Bacterial infections are a common cause of morbidity and mortality in pinnipeds, with bacteria acting both as primary and secondary pathogens (Dunn et al., 2001; Higgins, 2000), and members of the family Pasteurellaceae have been isolated both from healthy pinnipeds and from disease conditions in pinnipeds (Howard et al., 1983; Sweeney, 1978). The most frequently described species of the family Pasteurellaceae isolated from pinnipeds have been Pasteurella multocida (Dunn et al., 2001; Smith et al., 1978). However, recently, a new genus containing one species, Otariodibacter oris, was isolated from the oral cavity of healthy sea lions (Otarinae), fur seals (Arctocephalinae) and walruses (Odobenidae) (Hansen et al., 2012a, b). Similarly, a new genus containing one species, Bisgaardia hudsonensis, and one genomospecies, Bisgaardia genomospecies 1, has been isolated from both healthy and diseased seals (Phocidae) (Foster et al., 2011; Hansen et al., 2012a). B. hudsonensis has also been isolated from an infected seal bite in a human (Sundeep & Cleeve, 2011) and both Bisgaardia genomospecies 1 and O. oris have been isolated in pure culture from abscesses in a harbour seal (Phoca vitulina) and California sea lions (Zalophus californianus), respectively (Hansen et al., 2013).

Elephant seals are large, oceangoing seals in the genus Mirounga. The two species, the northern elephant seal...
(Mirounga angustirostris) and the southern elephant seal (Mirounga leonina), were both hunted to the brink of extinction by the end of the 19th century, but numbers have since recovered (Berta & Churchill, 2012). The bacteria most frequently isolated from inflammatory lesions in elephant seals are Escherichia coli, Klebsiella sp., Enterococcus sp., Salmonella sp. and Pseudomonas sp. (Stoddard et al., 2005; Thornton et al., 1998). However, descriptions of the bacterial flora of these animals in the literature are limited and to the authors’ knowledge, isolation of members of the family Pasteurellaceae from elephant seals has never previously been described.

The aim of the present investigation was to characterize a collection of 17 isolates representing the family Pasteurellaceae obtained from the oral cavity of northern elephant seals, to extend our knowledge of the phenotypic and genotypic diversity and possible host adaptation of these taxa and to improve classification and identification of the taxa demonstrated.

BBL culture swabs (BD Biosciences) were used for swabbing the gingival/dental fossa in the canine tooth area. Samples were collected opportunistically during admission exams when the seals arrived from the wild at The Marine Mammal Center (TMMC) in Sausalito, CA, USA. The northern elephant seals were evaluated as described by Greig et al. (2005) and examined clinically prior to sampling. Samples were obtained from a total of 15 juvenile wild northern elephant seals.

Swabs were kept in Stuart liquid medium (BD Biosciences) at 5 °C for up to 6 h before they were plated on 5 % bovine blood agar (BA) (Hardy Diagnostics). BA plates were incubated aerobically in sealed plastic bags for 24 h at 37 °C. Colonies typical of the family Pasteurellaceae were subsequently subcultured and characterized as described previously (Bisgaard, 1982, 1991). Phenotypic tests included Gram staining, motility, catalase, oxidase, Voges–Proskauer, ONPG, PNPG (x-glucosidase), indole, urease, arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, methyl red, nitrate reduction, gelatin hydrolysis, X-factor and V-factor dependency, growth in the presence of 10 % (v/v) CO2, growth under anaerobic conditions, growth at 20 °C, growth at 30 °C, growth at 42 °C and growth on MacConkey agar as well as acid production from L-arabinose, D-ribose, D-xylene, dulcitol, D-fructose, L-fucose, D-galactose, D-glucose, D-mannitol, D-mannose, melibiose, D-sorbitol, sucrose, L-rhamnose, inulin, lactose, maltose, raffinose, sucrose, salicin and trehalose. Acid production from glucose was tested in Hugh & Leifson’s medium. Pasteurella multocida subsp. multocida NCTC 10322T was included as a reference.

The Easy-DNA kit (Invitrogen) was used for DNA preparation according to the manufacturer’s instructions. The partial rpoB gene sequence of the 17 isolates (Table S1, available in the online Supplementary Material) was determined as reported previously (Korczak et al., 2004; Mollet et al., 1997) and covered the region 509–680 (positions refer to Escherichia coli K-12, association number U00096) of the deduced protein sequence as reported previously (Angen et al., 2003; Korczak et al., 2004). Based on the results from the phylogenetic analysis of the rpoB sequences, a subset of seven isolates were selected for 16S rRNA sequencing as described by Angen et al. (2003) and Christensen et al. (2002). Sequencing was performed by Macrogen Europe (Amsterdam, the Netherlands). The resulting sequences were compared to existing gene sequences in GenBank using BLAST (Altschul et al., 1997; Benson et al., 2007). Pairwise comparisons were performed by the program WATER included in EMBOS.

Multiple alignment was performed by CLUSTAL X (Thompson et al., 1997). Maximum-likelihood analysis including bootstrap analysis was performed by fastDNAml (Felsenstein, 1995; Olsen et al., 1994). The analysis was run with a transition/transversion ratio of 1.3 and 1.5 for rpoB and 16S rRNA gene sequences, respectively.

The isolates formed a monophyletic group closely related to the genus Bisgaardia within the family Pasteurellaceae (Figs 1 and S1). Within the group, gene sequence similarity was 97.2–100 % and 99.2–99.8 % for rpoB and 16S rRNA gene sequences, respectively. According to 16S rRNA gene sequence analysis, the most closely related species with a validly published name was B. hudsonensis with 96.9 % similarity, while the most closely related species based on rpoB sequence comparison was Bisgaardia genomospecies 1 with an rpoB sequence similarity of 90.9 %.

According to the minimal standards, 16S rRNA gene sequence similarity is greater than 89 % within the family Pasteurellaceae and within a genus is usually higher than 93 % (Korczak & Kuhnert, 2008), but generally below approximately 95 % between genera (Christensen et al., 2007). To separate species within a genus, other genotypic methods should be applied if the 16S rRNA gene sequence similarities to established species are higher than 97.0 % (Tindall et al., 2010). Likewise, the maximum similarity of partial rpoB gene sequences for delimiting genera of the family Pasteurellaceae has been suggested as 77 %, whereas 87 % similarity has been proposed for delimiting species within genera (Christensen et al., 2007).

Using these limits as criteria to define novel species of the family Pasteurellaceae, the strains from northern elephant seals are likely to represent a novel species in the genus Bisgaardia within the family Pasteurellaceae.

All 17 isolates were Gram-stain-negative, non-motile rods. All were catalase- and oxidase-positive and produced acid fermentatively from glucose without gas in Hugh & Leifson’s medium. Extended phenotypic characterization was performed for eight strains: Voodoo2, Topsy2, WildatricT, Makenna, Be Right1, Melba1, Be Right2 and Adagio2.

Phenotypic results are listed in the species description. The novel species demonstrates all the phenotypic
characteristics of the genus *Bisgaardia* (Foster et al., 2011) except for two. The novel species is negative for acid production from D-mannitol and trehalose, while members of the genus *Bisgaardia* are positive and, therefore, an emended description of the genus *Bisgaardia* is necessary. However, it is also noteworthy that the current genus description was only based on one species, *B. hudsonensis*. *Bisgaardia* genomospecies 1 definitely represents a potential third species of the genus *Bisgaardia*, but no differential phenotypic characteristics that separating it from *B. hudsonensis* have been observed yet. The novel species can be separated from *B. hudsonensis* by a total of seven phenotypic characteristics (Table 1).

It is evident from the phylogenetic analysis presented, that the northern elephant seal strains represent a distinct genotypic lineage within the genus *Bisgaardia*, which can be distinguished from the currently described members of the genus *Bisgaardia* by phenotypic characteristics shown in Table 1. On this basis, we propose that the strains should be classified as representatives of a novel species, *Bisgaardia miroungae* sp. nov., within the family *Pasteurellaceae*.

*Bisgaardia miroungae* sp. nov. was isolated from all the animals sampled in the study, suggesting that this species may be adapted to the tribe *Miroungini* (elephant seals) and the genus *Bisgaardia* to the family *Phocidae* (true seals), respectively. However, further studies including more seals and additional members, especially of the subfamily *Monachinae* (elephant-, monk- and lobodontine seals), are needed to support these host associations.

**Emended description of the genus *Bisgaardia***

Foster et al. 2011

In addition to the phenotypic characteristics given in the genus description by Foster et al. (2011), variable reactions are obtained for acid production from D-mannitol and trehalose.
Table 1. Phenotypic characteristics separating Bisgaardia miroungae sp. nov. from B. hudsonensis (Foster et al., 2011)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bisgaardia miroungae sp. nov.</th>
<th>B. hudsonensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at 42 °C</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Acid production</td>
<td>from:</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Trehalose</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

Description of Bisgaardia miroungae sp. nov.

Bisgaardia miroungae (mi.rou’gæe N.L. gen. n. miroungae of Mirounga, isolated from the northern elephant seal Mirounga angustirostris).

Colonies grown on BA are circular, grey, opaque, raised, entire, smooth, and pinpoint to 0.5 mm in diameter after 24 h of incubation at 37 °C. A very slight green discoloration of the agar is often apparent, particularly around colonies incubated for 48 h or more. In addition to the phenotypic characteristics given in the genus description, growth occurs in the presence of 10% (v/v) CO2, under anaerobic conditions and at 20 °C, 30 °C and 42 °C. Negative reactions are obtained for gelatin hydrolysis, methyl red and ONPG. Acid is produced from l-fucose, inulin, lactose, maltose, melibiose, raffinose, l-rhamnose, d-ribose and salicin, but not from l-arabinose, dulcitol, d-mannitol, sucrose or trehalose. Variable reactions are obtained for d-fructose (strains Voodoo2, Be Right2 and Melba1 are positive).

The type strain, Wildstrac7 (=CCUG 65148T=DSM 28141T), was isolated from the oral cavity of a wild northern elephant seal at The Marine Mammal Center, CA, USA in 2011. The pathogenic potential is unknown.

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