Evolutionary diversification of coral-dwelling gall crabs (Cryptochiridae)
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Chapter 5

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

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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.

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 fungiids (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 fungiids (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 fungiids, 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, 1990a). 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, 1990a; 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, 2014a). Based on host relations and molecular analyses, a cryptic species 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 Center in Leiden, the Netherlands (formerly Rijksmuseum van Natuurlijke Historie, collection coded as RMNH.Crus.D), and a paratype of *Fungicola syzygia* sp. nov. is deposited in the Lee Kong Chian Natural History Museum in Singapore (formerly Raffles Museum of 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.

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.
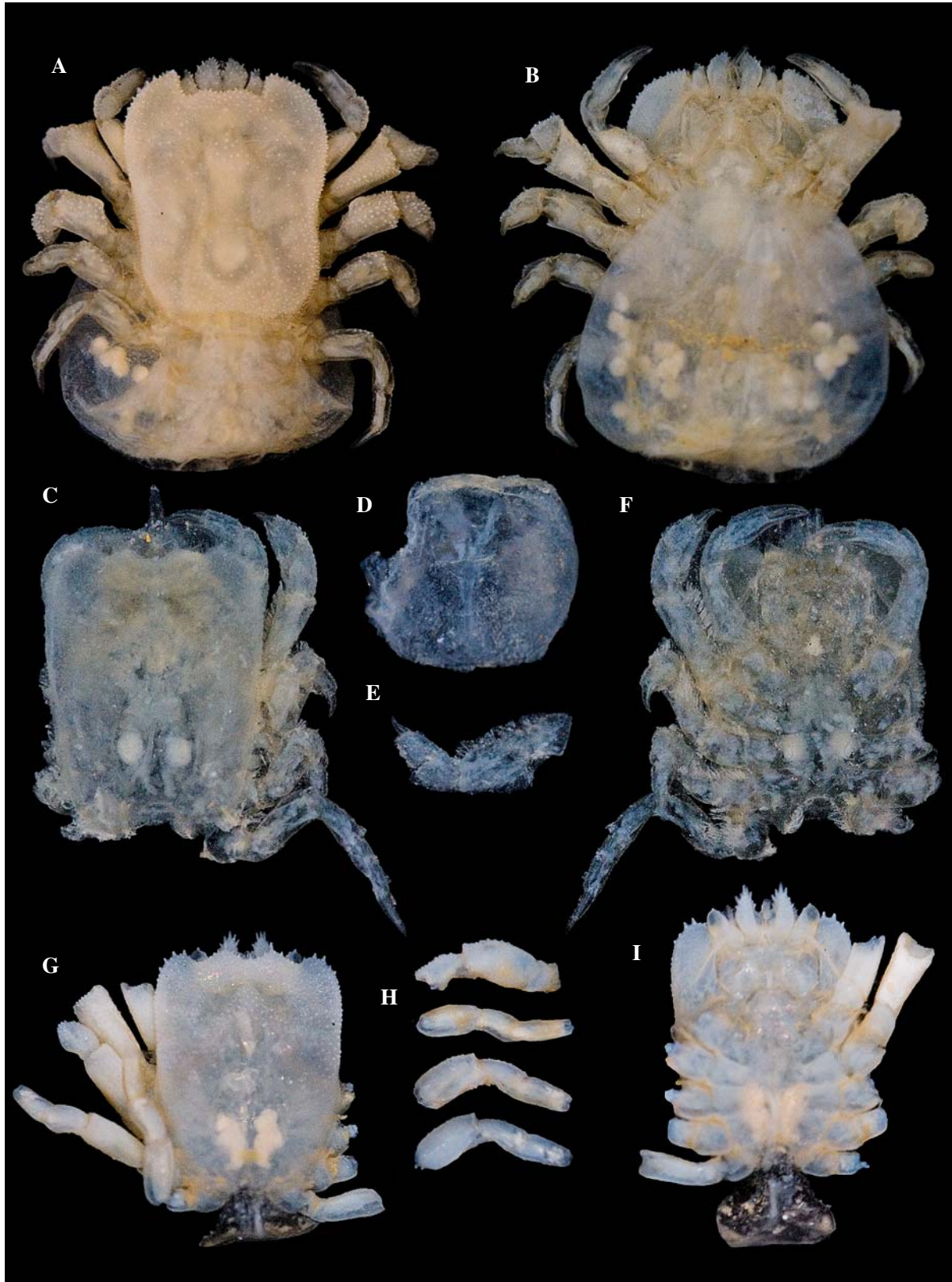
Fig. 1. Types in the Nha Trang collections; **A**, dorsal view and **B**, ventral view of the female holotype of *Troglocarcinus fagei* Fize and Serène, 1956 (E.38.444), carapace 4.5 × 4.0 mm; **C**, dorsal view, **D**, marsupium, **E**, pereiopod and **F**, ventral view of the female holotype of *T. 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.

Abbreviations used: ovigerous, ovig.; MXP, maxilliped; 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 (1956a, b, 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 lists of material examined 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'-GC-CGACMAGGTARGCCTCKG-'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 overnight for approximately 18 hrs. The final elution step was performed with 100 µl elution buffer. Polymerase chain reaction was carried out with standard PCR conditions (2.5 µl PCR buffer, 0.5 µl DNTP's, 1.0 µl primer FW, 1.0 µl primer RV, 0.3 µl Taq, 18.7 µl MilliQ and 1.0 µl DNA template). Thermal cycling for COI and 16S was as follows: initial denaturation at 95°C for five minutes, followed by 39 cycles of 95°C for five seconds, 47°C for one minute, and 72°C for one minute. This was followed by 10 minutes at 72°C. For H3 the standard PCR conditions were adjusted to 13.7 µl MilliQ and 5.0 µl Q-solution. Thermal cycling was as follows: initial denaturation at 95°C for three minutes, followed by 39 cycles of 95°C for ten seconds, 60°C for one minute, and 72°C for one minute. This was followed by 5 minutes at 72°C. Sequences were assembled and edited in Sequencer 4.10.1. A total of 1520 base pairs are included in the three marker dataset (n=50, including outgroups).

Sequences were aligned using ClustalW on the Guidance server (Penn *et al*., 2010), resulting in an alignment score of 0.991066. Prior to the model-based phylogenetic analysis, the best-fit model of nucleotide substitution was identified for each gene partition by means of the Akaike Information Criterion (AIC) calculated with jModeltest (Posada, 2008), resulting in GTR+I for 16S, GTR+G for COI and TIM2+G for H3. Because of the unavailability of TIM2 in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), I used the most complex GTR+G model of nucleotide substitution for H3. Bayesian inferences coupled with Markov chain Monte Carlo techniques (six chains) were run for 3,000,000 generations on the partitioned concatenated dataset, with a sample tree saved every 100 generations and the burnin set to 25%. Likelihood scores stabilized at a value of 0.005774. Consensus trees were visualized in FigTree v.1.3.1 (Rambaut, 2009). In addition, gene trees (H3 and concatenated 16S+COI) were constructed with the same parameters as for the concatenated three marker dataset (see online supplementary material Figs S1 and S2).

An analysis was also conducted in MEGA 6.06 (Tamura *et al*., 2013) to estimate the evolutionary divergence over sequence pairs between *a priori* determined groups (MCL model, 1000 bootstraps). Only specimens for which sequences for all three markers were obtained were included in the analysis (n=40).

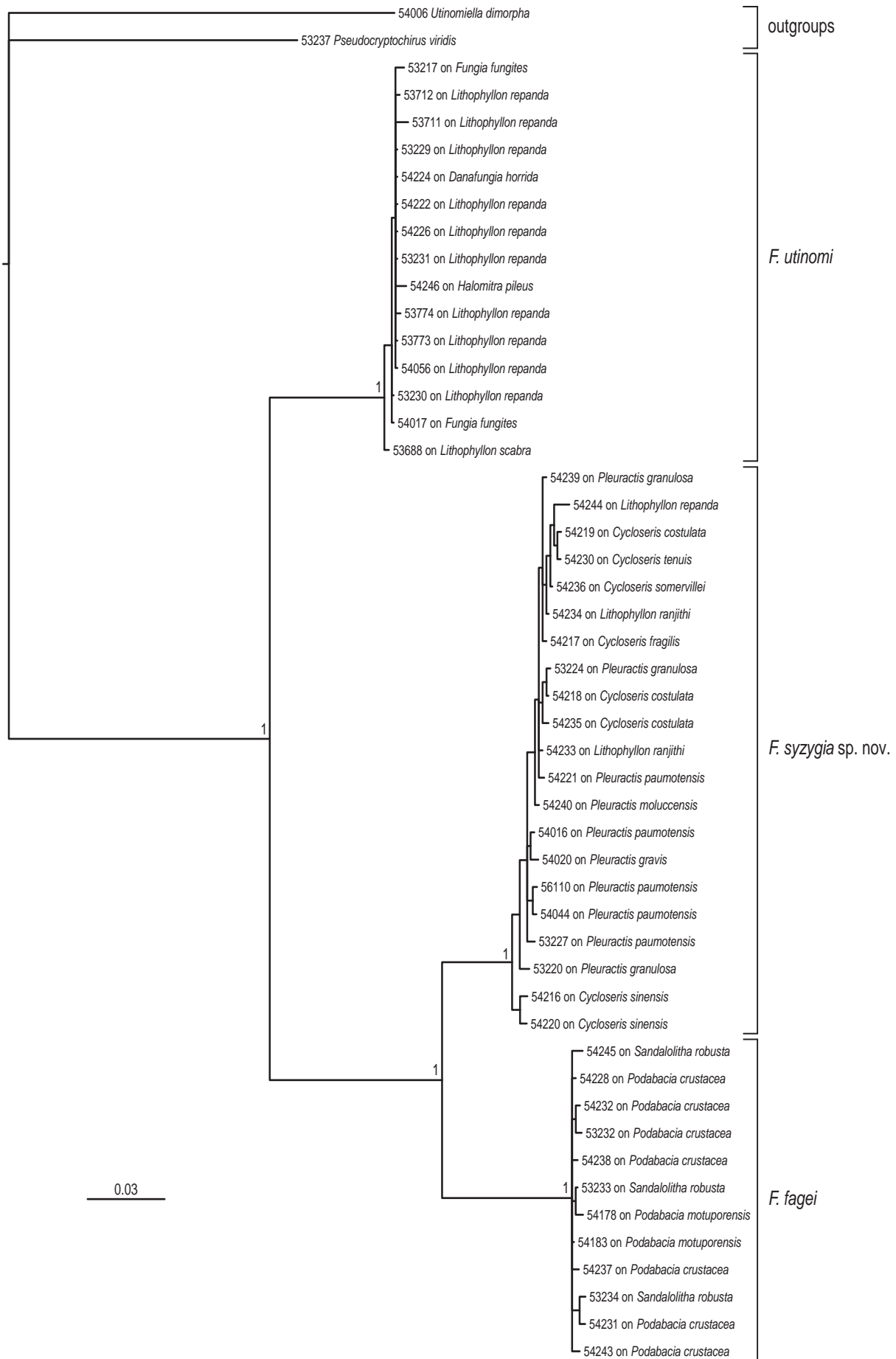## Results

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. 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.

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.

	F. fagei	*F. syzygia* sp. nov.	*F. utinomi*
Fungicola fagei (n=10)		0.015	0.025
F. syzygia sp. nov. (n=21)	0.064		0.022
F. utinomi (n=9)	0.110	0.092	

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.

	d.	SE
Fungicola fagei (n=10)	0.003	0.001
F. syzygia sp. nov. (n=21)	0.004	0.001
F. utinomi (n=9)	0.001	0.001

54006 *Utinomiella dimorpha*
53237 *Pseudocryptochirus viridis*
] outgroups

53217 on *Fungia fungites*
53712 on *Lithophyllon repanda*
53711 on *Lithophyllon repanda*
53229 on *Lithophyllon repanda*
54224 on *Danafungia horrida*
54222 on *Lithophyllon repanda*
54226 on *Lithophyllon repanda*
53231 on *Lithophyllon repanda*
54246 on *Halomitra pileus*
53774 on *Lithophyllon repanda*
53773 on *Lithophyllon repanda*
54056 on *Lithophyllon repanda*
53230 on *Lithophyllon repanda*
54017 on *Fungia fungites*
53688 on *Lithophyllon scabra*

F. utinomi

1

54239 on *Pleuractis granulosa*
54244 on *Lithophyllon repanda*
54219 on *Cycloseris costulata*
54230 on *Cycloseris tenuis*
54236 on *Cycloseris somervillei*
54234 on *Lithophyllon ranjithi*
54217 on *Cycloseris fragilis*
53224 on *Pleuractis granulosa*
54218 on *Cycloseris costulata*
54235 on *Cycloseris costulata*
54233 on *Lithophyllon ranjithi*
54221 on *Pleuractis paumotensis*
54240 on *Pleuractis moluccensis*
54016 on *Pleuractis paumotensis*
54020 on *Pleuractis gravis*
56110 on *Pleuractis paumotensis*
54044 on *Pleuractis paumotensis*
53227 on *Pleuractis paumotensis*
53220 on *Pleuractis granulosa*
54216 on *Cycloseris sinensis*
54220 on *Cycloseris sinensis*

F. syzygia sp. nov.

1

1

54245 on *Sandalolitha robusta*
54228 on *Podabacia crustacea*
54232 on *Podabacia crustacea*
53232 on *Podabacia crustacea*
54238 on *Podabacia crustacea*
53233 on *Sandalolitha robusta*
54178 on *Podabacia motuporensis*
54183 on *Podabacia motuporensis*
54237 on *Podabacia crustacea*
53234 on *Sandalolitha robusta*
54231 on *Podabacia crustacea*
54243 on *Podabacia crustacea*

F. fagei

1

1

0.03

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%.

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 *Lithophyllon*. *Fungicola utinomi* is mostly found in association with species of *Danafungia*, *Fungia*, *Halomitra*, and *Lithophyllon*, but its most common host is *L. repanda*.

Systematic account
Family Cryptochiridae Paul'son, 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, cardiointestinal 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; ventral thorax longer than broad; 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, 1990a).

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.

◀ **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 refer to collection numbers (RMNH. Crus.D).
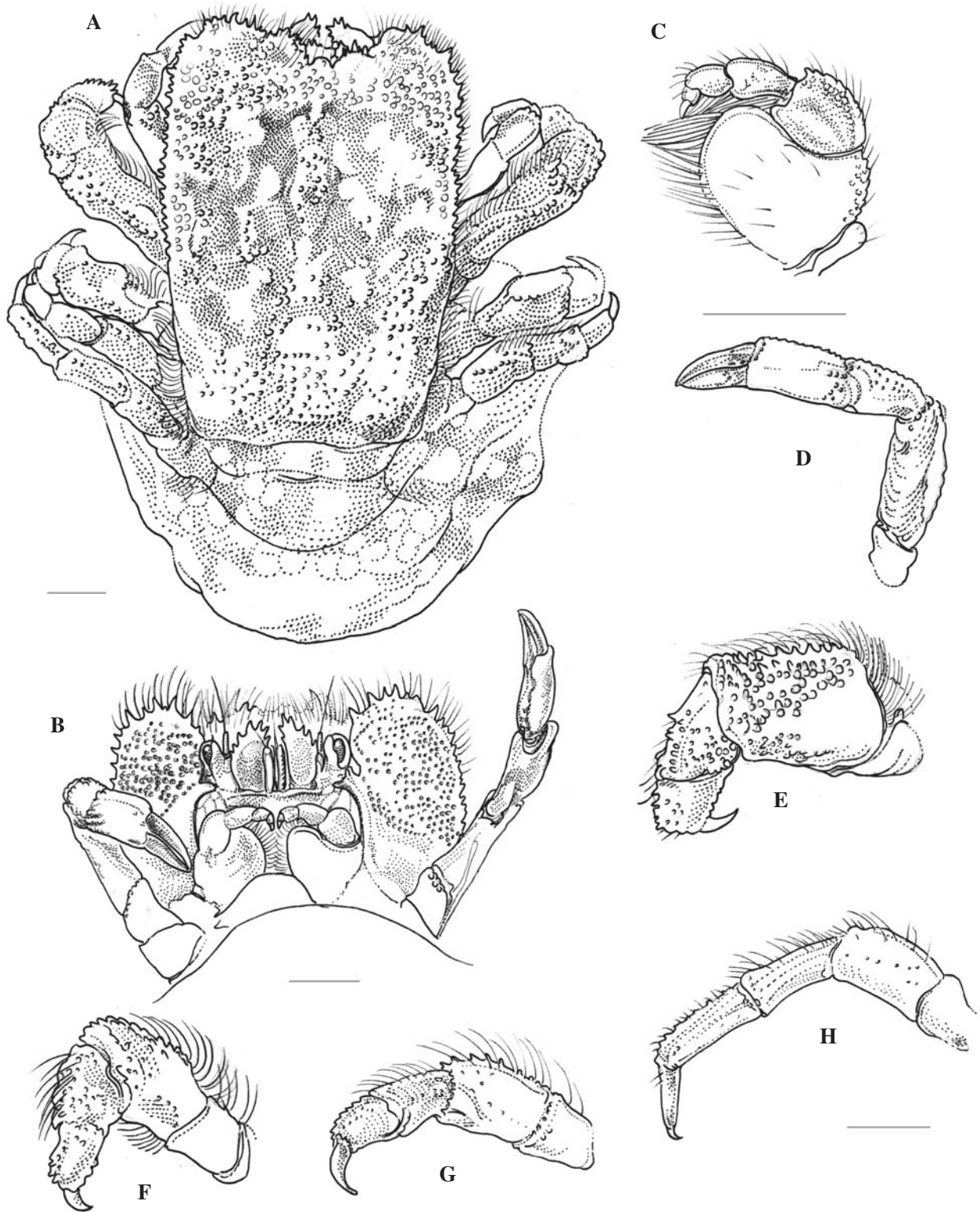
Fig. 3. *Fungicola utinomi* (RMNH.Crus.D.53229). **A**, habitus, dorsal view; **B**, anterolateral margin of carapace, ventral view; **C**, MXP3; **D**, left P1 (cheliped); **E**, left P2; **F**, left P3; **G**, left P4; **H**, left P5. Scale bars 1 mm, D-H share scale bar.

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

Troglocarcinus utinomi Fize and Serène, 1956: 377, fig. 2E
Troglocarcinus (*Fungicola*) *utinomi*.— Fize and Serène, 1957: 124
Fungicola utinomii.— 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 Ocean-ography in Nha Trang, Vietnam; allotype: E.37.766 (Fig. 2C-I), collected from *Fungia fungites* (Linnaeus, 1758).

    Material examined. Institute of Oceanography (Nha Trang, Vietnam): label E.37.277 (Rte 1560), in jar E.37.277, poor condition, **holotype**, 1 ovig. f; label E.37.183 (Rte 1555), in jar E.37.183 (Rte 1555) – E.37.776 (Rte 1569), good condition; label E.37.184 (Rte 1555), in jar E.37.183 (Rte 1555) – E.37.776 (1569), empty vial; label E.37.271 (Rte 1560), in jar E.37.898-38.276 (Rte 1589), residue; label E.37.272 (Rte 1560), in jar E.37.898- 38.276 (Rte 1589), empty vial; label E.37.275 (Rte 1560), in jar E.37.898-38.276 (Rte 1589), residue, on *Fungia* sp.; label E.37.276 (Rte 1560), in jar E.37.183 (Rte 1555) – E.37.776 (Rte 1569), reasonable condition; label E.37.283 (Rte 1560), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.37.554 (Rte 1569), in jar E.37.183 (Rte 1555) – E.37.776 (Rte 1569), reasonable condition; label E.37.672 (Rte 1579), in jar E.39.041 (1635) - E.39.217 (1641), fair/reasonable condition; label E.37.673 (Rte 1579), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.37.763 (Rte 1582), in jar E.39.041 (1635) - E.39.217 (1641), translucent but fair condition; label E.37.764 (Rte 1582), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.37.765 (Rte 1582), in jar E.39.041 (1635) - E.39.217 (1641), fair condition; label E.37.766 (Rte 1569), in jar E.37.183 (Rte 1555) – E.37.776 (Rte 1569), fair condition, some pereiopods missing, **allotype**; label E.37.818 (Rte 1585), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.37.898 (Rte 1589), in jar E.37.898-38.276 (Rte 1589), translucent and poor condition; label E.39.203 (Rte 1641), in jar E.37.183 (Rte 1555) – E.37.776 (1569), fair; label E.39.206 (Rte 1641), in jar E.39.041 (1635) - E.39.217 (1641), good condition; label E.39.207 (Rte 1641), in jar E.37.898- 38.276 (Rte 1589), residue, on *Fungia* sp.; label unreadable, in jar E.39.041 (1635) - E.39.217 (1641), good condition; jar E.39.041 (1635) - E.39.217 (1641) contains labelE.39.205 (Rte 1641) but no vial. **Muséum national d'Histoire naturelle (Paris, France)**: MNHNIU- 2014-10108 (was E.38.333 (Rte 1614)), good condition, 1 ovig. f, on *Fungia* [= *Lithophyllon*] *repanda*; MNHNIU- 2014-10107 (was E.38.425), good condition, 1 m, on *Fungia* [= *Lithophyllon*] *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 poste-rior 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 sep-arated 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
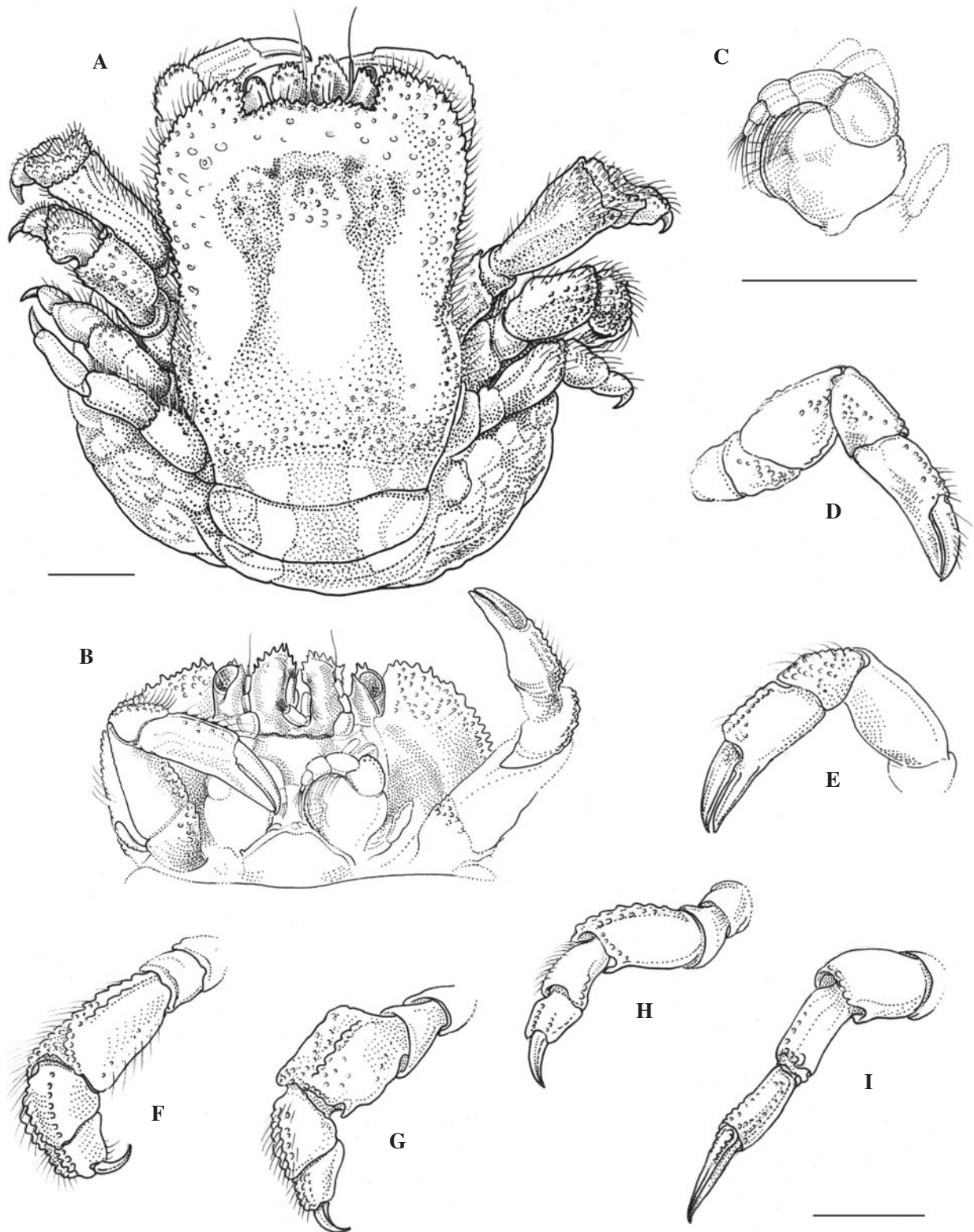
Fig. 4. *Fungicola fagei* (RMNH.Crus.D.53234). **A**, habitus, dorsal view; **B**, anterolateral margin of carapace, ventral view; **C**, MXP3; **D**, right P1 (cheliped); **E**, left P1 (cheliped); **F**, left P2; **G**, left P3; **H**, left P4; **I**, left P5. Scale bars 1 mm, B, D-I share a scale bar.

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 *Lithophyllon 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, 1990a). 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 (1990a) 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).

Material examined. Institute of Oceanography (Nha Trang, **Vietnam**): E.38.444 (Rte 1614), no label on jar, good condition, **holotype**, 1 ovig. f; E.37.458 (Rte 1569), in jar E.37.458, residue; E.37479 (Rte 1569), in jar E.37479-39.215 (Rte 1641), empty vial; E.37.727 (Rte 1581), in jar E.37.479-39.215 (Rte 1641), empty vial, **allotype**; E.39.210 (Rte 1641), in jar E.37.458, poor condition; E.39.213 (1641), in jar E.37.479-39.215 (Rte 1641), empty vial, on *Parahalomitra* [= *Sandalolitha*] *robusta*; E.39.215 (Rte 1641), in jar E.37.479-39.215 (Rte 1641), empty vial, on *Parahalomitra* [= *Sandalolitha*] *robusta*; jar E.37.479-39.215 (Rte 1641) contains one label without vial, E.38.334 (Rte 1614). **Muséum national d'Histoire naturelle (Paris**, **France)**: MNHN-IU-2009-2245 (was E.37.729 (Rte 1581)), pereiopods present but not attached to carapace, 1 m, on *Parahalomitra* [= *Sandalolitha*] *robusta*; MNHN-IU-2009-2246 (was E.37.728 (Rte 1581), good condition, 1 ovig. f, on *Parahalomitra* [= *Sandalolitha*] *robusta*. **Naturalis Biodiversity Center (Leiden**, **The Netherlands)**: see Table S3.

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 region, branchial regions separated from mid-gastric and cardio-intestinal regions by shallow furrows; front moderately concave; internal orbital angle falling much shorter than external; antero-lateral borders armed with spinules, moderately
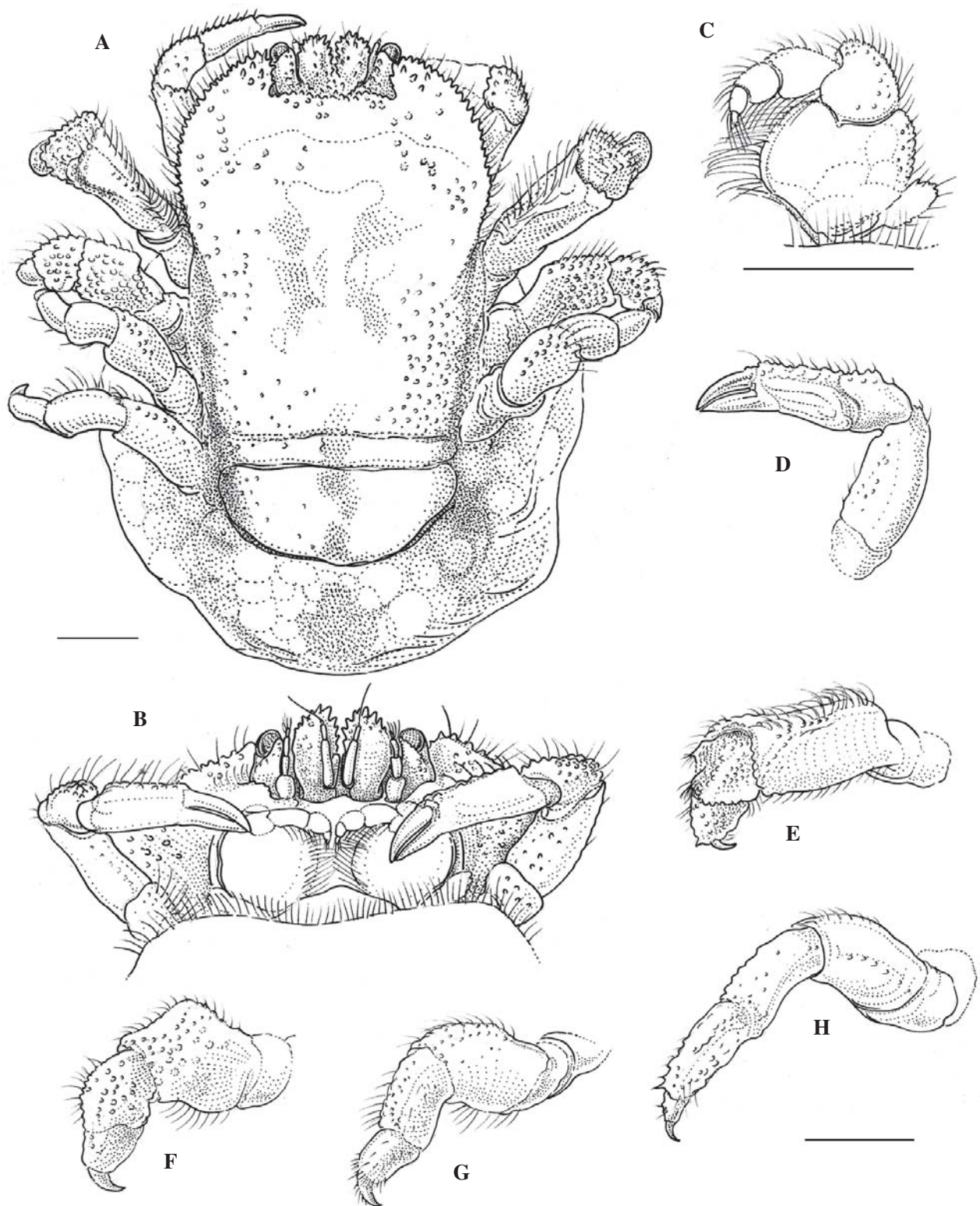
Fig. 5. Holotype *Fungicola syzygia* sp. nov. (RMNH.Crus.D.53220). **A**, habitus, dorsal view; **B**, anterolateral margin of carapace, ventral view; **C**, MXP3; **D**, left P1 (cheliped) **E**, left P2; **F**, left P3; **G**, left P4; **H**, left P5. Scale bars 1 mm, B, D-H share scale bar.

convex, lateral borders behind it slightly concave. Cheliped notstout; 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* [= *Sandalolitha 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* [= *Pleuractis*] *paumotensis*, most likely constitutes *F. syzygia* sp. nov. (see remarks in the species description of *F. syzygia* sp. nov).

Remarks. Kropp (1990a) 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.

Fungicola syzygia sp. nov.
Figs 5A-H, 6A-K, 7A-E

?Fungicola fagei.— Takeda and Tamura (1979: Fig. 2A-D)
?Fungicola sp.— Ng *et al.* (2008: Fig. 156)

Type locality. Sulamadaha Bay, Ternate, Indonesia (N 00°51'58", E 127°19'53")

Type material. Naturalis Biodiversity Center (Leiden, The Netherlands). **Holotype**: RMNH.Crus.D.53220, host *Pleuractis 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 *Pleuractis granulosa* (Klunzinger, 1 879), 0 2. xi.2009, 1 male (2.5 × 2.3), leg. SET van der Meij; **paratypes**: RMNH.Crus.D. 53225, Tanjung Ratemu (S of river), Ternate, Indonesia (N 00°54'24" E 127°29'17"), host *Pleuractis 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 *Pleuractis 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 2015.0006 (ex. RMNH.Crus.D.53219), Sulamadaha Bay, Ternate, Indonesia (N 00°51'58", E 127°19'53"), host *Pleuractis 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.
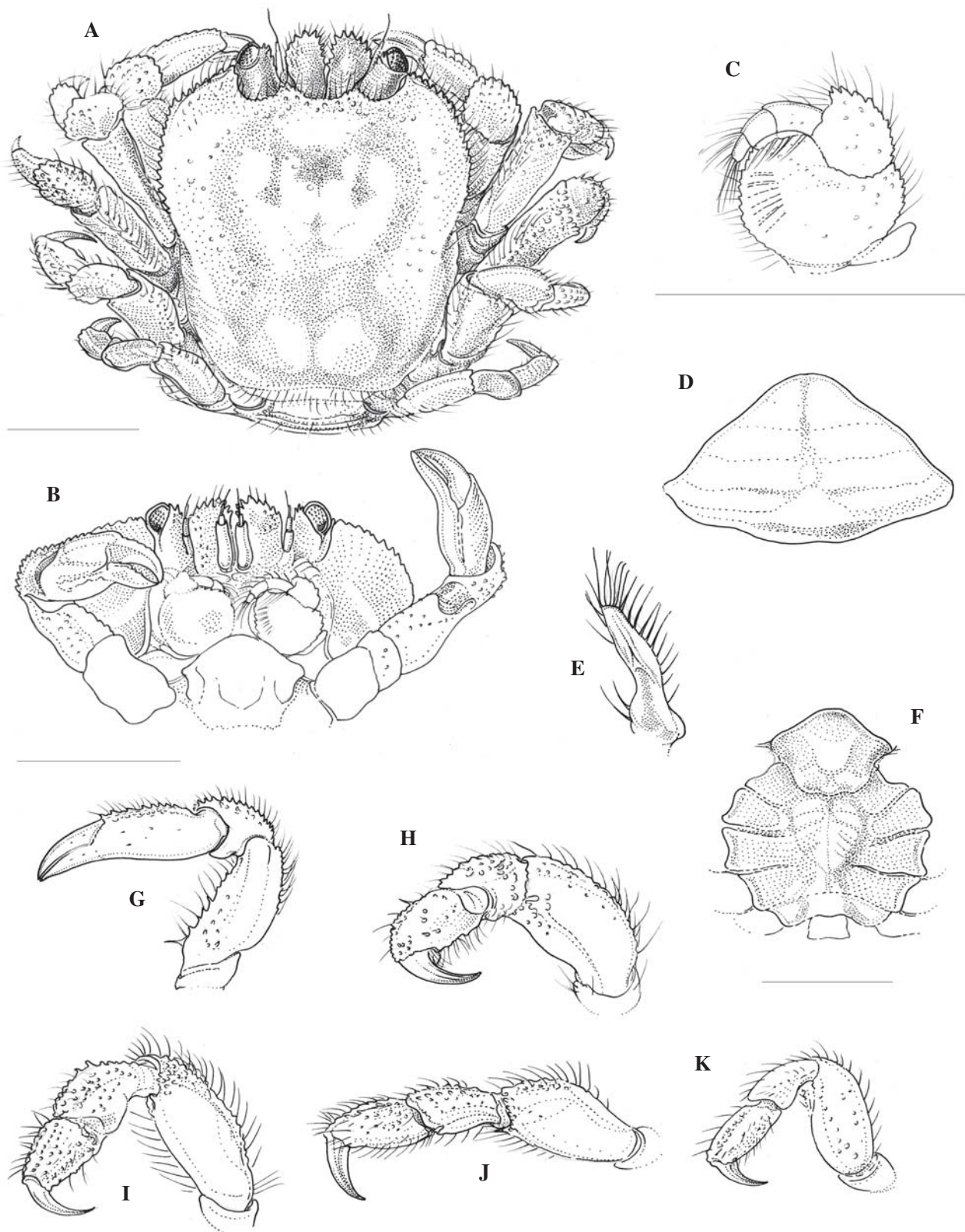
Fig. 6. Allotype *Fungicola syzygia* sp. nov. (RMNH.Crus.D.53224). **A**, habitus, dorsal view; **B**, anterolateral margin of carapace, ventral view; **C**, MXP3; **D**, abdomen; **E**, gonopod; **F**, thoracic sternites; **G**, left P1 (cheliped); **H**, left P2; **I**, left P3; **J**, left P4; **K**, left P5. Scale bars 1 mm, B, G-K as well as C-D and E-F share scale bar.

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 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. 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.

MXP3 (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.

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 halflength 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 to squaroid, 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 subquadrangular, smooth, mesial and distal margin slightly rounded, anteromesial
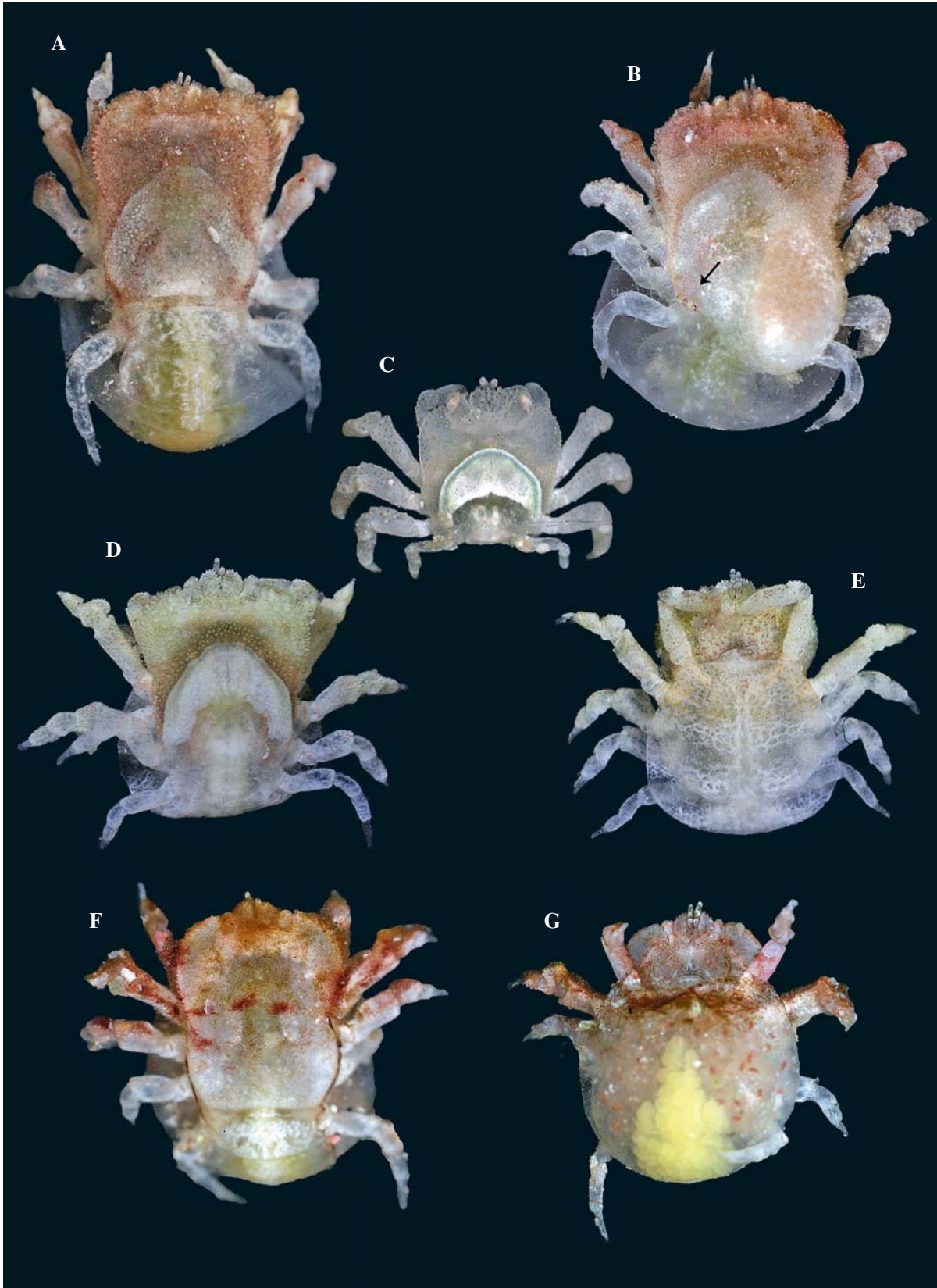
Fig. 7. *Fungicola syzygia* sp. nov., colouration in life. **A**, RMNH.Crus.D.53225, ovigerous female; **B**, RMNH.Crus.D.56481, non-ovigerous female parasitized by the isopod *Carcinione platypleura* Bourdon, 1983 (Isopoda: Bopyridae), male isopod indicated by arrow; **C**, RMNH.Crus.D.56479 male; **D-E**, RMNH.Crus.D.56480, non-ovigerous female. *Fungicola fagei*, colouration in life; **F-G**, RMNH.Crus.D.53234, ovigerous female.

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 ⅓ 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 *Lithophyllon repanda*, the most common host of *F. utinomi* (Fig. 2). This is the fourth species recorded from mushroom corals.

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

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

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 then in *F. fagei*. Based on the carapace length / carapace width ration, 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 (*Fungia paumotensis* [= *Pleuractis paumotensis*]) and the illustrations, possibly could be identified as *Fungicola syzygia* (National Museum of Nature and Science Tokyo, NSMTCr. 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 [suzugia], for a pair of related things or union, referring to the obligate symbiosis between the gall crab and its host coral.

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. 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.Crus.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 *Lithophyllon 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.*, 2012b; Budd *et al.*, 2012; Arrigoni *et al.*, 2014a; Huang *et al.*, 2014). The inconsistencies in the generic placement of the gall crabs based on host affinity, as observed by Kropp (1990a), are therefore likely related, at least in part, to past inconsistencies in scleractinian taxonomy.

Appendices S1-3 are available in the digital academic repository of Naturalis Biodiversity Center.