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Description of *Croceitalea* gen. nov. in the family *Flavobacteriaceae* with two species, *Croceitalea eckloniae* sp. nov. and *Croceitalea dokdonensis* sp. nov., isolated from the rhizosphere of the marine alga *Ecklonia kurome*

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Two novel bacterial strains, designated DOKDO 025^T and DOKDO 023^T, were isolated on Dokdo Island, Korea, from the rhizosphere of the brown alga *Ecklonia kurome*. The strains were subjected to a polyphasic taxonomy study and were found to be Gram-negative, aerobic, rod-shaped, non-motile and orange-coloured. The isolates shared 96.3 % 16S rRNA gene sequence similarity. They showed 93.8–95.6 % 16S rRNA gene sequence similarity with respect to members of the genus *Muricauda* in the family *Flavobacteriaceae*, but formed a distinct phyletic line. Moreover, the cellular appendages reported for all *Muricauda* species were absent from strains DOKDO 025^T and DOKDO 023^T. The predominant cellular fatty acids of strain DOKDO 025^T were iso-$C_{15:0}$, iso-$C_{15:1}$ and one with an equivalent chain-length of 13.565 and those of strain DOKDO 023^T were iso-$C_{15:0}$, iso-$C_{15:1}$ and iso-$C_{17:0}$ 3-OH. The DNA G+C content of strains DOKDO 025^T and DOKDO 023^T were 59.5 and 66.5 mol%, respectively, higher than any values found in recognized members of the family *Flavobacteriaceae*. The major respiratory quinone was MK-6. On the basis of evidence from the polyphasic study, strains DOKDO 025^T and DOKDO 023^T represent two novel species in a new genus, *Croceitalea* gen. nov., for which the names *Croceitalea eckloniae* sp. nov. (the type species) and *Croceitalea dokdonensis* sp. nov. are proposed. The type strain of *Croceitalea eckloniae* sp. nov. is DOKDO 025^T (=KCCM 42309^T =JCM 13827^T) and that of *Croceitalea dokdonensis* sp. nov. is DOKDO 023^T (=KCCM 42308^T =JCM 13826^T).

The family *Flavobacteriaceae* is one of the major branches of the phylum *Bacteroidetes* (Garrity & Holt, 2001), previously known as the *Cytophaga–Flavobacterium–Bacteroides* group. The family was first proposed by Jooste (1985) and the name was validly published by Reichenbach (1992); its description was subsequently emended by Bernardet *et al.* (1996, 2002). Members of this family are distributed in various terrestrial, marine and freshwater environments (Glöckner *et al.*, 1999; Bernardet & Nakagawa, 2006). Recently, many novel members of the family have been isolated from the surfaces of marine algae, e.g. *Maribacter ulvicola* from the green alga *Ulva fenestrata* (Nedashkovskaya *et al.*, 2004a), *Zobellia galactanivorans* from the red alga *Delesseria sanguinea* (Barbeyron *et al.*, 2001), *Zobellia amurskyensis* and *Zobellia laminariae* from the brown alga *Laminaria japonica* and *Zobellia russellii* from the green alga *Acrosiphonia sonderi* (Nedashkovskaya *et al.*, 2004b). This demonstrates that the surfaces of marine algae represent an important habitat for marine members of the family *Flavobacteriaceae*. In the present study, the taxonomic properties of two novel strains, DOKDO 023^T and DOKDO 025^T, originating from the rhizosphere of the marine macroalga *Ecklonia kurome*, were investigated. A novel genus and two novel species of the family *Flavobacteriaceae* are proposed for these strains.

Abbreviation: ECL, equivalent chain-length.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains DOKDO 023^T and DOKDO 025^T are DQ191182 and DQ191183, respectively.

Scanning electron micrographs of cells of strains DOKDO 023^T and DOKDO 025^T are available as supplementary material with the online version of this paper.

Strains DOKDO 023^T and DOKDO 025^T were isolated according to the procedure described by Bae *et al.* (2007). The isolates were further cultivated on marine agar 2216 (MA; Difco) or marine broth 2216 (MB; Difco) at 25 °C for morphological and biochemical characterization.

Unless otherwise stated, the minimal standards for describing new taxa in the family *Flavobacteriaceae* proposed by Bernardet *et al.* (2002) and previously described methods (Bae *et al.*, 2005, 2007; Sohn *et al.*, 2004) were used. Scanning electron micrographs were taken using a JSM-2000EXII (JEOL) electron microscope after bacterial cells had been dehydrated using a graded series of ethanol dilutions. The growth temperature was tested in MB at 12 different temperatures from 5 to 50 °C in a temperature gradient incubator (TVS126MA; Advantec) for up to 5 days. Growth under anaerobic conditions was determined after incubation in MB, supplemented with 20 mM each of sodium nitrate and sodium sulfate as electron acceptors, in serum vials for 2 weeks at 25 °C. Before inoculation, oxygen in the medium was removed by flushing with a mixture of oxygen-free H_2 and N_2 (1 : 2). The absence of oxygen was monitored using 0.001 % resazurin. The bacterial suspensions used to inoculate the API 20E, API 20NE, API 50 CH (all bioMérieux) and Microlog GN2 (Biolog) systems were prepared in 2 % sea salts (Sigma) solution. The tolerance range for salts was tested (from 0 to 7 % sea salts, w/v) in MB prepared with distilled water and supplemented with the same commercial sea salts solution. The tolerance range for pH was determined (from pH 4 to 10) in MB with the pH adjusted using 10 mM MES (pH 4–6), HEPES (pH 6–8) and AMPSO (pH 8–10) as biological buffers. The physiological, biochemical and morphological characteristics of strains DOKDO 023^T and DOKDO 025^T are given in the genus and species descriptions and in Table 1. Different inoculum volumes were tested, but results were

Table 1. Phenotypic characteristics that serve to differentiate strains DOKDO 025^T and DOKDO 023^T from closely related members of the family *Flavobacteriaceae*

Strains: 1, DOKDO 025^T; 2, DOKDO 023^T; 3, *Muricauda aquimarina* SW-63^T; 4, *Muricauda flavescens* SW-62^T; 5, *Muricauda ruestringensis* B1^T; 6, *Flagellimonas eckloniae* DOKDO 007^T; 7, *Costertonia aggregata* KOPRI 13342^T. Data are from Bae *et al.* (2007), Bruns *et al.* (2001), Kwon *et al.* (2006), Yoon *et al.* (2005) and this study. All of the strains are negative for hydrolysis of agar and starch and for production of urease and H_2S. All of the strains require NaCl for growth and have MK-6 as a major respiratory quinone. +, Positive; −, negative; ND, no data available; W, weakly positive; V, variable (different results obtained with different techniques).

Characteristic	1	2	3	4	5	6	7
Growth temperature (°C)							
Range	10–34	12–38	10–44	10–44	8–40	17–36	10–35
Optimum	29	35	30–37	30–37	20–30	26–29	26–32
Growth pH							
Range	6.5–10	7–10	ND	ND	6–8	7–9	6.5–9
Optimum	8	8.5–9	7	7	6.5–7.5	8	7.5–8.0
Growth with NaCl (%)							
Range	0.4–5.4*	0.8–5.4*	ND	ND	0.5–9	1.9–5.4*	1.2–9.3*
Optimum	1.6*	3.1*	2	2	3	2.7–3.1*	2.3*
Seawater requirement†	−	−	−	−	+	+	+
Gliding motility	−	−	−	−	+	−	−
Enzyme activity							
Oxidase/catalase	−/+	−/+	+/+	+/+	+/−	−/+	+/+
β-Glucosidase	−	+	ND	ND	ND	+	+
β-Galactosidase	−	+	ND	ND	ND	+	+
Nitrate reduction	−	−	−	−	−	−	+
Acid from carbohydrates	−	−	+	+	+	−	−
Hydrolysis of:							
Casein	−	−	−	−	−	+	−
Gelatin	+	−	−	−	−	−	+
Acetoin production	+	+	ND	ND	ND	W	−
Assimilation of:							
Glucose	+	V	+	+	−	+	+
Phenyl acetate	+	−	ND	ND	ND	−	ND
DNA G+C content (mol%)	59.5	66.5	44.1	45.2	41	56.3	35.8

*Sea-salt concentration was converted to NaCl concentration.

†Requirement for seawater indicates that Na^+ alone does not support growth: the strain requires additional cations present in seawater, such as Mg^{2+}, Ca^{2+} and/or K^+.

not obtained in the Microlog GN2 plate for strain DOKDO 025^T, because of a false-positive reaction in the control well.

Genomic DNA extraction and amplification of 16S rRNA gene sequences were conducted according to Sohn *et al.* (2004). Phylogenetic trees for strains DOKDO 023^T and DOKDO 025^T and closely related species were generated using the Jukes–Cantor and maximum-likelihood distance models with the neighbour-joining and maximum-parsimony algorithms, respectively. A total of 1328 unambiguously aligned bases were compared. Strains DOKDO 023^T and DOKDO 025^T shared 96.3 % sequence similarity. The closest neighbour with a validly published name was *Muricauda aquimarina* SW-63^T (95.3 and 95.6 % gene sequence similarity with respect to DOKDO 023^T and DOKDO 025^T, respectively), followed by *Muricauda ruestringensis* B1^T (95.0 and 95.3 %), *Muricauda flavescens* SW-62^T (93.8 and 94.1 %), *Flagellimonas eckloniae* DOKDO 007^T (92.6 and 94.1 %) and *Costertonia aggregata* KOPRI 13342^T (90.0 and 91.6 %). The neighbour-joining phylogenetic tree revealed that strains DOKDO 023^T and DOKDO 025^T formed a distinct branch in the phyletic line that comprised the genera *Muricauda*, *Flagellimonas* and *Costertonia* (Fig. 1). The maximum-likelihood tree showed essentially the same topology (data not shown).

Cellular fatty acid methyl esters were prepared from bacteria grown on MA for 3 days at 25 °C and were analysed according to the standard protocol of the Microbial Identification System (MIDI). In line with the other members of the same clade, strain DOKDO 023^T contained relatively large amounts (>5 %) of iso-$C_{15:0}$, iso-$C_{15:1}$, iso-$C_{17:0}$ 3-OH, summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), iso-$C_{15:0}$ 3-OH and $C_{15:0}$ (Table 2). Strain DOKDO 025^T also contained large amounts of iso-$C_{15:0}$, iso-$C_{15:1}$ and $C_{15:0}$, but the amounts of iso-$C_{17:0}$ 3-OH, summed feature 3 and iso-$C_{15:0}$ 3-OH were low. Instead, strain DOKDO 025^T contained relatively large amounts (>5 %) of summed feature 2 (comprising $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$) and an unknown fatty acid with an equivalent chain-length (ECL) of 13.565. *Zeaxanthinibacter enoshimensis* TD-ZE3^T is the only member of the family *Flavobacteriaceae* in which a large amount of this unknown fatty acid (ECL 13.565) had been reported previously (Asker *et al.*, 2007).

The major respiratory quinone in both strains was MK-6 (as determined using HPLC analysis according to the method of Collins, 1985). The DNA G+C contents of DOKDO 023^T and DOKDO 025^T were 66.5 and 59.5 mol%, respectively, as determined using a symmetry reversed-phase C18 column (Stackebrandt & Liesack,

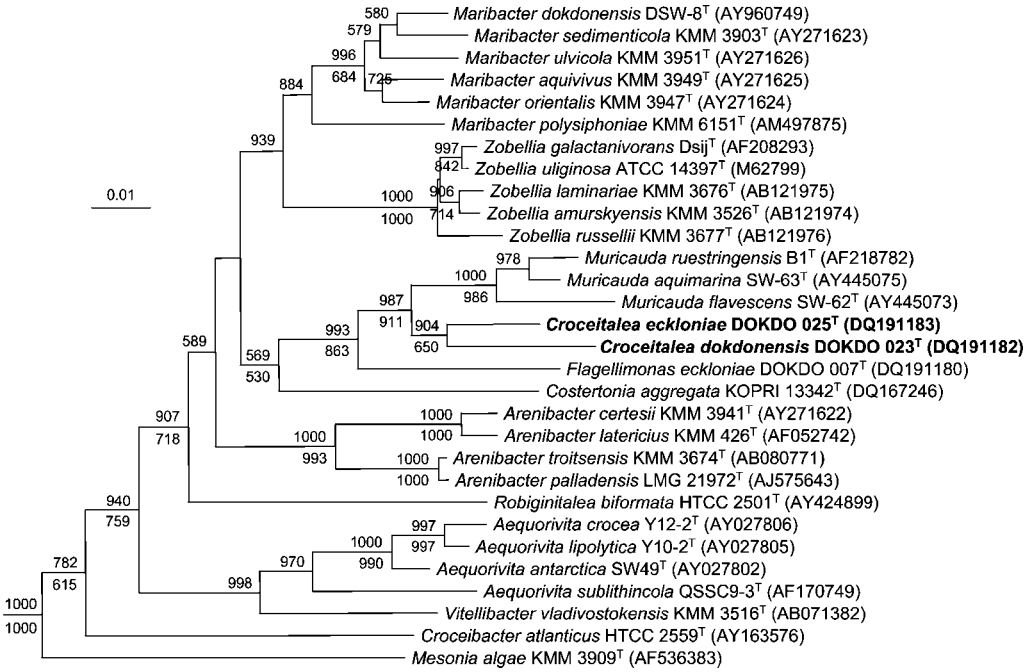

Fig. 1. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences (1328 unambiguously aligned base pairs), showing strains DOKDO 025^T and DOKDO 023^T and related members of the family *Flavobacteriaceae.* The 16S rRNA gene sequences of *Bacteroides fragilis* ATCC 25285^T (GenBank accession no. M61006) and *Sphingobacterium spiritivorum* DSM 2582^T (AJ459411) served as an outgroup (not shown). Bootstrap values (based on 1000 resampled datasets) greater than 500 are shown at nodes (upper value, Jukes–Cantor/neighbour-joining; lower value, maximum-likelihood/parsimony). Bar, 0.01 substitutions per nucleotide position.

Table 2. Fatty acid compositions (%) of strains DOKDO 025^T and DOKDO 023^T and closely related members of the family *Flavobacteriaceae*

Strains: 1, DOKDO 025^T; 2, DOKDO 023^T; 3, *Muricauda aquimarina* SW-63^T; 4, *Muricauda flavescens* SW-62^T; 5, *Muricauda ruestringensis* B1^T; 6, *Flagellimonas eckloniae* DOKDO 007^T; 7, *Costertonia aggregata* KOPRI 13342^T. Data are from Yoon *et al.* (2005) and this study. Some strains were not cultivated under the same conditions. Fatty acids amounting to <1 % in all strains were omitted. tr, Trace amounts (<1.0 %); –, not detected/not reported.

Fatty acid	1	2	3	4	5	6	7
Straight-chain							
$C_{14:0}$	tr	tr	–	–	–	tr	1.0
$C_{15:0}$	5.6	5.2	5.9	12.4	13.2	4.4	8.7
$C_{16:0}$	tr	tr	tr	tr	tr	tr	2.6
Branched							
iso-$C_{15:0}$	19.1	16.0	23.7	16.4	14.7	18.2	13.0
anteiso-$C_{15:0}$	1.4	tr	2.0	2.1	1.1	tr	tr
iso-$C_{16:0}$	tr	tr	tr	tr	–	tr	1.0
Unsaturated							
$C_{13:1}$ at 12–13	1.2	tr	–	–	–	–	tr
$C_{15:1}\omega6c$	tr	1.3	tr	tr	tr	tr	1.2
$C_{17:1}\omega6c$	tr	tr	tr	tr	1.0	tr	tr
iso-$C_{15:1}$ G*	31.2	21.9	21.6	19.9	20.5	24.0	16.6
iso-$C_{17:1}\omega9c$	2.9	1.4	1.5	1.3	1.4	2.1	4.3
Hydroxy							
$C_{15:0}$ 3-OH	–	2.1	1.4	1.0	1.8	tr	tr
iso-$C_{15:0}$ 3-OH	1.1	7.0	5.2	4.7	4.6	7.5	5.0
$C_{16:0}$ 3-OH	tr	4.6	tr	tr	tr	1.3	2.7
iso-$C_{16:0}$ 3-OH	1.8	2.2	4.0	2.9	1.7	1.0	tr
$C_{17:0}$ 2-OH	–	tr	tr	1.3	tr	tr	tr
$C_{17:0}$ 3-OH	–	–	tr	tr	1.3	tr	tr
iso-$C_{17:0}$ 3-OH	2.8	18.9	17.3	19.9	20.9	24.3	18.6
Summed features†							
2	6.1	tr	–	–	–	tr	tr
3	tr	7.3	2.3	4.1	4.2	8.5	14.3
Unknown							
ECL 11.543	2.4	tr	tr	–	tr	–	–
ECL 13.565	15.8	2.9	4.8	5.4	6.5	1.1	2.8
ECL 16.582	1.3	1.3	1.3	1.6	1.7	tr	1.1

*Double bond position indicated by an upper-case letter is unknown.
†Summed features represent fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2, $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$; summed feature 3, iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$.

1993); these values are higher than any reported previously for members of the family *Flavobacteriaceae* (Bae *et al.*, 2007). Cellular pigments were extracted with 3 ml methanol/acetone mixture (1 : 1, w/v) from culture grown on MA for 3 days; absorption spectra were measured with a spectrophotometer (UV-2410PC; Shimadzu). Flexirubin-type pigments were detected by placing a drop of 20 % KOH on colonies (Fautz & Reichenbach, 1980). Carotenoid pigments with absorption maxima at 474 and 504 nm were detected, but flexirubin-type pigments were not found.

Strains DOKDO 023^T and DOKDO 025^T shared many characteristics with closely related members of the family *Flavobacteriaceae*, including the type of major respiratory quinone, the temperature and pH ranges that support growth, the requirement for NaCl and oxygen and the absence of urease activity and H_2S production (Table 1). The two isolates displayed relatively high levels of 16S rRNA gene sequence similarity with respect to members of the genus *Muricauda*. However, the absence of long and relatively thick cellular appendages in DOKDO 023^T and DOKDO 025^T (see Supplementary Fig. S1, available in IJSEM Online) and the high DNA G+C contents clearly serve to differentiate these two strains from the recognized members of the genus *Muricauda*. Other characteristics that can be used to distinguish these strains from closely related members of the family *Flavobacteriaceae* are listed

in Table 1. Although the two isolates shared many common phenotypic features, they showed differences in some phenotypic characteristics listed in Table 1 and in the proportions of some fatty acids (Table 2). Consequently, strains DOKDO 023^T and DOKDO 025^T represent two novel species in a novel genus, *Croceitalea* gen. nov., of the family *Flavobacteriaceae*, for which the names *Croceitalea dokdonensis* sp. nov. and *Croceitalea eckloniae* sp. nov. are proposed.

Description of *Croceitalea* gen. nov.

Croceitalea (Cro.ce.i.ta′le.a. L. adj. *croceus* saffron-coloured, yellow, golden; L. fem. n. *talea* a slender staff, rod, stick; N.L. fem. n. *Croceitalea* a rod forming yellow–orange colonies).

Cells are Gram-negative rods devoid of appendages. Yellow–orange-coloured colonies are formed on MA. Irregular aggregates are formed during growth in liquid medium. NaCl and oxygen are required for growth. Non-diffusible carotenoid pigments are produced. Catalase activity is present but oxidase activity is absent. The major respiratory quinone is MK-6 and the major fatty acids are iso-$C_{15:1}$ and iso-$C_{15:0}$. The DNA G+C ratio is approximately 60–67 mol%. As determined by 16S rRNA gene sequence analysis, the genus *Croceitalea* is a member of the family *Flavobacteriaceae* in the phylum *Bacteroidetes*. The type species is *Croceitalea eckloniae*.

Description of *Croceitalea eckloniae* sp. nov.

Croceitalea eckloniae (eck.lo.ni′ae. N.L. fem. n. *Ecklonia* scientific genus name of a marine alga; N.L. gen. n. *eckloniae* of *Ecklonia*, referring to the isolation of the type strain from *Ecklonia kurome*).

Cells are usually 1.0–2.8 μm long and 0.3–0.5 μm wide, but longer and shorter cells also occur. Colonies are circular and 0.5–1.0 mm in diameter after 6 days incubation on MA at 25 °C. Carotenoid pigments with absorption maxima at 474 and 504 nm are produced, but flexirubin-type pigments are not produced. Gliding motility is absent. Growth occurs at 10–34 °C, at pH 6.5–10 and in the presence of 0.5–7.0 % sea salts (equivalent to 0.4–5.4 % NaCl). Optimal growth occurs in the presence of 2 % (w/v) sea salts (equivalent to 1.6 % NaCl), at pH 8 and at 29 °C. Nitrate is not reduced to nitrite. Gelatin is hydrolysed but agar, casein, starch and urea are not hydrolysed. Acetoin is produced but H_2S and indole are not produced. Phenyl acetate is assimilated. The following substrates are utilized in the API 50 CH strip: cellobiose, fructose, glucose, maltose, mannose, melibiose, sucrose, trehalose, ribose, galactose, *N*-acetylglucosamine, aesculin, salicin and glycogen. Mannitol and amygdalin are weakly utilized. No result is available from the Microlog GN2 strip, because of a false-positive reaction in the control well. The predominant fatty acids are iso-$C_{15:1}$, iso-$C_{15:0}$, an unknown fatty acid (ECL 13.565), summed feature 2 (comprising $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$) and $C_{15:0}$. The DNA G+C content of the type strain is 59.5 mol%.

The type strain, DOKDO 025^T (=KCCM 42309^T =JCM 13827^T), was isolated from the rhizosphere of the marine alga *Ecklonia kurome*, collected on Dokdo Island, Korea.

Description of *Croceitalea dokdonensis* sp. nov.

Croceitalea dokdonensis (dok.do.nen′sis. N.L. fem. adj. *dokdonensis* pertaining to Dokdo, the Korean island from where the type strain was isolated).

Cells are usually 1.4–3.1 μm long and 0.4–0.6 μm wide, but longer and shorter cells also occur. Colonies are circular and 1.0 mm in diameter after 6 days incubation on MA at 25 °C. Gliding motility is absent. Carotenoid pigments with absorption maxima at 474 and 504 nm are produced, but flexirubin-type pigments are not produced. Growth occurs at 12–38 °C, at pH 7–10 and in the presence of 1.0–7.0 % (w/v) sea salts (equivalent to 0.8–5.4 % NaCl). Optimal growth occurs in the presence of 4 % (w/v) sea salts (equivalent to 3.1 % NaCl), at pH 8.5–9 and at 35 °C. Nitrate is not reduced. Agar, casein, gelatin, starch and urea are not hydrolysed. Acetoin is produced but H_2S and indole are not produced. β-Glucosidase and β-galactosidase activities are present. The following substrates are utilized in the Microlog GN2 strip or in the API 50 CH strip: cellobiose, fructose, glucose, maltose, mannose, melibiose, sucrose, trehalose, dextrin, gentiobiose, α-D-lactose, lactulose, methyl β-D-glucoside, raffinose, turanose, acetic acid, α-ketobutyric acid, α-ketoglutaric acid, DL-lactic acid, L-alanine, L-alanyl glycine, L-glutamic acid, glycyl L-glutamic acid, L-proline, L-threonine, uridine and glucose 1-phosphate. The following substrates are weakly utilized: glycogen, D-psicose, L-rhamnose, D-gluconic acid, succinic acid, glycyl L-aspartic acid, hydroxy-L-proline, L-ornithine, L-serine and inosine. Glucose is utilized in the Microlog GN2 strip but not in the API 20NE strip. The predominant fatty acids are iso-$C_{15:1}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:0}$, summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), iso-$C_{15:0}$ 3-OH and $C_{15:0}$. The DNA G+C content of the type strain is 66.5 mol%.

The type strain, DOKDO 023^T (=KCCM 42308^T =JCM 13826^T), was isolated from the rhizosphere of the marine alga *Ecklonia kurome*, collected on Dokdo Island, Korea.

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