UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

CURSO DE GRADUAÇÃO EM BIOMEDICINA

JULIANO DE OLIVEIRA SILVEIRA

Ativação de Ureases: Inferências Evolutivas via Filogenia

Porto Alegre

Dezembro 2014

JULIANO DE OLIVEIRA SILVEIRA

Ativação de Ureases: Inferências Evolutivas via Filogenia

Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Bacharel em Biomedicina.

Orientador: Prof. Dr. Hugo Verli

Co-Orientador: Dr. Rodrigo Ligabue Braun

Porto Alegre

Dezembro 2014

Esse trabalho foi desenvolvido no Grupo de Bioinformática Estrutural do Centro de Biotecnologia da Universidade Federal do Rio Grande do Sul sob orientação do professor doutor Hugo Verli, do Departamento de Biologia Molecular e Biotecnologia durante os meses de janeiro a novembro de 2014, com o apoio financeiro do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e da Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS). O autor recebeu bolsa PROBIC FAPERGS durante o seu desenvolvimento.

"Nothing in Biology makes sense except in the light of Evolution"

Theodosius Dobzhansky

ÍNDICE

RESUMO

Ureases são enzimas de grande importância histórica, médica e agrícola que catalisam a hidrólise de ureia em amônia e carbamato. Estas enzimas são amplamente distribuídas em plantas, fungos e bactérias. Em todos esses organismos, a ligação de um grupo de proteínas acessórias é necessária para o real funcionamento da enzima, atuando na modificação do sítio ativo e permitindo a inserção dos íons de níquel essenciais para a catálise. O níquel é inserido no sítio ativo da urease, num processo dependente de GTP, com o auxílio de UreD / UreH, UreE, UreF, e UreG. Estas proteínas acessórias orquestram a ativação da apoproteína, fornecendo o metal apropriado e facilitando alterações conformacionais. O mecanismo de ativação e as funções de cada proteína acessória na maturação da urease não se encontram completamente elucidadas. Para obter-se uma visão geral das relações evolutivas que podem ser estabelecidas entre as proteínas acessórias e as próprias ureases, no presente estudo foi realizada uma ampla análise das sequências de aminoácidos de proteínas acessórias destas enzimas. Considerando que os resultados são preliminares, conclui-se que as relações filogenéticas aqui apresentadas demonstram que as árvores obtidas para proteínas acessórias de urease parecem seguir topologias similares àquelas obtidas para árvores da própria enzima. Além disso, os resultados de árvores quiméricas de UreG sugerem que a análise filogenética pode estar sofrendo Atração de Longos Ramos.

1. INTRODUÇÃO

1.1 Histórico: Ureases

Ureases (EC 3.5.1.5) são enzimas que catalisam a hidrólise de ureia em amônia e carbamato, que em seguida se decompõe em outra molécula de amônia e bicarbonato (**Figura *1***). Estas enzimas são amplamente distribuídas em plantas, fungos e bactérias. Em todos esses organismos, a ligação de um grupo de proteínas acessórias é necessária para a ativação da enzima (Yukl & Wilmot 2013), atuando na modificação do sítio alvo e permitindo a inserção dos íons de níquel essenciais para a catálise (Schenk et al. 2012).

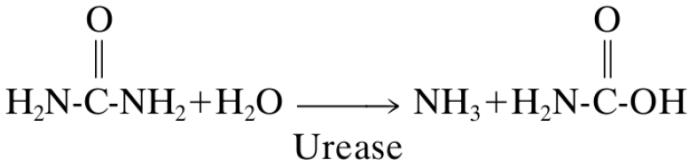


Figura 1: Representação simplificada da reação de hidrólise da ureia. Reação de hidrólise da ureia em amônia e carbamato, representando a catálise por urease (reação secundária de decomposição do carbamato em amônia e bicarbonato omitida). Adaptado de Mobley e colaboradores (Mobley et al. 1995).

Ureases são biomoléculas de grande importância em atividades como medicina e agricultura. Além de possuírem alto potencial de aplicabilidade tecnológica, possuem um papel histórico relevante para o desenvolvimento das ciências básicas em áreas como enzimologia, cristalografia e biologia molecular e estrutural.

Como exemplo de importância médica, pode se citar a bactéria Gram-negativa *Helicobacter pylori*, que se encontra presente no estômago de mais da metade da população humana (Kusters et al. 2006). Esse patógeno gástrico depende da urease para que a produção de amônia eleve localmente o pH no estômago e, dessa forma, se desfaçam as interações fracas no gel de mucina encontrado na parede do

estômago (Scott et al. 2002). Sem esse gel, o patógeno consegue acessar as células epiteliais do hospedeiro e a mucosa gástrica sofre lesão devido a exposição ao ácido estomacal. Também, tem sido demostrado que a urease pode ser um fator de virulência essencial para várias doenças, incluindo doenças de longa duração/crônicas (Konieczna et al. 2012) até mesmo de natureza extragástricas (Banić et al. 2012).

Na agricultura, ureases vegetais degradam a ureia, metabólito de plantas e fertilizante, agindo como importante fator no ciclo do nitrogênio (Krajewska 2009); no entanto, a ureia também pode ser metabolizada pelas bactérias do solo, o que pode levar a volatilização de amônia e consequente alquilação do solo, um processo danoso para o desenvolvimento de plantas (Bremner 1995). Recentemente, Polacco e colaboradores (Polacco et al. 2013), discutiram a relação entre o níquel e as ureases em plantas e constataram que os estudos devem ser expandidos a outras plantas, uma vez que até o momento muito do que se sabe das enzimas provém de estudos com soja, batata e Arabidopsis.

Também de interesse, a urease de sementes de feijão-de-porco (*Canavalia ensiformis*) foi a primeira enzima a ser cristalizada (Sumner 1926) e a primeira proteína que se mostrou conter níquel (Dixon 1975). Dessa forma, a urease é um modelo importante que fez avançar a nossa compreensão dos mecanismos de montagem dos sítios de inserção de metais em enzimas. No entanto, tais mecanismos continuam não completamente elucidados e ainda se encontram lacunas no estudo da ativação das ureases (Polacco et al. 2013).

1.2 Ativação: Proteínas Acessórias de Urease

A biossíntese do metalocentro de níquel em ureases geralmente requer a participação de diversas proteínas acessórias. O níquel é inserido no sítio ativo da urease, num processo dependente de GTP, com o auxílio de UreD / UreH, UreE, UreF, e UreG (Carter et al. 2009). Estas proteínas acessórias orquestram a ativação da apoproteína, fornecendo o metal apropriado, facilitando alterações conformacionais da proteína e, eventualmente, modificações pós-traducionais necessárias (Farrugia et al. 2013).

Os mecanismos propostos até agora para cada uma das proteínas acessórias, com exceção da UreG que também foi descrita em outros modelos, foram mais estudados em *Klebsiella aerogenes* e *Helicobacter pylori*. A seguir, são resumidos alguns mecanismos e atividades associados as proteínas acessórias de urease:

- UreD/UreH: Proteína *scaffold* que recruta outras proteínas e facilita a ligação das mesmas e a consequente formação do complexo de ativação. Estudos demonstram que UreD/UreH se liga à urease. Embora o complexo não possua cristal depositado em bancos de dados, um modelo foi proposto através da análise de outras evidencias (Ligabue-Braun, Real-Guerra, et al. 2013) (***Figura 2***).
- UreE: Principal candidata a metalochaperona, pode estar envolvida no fornecimento de níquel de urease devido a sequência da proteína em *K. aerogenes*, que revela 10 resíduos de histidina na porção C-terminal de 15 resíduos, sítio conhecido de ligação de Ni^{2+} e outros metais (Mulrooney & Hausinger 1990).
- UreF: A proteína age como um túnel da atividade de GTPase da UreG, de forma que integre a hidrólise de GTP a biossíntese do metalocentro,

processo que garante a fidelidade da ativação da urease (Boer & Hausinger 2012).

- UreG: Enquanto presente no complexo de ativação da urease, se observa atividade de GTPase. Quando ocorre a substituição de um resíduo chave na alça-P do motivo em que o GTP se liga em *K. aerogenes* ou *H. pylori,* a capacidade da célula produzir urease ativa é perdida (Moncrief & Hausinger 1997; Mehta et al. 2003).

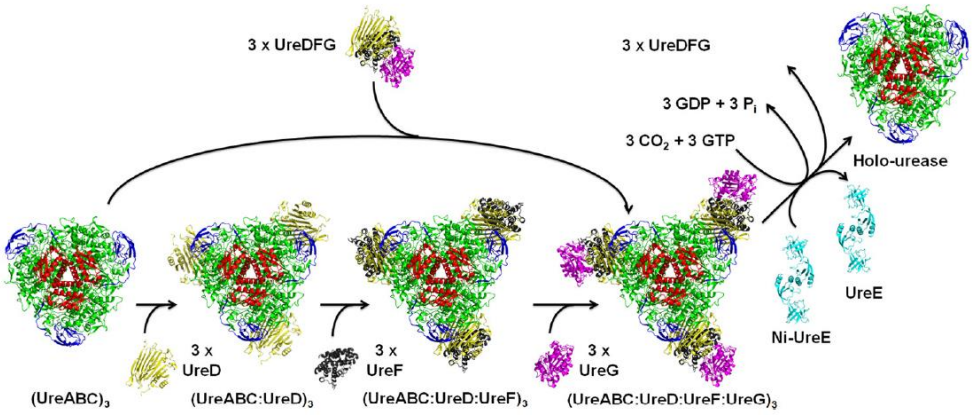


Figura 2: Modelo de ativação da urease através da formação do complexo UreABC:UreD:UreF:UreG:UreE. Modelo apresenta duas possíveis rotas de ativação: Através do acoplamento sequencial das proteínas acessórias de urease (representada nas reações abaixo na figura), ou da ligação do pré-complexo UreDFG diretamente a apoenzima (representada na reação acima na figura), seguido da ligação e transferência do Níquel pela UreE. (Farrugia et al. 2013) Baseado no modelo proposto por (Ligabue-Braun, Real-Guerra, et al. 2013).

1.3 Ancestralidade: Análise filogenética

São observadas algumas peculiaridades características nas ureases no que tange à sua estrutura. Geralmente, ureases de plantas e fungos apresentam estruturas de trímeros ou hexâmeros homo-oligoméricos, normalmente, com cerca de 840 resíduos de aminoácidos (Mobley et al. 1995). Ureases microbianas, por outro lado, são multímeros compostos de duas ou três subunidades (α/C, β/B, e γ/A, variando entre 100 e 570 resíduos), normalmente formando trímeros de αβγ ou αβ (Jabri et al. 1995; Mobley et al. 1995) (veja *Tabela 1*). Esse tipo de peculiaridade

chama atenção pela hipótese da possível fusão das subunidades, com a informação filogenética tida como ferramenta aliada na compreensão de seus possíveis caminhos evolutivos, como foi proposto por Ligabue-Braun et al. (2013).

K. *aerogenes*[a]	H. *pylori*	Plantas	Função
UreA/ γ			Subunidade da enzima
UreB/β	UreA/ γ [b]		Subunidade da enzima
UreC/ α	UreB/β[c]	Urease[d]	Subunidade da enzima
UreD	UreH	UreD	Proteína s*caffold*
UreE	UreE	—[e]	Metalochaperona
UreF	UreF	UreF	Possível potencializador de ligação
UreG	UreG	UreG	GTPase

Tabela 1: Proteínas necessárias para a ativação de ureases e suas potenciais funções. [a] Conjunto de proteínas comuns a maioria das bactérias. [b] Equivalente a fusão das subunidades A e C de *K. aerogenes*. [c] Equivalente a UreB de *K. aerogenes.* [d] Equivalente a fusão das subunidades A, B e C de *K. aerogenes*. [e] Nenhum ortólogo de UreE foi identificado em plantas. Adaptado de (Farrugia et al. 2013).

Para que potenciais caminhos evolutivos das proteínas acessórias de urease sejam inferidos, diferentes abordagens de análise filogenética podem ser utilizadas. A filogenética molecular, especificamente, usa como fonte de informação sequências de nucleotídeos ou aminoácidos. Esses dados são usados em diferentes modelos de algoritmos resultando em análises comparativas normalmente representadas na forma de uma árvore (filogenia ou árvore filogenética), que descreve as relações evolutivas entre as sequências. Dessa forma, é possível demonstrar a provável história evolutiva dos caracteres (proteínas, genes, genomas - chamados de unidades taxonômicas operacionais; OTUs, do inglês, *operational taxonomic units*) dos organismos incluídos nas análises. As árvores são representadas graficamente através de pontos (ou nós) ligados por linhas (ou ramos), de forma que a disposição dos mesmos defina a ancestralidade entre as OTUs (Figura 3). As OTUs, por sua vez, são representadas nos pontos terminais, unidas por linhas cujo nó interno representa

o ancestral comum mais recente desses *taxa*. Para que uma árvore evidencie o ancestral mais antigo do grupo a ser comparado, é necessária a identificação de uma raiz nas filogenias. Geralmente, o enraizamento se dá pela inclusão de uma ou diversas OTUs que representem grupos externos. Os grupos externos devem possuir ancestrais comuns com as OTUs em estudo, indicando caracteres presentes em organismos mais próximos aos ancestrais e provendo um direcionamento para a interpretação dos processos evolutivos (Braun et al. 2014)

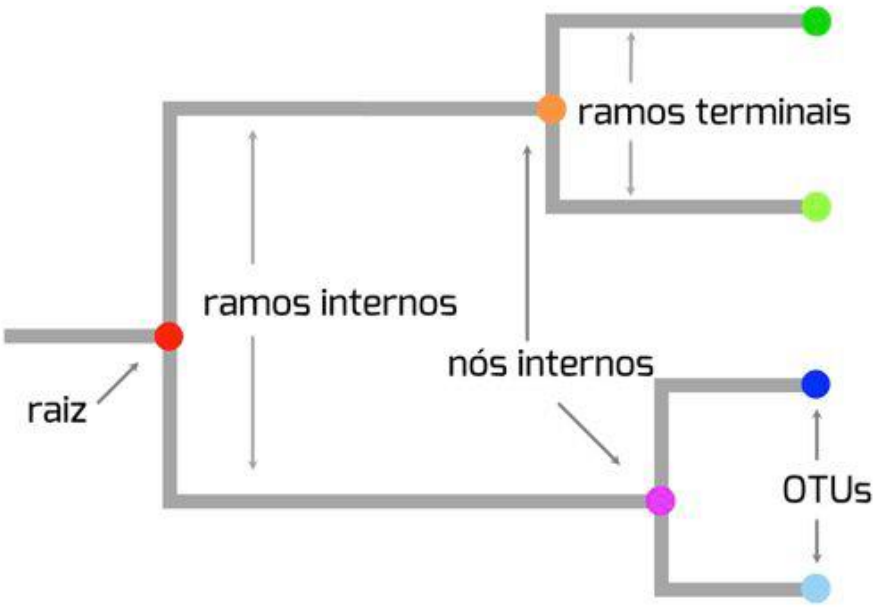


Figura 3: Modelo de nomenclatura de árvores filogenéticas. (Braun et al. 2014)

Finalmente, para que tais dados sejam analisados, é necessária a escolha dos algoritmos de reconstrução filogenética a serem usados. Vale ressaltar que a evolução é contínua e trata-se de um evento histórico, e não podemos observá-la exatamente como ocorreu ao compararmos sequências e outras características. Podemos, no entanto, inferir eventos de evolução através desses algoritmos, na chamada reconstrução filogenética. Para inferências de caráter evolutivo-molecular, podem ser

definidas quatro técnicas computacionais principais: agrupamento de vizinhos, máxima parcimônia, máxima verossimilhança e inferência Bayesiana (Yang 2006).

2. OBJETIVOS

O mecanismo de ativação e as funções de cada proteína acessória na maturação da urease não se encontram completamente elucidados e dependem da dedicação de diversas áreas de estudo, incluindo aplicações da bioinformática.

Para obter-se uma visão geral das relações evolutivas que podem ser estabelecidas entre as proteínas acessórias das ureases e as próprias ureases, na busca de padrões, similaridades e diferenças que possam auxiliar na predição e interpretação de resultados experimentais, o presente estudo pretende realizar uma ampla análise das sequências de aminoácidos de proteínas acessórias de ureases.

Especificamente, o trabalho visa buscar, extrair, editar e interpretar as sequências de diferentes proteínas acessórias de ureases em diferentes espécies. Dessa forma, objetiva-se construir árvores filogenéticas por meio de alinhamentos múltiplos de sequências, avaliar a similaridade e analisar possíveis regiões de conservação.

3. ARTIGO CIENTÍFICO

Urease Activation: Evolutionary Inferences via Phylogeny

Juliano de Oliveira Silveira[1], Rodrigo Ligabue-Braun[3], Célia Regina Carlini[2], Hugo Verli[3]

[1]Biomedical Sciences Undergraduate Program, Institute of Basic Health Sciences, UFRGS, Porto Alegre, RS, Brazil;

[2]InsCer, Brain Institute, Pontificia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil;

[3]Graduate Program in Cellular and Molecular Biology, Center of Biotechnology,
Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.

Corresponding Author: Prof. Hugo Verli, Ph.D., Center of Biotechnology and Faculty of Pharmacy, UFRGS, Av. Bento Gonçalves, 9500, Campus do Vale, Caixa postal 15005,
91501-970 Porto Alegre, RS, Brazil. Phone: +55 51 3308.7770; e-mail: hverli@cbiot.ufrgs.br.

Abstract

Ureases are enzymes are of great historic, agricultural, and medical importance. They catalyze the hydrolysis of urea to ammonia and carbamate. These enzymes are widely distributed in plants, fungi and bacteria. In all of these organisms, the binding of a group of accessory proteins is required for the proper functioning of the enzyme, acting in the modification of the active site and permitting the insertion of a nickel ion, which is essential for catalysis. The metal is inserted into the active site of urease, in a GTP-dependent process, with the aid of UreD/UreH, UreE, UreF, and UreG: accessory proteins which orchestrate the activation of the apoprotein. The mechanisms of activation and function of each urease accessory protein in urease maturation, are not fully understood. To obtain an overview of evolutionary relationships that can be established between the accessory proteins and urease itself, in this study an extensive analysis of the amino acid sequences of the accessory proteins is performed. Whereas the results are preliminary, we conclude that the phylogenetic relationships presented here demonstrate that the accessory proteins seem to follow similar tree topologies to those obtained for urease. Furthermore, the results of chimeric UreG trees phylogenetic analysis suggest that they may be suffering from Long Branch Attraction.

Keywords: Urease accessory proteins, Phylogeny, Molecular evolution, Urease activation

1. Introduction

Ureases (urea amidohydrolases, EC 3.5.1.5) are enzymes that catalyze the hydrolysis of urea to ammonia and carbamate, which then decomposes into bicarbonate and another ammonia molecule (Mobley et al. 1995). Ureases are biomolecules of great importance in technological activities such as medicine and agriculture. It has been shown, for example, that urease could be a key virulence factor for various long term and chronic diseases (Konieczna et al. 2012), (Banić et al. 2012). Besides having high potential for technological applicability, they have an important historical role in the development of basic sciences in areas such as enzymology, crystallography, molecular and structural biology. Urease from jack bean seeds (*Canavalia ensiformis*) was the first enzyme to be crystallized (Sumner 1926) and the first protein shown to contain nickel (Dixon 1975). Thus, urease is an important model for the advance of our understanding of the assembly mechanisms of metallocenters.

These enzymes are widely distributed in plants, fungi, archaea, and bacteria. In all urease positive organisms, the binding of a group of urease accessory proteins (UAP) is required for the activation of the enzyme (Yukl & Wilmot 2013), working on modifying the target site and allowing the insertion of nickel ions essential for catalysis (Schenk et al. 2012). Nickel is inserted into the active site of urease with the aid of the UAPs UreD/UreH, UreE, UreF, and ureG, through a GTP-dependent process (Carter et al. 2009). These accessory proteins orchestrate the activation of the apoprotein by providing the suitable metal and facilitating conformational changes of the protein and, eventually, necessary post-translational modifications (Farrugia et al. 2013).

The mechanism of activation and function of each accessory protein in the urease maturation are not fully elucidated and depend on the dedication of several areas of study, including bioinformatics applications. To obtain an overview of evolutionary relationships that can be established between the accessory proteins of urease and urease itself, this study intends to conduct a comprehensive analysis of the amino acid sequences of the urease accessory proteins through a phylogenetic approach. We expect that by searching for patterns, similarities, and differences, the results may assist in the prediction and interpretation of experimental data in the field of urease activation and metallocenter assembly.

2. Materials and Methods

The urease accessory proteins amino acid sequences were retrieved from the National Center for Biotechnology Information (Sayers et al. 2012) protein database. Lists of organisms representing a broad range of differences and shown to be effective for phylogenetic reconstruction for urease were used as a guide (Ligabue-Braun et al. 2013). The keyword search comprised the given UAP followed by the species or gender name (eg. UreD + *Canavalia ensiformis*). When the "UAP + species" combination did not return a valid sequence, the species segment would be deleted and a new search would be run looking for "UAP + genus" (eg. UreD + *Canavalia*). In case of another negative response, the organism would not be included in the study. In order to avoid prediction and annotation problems, manual filtering was conducted in the resulting sequences from the query. Sequences labeled as putative, predicted, or hypothetical were excluded, as well as sequences from uncultured organisms, or with incomplete sequences (number of AA lower than 50% of the protein consensus). Resulting sequences were grouped by protein: UreD/H, UreE, UreF, and UreG, and an abbreviation was created for each genus/species (Supp. Tables 1, 2, 3, and 4).

The ClustalW (McWilliam et al. 2013) algorithm was used for alignments. In order for the most appropriate model of amino acid substitution to be calculated for each group, the resulting alignments were subjected to analysis in the Evolutionary Molecular Genetic Analysis Package 6 (MEGA6) (Tamura et al. 2013) (Table 1). Following the AA substitution model calculated for each of the alignments, phylogenetic trees were estimated by the maximum likelihood (ML) method using MEGA6 with 1,000 replicates of bootstrap. All trees were rooted using proteins from BLAST searches (Altschul et al. 1990) of each accessory protein of *Klebsiella pneumoniae* with similarity below 30% (Table 2). The obtained trees were viewed and edited through the program FigTree (Rambaut 2009). For the analysis of conservation and sequence similarity the JALVIEW (Waterhouse et al. 2009) program was used. Chimeric sequences comprising only conserved sequence segments were built using Bioedit software (Hall 1999), using results from the sequence similarity analysis.

3. Results

Primarily, we were able to generate four phylogenetic trees, one for each UAP, through the application of ML method on the alignments for each set of full protein sequences (Figs. 1, 2, 3, and 4) with very low bootstrap values in the more internal nodes. Resulting models of amino acid substitution for the full sequence sets are shown in Table 1. Subsequently, a chimera set was created for UreG using conserved regions with at least 35% of conservation (Fig 5). Conservation of at least 35% was not evidenced when the analysis was carried in the other

protein sets, for that reason, the following analysis continued using only UreG. The set was submitted to ML analysis, using the LG + G model and resulted in a phylogenetic tree with similar low bootstrap values, especially in internal nodes (Fig 6). Finally, we decided to take a minimalistic approach, using less representatives from each group of organisms (3 from plants, 3 from archaea, 3 from fungi, and 3 from *Helicobacter*) for a new chimera set of UreG (Fig 7). This time, higher bootstrap values were shown resulting in a resolved tree.

4. Discussion

Because of low bootstrap values, trees with full AA sequences are not fully resolved. The overall topology found on the cited trees, however, resulted in groupings very similar to tree topology obtained for urease (Ligabue-Braun et al. 2013). In order to evaluate whether the low bootstrap values were due to lack of conservation on some regions and/or large number of gaps, we repeated the analysis in UreG using conserved areas only. The resulting tree still showed very low bootstrap values (Fig 6), suggesting that low conservation and gap-rich regions were not the bottleneck for the resolution of the tree. Finally, the results found in figure 7, in which a smaller number of UreG AA sequences was used is more resolved and shows higher reliability in terms of bootstrap.

We could observe in an extra tree (Figure 7) that as we include more sequences in the analysis, the bootstrap values tend to diminish. Such evidence suggests that urease accessory proteins, or at least UreG, are highly heterogeneous among species. Given that sequences vary so much among closely related groups, it can be difficult to separate them apart from further groups which do not share ancestors. Therefore, we propose that the phylogenetic analysis of urease accessory proteins could be under effect of the Long Branch Attraction (LBA) (Li et al. 2007) phenomenon. When this occurs, distant groups are considered to be more closely related, which causes the algorithm to investigate such relationship more frequently, resulting in a low bootstrap number (more topologies are erroneously considered likely to be representative, so the number of times the branch organization meets a certain criteria is divided by the other likely states.). Maximum likelihood inference, although robust and free from several biases when compared to other methods for phylogenetic reconstruction, still struggles with LBA. Also, there seems to exist few analytical solutions for ML, therefore the biases it suffers are not well understood (Parks & Goldman 2014). Autapomorphies rich samples, be it nucleotide or AA sequences, currently represent an obstacle when reconstructing phylogenies. Less sequences from brother groups diminish the inconsistency that high intra species variability may lead to. The very definition of inconsistency clearly states that adding more data will only strengthen the wrong tree (Sullivan & Swofford 1997), this way, having less sequences would lead to more information, or at least more precision in the obtained tree.

5. Conclusion

Given that the results are preliminary, we conclude that the phylogenies presented here demonstrate that urease accessory proteins seem to follow similar tree topologies when compared to urease. The results from chimera trees of UreG suggest that the phylogeny analysis can be suffering from Long Branch Attraction. In addition, we highlight the hardship found in working with such proteins, which seem to have accumulated many differences, even within closely related groups. Further analysis will replicate the methodology used in UreG, with less sequences, for the other accessory proteins.

6. Acknowledgements

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), MEC, Brasília-DF, Brazil.

7. Conflict of Interest

The authors declare no conflict of interest regarding the work presented.

References

Altschul, S.F. et al., 1990. Basic local alignment search tool. *Journal of molecular biology*, 215, pp.403–410.

Banić, M. et al., 2012. Extragastric Manifestations of Helicobacter pylori Infection. *Helicobacter*, 17, pp.49–55.

Dixon, N., 1975. Jack bean urease (EC 3.5.1.5). A metalloenzyme. Simple biological role for nickel? *Journal of the American Chemical Society*, (97), pp.4131–4133. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1159216.

Hall, T., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, pp.95–98. Available at: http://jwbrown.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf.

Konieczna, I. et al., 2012. Bacterial urease and its role in long-lasting human diseases. *Current protein & peptide science*, 13(8), pp.789–806. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3816311&tool=pmcentrez&rendertype=abstract.

Le, S.Q. & Gascuel, O., 2008. An improved general amino acid replacement matrix. *Molecular Biology and Evolution*, 25, pp.1307–1320.

Li, Y.-W., Yu, L. & Zhang, Y.-P., 2007. "Long-branch Attraction" artifact in phylogenetic reconstruction. *Yi chuan = Hereditas / Zhongguo yi chuan xue hui bian ji*, 29, pp.659–667.

Ligabue-Braun, R. et al., 2013. 3-To-1: Unraveling Structural Transitions in Ureases. *Die Naturwissenschaften*, 100(5), pp.459–67. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23619940 [Accessed April 30, 2014].

McWilliam, H. et al., 2013. Analysis Tool Web Services from the EMBL-EBI. *Nucleic acids research*, 41.

Mobley, H.L., Island, M.D. & Hausinger, R.P., 1995. Molecular biology of microbial ureases. *Microbiological reviews*, 59, pp.451–480.

Parks, S.L. & Goldman, N., 2014. Maximum Likelihood Inference of Small Trees in the Presence of Long Branches. *Systematic biology*, 0, pp.1–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24996414.

Rambaut, A., 2009. FigTree v1.3.1. 2006-2009. *Accessed on November 29, 2012*, p.Program package available at http://tree.bio.ed.ac.

Sullivan, J. & Swofford, D.L., 1997. Are guinea pigs rodents? The importance of adequate models in molecular phylogenetics. *Journal of Mammalian Evolution*, 4, pp.77–86.

Sumner, J.B., 1926. The Isolation And Crystallization Of The Enzyme Urease. *J. Biol. Chem.*, 69(2), pp.435–441. Available at: http://www.jbc.org/cgi/content/long/69/2/435 [Accessed November 4, 2014].

Tamura, K. et al., 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, pp.2725–2729.

Waterhouse, A.M. et al., 2009. Jalview Version 2-A multiple sequence alignment editor and analysis workbench. *Bioinformatics*, 25, pp.1189–1191.

Figures/Tables and legends

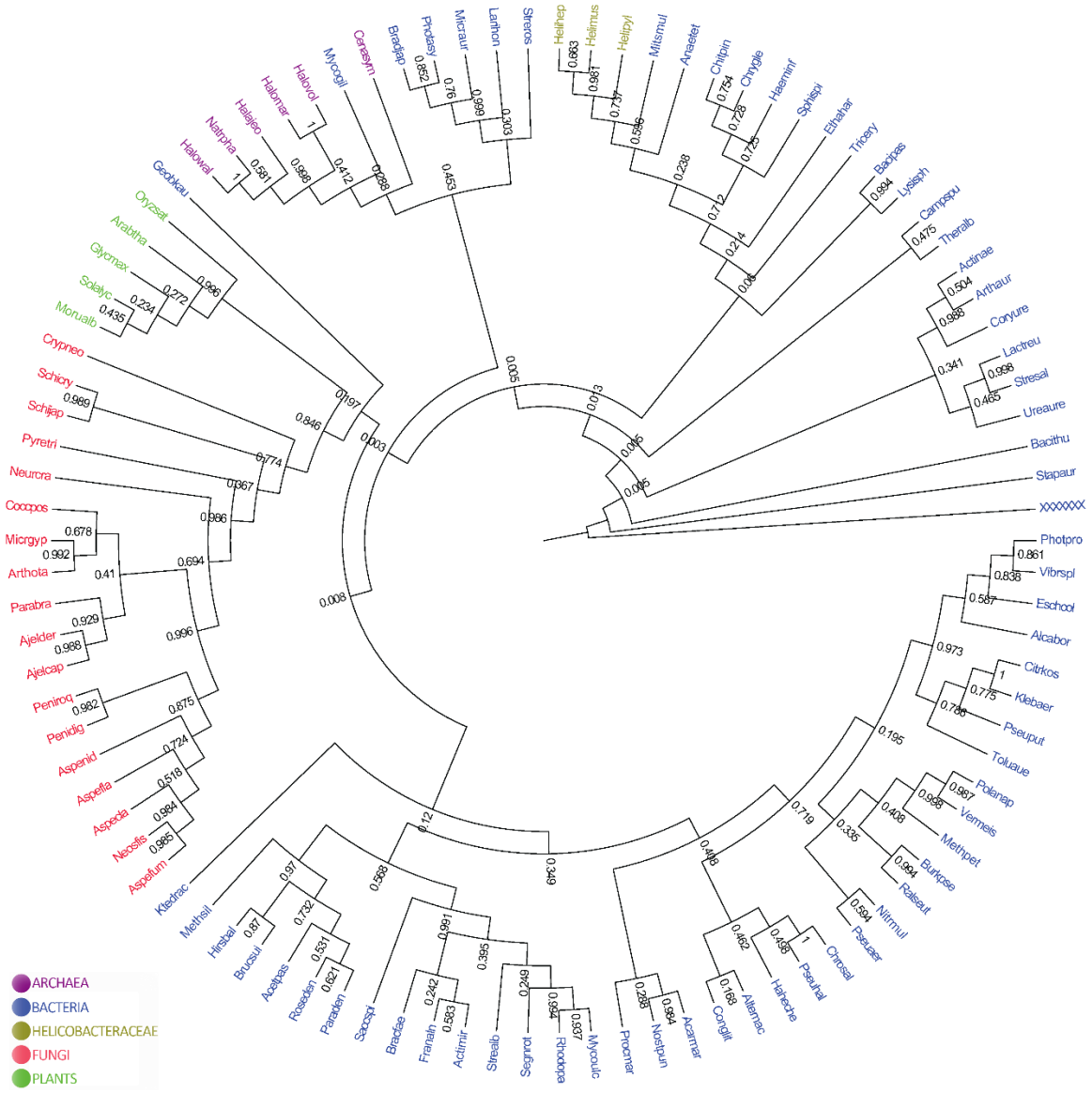


Figure 1: Molecular phylogenetic analysis of full UreD / H sequences by the Maximum Likelihood method. The evolutionary history was inferred using the ML method based on the model LG + G + F. Rooting sequence represented by "XXXXXXX" refers to *Methanobacterium sp.* Numbers on internodes represent the bootstrap values for the given topology.

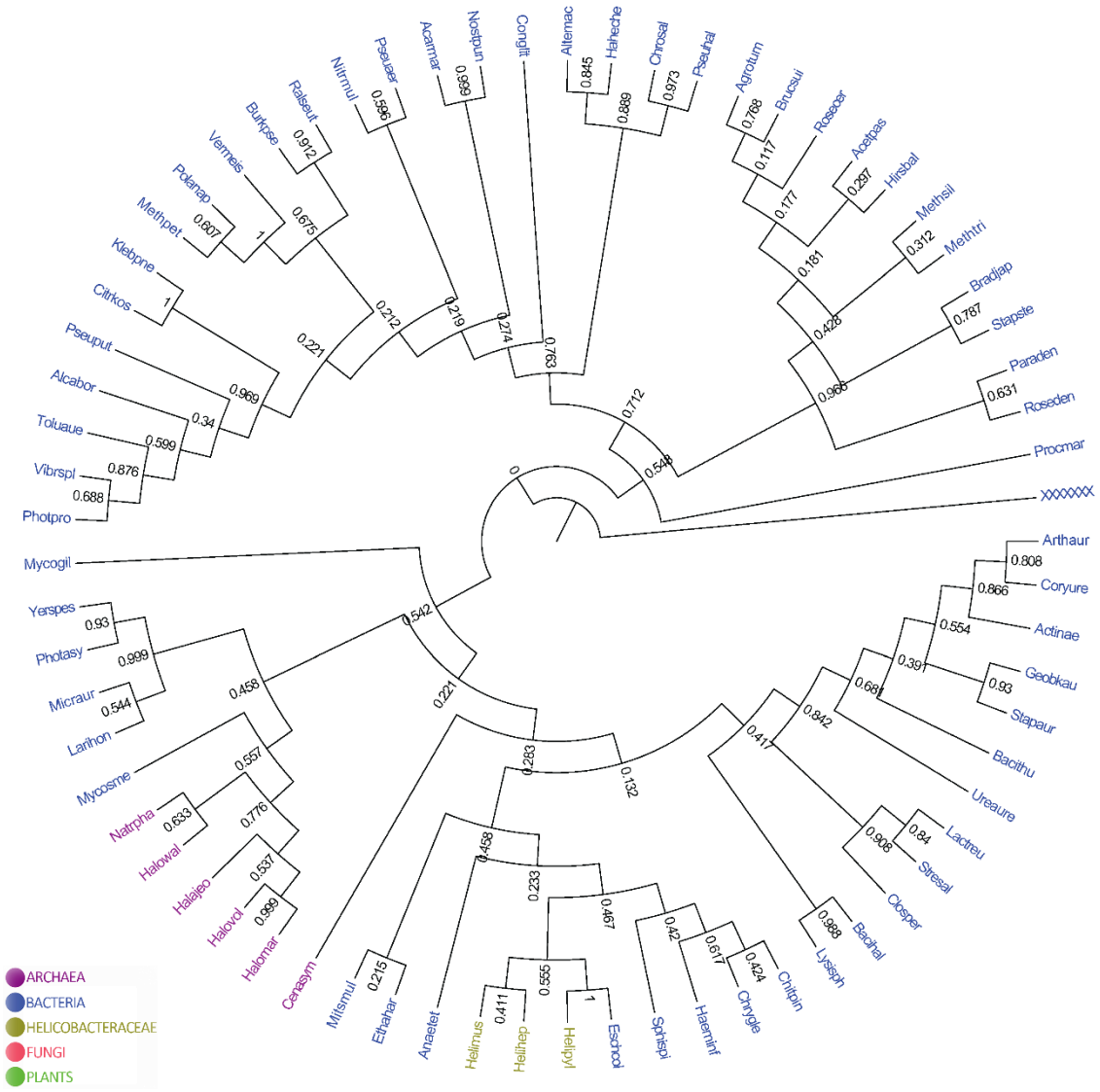


Figure 2: Molecular phylogenetic analysis of full UreE sequences by the Maximum Likelihood method. The evolutionary history was inferred using the ML method based on the model LG + G + I. Rooting sequence represented by "XXXXXXX" refers to *Aeromonas diversas.* Numbers on internodes represent the bootstrap values for the given topology.

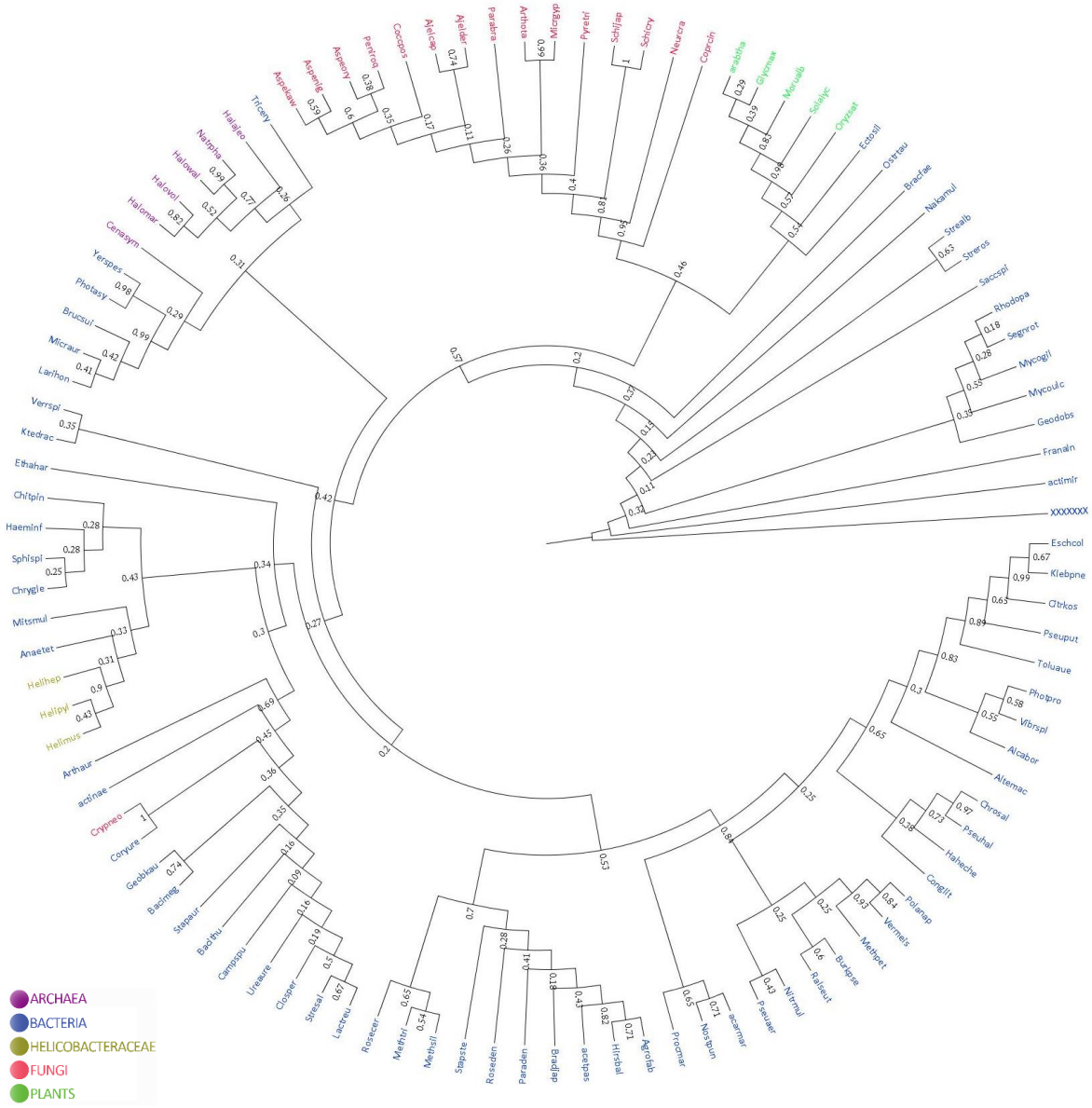


Figure 3: Molecular phylogenetic analysis of full UreF sequences by the Maximum Likelihood method. The evolutionary history was inferred using the ML method based on the model LG + G + I + F. Rooting sequence represented by "XXXXXXX" refers to *Aspergillus terreus*. Numbers on internodes represent the bootstrap values for the given topology.

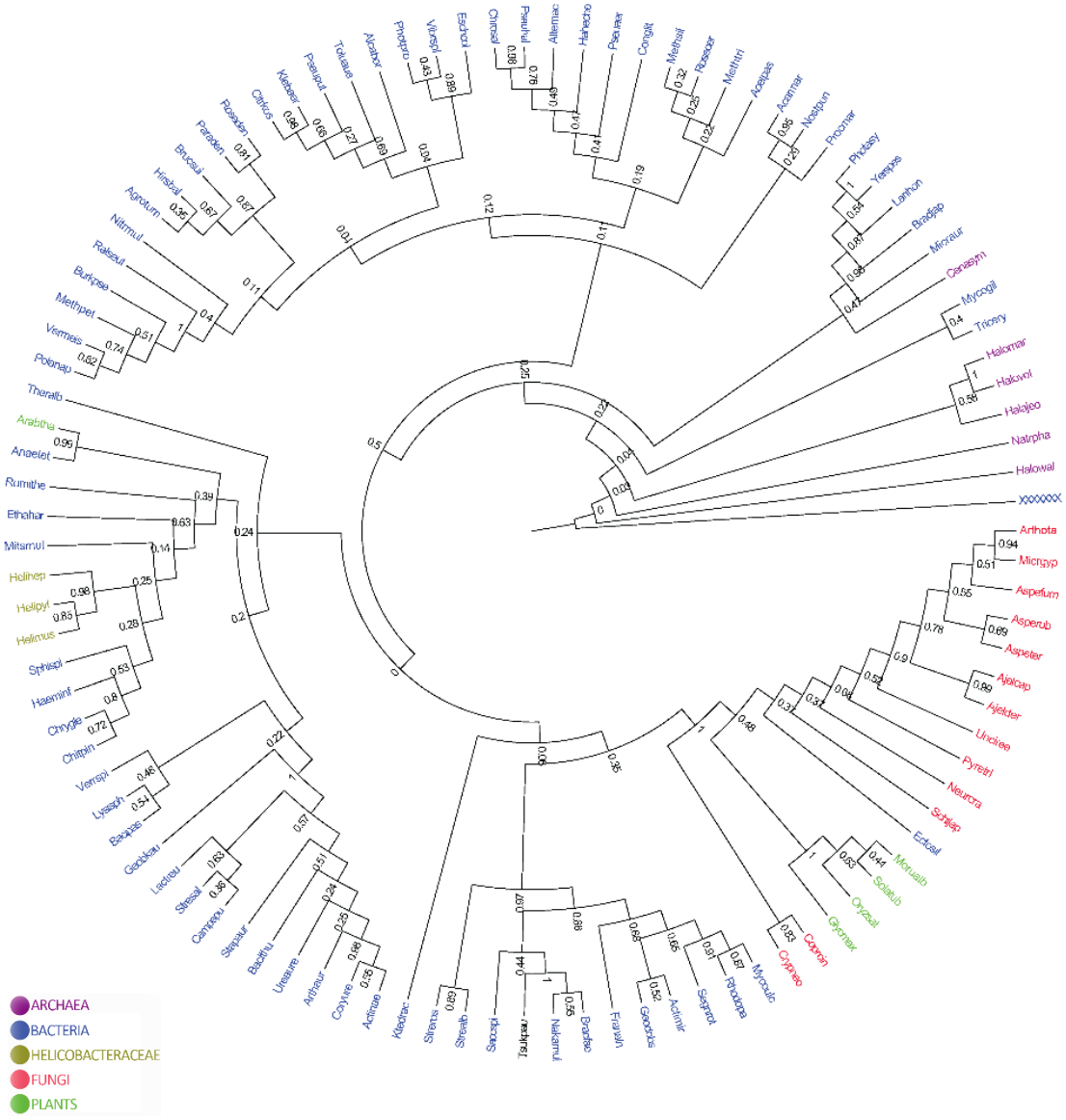


Figure 4: Molecular phylogenetic analysis of full UreG sequences by the Maximum Likelihood method. The evolutionary history was inferred using the ML method based on the model LG + G + I. Rooting sequence represented by "XXXXXXX" refers to *Bradyrhizobium sp.* Numbers on internodes represent the bootstrap values for the given topology.

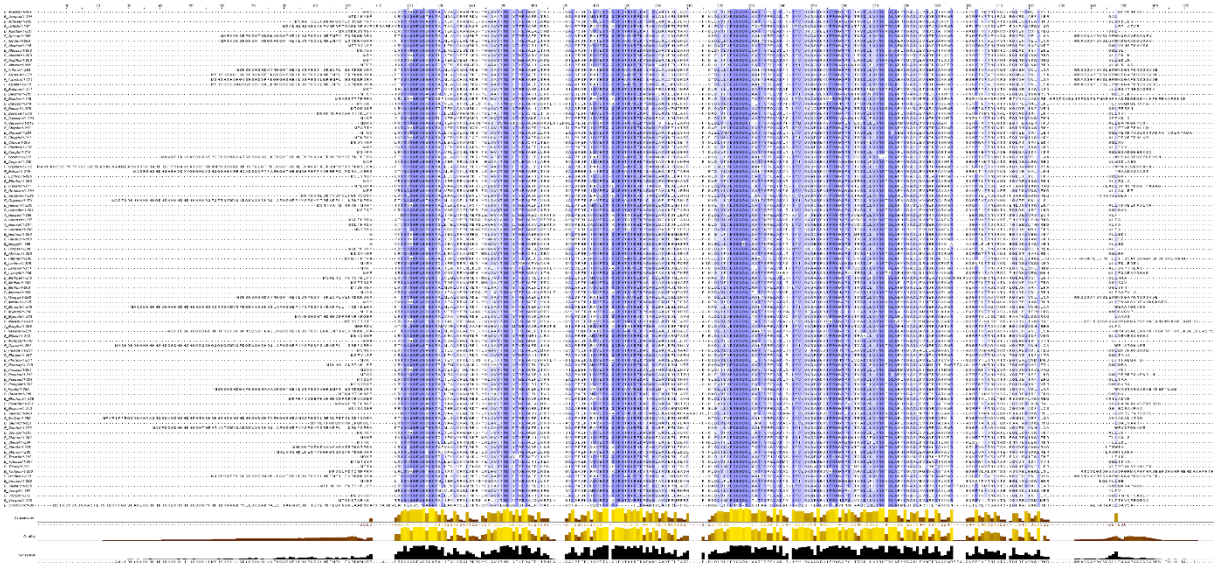


Figure 5: Analysis of conserved regions of the protein UreG. Regions hatched in blue represent minimum 35% conservation.

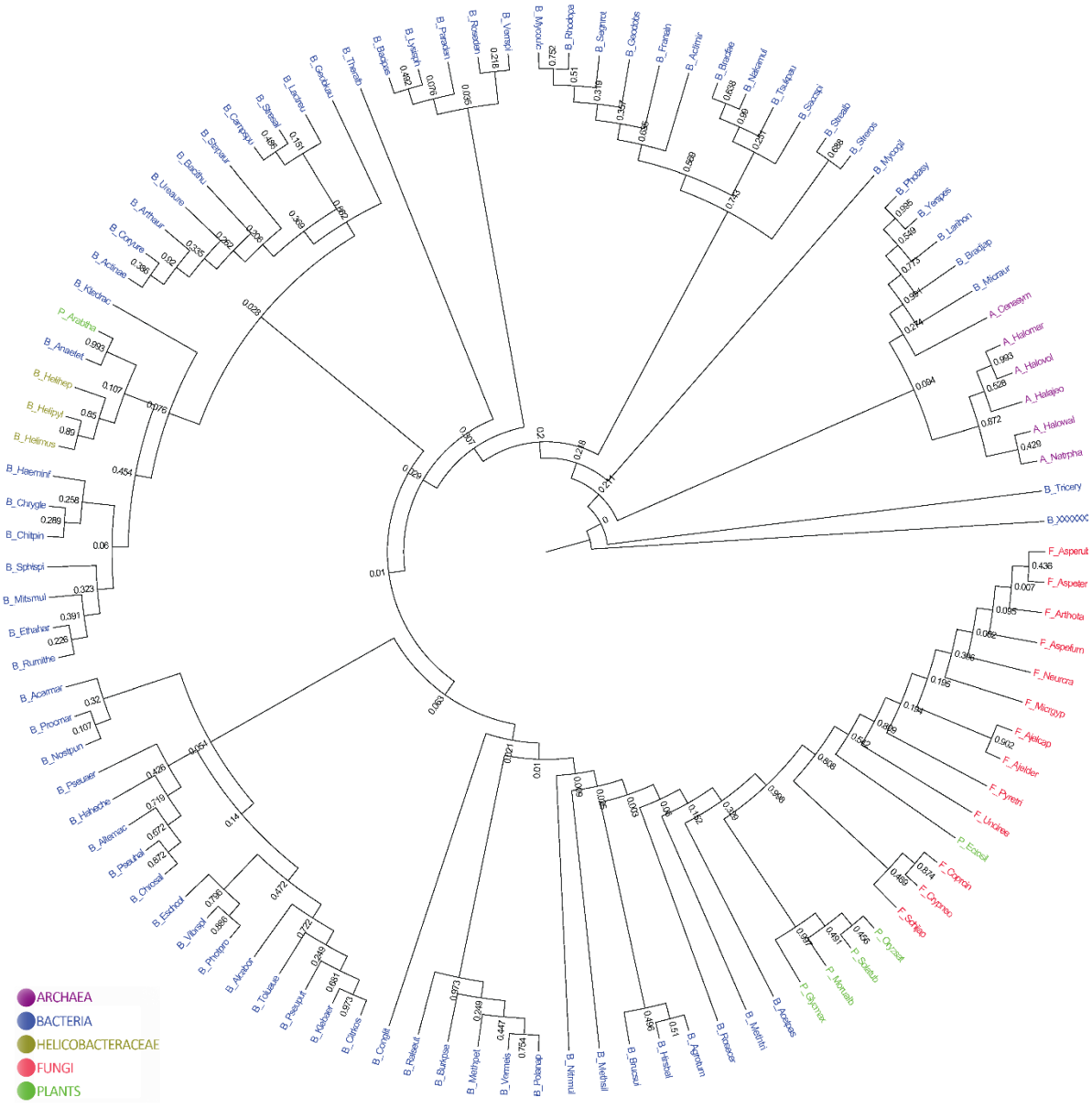


Figure 6: Molecular phylogenetic analysis of chimeric UreG sequences by the Maximum Likelihood method. The evolutionary history was inferred using the ML method based on the model LG + G . Rooting sequence represented by "XXXXXXX" refers to *Bradyrhizobium sp.* Numbers on internodes represent the bootstrap values for the given topology.

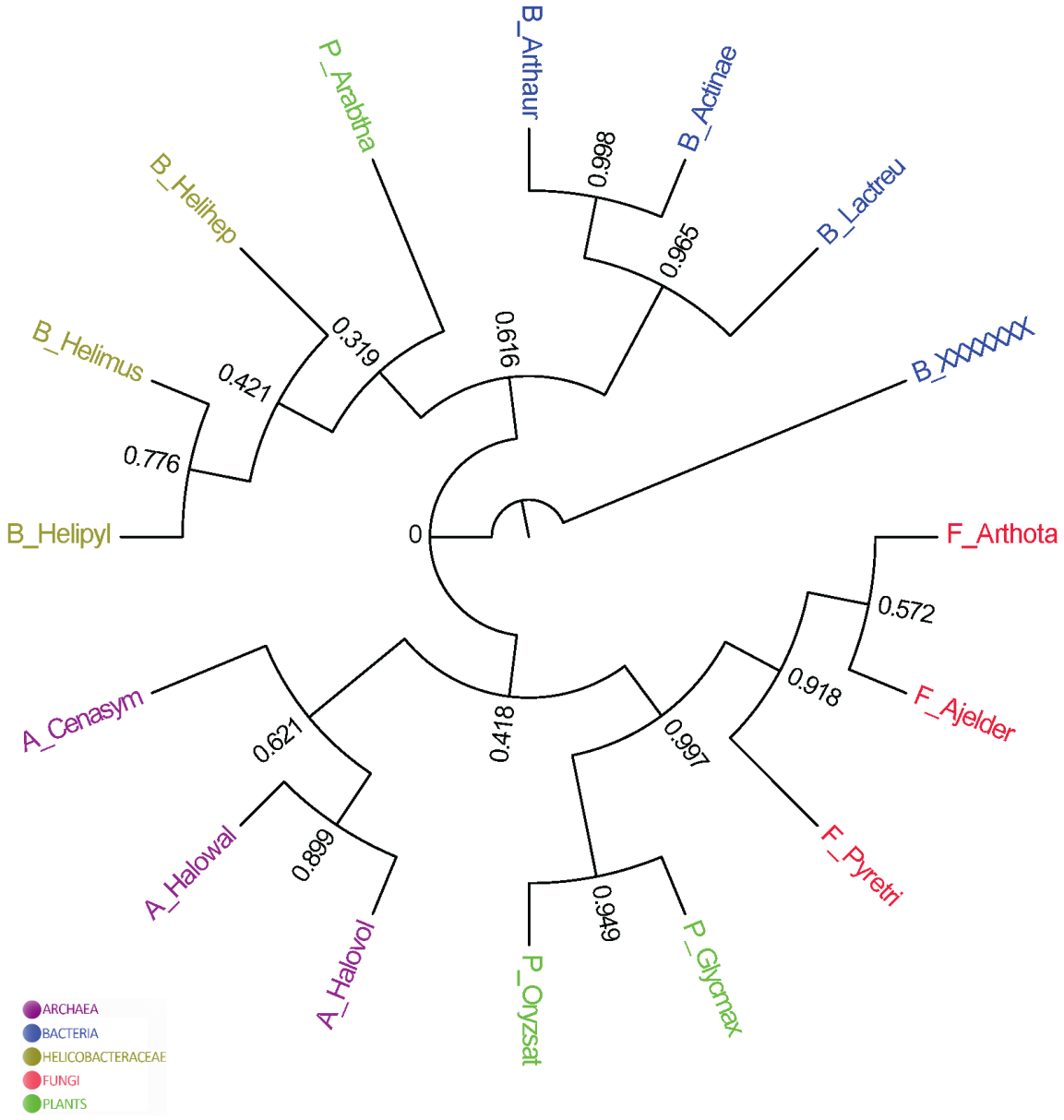


Figure 7: Molecular phylogenetic analysis of minimal chimeric UreG sequences by the Maximum Likelihood method. The evolutionary history was inferred using the ML method based on the model LG + G . Rooting sequence represented by "XXXXXXX" refers to *Bradyrhizobium sp*. Numbers on internodes represent the bootstrap values for the given topology.

Table 1: Models of amino acid substitution calculated in MEGA6 program for each accessory protein group. LG: Le & Gascuel general amino acid replacement matrix (Le & Gascuel 2008). G: Gamma distribution to account for evolutionary rate differences among sites. I: Considers some sites invariable. F: Takes in consideration observed AA frequencies.

Protein Group	Substitution Model
UreD/H	LG + G + F
UreE	LG + G + I
UreF	LG + G + I + F
UreG	LG + G + I
UreG Chimera	LG + G

Table 2: Sequences used as external groups for rooting of each accessory protein group.

Protein Group	Root Protein and Source Organism
UreD/H	Radical SAM domain-containing protein (*Methanobacterium sp.)*
UreE	ABC transporter ATP-binding protein (*Aeromonas diversas*)
UreF	Succinyl-CoA ligase beta-chain (*Aspergillus terreus)*
UreG and UreG Chimera	Hydrogenase nickel incorporation protein HypB (*Bradyrhizobium sp.*)

Supplementary Tables

Table 1: Table of used organisms from which UreD sequences were prospected. Sequence records are identified by GI numbers.

Species	*Abbreviation*	*GI*
Acaryochloris marina	Acarmar	190411570
Acetobacter pasteurianus	Acetpas	256652747
Actinosynnema mirum	Actimir	502426239
Actinomyces naeslundii	Actinae	7388355
Agrobacterium tumefaciens	Agrotum	635308168
Ajellomyces capsulatus	Ajelcap	240282260
Ajellomyces dermatitidis	Ajelder	327353434
Alcanivorax borkumensis	Alcabor	110835583
Alteromonas macleodii	Altemac	559062507
Anaerococcus tetradius	Anaetet	227216939
Arabidopsis thaliana	Arabtha	75145929
Arthrobacter aurescens	Arthaur	190411576
Arthroderma otae	Arthota	238845514
Aspergillus clavatus	Aspecla	119404049
Aspergillus flavus	Aspefla	220697881
Aspergillus fumigatus	Aspefum	666433420
Aspergillus nidulans	Aspenid	259487523
Bacillus pasteurii	Bacipas	205830126
Bacillus thuringiensis	Bacithu	595878206
Brachybacterium faecium	Bracfae	502488431
Bradyrhizobium japonicum	Bradjap	654722572
Brucella suis	Brucsui	190411640
Burkholderia pseudomallei	Burkpse	190411638
Campylobacter sputorum	Campspu	260162329
Cenarchaeum symbiosum	Cenasym	190411588
Chitinophaga pinensis	Chitpin	502446313
Chromohalobacter salexigens	Chrosal	122419607
Chryseobacterium gleum	Chrygle	300502356
Citrobacter koseri	Citrkos	673535173
Clostridium perfringens	Closper	170710936
Coccidioides posadasii	Coccpos	320036446
Congregibacter litoralis	Conglit	495571008
Corynebacterium urealyticum	Coryure	448277877
Cryptococcus neoformans	Crypneo	405120208
Escherichia coli	Eschcol	418163
Ethanoligenens harbinense	Ethahar	503251842
Frankia alni	Franaln	205830842
Geobacillus kaustophilus	Geobkau	81703969
Glycine max	Glycmax	351727327

Haemophilus influenzae	Haeminf	1174908
Hahella chejuensis	Haheche	190411635
Halalkalicoccus jeotgali	Halajeo	495693486
Haloarcula marismortui	Halomar	34419200
Haloferax volcanii	Halovol	291372705
Haloquadratum walsbyi	Halowal	121689508
Helicobacter hepaticus	Helihep	14579325
Helicobacter mustelae	Helimus	502787689
Helicobacter pylori	Helipyl	332672906
Hirschia baltica	Hirsbal	506308782
Klebsiella aerogenes	Klebaer	731078
Ktedonobacter racemifer	Ktedrac	495193921
Lactobacillus reuteri	Lactreu	659901712
Laribacter hongkongensis	Larihon	654310527
Lysinibacillus sphaericus	Lysisph	205830835
Methylibium petroleiphilum	Methpet	190411631
Methylocella silvestris	Methsil	501586734
Micromonospora aurantiaca	Micraur	503052676
Microsporum gypseum	Micrgyp	311343242
Mitsuokella multacida	Mitsmul	260850227
Morus alba	Morualb	222143566
Mycobacterium gilvum	Mycogil	190411605
Mycobacterium ulcerans	Mycoulc	118570527
Natronomonas pharaonis	Natrpha	121695537
Neosartorya fischeri	Neosfis	119409225
Neurospora crassa	Neurcra	28922161
Nitrosospira multiformis	Nitrmul	190411630
Nostoc punctiforme	Nostpun	205830823
Oryza sativa	Oryzsat	301344549
Paracoccidioides brasiliensis	Parabra	226291919
Paracoccus denitrificans	Paraden	190411606
Penicillium digitatum	Penidig	425769194
Penicillium roqueforti	Peniroq	584412396
Photorhabdus asymbiotica	Photasy	506314736
Photobacterium profundum	Photpro	493270411
Polaromonas naphthalenivorans	Polanap	190411607
Prochlorococcus marinus	Procmar	7839378
Pseudomonas aeruginosa	Pseuaer	81539701
Pseudoalteromonas haloplanktis	Pseuhal	123588265
Pseudomonas putida	Pseuput	81440943
Pyrenophora tritici_repentis	Pyretri	187982937
Ralstonia eutropha	Ralseut	7388345
Rhodococcus opacus	Rhodopa	226365152
Roseobacter denitrificans	Roseden	123172087
Saccharopolyspora spinosa	Saccspi	41350150

Schizosaccharomyces cryophilus	Schicry	528316377
Schizosaccharomyces japonicus	Schijap	213410395
Segniliparus rotundus	Segnrot	502903350
Solanum lycopersicum	Solalyc	350537735
Sphingobacterium spiritivorum	Sphispi	300760796
Staphylococcus aureus	Stapaur	384231297
Streptomyces albus	Strealb	664085801
Streptosporangium roseum	Streros	665590112
Streptococcus salivarius	Stresal	2501636
Thermocrinis albus	Theralb	502756781
Tolumonas auensis	Toluaue	506358985
Trichodesmium erythraeum	Tricery	122965194
Ureaplasma urealyticum	Ureaure	254797563
Verminephrobacter eiseniae	Vermeis	190411617
Vibrio splendidus	Vibrspl	518658520
Yersinia pestis	Yerspes	3901302

Table 2: Table of used organisms from which UreE sequences were prospected. Sequence records are identified by GI numbers.

Species	*Abbreviation*	*GI*
Acaryochloris marina	Acarmar	501111920
Acetobacter pasteurianus	Acetpas	256652743
Actinomyces naeslundii	Actinae	9789805
Agrobacterium tumefaciens	Agrotum	635308178
Alcanivorax borkumensis	Alcabor	110835579
Alteromonas macleodii	Altemac	226695780
Anaerococcus tetradius	Anaetet	490976022
Arthrobacter aurescens	Arthaur	167012849
Bacillus halodurans	Bacihal	81788163
Bacillus thuringiensis	Bacithu	595878209
Bradyrhizobium japonicum	Bradjap	654719640
Brucella suis	Brucsui	597795047
Burkholderia pseudomallei	Burkpse	157939456
Cenarchaeum symbiosum	Cenasym	503247054
Chitinophaga pinensis	Chitpin	256420520
Chromohalobacter salexigens	Chrosal	499826866
Chryseobacterium gleum	Chrygle	300502353
Citrobacter koseri	Citrkos	673535177
Clostridium perfringens	Closper	170662092
Congregibacter litoralis	Conglit	563352888
Corynebacterium urealyticum	Coryure	226695783
Escherichia coli	Eschcol	485333
Ethanoligenens harbinense	Ethahar	503250833
Geobacillus kaustophilus	Geobkau	81703968
Haemophilus influenzae	Haeminf	16272482
Hahella chejuensis	Haheche	83635257
Halalkalicoccus jeotgali	Halajeo	495693487
Haloarcula marismortui	Halomar	34419201
Haloferax volcanii	Halovol	291372442
Haloquadratum walsbyi	Halowal	385804940
Helicobacter hepaticus	Helihep	14579322
Helicobacter mustelae	Helimus	291276541
Helicobacter pylori	Helipyl	485333
Hirschia baltica	Hirsbal	506308777
Klebsiella pneumoniae	Klebpne	499532364
Lactobacillus reuteri	Lactreu	659901709
Laribacter hongkongensis	Larihon	654310525
Lysinibacillus sphaericus	Lysisph	226695790
Methylibium petroleiphilum	Methpet	189037565
Methylocella silvestris	Methsil	501586730
Methylosinus trichosporium	Methtri	489708565
Micromonospora aurantiaca	Micraur	503052679
Mitsuokella multacida	Mitsmul	260850224
Mycobacterium gilvum	Mycogil	500222475

Mycobacterium smegmatis	Mycosme	118472340
Natronomonas pharaonis	Natrpha	88909693
Nitrosospira multiformis	Nitrmul	123544644
Nostoc punctiforme	Nostpun	186686176
Paracoccus denitrificans	Paraden	226695792
Photobacterium profundum	Photpro	493270419
Photorhabdus asymbiotica	Photasy	506314739
Polaromonas naphthalenivorans	Polanap	189037567
Prochlorococcus marinus	Procmar	7839379
Pseudoalteromonas haloplanktis	Pseuhal	123588264
Pseudomonas aeruginosa	Pseuaer	15600084
Pseudomonas putida	Pseuput	313499134
Ralstonia eutropha	Ralseut	9789778
Roseobacter denitrificans	Roseden	109457062
Roseomonas cervicalis	Rosecer	296263240
Sphingobacterium spiritivorum	Sphispi	300760793
Staphylococcus aureus	Stapaur	384231294
Stappia stellulata	Stapste	656018176
Streptococcus salivarius	Stresal	363548486
Tolumonas auensis	Toluaue	259710157
Ureaplasma urealyticum	Ureaure	7316078
Verminephrobacter eiseniae	Vermeis	189037574
Vibrio splendidus	Vibrspl	518658517
Yersinia pestis	Yerspes	6686076

Table 3: Table of used organisms from which UreF sequences were prospected. Sequence records are identified by GI numbers.

Species	*Abbreviation*	*GI*
Acaryochloris marina	Acarmar	205830115
Acetobacter pasteurianus	Acetpas	256652742
Actinosynnema mirum	Actimir	502426242
Actinomyces naeslundii	Actinae	4249615
Agrobacterium fabrum	Agrofab	205830125
Ajellomyces capsulatus	Ajelcap	225554742
Ajellomyces dermatitidis	Ajelder	327354766
Alcanivorax borkumensis	Alcabor	499909253
Alteromonas macleodii	Altemac	522831428
Anaerococcus tetradius	Anaetet	227216937
Arabidopsis thaliana	Arabtha	15219116
Arthrobacter aurescens	Arthaur	205830117
Arthroderma otae	Arthota	238842926
Aspergillus kawachii	Aspekaw	358369688
Aspergillus niger	Aspenig	317035317
Aspergillus oryzae	Aspeory	317148887
Bacillus megaterium	Bacimeg	294499714
Bacillus thuringiensis	Bacithu	595878208
Brachybacterium faecium	Bracfae	502488432
Bradyrhizobium japonicum	Bradjap	658448727
Brucella suis	Brucsui	648132136
Burkholderia pseudomallei	Burkpse	81379229
Campylobacter sputorum	Campspu	260162334
Cenarchaeum symbiosum	Cenasym	503247053
Chitinophaga pinensis	Chitpin	502446311
Chromohalobacter salexigens	Chrosal	122419611
Chryseobacterium gleum	Chrygle	300502354
Citrobacter koseri	Citrkos	673535178
Clostridium perfringens	Closper	170662151
Coccidioides posadasii	Coccpos	320034975
Congregibacter litoralis	Conglit	495571013
Coprinopsis cinerea	Coprcin	169861560
Corynebacterium urealyticum	Coryure	501329375
Cryptococcus neoformans	Crypneo	448277875
Ectocarpus siliculosus	Ectosil	298711027
Escherichia coli	Eschcol	15830580
Ethanoligenens harbinense	Ethahar	503251844
Frankia alni	Franaln	111220897
Geobacillus kaustophilus	Geobkau	81703969
Geodermatophilus obscurus	Geodobs	502711312
Glycine max	Glycmax	351726506
Haemophilus influenzae	Haeminf	30995384

Hahella chejuensis	Haheche	123531909
Halalkalicoccus jeotgali	Halajeo	495693488
Haloarcula marismortui	Halomar	34419202
Haloferax volcanii	Halovol	291371470
Haloquadratum walsbyi	Halowal	385804941
Helicobacter hepaticus	Helihep	14579323
Helicobacter mustelae	Helimus	291276542
Helicobacter pylori	Helipyl	485334
Hirschia baltica	Hirsbal	506308776
Klebsiella pneumoniae	Klebpne	641638415
Ktedonobacter racemifer	Ktedrac	495193920
Lactobacillus reuteri	Lactreu	659901710
Laribacter hongkongensis	Larihon	254797573
Lysinibacillus sphaericus	Lysisph	659841406
Methylibium petroleiphilum	Methpet	205830511
Methylocella silvestris	Methsil	501586729
Methylosinus trichosporium	Methtri	639846881
Micromonospora aurantiaca	Micraur	503052678
Microsporum gypseum	Micrgyp	311344260
Mitsuokella multacida	Mitsmul	260850225
Morus alba	Morualb	222143564
Mycobacterium gilvum	Mycogil	503236987
Mycobacterium ulcerans	Mycoulc	118570529
Nakamurella multipartita	Nakamul	502530429
Natronomonas pharaonis	Natrpha	76557520
Neurospora crassa	Neurcra	28923098
Nitrosospira multiformis	Nitrmul	123727174
Nostoc punctiforme	Nostpun	501380802
Oryza sativa	Oryzsat	301344551
Ostreococcus tauri	Ostrtau	308812025
Paracoccidioides brasiliensis	Parabra	226294758
Paracoccus denitrificans	Paraden	205830515
Penicillium roqueforti	Peniroq	584407181
Photorhabdus asymbiotica	Photasy	253989857
Photobacterium profundum	Photpro	493270423
Polaromonas naphthalenivorans	Polanap	500124378
Prochlorococcus marinus subsp. pastoris	Procmar	7839380
Pseudomonas aeruginosa	Pseuaer	15600085
Pseudoalteromonas haloplanktis	Pseuhal	205830548
Pseudomonas putida	Pseuput	81440939
Pyrenophora tritici-repentis	Pyretri	187973256
Ralstonia eutropha	Ralseut	123329427
Rhodococcus opacus	Rhodopa	226365150
Roseomonas cervicalis	Rosecer	296263241
Roseobacter denitrificans	Roseden	123172086

Saccharopolyspora spinosa	Saccspi	498382100
Schizosaccharomyces cryophilus	Schicry	528315656
Schizosaccharomyces japonicus	Schijap	213410148
Segniliparus rotundus	Segnrot	502903714
Solanum lycopersicum	Solalyc	350535509
Sphingobacterium spiritivorum	Sphispi	300760794
Staphylococcus aureus	Stapaur	384231295
Stappia stellulata	Stapste	656018177
Streptomyces albus	Strealb	664085795
Streptosporangium roseum	Streros	665607552
Streptococcus salivarius	Stresal	2501638
Tolumonas auensis	Toluaue	506358979
Trichodesmium erythraeum	Tricery	123161225
Ureaplasma urealyticum	Ureaure	7272375
Verminephrobacter eiseniae	Vermeis	500133480
Verrucomicrobium spinosum	Verrspi	497646624
Vibrio splendidus	Vibrspl	518658515
Yersinia pestis	Yerspes	270338254

Table 4: Table of used organisms from which UreG sequences were prospected. Sequence records are identified by GI numbers.

Species	*Abbreviation*	*GI*
Acaryochloris marina	Acarmar	158304294
Acetobacter pasteurianus	Acetpas	256652741
Actinomyces naeslundii	Actinae	4249616
Actinosynnema mirum	Actimir	502426241
Agrobacterium tumefaciens	Agrotum	635308180
Ajellomyces capsulatus	Ajelcap	150411856
Ajellomyces dermatitidis	Ajelder	327348970
Alcanivorax borkumensis	Alcabor	123149169
Alteromonas macleodii	Altemac	226731813
Anaerococcus tetradius	Anaetet	227216938
Arabidopsis thaliana	Arabtha	227216938
Arthrobacter aurescens	Arthaur	119949672
Arthroderma otae	Arthota	238841889
Aspergillus ruber	Asperub	599158687
Aspergillus fumigatus	Aspefum	666427728
Aspergillus terreus	Aspeter	114196305
Bacillus pasteurii	Bacipas	75420971
Bacillus thuringiensis	Bacithu	595878207
Brachybacterium faecium	Bracfae	502488430
Bradyrhizobium japonicum	Bradjap	654722573
Brucella suis	Brucsui	673468322
Burkholderia pseudomallei	Burkpse	686978736
Campylobacter sputorum	Campspu	260162328
Cenarchaeum symbiosum	Cenasym	205830783
Chitinophaga pinensis	Chitpin	502446312
Chromohalobacter salexigens	Chrosal	499826864
Chryseobacterium gleum	Chrygle	300502355
Citrobacter koseri	Citrkos	673535179
Ruminoclostridium thermocellum	Rumithe	551234031
Congregibacter litoralis	Conglit	495571014
Coprinopsis cinerea	Coprcin	299743607
Corynebacterium urealyticum	Coryure	226731818
Cryptococcus neoformans	Crypneo	405118032
Ectocarpus siliculosus	Ectosil	299470337
Escherichia coli	Eschcol	418165
Ethanoligenens harbinense	Ethahar	503251843
Frankia alni	Franaln	123338675
Geobacillus kaustophilus	Geobkau	47076813
Geodermatophilus obscurus	Geodobs	502711313
Glycine max	Glycmax	351721504
Haemophilus influenzae	Haeminf	81336461
Hahella chejuensis	Haheche	123531908

Halalkalicoccus jeotgali	Halajeo	502964396
Haloarcula marismortui	Halomar	34419199
Haloferax volcanii	Halovol	291370919
Haloquadratum walsbyi	Halowal	121689509
Helicobacter hepaticus	Helihep	14579324
Helicobacter mustelae	Helimus	291276543
Helicobacter pylori	Helipyl	485335
Hirschia baltica	Hirsbal	506308775
Klebsiella aerogenes	Klebaer	137099
Ktedonobacter racemifer	Ktedrac	495193935
Lactobacillus reuteri	Lactreu	689724955
Laribacter hongkongensis	Larihon	226714896
Lysinibacillus sphaericus	Lysisph	226731825
Methylibium petroleiphilum	Methpet	205830793
Methylocella silvestris	Methsil	254797581
Methylosinus trichosporium	Methtri	489708561
Micromonospora aurantiaca	Micraur	302571835
Microsporum gypseum	Micrgyp	311340034
Mitsuokella multacida	Mitsmul	260850226
Morus alba	Morualb	222143562
Mycobacterium gilvum	Mycogil	205830796
Mycobacterium ulcerans	Mycoulc	226732038
Nakamurella multipartita	Nakamul	502530430
Natronomonas pharaonis	Natrpha	121721884
Neurospora crassa	Neurcra	28918042
Nitrosospira multiformis	Nitrmul	123544643
Nostoc punctiforme	Nostpun	186468626
Oryza sativa	Oryzsat	301344553
Paracoccus denitrificans	Paraden	205830798
Photobacterium profundum	Photpro	493270425
Photorhabdus asymbiotica	Photasy	253989856
Polaromonas naphthalenivorans	Polanap	226732041
Prochlorococcus marinus	Procmar	7839381
Pseudoalteromonas haloplanktis	Pseuhal	499647704
Pseudomonas aeruginosa	Pseuaer	674745349
Pseudomonas putida	Pseuput	313499131
Pyrenophora tritici_repentis	Pyretri	187983079
Ralstonia eutropha	Ralseut	123329424
Rhodococcus opacus	Rhodopa	254797584
Roseobacter denitrificans	Roseden	109457064
Roseomonas cervicalis	Rosecer	296263242
Saccharopolyspora spinosa	Saccspi	41350151
Schizosaccharomyces japonicus	Schijap	213409990
Segniliparus rotundus	Segnrot	502903349

Solanum tuberosum	Solatub	13161904
Sphingobacterium spiritivorum	Sphispi	300760795
Staphylococcus aureus	Stapaur	384231296
Streptococcus salivarius	Stresal	2501641
Streptomyces albus	Strealb	664085798
Streptosporangium roseum	Streros	665607548
Thermocrinis albus	Theralb	502756782
Tolumonas auensis	Toluaue	259710309
Trichodesmium erythraeum	Tricery	123161226
Tsukamurella paurometabola	Tsukpau	502893014
Uncinocarpus reesii	Unciree	237907169
Ureaplasma urealyticum	Ureaure	7272376
Verminephrobacter eiseniae	Vermeis	500133479
Verrucomicrobium spinosum	Verrspi	656243735
Vibrio splendidus	Vibrspl	515641537

3 CONCLUSÃO E PERSPECTIVAS

Dado que os resultados são preliminares, conclui-se que as relações filogenéticas apresentadas demonstram que as proteínas acessórias de urease parecem seguir as topologias das árvores obtidas na reconstrução filogenética para ureases. Os resultados de árvores quiméricas para UreG sugerem que a análise filogenética pode estar sofrendo atração de ramos longos (LBA*, long branch attraction*) (Li et al. 2007). Além disso, destacam-se as dificuldades encontradas no trabalho com essas proteínas, as quais parecem ter acumulado muitas alterações, dentro de seus próprios grupos homólogos.

Como perspectivas para este trabalho, uma análise mais aprofundada poderá replicar a metodologia utilizada para UreG, com menos sequências, para outras proteínas acessórias, mesmo que nestas não se encontrem regiões tão conservadas. Além disso, também poderão ser feitas análises de inferência bayesiana e a identificação caso a caso de OTUs que possam estar causando vieses nas análises (especialmente aquelas com potencial a causar LBA).

REFERÊNCIAS

Banić, M. et al., 2012. Extragastric Manifestations of Helicobacter pylori Infection. *Helicobacter*, 17, pp.49–55.

Boer, J.L. & Hausinger, R.P., 2012. Klebsiella aerogenes UreF: Identification of the UreG binding site and role in enhancing the fidelity of urease activation. *Biochemistry*, 51, pp.2298–2308.

Braun, R.L., Junqueira, D.M. & Verli, H., 2014. Filogenia Molecular. In H. Verli, ed. *Bioinformática: da Biologia à Flexibilidade Moleculares*. Porto Alegre, p. 282. Available at: http://www.ufrgs.br/bioinfo/ebook/.

Bremner, J.M., 1995. Recent research on problems in the use of urea as a nitrogen fertilizer. *Fertilizer Research*, 42, pp.321–329.

Carter, E.L. et al., 2009. Interplay of metal ions and urease. *Metallomics : integrated biometal science*, 1, pp.207–221.

Dixon, N., 1975. Jack bean urease (EC 3.5.1.5). A metalloenzyme. Simple biological role for nickel? *Journal of the American Chemical Society*, (97), pp.4131–4133. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1159216.

Farrugia, M. a, Macomber, L. & Hausinger, R.P., 2013. Biosynthesis of the urease metallocenter. *The Journal of biological chemistry*, 288(19), pp.13178–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23539618 [Accessed April 28, 2014].

Jabri, E. et al., 1995. The crystal structure of urease from Klebsiella aerogenes. *Science (New York, N.Y.)*, 268, pp.998–1004.

Konieczna, I. et al., 2012. Bacterial urease and its role in long-lasting human diseases. *Current protein & peptide science*, 13(8), pp.789–806. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3816311&tool=pmcentrez&rendertype=abstract.

Krajewska, B., 2009. Ureases I. Functional, catalytic and kinetic properties: A review. *Journal of Molecular Catalysis B: Enzymatic*, 59, pp.9–21.

Kusters, J.G., van Vliet, A.H.M. & Kuipers, E.J., 2006. Pathogenesis of Helicobacter pylori infection. *Clinical microbiology reviews*, 19, pp.449–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16847081.

Li, Y.-W., Yu, L. & Zhang, Y.-P., 2007. “Long-branch Attraction” artifact in phylogenetic reconstruction. *Yi chuan = Hereditas / Zhongguo yi chuan xue hui bian ji*, 29, pp.659–667.

Ligabue-Braun, R., Andreis, F.C., et al., 2013. 3-To-1: Unraveling Structural Transitions in Ureases. *Die Naturwissenschaften*, 100(5), pp.459–67. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23619940 [Accessed April 30, 2014].

Ligabue-Braun, R., Real-Guerra, R., et al., 2013. Evidence-based docking of the urease activation complex. *Journal of biomolecular structure & dynamics*, 31, pp.854–61. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22962938.

Mehta, N., Benoit, S. & Maier, R.J., 2003. Roles of conserved nucleotide-binding domains in accessory proteins, HypB and UreG, in the maturation of nickel-enzymes required for efficient Helicobacter pylori colonization. *Microbial Pathogenesis*, 35, pp.229–234.

Mobley, H.L., Island, M.D. & Hausinger, R.P., 1995. Molecular biology of microbial ureases. *Microbiological reviews*, 59, pp.451–480.

Moncrief, M.B.C. & Hausinger, R.P., 1997. Characterization of UreG, identification of a UreD-UreF-UreG complex, and evidence suggesting that a nucleotide-binding site in UreG is required for in vivo metallocenter assembly of Klebsiella aerogenes urease. *Journal of Bacteriology*, 179, pp.4081–4086.

Mulrooney, S.B. & Hausinger, R.P., 1990. Sequence of the Klebsiella aerogenes urease genes and evidence for accessory proteins facilitating nickel incorporation. *Journal of Bacteriology*, 172, pp.5837–5843.

Polacco, J.C., Mazzafera, P. & Tezotto, T., 2013. Opinion: nickel and urease in plants: still many knowledge gaps. *Plant science : an international journal of experimental plant biology*, 199-200, pp.79–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23265321 [Accessed September 17, 2014].

Schenk, G. et al., 2012. Binuclear Metallohydrolases : Complex. , 45(9), pp.1593–1603.

Scott, D.R. et al., 2002. Mechanisms of acid resistance due to the urease system of Helicobacter pylori. *Gastroenterology*, 123, pp.187–195.

Sumner, J.B., 1926. The Isolation And Crystallization Of The Enzyme Urease. *J. Biol. Chem.*, 69(2), pp.435–441. Available at: http://www.jbc.org/cgi/content/long/69/2/435 [Accessed November 4, 2014].

Yang, Z., 2006. *Computational molecular evolution*,

Yukl, E.T. & Wilmot, C.M., 2013. NIH Public Access. , 16, pp.612–624.

Formatação da Revista Naturwissenschaften - The Science of Nature

Instructions for Authors

Competition for space in Naturwissenschaften is keen, and the journal receives many more good manuscripts than can be accepted for publication. Current rejection rate reaches about 50% of all submitted work. Preference is thus given to scholarly works that present a compelling case for significant advances in a subject area or are of broader interest because of their interdisciplinary nature. Preliminary reports or work that just confirms previous findings, as well as articles that are likely to interest small specialist group only, will not be considered. All manuscripts received are checked for an initial suitability by an Editorial Board member and returned to the authors without in-depth peer review if the work is unlikely to match the scope of the journal. Prior to appearance in the print journal, all manuscripts are published as Online First article within ten days from receiving the corrected proofs from the authors.

The following article types are accepted:

Original Articles

must present scientific results that are essentially new and that have not been published or are being considered for publication elsewhere.

The length of Original Articles should ideally not exceed ten printed pages, which corresponds to about 30 to 35 manuscript pages (double-spaced, including everything from the title page to the last figure). For the publication of unusually long methodological descriptions and data or figures that are not core to the message of the publication, the publication of Electronic Supplementary Material is encouraged. It is generally acknowledged that the range of research areas covered by the journal may require variable article lengths; in the end manuscript length must be justified by contents! However, preference is given to concise manuscripts that are preferably even shorter than 10 print pages.

Reviews

are usually commissioned by the Editor or an Editorial Board member, but the submission of proposals for Reviews is very much encouraged. The initial submission of a proposal rather than a full manuscript is preferred. Proposals should not exceed 1500 words in length, provide a list of a few key supporting references, and advocate for the significance and timeliness of the topic. There is a general inflation of Reviews in the recent literature and the journal does not wish to support high-frequency publication of reviews from within the same research area. Proposals should be sent to the Editor-in-Chief via email (Sven Thatje; email: svth@noc.soton.ac.uk).

Reviews should cover a topic of current interest and present novel insights or conclusions for directing the respective research area(s). Manuscript length should not exceed 50 manuscript pages (double-spaced, including everything from the title page to the last figure). A Review may include up to seven figures and/or tables. The use of Electronic Supplementary Material is encouraged.

Concepts & Synthesis

comprise a new manuscript type in Naturwissenschaften, which aims to promote the conceptual advance of ideas across the natural sciences. Concepts & Synthesis articles present an evidence-supported opinion by established scientists on a research topic of ideally cross-disciplinary nature. This paper format should not be confused with a Review and does therefore not aim at a balanced revision of the topic. Concepts & Synthesis articles should stimulate thought on a controversial or widely ignored topic/problem, and at the same time

develop and/or direct new ideas for the research area(s) involved.

Concepts & Synthesis manuscripts should not exceed 30 manuscript pages (double-spaced, including everything from the title page to references) in length. Up to three additional figures or tables are allowed for this paper format explaining relevant theories and/or discuss outstanding questions in support of the manuscript's body text. Legends to figures/tables can be longer than in a standard article format but should be no longer than 300 words.

The reference list should not be review-like and Review articles should preferably be cited to reduce the number of references required. No more than 50 references are allowed; simply refer to key literature to raise awareness for the complexity of the subject rather than excessively reviewing the same.

Short Communications

are short papers that present significant new observations. Short Communications may present results that are often not sufficiently elaborated to justify an Original Article but provide compelling evidence for their potential significance. This paper format is not to promote minimal or otherwise insufficient data sets that do not justify an Original Article. Short Communications should be no longer than 2500 words. This includes everything from cover page, abstract, references, acknowledgements to figure/table legends. The length limit will be strictly enforced. The journal allows a maximum of three displays of which only two can be figures. No more than 30 references are allowed. The use of Electronic Supplementary Material is encouraged (see link).

Comments & Replies

give corrections, new analyses, provide critique or stimulate a more general oversight on contributions previously published in the journal. Comments should not refer to articles older than one year. Comments are usually not exceeding 600 words in length, with a maximum of 10 references. They should present a simple message that requires only one small figure or table if any. Comments do not function as a Corrigendum to an article. This article type does not include an Abstract, but the title page should contain a footnote, providing an explicit reference to the criticized article "This is a comment to Authors (Year) Title. Naturwissenschaften, Volume: pages or DOI".

Upon receipt of a Comment the authors of Original Articles are given the opportunity to reply. A Reply is usually published alongside the comment. Both Comments & Replies are subject to peer review, usually by the referees of the article to which they refer.

MANUSCRIPT SUBMISSION

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to

originate from the authors.

Online Submission

Authors should submit their manuscripts online. Electronic submission substantially reduces the editorial processing and reviewing times and shortens overall publication times. Please follow the hyperlink “Submit online” on the right and upload all of your manuscript files following the instructions given on the screen.

EDITORIAL PROCEDURE

Responses to reviewers’ comments

If your manuscript has been previously reviewed in Naturwissenschaften and you have been advised to revise it, you are asked to submit responses to reviewers’ comments together with the revised version of your work – that is a list of changes or a rebuttal against each point which has been raised by the reviewers. The response letter is to foster scientific discussion and to convince the editors of your views on the other hand (Without a response letter, the revised work will not be progressed any further).

Requirements for submitting the responses:

Use the special attachment category called “Authors' Response to Reviewers' Comments”, which is required for:

- all revised submissions (after minor or moderate revisions, submitted under the same manuscript number);

- all manuscripts that have been rejected but encouraged to resubmit, despite that the new version of your work will have a new number and will be treated as a new submission in EM.

Do not put the responses to reviewers into the cover letter to Editor, as the cover letter is addressed to the Editor only and none of the reviewers can access it.

TITLE PAGE

Title Page

The title page should include:

The name(s) of the author(s)
A concise and informative title
The affiliation(s) and address(es) of the author(s)
The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

TEXT

Text Formatting

Manuscripts should be submitted in Word.

- The text of a research paper should be divided into Introduction, Materials and Methods, Results, Discussion, Acknowledgements, Conflict of Interest, and

References.

- Materials and Methods must include statement of Human and Animal Rights.
- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

LaTeX macro package (zip, 182 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

SPECIFIC REMARKS

• Use a normal, plain font (e.g., 12-point Times Roman) for text.

• Please do not forget to add consecutive line-numbering throughout manuscript (not just page-by-page).

• The use of footnotes is discouraged

REFERENCES

Citation

Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson 1990).
This result was later contradicted by Becker and Seligman (1996).

This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work.

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329
- Article by DOI

  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086
- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London
- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257
- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007
- Dissertation

  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal’s name according to the ISSN List of Title Word Abbreviations, see

- ISSN.org LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

EndNote style (zip, 2 kB)

Please list all authors of a publication.

TABLES

- All tables are to be numbered using Arabic numerals.

- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

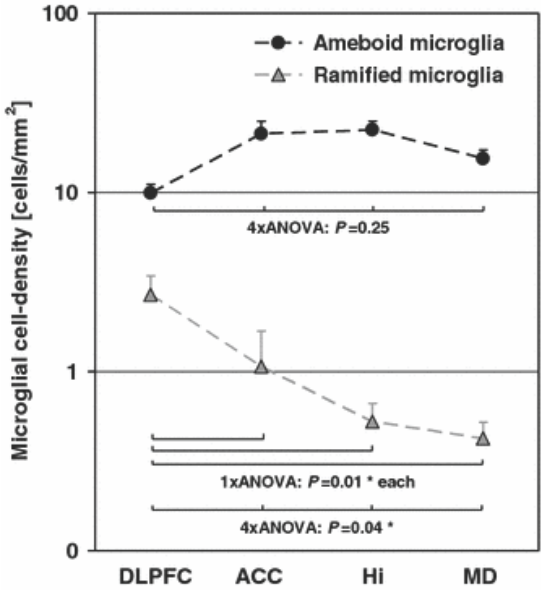
ARTWORK AND ILLUSTRATIONS GUIDELINES

For the best quality final product, it is highly recommended that you submit all of your artwork – photographs, line drawings, etc. – in an electronic format. Your art will then be produced to the highest standards with the greatest accuracy to detail. The published work will directly reflect the
quality of the artwork provided.

Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art

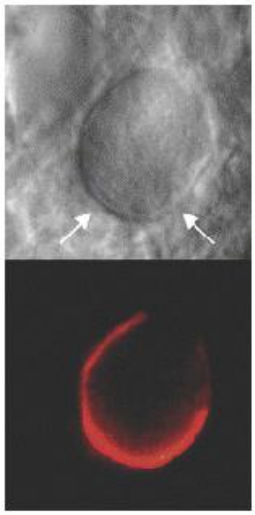


- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.

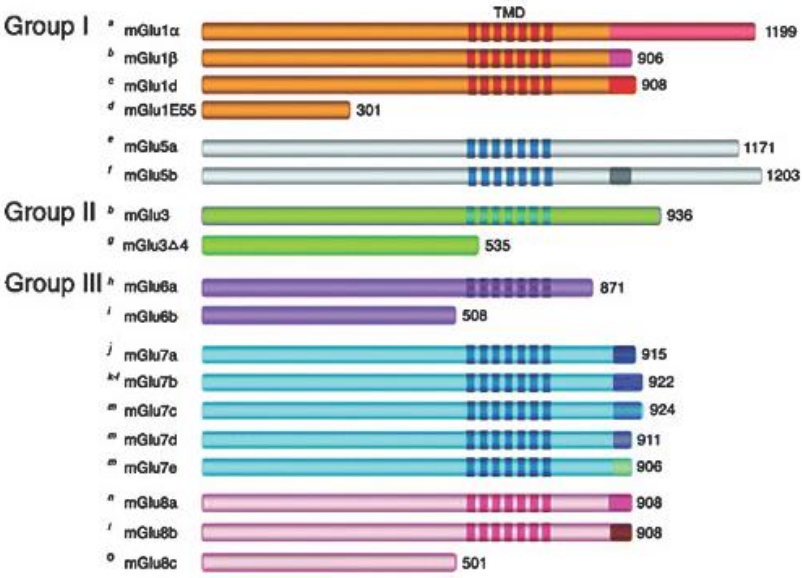
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art

Definition: Photographs, drawings, or paintings with fine shading, etc.
If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
Halftones should have a minimum resolution of 300 dpi.



Combination Art



Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
Combination artwork should have a minimum resolution of 600 dpi.

Color Art

Color art is free of charge for online publication.
If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one

another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
If the figures will be printed in black and white, do not refer to color in the captions. Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

All figures are to be numbered using Arabic numerals.
Figures should always be cited in text in consecutive numerical order.
Figure parts should be denoted by lowercase letters (a, b, c, etc.).
If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

When preparing your figures, size figures to fit in the column width.
For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.
For books and book-sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that
All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
Any figure lettering has a contrast ratio of at least 4.5:1

ELECTRONIC SUPPLEMENTARY MATERIAL

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Submission

Supply all supplementary material in standard file formats.
Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations

Always use MPEG-1 (.mpg) format.

Text and Presentations

Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
A collection of figures may also be combined in a PDF file.

Spreadsheets

Spreadsheets should be converted to PDF if no interaction with the data is intended.
If the readers should be encouraged to make their own calculations, spreadsheets should be submitted as .xls files (MS Excel).

Specialized Formats

Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.

Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource

4".
Name the files consecutively, e.g. "ESM_3.mpg", "ESM_4.pdf".

Captions

For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

The manuscript contains a descriptive caption for each supplementary material
Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

ETHICAL RESPONSIBILITIES OF AUTHORS

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation can be achieved by following the rules of good scientific practice, which include:

- The manuscript has not been submitted to more than one journal for simultaneous consideration.
- The manuscript has not been published previously (partly or in full), unless the new work concerns an expansion of previous work (please provide transparency on the re-use of material to avoid the hint of text-recycling ("self-plagiarism")).
- A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g. "salami-publishing").
- No data have been fabricated or manipulated (including images) to support your conclusions
- No data, text, or theories by others are presented as if they were the author's own ("plagiarism"). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks are used for verbatim copying of material, and permissions are secured for material that is copyrighted.

  Important note: the journal may use software to screen for plagiarism.
- Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities - tacitly or explicitly - at the institute/organization where the work has been carried out, **before** the work is submitted.
- Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

In addition:

- Changes of authorship or in the order of authors are not accepted **after** acceptance of a manuscript.
- Requesting to add or delete authors at revision stage, proof stage, or after publication is a serious matter and may be considered when justifiably warranted. Justification for changes in authorship must be compelling and may be considered only after receipt of written approval from all authors and a convincing, detailed explanation about the role/deletion of the new/deleted author. In case of changes at revision stage, a letter must accompany the revised manuscript. In case of changes after acceptance or publication, the request and documentation must be sent via the Publisher to the Editor-in-Chief. In all cases, further documentation may be required to support your request. The decision on accepting the change rests with the Editor-in-Chief of the journal and may be turned down. Therefore authors are strongly advised to ensure the correct author group, corresponding author, and order of authors at submission.
- Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results. This could be in the form of raw data, samples, records, etc.

If there is a suspicion of misconduct, the journal will carry out an investigation following the COPE guidelines. If, after investigation, the allegation seems to raise valid concerns, the accused author will be contacted and given an opportunity to address the issue. If misconduct has been established beyond reasonable doubt, this may result in the Editor-in-Chief's implementation of the following measures, including, but not limited to:

- If the article is still under consideration, it may be rejected and returned to the author.
- If the article has already been published online, depending on the nature and severity of the infraction, either an erratum will be placed with the article or in severe cases complete retraction of the article will occur. The reason must be given in the published erratum or retraction note.
- The author's institution may be informed.

COMPLIANCE WITH ETHICAL STANDARDS

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" before the References when submitting a paper:

- Disclosure of potential conflicts of interest
- Research involving Human Participants and/or Animals
- Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. double blind peer review) as well as per journal subject discipline. Before submitting your article check the Instructions for Authors carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Authors must disclose all relationships or interests that could have direct or potential influence or impart bias on the work. Although an author may not feel there is any conflict, disclosure of relationships and interests provides a more complete and transparent process, leading to an accurate and objective assessment of the work. Awareness of a real or perceived conflicts of interest is a perspective to which the readers are entitled. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate. Examples of potential conflicts of interests **that are directly or indirectly related to the research** may include but are not limited to the following:

- Research grants from funding agencies (please give the research funder and the grant number)
- Honoraria for speaking at symposia
- Financial support for attending symposia
- Financial support for educational programs
- Employment or consultation
- Support from a project sponsor
- Position on advisory board or board of directors or other type of management relationships
- Multiple affiliations
- Financial relationships, for example equity ownership or investment interest
- Intellectual property rights (e.g. patents, copyrights and royalties from such rights)
- Holdings of spouse and/or children that may have financial interest in the work

In addition, interests that go beyond financial interests and compensation (non-financial interests) that may be important to readers should be disclosed. These may include but are not limited to personal relationships or competing interests directly or indirectly tied to this research, or professional interests or personal beliefs that may influence your research.

The corresponding author collects the conflict of interest disclosure forms from all authors. In author collaborations where formal agreements for representation allow it, it is sufficient for the corresponding author to sign the disclosure form on behalf of all authors. Examples of forms can be found

- here:

The corresponding author will include a summary statement in the text of the manuscript in a separate section before the reference list, that reflects what is recorded in the potential conflict of interest disclosure form(s).

See below examples of disclosures:

Funding: This study was funded by X (grant number X).

Conflict of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stock in Company Y. Author C is a member of committee Z.

If no conflict exists, the authors should state:

Conflict of Interest: The authors declare that they have no conflict of interest.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

1) Statement of human rights

When reporting studies that involve human participants, authors should include a statement

that the studies have been approved by the appropriate institutional and/or national research ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that the independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study.

The following statements should be included in the text before the References section:

Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

For retrospective studies, please add the following sentence:

"For this type of study formal consent is not required."

2) Statement on the welfare of animals

The welfare of animals used for research must be respected. When reporting experiments on animals, authors should indicate whether the international, national, and/or institutional guidelines for the care and use of animals have been followed, and that the studies have been approved by a research ethics committee at the institution or practice at which the studies were conducted (where such a committee exists).

For studies with animals, the following statement should be included in the text before the References section:

Ethical approval: "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."

If applicable (where such a committee exists): "All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted."

If articles do not contain studies with human participants or animals by any of the authors, please select one of the following statements:

"This article does not contain any studies with human participants performed by any of the authors."

"This article does not contain any studies with animals performed by any of the authors."

"This article does not contain any studies with human participants or animals performed by any of the authors."

INFORMED CONSENT

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. Hence it is important that all participants gave their informed consent in writing prior to inclusion in the study. Identifying details (names, dates of birth, identity numbers and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scientific purposes and the participant (or parent or guardian if the participant is incapable) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity.

If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort scientific meaning.

The following statement should be included:

Informed consent: "Informed consent was obtained from all individual participants included in the study."

If identifying information about participants is available in the article, the following statement should be included:

"Additional informed consent was obtained from all individual participants for whom identifying information is included in this article."

AFTER ACCEPTANCE

Upon acceptance of your article you will receive a link to the special Author Query Application at Springer's web page where you can sign the Copyright Transfer Statement online and indicate whether you wish to order OpenChoice and offprints.

Once the Author Query Application has been completed, your article will be processed and you will receive the proofs.

Open Choice

In addition to the normal publication process (whereby an article is submitted to the journal and access to that article is granted to customers who have purchased a subscription), Springer now provides an alternative publishing option: Springer Open Choice. A Springer Open Choice article receives all the benefits of a regular subscription-based article, but in addition is made available publicly through Springer's online platform SpringerLink.

- Springer Open Choice

Copyright transfer

Authors will be asked to transfer copyright of the article to the Publisher (or grant the Publisher exclusive publication and dissemination rights). This will ensure the widest possible protection and dissemination of information under copyright laws.

Open Choice articles do not require transfer of copyright as the copyright remains with the author. In opting for open access, the author(s) agree to publish the article under the Creative Commons Attribution License..

Offprints

Offprints can be ordered by the corresponding author.

Color illustrations

Publication of color illustrations is free of charge.

Proof reading

The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.

After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

DOES SPRINGER PROVIDE ENGLISH LANGUAGE SUPPORT?

Manuscripts that are accepted for publication will be checked by our copyeditors for spelling and formal style. This may not be sufficient if English is not your native language and substantial editing would be required. In that case, you may want to have your manuscript edited by a native speaker prior to submission. A clear and concise language will help editors and reviewers concentrate on the scientific content of your paper and thus smooth the peer review process.

The following editing service provides language editing for scientific articles in all areas Springer

publishes in:

- Edanz English editing for scientists

Use of an editing service is neither a requirement nor a guarantee of acceptance for publication.

Please contact the editing service directly to make arrangements for editing and payment.

- Edanz English editing for scientists

For Authors from China

文章在投稿前进行专业的语言润色将对作者的投稿进程有所帮助。作者可自愿选择使用Springer推荐的编辑服务，使用与否并不作为判断文章是否被录用的依据。提高文章的语言质量将有助于审稿人理解文章的内容，通过对学术内容的判断来决定文章的取舍，而不会因为语言问题导致直接退稿。作者需自行联系Springer推荐的编辑服务公司，协商编辑事宜。

- 理文编辑

For Authors from Japan

ジャーナルに論文を投稿する前に、ネイティブ・スピーカーによる英文校閲を希望されている方には、Edanz社をご紹介しています。サービス内容、料金および申込方法など、日本語による詳しい説明はエダンズグループジャパン株式会社の下記サイトをご覧ください。

- エダンズグループジャパン

For Authors from Korea

영어 논문 투고에 앞서 원어민에게 영문 교정을 받고자 하시는 분들께 Edanz 회사를 소개해 드립니다. 서비스 내용, 가격 및

신청 방법 등에 대한 자세한 사항은 저희 Edanz Editing Global 웹사이트를 참조해 주시면 감사하겠습니다.