

# Untangling the evolution of the twisted-wing parasites (*Strepsiptera*)



---

## Sequencing the whole genome of *Xenos peckii* (*Strepsiptera*: Xenidae)

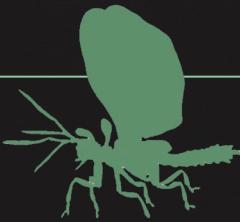


RJ Millena, Ethan Tolman, Jessica Ware

American Museum  
of Natural History



# Outline



01. History

02. Aims

03. Genome

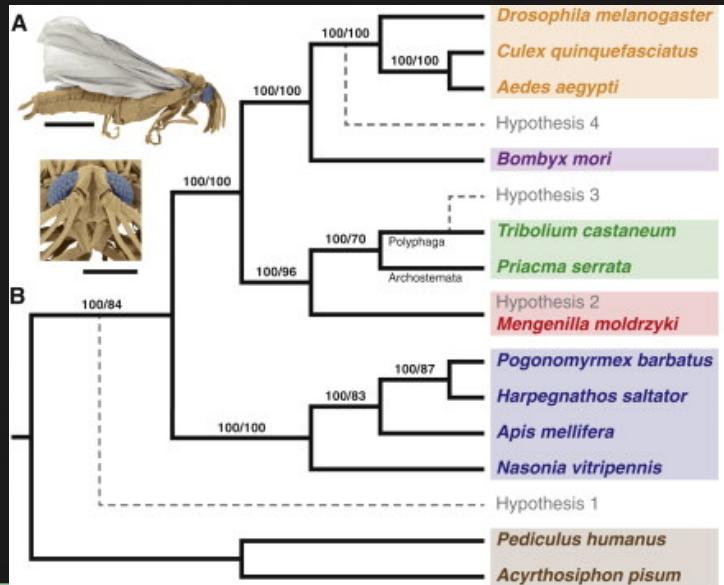
04. Plans

# What is Strepsiptera?



# Genomic and Morphological Evidence Converge to Resolve the Enigma of Strepsiptera

Oliver Niehuis,\* Gerrit Hartig, Sonja Grath, Hans Pohl, Jörg Lehmann, Hakim Tafer, Alexander Donath, Veiko Krauss, Carina Eisenhardt, Jana Hertel, Malte Petersen, Christoph Mayer, Karen Meusemann, Ralph S. Peters, Peter F. Stadler, Rolf G. Beutel, Erich Bornberg-Bauer, Duane D. McKenna, and Bernhard Misof\*



# Previous Work

## Whole genome

- ❖ One species (*Mengenilla moldrzyki*)
  - ❖ Niehuis et al., 2012
- ❖ Sequenced from 15 males
- ❖ For use only in context of insect radiation

## Mitochondrial genome

- ❖ Four species across three families; Xenidae, Mengenillidae, Halictophagidae
- ❖ Odd rearrangements of sequence and structure

## Transcriptome

- ❖ Two species (*Stylops melittae*, *Xenos vesparum*); 2015, 2019
  - ❖ Misof et al., 2014



# Previous Work

## Whole genome

- ❖ One species (*Mengenilla moldrzyki*)
  - ❖ Niehuis et al., 2012
- ❖ Sequenced from 15 males
- ❖ For use only in context of insect radiation

## Mitochondrial genome

- ❖ Four species across three families; Xenidae, Mengenillidae, Halictophagidae
- ❖ Odd rearrangements of sequence and structure

## Transcriptome

- ❖ Two species (*Stylops melittae*, *Xenos vesparum*); 2015, 2019
  - ❖ Misof et al., 2014

Comparative Study > Gene. 2006 Jul 19;376(2):248-59. doi: 10.1016/j.gene.2006.04.005.

## The mitochondrial genome of the entomophagous endoparasite *Xenos vesparum* (Insecta: Strepsiptera)

Antonio Carapelli <sup>1</sup>, Laura Vannini, Francesco Nardi, Jeffrey L Boore, Laura Beani, Romano Dallai, Francesco Frati

Research article | [Open access](#) | Published: 14 December 2009

## The mitochondrial genome of the 'twisted-wing parasite' *Mengenilla australiensis* (Insecta, Strepsiptera): a comparative study

Dino P McMahon , Alexander Hayward & Jeyaraney Kathirithamby

*BMC Genomics* 10, Article number: 603 (2009) | [Cite this article](#)



MITOCHONDRIAL DNA PART B  
2021, VOL. 6, NO. 2, 512–514  
<https://doi.org/10.1080/23802359.2021.1872443>

## MITOGENOME ANNOUNCEMENT

 OPEN ACCESS 

## The mitochondrial genome of one 'twisted-wing parasite' *Xenos cf. moutoni* (Insecta, Strepsiptera, Xenidae) from Gaoligong Mountains, Southwest of China

Ru Zhang<sup>a,b\*</sup>, Jun Li<sup>b\*</sup>, Chuyang Mao<sup>b</sup>, Zhiwei Dong<sup>b</sup>, Jinwu He<sup>a,b</sup>, Guichun Liu<sup>b</sup>, Ruoping Zhao<sup>b</sup> , Wen Wang<sup>a,b,c</sup> and Xueyan Li<sup>b</sup>

Article | [Open access](#) | Published: 10 January 2022

## Substantial rearrangements, single nucleotide frameshift deletion and low diversity in mitogenome of *Wolbachia*-infected strepsipteran endoparasitoid in comparison to its tephritid hosts

Sharon Towett-Kirui, Jennifer L. Morrow & Markus Riegler 

*Scientific Reports* 12, Article number: 477 (2022) | [Cite this article](#)

# Aims

---

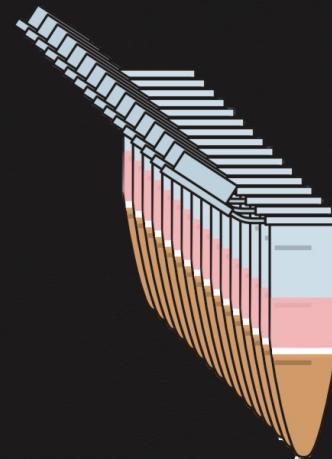
## This project:

- ❖ Sequence the whole genome of *Xenos peckii*
- ❖ Investigate structural elements of this genome

## Big picture:

- ❖ Evolution of the strepsipteran genome
- ❖ "The Strepsiptera Problem"





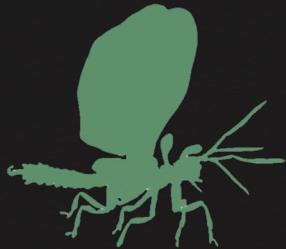
# Highly contiguous assemblies of 101 drosophilid genomes

Bernard Y Kim<sup>1†\*</sup>, Jeremy R Wang<sup>2†</sup>, Danny E Miller<sup>3</sup>, Olga Barmina<sup>4</sup>, Emily Delaney<sup>4</sup>, Ammon Thompson<sup>4</sup>, Aaron A Comeault<sup>5</sup>, David Peede<sup>6</sup>, Emmanuel RR D'Agostino<sup>6</sup>, Julianne Pelaez<sup>7</sup>, Jessica M Aguilar<sup>7</sup>, Diler Haji<sup>7</sup>, Teruyuki Matsunaga<sup>7</sup>, Ellie E Armstrong<sup>1</sup>, Molly Zych<sup>8</sup>, Yoshitaka Ogawa<sup>9</sup>, Marina Stamenković-Radak<sup>10</sup>, Mihailo Jelić<sup>10</sup>, Marija Savić Veselinović<sup>10</sup>, Marija Tanasković<sup>11</sup>, Pavle Erić<sup>11</sup>, Jian-Jun Gao<sup>12</sup>, Takehiro K Katoh<sup>12</sup>, Masanori J Toda<sup>13</sup>, Hideaki Watabe<sup>14</sup>, Masayoshi Watada<sup>15</sup>, Jeremy S Davis<sup>16</sup>, Leonie C Moyle<sup>17</sup>, Giulia Manoli<sup>18</sup>, Enrico Bertolini<sup>18</sup>, Vladimir Košťál<sup>19</sup>, R Scott Hawley<sup>20</sup>, Aya Takahashi<sup>9</sup>, Corbin D Jones<sup>6</sup>, Donald K Price<sup>21</sup>, Noah Whiteman<sup>7</sup>, Artyom Kopp<sup>4</sup>, Daniel R Matute<sup>6†\*</sup>, Dmitri A Petrov<sup>1†\*</sup>



## Single-fly assemblies fill major phylogenomic gaps across the Drosophilidae Tree of Life

Bernard Y. Kim, Hannah R. Gellert, Samuel H. Church, Anton Suvorov, Sean S. Anderson, Olga Barmina, Sofia G. Beskid, Aaron A. Comeault, K. Nicole Crown, Sarah E. Diamond, Steve Dorus, Takako Fujichika, James A. Hemker, Jan Hrcek, Maaria Kankare, Toru Katoh, Karl N. Magnacca, Ryan A. Martin, Teruyuki Matsunaga, Matthew J. Medeiros, Danny E. Miller, Scott Pitnick, Sara Simoni, Tessa E. Steenwinkel, Michele Schiffer, Zeeshan A. Syed, Aya Takahashi, Kevin H-C. Wei, Tsuya Yokoyama, Michael B. Eisen, Artyom Kopp, Daniel Matute, Darren J. Obbard, Patrick M. O'Grady, Donald K. Price, Masanori J. Toda, Thomas Werner, Dmitri A. Petrov



# Assembly Statistics

	BUSCO	N50	Size	Sequencer	Assembly
<i>Xenos peckii</i>	84.2%	421 kb	≤100Mb	ONT MinION + Illumina NovaSeq	Flye 2.9.1, w/AUGUSTUS (Mikhail et al., 2020, Stanke et al., 2006)
<i>Mengenilla moldrzyki</i> (Niehuis et al., 2012)	16.3%	4 kb	155.7 Mb	Roche 454 GS-FLX Titanium	Newbler v. 2.3 (Genivaldo et al., 2013)

	BUSCO	Sequencer	Assembly
<i>Stylops melittae</i> (Misof et al., 2014)	82.4%	Illumina	SOAPdenovo-Trans-31kmer 1.01 (Xie et al., 2014)
<i>Xenos vesparum</i> (Misof et al., 2014)	80.4%	Illumina	SOAPdenovo-Trans-31kmer 1.01 (Xie et al., 2014)



# Preliminary Comparisons

## Missing BUSCO genes

- ❖ *Stylops melittae* and *Xenos vesparum*: 43
  - ❖ *Stylops*: 43/153, 28%
  - ❖ *Xenos*: 43/173, 25%
- ❖ Transcriptomes + *Xenos peckii*: 23

## Hypothesis

- ❖ The BUSCO genes are still there, just difficult to assemble or identify!
- ❖ Unclassified repeats

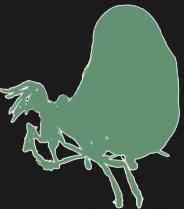
1	10008at50557	2	25673at50557	3	30640at50557
1	100475at50557	2	30640at50557	3	33009at50557
1	100579at50557	2	33009at50557	3	37475at50557
2	100854at50557	2	37475at50557	3	38406at50557
1	100957at50557	2	38406at50557	3	46955at50557
3	101417at50557	2	38523at50557	3	48837at50557
3	101448at50557	2	39032at50557	3	54603at50557
1	101501at50557	2	40442at50557	3	56896at50557
2	101659at50557	2	42982at50557	3	58479at50557
1	101800at50557	2	46955at50557	3	59985at50557
3	101869at50557	2	47233at50557	3	63888at50557
2	102324at50557	2	48837at50557	3	65980at50557
1	102414at50557	2	52049at50557	3	68094at50557
2	102433at50557	2	53250at50557	3	75685at50557
1	102531at50557	2	54603at50557	3	75864at50557
1	102539at50557	2	56276at50557	3	76005at50557
1	102553at50557	2	56896at50557	3	83433at50557
1	102616at50557	2	57602at50557	3	86747at50557
1	10268at50557	2	58479at50557	3	87750at50557
3	102771at50557	2	59985at50557	3	89079at50557
1	102774at50557	2	60720at50557	3	90358at50557
1	102920at50557	2	6239at50557	3	98887at50557
1	103040at50557	2	62733at50557	3	98900at50557
1	103576at50557	2	63888at50557		
1	10394at50557	2	65980at50557		
1	104016at50557	2	68094at50557		
1	104265at50557	2	69725at50557		
1	104460at50557	2	70072at50557		
1	104469at50557	2	73720at50557		
1	104526at50557	2	75685at50557		
1	104592at50557	2	75864at50557		
1	105092at50557	2	76905at50557		
1	105221at50557	2	78072at50557		
1	105548at50557	2	83433at50557		
1	105732at50557	2	83523at50557		
1	106023at50557	2	86747at50557		
1	106854at50557	2	87750at50557		
1	106896at50557	2	89079at50557		
3	106946at50557	2	90358at50557		
3	107013at50557	2	92708at50557		
1	107216at50557	2	95489at50557		
1	107380at50557	2	98887at50557		
1	107413at50557	2	98900at50557		
3	107457at50557				
1	107526at50557				
1	107564at50557				
1	107632at50557				
3	108060at50557				
1	108107at50557				
2	108140at50557				
1	108271at50557				
3	108301at50557				
2	108482at50557				

# Repeats

	number of elements*	length occupied	percentage of sequence
Retroelements	2027	1456668 bp	2.43 %
DNA transposons	2398	1120574 bp	1.87 %
Unclassified:	28989	10205828 bp	17.06 %
Total interspersed repeats:		12783070 bp	21.36 %

RepeatMasker v. 4.1.2 (Smit et al., 2013-2015)

# Next Steps



## Polishing, annotation

- ❖ Illumina reads
- ❖ reads2tree

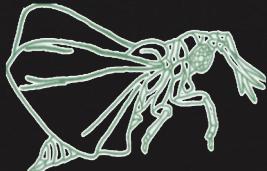
## More genomes with PacBio

- ❖ *Elenchus koebeliai* (Florida, USA)
- ❖ *Caenocholax fenesi* (Veracruz, Mexico)
- ❖ Resolution of repetitive elements

## Genomic collinearity analyses

- ❖ Intraordinal (Stylops, Mengenilla)
- ❖ Interordinal (Neuropterida, Coleoptera)





# Thanks!



Questions?  
[rmillena@amnh.org](mailto:rmillena@amnh.org)  
[rjmillena.com](http://rjmillena.com)

American Museum  
of Natural History

Oxford  
**NANOPORE**  
Technologies

BLACK ROCK  
FOREST

• BRIGHAM YOUNG UNIVERSITY •  
FOUNDED 1875  
**BYU**  
• PROVO, UTAH •



1. Kathirithamby, J. (2018). Biodiversity of Strepsiptera. *Insect biodiversity: science and society*, 2, 673-703. <https://doi.org/10.1002/9781118945582.ch22>
2. Pierce, W. D. (1909). *A monographic revision of the twisted winged insects comprising the order Strepsiptera Kirby* (No. 66-69). US Government Printing Office, 2-11.
3. Kathirithamby, J. (2022) World Strepsiptera Database. Accessed at <https://strepsiptera.aphia.org> on 2022-08-24. <https://doi.org/10.14284/484>
4. Niehuis, O., Hartig, G., Grath, S., Pohl, H., Lehmann, J., Tafer, H., ... & Misof, B. (2012). Genomic and morphological evidence converge to resolve the enigma of Strepsiptera. *Current Biology*, 22(14), 1309-1313. <https://doi.org/10.1016/j.cub.2012.05.018>
5. Carapelli, A., Vannini, L., Nardi, F., Boore, J. L., Beani, L., Dallai, R., & Frati, F. (2006). The mitochondrial genome of the entomophagous endoparasite *Xenos vesparum* (Insecta: Strepsiptera). *Gene*, 376(2), 248-259. <https://doi.org/10.1016/j.gene.2006.04.005>
6. McMahon, D. P., Hayward, A., & Kathirithamby, J. (2009). The mitochondrial genome of the 'twisted-wing parasite' *Mengenilla australiensis* (Insecta, Strepsiptera): a comparative study. *BMC genomics*, 10(1), 1-15. <https://doi.org/10.1186/1471-2164-10-603>
7. Zhang, R., Li, J., Mao, C., Dong, Z., He, J., Liu, G., ... & Li, X. (2021). The mitochondrial genome of one 'twisted-wing parasite' *Xenos cf. moutoni* (Insecta, Strepsiptera, Xenidae) from Gaoligong Mountains, Southwest of China. *Mitochondrial DNA Part B*, 6(2), 512-514. <https://doi.org/10.1080/23802359.2021.1872443>
8. Towett-Kirui, S., Morrow, J. L., Close, S., Royer, J. E., & Riegler, M. (2021). Host–endoparasitoid–endosymbiont relationships: concealed Strepsiptera provide new twist to Wolbachia in Australian tephritid fruit flies. *Environmental Microbiology*, 23(9), 5587-5604. <https://doi.org/10.1111/1462-2920.15715>
9. Towett-Kirui, S., Morrow, J. L., & Riegler, M. (2022). Substantial rearrangements, single nucleotide frameshift deletion and low diversity in mitogenome of Wolbachia-infected strepsipteran endoparasitoid in comparison to its tephritid hosts. *Scientific Reports*, 12(1), 1-13. <https://doi.org/10.1038/s41598-021-04398-y>
10. Gillespie, J. J., McKenna, C. H., Yoder, M. J., Gutell, R. R., Johnston, J. S., Kathirithamby, J., & Cognato, A. I. (2005). Assessing the odd secondary structural properties of nuclear small subunit ribosomal RNA sequences (18S) of the twisted-wing parasites (Insecta: Strepsiptera). <https://doi.org/10.1111/j.1365-2583.2005.00591.x>
11. Tolman, E. R., Beatty, C. D., Bush, J., Kohli, M., Moreno, C. M., Ware, J. L., ... & Frandsen, P. B. (2023). A chromosome-length assembly of the Black Petaltail (*Tanypteryx hageni*) Dragonfly. *Genome biology and evolution*, 15(3), evad024. <https://doi.org/10.1093/gbe/evad024>