Characterization and phylogenomic analysis of Breznakiella homolactica gen. nov. sp. nov. indicate that termite gut treponemes evolved from non-acetogenic spirochetes in cockroaches

Yulin Song,1 Vincent Hervé,1 Renate Radek,2 Fabienne Pfeiffer,1 Hao Zheng1 and Andreas Brune1*
1Research Group Insect Gut Microbiology and Symbiosis, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Str. 10, Marburg, 35043, Germany.
2Institute of Biology/Zoology, Free University of Berlin, Königin-Luise-Str. 1-3, Berlin, 14195, Germany.

Summary
Spirochetes of the genus Treponema are surprisingly abundant in termite guts, where they play an important role in reductive acetogenesis. Although they occur in all termites investigated, their evolutionary origin is obscure. Here, we isolated the first representative of ‘termite gut treponemes’ from cockroaches, the closest relatives of termites. Phylogenomic analysis revealed that Breznakiella homolactica gen. nov. sp. nov. represents the most basal lineage of the highly diverse ‘termite cluster I’, a deep-branching sister group of Treponemataceae (fam. ‘Termitinemataceae’) that was present already in the cockroach ancestor of termites and subsequently coevolved with its host. Breznakiella homolactica is obligately anaerobic and catalyses the homolactic fermentation of both hexoses and pentoses. Resting cells produced acetate in the presence of oxygen. Genome analysis revealed the presence of pyruvate oxidase and catalase, and a cryptic potential for the formation of acetate, ethanol, formate, CO₂ and H₂ - the fermentation products of termite gut isolates. Genes encoding key enzymes of reductive acetogenesis, however, are absent, confirming the hypothesis that the ancestral metabolism of the cluster was fermentative, and that the capacity for acetogenesis from H₂ plus CO₂ - the most intriguing property among termite gut treponemes - was acquired by lateral gene transfer.

Introduction
Spirochetes (phylum Spirochaetes) are morphologically distinct bacteria with highly motile, helical cells composed of a protoplasmic cylinder and periplasmic flagella that are enclosed in an outer sheath (Paster, 2015). They are widespread in a variety of environments but typically occur only in low numbers. A notable exception is the hindgut of termites, where spirochetes predominate the bacterial microbiota, particularly in the wood-feeding taxa (Brune, 2014; Mikaelyan et al., 2015a). Here, they occur either free-living or associated with cellulolytic protists and can make up more than one-half of the bacterial population (Breznak, 2002; Breznak and Leadbetter, 2006; Ohkuma and Brune, 2011).

The high abundance of spirochetes in the hindgut of termites was first described by Joseph Leidy (‘Vibrio termitis’; Leidy, 1881) and has puzzled biologists ever since. For a long time, termite gut spirochetes remained uncultured and (if at all) were classified only on a morphological basis; none of these names has been validly published (Breznak, 2002; Paster, 2018). However, with the advent of 16S rRNA-gene-based phylogenies, it became clear that almost all termite gut spirochetes fall into the radiation of the genus Treponema, where they form two distinct clades (Berchtold et al., 1994; Ohkuma and Kudo, 1996; Paster et al., 1996). The larger of the two clades, referred to as ‘termite cluster’ (Liblum et al., 1999) or ‘termite Treponema cluster I’ (Ohkuma et al., 1999), hereafter ‘termite cluster I’, is extremely diverse and seems to occur in all termite species (Mikaelyan et al., 2015b). It represents a separate line of descent in the Treponema complex that comprises the free-living Treponema caldarium and Treponema stenostreptum (Liblum et al., 1999; Ohkuma et al., 1999; Abt et al., 2013). The other clade, which has been referred to as ‘termite Treponema cluster II’ (Ohkuma et al., 1999; Noda et al., 2003), hereafter ‘termite cluster...
acetogenesis from H₂ contribute substantially to the high rates of reductive acetogenesis from H₂ + CO₂ via the Wood–Ljungdahl pathway (Ikeda-Ohtsubo, 2003; Matson et al., 2011; Noda et al., 2016). Consequently, it remains unsettled whether the putative ancestor of termite cluster I, which was probably present already in the common ancestor of Cryptocercus and termites, was already acetogenic, or whether this trait was acquired - possibly even more than once - only in individual lineages.

Almost nothing is known about the presence of treponemes in cockroaches. Members of termite clusters I and II were detected, albeit in low abundance, in short-read amplicon libraries of the bacterial gut microbiota of various cockroaches, including the wood-feeding Cryptocercus punctulatus (Dietrich et al., 2014; Schauer et al., 2015; Tai et al., 2015; Berlanga et al., 2016; Lampert et al., 2019). However, none of these phylotypes were represented in any of the 16S-rRNA-based clone libraries of the bacterial gut microbiota of cockroaches (Schauer et al., 2012; Bauer et al., 2015; Mikaelyan et al., 2015b), and their phylogenetic relationship to termite gut treponemes remains unclear.

Here, we report the isolation of the first representative of termite cluster I from the gut of an omnivorous Madeira cockroach (Rhyparobia maderae), using a combination of membrane filtration and deep-agar dilution series. We conducted a detailed ultrastructural and physiological characterization of strain RmG30, including its response to oxygen, and investigated its relationship to members of the termite cluster I and other members of the family Treponemataceae by phylogenomic analysis.

Results

Morphological characterization

Strain RmG30 showed visible growth in anoxic deep-agar tubes after 2 weeks of incubation. Initially, the colonies (<0.1 mm in diameter) had an irregular shape, but after 3 weeks, they became spherical with a well-defined edge (~0.2 mm in diameter). After 2–3 months, the colonies were white and cotton-ball-like with blurred edges and had a diameter of approximately 1–2 mm.

Phase-contrast microscopy of liquid cultures showed spiral-shaped cells with a length of 10–25 (some 3–90) μm (Fig. 1A). Cells were highly motile. Spherical bodies with a diameter of 2–4 μm were present in all cultures and increased in abundance during the stationary phase (Fig. 1B). Scanning electron microscopy revealed a cell width of 0.20 ± 0.01 μm and a wavelength of 1.0 ± 0.1 (some 0.8–1.6) μm (Fig. 1C). Transmission electron microscopy of ultrathin sections rarely showed two,
typically one, and sometimes no periplasmic flagella, indicating that the cells possess single periplasmic flagella at each end that do not always overlap at mid-cell (Fig. 1D).

**Phylogenetic analyses**

16S rRNA gene sequence analysis indicated that strain RmG30 falls into the radiation of the genus *Treponema* (Fig. 2). It represents a novel lineage that is the most basal member of termite cluster I. Together with *T. caldarium* and *T. stenostreptum*, they form a well-supported family-level clade that occupies a sister position to all other members of the genus *Treponema* (family *Treponemataceae*).

A phylogenomic analysis of all members of the order *Spirochaetales* with sequenced genomes confirmed the basal position of strain RmG30 in termite cluster I (Fig. 3 and Supplementary Fig. S1). As in the 16S rRNA-based analysis, the strain represents a line of descent that is separate from those of *T. primitia* and *T. azotonutricium* (the genome of *T. isoptericolens* has not been sequenced), and from numerous MAGs from higher termites (Hervé et al., 2020). The sister position of the ectosymbiotic spirochete of *Breznakiella homolactica* sp. from *Neotermes koshunensis* (NkOx-clu11) to *T. primitia* agrees with the original report (Utami et al., 2019). Notably, a MAG from the fungus-cultivating termite *Macrotermes natalensis* does not cluster with other MAGs from higher termites but represents the same lineage as strain RmG30.

The sequence similarities between the 16S rRNA genes of RmG30 and the other species of the clade range between 90.6% and 93.2% (Fig. 4), which is below the threshold typically observed among members of the same genus (Yarza et al., 2014). Likewise, the low average nucleotide identities between the genomes of strain RmG30 and the corresponding type strains (Fig. 4) justifies to consider each species a separate genus-level taxon. This agrees with the results obtained with the GTDB toolkit (Chaumeil et al., 2020), which classified *T. caldarium*, strain RmG30, and the termite gut isolates (*T. primitia* and *T. azotonutricium*) as separate genera in the family ‘Treponemataceae_B’ and elevated the family *Treponemataceae* to an order-level lineage (‘Treponematales’) separate from the other families in the Spirochaetales (Fig. 3; Supplementary Table S1). Also, members of the genus *Rectinema* (which are currently assigned to *Treponemataceae*; Hördt et al., 2020) were identified as a separate, family-level lineage (f__UBA8932) in the order ‘Treponematales’.

**Growth and physiology**

Strain RmG30 grew at temperatures between 20 and 37°C, with the highest growth rate at 35°C; growth yields showed a broad optimum between 25 and 35°C (Fig. 5A). The pH range of growth was between 6.5 and 7.8, with a broad optimum above pH 7.3 (Fig. 5B). Growth yields decreased drastically as soon as the pH fell below 7.3. Doubling times were similar above pH 7 (16–18 h) but increased at lower pH. Acid production stopped at a final pH of 6.0. No growth occurred at pH 8.5 (not shown).

Strain RmG30 grew fermentatively on D-glucose and a variety of other carbohydrates, including α-mannose, D-galactose, D-fructose, D-xylose, L-arabinose, D-ribose, D-trehalose and N-acetyl-glucosamine. Liquid culture on glucose showed no lag phase and reached maximal...
turbidity within 10 days. Cells precultivated on glucose showed a lag phase of 7–14 days when transferred on D-mannose, D-ribose and D-trehalose, and a lag phase of 14–28 days on D-galactose, D-fructose, L-arabinose and N-acetyl-glucosamine, indicating that the corresponding pathways require induction. No growth was observed on L-rhamnose, D-mannitol, D-gluconic acid, D-glucuronic acid, D-cellulobiose, D-maltose, D-sucrose, D-lactose, starch, cellulose, xylan, pyruvate, L-lactate, formate and H₂ + CO₂.

Lactate was the only product detected from all sugars tested. Acetate was formed only from N-acetyl-glucosamine and in stoichiometric amounts, indicating that it stems exclusively from deacetylation (Table 1). Ethanol, formate, or H₂ were not detected, and carbon and electron recoveries were complete on all substrates.

The lactate-to-substrate stoichiometries on glucose (1.96), N-acetyl-glucosamine (2.08) and xylose (1.73) matched those expected from homolactic fermentation of hexoses (2.00) and pentoses (1.67). Growth rates on xylose and N-acetyl-glucosamine were close to those on glucose, but growth yields were higher on N-acetyl-glucosamine and lower on xylose (Table 1).

Fumarate was not reduced to succinate in the presence of glucose, formate, or H₂. The addition of H₂ to the headspace did not affect growth and product formation on glucose. Cells did not grow in mineral medium without yeast extract and Casamino acids. Higher concentrations of yeast extract (0.2%) slightly stimulated the growth.

Strain RmG30 grew in non-reduced anoxic medium under N₂/CO₂, but no growth occurred if 0.5% O₂ was added to the headspace. Catalase and oxidase activity

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**Fig 2.** 16S rRNA gene tree illustrating the relationship of strain RmG30 (red) to other lineages of termite cluster I and other members of the family *Treponemataceae*. Type strains that represent genus-level taxa in the newly proposed family *Termitinemataceae* are shown in bold. Members of *Spirochaetaceae* were used as outgroup. Bullets indicate node support (● ≥ 95%; ○ ≥ 70%). [Color figure can be viewed at wileyonlinelibrary.com]
were not detected in cells grown on standard medium. However, cultures amended with hemin showed catalase activity. In washed cell suspensions incubated in the presence of oxygen, the turnover rate of glucose was significantly higher (0.99 ± 0.03 μmol mg⁻¹ h⁻¹) than under anoxic conditions (0.70 ± 0.08 μmol mg⁻¹ h⁻¹), and acetate accumulated (0.12 ± 0.01 μmol mg⁻¹ h⁻¹) in addition to lactate (Fig. 6). The amount of air that had to
be added to the tubes to maintain microoxic conditions indicated a considerable rate of O₂ consumption (roughly estimated at 0.2–0.3 μmol mg⁻¹ h⁻¹).

**Genome analysis**

Genome assembly of strain RmG30 resulted in a circular genome with a genome size of 4 646 109 bp and a G+C content of 52.9 mol%. Exploration of the annotated genes (Supplementary Table S2) fully explained the substrate spectrum of the strain and revealed further details of its homolactic metabolism (Fig. 7).

The genome encodes two possible mechanisms for the uptake and activation of glucose: (i) via glucose permease (GlcU) plus hexokinase, and (ii) via a phosphotransferase system (PTS) of the Man family, whose members have been shown to transport and phosphorylate glucose, mannose, fructose, N-acetyl-glucosamine and other substrates in *E. coli* (Plumbridge and Vimr, 1999). In addition, the genome contains numerous genes encoding putative transporters, including putative ATP-binding cassette transporters for galactose, xylose, ribose and trehalose, and putative major facilitator superfamily transporters for arabinose.

The glycolytic pathway oxidizing hexose phosphates to pyruvate is complete. The absence of glucose 6-phosphate dehydrogenase and phosphoketolase agree with the homolactic fermentation of both hexoses and pentoses. The absence of transaldolase, which catalyses a key step in the pentose phosphate pathway, indicates that pentoses are most likely shuttled into glycolysis via a bypass of the classic pentose phosphate pathway, which has been experimentally documented in *E. coli* mutants lacking transaldolase (Nakahigashi et al., 2009). It involves the phosphorylation of sedoheptulose 7-phosphate to sedoheptulose 1,7-bisphosphate and its subsequent cleavage to erythrose 4-phosphate and dihydroxyacetone phosphate. These activities are side reactions of 6-phosphofructokinase and fructose 1,6-bisphosphate aldolase, which are represented in the genome of strain RmG30 in multiple copies (Supplementary Table S2).

The genome encodes a \(\alpha\)-lactate dehydrogenase required to reduce pyruvate to lactate. In addition, there are homologues encoding pyruvate formate lyase (PFL) and pyruvate:ferredoxin oxidoreductase (PFOR), which catalyse the conversion of pyruvate to acetyl-CoA, enzymes for the production of ethanol (acetalddehyde dehydrogenase, alcohol dehydrogenase) and acetate (phosphate acetyltransferase, acetate kinase), and two [FeFe] hydrogenases, namely, a ferredoxin- and NAD⁺-dependent electron-bifurcating hydrogenase (HydABC) of group A and a ferredoxin-dependent homologue of group B (HydA2). The genome also encodes a ferredoxin:
NADP⁺ oxidoreductase (FNR) and a Na⁺-translocating ferredoxin:NAD⁺ oxidoreductase (Rnf) complex.

Key enzymes of the Wood–Ljungdhal pathway (hydrogen-dependent CO₂ reductase (HDCR), carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS)) are absent, confirming the inability of strain RmG30 to catalyse reductive acetogenesis. Genes indicating the capacity for aerobic respiration or anaerobic respiration with sulfate or nitrate as electron acceptor were not detected. The genome encodes several homologues of succinate dehydrogenase/fumarate reductase, although we found no evidence for fumarate respiration in strain RmG30 (see above). Genes encoding pyruvate oxidase, catalase and peroxiredoxin are present, but homologues of superoxide dismutase and glutathione peroxidase were not found.

Strain RmG30 possesses transporters for peptides, amino acids and ammonia (Supplementary Table S2). It encodes the biosynthetic pathways for almost all proteinogenic amino acids (Supplementary File 1). The pathway for tryptophan synthesis is absent. Meso-diaminopimelate, an essential precursor of lysin and peptidoglycan, is probably synthesized directly from tetrahydrodipicolinate via diaminopimelate aminotransferase (DapL) (McCoy et al., 2006), but all possible pathway variants lack at least one coding gene, it is unclear whether strain RmG30 can synthesize lysine (Supplementary Fig. S2). In the case of methionine synthesis, the acylhomoserine pathway that is used by most bacteria is incomplete, but strain RmG30 might produce homocysteine via an aspartate semialdehyde sulfur transferase (Ast, Supplementary Fig. S2), as experimentally documented in methanogenic archaea (Allen et al., 2015).

The pathways for the synthesis and breakdown of glyco- gen are complete, which indicates that strain RmG30 uses glycogen as an intracellular reserve compound. Genes encoding homologues of group IV nitrogenases (structural subunits D, H and K) are most likely not involved in dinitrogen fixation but seem to be part of an Fe³⁺-siderophore transport system (Supplementary Table S2; Ghebreamlak and Mansoorabadi, 2020).

**Discussion**

The isolation of the most basal member of termite cluster I from a cockroach provides important insights into the metabolic diversity of this clade and the evolutionary history of termite gut treponemes. Like the termite gut

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**Table 1. Growth parameters and fermentation balance of strain RmG30 on different substrates.**

| Substrate | Doubling time (h) | Turbidity (OD₅₇₈) | Yield coefficient (g mol⁻¹) | Substrate consumed (mM) | Lactate (mM) | Acetate (mM) | Carbon and electron recovery (%) |
|-----------|------------------|------------------|-----------------|-----------------------|--------------|--------------|-----------------------------|
| No substrate |                 |                  |                 |                       |              |              |                             |
| Glucose | 8.0 | 16 | 0.023d | 13.5 | 0.7 | 15.8 | 99 |
| Xylose | 8.0 | 14 | 0.142 | 9.0 | 0.6 | 14.3 | 104 |
| N-Acetyl-glucosaminea | 8.0 | 15 | 0.247 | 16.8 | 0.9f | 16.3 | 8.1 | 105 |

Unless indicated otherwise, precultures were grown on glucose. Values are means of results obtained with duplicate cultures (less than 10% deviation).

Based on consumed substrate and the conversion factor determined for glucose-grown cultures (60 ± 2 mg L⁻¹ at OD₅₇₈ = 0.1; n = 2).

Assuming an elemental composition of C₄H₈O₂N for bacterial cell mass (Mayberry et al., 1968).

Based on dissimilated substrate.

Turbidity without products formed in basal medium without glucose were subtracted in the subsequent calculations.

Preculture grown on N-acetyl-glucosamine.

Calculated as assimilated glucose.

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**Fig 6.** Effect of oxygen on glucose turnover by cell suspensions of strain RmG30. Washed cells (0.3 mg ml⁻¹) were incubated under a headspace of N₂/CO₂ under anoxic (1 mM DTT) or microoxic (0.2%–0.4% O₂) conditions. Values are means of four cultures (± standard deviation). [Color figure can be viewed at wileyonlinelibrary.com]
isolates *T. azotonutricium* and *T. isoptericolens*, strain RmG30 possesses a purely fermentative metabolism and lacks the ability to grow lithotrophically on H$_2$ + CO$_2$ (or homoaerobiotically on glucose), corroborating the hypothesis that the capacity for reductive acetogenesis in *T. primitia* has been acquired by lateral gene transfer from *Firmicutes* (Salmassi and Leadbetter, 2003; Matson et al., 2011; Ikeda-Ohtsubo et al., 2016). Moreover, the inability to grow on xylan agrees with the hypothesis that also the hemicellulolytic capacity attributed to uncultured members in the apical lineages of termite cluster I, which occur exclusively in higher termites, was acquired by lateral gene transfer (Tokuda et al., 2018).

**Evolutionary history of termite cluster I**

Members of termite cluster I are present in all termite species investigated so far and - unlike the cellulolytic flagellates - even maintained a prominent place in the microbial community after the evolutionary transition from a protist-based (lower termites) to a bacteria-based (higher termites) digestion of lignocellulose (Brune and Dietrich, 2015; Chouvenc et al., 2021). The internal phylogeny of the clade provides a strong coevolutionary signal with the major host groups (Mikaelyan et al., 2015b; Bourguignon et al., 2018), but their evolutionary origin is entirely obscure.
It is widely accepted that termites evolved from wood-feeding, subsocial cockroaches (Bourguignon et al., 2015). Although the gut microbiota of cockroaches and termites shows a strong coevolutionary pattern (Dietrich et al., 2014) and the presence of particular taxa is linked to host diet (Mikaelyan et al., 2015a), concrete evidence for an origin of termite gut treponemes in an ancestral cockroach is so far lacking.

Hence, the genome sequence of strain RmG30 is of great significance for the reconstruction of the evolutionary history of termite cluster I. The monophyly of the cluster, its exclusive presence in termites, and the striking host specificity of its members (the phylotypes from a particular termite species are, in general, more closely related to each other than to any phylotype from other termites), had been recognized already in the early studies of termite gut treponemes (Lilburn et al., 1999; Ohkuma et al., 1999). Our phylogenomic analysis, corroborated by the corresponding 16S rRNA-based phylogeny, provides unequivocal support for a basal position of strain RmG30 (from a cockroach), an intermediate position of the isolates from lower termites, and an apical position of clones (there are no isolates) from higher termites, which is in full agreement with a coevolutionary scenario (Fig. 2).

An interesting exception is the single clone (AB234368) obtained from Macrotermes gilvus (Hongoh et al., 2006) and a corresponding MAG (g_UBA937) from Macrotermes natalensis (Parks et al., 2017), which are more closely related to strain RmG30 than to all other members of termite cluster I. Apparently, the fungus-feeding Macrotermes spp. have independently acquired a lineage of termite cluster I that is separate from the main line of descent - possibly from a cockroach. This was corroborated by phylogenetic placement of the short reads previously obtained from amplicon libraries of cockroach gut microbiota (Lampert et al., 2019), where reads from several cockroaches that had been classified as Treponema caldarium or termite cluster I clustered with the 16S rRNA gene of strain RmG30 and the clone from Macrotermes gilvus (details not shown). Such a horizontal acquisition of termite gut microbiota from the environment, including the guts of other animals, is not unusual (Bourguignon et al., 2018), and agrees with the observation that the gut microbiota of Macrotermitinae (fungus-feeding higher termites) is more similar to that of cockroaches than to termites from other diet groups (Dietrich et al., 2014; Otani et al., 2014; Lampert et al., 2019).

These observations provide strong evidence that members of termite cluster I were already present among the gut microbiota of omnivorous cockroaches before the common ancestor of termites and Cryptocercidae acquired cellulolytic flagellates. Since Rhyparobia maderae (superfam. Blaberoidae), the host of strain RmG30, is only distantly related to the lineage that gave rise to termites (superfam. Blattoidea) (Djernæs et al., 2020), host specificity and transmission mode of the basal members of termite cluster I in cockroaches remain unclear. The same is true for the ultimate origin of the cluster and their relationship to the isolates from freshwater sediments (T. stenostreptum and T. caldarium). Clearly, more information is needed on the diversity and distribution of the cluster in cockroaches, other insects and their environments.

**Energy metabolism**

Since the isolation of *T. primitia* (Leadbetter et al., 1999; Graber et al., 2004), evidence has accumulated that members of termite cluster I are responsible for the high rates of reductive acetogenesis in termite guts. However, *T. primitia* and possibly also the closely related strain NkOxocl11, an uncultured ecytobiont of a termite gut flagellate, are the only representatives of the cluster that possess a complete Wood–Ljungdahl pathway (Rosenthal et al., 2011; Utami et al., 2019). Their energy metabolism resembles that of *Acetobacterium woodii* and involves an HDCR, a CODH/ACS complex, an electron-bifurcating hydrogenase (HydABC), and an Rnf complex, which balance the reducing equivalents and generate the membrane potential for ATP synthesis (Schuchmann and Müller, 2014).

The presence of a fermentative metabolism in the ancestral *T. caldarium* and *T. stenostreptum*, in strain RmG30, and in two of the three species isolated from termite guts is a strong indication that reductive acetogenesis in *T. primitia* and possibly other uncultured members of termite cluster I is an apomorphic trait. This agrees with the absence of HDCR and CODH/ACS from *T. azotonutricium* and strain RmG30 - the only other isolates of termite cluster I with sequenced genomes - and from the MAG from *Macrotermes natalensis*, the closest relative of strain RmG30 in the phylogenomic tree (Fig. 3). The gene functions of the Wood–Ljungdahl pathway that are encoded by strain RmG30 (Fig. 7) and all other basal members of the clade probably serve in the interconversion of C1 compounds and the provision of formate and methyl groups for the biosynthesis of purines (Sah et al., 2015), methionine and serine (Zhuang et al., 2014) and were most likely present already in the common ancestor of termite cluster I. The Wood–Ljungdahl pathway was most likely complemented during the radiation of the clade by the lateral acquisition of HDCR and CODH/ACS from acetogenic *Firmicutes*, rendering certain lineages acetogenic. It remains to be clarified whether this has happened more than once.

There is circumstantial evidence that also the apical lineages of termite cluster I, which occur exclusively in higher termites (Fig. 3), may not be acetogenic.
Metagenome analysis of the hindgut fluid of a *Nasutitermes* sp. revealed numerous gene homologues encoding FTHFS and CODH/ACS that were assigned to treponemes, but homologues of HDCR were conspicuously absent (Warnecke et al., 2007). Comparative analysis of the gene functions encoded by the uncultured representatives of termite cluster I, including also MAGs from lower termites, will allow to clarify the situation (Y. Song, V. Hervé, and A. Brune; unpublished results).

An unusual feature of strain RmG30 is the homolactic fermentation of pentoses. While homolactic fermentation which uses the glycolytic pathway and yields two lactate per sugar, is typical for hexoses, pentoses are usually fermented heterolactically via the phosphoketolase pathway, which yields one lactate and one acetate (Kandler, 1983). Homolactic fermentation of pentoses is quite rare in nature but common in biotechnological applications, where a high yield of lactate is achieved by genetic engineering (Tarraran and Mazzoli, 2018), which usually involves the substitution of phosphoketolase with a heterologous expressed transketolase (Okano et al., 2009).

The absence of both phosphoketolase and transaldolase from the genome of strain RmG30 and the formation of about 1.7 lactate per xylose, which matches the theoretical stoichiometry of a homolactic fermentation, indicates that pentoses are converted to hexoses via a modified pentose phosphate pathway that involves a sedoheptulose 1,7-bisphosphate shunt (Nakahigashi et al., 2009; see above). While the few lactic acid bacteria that ferment pentoses via pentose phosphate pathway/glycolysis still form appreciable amounts of acetate, strain RmG30 is the first strain that conducts a purely homolactic fermentation of pentoses. Although the strain has the genomic capacity to convert pyruvate to acetyl-CoA (via PFL or PFOR) and to form acetate, formate, hydrogen or ethanol, these enzymes are obviously not involved in catabolic reactions.

**Relationship to oxygen**

Insect guts are gradient systems characterized by the continuous influx of oxygen across the gut epithelium (Brune, 2014). Therefore, even strictly anaerobic members of the gut microbiota may be at least temporarily exposed to oxygen. Typical adaptations to microoxic conditions are pathways for a non-respiratory reduction of oxygen and the removal of reactive oxygen species, which can be found even in strict anaerobes (e.g. Condon, 1987; Brioukhanov and Netrusov, 2007; Imlay, 2019). Strain RmG30 is no exception. Although it has a purely fermentative metabolism and does not grow under microoxic conditions (0.5% O₂, vol/vol.), resting cells consume oxygen and accumulate acetate at appreciable rates. An oxygen-dependent accumulation of acetate is common among lactic acid bacteria, which reduce oxygen in non-respiratory pathways, which shifts fermentation products from lactate to acetate and allows to gain additional ATP (e.g. Condon, 1987; Bauer et al., 2000). In strain RmG30, oxygen consumption is most likely catalysed by pyruvate oxidase; homologues of lactate oxidase or NADH oxidase were not detected.

Like most non-respiratory oxidases, pyruvate oxidase produces acetyl-CoA and H₂O₂. The latter is cytotoxic but may be detoxified by catalase, which ameliorates the effects of H₂O₂ production and increases oxygen tolerance (Whittenbury, 1960; Engesser and Hammes, 1994). Also, the genome of strain RmG30 encodes the apoenzymne, catalase activity requires the presence of hemin. The dependence of catalase activity on an external hemin supply is common in lactic acid bacteria (Baureder and Hederstedt, 2013) but has been reported also for methanogens (Brioukhanov and Netrusov, 2012). In strain RmG30, hemin auxotrophy is explained by the absence of genes required for porphyrin biosynthesis, which indicates a requirement also for vitamin B₁₂. Such cross-feeding of growth factors is common in gut environments (Sokolovskaya et al., 2020), exemplified by the dependence of *T. primitia* on the provision of folinate by other members of the termite gut microbiota (Graber and Breznak, 2005).

Except for the microaerophilic *Treponema pallidum*, which requires low levels of oxygen for survival and multiplication, all other members of the genus *Treponema* are considered strict anaerobes (Norris et al., 2015). Nevertheless, cultures of *T. primitia* tolerate exposure to low levels of oxygen, and cell extracts of strain ZAS-2 show relatively high levels of NADH oxidase and NADH peroxidase but no catalase activity (Graber and Breznak, 2004). In addition, and quite surprisingly, *T. primitia* encodes a catechol-2,3-dioxygenase (Lucey and Leadbetter, 2014), an enzyme that typically occurs in aerobic bacteria degrading aromatic compounds; it is absent from the other members of the clade. The genomes of *T. primitia* ZAS-2 and *T. azotonutricium* encode homologues of NADH oxidase, but strain RmG30 is the only isolate of termite cluster I (with sequenced genome) that encodes catalase and pyruvate oxidase. It remains to be investigated whether external hemin affects the aerotolerance of strain RmG30, its capacity for fumarate reduction, or its cell yields on glucose.

**Taxonomic implications**

For the longest time, all members of the phylum ‘*Spirochaetes*’ were classified in a single order (*Spirochaetales*) (Paster, 2015). However, 16S rRNA-based and phylogenomic analyses indicated that the
taxonomic ranks did not appropriately reflect the diversity of the phylum, and numerous taxa have been subsequently elevated to higher ranks (Gupta et al., 2013; Yarza et al., 2014; Hördt et al., 2020). In that course, the family Treponemataceae has been reinstated; it currently contains the genera Treponema and Rectinema (Hördt et al., 2020).

However, members of the genus Treponema are phylogenetically highly divergent (Norris et al., 2015; Paster, 2018). Based on the clear separation of termite cluster I (together with T. stenostreptum and T. caldarium) from all other members of the genus Treponema, Lilburn et al. (1999) already considered it inevitable that ‘a new genus or genera will ultimately be created to accommodate spirochaetes of the termite cluster.’ This notion is substantiated by the current GTDB taxonomy, which takes into account phylogeny, average nucleotide identity and relative evolutionary distance (Parks et al., 2018; Parks et al., 2020). The GTDB taxonomy distinguishes two separate, family-level lineages in the order ‘Treponematales’: (i) Treponemataceae, which comprise the majority of all Treponema species but distinguishes several genus-level taxa, and (ii) ‘Treponemataceae_B’, which include T. caldarium, strain RmG30, and members of termite cluster I as separate genus-level taxa (Fig. 3). The membership of T. stenostrepta and T. isoptericolens, whose genomes have not been sequenced, in ‘Treponemataceae_B’ is highly supported by the 16S rRNA-based phylogeny (Fig. 2).

The nucleotide identities of the genomes and/or the respective 16S rRNA genes (Fig. 4) corroborate that each species in the family ‘Treponemataceae_B’ represents separate genus-level lineages. This agrees with the considerable phenotypic differences between the species, concerning not only cell shape, flagellar arrangement, genome size and G + C content but also metabolic traits such as substrate and product range, response to oxygen, and the capacity for reductive acetogenesis (Tables 2 and 3).

Based on these results, we describe strain RmG30 as the type strain of Breznakiella homolactica gen. nov. sp. nov. and propose classification of all members of the ‘Treponemataceae_B’ in the new family ‘Termitinemataceae’. The formal description of this family and the reclassification of all Treponema spp. in its radiation into separate genera, with ‘Termitinema’ [Treponema primitia] as type genus, will be addressed in a separate publication. In addition, the taxonomic ranks suggested by GTDB will require further taxonomic acts, such as elevation of the genus Rectinema to family level (‘Rectinemataceae’), and the reclassification of Treponemataceae, Termitinemataceae and ‘Rectinemataceae’ in the new order ‘Treponematales’.

**Description of Breznakiella homolactica gen. nov.**

Etymology: Brez.na.ki.el'a. N. L. dimin. fem. n. Breznakiella, named after the American microbiologist John A. Breznak, in recognition of his fundamental contributions to the studies on termite gut treponemes.

The description is as given for Breznakiella homolactica sp. nov., which is the type species. The genus is monospecific and has been separated from other members of the family ‘Termitinemataceae’ based on physiological and phylogenetic analyses of genome and 16S rRNA gene sequences.

**Description of Breznakiella homolactica sp. nov.**

Etymology: ho.mo.lac.ti.ca. Gr. adj. homoios, the same, similar; N.L. neut. n. acidum lacticum, lactic acid; N.L. fem. adj. homolactica, referring to a metabolic analogy to other bacteria with homolactic fermentation.

Cells are helical, with a diameter of 0.2 μm, a length of 10–25 μm, and a wavelength of about 1.0 μm. Motile by two periplasmic flagella inserted at opposite ends of the cytoplasmic cylinder. Spherical bodies with a diameter of 2–4 μm are formed in stationary phase cultures. Mesophilic, grows optimally at 35°C (range 20–37°C); no growth at 40°C. Optimum pH for growth is 7.3–7.8 (range 6.5–7.8). Fermentative metabolism. Energy sources include D-glucose, D-mannose, D-galactose, D-fructose, D-xyllose, L-arabinose, D-ribose, D-trehalose and N-acetylglucosamine. No growth on L-rhamnose, D-gluconic acid, D-glucuronic acid, D-cellobiose, D-maltose, D-sucrose, D-lactose, starch, cellulose, xylan, pyruvate, lactate, formate, or H2 + CO2. Lactate is the only product from all sugars tested. Requires yeast extract and Casamino acids. Obligately anaerobic, but resting cells reduce oxygen. Genome size is 4.65 Mbp, G + C content is 52.9 mol.% (based on the type strain).

Source: The intestinal tract of the Madeira cockroach, Rhyparobia maderae (Fabricius 1781).

Type strain: strain RmG30 = DSM 111054 = JCM 39135. GenBank accession numbers: MW396710 (16S rRNA gene); CP067089 (genome).

**Experimental procedures**

**Microbiological media**

Cultures were routinely grown in medium AM-5, an anoxic, bicarbonate-buffered mineral medium supplemented with vitamins and other growth factors (Tegtmeyer et al., 2016), which was amended with yeast extract and Casamino acids (0.1% each), cysteine and DTT (1 mM each) as reducing agents, and resazurin.
(0.8 mg L\(^{-1}\)) as redox indicator. Non-reduced anoxic medium received cystine (0.5 mM) from an acidic stock solution (250 mM in 1 M HCl) as sulfur source. Unless otherwise indicated, this ‘basal medium’ was amended with glucose (8 mM), dispensed (5 ml) into 16-ml rubber-stoppered culture tubes, gassed with a headspace of N\(_2\)/CO\(_2\), and inoculated with a fresh preculture (0.1 ml).

To assess the pH range of growth, the initial pH of the medium was adjusted by varying the mixing ratio of N\(_2\) and CO\(_2\) in the headspace gas; Widdel and Bak, 1992). The N\(_2\)/CO\(_2\) ratio routinely used during isolation and initial experiments was 80:20 (vol./vol.), resulting in a medium pH of 7.0. It was changed to 90:10 (vol./vol.), resulting in a medium pH of 7.3, after we realized that

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### Table 2. Characteristics that differentiate strain RmG30 from other species in the radiation of the new family ‘Termitinemataceae’.

| Characteristics | Strain RmG30 | Treponema primitia\(^a\) | Treponema azotonutricium\(^a\) | Treponema isoptericolens\(^b\) | Treponema caldarium\(^c\) | Treponema stenostreptum\(^d\) |
|----------------|-------------|--------------------------|-----------------------------|-----------------------------|--------------------------|-----------------------------|
| Habitat        | Cockroach gut | Termite gut              | Termite gut                 | Hot spring sediment         | Freshwater sediment      |
| Cell diameter (μm) | 0.2       | 0.2–0.3                  | 0.2–0.3                     | 0.4–0.5                     | 0.2–0.3                  | 0.2–0.3                     |
| Cell length (μm)  | 10–25     | 3–7                      | 10–12                       | 12–20                       | 15–45                    | 15–45                       |
| Wavelength (μm)   | 1.0        | 2.3                      | 1.2                         | 6.5                         | 0.6\(^{g}\)              | 1.2                         |
| Flagellar arrangement | 1:2:1    | 1:2.1                     | 1:2.1                       | 3:6:3                       | 1:2:1                    | 1:2:1                       |
| Genome size (Mbp) | 4.65       | 4.06                     | 3.86                        | NA                         | 3.24                     | NA                          |
| G + C content (mol.%\(^{f}\)) | 52.9      | 50.8                     | 49.8                        | 47.7\(^{g}\)               | 45.6                     | 60.2\(^{b}\)               |
| pH optimum        | 7.3–7.8   | 7.2                      | ND                          | 7.2–7.4                     | 7.2–7.5                  | 7.0–7.5                     |
| Temp. optimum (°C) | 35        | 30                       | 30                          | 30                          | 48–52                    | 35–37                       |
| Catalase          | +\(^{i}\)  | –                        | –                           | –                           | ND\(^{j}\)               | –                           |
| Fermentation products\(^{k}\) | Lactate | Acetate                  | Acetate, ethanol, CO\(_2\), H\(_2\) | Ethanol, CO\(_2\)           | Acetate, lactate, CO\(_2\), H\(_2\) | Ethanol, acetate, lactate, CO\(_2\), H\(_2\) |
| Growth on H\(_2\) + CO\(_2\) | –        | +                        | +                           | –                           | –                        | –                           |

\(-\), no activity; NA, not available; ND, not determined.

\(^{a}\)Data from Graber and Breznak (2004), Graber et al. (2004), Norris et al. (2015).

\(^{b}\)Data from Dröge et al. (2008).

\(^{c}\)Data from Pohlschroeder et al. (1994).

\(^{d}\)Data from Zuelzer (1912) and Norris et al. (2015).

\(^{e}\)Estimated from buoyant density of DNA in CsCl (Canale-Parola et al., 1968).

\(^{f}\)Based on genome sequences.

\(^{g}\)Estimated from Fig. 1 in Pohlschroeder et al. (1994).

\(^{h}\)Expressed only in the presence of hemin.

\(^{i}\)Not encoded in genome.

\(^{k}\)Product(s) from glucose (maltose in T. isoptericolens), in stoichiometric order.

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### Table 3. Differences in substrate utilization by strain RmG30 and other species in the new family ‘Termitinemataceae’.

| Substrate     | Strain RmG30 | Treponema primitia\(^a\) | Treponema azotonutricium\(^a\) | Treponema isoptericolens\(^b\) | Treponema caldarium\(^c\) | Treponema stenostreptum\(^d\) |
|---------------|-------------|--------------------------|-----------------------------|-----------------------------|--------------------------|-----------------------------|
| Glucose       | +           | +                        | –                           | –                           | +                        | +                           |
| Mannose       | +           | +                        | –                           | –                           | +                        | +                           |
| Fructose      | +           | +                        | –                           | +                           | –                        | +                           |
| Galactose     | +           | +                        | –                           | +                           | –                        | +                           |
| Xylose        | +           | +                        | +                           | +                           | +                        | +                           |
| Arabinose     | +           | +                        | –                           | +                           | –                        | +                           |
| Ribose        | +           | +                        | –                           | –                           | +                        | +                           |
| Mannitol      | –           | +                        | –                           | –                           | +                        | +                           |
| Maltose       | –           | +                        | +                           | +                           | –                        | +                           |
| Cellobiose    | –           | +                        | –                           | –                           | +                        | +                           |
| Trehalose     | +           | +                        | –                           | +                           | +                        | +                           |
| Sucrose       | –           | –                        | –                           | –                           | +                        | –                           |
| Lactose       | –           | –                        | –                           | –                           | –                        | –                           |
| Starch        | –           | –                        | –                           | –                           | –                        | –                           |

\(+\), utilized; \(-\), not utilized.

\(^{a}\)Data from Graber and Breznak (2004) and Graber et al. (2004).

\(^{b}\)Data from Dröge et al. (2008).

\(^{c}\)Data from Pohlschroeder et al. (1994).

\(^{d}\)Data from Zuelzer (1912) and Norris et al. (2015).
strain RmG30 grew better if the medium was slightly alkaline. For growth tests at pH 8.5, bicarbonate buffer was replaced with N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid at a final concentration of 20 mM, and N₂ was the headspace gas.

**Enrichment and isolation**

*Rhyparobia maderae* was obtained from a commercial breeder (Jörg Bernhardt, Helbigsdorf, Germany; http://www.schaben-spinnen.de) and maintained as previously described (Schauer et al., 2012). An adult female cockroach was dissected, and the whole gut was placed in a culture tube containing 2-mm glass beads (2 g). After addition of 5 ml basal medium, the tube was closed with a rubber stopper, the headspace was gassed with N₂/CO₂ (80:20, vol./vol.), and the gut was homogenized by vortexing for 2 min. The gut homogenate was passed through a cellulose ester membrane filter (Merck Millipore) with pore diameter of 0.3 μm, and the filtrate was serially diluted in deep-agar tubes containing basal medium with 1% agar under an N₂/CO₂ headspace. The series was tested by adding 16 mM fumarate to basal medium supplemented with the respective substrates (20 mM, except 4 mM for disaccharides); carboxylic acids were supplied as sodium salts. Polysaccharides (6 mg ml⁻¹) were autoclaved in the tubes before basal medium was added. Growth on H₂ + CO₂ was tested by adding 5 ml H₂ to the headspace of culture tubes with (bicarbonate-buffered) basal medium. Fumarate reduction was tested by adding 16 mM fumarate to basal medium with glucose, sodium formate, or H₂.

Oxygen tolerance was tested in culture tubes with non-reduced basal medium with 8 mM glucose under N₂/CO₂, which received different volumes of air in the headspace and were incubated on a roller mixer (60 rpm). The effect of oxygen on glucose turnover was tested with washed cell suspensions. Cells were grown on the same medium (100 ml) in rubber-stopped, centrifugable glass bottles (150 ml) under N₂/CO₂, harvested in the exponential growth phase by centrifugation (3000g; 30 min) and resuspended in 10 ml anoxic buffer (30 mM NaHCO₃, 40 mM NaCl, pH 7.0) under N₂/CO₂. The cell suspensions (0.3 mg ml⁻¹) were amended with 8 mM glucose and divided into two cohorts: one was supplemented with 1 mM DTT and was incubated under anoxic conditions, the other was supplemented with air at a headspace concentration of 0.4% O₂ to create microoxic conditions. The cell suspensions were incubated on a shaker (100 rpm) at 30°C; O₂ concentration was monitored with a Fibox 4 trace meter and SP-PIX-QAU sensor spots (Presens; https://www.presens.de/) and maintained between 0.2% and 0.4% throughout the incubation period by adding defined volumes of air at regular intervals.

Oxidase activity was tested with glucose-grown cultures in basal medium using oxidase test strips (Bactident, Merck, Darmstadt, Germany); *Bacillus subtilis* and *Escherichia coli* (oxidase-positive) and *Elusimicrobium minutum* (catalase-negative; Geissinger et al., 2009) were used as controls. Catalase activity was tested by checking the formation of gas bubbles after adding a drop of H₂O₂ (3%) to cell pellets of glucose-grown cultures; *E. coli* (catalase-positive) and *Elusimicrobium minutum* (catalase-negative; Geissinger et al., 2009) were used as controls. The effect of hemin on catalase expression was tested by adding hemin (2 μg ml⁻¹; Sigma-Aldrich) from a stock solution (5 mg ml⁻¹ in 50 mM NaOH). To avoid false-positive reactions, the suspended cells were separated from precipitated hemin before centrifugation and washed twice with phosphate-buffered saline (10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 137 mM NaCl, 2.7 mM KCl, pH 7.2).

**Metabolic products**

Hydrogen was analysed in the culture headspace by gas chromatography, using a molecular sieve column and a thermal conductivity detector (Schuler and Conrad, 1990). Other fermentation products were analysed in the culture supernatant by high-performance liquid chromatography after centrifugation at 10 000g for 10 min and acidification with H₂SO₄ to 50 mM final concentration, using a system equipped with an ion-exclusion column and a refractive index detector (Schauer et al., 2012). For the calculation of electron recoveries, all metabolites were formally oxidized to CO₂, and the number of valence electrons theoretically released during product formation was compared with that of the dissimilated substrate (Tholen et al., 1997).

**Light and electron microscopy**

Cultures were examined by light microscopy using an Axioshot photomicroscope (Zeiss, Oberkochen, Germany).
Germany). Non-stained cultures were routinely examined using phase-contrast illumination (100 × objective).

For electron microscopy, cells were fixed with glutaraldehyde and postfixed with osmium tetroxide before dehydrating in a graded series of ethanol and embedding in Spurr’s resin (Zheng et al., 2016). Alternatively, 2-μl samples of concentrated cell suspensions were high-pressure frozen, freeze-substituted with HUGA (0.5% uranyl acetate, 0.5% glutaraldehyde, 5% H2O in acetone), and embedded in Epon 812 substitute resin, as previously described (Renicke et al., 2017). Ultrathin sections were cut with a microtome equipped with a diamond knife and contrasted with uranyl acetate and lead citrate. The sections were examined with a Philips EM 208 transmission electron microscope.

Genomic DNA was prepared using cetyltrimethylammonium bromide extraction (Winneppeninckx et al., 1993) and commercially sequenced (GATC-Eurofins, Konstanz, Germany) on a PacBio RS platform using one SMRT cell (insert size up to 10 kb). Reads were assembled with the PacBio SMRT Portal software (version 2.3.0) using the Hierarchical Genome Assembly Process for assembly and Quiver for polishing (Chin et al., 2013). The polished single contig was circularized with Circulator (Hunt et al., 2015).

The genome sequence of strain RmG30 has been submitted to GenBank (https://www.ncbi.nlm.nih.gov; accession number CP067089; circularized) and to the Integrated Microbial Genomes and Microbiomes database (IMG/M) of the Joint Genome Institute (JGI) (https://img.jgi.doe.gov; taxon ID 2772190975; not circularized). The genome was annotated via the IMG annotation pipeline (v.4.16.1; Chen et al., 2019). For the analysis of the metabolic pathways, annotation results were verified, and missing functions were identified using Blast with a threshold E-value of 1E–5. Hydrogenases were classified using the HydDB reference database (https://services.birc.au.dk/hyddb/; Søndergaard et al., 2016).

The 16S rRNA gene of strain RmG30 was amplified with Bacteria-specific primers and sequenced by Sanger sequencing as previously described (Strassert et al., 2010); the sequence has been submitted to GenBank (ID: MW396710). The sequence was aligned with the SINA aligner (Pruesse et al., 2012) and imported into the reference alignment of the Silva database (version 132; Pruesse et al., 2007); additional sequences were downloaded from GenBank. The alignments were manually curated using the ARB software package (version 6.0.6; Ludwig et al., 2004). A maximum-likelihood tree of the 16S rRNA genes was inferred from 1275 unambiguous aligned positions (sites with more than 50% gaps were masked) using the PhyML algorithm (version 3.3; Guindon et al., 2010) with GTR model and aBayes branch supports (Anisimova et al., 2011) included in ARB. Pairwise sequence identities of 16S rRNA genes are based on a distance matrix of the unfiltered alignment generated in ARB.

The genomes of strain RmG30 and other members of termite cluster I were phylogenetically classified within the taxonomic framework of the Genome Taxonomy Database (GTDB, release 89) using the GTDB toolkit (GTDB-tk, version 1.3.0; Chaumil et al., 2020). A maximum-likelihood tree based on the genomes was inferred from a concatenated alignment of 120 bacterial single-copy genes (5040 amino acid positions) using the PhyML algorithm with LG model and aBayes branch supports. The average nucleotide identities of the genomes were calculated with FastANi (version 1.3; Jain et al., 2018).

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Fig. S1.** Expanded version of the phylogenomic tree in the manuscript (Fig. 3), illustrating the relationship of strain RmG30 (red) and other members of the proposed ‘Termitinemataceae’ to other lineages in the *Spirochaetales*. Type species of current genera are marked in bold. The tree was rooted using other lineages of *Spirochaeta* as out-group. Genome sequences were downloaded from GenBank (accession numbers are given), except for strain NkOx-clu11 (Utami *et al.*, 2019) and the genomes in UBA8932 (Dong *et al.*, 2018), which were provided by the authors.

**Fig. S2.** The pathways for lysine and methionine biosynthesis in the genome of strain RmG30. Missing genes and the respective reactions are shown in grey. Gene abbreviations and the encoded functions: *asd*, aspartate-semialdehyde dehydrogenase; *ast*, α-aspartate semialdehyde sulfurtransferase; *dapA*, 4-hydroxy-tetrahydrodipicolinate synthase; *dapB*, dihydrodipicolinate reductase; *dapC*, succinyl-diaminopimelate aminotransferase; *dapD*, tetrahydrodipicolinate succinylase; *dapE*, succinyl-diaminopimelate desuccinylase; *dapF*, diaminopimelate epimerase; *dapH*, tetrahydrodipicolinate acetylase; *dapL*, LL-diaminopimelate amidotransferase; *ddh*, diaminopimelate dehydrogenase; *lysA*, LL-diaminopimelate decarboxylase; *lysC*, aspartate kinase; *metA*, homoserine O-succinyltransferase/O-acetyltransferase; *metB*, cystathionine gamma-synthase; *metC*, cystathionine beta-lyase; *metH*, 5-methyltetrahydrofolate–homocysteine methyltransferase; *metK*, S-adenosylmethionine synthase; *metT*, homoserine O-acetyltransferase; *metY*, O-acetylhomoserine aminocarboxypropyltransferase; *metZ*, O-succinylhomoserine sulfhydrylase; *nifS*, cysteine desulfurase; *patA*, N-acetyl-LL-diaminopimelate aminotransferase; *ykuR*, N-acetyl-LL-diaminopimelate deacetylase.

**File S1.** KEGG maps showing detailed results on the pathways for the biosynthesis of amino acids in RmG30.

**Table S1.** GTDB-Tk classification of strain RmG30 and other members of termitic cluster I (shown in blue).

**Table S2.** Annotation details of the genes encoding metabolic pathways of strain RmG30 (Fig. 7). The genes are sorted by functional groups (in blue) and subgroups (in grey). Genes without gene ID were not found in the genome.