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Laboratório de Genômica e Biodiversidade

Pré-Graduação

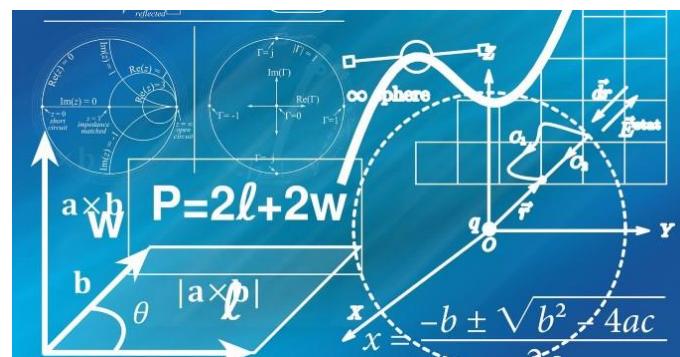
- Origem: Viçosa-MG (UFV)
- Trajetória: Escolas públicas



- Videogames:
 - Inglês
 - Tecnologia
- Ensino Médio:
 - Exatas X Biológicas



- Lavouras:
 - Animais (insetos)
 - Plantas



Graduação

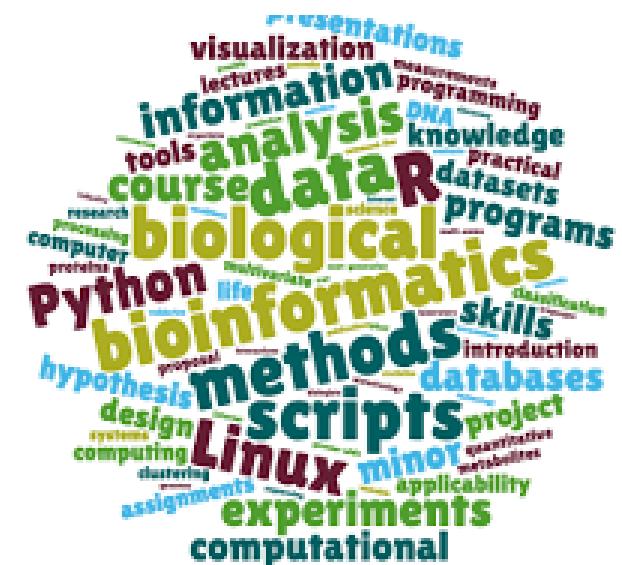


- Ciências Biológicas – UFV
- Bancada: Estudo molecular de insetos vetores
- Campo: Coleta e triagem de espécimes (Formigas)
- Ensino: Monitor de genética



Graduação

- III Curso de Verão em Biotecnologia da UFOP
 - Bioinformática (Exatas E Biológicas)
 - Capacitação na área
 - Eventos
 - Bioinformática Básica
 - Genética + bioinfo = Montagem de genomas
 - Procura por laboratórios
 - LAMPADA – Francisco Prosdocimi
 - Ensino e Extensão
 - Instituto de Bioquímica Médica Leopoldo de Meis
 - Aprovação no mestrado



MONTAGEM E ANOTAÇÃO

- Sequenciamento:

Obtenção de sequências curtas de DNA (*reads*)

- Fragmentos sequenciados:

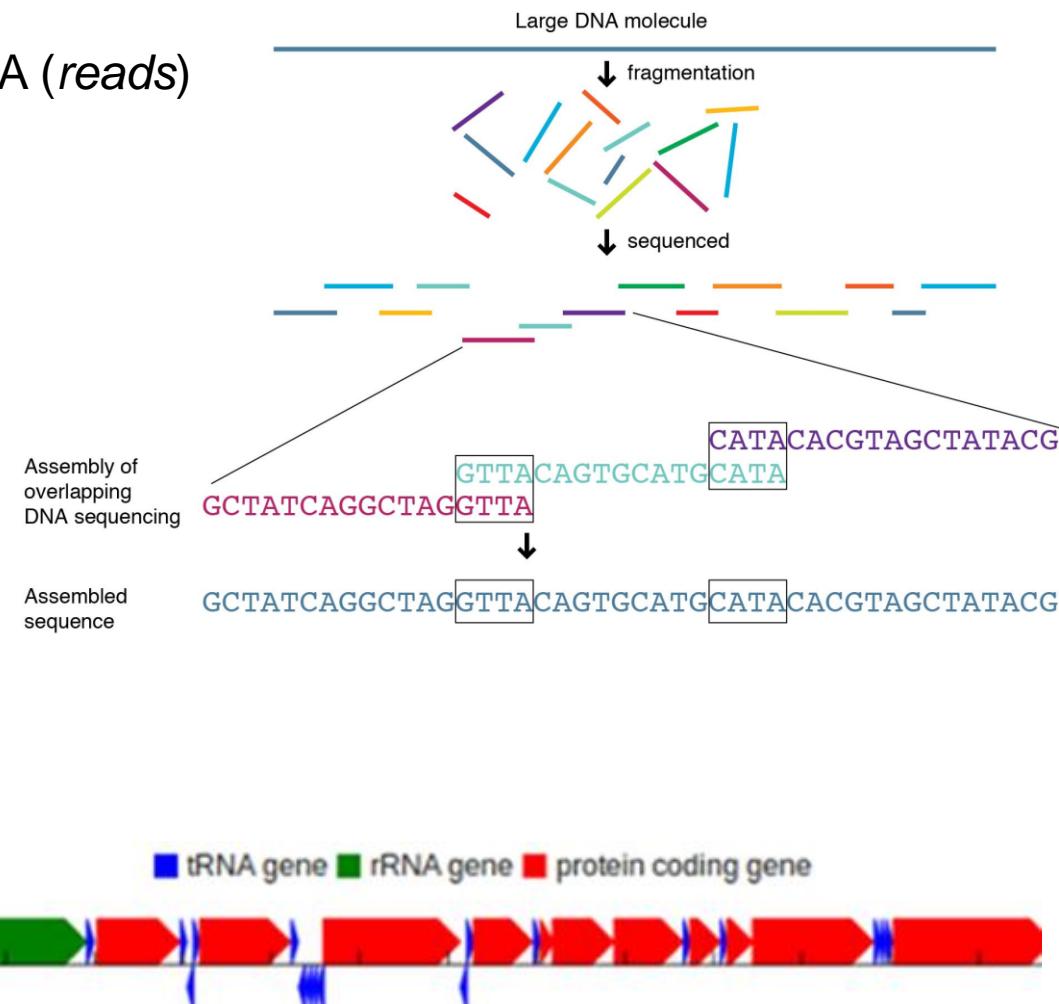
- Não Pareados – 1 extremidade
- Pareados – 2 extremidades
 - Distância entre extremidades
 - Fechamento de gaps
 - Uso de ≠s distâncias

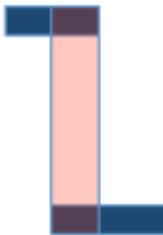
- Montagem

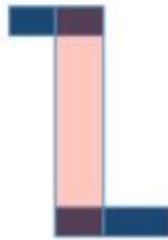
- Sobreposição das *reads*

- Anotação

- Identificar regiões no genoma (Ex: genes)

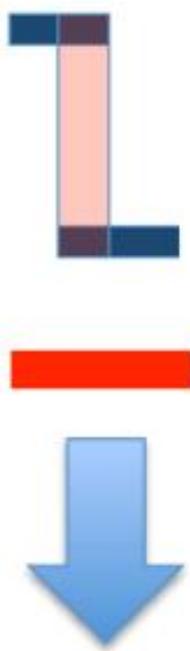






Sequência contínua ou contig





Fragmentos
alinhados

ACGCGATTCA~~G~~GTACCA~~C~~CG
 GCGATTCA~~G~~GTACCA~~C~~CGCG
 GATTCA~~G~~GTACCA~~C~~CGCGTA
 TTCA~~G~~GTACCA~~C~~CGCGTAGC
 CA~~G~~GTACCA~~C~~CGCGTAGCGC
 GGTTACCA~~C~~CGCGTAGCGCATT
 TTACCA~~C~~CGCGTAGCGCATT
 ACCACGCGTAGCGCATTACA
 CACGCGTAGCGCATTACACACA
 CGCGTAGCGCATTACACAGA
 CGTAGCGCATTACACAGATT
 TAGCGCATTACACAGATTAG

Contig consenso

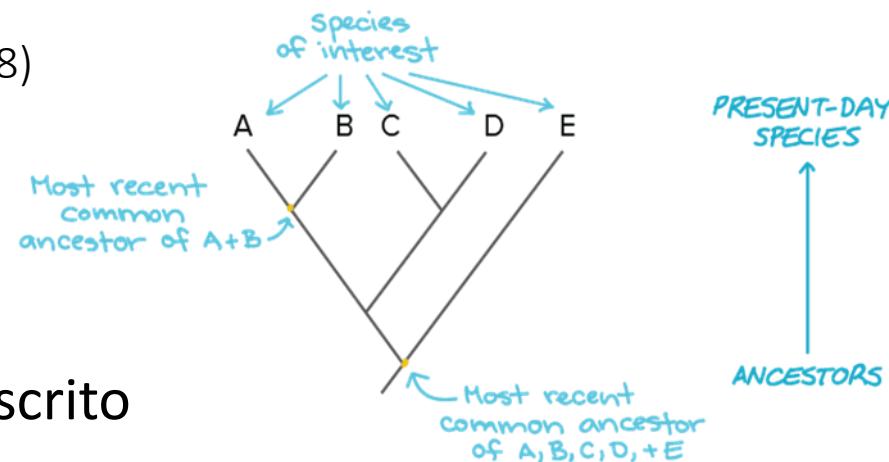
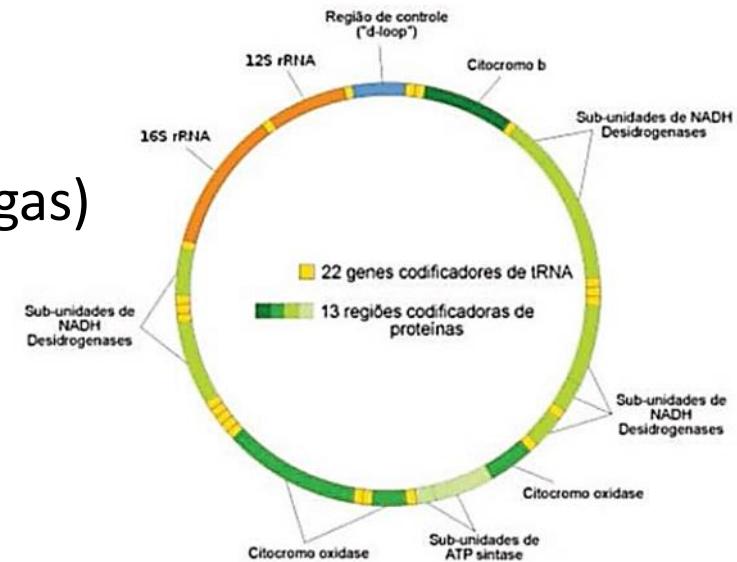
ACGCGATTCA~~G~~GTACCA~~C~~CGCGTAGCGCATTACACAGATTAG

TCTACCGCAAACAACACCGTCGAGGTGACTTACGAGGAACTCTCCCCAAGGTTGACCGACTAACGCAGAGTGAAAGTGGTCGTGAGGCCGGAGAGAGAGGG
GGGGGTGAGGAGTTGGTATGCTTCCCTACCTGTGCGTCTACCATTGGCAGTGATGCTGAGATGCCTCATTGGCGGTACTCTGACCTCGCTTCTCTCTC
CCGTTCGCTGAGCGTGACTTCTGCTCTGGTTGGCGGTAGGCTAGGGGAGGGAGGGGGACGGCGGTGTCGTGCCTCCCCCTTGTGCTTGTGGA
GATGCAGGCATGTTTCATGTGCTGAGGACAACGATGTAACACTGGGCTGCAACCTGGAAGTGTGGGGCAGAGAGAGAGGGCTGAGGAATGGAGCATGGAGG
CTCATGGAGAGGGCTTGTGGTGCCTTGATGTGGCATCACACACCTCACTGAAAACACTCGCCAGCGAGCCTCTCCTTCCCAGTCGTGTTGGCTCT
TCCTCTCTCTTCCGGTCTGGCTCTTCTCAGGGCAGCGCAACCAGCGCAAAAACAAAGCGAGGCACAGAGGAGGAGTACTCACGACACGAGTAATGCC
GAAGCAGGTCAATTGATGCAGCAAACATGCCCGCAGGAGAAACGCCATGGCGCTGTCGCCGGACTGCAGGTGTGCCTATGGCGAGCGCCCCCTCCCCCTCA
CTGAGCGCGTGCCTGCCGGCACGCCGACCGCACCACGCCACGCCCTTTGGTTGCAGGCATTGGCTCTCGCTCAACCTCACGACATTGCGC
CTCCGTCTCCTGCCTCGCACACTCCCTCACCTCCCTCCCTCTTCTCTTCCCTCCCTCGACGCCGGCCACCGCACACAGGCACAGGGACAGAC
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GGTGTGAAAGTCTCGACGGCGCGCTACACCGCAACTCGAGCCTCGTCGCCAAACCAACAGCCTCTGCATCTCGAAAACCTCGTCAGAGGAATCACT
CGCTCGTGGCTTCCCTGACAAGACGCAAGCGCAGCGGCTGCTCCTCTTACGTGAACGCCCTCGCTGTTGGCGCAACCAGAACCTCTCCAGGAGGTTGC
GCTGCCGGCGGCGGCTGCCGACGGCGTTCCGCTGCCGTGCCCTGGCGGTGGCGCCGTCTTCATGACGAGGGGAACCTCGCTCTGCTTCATCACGC
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GACCTCTTGGACGGAAAAAAGATCACCGGATCTGCAATGCCGGTGCAGCGCAAATTGCGTACCAACTGCACGCTGCTCGATAACCCACTGGCGTC
GCTCGGTGCTACCTCACCCGAAGGGGAGTACGTGGCGTTCAAGACGTCGTCGGTGGGCTGGTGCAGGCCCTGTCACCACACTCGGGAGTCGGTCCACA
TTGCGAGTGGCAGGGCGCCATGGCCTCACTCAAGAGGAACATGGCGGAGTTTTCTAAGTGGGTGATCGAGTGCTGGAAGCAGCGGACCGTGGAGCT
CGACGTGAGGGAGCTGCCAGTCCTCGCCACCGCACGTCACTGCTGCCGGACACGCCCTCTTCCCTGATGTTGTCGGAGGCCGTCGCCGGACATGTC
TTTCATCGAGGGCGAGGGCAGGCAGGGCGGCACTGACCTCGCCACCCCTGGCGAGGCGGTTACAAGGCTGCGTCAAGGACTGGGCTACGCGATGCC
AGCCTTCACATCCACGGTGTCTCAGCAGCGGTGAGCTCCAGCGGCGCTACAAGCGCTTCCGTTGGCGGATGTAGTGCAGGCTATCCTCTCGCGGAGGA
GCAGTTGCTGGCGGACTGCAGCAATGTGCTTGGACTTGGCGCTGCCGGAGTCGTGGCAGAGACCGAAGTGGGTTGCACCTGCTTACACAGTC

TCTACCGCAAACAACACCGT**CGAGGTACTTACGAGGA** Regiões reguladoras **CTAACGCAGAGTGAAGTGGTCGTAGGCGCGAGAGAGAGGG**
GGGGGTGAGGAGTTGGTATGCTTCCCTACCTGTGCGTCTACCATTGGCAGTGTAGTCCTCTGAGATGCCCTATTGGCGGTACTCTGACCTCGCTTTCTCTC
CCGTCGCTGAGCGTGACTTCTGCTCTGGTTGGCGGTAGGCTAGGGGAGGGAGGGGGACGGCGGTTCGTGCCTCCCC**CTCTGCTTGGA**
GA CDS **GAGGACAACGATGTACACTGGGCTGCAACCTGGAAGTGTGGGGCAGAGAGAGAGGCTCGAGGAATGGAGCATGGAGG**
CTCATGGAGAGGTCTTGTGGTGCCTTGTGGATGTGGTGGCATCACACACCTCACTCGAAAACTACTGCCAGCGAGCCTCCCTTCCGCATGCGTGTGGCTCTC
TCCTCTCTCTTCCGGTCTGGCTCTTCTTCAAGGCAGCGCCAACCAGCGCAAAAACAAAGCGAGGCAC**AGAGGAGGAGTACTCACGACACGAGTAATGCC**
GAAGCAGGTATTCATGCAGCAAACATGCCCGGCAGGAGAAACGCCATGGCGCTGCCGGACTGCAGGTGTGCCTATGGCGAGCGCCCCCTCCCCCTCA
CTGAGCGCGTGCCTGCCGGCACGCCCTGCGCACCATGCCACGCGCTCTTTGGTTGCAGGCATTGGCTTTGCCTCTCACGACATTGCGC
CTCCGTCTCCTGCCCTGCACACTCCCTCACCTC**CTCCCTCCC** rRNA **TCGACGCCGGGCCACGCGCACACAGGCACAGGGACAGAC**
ACACCTATGGACAGCGTGCCTGTGCAAACATGTGGCAGACTCTGCCAGCGATATCGTCTTCAGCCCACATCCCTCGCAGCCTCCTGGAGTCAAATGCAC
GGTGTGAAGTCTCCGACGGCGCGCTACACCGCAACTCGAGCCTCGTCGCCGAAACCAACAGCCTCTGCATCTCGAAAACCTCGCAGAGGAATCACT
CGCTCGTGGCTTCCCTGACAAGACGCAGCGGCTGCCCTTACGTGAACCGCCCTGCGTGGTGGCCGCAACCAGAACCTTCCAGGAGGTGGC
GCTGCCGGCGGCGCTGCCGACGGCGTTCCGTCGCTGCCGTGCCCTGGCGGTGGCGCCGCTTTCATGACGAGGGAACCTCTGCCCT**TGCTTCATCACGC**
tRNA **GAAGACAATCCAGCTGATCCGCCCTGGCTCTGTGAATTATGCGATCGACCCCTGCACGGCTGACCACGACAAGGCGGCAC**
GACCTCTCTGGACGGAAAAAAAGATCACCGGATCTGCAATGCCGGTGCAAGCGCAAATTGCGTACCAACTGCACGCTGCTCGCATACCCACTGGCGTC
GCTCGGTCGCTACCTCACCCGAAGGGAGTACGTGGCGTTCAAGACGTCGTCGGTGGCTCGTGAAG**CCC** Padrões específicos **TCCACA**
TTGCGAGTGGGCAAGGCGCCATGGCCTCACTCAAGAGGAACATGGCGGAGTTTTCTAAGTGGGTGATCGAGTGTGGAAAGCAGCGGCACCGTGGGAGCT
CGACGTGAGGGAGCTGCCAGTCCTCGCCACCGCACGTCACTGCTGCCGGACACGCCCTCTTTCCCTGATGTTGCGGAGCCGTCGCCGGGACATGTC
TTTCATCGAGGGCGAGGGCAGGCCGGGGCCAGTGGTGAACCTGCCACCCCTGGCGAGGCCGGTTACAAGGCTCGTGAAGGACTGGGCCTACGCGATGCC
AGCCTTCACATCCACGGTGCTCCTCAGCAGCGGTGAGCTCCAGCGCGCCTACAAG Elementos repetitivos **GTGCGGCTATCCTCTCGCGGAGGA**
GCAGTTGCTGGCGGCACTGCAGCAATGTGTCTTGCAGCTGGCTGCCGGGGAAAGTCGTGGCAGAGACCGAAGTGGGGTTGCACCTGCTTACACAGTC

Mestrado

- Montagem de genomas mitocondriais (Formigas)
 - Menor genoma da célula (≈ 16 kbp)
 - Excelente treino
 - Diversos estudos:
 - Filogenética (Krzemińska et al., 2017)
 - Filogeografia (Fields et al., 2018)
 - Genética populacional (Kilinç et al., 2018)
 - Conservação (Rosel et al., 2017)
- Dados públicos
 - Várias espécies sem mitogenoma descrito



Formigas (Hymenoptera: Formicidae)

- Mais de 13000 spp.
- Grande quantidade de informação para o clado:
 - ≈ 3.91 Terabytes
- 15 mitogenomas completos
- Todas as subfamílias
 - Exceto Martialinae
- Pseudomyrmecinae
 - Sem mitogenomas
 - Mutualismo com plantas
 - Abundância de dados

NCBI Taxonomy Browser

Search for: Formicidae

Display: 1 levels using filter: none

Lineage (full): cellular organisms; Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Bilateria; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Holometabola; Hymenoptera; Apocrita; Formicidae

- [Formicidae](#) 3,368 Click on organism name to get more information.
 - [Agroecomyrmecinae](#) 1
 - [Amblyoponinae](#) 3
 - [Aneuretinae](#) 2
 - [Apomyrminae](#) 1
 - [Dolichoderinae](#) 201
 - [Dorylinae](#) 388
 - [Ectatomminae](#) 4
 - [Formicinae](#) 863
 - [Heteroponerinae](#) 2
 - [Leptanillinae](#) 1
 - [Martialinae](#)
 - [Myrmeciinae](#) 3
 - [Myrmicinae](#) 1,580
 - [Nothomyrmeciinae](#) 2
 - [Paraponerinae](#) 1
 - [Ponerinae](#) 269
 - [Proceratiinae](#) 2
 - [Pseudomyrmecinae](#) 45
 - [unclassified Formicidae](#)

Mestrado

- 14 mitogenomas
- Montagem:
 - NOVOPlasty, MIRA, MITObim
- Anotação:
 - MITOS Web Server (automática)
 - Artemis (manual)
- Submissão ao TPA (Genbank)
- Análises adicionais:
 - Ordem gênica (Sintenia)
 - Genômica comparativa
 - Filogenômica



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Declarations can be found on
page 17

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OPEN ACCESS

Accessible molecular phylogenomics at no cost: obtaining 14 new mitogenomes for the ant subfamily Pseudomyrmecinae from public data

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ABSTRACT

The advent of Next Generation Sequencing has reduced sequencing costs and increased genomic projects from a huge amount of organismal taxa, generating an unprecedented amount of genomic datasets publicly available. Often, only a tiny fraction of outstanding relevance of the genomic data produced by researchers is used in their works. This fact allows the data generated to be recycled in further projects worldwide. The assembly of complete mitogenomes is frequently overlooked though it is useful to understand evolutionary relationships among taxa, especially those presenting poor mtDNA sampling at the level of genera and families. This is exactly the case for ants (Hymenoptera:Formicidae) and more specifically for the subfamily Pseudomyrmecinae, a group of arboreal ants with several cases of convergent coevolution without any complete mitochondrial sequence available. In this work, we assembled, annotated and performed comparative genomics analyses of 14 new complete mitochondria from Pseudomyrmecinae species relying solely on public datasets available from the Sequence Read Archive (SRA). We used all complete mitogenomes available for ants

- Publicado na PeerJ (01/2019)
 - Manuscrito e revisão por mim
 - Supervisão do Francisco

Mestrado – Atividades paralelas

- Crédito Didático – “Projetinho” – Prof. Andrea Cheble
 - Montagem mitogenomas
- Curso de Férias - Prof. Wagner
- Apresentações:
 - SIAc (2017 e 2018)
 - No-Budget Science (2017)
 - Apresentação VII Semana Bioqmed (2017)
 - 2º lugar da categoria “Mestrado”



Mestrado – Atividades paralelas

- Orientações:
 - Montagens de mitogenomas com dados públicos

- Gabriela Warwar

- Lagartos
- 3 mitogenomas + Análise filogenômica
- Publicado na Mitochondrial DNA Part B



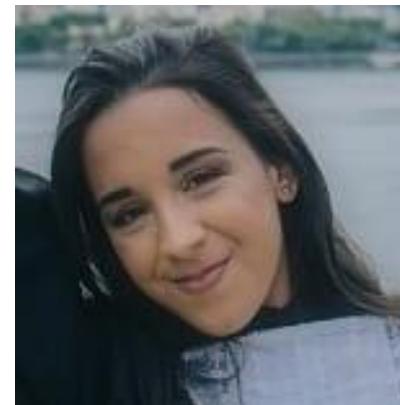
Mitochondrial DNA Part B
Resources

ISSN: (Print) 2380-2359 (Online) Journal homepage: <https://www.tandfonline.com/loi/tmdn20>
Complete mitochondrial genomes for three lizards (*Anolis punctatus*, *Sceloporus woodi*, and *S. grammicus*): a contribution to mitochondrial phylogenomics of Iguanoidea

Ana Teresa Nogueira Dumanas, Gabriela Warwar Teixeira, Gabriel Alves Vieira, Deise Schroder Sarzi, Carolina Furtado, W. Bryan Jennings & Francisco Prosdocimi

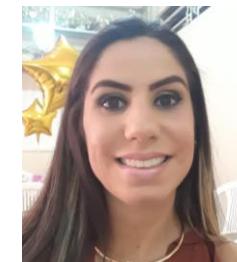
- Cristiane Esteves

- Co-Orientador TCC
- Borboletas
- 2 mitogenomas
- Análises adicionais



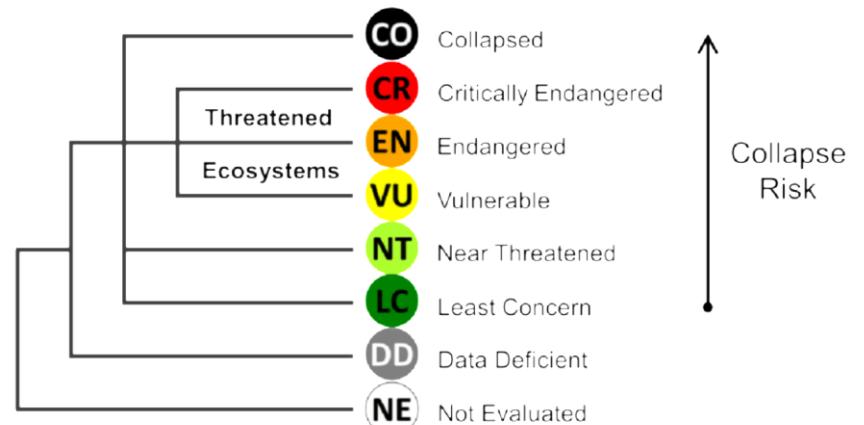
Mestrado – Atividades paralelas

- Mitogenomas:
 - Seq. curtas – Poucos dados
 - Montagem em computadores normais
- **MitoFree** = Adaptação do *pipeline* usado no crédito didático
- Otimização do consumo de RAM
 - Não usa MIRA
 - NOVOPlasty e MITObim
- Prova de conceito:
 - Montar 100 mitocôndrias usando no máximo 8 GB de RAM (32 GB)
 - 40 já montadas
 - 6 alunos de graduação envolvidos
 - Finalizado no doutorado



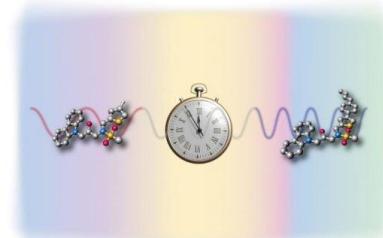
DOUTORADO

- Fuselo (*Limosa lapponica baueri*)
 - Pássaro migratório – Nova Zelândia
- Resiliência física
 - Maior vôo sem paradas: ≈ 12000 km (Gill Jr et al., 2008)
 - Nova Zelândia – China
- Consistência temporal
 - Migração geralmente no mesmo dia (14 de Março)
 - Data pouco afetada por condição metereológica (Battley, 2006)
- Declínio populacional
 - Quase ameaçada



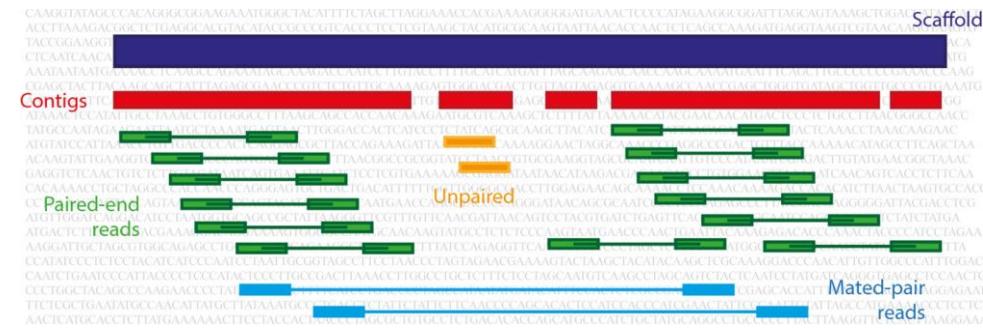
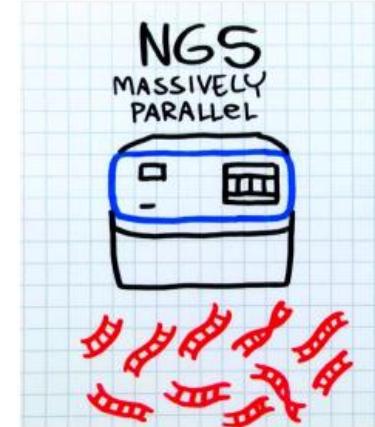
OBJETIVOS

- Montar e anotar o genoma nuclear do Fuselo
 - Divisor de águas
 - Evolução, Metabolismo e Conservação
- Estudar genes de desempenho físico
 - MLCK (*Myosin Light Chain Kinase*); ACE (*Angiotensin I Converting Enzyme*)
 - Mutações ou duplicações
 - Mecanismos moleculares – Proteção à dano muscular
- Estudar clock genes (ritmo circadiano) – Data de migração
- Extra: Predição de novos genes
 - Implicações biomédicas e/ou biotecnológicas



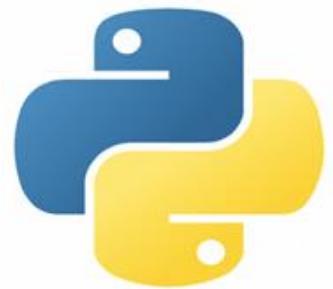
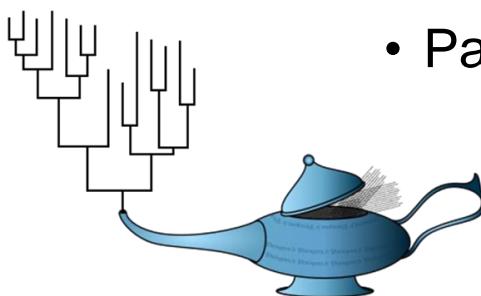
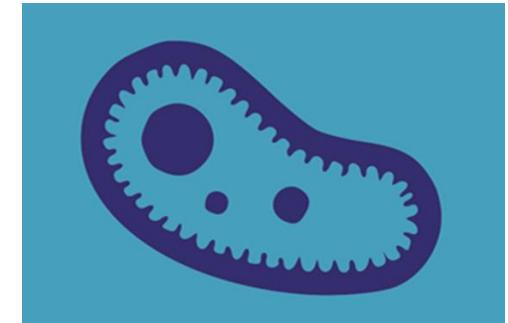
Metodologia

- Dados de sequenciamento pareado – Já em nosso servidor:
 - **NÃO PÚBLICOS**
 - $\approx 1,3$ bilhões de *reads* – 100 bp cada
 - Bibliotecas genômicas - 4 tamanhos diferentes:
 - 170, 500, 800 e 4000 bp
 - Fechamento de gaps
- Revisão de literatura: Metodologia
- Montagem:
 - Abyss, Soapdenovo, MIRA e SPAdes
- Predição gênica/Anotação automática:
 - AUGUSTUS, SABIA
- Anotação manual:
 - Artemis
- Genômica comparativa e Filogenômica:
 - ACT, Circos, MEGA7 e Beast



Projetos paralelos

- Coordenar alunos de graduação:
 - Montar e anotar mais mitogenomas (MitoFree)
 - Otimizar a amostragem dessas sequências:
 - Artrópodes
 - Formigas
- Meta pessoal - Dois projetos simultâneos:
 - Estudo do genoma do Fuselo (Biológicas)
 - Programação (Exatas):
 - Automatizar o MitoFree
 - PartitionFinder + Phylomito: Árvores filogenômicas



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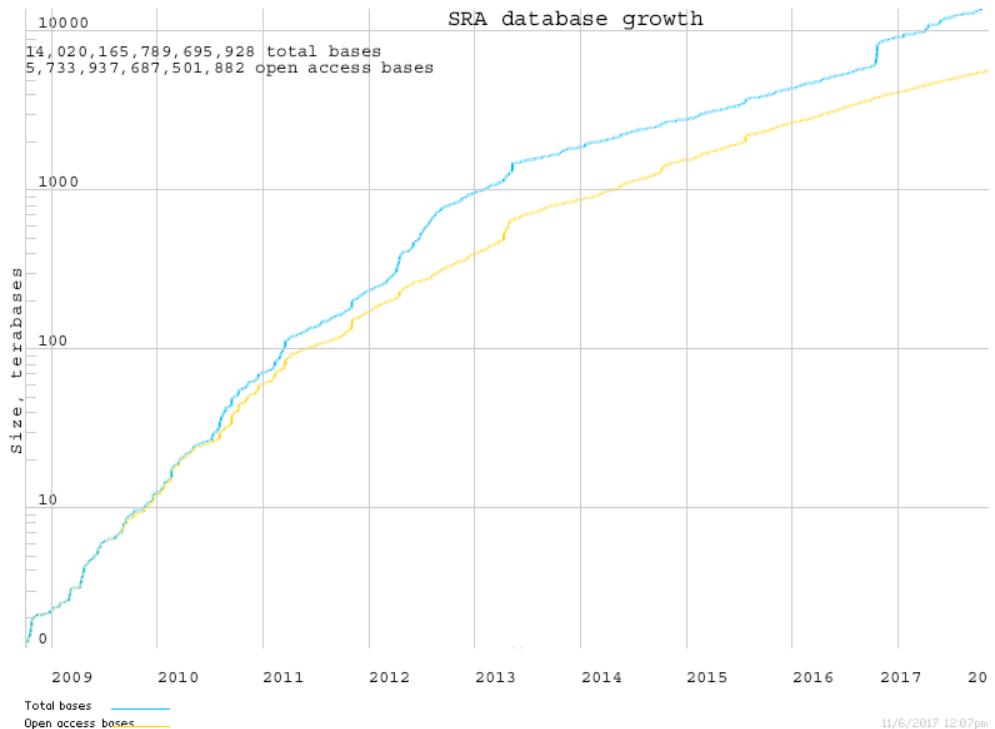
OBRIGADO!



Ilustração por Camille Prado

International Nucleotide Sequence Database Collaboration

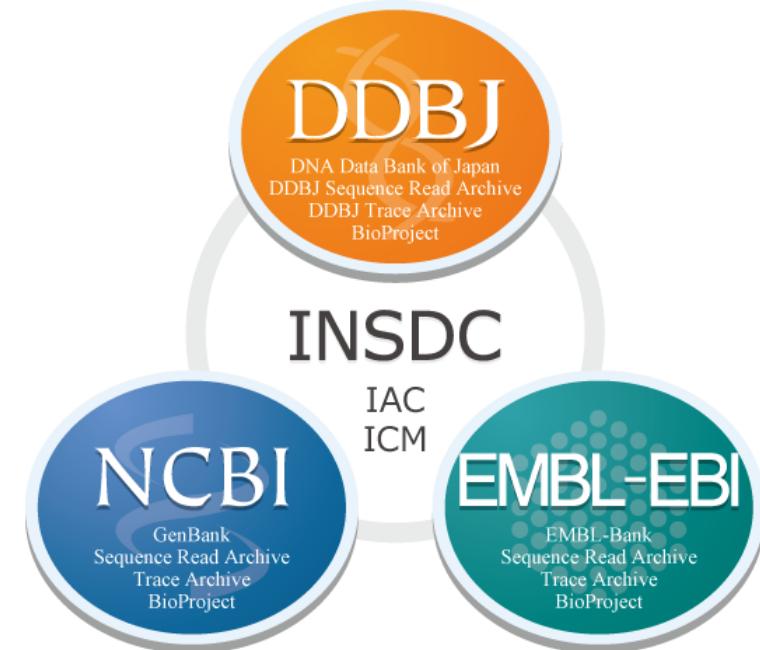
- Sequenciamento de Nova Geração (NGS) : 2005
 - ↑ produção de dados
 - ↓ custo por base
- Crescimento dos bancos de dados públicos de sequenciamento
- Revistas: Incentivam disponibilização dos dados
 - Reprodutibilidade



Next Generation
Sequencing

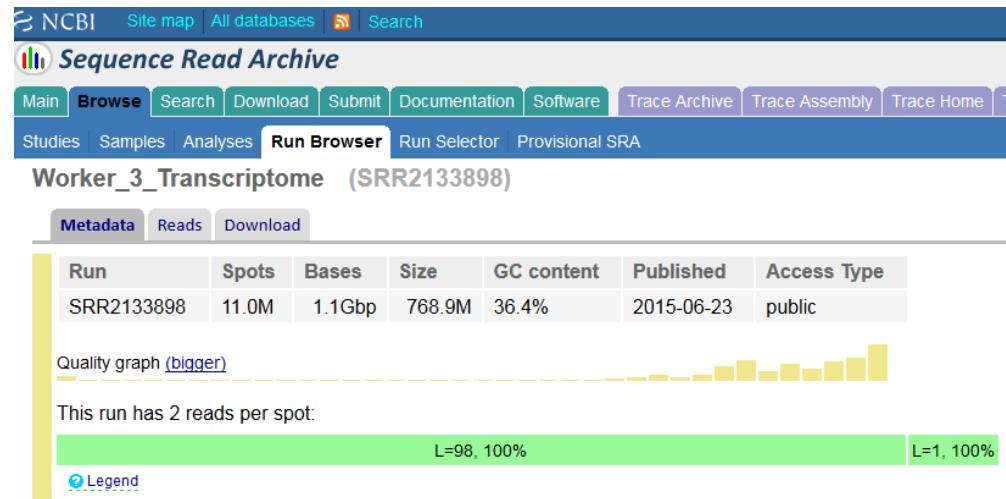
Sequence Read Archive (SRA)

- Parte de colaboração internacional
 - 3 bancos de dados:
 - SRA: NCBI Sequence Read Archive
 - ERA: EBI Sequence Read Archive
 - DRA: DDBJ Sequence Read Archive
 - Sincronizados semanalmente
- Diferentes tipos de dados:
 - Um pouco de reflexão e imaginação
 - Gerar novos conhecimentos



Dados públicos

- Dados usados para responder uma pergunta
 - Sem relação com a mitocôndria
- Sequenciamento
 - Reads mitocondriais presentes
 - 1 em cada 200 / 1 em cada 1000 reads é mitocondrial
 - Espécies com dados públicos e sem mitogenoma descrito
- Esses dados podem ser usados para obter o mitogenomas
 - Além de outras análises...



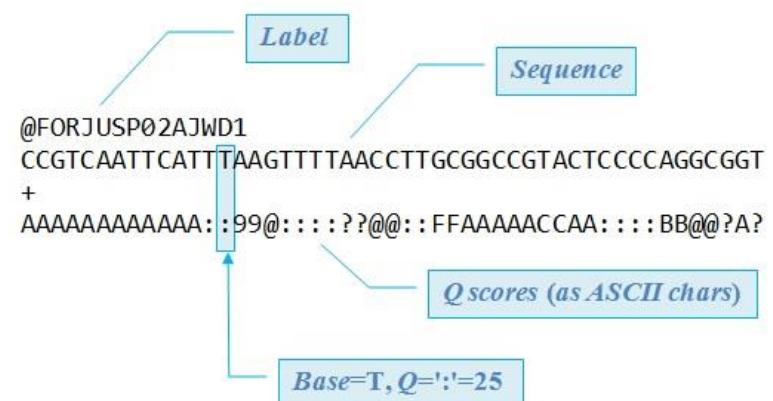
Obtenção dos dados

● Arquivos .sra

- Utilizado pelos 3 bancos
- Disponíveis para download direto
- Precisa ser convertido (SRA Toolkit) para .fastq

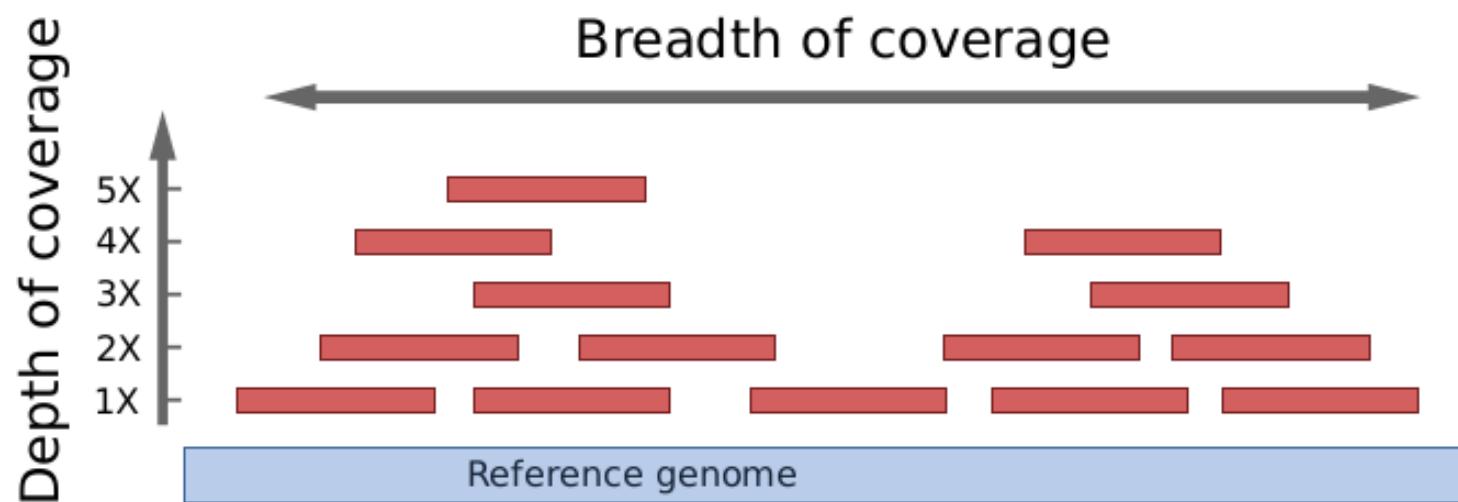
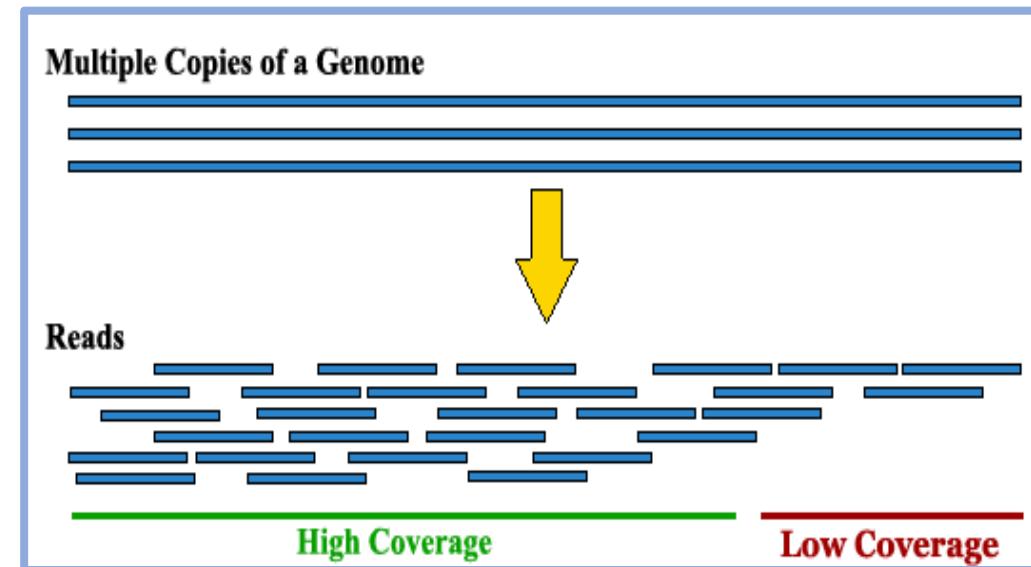
Study accession	Sample accession	Secondary sample accession	Experiment accession	Run accession	Tax ID	Scientific name	Instrument model	Library layout	FASTQ files (FTP)	FASTQ files (Galaxy)	Submitted files (FTP)	Submitted files (Galaxy)	NCBI SRA file (FTP)	NCBI SRA file (Galaxy)	CRAM Index files (FTP)	CRAM Index files (Galaxy)
PRJNA360290	SAMN06208930	SRS1901018	SRX2468701	SRR5150611	55425	Paraponera clavata	Illumina HiSeq 2500	PAIRED	File 1 File 2	File 1 File 2			File 1	File 1		

- Cabeçalho, sequência e qualidade
- Aceitos pela maioria dos programas

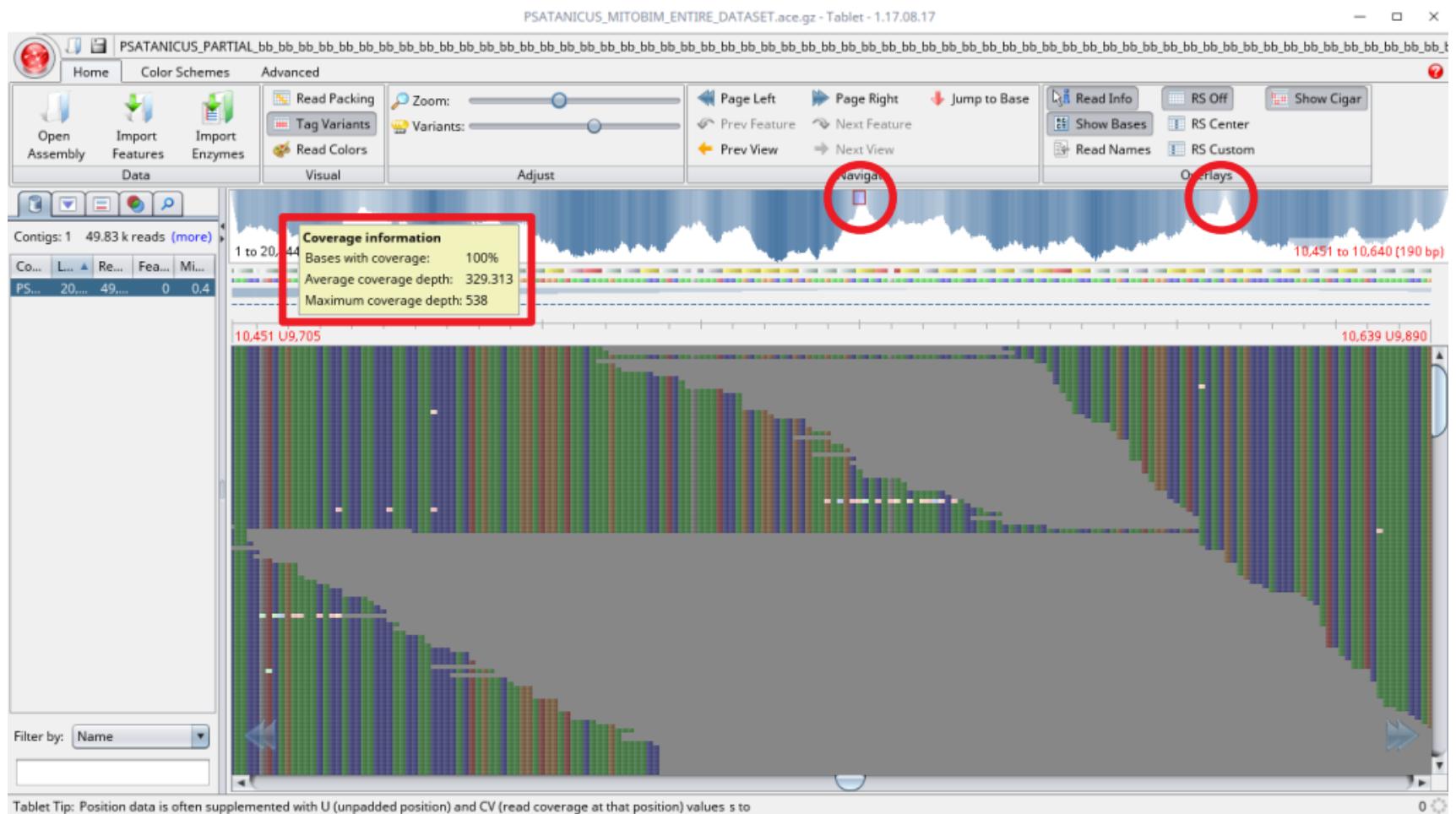


Cobertura

- Checagem
 - Cobertura
 - Quantas sequências corroboram a montagem

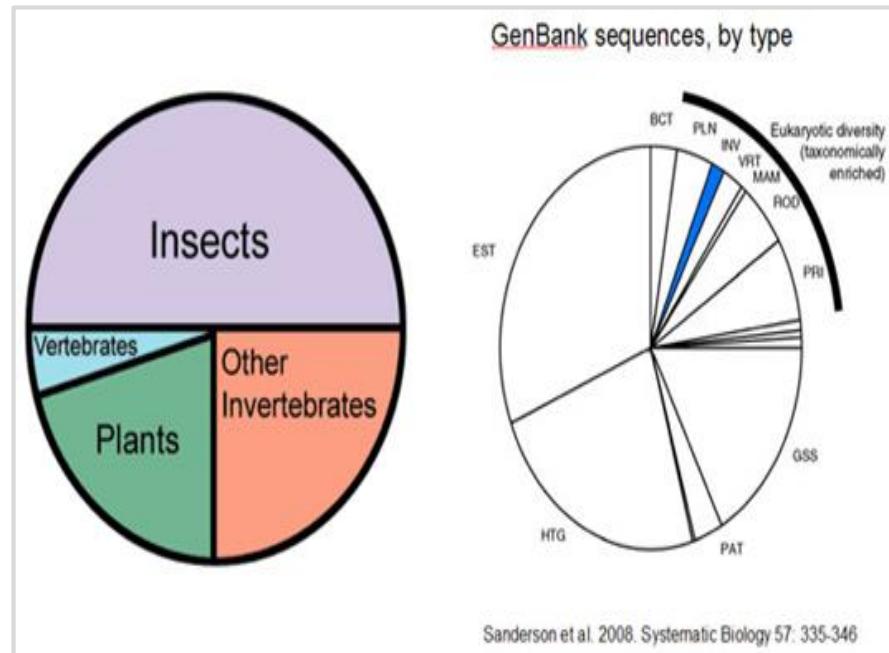


TABLET



Formigas (Hymenoptera: Formicidae)

- Engenheiras ecossistêmicas
 - Alteram características do solo
 - Serviços ecossistêmicos
- Importância econômica
 - Pragas
 - Agentes de controle biológico
- Grande biodiversidade
 - ≈ 13.000 spp.
 - GenBank
 - 15 mitogenomas completos descritos



Pseudomyrmecinae

- 231 espécies – Novo Mundo
- 3 gêneros:
 - *Pseudomyrmex* (~137 spp.)
 - Mais diverso e estudado
 - Dividido em 10 grupos (morfologia)
 - *Tetraponera* (93 spp.)
 - Únicas não encontradas nas américas
 - Paleotrópicas
 - *Myrcidris* (1 espécie)
- Dois grupos principais:
 - Generalistas (geralmente arbóreas)
 - Mutualistas obrigatórios (plant-ant)

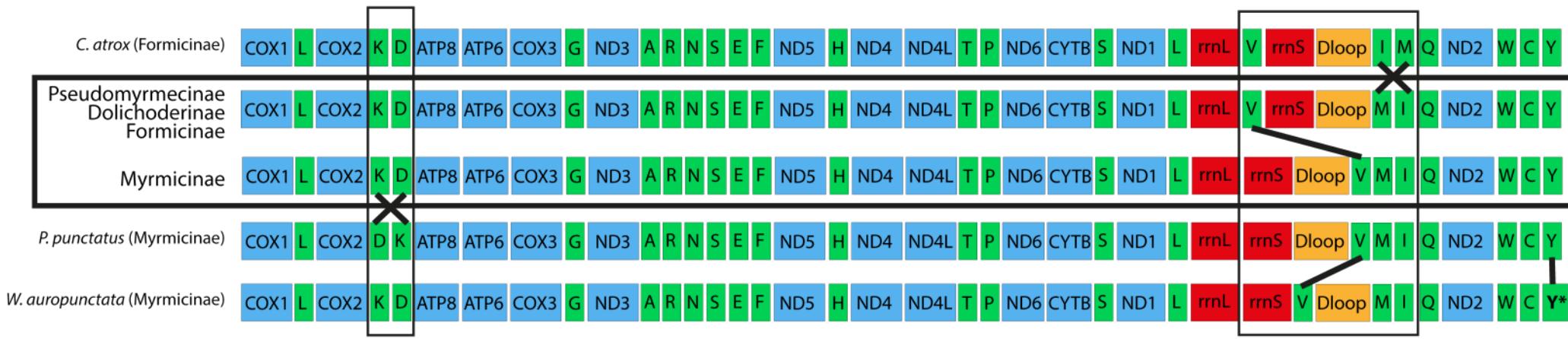


Generalista: *Pseudomyrmex gracilis*



Mutualista: *Tetraponera aethiops*

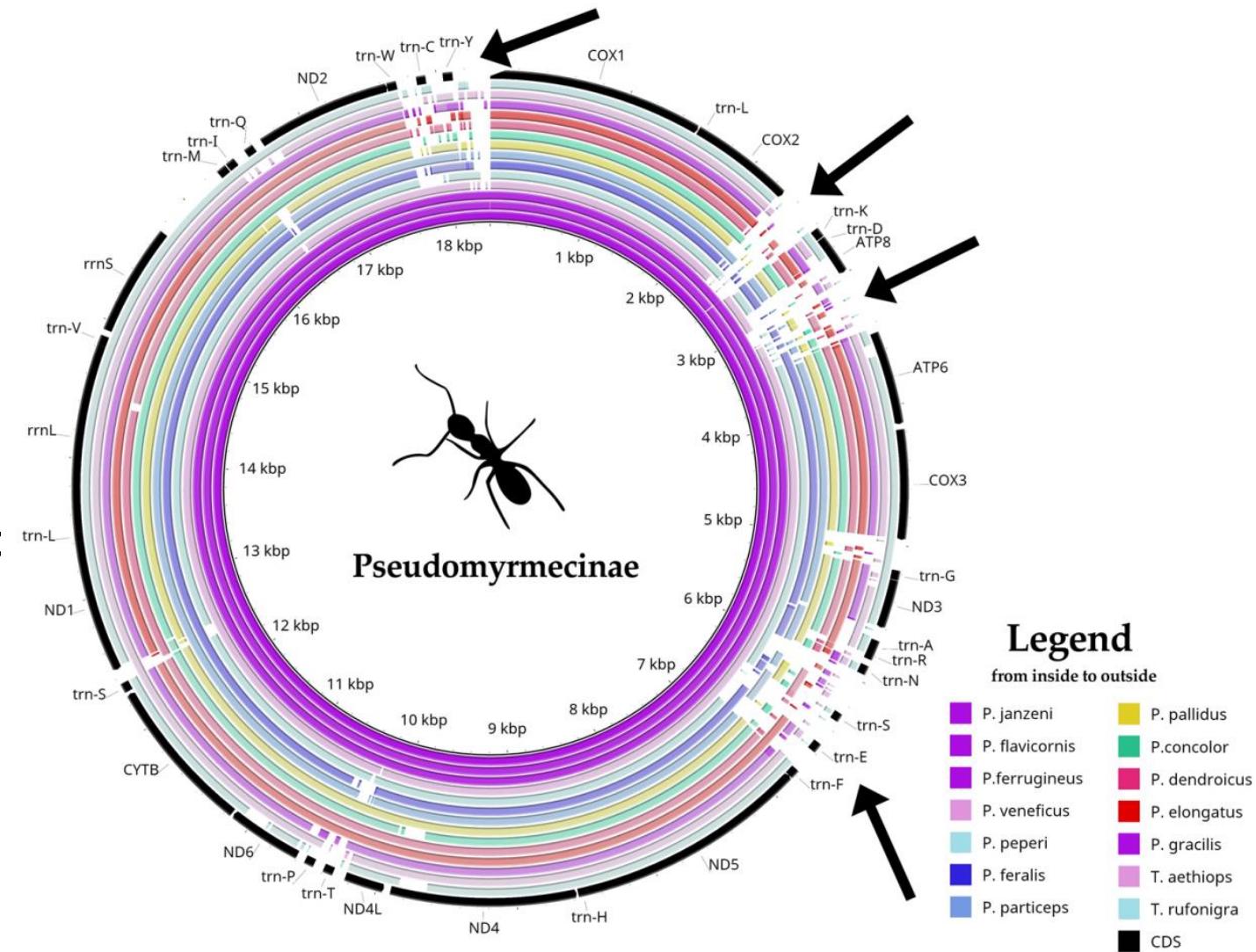
Sintenia



Synteny of all complete Formicidae mitogenomes available on Genbank. The gene arrangements inside the horizontal rectangle are present in most species analyzed and believed to be an ancestral feature for their clades, while the ones outside correspond to derivate unique gene orders, encountered in a single species. Vertical rectangles and arrows indicate regions where synteny changes occurred and the asterisk (*) and arrow in the trn-Y of *W. auropunctata* indicates that it is the only feature in Formicinae mitochondria that changed its coding strand and transcription direction.

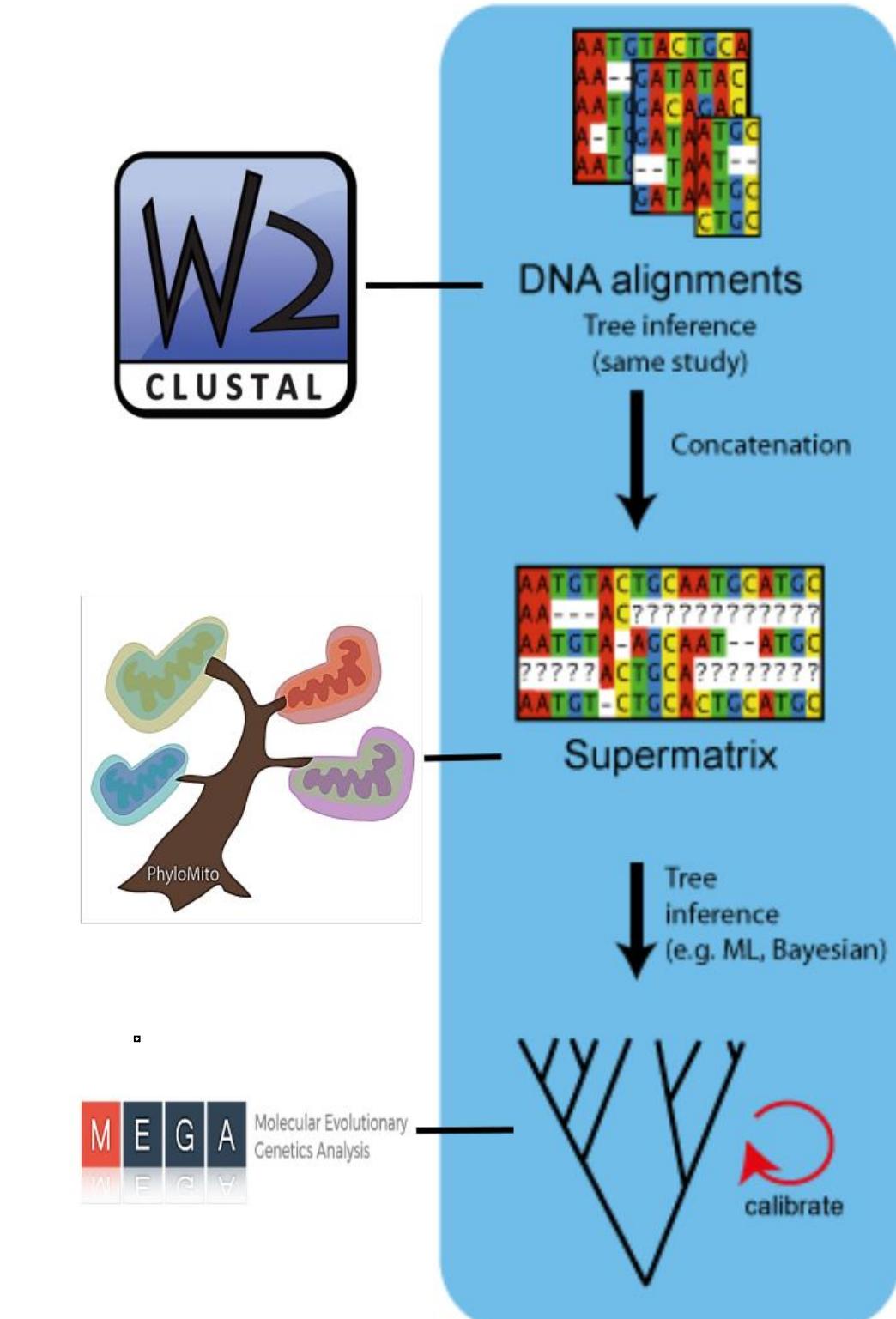
Genômica comparativa

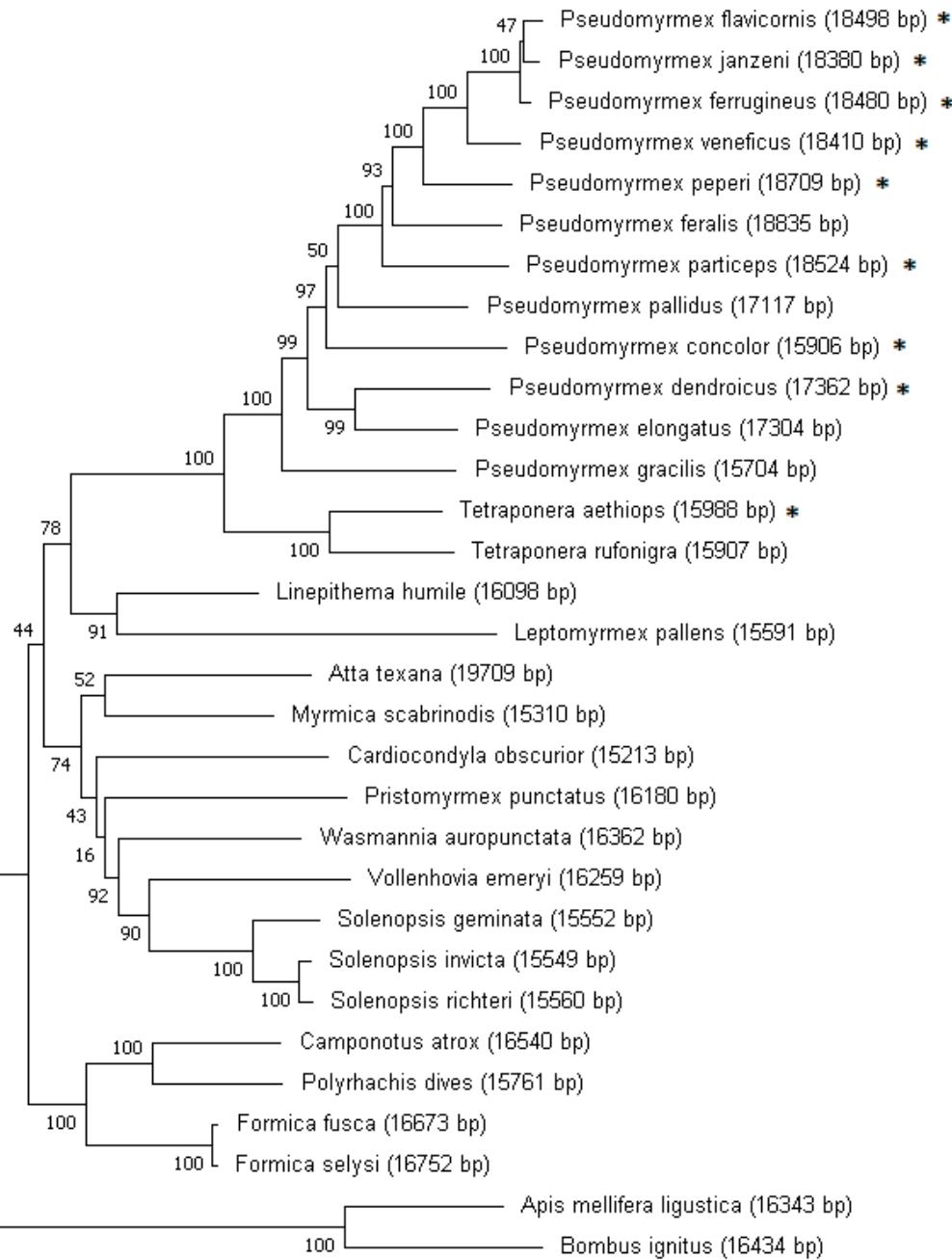
- Tamanho de sequência (bp) variável em Pseudomyrmecinae:
 - 15704 (*Pseudomyrmex gracilis*) até 18835 (*Pseudomyrmex feralis*)
- Hipótese: Inserções de nucleotídeos
- Comparação por BLAST:
 - 4 possíveis regiões de inserção



Filogenômica

- 29 mitogenomas de formiga
 - 14 novos
 - 15 já descritos
 - +2 outgroups (abelhas)
- Supermatriz:
 - 13 genes mitocondriais
 - Alinhados e concatenados
- Inferência filogenômica
 - Máxima verossimilhança
 - Reamostragem: Bootstrap (1000 repetições)





* = MUTUALIST

P. ferrugineus group

P. pallidus group

P. viidus group

P. oculatus group

P. gracilis group

Pseudomyrmecinae

FORMICIDAE

Dolichoderinae

Myrmicinae

Formicinae

Apinae

Bombinae

APIDAE

0,20

