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Coetzeea brasiliensis gen. nov., sp. nov. isolated from larvae of *Anopheles darlingi*

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A Gram-stain-negative, rod-shaped strain, Braz8^T, isolated from larvae of *Anopheles darlingi* was investigated using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain Braz8^T was related most closely to species of the genus *Thorsellia*, with 95.6, 96.5 and 96.6 % similarity to the type strains of *Thorsellia anophelis*, *Thorsellia kandunguensis* and *Thorsellia kenyensis*, respectively, and formed a separate branch in the phylogenetic tree next to the monophyletic cluster of the genus *Thorsellia*. Chemotaxonomic data supported the allocation of the strain to the family *Thorselliaceae*. The major fatty acids were $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{14:0}$. The quinone system was composed of ubiquinones Q-8 and Q-7 (1 : 0.3), the predominant polar lipids were diphosphatidylglycerol and phosphatidylglycerol, and the polyamine pattern showed the major compound putrescine. However, qualitative and quantitative differences in the major polyamine, polar lipid profile and fatty acid patterns distinguished strain Braz8^T from species of the genus *Thorsellia*. Phylogenetic analysis based on 16S rRNA gene sequences, average nucleotide identity, DNA–DNA hybridization, multilocus sequence analysis as well as physiological and biochemical tests distinguished strain Braz8^T both genotypically and phenotypically from the three *Thorsellia* species but also showed its placement in the family *Thorselliaceae*. Thus, strain Braz8^T is considered to represent a novel species of a new genus most closely related to the genus *Thorsellia*, for which the name *Coetzeea brasiliensis* gen. nov., sp. nov. is proposed. The type strain of *Coetzeea brasiliensis* is Braz8^T (=LMG 29552^T=CIP 111088^T).

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; MLSA, multilocus sequence analysis; oNP, *ortho*-nitrophenyl; pNA, *para*-nitroanilide; pNP, *para*-nitrophenyl.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and protein coding gene sequences of strain Braz8^T are KU748636 (16S rRNA gene), KU748637 (*recA*), KU748638 (*recN*), KU748639 (*rpoA*), KU748640 (*rpoB*), KU748641 (*rpoD*) and KU748642 (*gyrB*). Raw reads for *Coetzeea brasiliensis* Braz8^T were submitted to the NCBI Sequence Read Archive under accession SRL1712517. Raw-reads accession numbers are: for *Thorsellia anophelis* (CCUG 49520^T) SRX712852, for *Thorsellia kandungensis* (W5.1.1^T) SRX734467 and for *Thorsellia kenyensis* (T2.1^T) SRX719216.

One supplementary figure is available with the online Supplementary Material.

The genus *Thorsellia* was initially proposed by Kämpfer *et al.* (2006) based on the single species *Thorsellia anophelis* as a member of the class *Gammaproteobacteria*. Two additional species, *Thorsellia kenyensis* and *Thorsellia kandunguensis,* were subsequently described and a novel family *Thorselliaceae* was proposed for these unusual organisms (Kämpfer *et al.*, 2015). All species were isolated from the midgut of mosquitoes originating from Kenya (Lindh *et al.*, 2005; Kämpfer *et al.*, 2015). Here, we characterize an additional strain, Braz8^T, isolated from the larvae of *Anopheles darlingi*, which is the major neotropical malaria vector (Marinotti *et al.*, 2013; Laporta *et al.*, 2015). The strain showed a close relationship to, but a clear distinction from, species of the genus *Thorsellia*.

Strain Braz8^T was isolated after plating supernatant from crushed mosquito larvae on Luria Bertani agar plates (LB; Sigma-Aldrich). The plates were incubated at 30 °C for 2 days. Subcultivation was done on nutrient agar (NA; Oxoid) at 28 °C for 48 h. Gram-staining with the Hucker method was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at 1000× magnification using cultures grown on NA for 2 days at 28 °C. Oxidase activity was tested using oxidase reagent (bioMérieux) according to the manufacturer's instructions. Catalase activity was tested by gas formation after dropping 2 % (v/v) H_2O_2 on the fresh biomass grown on NA for 48 h at 28 °C. Results are given in the species description.

Nearly full-length 16S rRNA gene sequences were PCR-amplified using universal primers 8F (5′-AGAGTTTGA TCCTGGCTCAG-3′) and 1492R (5′-ACGGCTACCTTG TTACGACTT-3′; Lane, 1991) from cell lysates of a loop of biomass generated by three freezing (−20 °C) and heating (100 °C, 1 min) cycles. PCR products were sequenced by the Sanger sequencing method using primers 27F (5′-GAG TTTGATCMTGGCTCAG-3′) and E786F (5′-GATTAGA TACCCTGGTAG-3′; Coloqhoun, 1997). Sequence processing and manual correction based on electropherograms was performed in MEGA 5 (Tamura *et al.*, 2011). The obtained 16S rRNA gene fragment of strain Braz8^T was a continuous stretch of 1391 bp spanning gene termini 82–1481 (according to *Escherichia coli rrnB*; Brosius *et al.,* 1978).

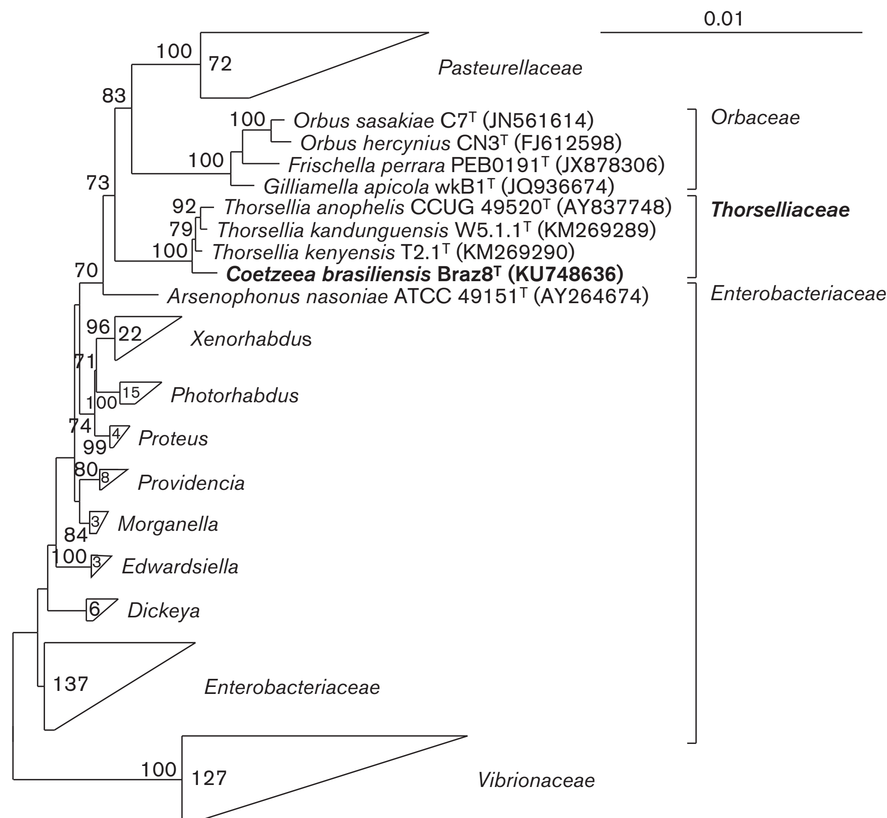

Fig. 1. Maximum-likelihood tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic position of strain Braz8^T next to species of the genus *Thorsellia* within the family *Thorselliaceae*. The phylogenetic tree was generated in ARB using RAxML with GTR-GAMMA and 100 re-samplings (bootstrap analysis) and based on sequences between sequence positions 107 and 1408 according to the *E. coli* numbering (Brosius *et al.*, 1978). Only bootstrap values >70 % are given at nodes. Numbers in clusters represent the number of type strains included in the cluster shown. Bar, 0.01 nucleotide substitutions per site.

Phylogenetic analysis based on nearly full-length 16S rRNA gene sequences was performed in ARB (Ludwig *et al.*, 2004) using the 'All-Species Living Tree' (LTP) ARB database (Yazar *et al.*, 2008) release LTPs111 (February 2013). Sequences not present in the database were added to the database after pre-alignment with SINA (v. 1.2.11; Pruesse *et al.*, 2012). The final alignment was trimmed to keep only the region spanning *E. coli* positions 107–1408, the gene region which was covered by all sequences included in the analysis. Phylogenetic trees were inferred by maximum-likelihood using RAxML v7.04 (Stamatakis, 2006) with the evolutionary model GTR-GAMMA and rapid bootstrap analysis, and by neighbour-joining using ARBNeighbor-joining, with the Jukes–Cantor substitution model, both using bootstrap analysis based on 100 replications. Pairwise sequence similarities were calculated with the ARBNeighbor joining tool without the application of an evolutionary model. Strain Braz8^T shared 95.6, 96.5 and 96.6 % 16S rRNA gene sequence similarity with the type strains of *T. anophelis*, *T. kandunguensis* and *T. kenyensis*, respectively. Sequence similarities to type strains of species in the next closest related families were below 93 %.

Phylogenetic trees, the maximum-likelihood tree in Fig. 1 and a neighbour-joining tree (data not shown), showed that strain Braz8^T formed a separate branch next to the monophyletic cluster of the three *Thorsellia* species. The broader cluster containing all four strains was clearly separated from all other families within the *Gammaproteobacteria*, indicating the assignment of strain Braz8^T to the family *Thorselliaceae* on the basis of 16S rRNA gene sequence phylogeny.

Draft genome sequences of strain Braz8^T, *T. kandunguensis* W5.1.1^T, *T. kenyensis* T2.1^T and *T. anophelis* CCUG 49520^T were used for detailed genotypic comparison. Pairwise average nucleotide identity (ANI) values between the draft genomes of strain Braz8^T and the three *Thorsellia* type strains were calculated in the EzGenome ANI tool (http://ezgenome.ezbiocloud.net/ezg_ANI) using the algorithm of Goris *et al.* (2007). Pairwise ANI values for strain Braz8^T and *T. anophelis* CCUG 49520^T were 70.9 % (reciprocal 70.9 %), for Braz8^T and *T. kandunguensis* W5.1.1^T 70.8 % (reciprocal 71.1 %), and for Braz8^T and *T. kenyensis* T2.1^T 70.1 % (reciprocal 70.3 %). *In silico* DNA–DNA hybridization (DDH) values were calculated using GGDC 2.0 (Meier-Kolthoff *et al.*, 2013) and found to be 25.5, 25.3 and 27.9 % between strain Braz8^T and *T. anophelis* CCUG 49520^T, *T. kandunguensis* W5.1.1^T and *T. kenyensis* T2.1^T, respectively. All ANI values were clearly below the proposed species boundary cut-off of 95–96 % (Richter &

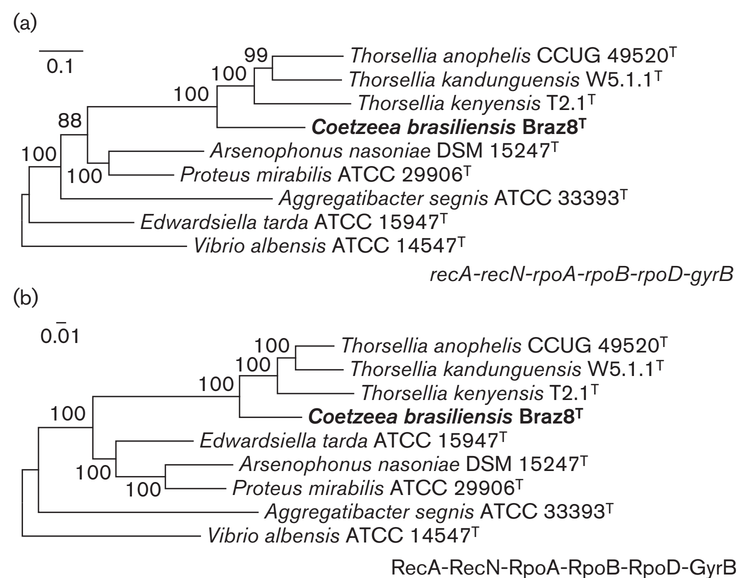

Fig. 2. Maximum-likelihood trees based on concatenated nucleotide (a) and amino acid (b) sequences of six housekeeping genes (*recA*, *recN*, *rpoA*, *rpoB*, *rpoD* and *gyrB*) representing the phylogenetic placement of strain Braz8^T next to species of the genus *Thorsellia* within the family *Thorselliaceae*. A total of 11 883 nucleotide or 3961 amino acid positions were included in the analysis. The trees were reconstructed in MEGA5. The type strain of *Vibrio albensis* was used as an outgroup. Accession numbers of gene sequences for strain Braz8^T, *T. anophelis* CCUG 49520^T, *T. kenyensis* T2.1^T and *T. kandunguensis* W5.1.1^T are KU748637, KM350519–KM350521 (*recA*), KU748638, KM350522–KM350524 (*recN*), KU748639, KM350525–KM350527 (*rpoA*), KU748640, KM350528–KM350530 (*rpoB*), KU748641, KM350531–KM350533 (*rpoD*) and KU748642, KM350516–KM350518 (*gyrB*), respectively. Gene sequences for *Arsenophonus nasoniae* DSM 15247^T, *Proteus mirabilis* ATCC 29906^T, *Aggregatibacter segnis* ATCC 33393^T, *Edwardsiella tarda* ATCC 15947^T and *Vibrio albensis* ATCC 14547^T were taken from genome data published under following NCBI bio project accession numbers PRJNA185551, PRJNA31523, PRJNA53019, PRJNA39897 and PRJNA224116, respectively. Numbers at nodes represent bootstrap values. Values <70 % are not indicated. Bar, 0.1 nucleotide (a) and 0.01 amino acid (b) substitutions per site.

Table 1. Differential characteristics between strain Braz8^T and the type strains of *T. kenyensis*, *T. kandunguensis* and *T. anophelis* by MLSA based on six concatenated protein coding housekeeping genes

Nucleotide and amino acid sequence similarities as well as the number of signature amino acids differentiating Braz8^T from the tree *Thorsellia* type strains. A detailed overview of the signature amino acids is given in Fig. S1 (available in the online Supplementary Material).

	Length (nt)	Nucleotide sequence similarities (%) of Braz8^T to *Thorsellia* type strains	Nucleotide sequence similarities (%) of *Thorsellia* type strains among each other	Length (aa)	Amino acid sequence similarities (%) of Braz8^T to *Thorsellia* type strains	Amino acid sequence similarities (%) of *Thorsellia* type strains among each other	No. of variable amino acids	No. of signature amino acids
Concatenated protein coding sequences	11 924	76.2–77.7	78.5–81.1	3 973	85.8–87.7	88.4–91.5	–	–
recA	990	78.8–81.8	80.4–81.9	330	91.5–93.0	93.3–93.9	38	7
recN	1 661	64.7–66.8	69.1–75.2	553	69.8–68.5	70.7–81.0	242	38
rpoA	990	80.9–81.7	87.7–89.5	329	91.2–94.1	93.9–96.0	34	12
rpoB	4 025	80.1–80.8	81.8–83.5	1 341	90.8–91.6	92.7–95.3	170	48
rpoD	1 917	76.6–77.9	77.3–79.5	604	86.6–88.4	88.8–90.6	118	26
gyrB	2 405	74.6–76.0	76.1–79.9	801	85.1–88.8	87.5–91.3	146	18

Rosselló-Móra, 2009). DDH values were all very low and clearly below the species cut-off value. Slightly higher pairwise ANI (72.3–73.6 %) and *in silico* DDH (27.630.5 %) values among *Thorsellia* type strains supported the genetic distance of strain Braz8^T to the recognized *Thorsellia* species (Kämpfer *et al.*, 2015).

A six-proteine-coding housekeeping gene-based multilocus sequence analysis (MLSA) including full-length *recA*, *recN*, *rpoA*, *rpoB*, *rpoD* and *gyrB* gene sequences was performed to confirm the assignment of strain Braz8^T to the family *Thorselliaceae* and to differentiate it from the three described *Thorsellia* species. Full-length gene sequences were obtained from draft genomes of strain Braz8^T. The analysis was performed as described in detail previously (Kämpfer *et al.*, 2015). Accession numbers of gene sequences used for MLSA are summarized in the legend to Fig. 2. The placement of strain Braz8^T in the individual and concatenated nucleotide and amino acid sequence-based trees confirmed the topology of the 16S rRNA gene sequence-based tree. Strain Braz8^T always formed a separate branch next to the monophyletic cluster containing all *Thorsellia* species. Using MLSA, the four strains together also formed a broader distinct cluster supported by high bootstrap values at the level of nucleotide and amino acid sequences (Fig. 2). This indicated the distinction of members of the *Thorselliaceae* from next closest related species of other families. Pairwise sequence similarities (calculated for concatenated sequences without an evolutionary model) showed that strain Braz8^T shared lower nucleotide (76.2–77.0 %) and lower amino acid (85.8–87.7 %) sequence similarities to the type strains of *T. anophelis*, *T. kandunguensis* and *T. kenyensis* than those among each other (78.5–81.1 % for concatenated nucleotide sequences and 88.4–91.5 % for amino acid sequences). Sequence similarities to next closest related species of other families included in the analysis (Fig. 2) were clearly lower: ≤72 % for concatenated nucleotide sequences and <80 % for concatenated amino acid sequences. Single gene-based nucleotide sequence similarities between strain Braz8^T and the three *Thorsellia* type strains are given in Table 1. Comparison of variable amino acids in the amino acid sequences of the individual genes of strain Braz8^T and the *Thorsellia* species type strains showed that between seven and 48 signature amino acids were present in the amino acid sequences of the individual genes that clearly differentiated strain Braz8^T from the three *Thorsellia* type strains. A signature amino acid position was thereby defined as an amino acid position of the investigated housekeeping genes with identical amino acids for the *Thorsellia* type strains and a different amino acid for strain Braz8^T.

The genomic G+C content obtained from the genome sequence for strain Braz8^T was 38.3 mol%, which was slightly higher than for *T. anophelis* CCUG 49520^T, *T. kandunguensis* W5.1.1^{T}and *T. kenyensis* T2.1^T, which had G+C values of 36, 35 and 35 mol%, respectively (Kämpfer *et al.*, 2015).

Fatty acids were analysed as described by Kämpfer & Kroppenstedt (1996) from total cell hydrolysates after growth on triptone soy agar for 48 h at 28 °C. Growth was

assessed and colony sizes were observed at 12 h intervals before selecting the time points for generating biomass. Growth (and colony expansion) could be clearly observed after 48 h of incubation. The fatty acid profile of strain Braz8^T was compared to those from *Thorsellia* species. Detailed fatty acid profiles are given in Table 2 and are summarized in the species description.

Extraction and analyses of quinones, polar lipids and polyamines were carried out as described previously (Tindall, 1990a, b; Altenburger *et al.*, 1996; Busse & Auling, 1988; Busse *et al.*, 1997; Stolz *et al.*, 2007). The quinone system of strain Braz8^T was composed of ubiquinones Q-8 (78 %) and Q-7 (22 %), which was similar to the quinone systems of recognized *Thorsellia* species but most similar to that of *T. kenyensis* T2.1^T, which was reported to contain relatively high amounts of Q-7 in addition to the major ubiquinone Q-8 (Kämpfer *et al.*, 2006, 2015). The polar lipid profile of strain Braz8^T contained the major lipids phosphatidylethanolamine and phosphatidylglycerol. In addition, we found minor amounts of an unidentified aminolipid (AL1), an unidentified phospholipid (PL1) and an unidentified lipid (L1) only detectable after staining for total lipids (Fig. 3). The presence of phosphatidylglycerol and absence of the major lipid AL1 distinguished strain Braz8^T from *T. kenyensis*, and the polar lipid profile allowed its differentiation from *T. anophelis* and *T. kandunguensis* based on the presence of some minor lipids. The polyamine pattern of strain Braz8^T contained 51.0 μmol putrescine (g dry weight)$^{-1}$, 3.0 μmol spermidine (g dry weight)$^{-1}$, 1.2 μmol 1,3-diaminopropane (g dry weight)$^{-1}$ and 0.2 μmol spermine (g dry weight)$^{-1}$. This polyamine pattern clearly distinguished strain Braz8^T from the three recognized *Thorsellia* species, which were reported to contain the major polyamine 1,3-diaminopropane (Kämpfer *et al.*, 2006, 2015).

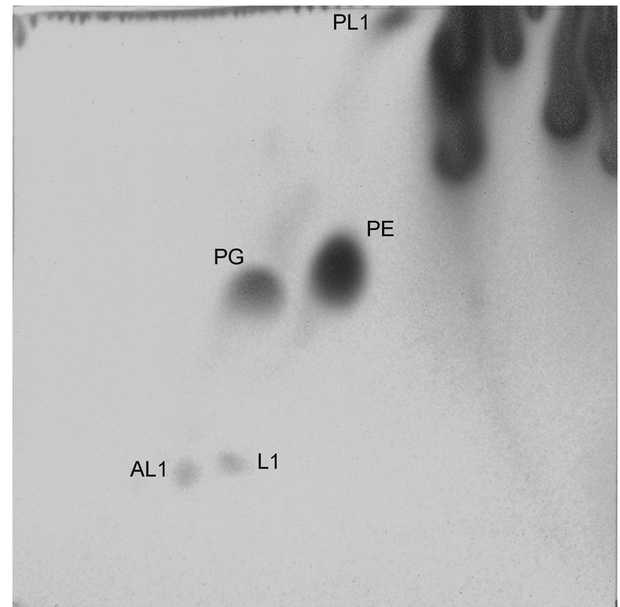

Fig. 3. Polar lipid profile of strain Braz8^T after separation by two-dimensional TLC and detection using 5 % ethanolic molybdatophosphoric acid. PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL1, unidentified aminolipid; PL1, unidentified phospholipid; L1, unidentified polar lipid not detectable with any of the spray reagents specific for lipids containing a phosphate group, an amino group or a sugar moiety.

Table 2. Cellular fatty acid content (%) of strain Braz8^T and the three *Thorsellia* type strains

Strains: 1, Braz8^T; 2, *T. kenyensis* T2.1^T; 3, *T. kandunguensis* W5.1.1^T; 4, *T. anophelis* CCUG 49520^T. Biomass of all strains was grown under the same conditions in the same laboratory. The fatty acid patterns were generated using the same method as described by Kämpfer & Kroppenstedt (1996) using an HP 6890 gas chromatograph with Sherlock MIDI software version 2.11 and a TSBA peak naming table version 4.1.

Fatty acid	1	2	3	4
Summed feature 1*	–	–	–	2.7
$C_{12:0}$	–	0.4	–	–
$C_{12:0}$ 3-OH	1.1	1.2†	–†	0.7†
$C_{14:0}$	8.1	15.7	23.7	12.2
Unknown 14.502	0.9	0.6	–	1.2
Summed feature 2*	6.0	5.0	25.5	5.4
Summed feature 3*	15.7	8.1	26.2	8.0
$C_{16:0}$	25.7	18.7	17.2	32.9
$C_{18:1}\omega 7c$	39.3	48.8	7.6	35.9
$C_{18:0}$	1.0	–	–	1.0
Summed feature 7*	2.2	1.5	–	–

*Summed features are groups of two or three fatty acids that cannot be separated using the MIDI System. Summed feature 1 contained 12:0 ALDE; summed feature 2 contained iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH; summed feature 3 contained $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH; summed feature 7 contained $C_{19:1}\omega 6c$ and/or $C_{19:0}$ cyclo $\omega 10c$. Since branched fatty acids were not detected it appears likely that summed features 2 and 3 represent $C_{14:0}$ 3-OH and $C_{16:1}\omega 7c$, respectively.

†Data were erroneously reported as $C_{12:0}$ 2-OH by Kämpfer *et al.* (2015).

The results of the physiological characterization, performed using previously described methods (Kämpfer *et al.*, 1991), are given in Table 3 and in the species description.

Genotypic analysis based on the genome sequence and physiological, biochemical and chemotaxonomic data clearly indicates that strain Braz8^T represents a novel species of the family *Thorselliaceae* but shows distinct features indicating that it represents a new genus most closely related to the genus *Thorsellia*. We propose the name *Coetzeea brasiliensis* gen. nov., sp. nov. to accommodate strain Braz8^T.

Description of *Coetzeea* gen. nov.

Coetzeea (Coet.zee′a. N.L. fem. n. Coetzeea named in honour of Maureen Coetzee from South Africa, an expert on African malaria mosquitoes).

Table 3. Distinguishing characteristics between strain Braz8^T and members of the genus *Thorsellia*

Strains: 1, Braz8^T; 2, *T. kenyensis* T2.1^T; 3, *T. kandunguensis* W5.1.1^T; 4, *T. anophelis* CCUG 49520^T. All data were obtained in this study. All strains were positive for acid production from D-glucose, D-lactose, sucrose and maltose and negative for acid production from D-sorbitol. All strains were negative for the utilization of *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, cellobiose, D-fructose, D-galactose, D-gluconate, D-glucose, D-mannose, maltose, α-melibiose, L-rhamnose, D-ribose, salicin, sucrose, D-xylose, D-adonitol, i-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, acetate, propionate, adipate, suberate, azelate, glutarate, DL-3-hydroxybutyrate, oxoglutarate, L-aspartate, *cis*-aconitate, *trans*-aconitate, L-malate, pyruvate, β-alanine, D-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzate and phenylacetate. All strains hydrolysed: aesculin, L-alanine-pNA (pNA = *para*-nitroanilide), Bis-pNP-phosphate, pNP-phenyl-phosphonate and L-proline-pNA. None of the strains hydrolysed: oNP-β-D-galactopyranoside (oNP = *ortho*-nitrophenyl-), pNP-β-D-glucuronide (pNP=*para*-nitrophenyl-), *p*-phosphoryl-choline and L-glutamate-γ-3-carboxy-pNA.

Test	1	2	3	4
Acid production from:				
D-Mannitol	+	–	–	+
Dulcitol	–	–	–	+
Salicin	+	–	+	+
D-Adonitol	–	–	–	+
i-Inositol	–	–	–	+
L-Arabinose	+	–	+	+
L-Rhamnose	(+)	–	–	+
Raffinose	(+)	–	–	–
Trehalose	–	–	+	+
Cellobiose	+	–	(+)	(+)
Erythritol	–	–	–	(+)
Melibiose	+	–	–	(+)
D-Arabitol	–	–	–	+
D-Mannose	+	–	+	+
Hydrolysis of:				
pNP-α-D-glucopyranoside	(+)	–	–	–
pNP-β-D-glucopyranoside	(+)	–	–	–
pNP-β-D-xyloside	(+)	–	–	–

Cells are Gram-stain-negative, facultatively anaerobic, motile and rod-shaped. Cells are negative for cytochrome oxidase and catalase. The fatty acid profile consists of the major fatty acids $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{14:0}$ followed by minor amounts of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH and iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH. The quinone system consists of ubiquinones Q-8 and Q-7 (1 : 0.3). The predominant polar lipids are diphosphatidylglycerol and phosphatidylglycerol. Minor amounts of three unidentified lipids are present as well (phospholipid PL1, aminophospholipid AL1 and polar lipid L1). In the polyamine pattern, putrescine is the major component. The genomic DNA G+C content is 38.3 %. The type species is *Coetzeea brasiliensis*.

Description of *Coetzeea brasiliensis* sp. nov.

Coetzeea brasiliensis (bra.si.li.en′sis. N.L. fem. adj. *brasiliensis* of or pertaining to Brazil).

Shares all characteristics given in the genus description. Cells are approx. 1 µm in width and 2 µm in length. Growth occurs after 48 h of incubation at 28 °C on tryptone soy agar and NA. In addition to the major polyamine putrescine, minor amounts of 1,3-diaminopropane, cadaverine, spermidine and spermine are present. Cells are positive for acid production from L-arabinose, cellobiose, D-glucose, D-lactose, sucrose, maltose, D-mannitol, raffinose, D-mannose and D-xylose but negative for acid production from D-sorbitol, methyl α-D-glucoside, dulcitol, salicin, D-adonitol, i-inositol, L-rhamnose, trehalose, erythritol, melibiose and D-arabitol. Unable to catabolize *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, cellobiose, D-fructose, D-galactose, D-gluconate, D-glucose, D-mannose, D-maltose, α-melibiose, L-rhamnose, D-ribose, salicin, sucrose, D-xylose, D-adonitol, i-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, acetate, propionate, adipate, suberate, azelate, glutarate, DL-3-hydroxybutyrate, oxoglutarate, L-aspartate, *cis*-aconitate, *trans*-aconitate, L-malate, pyruvate, β-alanine, D-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate. Hydrolyses aesculin, L-alanine-pNA, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-β-D-xyloside, bis-pNP-phosphate, pNP-phenyl-phosphonate and L-proline-pNA, but not oNP-β-D-galactopyranoside, pNP-β-D-glucuronide, *p*-phosphoryl-choline or L-glutamate-γ-3-carboxy-pNA.

The type strain, Braz8^T (=LMG 29552^T=CIP 111088^T), was isolated from larvae of *Anopheles darlingi.* The genomic DNA G+C content of the type strain is 38.3 % (genome sequence).

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