzygia* sp. nov. (see Appendix). The concatenated 16S + COI mtDNA (Fig. S1) has the same topology as the three marker phylogeny reconstruction, the H3 gene tree (Fig. S2) recovers *F. utinomi* as monophyletic, but does not distinguish between *F. fagei* and *F. syzygia* sp. nov.

The evolutionary divergence over sequence pairs was estimated for *Fungicola fagei*, *F. syzygia* sp. nov. and *F. utinomi*. In this analysis, the groups were determined *a priori* based on Fig. 2. There is about a 6% difference between *F. fagei* and *F. syzygia* sp. nov. and an 11% difference between *F. fagei* and *F. utinomi*. *F. syzygia* sp. nov. and *F. utinomi* have a difference of 9.2% (Table 1). Table 2 shows the intraspecific difference within the three species, which ranges from 0.1 to 0.4%.

### Table 1. Estimates of evolutionary divergence based on 16S, COI and H3. The number of base substitutions per site averaging over all sequence pairs between groups determined *a priori* is shown below the diagonal. Standard error (SE) estimates are shown above the diagonal.

<table>
<thead>
<tr>
<th></th>
<th><em>F. fagei</em></th>
<th><em>F. syzygia</em> sp. nov.</th>
<th><em>F. utinomi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fungicola fagei</em> (n=10)</td>
<td>0.064</td>
<td>0.015</td>
<td>0.025</td>
</tr>
<tr>
<td><em>F. syzygia</em> sp. nov. (n=21)</td>
<td>0.110</td>
<td>0.092</td>
<td></td>
</tr>
<tr>
<td><em>F. utinomi</em> (n=9)</td>
<td>0.110</td>
<td>0.092</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Estimates of evolutionary divergence based on 16S, COI and H3. The number of base substitutions per site averaging over all sequence pairs within groups determined *a priori* and the standard error (SE) estimates are shown.

<table>
<thead>
<tr>
<th></th>
<th>d.</th>
<th>SE</th>
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<tbody>
<tr>
<td><em>Fungicola fagei</em> (n=10)</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td><em>F. syzygia</em> sp. nov. (n=21)</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td><em>F. utinomi</em> (n=9)</td>
<td>0.001</td>
<td>0.001</td>
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Host specificity

Different host occurrences for *Fungicola fagei* and its sibling species *F. syzygia* sp. nov. (Fig. 2, Table S3) were observed. *Fungicola fagei* occurs only in corals belonging to *Podabacia* or *Sandalolitha*. *Fungicola syzygia* sp. nov. is predominantly associated with *Cycloseris* and *Pleuractis*, with a few records from *Lithophyllum*. *Fungicola utinomi* is mostly found in association with species of *Danafungia*, *Fungia*, *Halomitra*, and *Lithophyllum*, but its most common host is *L. repanda*.

Discussion

Species having a small size or hidden habitat are often referred to as ‘cryptic’. Although gall crabs are small and live a hidden life inside their corals hosts and hence ‘cryptic’, cryptic species is herein referred to as two or more species that were previously classified as a single one because of (at least superficial) morphological similarities (Bickford *et al.*, 2007). This definition is applicable to *F. syzygia* sp. nov., which is difficult to separate from *F. fagei* based on morphology alone (see description in Appendix). DNA taxonomy is becoming an indispensable tool for unravelling cryptic speciation. This especially holds true for endosymbionts which have prolonged relationships with their hosts that can lead to morphological stasis (e.g. Gittenberger and Gittenberger, 2010).

*Fungicola syzygia* sp. nov. and *F. fagei* are clearly separated based on DNA and host specificity (Fig. 2). *Fungicola syzygia* sp. nov. and *F. fagei* are more closely related to each other than to *F. utinomi*. The intraspecific variation is low (Tables 1-2). The mitochondrial markers 16S and COI showed high resolution at species level (Fig. S1), contrary to the nuclear marker H3 (Fig. S2). The latter marker recovered *F. utinomi* as a monophyletic clade, except for RMNH. Crs.D.54246, which clustered basally to all other specimens. H3 did not distinguish between *F. fagei* and *F. syzygia* sp. nov. A study by Dinapoli *et al.* (2007) showed that H3 is most informative on genus level in heterobranch Gastropoda, making this marker a possible candidate for further reconstructions of the deeper phylogenetic relationships within the Cryptochiridae.

*Fungicola syzygia* sp. nov. and *F. fagei* can also be separated based on their host occurrence. The usual pattern among host-specific organisms is a common or ‘preferred’ host, with a number of other hosts less frequently inhabited (Norton and Carpenter, 1998). This seems to be the case in fungiid-associated gall crabs as well. The present results showed that *Fungicola fagei* was strictly associated with the sister genera *Podabacia* and *Sandalolitha*, with *P. crustacea* as the most common host. *Fungicola syzygia* sp. nov. is mostly associated with the genera *Cycloseris* and *Pleuractis*, with *P. granulosa* and *P. paumotensis* as most common hosts. *Fungicola utinomi* is associated with a wider range of related genera but is most often encountered in *Lithophyllum repanda* (Fize and Serène, 1957; van der Meij and Hoeksema, 2013; Fig. 2, Table S3). Hoeksema *et al.* (2012) mentioned that gall crabs are not very host specific, but at the time of writing, the new sibling species of *Fungicola fagei* was not yet discovered. It turns out that *Fungicola* species are specific to at least genus level. The specimens referred to as ‘cryptochirid sp.’ in Hoeksema *et al.* (2012) belonged to one of the, now four, known species associated with Fungiidae. The identifications of these specimens was hampered by being juvenile stages or needed back-up from molecular work.

Several fungiid species have so far not been found occupied by a gall crab, possibly because their coral morphology makes for an unsuitable habitat for gall crabs (e.g. long tentacles in *Heliofungia actiniformis* or very thin plate-like morphology in *Halomitra clavator* and *Zoopilus echinatus*). Other species have distributions outside the range in which the fieldwork was carried out and might therefore not yet have been found in association with gall crabs.

Kropp and Manning (1987) removed the concept of host specificity as a generic character for gall crabs, which was previously believed to be a reliable character to distinguish genera (Fize and Serène, 1957). Many changes have been recently made and proposed in scleractinian phylogeny and taxonomy, largely based on new insights coming from molecular data and microstructures (e.g. Gittenberger *et al.*, 2011; Benzoni *et al.*, 2012; Budd *et al.*, 2012; Arrigoni *et al.*, 2014; Huang *et al.*, 2014). The inconsistencies in the generic placement of the gall crabs based on host affinity, as observed by Kropp (1990), are therefore likely related, at least in part, to past inconsistencies in scleractinian taxonomy.

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for locating the French specimens of Fize and Serène, to Paul Clark (Natural History Museum, London, UK) for his help during my short visit, and to Karen Reed and Rafael Lemaître (National Museum of Natural History, Smithsonian Institution, Washington D.C., USA) for their assistance during a collection visit in 2009. Bert Hoeksema (Naturalis) collected cryptochirids from Fungiidae over the last years, many of which were used in this study. Charles Fransen (Naturalis) helped to search for morphological characters distinguishing *Fungicola fagei* and *F. syzygia* sp. nov. Chris Boyko (Dowling College and American Museum of Natural History) helped to identify the epicaridean parasite. Bastian Reijnen (Naturalis) is thanked for all his help during our visit to the Institute of Oceanography in Nha Trang, as well as help with photography and lab work. The beautiful line drawings in this manuscript were made by Inge van Noortwijk (Naturalis). The COI sequences were produced as part of the Naturalis Barcoding project. The fieldwork in Indonesia was organized by Naturalis and the Indonesian Institute of Sciences (LIPI), under the umbrella of Ekspedisi Widyay Niwasanta (E-Win). Fieldwork in Lembeh Strait in 2012 took place during a Marine Biodiversity Workshop based at the Bitung Field Station (LIPI), co-organized by Universitas Sam Ratulangi in Manado, N Sulawesi (Indonesia). I am grateful to LIPI and RISTEK for granting research permits. Bert Hoeksema (Naturalis) and Yosephine Tuti Hermanlimianto (RCO-LIPI) are acknowledged for all their efforts in organizing the various expeditions in Indonesia. The 2010 Semporna Marine Ecological Expedition was jointly organized by WWF-Malaysia, Universiti Malaysia Sabah’s Borneo Marine Research Institute, Universiti Malaya’s Institute of Biological Sciences and Naturalis, and was funded through WWF-Malaysia. The research permits for Malaysia were granted by the Economic Planning Unit, Prime Minister’s Department, Sabah Parks and Department of Fisheries Sabah. The Tun Mustapha Park Expedition (TMPE) 2012 was jointly organized by WWF-Malaysia, Universiti Malaysia Sabah (UMS), Sabah Parks and Naturalis. TMPE was funded by the Ministry of Science, Technology and Innovation (MOSTI) and USAID Coral Triangle Support Partnership (CTSP). The research permits were granted by the Economic Planning Unit, Prime Minister’s Department and Sabah Biodiversity Centre. Permits to sample from Payar Isl. were granted to Zarinah Waheed (Naturalis & Universiti Malaysia Sabah) by the Economic Planning Unit, Prime Minister’s Department Malaysia, and the Department of Marine Park Malaysia. Collecting in New Caledonia (2012) was done during the mission CORALCAL 4. Provinces Sud and Nord of New Caledonia provided sampling permits. Loyalty Island samples were collected during the BIBELOT campaign in 2014 onboard RV Alis of IRD at Nouméa. Sampling permits were granted by the Loyalty Islands Province, New Caledonia. For the samples from the Maldives the help of the University of Milano-Bicocca Marine Research and High Education Centre in Magoodhoo, the Ministry of Fisheries and Agriculture, Republic of Maldives and the community of Magoodhoo, Faafu Atoll is gratefully acknowledged. Funding for the several fieldwork trips was provided by the AM Buitendijksfonds, LB Holthusifonds, JJ ter Pelkikwifonds (all Naturalis), Schure-Beijerincck-Poppingfonds (KNAW), Stichting Fonds Dr C van Tussenbroek (N Ongerboerfonds), LUF International Study Fund (Leiden University) and the Van Tienhoven Foundation for International Nature Protection. Travel to the Smithsonian Institution was funded by a 2009 EDIT Women in Science Fellowship. I am grateful to Peter Castro and Roy Kropp for their constructive comments on an earlier version of this manuscript.

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Van der Meij – A cryptic new gall crab species from Fungiidae


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S1. Phylogeny reconstruction of the genus *Fungicola* based on 16S and COI mtDNA of 47 taxa (including outgroups). Topology derived from Bayesian inference 50% majority rule, significance values are posterior probabilities. Numbers refers to collection numbers (RMNH.Crus.D, see Table S3). Likelihood scores stabilized at a value of 0.008292.

S2. Phylogeny reconstruction of the genus *Fungicola* based on H3 nDNA of 45 taxa (including outgroups). Topology derived from Bayesian inference 50% majority rule, significance values are posterior probabilities. Numbers refers to collection numbers (RMNH.Crus.D, see Table S3). Likelihood scores stabilized at a value of 0.007425.

S3. List of material examined in Naturalis Biodiversity Center (Leiden, The Netherlands), including GenBank Accession numbers.
Fig. 3. *Fungicola utinomi* (RMNH.Crus.D 53229). A, habitus, dorsal view; B, anterolateral margin of carapace, ventral view; C, MXP3; D, left P1 (cheliped); E, left P2; F, left P3; G, left P4; H, left P5. Scale bars 1 mm, D-H share scale bar.
Appendix

Systematic account

Family Cryptochiridae Paulson, 1875
Genus Fungicola Serène, 1967

Fungicola.— Fize and Serène, 1957: 122 [name unavailable]
Fungicola Serène, 1967: 396
Fungicora.— Takeda and Tamura, 1986: 64 [erroneous spelling]

Type species. Troglocarcinus utinomi Fize and Serène, 1956: 377.

Diagnosis. Carapace rectangular to squaroid, longer than broad, widest anterior to midlength, flat in lateral view, not deflected anteriorly, surface covered with granules, mesogastric region slightly inflated, cardionicestinal region outlined; pterygostomial region fused to carapace; epistome with parallel lateral ridges; lateral lobe of antennule oval, extending slightly beyond eyestalk; antennal segment two longer than broad, distal margin lacking lateral spine; MXP3 with exopod, merus with small distolateral projection, mesial margin granulated, with setae; lateral lobe of antennule oval, extending slightly beyond eyestalk; dorsal surface in the shape of a spatula without much relief, anterior-lateral angle shaped as a large round lobe, rhomboid outline of the male abdomen, with the segments 3-5 enlarged. P1 cutting edges entire; P2 merus with distomesial projection; P3-4 coxae with well-developed anterior lobes; PLP3 of female uniramous; male abdomen triangle-shaped (adapted from Kropp, 1990).

Remarks. Fize and Serène (1957) described the subgenus Fungicola in the genus Troglocarcinus based on the following characteristics: 1) flat carapace on the dorso-ventral side, 2) flat dorsal surface in the shape of a spatula without much relief, 3) anterior-lateral angle shaped as a large round lobe, 4) rhomboid outline of the male abdomen, with the segments 3-5 enlarged.

Fize and Serène unfortunately did not designate a type species. Serène (1967) elevated the subgenus to genus level and designated T. utinomi as the type species. Species in the genus Fungicola are only known to occur in association with fungiid corals.

Troglocarcinus (Fungicola) utinomi.— Fize and Serène, 1957: 124
Fungicola utinomi.— Serène, 1967: 396 [unjustified emendation]
Pseudocryptochirus ishigakiensis Takeda and Tamura, 1979: 188
Hiroia ishigakiensis.— Takeda and Tamura, 1981: 20

Type locality. Nha Trang, Vietnam
Holotype. E.37.277 (‘Type’ according to Fize and Serène, 1957) in the Institute of Oceanography in Nha Trang, Vietnam; allotype: E.37.766 (Fig. 2C-I), collected from Fungia fungites (Linnaeus, 1758).


Fungicola utinomi (Fize and Serène, 1956)
Figs 1C-I, 3A-H

Troglocarcinus utinomi Fize and Serène, 1956: 377, fig. 2E
Fig. 4. *Fungicola fagei* (RMNH.Crus.D 53234). A, habitus, dorsal view; B, anterolateral margin of carapace, ventral view; C, MXP3; D, right P1 (cheliped); E, left P1 (cheliped); F, left P2; G, left P3; H, left P4; I, left P5. Scale bars 1 mm, B, D-I share a scale bar.
E.39.205 (Rte 1641) but no vial. Muséum national d’Histoire naturelle (Paris, France): MNHN-IU-2014-10108 (was E.38.333 (Rte 1614)), good condition, 1 ovig. f, on Fungia [= Lithophyllum] repanda; MNHN-IU-2014-10107 (was E.38.425), good condition, 1 m, on Fungia [= Lithophyllum] repanda. Natural History Museum (London, UK): BMNH.1958.1020.17-18 contains: E.39.336 (Rte 1644), good condition, 1 ovig. f, on Fungia sp.; E.39.337 (Rte 1644), good condition, 1 m, on Fungia sp. Naturalis Biodiversity Center (Leiden, The Netherlands): see Table S3.

Diagnosis. Carapace rectangular, longer than broad, depressed, anterior half broader than posterior half, surface covered with small, low granules in posterior half, larger and rather spiniform in anterior; median gastric region moderately convex and its posterior part only indistinctly separated from cardio-intestinal region, branchial regions hardly separated from both, mid-gastric, cardio-intestinal regions by very shallow furrows; front moderately concave, armed with spines of different size; internal orbital nearly reaching level of external; antero-lateral borders armed with spines and forming a convex lobe, lateral borders behind it straight or slightly converging. Cheliped slender; upper and external faces of merus, carpus and palm covered with fine granules. Upper face of coxae of pereiopods 3-4 with small forward protruding lobe. Carapace with striking brown, white patterns (visible in ethanol), pereiopods banded. For a full description see Fize and Serène (1957); for a colour figure of F. utinomi see Van der Meij and Schubart (2014: Fig. 1).

Host corals. Fungicola utinomi inhabits at least nine coral species belonging to eight genera, but is most often recorded from Lithophyllum repanda (Fig. 2, Table S3). In L. repanda, F. utinomi lives in an oval cavity with overhanging canopy, which can be raised above the coral surface (see Fize and Serène, 1957: Pl. XIII A-C; Takeda and Tamura, 1979: Pl. 1A-D, Pl 2A-D).

Distribution range. Fungicola utinomi has so far been documented from Nha Trang, Vietnam (Fize and Serène, 1957); Maluku, Indonesia (Rumphius I expedition, Serène et al., 1974); Semporna, Malaysia (van der Meij and Hoeksema, 2013); Ishigaki-jima and Kuroshima, Okinawa, Japan (Takeda and Tamura, 1979); and Palau and Guam in Micronesia (Kropp, 1990). New records from this study include Raja Ampat, Manado, Lembeh and Ternate in Indonesia, and Payar Isl. and Kudat in Malaysia.

Remarks. According to Kropp (1990) the location of the holotype is unknown; however, the holotype and the allotype were located in the Institute of Oceanography in Nha Trang (Fig. 2C-F).

Fungicola fagei (Fize and Serène, 1956)
Figs 1A-B, 4A-I, 7F-G

Troglocarcinus fagei Fize and Serène, 1956: 378, fig. 2F
Troglocarcinus (Fungicola) fagei.— Fize and Serène 1957: 131

Type locality. Nha Trang, Vietnam
Holotype. E.38.444 (‘Type’ according to Fize and Serène, 1957) in the Institute of Oceanography in Nha Trang, Vietnam (Fig. 2A-B); allotype: E.37.727, collected from Sandalolitha dentata Quelch, 1884 (but see section on host corals).


Diagnosis. Carapace subrectangular, longer than broad, depressed, anterior half broader than posterior half, surface covered with fine granules; median gastric region feebly convex, its posterior part not separated from cardio-intestinal and branchial regions by very shallow furrows; front moderately concave; internal orbital angle falling much shorter than external; antero-lateral borders armed with spines, moderately convex, lateral borders behind it slightly concave. Cheliped not
Fig. 5. Holotype *Fungicola syzygia* sp. nov. (RMNH.Crus.D.53220). A, habitus, dorsal view; B, anterolateral margin of carapace, ventral view; C, MXP3; D, left P1 (cheliped); E, left P2; F, left P3; G, left P4; H, left P5. Scale bars 1 mm, B, D-H share scale bar.
stout; merus as well as upper borders of carpus, palm covered with fine granules. Upper face of coxae of third to fifth pereiopods with small forward protruding lobe. Carapace, pereiopods a dull beige-grey, semi-translucent. For a complete description of *F. fagei* see Fize and Serène (1957).

**Host corals.** *Fungicola fagei* can be found inhabiting flattened pits lodged between septae (see Fize and Serène, 1957: Pl. XIII D-F). According to Fize and Serène (1957) *Fungicola fagei* is associated with *Parahalomitra robusta* (= *Sandalolititha robusta*). Based on Pl. XIII, fig. D-E in Fize and Serène (1957), however, this coral species should instead be identified as *Sandalolitha dentata* (B.W. Hoeksema, pers. comm.). The host genera of *F. fagei* are the attached *Podabacia* and the free-living *Sandalolitha*, two closely related genera. *Podabacia crustacea* is the most common host, and *P. sinai* is a new host for *F. fagei* (Table S3).

**Distribution range.** *Fungicola fagei* has is so far known from Nha Trang, Vietnam (Fize and Serène, 1957); Maluku, Indonesia (Serène et al., 1974); Semporna, Malaysia (van der Meij and Hoeksema, 2013); and Palau and Guam in Micronesia (Kropp, 1990). New records herein are New Caledonia and Raja Ampat and Ternate in Indonesia. A record of *F. fagei* from Japan by Takeda and Tamura (1979), collected from *Fungia paumotensis* (see remarks in the species description).

**Remarks.** Kropp (1990) stated that the location of the holotype was unknown; however, the type was located in the Institute of Oceanography in Nha Trang and is in relatively good condition. The allotype was not found and is considered lost. Out of the other material mentioned by Fize and Serène only sample E. 39.210 was located, samples E. 37.458, E.37.471, E.37.727, E.37.729, E.38.334 and E.39.211-215 appear to be lost.

**Type material.** Naturalis Biodiversity Center (Leiden, The Netherlands). Holotype: RMNH. Crus.D.53220, host *Pleuraactis granulosa* (Klunzinger, 1879), 26.x.2009, 1 ovig. female (4.0 × 3.8), leg. SET van der Meij / BW Hoeksema (the holotype will be deposited in the Museum Zoologicum Bogoriense, Bogor, Indonesia with catalogue number MZB Cru 4130); allotype: RMNH.Crus.D.53224 (male), Tanjung Pasir Putih, Ternate, Indonesia (N 00°51’50”, E 127°20’36”), host *Pleuraactis granulosa* (Klunzinger, 1879), 02. xi.2009, 1 male (2.5 × 2.3), leg. SET van der Meij; para-types: RMNH.Crus.D. 53225, Tanjung Ratemu (S of river), Ternate, Indonesia (N 00°54’24" E 127°29’17"), host *Pleuraactis granulosa* (Klunzinger, 1879), 05. xi.2009, 1 non-ovig. female (4.3 × 4.3), leg. SET van der Meij; RMNH.Crus.D.53226, Pilongga N, Tidore, Indonesia (N 00°42’49” E 127°28’45”), host *Pleuraactis granulosa* (Klunzinger, 1879), 12.xi.2009, 1 ovig. female (5.1 × 4.6), leg. SET van der Meij; Lee Kong Chian Natural History Museum (Singapore). ZRC 2015.006 (ex. RMNH.Crus.D.53219), Sulamadaha Bay, Ternate, Indonesia (N 00°51’58”, E 127°19’53”), host *Pleuraactis granulosa* (Klunzinger, 1879), 26.x.2009, 1 ovig. female (5.3 × 5.3), leg. BW Hoeksema.

**Material examined.** Naturalis Biodiversity Center (Leiden, The Netherlands): see Table S3.

**Description of holotype.** Carapace (Fig. 5A) subrectangular to squaroid, longer than broad, anterior half rounded, broader than posterior half, surface covered with fine granules; flat in lateral view, not deflected anteriorly; cardio-intestinal region outlined; sharp internal orbital angle; frontal margin, anterolateral border moderately convex; lateral borders not clearly defined, somewhat concave. Pterygostomial region fused to carapace.

Ocular peduncles (Fig. 5B) granulated on distal margin, cornea round to oval, longer than broad; antennule same length as ocular peduncles; antennal segment 2.5 times longer than broad, extending beyond eyestalk, distal margin with several lateral spines.

MPX3 (Fig. 5C) exopod subrectangular, reaching approx. ½ length of ischium, with tubercles and setae; ischium subquadrangular, smooth, mesial and distal margin slightly rounded, anteromesial lobe with setae, distal margin with tubercles; merus with distolateral projection, anterolateral margin of merus with tubercles and setae; distal portion of carpus with tubercles and setae; dactylus with bundle of long setae.
Fig. 6. Allotype *Fungicola syzygia* sp. nov. (RMNH.Crus.D.53224). A, habitus, dorsal view; B, anterolateral margin of carapace, ventral view; C, MXP3; D, abdomen; E, gonopod; F, thoracic sternites; G, left P1 (cheliped); H, left P2; I, left P3; J, left P4; K, left P5. Scale bars 1 mm, B, G-K as well as C-D and E-F share scale bar.
P1 (chelipeds, Fig. 5D) slender; ischium length ½ height; merus length twice height with scattered small tubercles and few short setae; carpus with pronounced granules on distal margin, propodus with granules on distal margin, fingers slender, mesial surfaces of fingers smooth, cutting edge entire.

P2 (Fig. 5E) coarser than P1; ischium without setae; merus stout, two times longer than broad, few, small conical tubercles on distal half of dorsal surface, simple short setae on lateral, dorsal surface; carpus surface granulated, with setae; propodus about as long as carpus, surface granulated, fine scattered setae, dactylus smooth, sharp, curved ventrally.

P3 (Fig. 5F) ischium without setae; merus stout and rounded, prominent distomesial projection, surface with tubercles, simple setae; carpus not extending more than at right angle; carpus bend; carpus, propodus roughly of equal length, rounded tubercles on dorsal surface, simple setae on lateral, dorsal surface; dactylus half-length of propodus, smooth, sharp, curved ventrally.

P4 (Fig. 5G) ischium without setae; merus stout and rounded, small distomesial projection, surface with tubercles and simple setae; carpus not extending more than at right angle; carpus bend; carpus, propodus roughly of equal length, rounded tubercles on dorsal surface, simple setae on lateral, dorsal surface; dactylus half-length of propodus, smooth, sharp, curved ventrally, with setae.

P5 (Fig. 5H) slender; ischium without setae; merus, carpus, propodus of equal length; merus slightly bend, very few scattered tubercles and setae, carpus, propodus with sharp tubercles on dorsal surface; dactylus ⅓ length of propodus, smooth, sharp, straight, with few setae. P-5 right sampled for DNA analysis.

Abdomen (= pleon) enlarged, lateral margin fringed with setae (Fig. 5A-B).

Description allotype. Carapace (Fig. 6A) subrectangular, slightly longer than broad, anterior half broader than posterior half, surface covered with fine granules; flat in lateral view, not deflected anteriorly; cardio-intestinal region outlined; internal orbital angle rounded; frontal margin and anterolateral borders armed with spinules, few setae; anterolateral border moderately convex; lateral borders not clearly defined, somewhat concave. Pterygostomial region fused to carapace.

Ocular peduncles (Fig. 6A-B) with small spines on distal margin, cornea round to oval, longer than broad; antennule shorter than ocular peduncles; antennal segment two longer than broad, slightly extending beyond eyestalk, distal margin with several lateral spines.

MXP3 (Fig. 6C) exopod subrectangular, reaching approx. ½ length of ischium, with tubercles, setae; ischium subquadangular, smooth, mesial and distal margin slightly rounded, anteromesial lobe with setae, distal margin with tubercles; merus with distolateral projection, anterolateral margin of merus with tubercles, setae; distal portion of carpus with tubercles, setae; dactylus with bundle of long setae.

P1 (chelipeds, Fig. 6G) slender; ischium length ½ height; merus with scattered small tubercles, few simple setae; carpus, propodus with pronounced granules on distal margin, fingers slender, mesial surfaces of fingers smooth, cutting edge entire.

P2 (Fig. 6H) ischium without setae; merus stout, two times longer than broad, few, small conical tubercles on distal half of dorsal surface, simple short setae on lateral, dorsal surface; carpus surface granulated, with setae; propodus about as long as carpus, surface granulated, fine scattered setae, dactylus smooth, sharp, curved ventrally.

P3 (Fig. 6I) ischium without setae; merus surface with tubercles and setae; joint between merus, carpus not extending more than at right angle; carpus and propodus roughly of equal length, conical tubercles on spread over surface, with setae; dactylus smooth, sharp, curved ventrally.

P4 (Fig. 6J) similar to P3; ischium without setae; merus stout with distomesial projection, surface with tubercles and simple setae; joint between merus, carpus not extending more than at right angle; carpus and propodus roughly of equal length, conical tubercles on spread over surface, with setae; dactylus smooth, sharp, curved ventrally.

P5 (Fig. 6K) slender; ischium without setae; merus length twice height; carpus, propodus of equal length; merus, carpus, propodus with few scattered tubercles and setae; dactylus ½ length of propodus, smooth, sharp, curved ventrally.

Abdomen rhomboid, longest and widest at 5th segment; telson rounded (Fig. 6D).

Gonopod slightly curved laterally, broad shoulder, apex rounded, long setae. (Fig. 6E)

Colour. An overall pale beige-grey. On some specimens a horseshoe shaped pattern is visible on the carapace, more pronounced in males than in females (Fig. 7A-E). A similar pattern can occur in specimens of F. fagei, hence colour pattern is not a diagnostic character.

Host corals. Fungicola syzygia can be found inhabiting flattened pits lodged between septae (see Vehof et al.,
in press: Fig. 1A). Main host genera are *Cycloseris* and *Pleuractis*, but there are single records from other species (Fig. 2, Table S3). *Fungicola syzygia* sp. nov. (RMNH. Crus.D.54244) was found once in *Lithophyllum repanda*, the most common host of *F. utinomi* (Fig. 2). This is the fourth species recorded from mushroom corals.

**Diagnostic characters.** *Fungicola fagei* and *F. syzygia* sp. nov. can be separated based on their host specificity and DNA. For the latter, based on the results of this study, a positive identification can be achieved by analysis of a single mitochondrial marker. *F. fagei* and *F. syzygia* sp. nov. are more difficult to separate based on morphology. Both species show considerable intraspecific variation in the shape (angle) of the external orbital angle and the border between the orbital angles. Intraspecific variation is also observed on the lateral margins. In general, in *F. syzygia* sp. nov. the lateral margins are more concave than in *F. fagei*. Based on the carapace length / carapace width ratio, it is clear that *F. fagei* is more rectangular than *F. syzygia* sp. nov., which is almost squaroid. The one available male of *F. fagei* is somewhat of an outlier, perhaps the results of sexual dimorphism. After removal of this single male from the CL / CW (all) category the mean ratio becomes 1.10 ± 0.08 (n=9) (Table 3).

**Distribution range.** *Fungicola syzygia* is so far known from various locations in Indonesia, Malaysia and the Maldives. The male specimens of Takeda and Tamura (1979) may belong to the new species *F. syzygia* sp. nov. (see remarks), which would add Japan to the known distribution range if the identification were confirmed. The figured specimen in Ng *et al.* (2008) was collected in Vanuatu (see remarks). The distribution of *F. syzygia* sp. nov. possibly overlaps the distribution of their host corals (for fungiid distributions see Hoeksema (1989)), but large areas of their host’s distribution have not yet been examined for gall crabs.

**Remarks.** A sibling species is a cryptic sister species: two species that are the closest relative of each other and have not been distinguished from one another taxonomically (Bickford *et al.*, 2007). *Fungicola syzygia* sp. nov. is therefore a sibling species of *F. fagei*. It is the third species assigned to the genus *Fungicola*.

Previous workers most likely did not encounter this new species in their material. Fize and Serène (1957) collected *F. fagei* specimens from *S. dentata* (and possibly *S. robusta*; see host corals section of *F. fagei*), and did not collect gall crabs from *Cycloseris* or *Pleuractis*. Their material therefore most likely consists of solely *F. fagei*. Specimens of *F. fagei* were furthermore collected during the Rumphius I and II expeditions to Indonesia. Kropp (1994) re-examined the Rumphius material, but mentioned that the specimens of *F. fagei* were no longer available and that the host corals were not included. Takeda and Tamura (1979) did collect two male specimens, which, based on the host specificity data (*Funga paumotensis* [= *Pleuractis paumotensis*]) and the illustrations, possibly could be identified as *Fungicola syzygia* (National Museum of Nature and Science Tokyo, NSMT-Cr. 5896/7). Ng *et al.* (2008: Fig. 156) illustrated a specimen of *Fungicola* sp., collected from *P. paumotensis* (B.W. Hoeksema, pers. comm.) in Santo, Vanuatu. *Pleuractis paumotensis* is only known to host *Fungicola syzygia*, therefore this material is tentatively included in the synonymy of this species.

**Etymology.** *Syzygia* in reference to the type locality Ternate, once the world’s major producer of cloves (*Syzygium aromaticum* L. Merr. and Perry). The Latin *syzygia* is derived from the ancient Greek συζυγία (syzugia), for a pair of related things or union, referring to the obligate symbiosis between the gall crab and its host coral.

### Table 3. Characters distinguishing *Fungicola fagei* and *F. syzygia* sp. nov.

<table>
<thead>
<tr>
<th>Character</th>
<th><em>F. fagei</em></th>
<th><em>F. syzygia</em> sp. nov.</th>
</tr>
</thead>
<tbody>
<tr>
<td>host genus</td>
<td>Podabacia, <em>Sandalolitha</em></td>
<td><em>Cycloseris</em>, <em>Pleuractis</em></td>
</tr>
<tr>
<td>carapace shape</td>
<td>subrectangular</td>
<td>subrectangular to squaroid</td>
</tr>
<tr>
<td>CL / CW (all)</td>
<td>1.12 ± 0.10 (n=10)</td>
<td>1.02 ± 0.07 (n=20)</td>
</tr>
<tr>
<td>CL / CW (ovig. f)</td>
<td>1.13 ± 0.06 (n=7)</td>
<td>1.02 ± 0.08 (n=11)</td>
</tr>
<tr>
<td>CL / CW (m)</td>
<td>1.33 (n=1)</td>
<td>1.02 ± 0.03 (n=5)</td>
</tr>
<tr>
<td>border between orbital angles (f)</td>
<td>(mildly) undulating somewhat defined, less concave than <em>F. syzygia</em> sp. nov.</td>
<td>straight to mildly undulating not well-defined, more concave than <em>F. fagei</em></td>
</tr>
<tr>
<td>lateral margin carapace</td>
<td>modest distolateral projection</td>
<td>pronounced distolateral projection</td>
</tr>
<tr>
<td>merus MXP3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>