# The genus Iare Barbalho and Penteado-Dias (Hymenoptera: Braconidae: Doryctinae) in Mexico, with the description of two new species 

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#### Abstract

Two new species of the doryctine genus Iare, I. mexicanus sp. nov. and I. cheguevarai sp. nov., are genetically and morphologically described from a tropical dry forest in Jalisco, Mexico. Iare belokobylskiji Marsh is also reported for the latter region. These species represent the northernmost distribution records for the genus. A simultaneous Bayesian analysis with COI and 28 S DNA sequence data recovered the three examined species of Iare within a single clade, though with low support. This genus appears nested within a large doryctine Neotropical clade as sister group of a cluster containing species of Callihormius Ashmead, Leluthia Cameron, Histeromeroides Marsh and Panama Marsh.


Key words: Apocrita, parasitoid wasps, Jalisco, tropical dry forest


#### Abstract

\section*{Resumen}

Dos especies nuevas del género de doryctinos Iare, I. mexicanus sp. nov. y I. cheguevarai sp. nov., se describen genética y morfologicamente de un bosque tropical caducifolio en Jalisco, México. Iare belokobylskiji Marsh es registrada también para la misma región. Estas especies representan los registros más norteños en la distribución del género. Un análisis bayesiano simultaneo empleando secuencias de ADN de los genes COI y 28 S reconstruyó un árbol con las tres especies examinadas de Iare dentro de un mismo clado, aunque con un soporte bajo. Este género aparece dentro de un clado mayor representado por doryctinos del Neotrópico como grupo hermano de un subclado con las especies de los géneros Callihormius Ashmead, Leluthia Cameron, Histeromeroides Marsh and Panama Marsh.


## Introduction

The braconid wasp genus Iare Barbalho \& Penteado-Dias was recently erected within the subfamily Doryctinae to include two species from Brazil, I. rochae Barbalho \& Penteado-Dias and I. ariquemes Barbalho \& Penteado-Dias (Barbalho \& Penteado-Dias, 2002). Almost simultaneously, two additional species of this genus were described both from Costa Rica and Panama, I. belokobylskiji Marsh and I. gauldi Marsh (Marsh, 2002). Specimens of this genus were also reported in the latter work for Mexico and Bolivia, although neither species nor localities were specified for them. Species of Iare are clearly distinguishable from all other New World doryctines by the fusion of the first three metasomal tergites. This feature is only found within Doryctinae in species of two other distantly related genera, the Australian, Oriental and East Palaearctic Arhaconotus Belokobylskij and Mimipodoryctes Belokobylskij (Belokobylskij, 2000, Belokobylskij et al., 2004).

Recently, several specimens assigned to Iare were collected in the Chamela-Cuixmala Biosphere Reserve in Jalisco, Mexico, as part of a DNA barcoding study of the doryctine fauna from that region. Here we present
the descriptions of two new species of Iare and provide a new geographical record of I. belokobylskiji based on the above specimens, and reconstruct the phylogenetic relationships of the genus based on two broadly used DNA sequence markers.

## Methods

Specimens and terminology. All the specimens assigned to Iare included in this work were collected during a field trip carried out in the Chamela-Cuixmala Biosphere reserve in Jalisco, Mexico, between the 23rd and 27th of June 2009. All specimens were collected with light traps, preserved in $100 \%$ ethanol and subsequently taken to the laboratory, where they were kept at $-20^{\circ} \mathrm{C}$ until they were processed for DNA sequencing (see below) and then air-dried and mounted. The examined material is deposited in the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México (IB-UNAM CNIN), and in the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN).

The terminology employed follows Sharkey and Wharton (1997), except for the surface sculpture, which follows Harris (1979). Photographs were taken and edited with a Leica ${ }^{\circledR}$ Z16 APO-A stereoscopic microscope, a Leica ${ }^{\circledR}$ DFC295/DFC290 HD camera, and the Leica Application Suite ${ }^{\circledR}$ program. All photographs were uploaded to the Morphbank web site (www.morphbank.org).

Laboratory procedures. A single leg was removed from each of the examined specimens, placed in a 96well lysis plate and posted to the University of Guelph for DNA extraction, amplification and sequencing. A detailed description of the laboratory protocols employed is found in Smith et al. (2009). Two gene fragments were obtained to assess the phyogenetic placement of the specimens assigned to Iare: 615-658 bp of the standard DNA barcoding marker [cytochrome oxidase I mitochondrial DNA gene (COI)], and a $\sim 650 \mathrm{bp}$ fragment corresponding to the second and third domain regions of the nuclear 28 S rDNA gene. Sequences of specimens assigned to Callihormius and Panama were also included in this work. 28S primers were designed by Belshaw and Quicke (1997) (fwd: 5' GCG AAC AAG TAC CGT GAG GG 3') and Mardulyn and Whitfield (1999) (rev: 5' TAG TTC ACC ATC TTT CGG GTC CC 3'). COI amplifications were carried out using both the LepF1/LepRI (Hebert et al., 2004) (LEP-F1: 5'-ATT CAA CCA ATC ATA AAG ATA T-3'; LEP-R1: 5'-TAA ACT TCT GGA TGT CCA AAA A-3') or the LCO1490/HCO2198 (Folmer et al., 1994) (LCO: 5' GGT CAA CAA ATC ATA AAG ATA TTG G-3'; HCO: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') primers. COI sequences were edited with Sequencher version 4.0.5 (Gene Codes).

All the sequences generated for this study are deposited in GenBank (accession numbers HM434313,15, GU715218, 28, 43-52, 81, 84, HQ200864-74). These sequences will be also available in the project file 'Parasitoid Wasps (Braconidae: Doryctinae) of Chamela-Cuixmala Biosphere Reserve' (ASDOR project) in the projects section of the Barcode of Life Data System (www.barcodinglife.org).

Genetic distances and phylogenetic placement of Iare. Genetic distances and number of nucleotidic differences among the specimens assigned to Iare were calculated for the two examined markers with the $p$ uncorrected distances using PAUP version version 4.0 b 10 (Swofford, 20002). The phylogenetic placement of Iare within the Doryctinae was assessed by incorporating all the COI and 28 S sequences generated in this study into an existing alignment analysed in a previously published phylogenetic study that contains 94 specimens belonging to 64 different doryctine genera (Zaldivar-Riverón et al., 2008). COI sequences were aligned manually based on their translated aminoacids, whereas 28 S sequences were aligned based on the braconid secondary structure model proposed by Gillespie et al. (2005). All the ambiguously aligned regions of the 28 S data set were excluded from the analysis. The CO1 fragment was partitioned into first, second and third codon positions. The appropriate model of nucleic acid substitution was chosen for each partition based on the akaike information criterion using MrModeltest version 2.3 (Nylander, 2004) and PAUP version 4.0b10 (Swofford, 20002).

A bayesian MCMC partitioned analysis was run with Mrbayes version 3.1.2 (Ronquist and Huelsenbeck 2003) using the University of Oslo bioportal (http://www.bioportal.uio.no/). The bayesian analysis consisted of two simultaneous runs of 20 million generations each using four chains and default priors. Stationarity was
reached before 1 million generations, though we followed a conservative approach and built a $50 \%$ majority rule consensus tree only using the trees sampled after the last ten million generations. Clades were considered as significantly supported when they had a posterior probability ( PP ) $\leq 0.95$ (Huelsenbeck and Ronquist, 2001).

## Results and discussion

## Taxonomy

## Iare Barbalho \& Penteado-Dias

Iare Barbalho \& Penteado-Dias, 2002: 180.
Type species: Iare rochae Barbalho \& Penteado-Dias.

Diagnosis. Species ranging in size from 2.0 to 4.0 mm long; colour uniformly dark brown to black; head and mesosoma mostly coriaceous; occipital carina present and complete; antenna with 18 to 25 antennomeres; mesoscutum usually without complete notauli, foretibia with a row of spines along the anterior edge; hind coxa with a distinct, though not sharp, basoventral tooth; fore wing with the second submarginal cell closed by $\mathrm{r}-\mathrm{m}$, first subdiscal cell open apically, $\mathrm{RS}+\mathrm{Mb}$ short but distinguishable; hind wing with $\mathrm{M}+\mathrm{CU}$ slightly longer than 1 M ; metasoma not petiolated, first three metasomal tergites immovably fused; males with a stigma in the hind wing.

Distribution. New World.-Bolivia, Brazil, Costa Rica, Panama and Mexico (Barbalho \& Penteado-Dias, 2002; Marsh, 2002).

## Iare mexicanus sp. nov.

Figs 1A, 2A-D

Diagnosis. This species can be distinguished from the other species of the genus by the proportions of the mesosoma, 1.7 times longer than high and slightly tapering to rear, with the scutellum slightly convex. In other species of the genus the mesosoma is at least 2.0 times longer than high and not tapering to rear, and with the scutellum distinctly flat.

Description. Female. Body length 2.5-2.7 mm. Head: 1.7-2.0 times wider than median length. Occipital carina complete and joining hypostomal carina before mandible. POL 1.3 times Od, 0.4 times OOL. Eye 1.2 times longer than wide, transverse diameter of eye 1.6-1.7 times longer than temple (dorsal view). Malar space 0.5 times eye height. Width of hypoclypeal depression $0.8-1.0$ times distance from edge of depression to eye. Antenna filiform, with 19-21 antennomeres. First flagellar segment 3.8-4.0 times longer than wide, 1.2-1.3 longer than second segment. Penultimate segment 1.5-2.0 times as longer than wide, 0.5 times as long as first segment.

Mesosoma: 1.7 times longer than high, 1.6-1.7 times longer than wide; higher anteriorly and slightly tapering to rear in lateral view. Mesoscutum 0.7 times as long as wide and uniformly rounded. Notauli almost completely absent, barely distinguishable on anterior edge of mesoscutum. Prescutellar depression with four transverse carinae. Sternaulus about 0.5 times as long as mesopleuron.

Wings:Fore wing with pterostigma about as long as R. Vein r 0.8 times as long as $3 \mathrm{RSa}, 0.3$ times as long as 3 RSb , and 0.6 times as long as 2 RS . ( $\mathrm{RS}+\mathrm{M}$ ) a slightly sinuate. Discal cell 2.3 times longer than wide.

Legs: Hind coxa with distinct basoventral tooth. Hind femur 2.8 times longer than wide; hind tibia 5.3 times longer than wide and 2.7 times longer than basitarsus.

Metasoma: Almost as long as head and mesosoma combined (Fig. 2A). First tergite wider than long, about 0.7 times as long as its apical width; second tergite 0.4 times as long as wide and 1.3 times longer than third tergite. Ovipositor sheath 0.7 times as long as metasoma.


FIGURE 1. A. Iare mexicanus (holotype, female; IB-UNAM CNIN, voucher no. ASDOR033); B. Iare cheguevarai (female, IB-UNAM, voucher no. ASDOR028). Habitus of female, lateral view.

Sculpture and pubescence: Head mostly coriaceous except malar space, which is almost smooth, and median and lower face, which is weakly rugulose. Pronotum coriaceous, pronotal furrow scrobiculate, mesoscutum strongly coriaceous, notauli absent, only indicated by a few rugose sculptures on antero-lateral edges of mesoscutum; scutellum coriaceous. Mesopleuron strongly and uniformly coriaceous; subalar groove and sternaulus scrobiculate. Metapleuron coriaceous, turning weakly rugose on posterior edge. Propodeum with basolateral areas coriaceous; areolar carinae present, enclosing areola crossed by several carinae. Coxae mostly smooth except hind coxa, which is dorsally coriaceous; femora and tibiae coriaceous. First metasomal tergite with median area clearly delimited by carinae, anterior surface mostly coriaceous, turning striate apically. First three metasomal tergites longitudinally striate (except anterior area of first tergite), with coriaceous sculpture between ridges, third tergite turning smooth on posterior edge. Remaining tergites coriaceous, turning smooth on posterior edge.

Male: Unknown.

## Biology. Unknown.

Etymology. the specific name refers to Mexico, country were the species was collected.
Material examined. Holotype (IB-UNAM CNIN): Voucher no. ASDOR033 (Cham-033), female, Mexico, Jalisco, Estación de Biología de Chamela, UNAM, near lab, $19.49814 \mathrm{~N}-105.0444 \mathrm{~W}, 95 \mathrm{~m}, 23-24$ June 2009, light trap, tropical dry forest. Hans Clebsch, Alejandro Zaldívar-Riverón, Andrew Polaszek collectors. Paratype (IB-UNAM CNIN): ASDOR030 (Cham-030), female, same data as holotype.


FIGURE 2. Iare mexicanus (holotype, female; IB-UNAM CNIN, voucher no. ASDOR033). A) head, frontal view; B) mesosoma, lateral viev; C) mesoscutum, dorsal view; D) first three metasomal tergites, lateral view.

## Iare cheguevarai sp. nov.

Figs 1B, 3A-D

Diagnosis. This species is similar to I. gauldi Marsh. It can be distinguished from the other species of the genus by the sinuate transverse furrow on the second metasomal tergite, which is placed close to its anterior margin and is sometimes only very weakly indicated, and by the median longitudinal furrow on posterior edge of mesoscutum.

Description. Female. Body length $2.5-2.7 \mathrm{~mm}$. Head: 1.8 times wider than median length. POL 1.3-1.5 times Od, $0.4-0.5$ times OOL. Eye 1.1-1.2 times longer than wide, transverse diameter of eye 2.0 longer than temple (dorsal view). Malar space 0.4 times eye height. Width of hypoclypeal depression $0.9-1.1$ times distance from edge of depression to eye. Antenna filiform, with 20 antennomeres. First flagellar segment 3.84.0 times longer than wide, $1.0-1.1$ times longer than second segment. Penultimate segment 2.5 times longer than wide, 0.7 times as long as first segment.

Mesosoma: 2.0 times longer than high, 1.7 times longer than wide. Mesoscutum 0.7 times as long as wide and uniformly rounded. Notauli barely distinguishable on antero-lateral edge of mesoscutum as scrobiculate sculptures, and very weakly indicated by two furrows on dorsal surface of mesoscutum, only visible at some angles of light. Prescutellar depression with five transverse carinae. Sternaulus about 0.6 times as long as mesopleuron.

Wings: Fore wing with pterostigma about as long as R. Vein r $0.8-0.9$ times as long as 3 RSa , $0.3-0.4$ times as long as 3 RSb , and 0.7 times as long as 2 RS . Vein (RS+M) a slightly sinuate. Discal cell 2.0-2.1 times longer than wide.

Legs: Hind coxa with distinct basoventral tooth. Hind femur 2.6 times longer than wide; hind tibia 4.0 times longer than wide and 2.8 times longer than basitarsus.

Metasoma: Almost as long as head and mesosoma combined. First tergite wider than long, 0.7 times as long as its apical width; second tergite 0.3 times as long as wide and 1.1 times longer than third tergite. Ovipositor sheath 0.8 times as long as metasoma.

Sculpture and pubescence: Head mostly coriaceous except malar space, which is almost smooth. Pronotum coriaceous, pronotal furrow scrobiculate. Mesoscutum strongly coriaceous, with a single median impression on posterior edge; notauli absent, only indicated by a few rugose sculptures on antero-lateral edges of mesoscutum, which continue as two very weak furrows barely visible at some angles of light. Scutellum coriaceous. Mesopleuron strongly and uniformly coriaceous; subalar groove and sternaulus scrobiculate. Metapleuron coriaceous, turning weakly rugose on posterior edge. Propodeum with basolateral areas coriaceous; areolar carinae present, enclosing median areola crossed by several carinae. Coxae mostly smooth, mid and hind coxae dorsally coriaceous. Hind femur slightly coriaceous, turning smooth ventrally. First metasomal tergite with median area clearly delimited by carinae; median area mostly coriaceous, turning striate on posterior edge, lateral areas striate; second tergite longitudinally striate, occasionally with weak granular sculpture between striae, with a transverse and sinuate furrow very close to anterior margin of tergite on lateral areas; third tergite mostly coriaceous, striate on anterior edge; remaining tergites coriaceous, turning smooth on posterior edge. Head except eyes, mesoscutum, and pronotum covered by short, erect setae. Mesopleuron glabrous medially. Propodeum and metapleuron sparsely setose. Hind tibia with short semi-erect setae, more dense ventrally. Metasoma with first and second tergites with sparsely and uniformly distributed short setae, remaining tergites mostly glabrous, only with a transverse row of sparse setae subapically. Ovipositor sheath uniformly covered by long, erected setae.

Male: Similar to female. Antennae with 18 antennomeres. Hind wing with pterostigma at junction of $\mathrm{SC}+\mathrm{R}$ and $\mathrm{r}-\mathrm{m}$.


FIGURE 3. Iare cheguevarai (holotype, female; IB-UNAM CNIN, voucher no. ASDOR028). A) head, frontal view; B) mesosoma, lateral viev; C) mesoscutum, dorsal view; D) first three metasomal tergites, lateral view.

Biology. Unknown.
Etymology. this species was named in honour of the Argentine revolutionary Ernesto "Che" Guevara.
Material examined. Holotype (IB-UNAM CNIN): voucher no. ASDOR028 (Cham-028), female, Mexico, Jalisco, Estación de Biología de Chamela, UNAM, near lab, 19.49814 N-105.0444 W, 95 m, 23-24 June 2009, light trap, tropical dry forest. Hans Clebsch, Alejandro Zaldívar-Riverón, Andrew Polaszek collectors. Paratypes (IB-UNAM CNIN, MACN): ASDOR020, ASDOR021, ASDOR027, ASDOR096 (Cham-020, Cham-021, Cham-027, Cham-096, respectively), two females, three males, same data as holotype.

## Iare belokobylskiji Marsh

Iare belokobylskiji Marsh, 2002: 108.
Our phylogenetic analysis recovered three main clusters with specimens assigned to Iare, two of which belong to the species described above (fig. 4). We compared the specimens of the third recovered cluster with some of the paratypes of $I$. belokobylskiji and we were not able to recognise fixed differences between them. We have therefore decided to assign the specimens of this cluster to the latter species until additional morphological and molecular information is gathered. Iare belokobylskiji was previously known only from Costa Rica and Panama (Marsh, 2002), and thus the below specimens represent the first records of the species for Mexico.

Material examined. five females, two males, Mexico, Jalisco, Estación de Biología de Chamela, UNAM, near lab, 19.49814 N-105.0444 W, $95 \mathrm{~m}, 23-24$ June 2009, light trap, tropical dry forest. Hans Clebsch, Alejandro Zaldívar-Riverón, Andrew Polaszek collectors. Voucher numbers (IB-UNAM CNIN, MACN): ASDOR022, ASDOR023, ASDOR025, ASDOR026, ASDOR029, ASDOR031, ASDOR035 (Cham-023, Cham-022, Cham-025, Cham-026, Cham-029, Cham-031, Cham-035, respectively).

## Genetic distances and phylogenetic placement of Iare

Specimens of each of the three examined species of Iare share a same 28 S haplotype except one sequence of I. cheguevarai, which has one nucleotide difference ( $0.28 \%$ ) with respect to the remaining sequences of the species. Intraspecific $p$ uncorrected distances for COI ranged from 0 to $6 \mathrm{bp}(0$ to $0.96 \%)$ in the three species except for the distances of one of the sequences of I. belokobylskiji (ASDOR031), which varied between 48 and $49 \mathrm{bp}(7.3-7.4 \%)$. We carefully checked this COI sequence and did not detect any mistake. Since this specimen is morphologically indistinguishable from the other specimens assigned to $I$. beolokobylskiji and it has their same 28 S haplotype, we have therefore decided tentatively to assign it to the latter species. Interespecific distances among the three species ranged from 8 to 16 (1.4 to $4.1 \%$ ) and from 47 to 71 (7.1 to $10.8 \%$ ) bp for the 28 S and COI markers, respectively.

The COI +28 S Bayesian analysis (fig. 4) recovered all the specimens assigned to Iare within a single clade, though with low PP support (0.36). Three significantly supported clusters were recovered within the above clade, which correspond to I. mexicanus, I. cheguevarai and the specimens assigned to I. belokobylskiji, respectively. The clade with the specimens of Iare is nested within a large Neotropical clade that was also recovered in Zaldívar-Riverón et al.'s (2008) study. The sister group of Iare was a clade represented by the species of Callihormius Ashmead, Leluthia Cameron, Histeromeroides Marsh, and Panama Marsh. Interestingly, Marsh (2002) pointed out that Iare might be related to Callihormius based on the absence of notauli and general habitus in both genera.

## Key to described species of Iare

1. Notauli present and distinguishable along the entire length of mesoscutum
I. ariquemes Barbalho \& Penteado-Dias

- Notauli absent posteriorly, indicated only as a few rugae on anterolateral areas of mesoscutum, sometimes also very poorly indicated furrows only distinguishable at some angles of light. . 2

2. Mesoscutum with a posteromedian furrow. $\qquad$ I. cheguevarai sp. nov.

- Posteromedian area of mesoscutum entirely coriaceous, without a median furrow. .3

3. Mesosoma 1.7 times longer than high and slightly tapering to rear ........................................... I. mexicanus sp. nov.

- Mesosoma 1.8 to 2.1 times longer than high, with dorsal and ventral surfaces approximately parallel. $\qquad$

4. Second metasoma tergite with a straight transversal furrow
I. gauldi Marsh

- Second metasoma tergite with a sinuate transversal furrow........................................................................................ 5

5. Ovipositor as long as metasoma, species from Brazil .........................................I. rochae Barbalho \& Penteado-Dias

- Ovipositor about 0.5 times as long as metasoma, sometimes shorter, species known from Central America and Mexico.
I. belokobylskiji Marsh


FIGURE 4. Bayesian phylogram ( 20 million generations, two simultaneous runs, 10,000 sampled trees per run) showing the phylogenetic relationships recovered for the species of Iare included in this study. Black circles below nodes represent clades that are significantly supported (posterior probability $\leq 0.95$ ).

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