

Taxonomic errors in the mitogenomes of two scale insects

Han XU, San'an WU^①

The Key Laboratory for Silviculture and Conservation of Ministry of Education, Beijing Forestry University, Beijing 100083, China

Abstract: The mitogenome is a double strand molecule that has become an extensively studied molecular marker used in the study of the phylogeny of insects. A recent study investigated the mitogenomes of *Planococcus citri* (Risso, 1813) and *Unaspis yanonensis* (Kuwana, 1923) published by *PeerJ* (Liu *et al.* 2020). In our study, we determine that the putative “*P. citri* and *U. yanonensis*” mitogenomes did not originate from Coccoidea but from parasitic wasps in the Chalcidoidea. These results are based on the genetic distances of the barcoding sequences of cytochrome *c* oxidase I (*COI*) and on molecular identifications performed against the Basic Local Alignment Search Tool (BLAST) in NCBI.

Key words: Molecular phylogenetics; correction; parasitic wasps; taxonomy

关于 2 种蚧虫线粒体基因组分类的错误

徐晗, 武三安^①

北京林业大学省部共建森林培育与保护教育部重点实验室, 北京 100083

摘要: 线粒体基因组作为稳定的双链大分子, 目前被广泛应用于昆虫系统进化分析中。*PeerJ* 杂志 2020 年发表了柑橘臀纹粉蚧 *Planococcus citri* (Risso, 1813) 和矢尖蚧 *Unaspis yanonensis* (Kuwana, 1923) 的线粒体基因组。然而, 本研究通过 *COI* 条形码序列的遗传距离分析以及基于 NCBI 上的 BLAST 功能进行分子鉴定, 结果发现这 2 种所谓的蚧虫线粒体基因组并非取自真正的蚧虫标本, 而是来自其寄生蜂, 从而导致鉴定错误。

关键词: 蚧虫; 指正; 寄生蜂; 分类

Introduction

The mitogenome is a double-strand circular molecule, ranging from 14 kb to 20 kb in size, and usually consists of 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs), and one or more non-coding regions (also known as the control region, CR) (Wolstenholme 1992; Boore 1999). Because of relatively high evolutionary rates and rare recombination, mitogenomes have been used recently as molecular markers for molecular evolution, population genetics and reconstruction of phylogenetic relationships at various taxonomic levels (Cameron 2014). Advanced sequencing methods have resulted in the publication of a greater number of mitogenomes (Cameron 2014; Deng *et al.* 2019; Lu *et al.* 2020; Xu *et al.* 2021). Liu *et al.* (2020) recently published the mitogenomes of three species assigned as coccoid species, namely *Ceroplastes rubens* Maskell, 1893, *Planococcus citri* (Risso, 1813), and *Unaspis yanonensis* (Kuwana, 1923).

Accepted 29 December 2021. Published online 22 February 2022. Published 25 March 2022.

① Corresponding author, E-mail: Sananwu@bjfu.edu.cn

In this study, we examined if the molecular data published by Liu *et al.* (2020) originated from the *P. citri* and *U. yanonensis* by analysis of genetic distance based on the barcoding sequences of *COI* and by molecular identification against the Basic Local Alignment Search Tool (BLAST) in NCBI.

Material and methods

Taxon sampling. We downloaded the molecular sequences from the NCBI (<https://www.ncbi.nlm.nih.gov/>) and selected 651 bp of barcoding sequences of cytochrome c oxidase I (*COI*) of mitogenomes from the nominal species “*P. citri*” (Accession Number MT611526) (position: 5297 bp – 5947 bp) and “*U. yanonensis*” (Accession Number MT611525) (position: 10001 bp – 10651 bp). In addition, we selected an additional 48 and 11 barcoding sequences of actual species *P. citri* and *U. yanonensis*, respectively, to analyse the genetic divergence between them and two nominal coccoid species (Table 1). The sequences we adopted are submitted by different researchers in order to reduce the potential impact of any incorrect sequences.

Table 1. Taxon used in genetic distance analysis

Species	Accession Number	Species	Accession Number
" <i>Planococcus citri</i> "*	MT611526	<i>Planococcus citri</i>	KY373077
" <i>Unaspis yanonensis</i> "*	MT611525		KY373079
<i>Planococcus citri</i>	GU936938		KY373081
	HM474278		KY373107
	HM474279		KY373108
	HM474280		MF952472
	HM474281		MF952477
	HM474283		MF952478
	HM474285		MF952535
	HM474286		MG437485
	HM474287		MG813757
	HM474288		MG813759
	JF905461.1		MG813760
	KJ187500		MG813761
	KP692643		MG813762
	KP692644		MG813763
	KP692645		MG813766
	KP692646		MG813767
	KP692647		MG813768
	KP692648	<i>U. yanonensis</i>	HM474407
	KU254182		HM474408
	KU254185		HM474409

Continued Table 1.

Species	Accession Number	Species	Accession Number
	KY211612		KP981077
	KY372905		KP981078
	KY372939		KP981079
	KY372948		KP981075
	KY372979		KP981076
	KY373012		KR001888
	KY373051		KR001889
	KY373073		KR001890

* The two nominal coccoid species are indicated with quotation marks.

Genetic distance analysis. The genetic distances of the *COI* barcoding sequences between the two nominal coccoid species with other orders and with other specimens of Coccoidea were calculated by MEGA7 using the Kimura 2-parameter (K2P) model (Kimura 1980; Kumar *et al.* 2016).

Molecular identification. “*P. citri*” and “*U. yanonensis*” *COI* barcoding sequences were queried against the Basic Local Alignment Search Tool (BLAST) in NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul *et al.* 1990).

Results

Genetic divergence of the barcoding sequences. The genetic divergence between the nominal “*P. citri*” and other specimens of *P. citri*, ranged from 51.5% to 94.4% with an average value of 58.5% (Table 2). And the genetic divergence between the nominal “*U. yanonensis*” and other specimens of *U. yanonensis*, ranged from 45.3% to 52.5% with an average value of 50.2% (Table 2).

Molecular identification. The nominal “*P. citri*” sequence was determined to originate from Hymenoptera with high similarity, ranging from 83.87% with Platygasteridae to 99.45% with Encyrtidae (Table 3). And “*U. yanonensis*” was determined to originate from within Chalcidoidea in Hymenoptera with a high similarity, ranging from 88.79% with Aphelinidae to 89.42% with Pteromalidae (Table 4).

Table 2. The genetic divergence between the nominal ‘*Planococcus citri*’ and ‘*Unaspis yanonensis*’ and other conspecific individuals, respectively (%)

	Min	Max	Ave.
“ <i>P. citri</i> ”*	51.5	94.4	58.5
“ <i>U. yanonensis</i> ”*	45.3	52.5	50.2

* The two nominal coccoid species are indicated with quotation marks.

Table 3. Top 10 records of matching results based on the barcoding sequence of *COI* of the nominal “*P. citri*” from BLAST in NCBI

Order	Superfamily	Family	Species	Similarity (%)	GenBank Accession Number
Hymenoptera	Chalcidoidea	Encyrtidae	<i>Anagyrus jenniferae</i>	99.45	KY211367
Hymenoptera	Chalcidoidea	Encyrtidae	<i>Aenasius arizonensis</i>	99.45	KY211366
Hymenoptera	Chalcidoidea	Encyrtidae	<i>Anagyrus jenniferae</i>	99.09	KY211364
Hymenoptera	Chalcidoidea	Encyrtidae	<i>Anagyrus thailandicus</i>	98.91	KY211369
Hymenoptera	Chalcidoidea	Encyrtidae	<i>Clausenia purpurea</i>	94.9	KU499516
Hymenoptera	-	-	Hymenoptera sp.	86.36	KM568188
Hymenoptera	-	-	Hymenoptera sp.	86.01	KM557055
Hymenoptera	Chalcidoidea	Eurytomidae	Eurytomidae sp.	84.26	KY845423
Hymenoptera	Chalcidoidea	Megastigmidae	<i>Bootanomyia</i> sp.	84.13	MF956341
Hymenoptera	Platygaстроidea	Platygastridae	Platygastridae sp.	83.87	KR802803

Table 4. Top 10 records of matching results based on the barcoding sequence of *COI* of the nominal “*U. yanonensis*” from BLAST in NCBI

Order	Superfamily	Family	Species	Similarity (%)	GenBank Accession Number
Hymenoptera	Chalcidoidea	Pteromalidae	Pteromalinae sp.	89.42	KR892750
Hymenoptera	Chalcidoidea	Trichogrammatidae	<i>Trichogramma platneri</i>	89.11	KX512841
Hymenoptera	Chalcidoidea	Trichogrammatidae	<i>Trichogramma platneri</i>	89.11	KX512840
Hymenoptera	Chalcidoidea	Trichogrammatidae	<i>Trichogramma platneri</i>	89.11	KX512839
Hymenoptera	Chalcidoidea	Trichogrammatidae	<i>Trichogramma</i> sp.	89.11	KR794810
Hymenoptera	Chalcidoidea	Aphelinidae	<i>Aphytis melinus</i>	88.79	MH456570
Hymenoptera	Chalcidoidea	Aphelinidae	<i>Aphytis melinus</i>	88.79	MH456569
Hymenoptera	Chalcidoidea	Aphelinidae	<i>Aphytis melinus</i>	88.79	MH456568
Hymenoptera	Chalcidoidea	Aphelinidae	<i>Aphytis melinus</i>	88.79	MH456567
Hymenoptera	Chalcidoidea	Aphelinidae	<i>Aphytis melinus</i>	88.79	MH456566

Discussion

Previous studies indicated that the genetic distance of barcoding sequence of *COI* among conspecific individuals in Diaspididae and Pseudococcidae ranged from 0.00% to 5.98% at least (Park *et al.* 2011; Wang *et al.* 2016). Had the molecular data from Liu *et al.* (2020) been correctly assigned as those of *P. citri* and *U. yanonensis*, these two nominal coccoid specimens would have been the closest genetic distance with each conspecific individual based on the barcoding sequence of *COI*. But they both had an extraordinarily high genetic

divergence with each conspecific specimen, with an average genetic distance of 58.5% and 50.2%, respectively, far exceeding the range suggested by previous studies. Moreover, they should also be identified as corresponding coccoid species by the BLAST on NCBI, because of numerous records of the barcoding sequences of *COI* of the actual *P. citri* and *U. yanonensis* submitted from different researchers on NCBI. Instead, they were both identified as parasitic wasps Chalcidoidea by the BLAST on NCBI. The Chalcidoidea is unquestionably one of the most key natural enemies of scale insects and extensively adopted as a biocontrol agent (Dean *et al.* 1978; Noyes 1985; Neuenschwander *et al.* 1989; García Morales *et al.* 2016; Ramos *et al.* 2018). There have been 29 species belonging to five chalcidoid families reported as a natural enemy of *P. citri*, including *Anagyrus aurantifrons*, *A. bohemani*, and *A. dactylopii* (ScaleNet; García Morales *et al.* 2016). Likewise, there have also been 16 species belonging to three chalcidoid families as a natural enemy of *U. yanonensis*, including *Aphytis melinus*, *Ap. proclia*, and *Ap. unaspidis* (ScaleNet; García Morales *et al.* 2016).

Accordingly, the molecular data from Liu *et al.* (2020) was erroneously assumed by the original authors to originate from coccoid species. However, they are in fact from parasitic Chalcidoidea of Hymenoptera. An incorrect species assignment may cause confusion in the phylogenetic analysis of Coccoidea and lead to mistaken conclusions in subsequent studies. Therefore, researchers must ensure the identity of specimens and their molecular data.

Acknowledgements

We would like to thank the editor and reviewers for their valuable input. This project was supported by the National Natural Science Foundation of China (31772488).

References

- Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology*, 215(3): 403–410.
- Boore JL. 1999. Animal mitochondrial genomes. *Nucleic Acids Research*, 27(8): 1767–1780.
- Cameron SL. 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. *Annual Review of Entomology*, 59: 95–117.
- Dean HA, Schuster MF, Boling JC & Rihard PT. 1979. Complete biological control of *Antonina graminis* in Texas with *Neodusmetia sangwani* (a classic example). *Bulletin of the Entomological Society of America*, 25: 262–267.
- Deng J, Lu C & Huang X. 2019. The first mitochondrial genome of scale insects (Hemiptera: Coccoidea). *Mitochondrial DNA Part B*, 4(2): 2094–2095.
- García Morales M, Denno BD, Miller DR, Miller GL, Ben-Dov Y & Hardy NB. 2016. ScaleNet: a literature-based model of scale insect biology and systematics. *Database*, 2016.
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16: 111–120.
- Kumar S, Stecher G & Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7): 1870–1874.

- Liu HL, Chen QD, Chen S, Pu DQ, Chen ZT, Liu YY & Liu X. 2020. The highly rearranged mitochondrial genomes of three economically important scale insects and the mitochondrial phylogeny of Coccoidea (Hemiptera: Sternorrhyncha). *PeerJ*, 8: e9932.
- Lu C, Huang X & Deng J. 2020. The challenge of Coccidae (Hemiptera: Coccoidea) mitochondrial genomes: The case of *Saissetia coffeae* with novel truncated tRNAs and gene rearrangements. *International Journal of Biological Macromolecules*, 158: 854–864.
- Neuenschwander P, Hammond WNO, Gutierrez AP, Cudjoe AR, Adjakloe R, Baumgärtner JU & Regev U. 1989. Impact assessment of the biological control of the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) by the introduced parasitoid *Epidinocarsis lopezi* (De Santis) (Hymenoptera: Encyrtidae). *Bulletin of Entomological Research*, 79: 579–594.
- Noyes JS. 1985. Chalcidoids and biological control. *Chalcid Forum*, 5: 5–10.
- Park DS, Suh SJ, Hebert PD, Oh HW & Hong KJ. 2011. DNA barcodes for two scale insect families, mealybugs (Hemiptera: Pseudococcidae) and armored scales (Hemiptera: Diaspididae). *Bulletin of Entomological Research*, 101(4): 429–434.
- Ramos ASDJC, de Lemos RNS, Costa VA, Peronti ALBG, da Silva EA, Mondego JM & Moreira AA. 2018. Hymenopteran parasitoids associated with scale insects (Hemiptera: Coccoidea) in tropical fruit trees in the eastern Amazon, Brazil. *Florida Entomologist*, 101(2): 273–278.
- Wang XB, Zhang JT, Deng J, Zhou QS, Zhang YZ & Wu SA. 2016. DNA barcoding of mealybugs (Hemiptera: Coccoidea: Pseudococcidae) from mainland China. *Annals of the Entomological Society of America*, 109(3): 438–446.
- Wolstenholme DR. 1992. Animal mitochondrial DNA: structure and evolution. *International Review of Cytology*, 141: 173–216.
- Xu H, Liu X, Li H & Wu S. 2021. The mitogenome of the scale insect *Didesmococcus koreanus* Borchsenius, 1955 (Coccoidea: Coccidae). *Mitochondrial DNA Part B*, 6(4): 1298–1299.