The genus *Maribacter* was established in 2004 by Nedashkovskaya *et al.* (2004) and was subsequently emended by Barbeyron *et al.* (2008), Nedashkovskaya *et al.* (2010), Lo *et al.* (2013), Weerawongwiwat *et al.* (2013) and Hu *et al.* (2015). At the time of writing, the genus comprises thirteen species with validly published names, which form a monophyletic clade within the family *Flavobacteriaceae* of the phylum Bacteroidetes. The type strain most closely related to strain W13M1AT is *Maribacter forsetii* DSM 18668T with a gene sequence similarity of 96.5%. The closest related type strain to strain W15M10T is *Maribacter orientalis* DSM 16471T with a gene sequence similarity of 98.3%. Phylogenetic inference and phenotypic data combined indicate that the isolates represent two novel species of the genus *Maribacter*, for which the names *Maribacter spongiicola* sp. nov. with type strain W13M1AT (=NCIMB 14725T =DSM 25233T) and *Maribacter vaceletii* sp. nov. with type strain W13M1AT (=NCIMB 14724T =DSM 25230T), are proposed.

Isolation of culturable bacteria from marine sponges to discover novel bioactive compounds was performed, and here we report on the phenotypic and phylogenetic characteristics of two novel sponge-derived *Maribacter* strains; one (W13M1AT) was isolated from the marine sponge *Suberites carnosus* and the other (W15M10T) from the marine sponge *Leucosolenia sp.* respectively, which were sampled from Lough Hyne, Co. Cork, Ireland. Analysis of the 16S rRNA gene sequences of these isolates revealed that they are members of the genus *Maribacter*, in the family *Flavobacteriaceae* of the phylum Bacteroidetes. The type strain most closely related to strain W13M1AT is *Maribacter forsetii* DSM 18668T with a gene sequence similarity of 96.5%. The closest related type strain to strain W15M10T is *Maribacter orientalis* DSM 16471T with a gene sequence similarity of 98.3%. Phylogenetic inference and phenotypic data combined indicate that the isolates represent two novel species of the genus *Maribacter*, for which the names *Maribacter spongiicola* sp. nov. with type strain W13M10T (=NCIMB 14724T =DSM 25230T) and *Maribacter vaceletii* sp. nov. with type strain W13M1AT (=NCIMB 14725T =DSM 25233T), are proposed.

Abbreviation: ASW, artificial seawater.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Maribacter vaceletii* sp. nov. W13M1AT and *Maribacter spongiicola* sp. nov. W15M10T are JX050190 and JX050191, respectively.

A supplementary figure is available with the online Supplementary Material.
Strain W15M10T shared species of the genus Maribacter gene sequence similarity with five other type strains of strain to strain W13M1AT was 

Genomic DNA was extracted from pure bacterial cultures for 16S rRNA gene sequencing. Near full-length 16S rRNA genes (W13M1AT, 1332 bp; W15M10T, 1331 bp) were amplified using Taq polymerase (Fermentas) and primers 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492r (5′-ACCTTGTTACGACTT-3′) (Lane, 1991). The amplified genes were gel purified and sequenced by capillary electrophoresis, single extension sequencing (Macrogen) using a 3730xl DNA Analyser. Sequence similarity searches were performed by BLAST searches (Altschul et al., 1990). Reference sequences were downloaded from the Ribosomal Database Project (Wang et al., 2007). Sequences were analysed further by alignments using CLUSTAL W (Thompson et al., 1994) and tree reconstruction using neighbour-joining (Saitou & Nei, 1987; Tamura et al., 2004) algorithms in MEGA5 software (Tamura et al., 2011). Bootstrap tests (Felsenstein, 1985) were performed 1000 times to produce a consensus tree, which is taken to represent the evolutionary history of the taxa herein (Fig. 1).

The closest related type strains, by percentage 16S rRNA gene sequence similarity, to strain W15M10T were M. stanieri DSM 19891T and M. sedimenticola DSM 19840T (both 98.3 %). Strain W15M10T shared >97 % gene sequence similarity with five other type strains of species of the genus Maribacter. The closest related type strain to strain W13M1A T was M. forsetii DSM 18668T (96.5 % sequence similarity). Consequently, M. stanieri DSM 19891T, M. orientalis DSM 16471T and M. forsetii DSM 18668T were obtained from the Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkultur (DSMZ), Braunschweig, Germany and used as reference strains in further characterization studies. Alignment of near full-length 16S rRNA sequences from strain W15M10T and strain W13M1A T revealed a sequence similarity of 95.6 %, confirming that the two strains are not of the same species.

The following phenotypic tests were performed on the novel isolates only, with the exception of API test strips and fatty acid profiling, which were performed in parallel on the reference strains. Gram staining was performed using standard methods (Beveridge, 2001). Cell sizes and morphologies were determined using a light microscope (DM3000; Leica). Production of flexirubin-type pigments was determined as previously described at the DSMZ (Bernardet et al., 2002). Gliding motility was assessed from 24–28 h cultures in Marine Broth 2216 (Difco) using phase-contrast microscopy, Carl Zeiss Universal.

Anaerobic growth was tested by incubation on Marine Agar 2216 (Difco) plates in an anaerobic jar at 25 °C for 14 days. The temperature range for growth was determined on MA plates incubated at 4 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 37 °C and 45 °C and monitored for 6 weeks. The pH range for growth was monitored in Marine Cytophaga (MC) broth [0.2 % tryptone (w/v, Oxoid), 0.05 % Lab Lemco powder (w/v, Oxoid), 0.05 % yeast extract (w/v, Oxoid) and 0.02 % sodium acetate (w/v, Sigma)] containing 4 % (w/v) artificial sea salts (Sigma) with a pH ranging from 4–11 at intervals of 1 pH unit and incubated at 25 °C for 6 weeks. The pH values were attained in MC Broth by the addition of 10 mM MES buffer, PIPES buffer, HEPES buffer and Tris/HCl. Salt requirement for growth was tested on ZoBell (1941) and MC agars containing 0–10 % (w/v) NaCl. The composition of MC agar was the same as MC broth with 1.5 % (w/v) agar added. Plates were monitored for growth for six weeks. As growth of both strains was unsatisfactory, NaCl was replaced in the MC agar with artificial sea salts (Instant Ocean).

Biochemical profiles were obtained using API 20 E and API 20 NE kits following the manufacturer’s instructions (bio-Mérieux), except that they were incubated at 25 °C and, because no significant colour changes were observed after 24 h, the API strips were incubated and read after 48 and 72 h.

Penicillin G susceptibility was tested by spreading the isolate onto MA plates with a 1 μg penicillin G disc (MAST) placed on the surface. The plates were incubated at 25 °C for 7 days and checked periodically for a zone of inhibition. Other biochemical tests (for catalase and oxidase activities, Congo red adsorption, hydrolysis of Tween 80, casein, starch and agar, and DNase activity) were investigated as described in previous studies (Bernardet et al., 2002; Cowan & Steel, 1993; Johnson & Chilton, 1966). Acid production from glucose and starch was tested as described in previous studies (Cowan & Steel, 1993) and also with modified media incorporating 4 % artificial sea salts (w/v, Sigma). The phenotypic characteristics of strains W13M1A T and W15M10T are listed in Table 1 and in the species descriptions.

The fatty acid methyl ester profiles of strains W13M1A T, W15M10T, M. orientalis DSM 16471T and M. forsetii DSM 18668T were determined at the DSMZ using the Sherlock Microbial Identification System version 6.1 (MIDI, Microbial Inc.) using the standard protocol. The analysis was performed by GC (Agilent 6890N) and identified by comparison to the TSBA40 database of the Microbial Identification System (Sasser, 1990). These profiles were generated from cultures standardized for their physiological age using the method described by Sasser (1990), and grown at 25 °C on MA. The major fatty acids in strain W13M1A T were iso-C_{17:0} 3-OH, C_{15:0}, iso-C_{15:0} summed feature.
Fig. 1. Neighbour-joining phylogenetic tree of *Maribacter spongiicola* sp. nov., *Maribacter vaceletii* sp. nov. and related members of the family *Flavobacteriaceae* based on 16S rRNA gene sequence analysis. The tree was reconstructed using the neighbour-joining method with 1000 bootstrap replicates. Numbers at nodes indicate % bootstrap values, only values ≥ 70% are shown. All positions containing gaps and missing data were eliminated from the dataset. Bar, 0.05 substitutions per nucleotide position.
Table 1. Differential characteristics of strains W15M10T and W13M1A7, and type strains of closely related species

<table>
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<tr>
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<td>+</td>
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<td>4–32</td>
<td>4–35</td>
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<td>−</td>
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<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<td>−</td>
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<td>+</td>
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<td>−</td>
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</tr>
<tr>
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<td>V</td>
<td>−</td>
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<td>−</td>
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<td>34.4</td>
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<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Temperature range for growth (°C)</td>
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<td>1.5–8</td>
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<td>+</td>
<td>−</td>
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<td>Urease</td>
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<td>−</td>
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<tr>
<td>Capric acid</td>
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<td>+</td>
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<td>−</td>
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</tr>
<tr>
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<td>−</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>+</td>
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<td>−</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>35.7</td>
<td>32.6</td>
<td>34.4</td>
<td>36.6</td>
<td>37.0</td>
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</table>

3 (iso-C15 : 0 2-OH and/or C16 : 1ω7c), iso-C15 : 1G and ECL 13,565 and those in strain W15M10T were iso-C15 : 1G, iso-C17 : 0 3-OH, summed feature 3, ECL 13,565, iso-C17 : 1ω9c and iso-C15 : 0 (Table 2). These fatty acid profiles are consistent with other members of the genus Maribacter.

Determination of the respiratory quinones was performed by the DSMZ, by the organic extraction of freeze-dried materials and subsequent TLC and HPLC analyses (Tindall, 1990a, b). Consistent with all type strains of species of the genus Maribacter, the only respiratory quinone identified in strains W13M1A7 and W15M10T was MK-6. Polar lipids were extracted by DSMZ from freeze-dried materials (Bligh & Dyer, 1959) and separated by 2-D silica gel TLC. Total lipids, phospholipids, aminolipids and glycolipids were determined using specific stains (Tindall et al., 2007). This analysis identified phosphatidylethanolamine, one unidentified aminolipid and four unidentified polar lipids in strain W13M1A7. Strain W15M10T displayed the same polar lipid profile as strain W13M1A7 with an additional unidentified aminolipid as seen in the TLC plates shown in Fig. S1 (available in the online Supplementary Material), which shows the total lipids stained with ethanolic phosphomolybdic acid. This profile is consistent with the emended description of the genus (Weerawongwiwat et al., 2013).

DNA–DNA hybridization experiments were conducted between strain W15M10T and the most closely related species at DSMZ. DNA–DNA hybridization was carried out as described by De Ley et al. (1970) and as modified by Huss et al. (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multiecell changer and a temperature controller with an in-situ temperature probe (Varian). The DNA–DNA relatedness was 21.6 % between strain W15M10T and M. stanieri DSM 19891T and 25.8 % between strains W15M10T and M. orientalis DSM 16471T confirming that strain W15M10T belongs to neither of those species.

Genomic DNA was isolated from strains W13M1A7 and W15M10T and the G+C content was determined at the DSMZ by HPLC (Mesbah et al., 1989; Tamaoka & Komagata, 1984; Visuvanathan et al., 1989). The DNA G+C content of strain W13M1A7 was 32.6 mol% (a value slightly below the range of 34–41.1 % reported for the genus), while that of strain W15M10T was 35.7 mol%.

Many phenotypic and biochemical features previously described for the genus Maribacter were observed in these
Table 1. Percentage whole-cell fatty acid composition of strains W15M10T and W13M1AT and type strains of the most closely related species

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<th>3</th>
<th>4</th>
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<tr>
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<td>TR</td>
<td>TR</td>
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<tr>
<td>C&lt;sub&gt;13&lt;/sub&gt;:0</td>
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<td>0.7</td>
<td>0.7</td>
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<tr>
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<td>TR</td>
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<td>0.7</td>
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<td>9.4</td>
<td>13.8</td>
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<td>–</td>
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<td>3</td>
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<tr>
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<td>11.2</td>
</tr>
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<td>14.1</td>
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<td>–</td>
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Summed features: 3% and 4%

*Summed features are groups of two or three fatty acids treated together for the purposes of evaluation in the MIDI system (Montero-Calasanz et al., 2013) and include both peaks with discrete ECLs as well as those where ECLs are not reported separately. Summed feature 3 was reported to be iso-C<sub>15</sub>:0 2-OH and/or C<sub>16</sub>:1 o9c; summed feature 4 was reported to be anteiso-C<sub>17</sub>:1B and/or iso-C<sub>17</sub>:1I.

The description of the genus is as given by Nedashkovskaya et al. (2004, 2010), Barbeyron et al. (2008, Lo et al. (2013), Hu et al. (2015) and Weerawongwiwat et al. (2013), except that gliding motility, production of flexirubin-type pigments and indole production are species-dependent.

The DNA G + C content is 32.6–41.1 mol%.

**Description of Maribacter spongicola sp. nov.**

Maribacter spongicola [spon.gi.čo’la] L. fem. n. spongia sponge; L. suff. -cola (from L. n. incola) inhabitant, dweller; N.L. n. spongicola sponge inhabitant].

Cells are rod-shaped, approximately 0.44–0.69 μm wide and 8.0–12.3 μm long, Gram-stain-negative and non-motile. Colonies, when grown on MA, are orange, entire, convex, circular, smooth and shiny. The pigments are non-diffusible and of a non-flexirubin-type. Cells are heterotrophic, strictly aerobic and require 1–6 % sea-salts (w/v) for growth. Growth does not occur with NaCl as the only added salt, however, growth occurs when a 2–5 % artificial sea salts (w/v, Instant Ocean) concentration is present in the media; the optimum sea salts concentration is 3 % (w/v). Growth is observed in the temperature range of 4–30 °C and at a pH range from 6–10. Optimum growth occurs at 25–30 °C and at pH 6–7. Catalase-, oxidase- and alkaline phosphatase-positive. Hydrolyses starch with acid production only when sea salts are added. Similarly, acid production from glucose only occurs when sea salts are added. Does not hydrolyse agar, DNA or gelatin. Hydrolyses Tween 80 and aesculin, Penicillin G resistant and displays ß-galactosidase activity. According to API 20 E strips, acid is not produced from glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, mannose, mannan, N-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid are all observed. According to API ZYM strips, alkaline phosphatase, esterase and esterase lipase activities are present, but lipase activity is absent. Leucine-, valine- and cystine-arylamidase activities, trypsin, ß-chymotrypsin, acid-phosphatase and naphthol-AS-BI-phosphohydrolase activities are noted as are ß-galactosidase, ß-galactosidase, ß-glucuronidase, ß- and ß-glucosidase, N-acetyl-ß-glucosaminidase and ß-mannosidase activities, but ß-fucosidase activity is absent. Utilizes citrate and novel strains (Table 1), however some important differences were observed and so an emended description of the genus is now required. These strains did not produce acid from glucose or starch in the presence of only NaCl. However, when sea salts were included in the media, strain W15M10T produced acid from glucose and starch. Contrary to the original and emended descriptions of the genus, these strains were non-motile. Therefore, on the basis of phylogenetic inference and phenotypic characteristics, we propose that strains W15M10T and W13M1AT represent two novel species of the genus Maribacter with the names Maribacter spongicola sp. nov. and Maribacter vaceletii sp. nov., respectively. In addition, an emended description of the genus Maribacter is proposed on the basis of some of the data obtained in this study.
produces acetoin. No nitrite reduction, nitrate reduction, indole production or H₂S production is observed. Urease activity is noted. Arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase activities are absent. Tryptophan deaminase activity is observed after 72 h. The main fatty acids (>9% of the total fatty acids) are iso-C₁₅:₁G, iso-C₁₇:₀ 3-OH, summed feature 3 (iso-C₁₅:₀ 2-OH and/or C₁₆:₁ω₇c), ECL 13.565, iso-C₁₇:₁ω₉c and iso-C₁₅:₀. The only respiratory quinone is MK-6. The main polar lipids are phosphatidyethanolamine, two unidentified aminolipids and four unidentified polar lipids.

The type strain is W15M10ᵀ (=NCIMB 14725ᵀ=DSM 25233ᵀ) isolated from the marine sponge *Leucosolenia* sp. collected from Lough Hyne Marine Nature Reserve, Co. Cork, Ireland. The DNA G+C content of the type strain is 35.7 mol%.

**Description of *Maribacter vaceletii* sp. nov.**

*Maribacter vaceletii* (va.ce.let’i. N.L. gen. n. *vaceletii* acknowledging the work of Jean Vacelet in the field of sponge-microbiology).

Cells are rod-shaped, approximately 0.5–0.54 μm wide and 3.0–3.3 μm long, Gram-stain-negative, non-motile, heterotrophic and strictly aerobic. Colonies, when grown on MA are entire, convex, circular, smooth and shiny with dark orange, non-diffusible, flexirubin-type pigments. Growth occurs at 10–30 °C with an optimum growth temperature of 25 °C. Growth occurs at pH 6–9 with optimum growth at pH 6–7. Growth does not occur with NaCl as the only added salt, however, growth occurs when 2–5 % (w/v, final concentration) artificial sea salts (Instant Ocean) are added to the media; the optimum sea salts concentration is 2 % (w/v). Catalase-, oxidase- and alkaline phosphatase- positive. Does not hydrolyse starch, DNA, gelatin or agar. Hydrolyses Tween 80 and aesculin, and is Penicillin G resistant. Does not reduce nitrite or nitrate, does not produce H₂S and is urease-negative. Does not display β-galactosidase activity when diluted in 0.85 % saline, but does when diluted in 3.33 % (w/v) sea salts (Instant Ocean). Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are all observed as is indole production (after 72 h of incubation). According to API 20 NE strips, utilization of glucose, arabinose, mannose, mannitol, N-acetylgalactosamine, maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid are all observed. According to API ZYM strips, alkaline phosphatase, esterase and esterase lipase activities are present but lipase activity is absent. Leucine- valine- and cystine-arylamidase activities, trypsin, α-chymotrypsin, acid-phosphatase and naphthol-AS-Bl-phosphohydrolase activities are noted as are α-galactosidase, β-galactosidase, β-glucuronidase, α- and β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase activities, but α-fucosidase activity is absent. Acetoin production is not observed. According to API 20 E strips, acid is not produced from glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin or L-arabinose. In contrast to the results from the API 20 E strips, acid production from glucose was observed in a stand-alone assay, so discrepancies may be observed depending on the method used. The main fatty acids (>10 % of the total) are iso-C₁₇:₀ 3-OH, C₁₅:₀, iso-C₁₅:₀ 2-OH and/or C₁₆:₁ω7c, iso-C₁₇:₁ω9c and iso-C₁₅:₀. The only respiratory quinone is MK-6. The main polar lipids are phosphatidyethanolamine, one unidentified aminolipid and four unidentified polar lipids.

The type strain is W13M1Aᵀ (=NCIMB 14724ᵀ=DSM 25230ᵀ) isolated from the marine sponge *Suberites carnosus* sampled from Lough Hyne Marine Nature Reserve, Co. Cork, Ireland. The DNA G+C content of the type strain is 32.6 mol%.

**Acknowledgements**

We would like to thank Bernard Picton (Ulster Museum) and Dr Robert McAllen (UCC) for assistance with sponge-sampling and identification. This work was funded by the Beaufort Marine Research Award, part of the Sea Change Strategy and the Strategy for Science Technology and Innovation (2006–2012), with the support of the Marine Institute under the Marine Research SubProgramme of the National Development Plan 2007–2013.

**References**


