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Corynebacterium pelargi sp. nov., isolated from the trachea of white stork nestlings

Peter Kämpfer,[1] Leszek Jerzak,[2] Marcin Bochenski,[2] Mariusz Kasprzak,[2] Gottfried Wilharm,[3] Jan Golke,[4] Hans-Jürgen Busse[4] and Stefanie P. Glaeser[1]

Correspondence
Peter Kämpfer
peter.kaempfer@umwelt.uni-giessen.de

[1]Institut für Angewandte Mikrobiologie, Universität Giessen, Giessen, Germany

[2]Faculty of Biological Sciences, University of Zielona Góra, Poland

[3]Robert Koch-Institut, Bereich Wernigerode, Wernigerode, Germany

[4]Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

A Gram-stain-positive, pleomorphic, oxidase-negative, non-motile isolate from the trachea of a white stork from Poland, designated strain 136/3^T, was subjected to a comprehensive taxonomic investigation. A comparative analysis of the 16S rRNA gene sequence showed highest similarities to *Corynebacterium mustelae*, *Corynebacterium pseudotuberculosis*, *Corynebacterium vitaeruminis* and *Corynebacterium ulcerans* (96.0–96.3 %). The quinone system consisted of major amounts of MK-8(H_2), minor amounts of MK-9(H_2) and traces of MK-8 and MK-9. The polar lipid profile of strain 136/3^T contained phosphatidylinositol and phosphatidylinositol-mannoside as major lipids and phosphatidylglycerol and an acidic glycolipid in moderate amounts. In addition small amounts of diphosphatidylglycerol, a phospholipid, an aminolipid and two lipids of unknown group affiliation were found. The polyamine pattern was composed of the major components spermidine and spermine. Putrescine, 1,3-diaminopropane, cadaverine, *sym*-homospermidine and tyramine were found in minor or trace amounts. The diamino acid of the peptidoglycan was *meso*-diaminopimelic acid. In the fatty acid profile straight-chain, saturated and mono-unsaturated fatty acids predominated ($C_{18:1}\omega9c$, $C_{16:1}\omega7c$, $C_{16:0}$, $C_{18:0}$). Corynemycolic acids were detected. Physiological traits as well as unique traits of the polar lipid profile and the fatty acid pattern distinguished strain 136/3^T from the most closely related species. All these results indicate that strain 136/3^T represents a novel species of the genus *Corynebacterium* for which we propose the name *Corynebacterium pelargi* sp. nov. The type strain is 136/3^T (=CIP 110778^T=CCM 8517^T=LMG 28174^T).

The genus *Corynebacterium* comprises, at the time of writing, more than 80 recognized species with validly published names, many of them isolated from animals and human clinical material including *Corynebacterium canis* (Funke *et al.*, 2010a), *Corynebacterium epidermidicanis* (Frischmann *et al.*, 2012), *Corynebacterium mustelae* (Funke *et al.*, 2010b), *Corynebacterium pilbarense* (Aravena-Roman *et al.*, 2010) and others. The majority of these species contain corynemycolic acids (mycolic acids with 22–38 carbons; Collins *et al.*, 1982a) but in some of the species mycolic acids are absent, among them *Corynebacterium amycolatum*, *Corynebacterium atypicum*, *Corynebacterium caspium*, *Corynebacterium ciconiae* and *Corynebacterium kroppenstedtii* and *Corynebacterium lactis* (Collins *et al.*, 1988, 1998, 2004; Fernández-Garayzábal *et al.*, 2004; Hall *et al.*, 2003; Wiertz *et al.*, 2013). The major quinone types represent dihydrogenated menaquinones with eight or nine isoprenoic units in the side chain [MK-8(H_2), MK-9(H_2) or a mixture of both] (Collins & Jones, 1981).

In the fatty acid patterns most often straight-chain saturated and mono-unsaturated fatty acids are found. Some species possess tuberculostearic acid as well (Collins *et al.*, 1982b). The peptidoglycan type is A1γ with the diagnostic diamino acid *meso*-diaminopimelic acid (Schleifer & Kandler, 1972).

In the course of a study dealing with the classification of bacteria isolated from nestlings of white stork (*Ciconia ciconia*) in Poland in the year 2013, strain 136/3^T was isolated from a choana (posterior nasal aperture) swab.

Strain 136/3^T grew well aerobically on nutrient rich media such as tryptone soy agar and nutrient agar (both Oxoid),

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 136/3^T is KF986249.

3.3 × PYE agar (0.99 % peptone from casein, 0.99 % yeast extract, 1.5 % agar, pH 7.2) at 28 °C and in the corresponding liquid medium and was also able to grow on Columbia III agar (Becton Dickinson) supplemented with 5 % sheep blood but not on MacConkey II agar (Becton Dickinson). Anaerobic growth on this medium was also observed.

Gram staining was done as previously described according to the method of Gerhardt *et al.* (1994). Cell morphological characteristics were observed under a Zeiss light microscope at × 1000 magnification. The ability of the strain to grow at various pH values was determined in nutrient broth (Oxoid) which was adjusted prior to sterilization to pH 3–11 (at 0.5 pH unit intervals). Growth at different temperatures in the range of 10–55 °C (in 5 °C intervals) was also investigated in nutrient broth. The strain grew well at temperatures between 10 °C and 35 °C and not at 5 °C and below or 40 °C and above. In addition, the strain grew well at a pH range of 5.5 to 9.5. Weak growth was observed at a pH of 4.5.

The morphological, physiological and biochemical characteristics of strain 136/3^T are given in detail in the species description and in Table 1. Physiological/biochemical characterization was performed to assess the carbon source utilization pattern and hydrolysis of chromogenic substrates according to the methods of Kämpfer *et al.* (1991) at 30 °C and in addition by using the API Coryne kit (bioMérieux) at 35–37 °C. The CAMP test with *Staphylococcus aureus* ATCC 25923 was performed according to the protocol of Gerhardt *et al.* (1994).

For 16S rRNA gene-based phylogenetic analysis the nearly full-length 16S rRNA gene of strain 136/3^T was PCR-amplified and sequenced by the Sanger method with universal primers fd1 and rp1 (Weisburg *et al.*, 1991). Sequence similarities were calculated and phylogenetic trees were reconstructed with ARB release 5.2 (Ludwig *et al.*, 2004) using the 'All-Species Living Tree' Project (LTP; Yarza *et al.*, 2008) database release LTPs115 (March 2014). Sequences not included in the database were aligned with SINA (v1.2.9) according to the SILVA seed alignment (Pruesse *et al.*, 2012) and imported into the database. The alignment of sequences selected for the phylogenetic analysis was checked manually including secondary structure information. Sequence similarities were calculated with the ARB neighbour-joining tool without the use of an evolutionary substitution model. Phylogenetic trees were reconstructed with the neighbour-joining method and the Jukes–Cantor correction model (Jukes & Cantor, 1969), the maximum-likelihood method using RAxML v7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis, and the maximum-parsimony method using DNAPARS v 3.6 (Felsenstein, 2005). Final trees were based on 100 resamplings (bootstrap analysis; Felsenstein, 1985) and 16S rRNA gene sequences between *Escherichia coli* positions 108 and 1364 (*E. coli* numbering according to Brosius *et al.*, 1978) and included all type strains of species in the genus *Corynebacterium*.

Table 1. Cellular fatty acid composition of strain 136/3^T and related strains of species of the genus *Corynebacterium*

Strains: 1, 136/3^T; 2, *C. epidermidicanis* 410^T; 3, *C. mustelae* DSM 45274^T; 4, *C. diphtheriae* NCTC 11397^T; 5, *C. pseudotuberculosis* DSM 20689^T; 6, *C. ulcerans* DSM 46325^T; 7, *C. glutamicum* DSM 20300^T. Data were obtained with the Sherlock MIDI version 2.1 (TSBA version 4.1). TR, Traces (fatty acid amounts <1 %).

Fatty acid	1	2	3	4	5	6	7
$C_{13:0}$	–	0.3	–	–	–	–	–
$C_{14:0}$	–	1.9	–	–	2.9	4.6	TR
$C_{15:0}$	–	15.4	–	TR	–	–	–
iso-$C_{15:0}$	3.6	–	–	1.0	–	–	–
anteiso-$C_{15:0}$	2.5	–	–	–	–	–	–
iso-$C_{17:0}$	1.6	–	–	–	–	–	–
anteiso-$C_{17:0}$	1.6	–	–	1.0	–	–	–
$C_{15:1}\omega6c$	–	–	–	1.0	–	–	–
$C_{16:1}\omega5c$	–	–	–	3.1	–	–	–
$C_{16:1}\omega9c$	–	–	–	–	–	–	TR
$C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH	6.1	7.9	25.0	25.3	33.6	30.6	–
anteiso-$C_{17:1}\omega9c$	–	–	–	1.5	–	–	–
$C_{16:0}$	30.1	13.3	38.0	18.0	51.6	45.2	42.6
$C_{18:1}\omega9c$	40.0	8.2	22.9	30.6	9.4	17.6	53.6
$C_{18:1}\omega7c$	–	–	–	2.6	–	–	–
$C_{18:2}\omega9,11c$	–	12.3	4.0	–	–	–	–
$C_{17:0}$	–	6.7	3.6	–	–	–	1.1
$C_{17:0}\omega8c$	–	29.3	3.8	7.9	–	–	–
$C_{17:0}\omega6c$	–	–	–	3.2	–	–	–
$C_{18:0}$	12.8	3.6	1.9	1.3	2.6	2.3	1.0
10-Methyl $C_{18:0}$	2.5	–	–	–	–	–	–

The 16S rRNA gene sequence of strain 136/3^T represented a continuous stretch of 1419 bp in length spanning *E. coli* nucleotide positions 60 to 1497 according to Brosius *et al.* (1978). Sequence similarities for strain 136/3^T and type strains of the genus *Corynebacterium* ranged between 91.2 and 96.1 % with highest 16S rRNA gene sequence similarities to the type strains of the species *C. vitaeruminis* (96.2 %) and *C. mustelae* (96.1 %) followed by *C. ulcerans* and *C. pseudotuberculosis* (both 96 %). Phylogenetic analysis using the LTP database clearly placed strain 136/3^T into the genus *Corynebacterium* (Fig. 1). The strain clustered with *C. epidermidicanis* (95.7 % sequence similarity), *C. vitaeruminis*, *C. mustelae*, *C. ulcerans*, *C. pseudotuberculosis* and also the type species of the genus, *C. diphtheriae*. Variation in the clustering obtained by the different treeing methods and the low bootstrap values indicated that a clearer phylogenetic relationship to different species of the genus *Corynebacterium* cannot be obtained using the 16S rRNA gene sequence approach.

For production of biomass for analyses of mycolic acids, quinones, polar lipids and polyamines, strain 136/3^T was grown at 28 °C in 3.3 × PYE broth. Mycolic acids were extracted and analysed according to the protocol of Frischmann *et al.* (2012). Polar lipids and quinones were

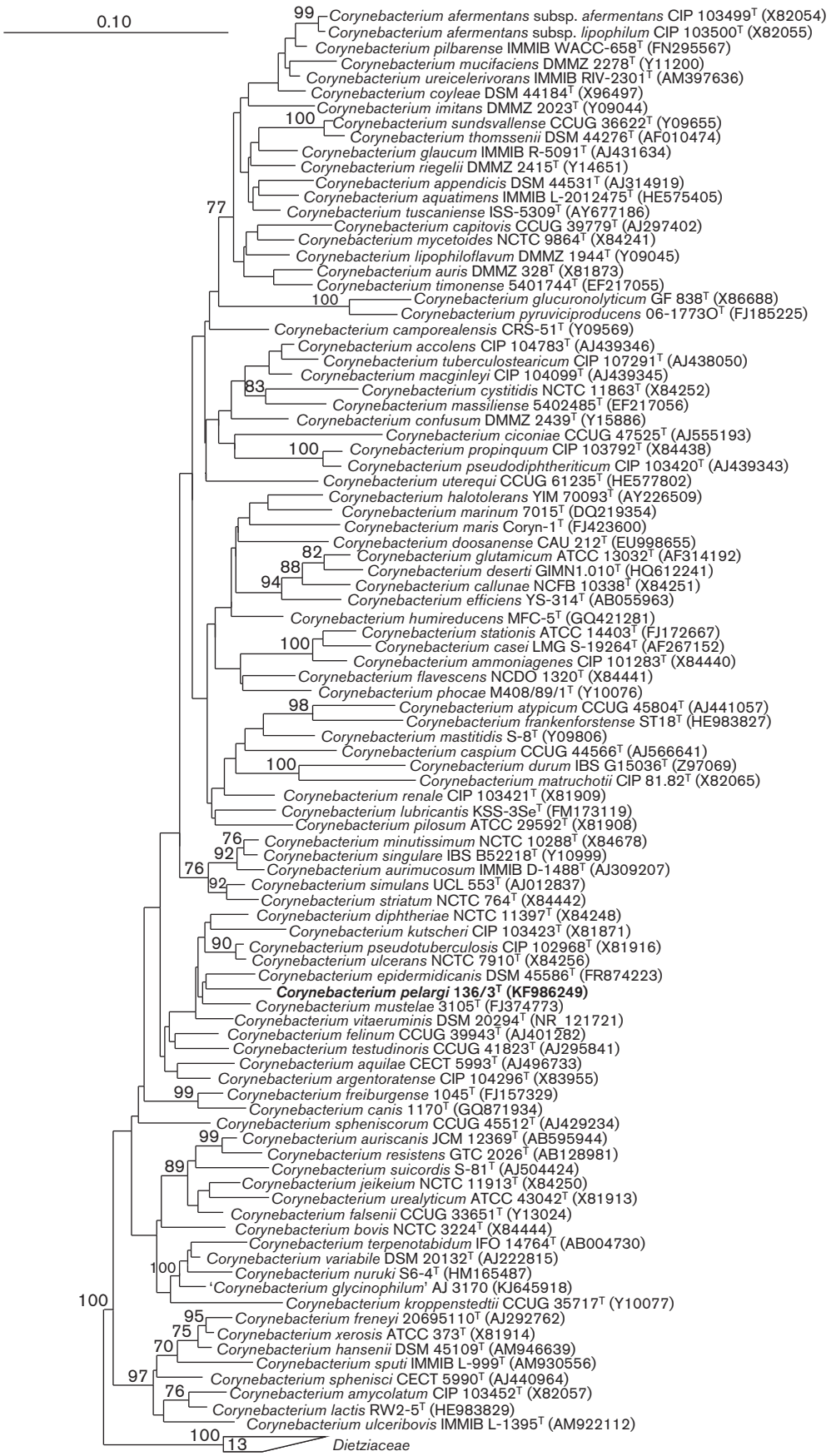
0.10
Corynebacterium afermentans subsp. afermentans CIP 103499T (X82054)
Corynebacterium afermentans subsp. lipophilum CIP 103500T (X82055)
Corynebacterium pilbarense IMMIB WACC-658T (FN295567)
Corynebacterium mucifaciens DMMZ 2278T (Y11200)
Corynebacterium ureicelerivorans IMMIB RIV-2301T (AM397636)
Corynebacterium coyleae DSM 44184T (X96497)
Corynebacterium imitans DMMZ 2023T (Y09044)
Corynebacterium sundsvallense CCUG 36622T (Y09655)
Corynebacterium thomssenii DSM 44276T (AF010474)
Corynebacterium glaucum IMMIB R-5091T (AJ431634)
Corynebacterium riegelii DMMZ 2415T (Y14651)
Corynebacterium appendicis DSM 44531T (AJ314919)
Corynebacterium aquatimens IMMIB L-2012475T (HE575405)
Corynebacterium tuscaniense ISS-5309T (AY677186)
Corynebacterium capitovis CCUG 39779T (AJ297402)
Corynebacterium mycetoides NCTC 9864T (X84241)
Corynebacterium lipophiloflavum DMMZ 1944T (Y09045)
Corynebacterium auris DMMZ 328T (X81873)
Corynebacterium timonense 5401744T (EF217055)
Corynebacterium glucuronolyticum GF 838T (X86688)
Corynebacterium pyruviciproducens 06-1773OT (FJ185225)
Corynebacterium camporealensis CRS-51T (Y09569)
Corynebacterium accolens CIP 104783T (AJ439346)
Corynebacterium tuberculostearicum CIP 107291T (AJ438050)
Corynebacterium macginleyi CIP 104099T (AJ439345)
Corynebacterium cystitidis NCTC 11863T (X84252)
Corynebacterium massiliense 5402485T (EF217056)
Corynebacterium confusum DMMZ 2439T (Y15886)
Corynebacterium ciconiae CCUG 47525T (AJ555193)
Corynebacterium propinquum CIP 103792T (X84438)
Corynebacterium pseudodiphtheriticum CIP 103420T (AJ439343)
Corynebacterium uterequi CCUG 61235T (HE577802)
Corynebacterium halotolerans YIM 70093T (AY226509)
Corynebacterium marinum 7015T (DQ219354)
Corynebacterium maris Coryn-1T (FJ423600)
Corynebacterium doosanense CAU 212T (EU998655)
Corynebacterium glutamicum ATCC 13032T (AF314192)
Corynebacterium deserti GIMN1.010T (HQ612241)
Corynebacterium callunae NCFB 10338T (X84251)
Corynebacterium efficiens YS-314T (AB055963)
Corynebacterium humireducens MFC-5T (GQ421281)
Corynebacterium stationis ATCC 14403T (FJ172667)
Corynebacterium casei LMG S-19264T (AF267152)
Corynebacterium ammoniagenes CIP 101283T (X84440)
Corynebacterium flavescens NCDO 1320T (X84441)
Corynebacterium phocae M408/89/1T (Y10076)
Corynebacterium atypicum CCUG 45804T (AJ441057)
Corynebacterium frankenforstense ST18T (HE983827)
Corynebacterium mastitidis S-8T (Y09806)
Corynebacterium caspium CCUG 44566T (AJ566641)
Corynebacterium durum IBS G15036T (Z97069)
Corynebacterium matruchotii CIP 81.82T (X82065)
Corynebacterium renale CIP 103421T (X81909)
Corynebacterium lubricantis KSS-3SeT (FM173119)
Corynebacterium pilosum ATCC 29592T (X81908)
Corynebacterium minutissimum NCTC 10288T (X84678)
Corynebacterium singulare IBS B52218T (Y10999)
Corynebacterium aurimucosum IMMIB D-1488T (AJ309207)
Corynebacterium simulans UCL 553T (AJ012837)
Corynebacterium striatum NCTC 764T (X84442)
Corynebacterium diphtheriae NCTC 11397T (X84248)
Corynebacterium kutscheri CIP 103423T (X81871)
Corynebacterium pseudotuberculosis CIP 102968T (X81916)
Corynebacterium ulcerans NCTC 7910T (X84256)
Corynebacterium epidermidicanis DSM 45586T (FR874223)
Corynebacterium pelargi 136/3T (KF986249)
Corynebacterium mustelae 3105T (FJ374773)
Corynebacterium vitaeruminis DSM 20294T (NR_121721)
Corynebacterium felinum CCUG 39943T (AJ401282)
Corynebacterium testudinoris CCUG 41823T (AJ295841)
Corynebacterium aquilae CECT 5993T (AJ496733)
Corynebacterium argentoratense CIP 104296T (X83955)
Corynebacterium freiburgense 1045T (FJ157329)
Corynebacterium canis 1170T (GQ871934)
Corynebacterium spheniscorum CCUG 45512T (AJ429234)
Corynebacterium auriscanis JCM 12369T (AB595944)
Corynebacterium resistens GTC 2026T (AB128981)
Corynebacterium suicordis S-81T (AJ504424)
Corynebacterium jeikeium NCTC 11913T (X84250)
Corynebacterium urealyticum ATCC 43042T (X81913)
Corynebacterium falsenii CCUG 33651T (Y13024)
Corynebacterium bovis NCTC 3224T (X84444)
Corynebacterium terpenotabidum IFO 14764T (AB004730)
Corynebacterium variabile DSM 20132T (AJ222815)
Corynebacterium nuruki S6-4T (HM165487)
'Corynebacterium glycinophilum' AJ 3170 (KJ645918)
Corynebacterium kroppenstedtii CCUG 35717T (Y10077)
Corynebacterium freneyi 20695110T (AJ292762)
Corynebacterium xerosis ATCC 373T (X81914)
Corynebacterium hansenii DSM 45109T (AM946639)
Corynebacterium sputi IMMIB L-999T (AM930556)
Corynebacterium sphenisci CECT 5990T (AJ440964)
Corynebacterium amycolatum CIP 103452T (X82057)
Corynebacterium lactis RW2-5T (HE983829)
Corynebacterium ulceribovis IMMIB L-1395T (AM922112)
Dietziaceae

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences showing the relatedness of strain 136/3^T to type strains of other species of the genus *Corynebacterium*. The tree was generated in ARB using RAxML (GTR-GAMMA/Rapid bootstrap analysis) and based on 100 replications. Type strains of species of the family *Dietziaceae* were used as the outgroup. Numbers at nodes represent bootstrap values of 70 % and above. Bar, 0.1 substitutions per nucleotide position.

extracted and analysed applying the integrated procedure reported by Tindall (1990a, b) and Altenburger *et al.* (1996). Polyamines were extracted as reported by Busse & Auling (1988) and modified by Altenburger *et al.* (1997). For HPLC analyses of quinones and polyamines the equipment used was as reported by Stolz *et al.* (2007). Corynemycolic acids could be detected (results not shown).

The polyamine pattern was composed of spermidine [1.48 μmol (g dry weight)$^{-1}$], spermine [1.04 μmol (g dry weight)$^{-1}$], putrescine [0.09 μmol (g dry weight)$^{-1}$], 1,3-diaminopropane [0.02 μmol (g dry weight)$^{-1}$], cadaverine [0.04 μmol (g dry weight)$^{-1}$] and tyramine [0.08 μmol (g dry weight)$^{-1}$]. This polyamine pattern is in agreement with characteristics reported for several species of the genus *Corynebacterium*, which also were reported to contain predominantly spermidine and spermine (Altenburger *et al.*, 1997).

The quinone system of strain 136/3^T was composed of MK-8(H_2) (91.1 %), MK-9(H_2) (7.9 %), MK-8 (0.8 %) and traces of MK-9 (<0.1 %), which supported the affiliation to the genus *Corynebacterium* and was similar to those of the close relatives *C. pseudotuberculosis* and *C. ulcerans* both reported to contain predominantly menaquinone MK-8(H_2) (Collins *et al.*, 1977).

Strain 136/3^T showed a polar lipid profile typical for a species of the genus *Corynebacterium* (Fig. 2). The major compounds were phosphatidylinositol, a phosphatidylinositol-mannoside, phosphatidylglycerol and an acidic glycolipid which has been reported to be present in several species of the genus *Corynebacterium* (Minnikin *et al.*, 1977). In addition, small amounts of diphosphatidylglycerol, a phosholipid, an aminolipid and two lipids of unknown group affiliation (L1 and L2) were detected. Qualitatively, this polar lipid profile resembled that of *C. ulcerans* but the major compound diphosphatidylglycerol in the latter species was a major distinguishing trait (Frischmann *et al.*, 2012). Less similarity in the polar lipid profile was detected with *C. pseudotuberculosis*, which has been reported to contain two highly hydrophobic glycolipids (Frischmann *et al.*, 2012) not detected in strain 136/3^T.

Production of biomass and extraction and analysis of fatty acids was carried out as described by Kämpfer & Kroppenstedt (1996). The fatty acid profile (Table 1) was composed predominantly of straight chain, saturated and mono-unsaturated fatty acids and major fatty acids were $C_{18:1}\omega9c$, $C_{16:1}\omega7c$ /iso-$C_{15:0}$ 2-OH, $C_{16:0}$ and $C_{18:0}$. Tuberculostearic acid could also be detected. This fatty acid profile was qualitatively quite similar to those of other species of the genus *Corynebacterium* such as *C. diphtheriae* NCTC 11397^T, *C. pseudotuberculosis* DSM 20689^T, *C. ulcerans* DSM 46325^T and *C. glutamicum* DSM 20300^T but significant quantitative differences in the contents of several fatty acids were detected between the strains under comparison (Table 1).

The results of the comparative physiological characterization using identical test conditions (Kämpfer *et al.*, 1991) for the type strains of the species *C. epidermidicanis* 410^T, *C. mustelae* DSM 45274^T, *C. pseudotuberculosis* DSM 20689^T, *C. ulcerans* DSM 46325^T and *C. glutamicum* DSM 20300^T are shown in Table 2. API Coryne code: 3-1-4-0-1-0-4.

The results from 16S rRNA gene sequence analyses and the differences in phenotype (polar lipid profiles and physiology) indicate that strain 136/3^T is different from strains of other species of the genus *Corynebacterium*.

In conclusion, strain 136/3^T is a representative of a novel species of the genus *Corynebacterium* for which we propose the name *Corynebacterium pelargi* sp. nov.

Description of *Corynebacterium pelargi* sp. nov.

Corynebacterium pelargi [pe′lar.gi. Gr. n. *pelargos* a stork; N.L. gen. n. *pelargi* of a stork, isolated from a white stork in Poland].

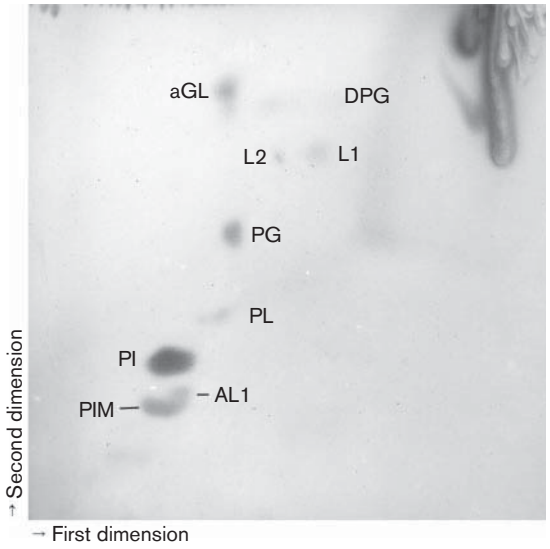

Fig. 2. Polar lipid profile of strain 136/3^T after two-dimensional TLC and detection with molybdatophosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol-mannoside; aGL, acidic glycolipid, L1–2, unidentified lipids; PL, unidentified phospholipid; AL1, unidentified aminolipid.

Table 2. Physiological test results for strain 136/3^T and related strains of species of the genus *Corynebacterium*

Strains: 1, 136/3^T; 2, *C. epidermidicanis* 410^T; 3, *C. mustelae* DSM 45274^T; 4, *C. diphtheriae* NCTC 11397^T; 5, *C. pseudotuberculosis* DSM 20689^T; 6, *C. ulcerans* DSM 46325^T; 7, *C. glutamicum* DSM 20300^T. +, Positive reaction; (+) weakly positive reaction; −, negative reaction. Test results are based on tests performed with the API Coryne system. The tests were performed according to the manufacturer's instructions. All strains were negative for pyrolidonyl arylamidase, β-glucuronidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, gelatin hydrolysis, and acid production from xylose, mannitol and lactose and positive for acid production from glucose.

Test	1	2	3	4	5	6	7
Nitrate reduction	+	−	−	+	−	−	+
Pyrazinamidase	+	+	(+)	−	−	−	+
Alkaline phosphatase	(+)	+	(+)	−	−	+	−
α-Glucosidase	−	−	(+)	+	(+)	+	−
β-Glucosidase (aesculin)	+	−	+	−	−	−	−
Urease	−	−	−	−	+	+	+
Acid production from:							
Ribose	−	−	+	+	+	+	+
Maltose	−	+	+	+	+	+	+
Sucrose	−	−	+	−	−	−	+
Glycogen	−	−	−	−	−	+	−
Catalase	+	−	+	(+)	+	+	+

Cells are Gram-staining-positive, non-spore-forming, coccoid, sometimes irregular, non-motile rods, which grow both aerobically and anaerobically. On tryptone soy agar the strain produces creamy-whitish to beige, non-translucent colonies with a diameter of approximately 0.5 mm. The cells are not typically rods but coccoid to irregular rods. Oxidase-negative. Catalase-positive. Good growth occurs on nutrient-rich media, like tryptone soy and nutrient agar, PYE agar and blood agar but not on MacConkey agar. CAMP-negative with *S. aureus*. On nutrient agar growth is observed at temperatures between 10 °C and 35 °C and not at 5 °C or below or at 40 °C or above. Grows well at a pH range of 5.5 to 9.5. Weak growth is observed at a pH of 4.5. Corynemycolic acids are present. The quinone system consists of major amounts of MK-8(H_2), minor amounts of MK-9(H_2) and traces of MK-8 and MK-9. The polar lipid profile of strain 136/3^T is composed of phosphatidylinositol and a phosphatidylinositol-mannoside as major lipids and phosphatidylglycerol and an acidic glycolipid in moderate amounts. In addition small amounts of diphosphatidylglycerol, a phosholipid, an aminolipid and two lipids of unknown group affiliation are detected. The polyamine pattern is composed of the major compounds spermidine and spermine and minor or trace amounts of 1,3-diaminopropane, putrescine, cadaverine, *sym*-homospermidine and tyramine. The diamino acid in the cell is *meso*-diaminopimelic acid. The fatty acid profile contains large amounts of $C_{18:1}\omega9c$, $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH, $C_{18:0}$ and $C_{16:0}$. Tuberculostearic acid is present. API Coryne results: nitrate reduction, pyrazinamidase, aesculin hydrolysis and acid formation from D-glucose are positive. α-Glucosidase, pyrolidonyl amidase, β-glucuronidase, β-galactosidase, *N*-actetyl-β-D-glucosaminidase, urease, gelatinase and acid production from ribose, maltose, sucrose, xylose, mannitol, lactose and glycogen are negative. The API coryne code is 3-1-4-0-1-0-4. On the basis of the methods according to Kämpfer *et al.* (1991), *N*-acetyl-D-glucosamine, D-fructose, D-galactose, D-gluconate, D-glucose, D-mannose, D-ribose, acetate, propionate, fumarate, citrate and pyruvate are assimilated but *N*-acetyl-D-galactosamine, L-arabinose, *p*-arbutin, cellobiose, α-melibiose, L-rhamnose, sucrose, salicin, D-xylose, adonitol, i-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, *cis*-aconitate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, glutarate, DL-3-hydroxybutyrate, itaconate, mesaconate, oxoglutarate, suberate, L-alanine, β-alanine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate are not. Acid is produced (weakly) from glucose, but not from lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl-D-glucoside, erythritol, melibiose, D-arabitol or D-mannitol. Aesculin, bis-*p*-nitrophenyl (NP) phosphate, *p*NP-phenylphosphonate and 2-deoxythymidine-5′-*p*NP phosphate are hydrolysed. ONPG, *p*NP-β-D-glucuronide, *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, *p*NP-β-D-xylopyranoside, *p*NP-phosphorylcholine, 2-deoxythymidine-5′-*p*NP phosphate and L-glutamate-γ-3-carboxy-*p*NA are not hydrolysed.

The type strain is 136/3^T (=CIP 110778^T=CCM 8517^T=LMG 28174^T), isolated from the trachea of a white stork (*Ciconia ciconia*) from Poland.

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