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Informe Escrito Final

Sistemática, evolución y conservación de los onicóforos de la Cordillera Volcánica Central de Costa Rica

Proyecto de graduación presentado como requisito parcial para optar al grado de Licenciatura en Biología con énfasis en Manejo de Recursos Naturales

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RESUMEN

El género Peripatidae posee una limitada diversidad morfológica, la cual ha sido un obstáculo para establecer una adecuada taxonomía. El uso de análisis de ADN, en lugar de ayudar a resolver este problema, hizo surgir nuevas preguntas sobre la validez de los géneros existentes, principalmente en los peripatidos neotropicales (Neopatida). En el caso de Costa Rica, las especies descritas necesitan una urgente revisión taxonómica, haciendo uso de tanto metodologías en genética como en morfología. En este estudio analizamos dos especies de la Cordillera Volcánica Central de Costa Rica y proponemos un nuevo género: *Picadopatus*, el cual es identificado por poseer un tubérculo nefridial que indenta tanto la tercera como la cuarta almohadilla de las patas cuatro y cinco; tubérculos crurales pareados unidos por un pliegue de piel, los cuales poseen un ápice con escamas de textura suave y una sola abertura; glándulas anales pareadas con sus aberturas curvadas en la parte distal y rectas en la proximal; y por sus secuencias de ADN. También re-describimos ambas especies que estaban erróneamente asignadas a *Epiperipatus* (*P.biolleyi*) y *Peripatus* (*P. ruber*); estas se distinguen por la forma de ambas papilas de cráter, la forma de sus estructuras interpedales y de las escamas de las papilas primarias. En este estudio demostramos la presencia de una baja variabilidad morfológica, característica de especies crípticas, que solo pueden ser identificadas utilizando caracteres morfológicos puntuales y análisis genéticos. La localidad muestreada es habitada por dos especies, separadas por apenas 10 kms, por lo tanto debería de considerarse esta como un hábitat que promueve la especiación, donde el endemismo de corto rango está presente y con un valor evolutivo. Revisiones similares se requieren en todas las especies de Neopatida, para elucidar la correcta taxonomía de la familia y descubrir sitios que promueven procesos de especiación.

Palabras clave: Filogenética, Onychophora, redescripción de especies, especies crípticas, conservación de invertebrados.

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ABREVIATURAS

UCR	Universidad de Costa Rica
UNA	Universidad Nacional de Costa Rica
SINAC	Sistema Nacional de Áreas de Conservación
NCBI	National Center for Biotechnology Information
DNA	Deoxyribonucleic Acid
COI	Cytochrome c oxidase subunit I
BR	Bajo la Rosa
CA	Cascajal
LJ	Los Juncos
PA	Paja de Agua
RR	Rancho Redondo
SP	San Pedro
MAFFT	Multiple Alignment using Fast Fourier Transform
G-INS-i	Globalpair maxiterate 1000 Alignment
E-INS-i	Genafpair maxiterate 1000 Alignment
OsO_4	Tetróxido de Osmio
MZUCR	Museo de Zoología de la Universidad de Costa Rica
TA	Tip of the antennae
BA	Antennae's base
MO	Unpaired-lipped mouth
SP	Slime papillae
FO	First foot
N	Number

Capítulo 1.

MARCO TEÓRICO.

Pocos invertebrados han llamado la atención de tanto científicos como del público en general como los onicóforos (mal llamados gusanos de terciopelo o peripatos), sus características biológicas los han hecho blanco de numerosas investigaciones en diversos ámbitos (Podsiadlowski, Braband & Mayer, 2008; Sampaio-Costa, Chagas-Junior & Baptista, 2009; Braband et al. 2010a, 2010b; Brito et al. 2010; Rota-Stabelli et al. 2010; Lacorte, Oliveira & Fonseca, 2011; Chagas-Júnior & Sampaio-Costa, 2014), es importante recalcar que por su historia de vida (que los restringe a ambientes con alto nivel de humedad) presentan un endemismo muy marcado (Oliveira et al. 2011; Cunha et al. 2017), adicionalmente son considerados como fósiles vivientes, cuyos ancestros han sido documentados en el periodo cámbrico en China y Canadá (Monge-Nájera & Hou, 1999). El filo Onychophora presenta características primitivas de gusanos, como Nematoda y Nematomorpha, mezcladas con algunas propias de artrópodos (Grimaldi & Engel, 2005), incluso llegando a presentar estructuras cerebrales que permiten comportamientos sociales propios de los artrópodos más avanzados (Reinhard & Rowell, 2005). Los onicóforos actuales se clasifican en dos familias, Peripatopsidae, propia de zonas templadas y Peripatidae de zonas tropicales, en estos lugares se pueden encontrar en troncos en descomposición, paredones de tierra cerca de quebradas, bajo las rocas, en cavernas, hojarasca e incluso entre las bromelias (Picado 1911; Carvalho, 1941; Monge-Nájera, 1995) y briófitos arbóreos (Zitani et al. 2018). Dicha distribución geográfica antecede la ruptura del súper continente Pangea, una vez que ocurrido dicho evento (Murienne, Daniels, Buckley, Mayer, & Giribet, 2014).

El concepto biológico de especie ha sido el más utilizado en biología de poblaciones, genética evolutiva y biología de conservación. Esta define una especie como un grupo de individuos o poblaciones naturales que actualmente o potencialmente pueden reproducirse entre ellos, que no pueden entrecruzarse con individuos de todos los otros grupos (Simpson 1961; Mayr 1963). Reconoce que los individuos pueden intercambiar alelos, lo cual la hace muy útil para delinear especies genéticamente. Las especies surgen de dos formas principalmente: por diversificación, donde una

especie anterior da origen a dos o más especies descendientes, esto ocurre cuando las poblaciones se diferencian genéticamente, y se aíslan reproductivamente; y cuando ocurre un cambio gradual en un linaje en el tiempo, de modo que una especie previa se nombra como una especie diferente tiempo después (Patterson et al. 2006). La especiación puede también ocurrir cuando ocurre una hibridización entre especies diploides existentes lo cual permite la adaptación a un nuevo ambiente, como ha sido documentado en plantas e insectos (Rieseberg et al., 2003; Schawarz et al., 2005; Mavárez et al., 2006). Los factores de aislamiento físico usualmente son el resultado de cambios geográficos (levantamiento de montañas, desertificación, división de ríos, cambios en el nivel del mar y deriva continental) o de la propagación de organismos en nuevos territorios. Si la población aislada se hace tan distinta genéticamente que no puede entrecruzarse cuando ocurre un contacto secundario, entonces se le llama especiación alopátrica. Esta es la forma más común de especiación en los animales (Coyne & Orr 2004; Gavrilets 2004). Las poblaciones simpátricas comparten la misma distribución geográfica, llegando en casos a solaparse. De acuerdo al concepto biológico de especie, las poblaciones simpátricas de la misma especie podrían intercambiar alelos, mientras que especies distintas que comparte la misma área geográfica no podrían. Consecuentemente si algún marcador genético muestra falta de intercambio entre linajes, dos poblaciones simpátricas pertenecientes a distintas especies se habrían identificado, pero se requieren varios loci para tales diagnósticos, de modo que se refleje la historia evolutiva de varios genes. Por ejemplo onicóforos hallados del mismo tronco en las Montañas Azules en Sidney, Australia, que eran morfológicamente idénticos, en realidad presentaban diferencias de un 86% de 21 loci de aloenzimas, por lo que constituían especies distintas (Briscoe & Tait 1995).

El ADN mitocondrial es uno de los marcadores genéticos más usados para delimitar taxas. Sin embargo son de herencia materna, por lo que sus diferencias podrían ser producto de falta de dispersión de las hembras, por lo que patrones en distintas poblaciones pueden ser engañosos, ya que son resultado de la selección o polimorfismo de especies ancestrales (Nei 1996). Se debe utilizar ADN ribosomal mitocondrial codificante combinado con otros genes, preferiblemente el marcador nuclear 18S que es muy conservado, para la delimitación adecuada del estatus taxonómico.

Tradicionalmente se han utilizado los árboles filogenéticos como una medida de la diferencia evolutiva entre especies, lo cual es muy útil a la hora de justificar la asignación de recursos de conservación e investigación (Crozier, Dunnett, & Agapow, 2005). Los árboles filogenéticos pueden construirse utilizando datos morfológicos y genéticos (Avise 2000; Nei & Kumar 2000). Un gran número de métodos estadísticos están disponibles para realizar dichos estudios incluyendo métodos como la distancia entre matrices (UPGMA), máxima parsimonia, máxima verosimilitud y métodos bayesianos (Nei & Kumar 2000; Felsenstein 2004; Hall, Iltis, & Sytsma, 2004). El método de máxima parsimonia simplemente construye un árbol basado en el mínimo número de cambios, sin importar cuales cambios sean estos. Estos métodos generalmente arrojan árboles confiables y congruentes si hay suficiente información disponible (Nei 1996). Las especies del neotrópico han sido poco estudiadas, principalmente debido a lo difícil que resulta encontrarlos, estudiarlos y principalmente identificarlos (Read, 1988). Respecto a este punto la morfología no ha resultado ser la herramienta precisa que se esperaba, ni siquiera los análisis de ADN más recientes han logrado solventar las dudas acerca de las especies o géneros (Van der Lande, 1991; Giribet et al. 2018)

La determinación de especies de onicóforos es problemática debido a la presencia de variaciones intraespecíficas en morfología (Ruhberg, 1985; Hamer, Samways & Ruhberg, 1997). Cuando se han sometido a un escrutinio sistemático, se ha revelado una diversidad taxonómica considerable (Reid, 1996, 2000a, b; Ruhberg & Hamer, 2005; Daniels et al. 2009; Cunha et al. 2016; Giribet et al. 2018). La limitación de los caracteres taxonómicos convencionales (como el número de pares de patas, patrones del tegumento dorsal, forma y número de dientes, etc.) ha sido resaltada, al ser infructuosas para diferenciar linajes como en el caso del género *Peripatopsis* (Daniels et al., 2009); otras características como la coloración resultó en un moderado éxito para esta tarea (Daniels & Ruhberg 2010). Específicamente en las especies del Neotrópico, al analizar los caracteres descritos en investigaciones recientes (Oliveira et al. 2012) se podría clarificar la diversidad y filogenia de algunos géneros de onicóforos (Oliveira et al., 2011) Los esfuerzos taxonómicos son importantes considerando el alto nivel de endemismo entre estos invertebrados, promoviendo un mayor esfuerzo de conservación para estas especies (Reid, 1996, 2000a, b; Trewick, 1998, 1999, 2000; Daniels et al.

2009). Existe una diversidad de caracteres que no ha sido explorada suficientemente en Onychophora y que podría resultar ser útil para resolver las incertidumbres taxonómicas (Oliveira et al. 2012). Por ejemplo, en Australia una especie que se creía poseía una amplia distribución, resultó en su lugar, ser varias especies. Esta situación demuestra la necesidad de estudios paralelos en otras regiones del mundo para considerar si variaciones similares ocurren en otros lugares (Tail et al. 1990).

Debido a su alta dependencia de la humedad, los onicóforos son considerados como especies vulnerables, clasificación que abarca varios rangos de potenciales asignaciones de algunas especies a diferentes categorías de la UICN (Wells et al. 1983). Los onicóforos normalmente ocupan hábitats susceptibles a alteraciones antropogénicas; sus poblaciones parecen ser pequeñas o de poca densidad (a excepción de algunas especies australianas); como ya se mencionó muchos están restringidos a localidades puntuales (endémicos); también comparten sus hábitats con invertebrados poco estudiados y con características de vida similares, como arañas, escarabajos, platelmintos de vida libre, cucarachas silvestres, grillos, entre otros; y al ser depredadores de muchos de estos animales anteriormente mencionados, su presencia se relaciona con poblaciones saludables de presas. Estos factores, aunados a su atractiva apariencia y haber sido utilizada como especie bandera (Sato et al., 2018; Barnes & Daniels, 2018), reflejan la importancia de conservación del filo (New, 1995).

La falta de resiliencia de los onicóforos a ambientes severos y sus pequeñas poblaciones refuerzan la necesidad de preservar sus hábitats, este esfuerzo podría desencadenar una amplia conservación de ecosistemas, fundada por requerimientos ecológicos precisos de fauna local y su importancia para mantener los procesos ecológicos (Vasconcellos, 2006), conjeturas sobre la respuesta de Onychophora a la urbanización puede limitar el ámbito de los estudios y la conservación de los peripatos, considerando la actual y futura expansión de las ciudades (Heilig, 2012). Investigar y manejar las poblaciones de onicóforos, inclusive en áreas improbables y perturbadas también es importante para la conservación de este filo vulnerable (Barrett et al., 2016), es especialmente relevante extender este tipo de estudios a las especies del Neotrópico, en este caso las especies de Costa Rica representan una oportunidad inigualable para realizarlos. Para este trabajo de graduación

se investigó la sistemática de los onicóforos de la Cordillera Volcánica Central de Costa Rica desde una perspectiva morfológica y molecular, se definió la distribución de las especies, para la toma de decisiones respecto a su manejo y conservación (Barquero-González, Villalobos-Brenes, Monge-Nájera & Morera-Brenes, 2019), adicionalmente se presenta la primer nota corta sobre el estudio de comportamiento de las especies del país (Barquero-González, Vega-Hidalgo & Monge-Nájera, 2019).

Capítulo 2.

SISTEMÁTICA, EVOLUCIÓN Y CONSERVACIÓN DE LOS ONICÓFOROS DE LA CORDILLERA VOLCÁNICA CENTRAL DE COSTA RICA

Systematics, evolution and conservation of two cryptic Onychophoran species from the Costa Rican Central Volcanic Mountain Range

INTRODUCTION

Onychophorans are amongst the most fascinating terrestrial invertebrates, they called the attention of investigators due to their extraordinary biology (Podsiadlowski, Braband & Mayer, 2008; Sampaio-Costa, Chagas-Junior & Baptista, 2009; Braband et al. 2010a, 2010b; Brito et al. 2010; Rota-Stabelli et al. 2010; Lacorte, Oliveira & Fonseca, 2011; Chagas-Júnior & Sampaio-Costa, 2014) that includes a high endemism (Oliveira et al. 2011; Cunha et al. 2017), the presence of feeding hierarchies (Reinhard & Rowell, 2005), the capacity to expel a self-assembled proteinaceous adhesive net (Bouvier, 1905; Haritos et al. 2010; Concha et al. 2015; Corrales-Ureña et al. 2017; Baer et al. 2018) and that are considered as living fossils, since they inhabit the planet since 300 million years ago (Garwood et al. 2016). Such invertebrates live in moist microhabitats as leaf litter, moss, rotten logs, caves, bromeliads (Picado, 1911; Carvalho, 1941; Monge-Nájera, 1995) and arboreal bryophytes (Zitani et al. 2018); and present marked biogeographic patterns (Monge-Nájera, 1995).

These patterns explain the distribution of the two families of the phylum: the temperate distributed Peripatopsidae and the pantropical Peripatidae (Brinck, 1957; Ruhberg, 1985; Reid, 1996; Mayer, 2007; Allwood et al. 2010; Braband et al. 2010). Neotropical species have been assigned to the subfamily Neopatida (*sensu* Oliveira et al. 2016). Within Neopatida, the Central American species are the lesser known, except the ones that inhabit Costa Rica, even so taxonomic uncertainties occurs (Barquero-González et al. 2016a; Barquero-González et al. 2016b).

The known low morphological diversity among taxa has been recognised as one main obstacle for Onychophoran taxonomy. In such context, the Scanning Electron Microscopy (SEM) arose as a promising useful technique for the revision of the New World Peripatidae (Read 1988a; Oliveira et al. 2012a, 2014, 2018). Up to this moment, SEM has shown us remarkable images of the external morphology of some few species, but it has not help to resolve the taxonomy in a convincing way. For example, it is still impossible to recognize between both genera *Peripatus* and *Epiperipatus*; Read (1988a, 1988b) studied the primary papillae apical piece of several species (mainly of the Caribbean Islands), examined specimens of *Oroperipatus* and *Peripatus* had more than three scale ranks in the primary papillae apical piece, while *Epiperipatus*, *Macroperipatus* and others had three or fewer scale ranks, but that distinction may be valid only to the studied species. Since then not a single revision of the genus *Peripatus* has been done, while only one revision of the genus *Epiperipatus* has been published (Sampaio-Costa, Chagas-Junior & Pinto-da-Rocha, 2018), but had not contributed much in that sense, and instead have contradicted Read's (1988a) findings; moreover the new diagnosis characters proposed for the genus allows to allocate almost every known species within it, making taxonomic confusion even higher. SEM has been undoubtedly useful only to recognize *Plicatoperipatus* (from Jamaica), and *Macroperipatus* (from Trinidad), both insular genera and both supposedly monotypical. Thirty years later, a sound Onychophoran systematics based on morphology (Ruhberg, 1985), or even a simple distinction between neopatids, remains elusive.

The problem now is even more complex, because the discovery of cryptic species in South African genera *Peripatopsis* (Daniels et al. 2009; Daniels & Ruhberg, 2010; Daniels, McDonald & Picker, 2013; Ruhberg & Daniels, 2013; Myburgh & Daniels, 2015) and *Opishtopatus* (Daniels et al. 2016; Daniels, 2011; Ruhberg & Hamer, 2005), in Brazilian genus *Epiperipatus* (Oliveira et al. 2011; Cunha et al. 2017), Australian (Briscoe & Tait, 1995; Sato et al. 2018) and New Zealand genus *Peripatoides* (Trewick, 1998,1999, 2000); the life history of onychophorans is constrained by a low vagility and dependence on high humidity habitats, which favors the presence of cryptic speciation in wide distributed taxa (Daniels et al. 2008); in these cases species are only identified by genetic analyses or detailed gross morphology, given their low character variation. Giribet et al. (2018) recent

genetic analysis showed that most peripatids are not correctly classified; this includes the inhabitants of Costa Rica.

An appropriate systematic based on taxonomic identification and the definition of the localities where neopatids live in Costa Rica, is the first step to develop appropriate conservation policies for these invertebrates (Wells, Pyle & Collins, 1983; New, 1995; Barrett et al. 2016) and to discover the occurrence of speciation hotspots (Sato et al. 2018). The starting point of this process is the redescription of the known species and their habitats; amongst the first ones described in the country are *Epiperipatus biolleyi* (Bouvier, 1902) and *Peripatus ruber* (Furhmann, 1913); considered as two different species of different genera. We hypothesized that due to the proximity of the type localities, similar coloration and the morphological stasis on both species, they must constitute cryptic members of the same genus. In this study we erect a new onychophoran genus endemic to Costa Rica: *Picadopatus,* we redescribed *P. biolleyi* and *P. ruber,* and evaluate the importance of their habitat for invertebrate speciation and conservation.

MATERIALS AND METHODS

TABLE 1
Geographic coordinates for the sampled specimens of *Picadopatus*.

Locality	Coordinates
Alto la Palma*	10° 2'44.39" N and 83°59'11.74" W
Bajo la Rosa "BR"	10° 1'10.96" N and 83°56'58.46" W
Cascajal "CA"	10° 1'14.39" N and 83°56'51.73" W
La Julieta	9°59'30.20" N and 83°57'9.67" W
La Julieta "b"	9°59'29.47" N and 83°57'4.28" W
Los Juncos "LJ"	10° 1'27.46" N and 83°56'30.29" W
Paja de Agua "PA"	10° 0'59.42" N and 83°56'50.76" W
Rancho Redondo "RR"*	9°57'47.20" N and 83°56'33.14" W
San Pedro "SP"	10° 0'10.69" N and 83°57'59.26" W

*=Specimens from the types localities.

Study sites:

We surveyed from the year 2016 to 2018, the type and nearby localities on the districts of Moravia and Vasquez de Coronado, San José, Costa Rica, in search of *Epiperipatus biolleyi* and *Peripatus ruber* (Table 1). Specimens were collected with SINAC's licenses (SINAC-SE-CUSBSE-

PI-R-133-2016 and SINAC-SE-CUSBSE-PI-R-015-2017) and we mapped their geographic coordinates (Fig 1).

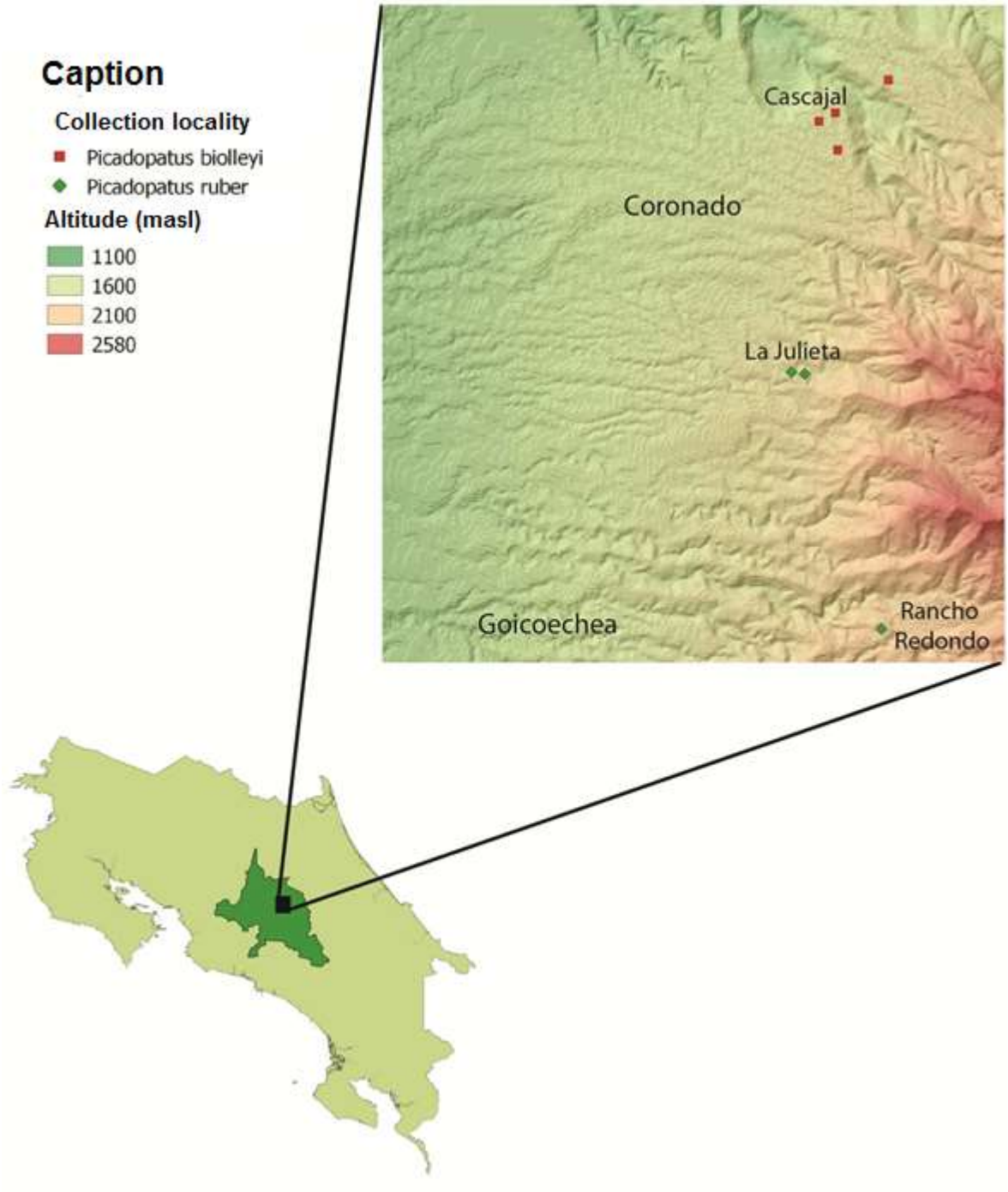

Fig. 1. Map of Costa Rica illustrating the locality of the studied *Picadopatus* species.

DNA sequences:

Corresponding sequences of cytochrome oxidase I (COI) and 12S sequences of three localities from studied specimens were generated and donated by colleagues from Universidad de Costa Rica (UCR) on February 2017; other sequence data of representative species (Oliveira, Read & Mayer, 2012b) from some Latin American countries were acquired from GeneBank (Morera-Brenes & Monje-Nájera, 2010; Oliveira et al. 2012b; Murienne et al. 2014; Giribet et al. 2018). We created two data matrices: one consisted in 53 species of Neopatida plus the closest representative of the subfamily *Mesoperipatus tholloni* (Giribet et al., 2018) as outgroup (Peripatidae matrix), bearing 53 sequences of COI, complemented with the following ones: 12S (45 species), 16S (35 species) and 18S (34 species); the second matrix only contained Neopatida species (Neopatida matrix) due to the long branches between *Mesoperipatus* and them (Giribet et al. 2018), comprising 52 species sequences of COI, 12S (44 species), 16S (34 species) and 18S (33 species). Sequences were aligned in the MAFFT web server (Kuraku et al. 2013; Katoh, Rozewicki & Yamada, 2017), the G-INS-i strategy for COI, 12S and 16S genes were used, while for the 18S gene the E-INS-i strategy was chosen (Sato et al., 2018). Each gene alignment was trimmed using Gblocks web server (Castestrana, 2000). Default parameters settings for DNA global alignment with free end gaps were used. Sequences were concatenated using SequenceMatrix 1.8 (Vaidya et al. 2011). Partitions were found with Jmodeltest 2.1.10 (Posada, 2008) according to the used genes (Table S1).

TABLE 2
Taxon IDs with Genbank collection accession numbers for studied specimens.

Species	Catalog no.	Region	COI	12S	16S	18S
*Epiperipatus adenocryptus**	ONY-SBL011	Brazil	HQ236114	HQ236140	-	-
*Epiperipatus adenocryptus**	ONY-SBL008	Brazil	JN564575	-	-	-
Epiperipatus acacioi	ONY-ITA001 - MNRJ:0044	Brazil	HQ404904	HQ404922	MG973517	MG973554
Epiperipatus acacioi	Tripui1	Brazil	HQ655588	HQ404920	-	-
Epiperipatus cf. edwardsii	MCZ 131427	French Guiana	MH107336	MG973702	MG973468	MG973561
Epiperipatus edwardsii	GF180312HC003-06 - MCZ:141306	French Guiana	HG531958	HG531961	HG531962	MG973542
*Epiperipatus diadenoproctus**	ONY-MDS010	Brazil	HQ236095	HQ236121	-	-
Epiperipatus paurognostus	UFMT	Brazil	MH107346	MG973696	MG973516	MG973553
Epiperipatus sp.	MZUSP 0090	Brazil	MH107338	MG973653	MG973479	MG973550
Epiperipatus sp.	MCZ 131428	Colombia	MH107352	MG973706	MG973525	MG973590
Epiperipatus sp.	MCZ 131430	Colombia	MH107354	MG973708	MG973527	MG973592
Epiperipatus sp.	MZUP 0112	Panama	MH107360	MG973684	MG973490	MG973577
Epiperipatus sp.	MZUP 0111	Panama	MH107359	MG973683	MG973489	MG973576
Epiperipatus sp.	UNACHI	Panama	MH107365	MG973685	MG973491	MG973578
Epiperipatus vagans	MZUSP 0114	Panama	MH107350	MG973665	MG973484	MG973547

Epiperipatus vagans	MZUSP 0101	Panama	MH107349	MG973663	MG973482	MG973544
Epiperipatus vagans	MZUSP 0113	Panama	MH107348	MG973664	MG973483	MG973545
Epiperipatus vagans	MZUSP 0115	Panama	MH107347	MG973666	MG973485	MG973546
Macroperipatus valerioi	MCZ 130841	Costa Rica	MH107341	MG973681	MG973496	MG973558
Macroperipatus valerioi	MCZ 130842	Costa Rica	MH107342	MG973682	MG973497	MG973569
Macroperipatus torquatus	MCZ 143928	Trinidad	MH107344	MG973699	MG973504	MG973562
Oroperipatus eisenii	MCZ 71297	Mexico	MH107369	MG973712	MG973531	-
Oroperipatus sp	MCZ 43394	Galapagos	MH107372	MG973716	MG973532	MG973603
Oroperipatus sp	MNRJ 0069 - MCZ131387	Ecuador	MH107368	MG973711	MG973530	MG973604
Peripatus solorzanoi (type)	PE11 (red)	Costa Rica	KM095130	KM095128	-	-
Peripatus solorzanoi	PE12	Costa Rica	KM095131	KM095129	-	-
Peripatus solorzanoi	MCZ 130840	Costa Rica	-	MG973679	MG973486	MG973580
Peripatidae sp.	MZUSP 0019	Brazil	MH107356	MG973655	MG973478	MG973548
Peripatus sp.	MCZ 46445	Guyana	MH107337	MG973703	MG973470	MG973583
Peripatidae sp.	MCZ32028	Venezuela	MH107366	MG973676	MG973524	MG973581
Peripatus sp.	MZUSP 0102	Panama	MH107357	MG973677	MG973522	MG973571
Peripatus sp.	MZUSP 0103	Panama	MH107358	MG973678	MG973523	MG973572
Peripatidae sp.	MZUSP 0106	Panama	MH107364	MG973686	MG973493	MG973573
Peripatidae sp.	MZUSP 0108	Panama	MH107361	MG973687	MG973495	MG973575
Peripatidae sp.	MZUSP 0110	Panama	MH107363	MG973688	MG973494	MG973579
Picadopatus biolleyi	NC009082	Costa Rica	NC009082	NC009082	NC009082	AF370782 - AF370783
Picadopatus ruber	HM600781	Costa Rica	HM600781	HM600781	HM600781	MG973570
Picadopatus biolleyi (neotype)	**Oni-074 La Palma	Costa Rica	Oni074**	-	-	-
Picadopatus biolleyi "BR"	**Oni-073 La Rosa	Costa Rica	Oni073**	-	-	-
Picadopatus biolleyi "PA"	**Oni-152 Paja Agua	Costa Rica	Oni152**	-	-	-
Picadopatus biolleyi "ET"	**Oni-149 El Terrón	Costa Rica	Oni149	-	-	-
Picadopatus biolleyi "SP"	**Oni-151 San Pedro	Costa Rica	Oni151**			
Picadopatus biolleyi "SP"	**Oni-061 San Pedro	Costa Rica	Oni061**	Oni061**		
Picadopatus ruber (neotype)	**Oni-173 Rancho Redondo	Costa Rica	Oni173**	-	-	-
Picadopatus ruber "RR"	**ADN-A Rancho	Costa Rica	ADN-A	ADN-A	-	-
Picadopatus ruber "RR"	**Oni-170 near Rancho Redondo	Costa Rica	Oni170	-	-	-
Picadopatus ruber "LJ"	**Oni-218 La Julieta	Costa Rica	Oni218**	-	-	-
Picadopatus ruber "CA"	**Ony_060 Cascajal	Costa Rica	Ony_060	Ony_060	Ony_060	-
Principapillatus hitoyensis	MCZ131340	Costa Rica	MH107340	MG973680	MG973488	MG973555
Principapillatus hitoyensis	DNA103564	Costa Rica	KC754642	KC754476	KC754525	KC754575
Principapillatus hitoyensis	PH1 - MCZ131339	Costa Rica	JX568983	JX568960	-	MG973556
Principapillatus hitoyensis	PH12	Costa Rica	JX568994	JX568971	-	-
Principapillatus hitoyensis	PH13	Costa Rica	JX568995	JX568972	-	-

*=Sequences reversed for the 12S alingment on MAFFT.

**=Sequences awaiting for the assignation of a genbank code.

Phylogenetic analysis:

We conducted a Bayesian analysis using MrBayes 3.2.1 (Ronquist et al. 2012), Markov Monte Carlo chains (MCMC) with two independent runs of 10000000 generations and four chains each one were done. We sampled trees every 1000 generations with a burn-in of 50. Convergence in MCMC chains and sample size were checked in Tracer 1.7.0. (Rambaut et al.2018). The analysis was made in the CIPRES web server (Miller, Pfeiffer, & Schwartz, 2010). Obtained trees were edited and visualized in Figtree v1.4.1; support of more than 85 percent was considered as high and indicative of monophyly.

Scanning Electron Microscopy:

Specimens were preserved in 70% ethanol after distention in 20% ethanol. An Hitachi S3700 Scanning Electron Microscope (SEM) was used, small pieces were treated as follows: gradually rehydrated and re-distended using 50, 30, 15 and 5 % ethanol and distilled water for about 15 min each sample, in OsO_4 for two hours, then in distilled water for 15 min and with tannic acid followed by two hours in OsO_4, after this two rinses in distilled water were done (15 min each). After a 5, 15, 30, 50, 70, 80, 90, 95 and 100% ethanol series (15 min each, the 100% treatment repeated three times) and critical point drying, samples were coated in gold for 10-15 min (Morera-Brenes & Monge-Nájera, 1990), all preparations were deposited as neoparatypes.

Morphological characters:

We used the terminology of Oliveira, Wieloch, & Mayer, 2010. We analyzed a total of 9 specimens from only the type locations with the use of SEM (Tab.S2), as well as individuals of the collection of Laboratorio de Sistemática, Genética y Evolución, Universidad Nacional, Heredia, Costa Rica. We recorded the following characters for the cited specimens: number of legs, sex, antennal rings (Tab. S3) and register their coloration (Fig. 2). Then compared then autapomorphies of each extant genera of Peripatidae (Oliveira et al. 2012a, 2014) with our specimens in order to assign them to a valid genus (Table 3); we also compared both species based on the results of our phylogenetic tree (Table 4).

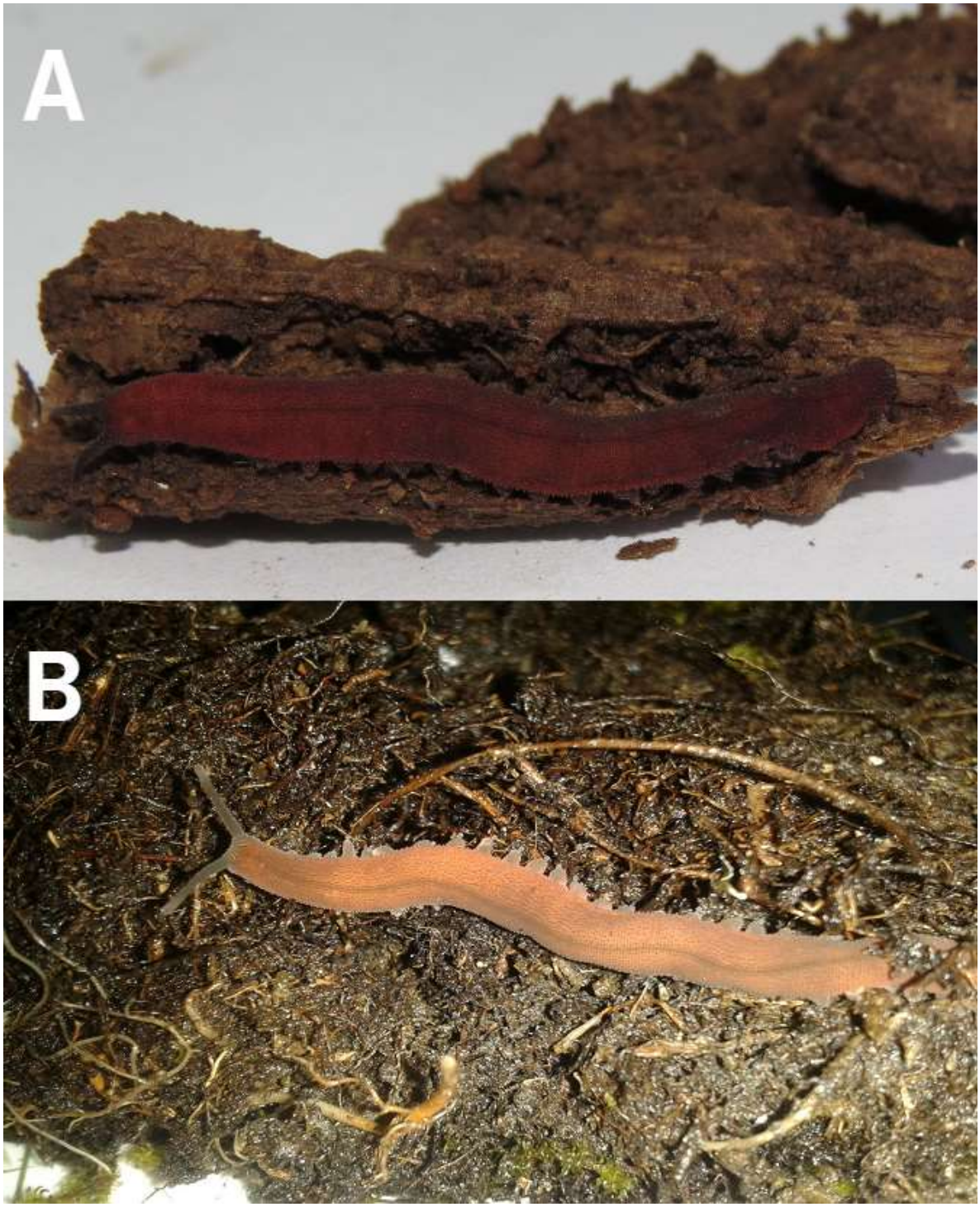

Fig. 2. **A**. Coloration of individuals of *P. biolleyi* on low light and **B**. of *P. ruber* on bright light, note that coloration vary depending on the light available. Individuals photographed belong only to the neotype localities.

Deposition of type specimens:

Type specimens of the new genus species were deposited in the collections of the Museo de Zoología of the Universidad de Costa Rica (MZUCR).

RESULTS

Morphological characters:

Our morphological comparison showed that *Picadopatus* do not share autapomorphies with any genera (*Epiperipatus* were not compared, details in discussion), and hence cannot be assigned to an established genus (Table 3). Therefore we erected a new one according to the unique character combination. Most characters from both species were similar, but the following ones differed: number of leg pairs in females varied from 29-31 in *P. biolleyi* while they were fixed at 31 in *P. ruber*, in males of *P. biolleyi* it ranged from 27-28, on *P. ruber* they were 27; interpedal structures are fused with smooth texture small scales on *P. biolleyi*, whereas they are separated and showed big scales in *P. ruber*; type I crater shaped papillae are absent in *P. biolleyi*, on *P. ruber* they bear a 9 scale collar around the crater, with a rudimentary apical piece; type II crater shaped papillae in *P. biolleyi* have smooth small scales, in *P. ruber* scales are larger and rugous; *P. biolleyi* primary papillae scales have a flattened apex from which thin roots and with few ramifications emerge, *P. ruber* scales have a conical apex from which thick, long roots with numerous branches appear (Table 4). As the majority of characters are equal in both species, some images only correspond to the structure of *P. biolleyi* or *P. ruber*, as a comparison was unnecessary.

Phylogenetic analysis:

The final alignment for the Peripatidae matrix consisted of 755 nucleotide positions for COI, 322 for 12S, 354 for 16S and 1774 for 18S; the Neopatida matrix consisted of 720 positions for COI, 322 for 12S, 354 for 16S and 1776 for 18S. Bayesian analyses revealed congruent geographic clades. For the Neopatida matrix we placed the root between Bouvier's "Andean Clade" species (*Oroperipatus*, Cockerell, 1908) and the rest of the Neopatida ("Caribbean Clade"). In both analyses *Picadopatus* gen. nov. is retrieved as a monophyletic clade, showing a Bayesian Markov Chain

Monte Carlo sampling support of 100, forming a sister group with species from Panama (Fig 3, 4) except for Peripatidae sp. MZUSP0102, Peripatidae sp. MZUSP0103 and *Epiperipatus vagans* (see discussion). This clade is subdivided in two monophyletic groups one containing the *P. ruber* neotype, specimens from La Julieta and Cascajal from Vásquez de Coronado, and "*E. biolleyi*" from near Rancho Redondo, Goicochea (HM600781; Rota-Stabelli et al. 2010), with a support 86% on the Neopatida matrix (Fig. 3) and 84% on the Peripatidae one (Fig. 4). The second monophyletic group consist in the *P. biolleyi* neotype from Alto la Palma as well as localities near the neotype: Bajo la Rosa, El Terron, San Pedro de Cascajal, Paja de Agua all from Vásquez de Coronado, and specimens labeled as "*E. biolleyi*" (NC_009082; Podsiadlowski et al. 2008; Oliveira et al 2012a), bearing a support of 89% on the Neopatida matrix (Fig. 3) and a 81% on the Peripatidae matrix (Fig. 4). The rest of both topologies are similar, grouping species from close geographic regions together (Fig 3, 4). However, some relationships within these groups differ. The Brazilian clade in the Neopatida matrix (Fig. 3) show a relationship of 82% support between *E. diadenoproctus* and *E. acacioi*, whereas on the Peripatidae one (Fig. 4) it groups *E. diadenoproctus* with *Epiperipatus adenocryptus*, albeit with low support (56%). *E. vagans* on the Neopatida matrix is sister group of the Costa Rican-Panamanian clade (Fig. 3) –with Venezuelan Peripatidae sp. MCZ32028 grouped there– but with low support value (36%), on the Peripatidae topology (Fig. 4) it joins with South American species from Brazil, French Guiana and Guyana, with a support 86%. It must be noted that the support of the branch that split South from Central American samples is low on our topologies (29% and 59% respectively), and connected with *Macroperipatus torquatus* from Trinidad, which in turn is a sister group of the Colombian samples (*Epiperipatus* sp MCZ131428 and *Epiperipatus* sp. MCZ131430) with 100% support, this clade is the sister group of the Bouvier's "Andean clade" samples of *Oroperipatus* from Ecuador (*Oroperipatus* sp. MNRJ0069), Galápagos (*Oroperipatus* sp. MCZ43394) and México (*Oroperipatus eisenni*), with 100% support.

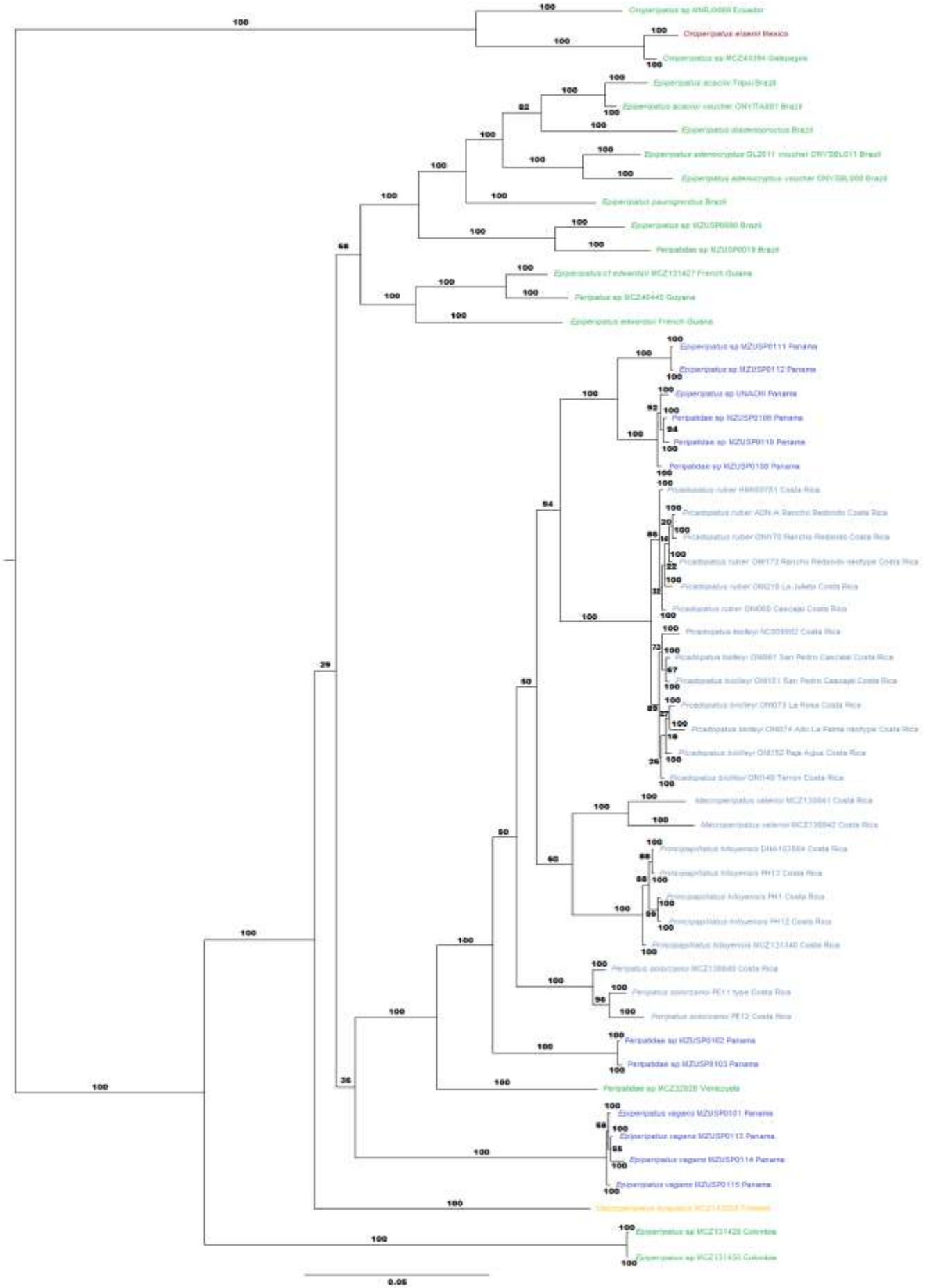
Epiperipatus sp MZUSP0111 Panama
Epiperipatus sp MZUSP0112 Panama
Epiperipatus sp UNACHI Panama
Peripatidae sp MZUSP0108 Panama
Peripatidae sp MZUSP0110 Panama
Peripatidae sp MZUSP0109 Panama
Peripatidae sp MZUSP0102 Panama
Peripatidae sp MZUSP0103 Panama
Peripatidae sp MCZ32026 Venezuela
Epiperipatus vagans MZUSP0101 Panama
Epiperipatus vagans MZUSP0113 Panama
Epiperipatus vagans MZUSP0114 Panama
Epiperipatus vagans MZUSP0115 Panama
Epiperipatus sp MCZ131428 Colombia
0.05

Fig. 3. Bayesian analysis of the Neopatida dataset. Percentage of Bayesian support is offered near every branch. Distinct geographic regions are represented by different colors: South-America (green), Costa Rica-Panama (gray blue and blue), Caribbean Islands (yellow) and Mexico (brown).

Taxonomy

Family **PERIPATIDAE** Bouvier, 1902

Genus *Picadopatus*, gen. nov. Barquero-González, Sánchez-Vargas, Rodríguez-Arrieta, Villalobos-Brenes, Monge-Nájera & Morera-Brenes

Previously referred to as *Peripatus* (Bouvier 1902, 1905; Furhmann, 1913) and *Epiperipatus* (Peck, 1975).

Type species: *Picadopatus biolleyi*, gen. nov. by designation.

Diagnosis

Two types of dermal papillae in the dorsal integument: primary and accessory (Fig 6C,D); conical shaped primary papillae with roundish bases (Fig 6E,F), larger primary papillae with six-eight scale ranks, base surrounded by 24-33 scale collar, rarely 38 on the biggest papilla; medium sized primary papillae with four-five scale ranks surrounded by a 18-23 scale collar. The conical apical piece has two-four scales, with a straight medium sized bristle. Rosette-like shaped accessory papillae, usually in numbers of three between each primary papilla (Fig. 6C,D). Plicae with no obvious pattern (Fig. 6C,D). Four spinous pads on each leg (Fig. 7C,D), except for the last pair that only have two pads (Fig. 10C,E); first pad smaller than the two subsequent, that are similar in size, fourth pad narrower; bristles of spinous pads have low developed conical bases with prominent scales (Fig. 9D). Nephridial tubercle in fourth and fifth pairs of legs pulled to the posterior side of the pad, indenting the lower part of the third and the upper of the fourth pad (Fig. 7A,B,C,D), which in turn sometimes is deformed. Two posterior and one anterior foot papillae (Fig. 7E,F). Bean shaped papillae present on the dorsal part of each leg, next to the claw base. Paired crural tubercles in two

pregenital leg pairs with their bases joined by a dermal fold (Fig. 10A,B,C,D), tubercles covered with smooth scales on the surface apices and with a single slit-like opening. A couple of anal glands present, with their openings curved in its distal part and straight the proximal part (Fig. 10A,B). 27-28 pairs of legs on males and 29-31 on females. COI sequences as in the studied specimens (Table 2).

Distribution

Costa Rica: Goicochea and Vázquez de Coronado, San José.

Etymology

The genus *Picadopatus* is dedicated to the Costa Rican scientist Clodomiro Picado Twight, a pioneer in the research of snake toxins. His most internationally recognized achievements comprises the development of several antivenins; his work on molds is considered as a precursor of the formal penicillin's discovery, which resulted in compounds used as treatments for human patients at least one year before the re-discovery of penicillin by Alexander Fleming. Over his lifetime he wrote about 115 works, mostly books and monographs in topics ranging from zoology, bromeliads (which led him to the finding of onychophorans), ophidism, physiology, phytopathology, industrial microbiology, medical microbiology and immunology.

Species description.

Picadopatus biolleyi, comb. nov.

Synonyms. *Peripatus biolleyi* (Bouvier, 1902: 258); *Epiperipatus biolleyi* (Peck, 1975: 345).

Neotype. MZUCR 70-01 one female in 70% ethanol (Costa Rica, San José Province, Alto de la Palma, border between cantón of Moravia and cantón of Vázquez de Coronado, besides the ancient Carrillo road, Lower Montane Rain Forest in Holdridge (1978) system, approximately 10°2'52.84" N - 83°59'11.79" W, approximately 1 530 m.a.s.l., B. Morera-Brenes & J. Monge-Nájera col., i. 2017).

Additional material: One female and one male, prepared for SEM [as neotype], which will be deposited as neoparatypes.

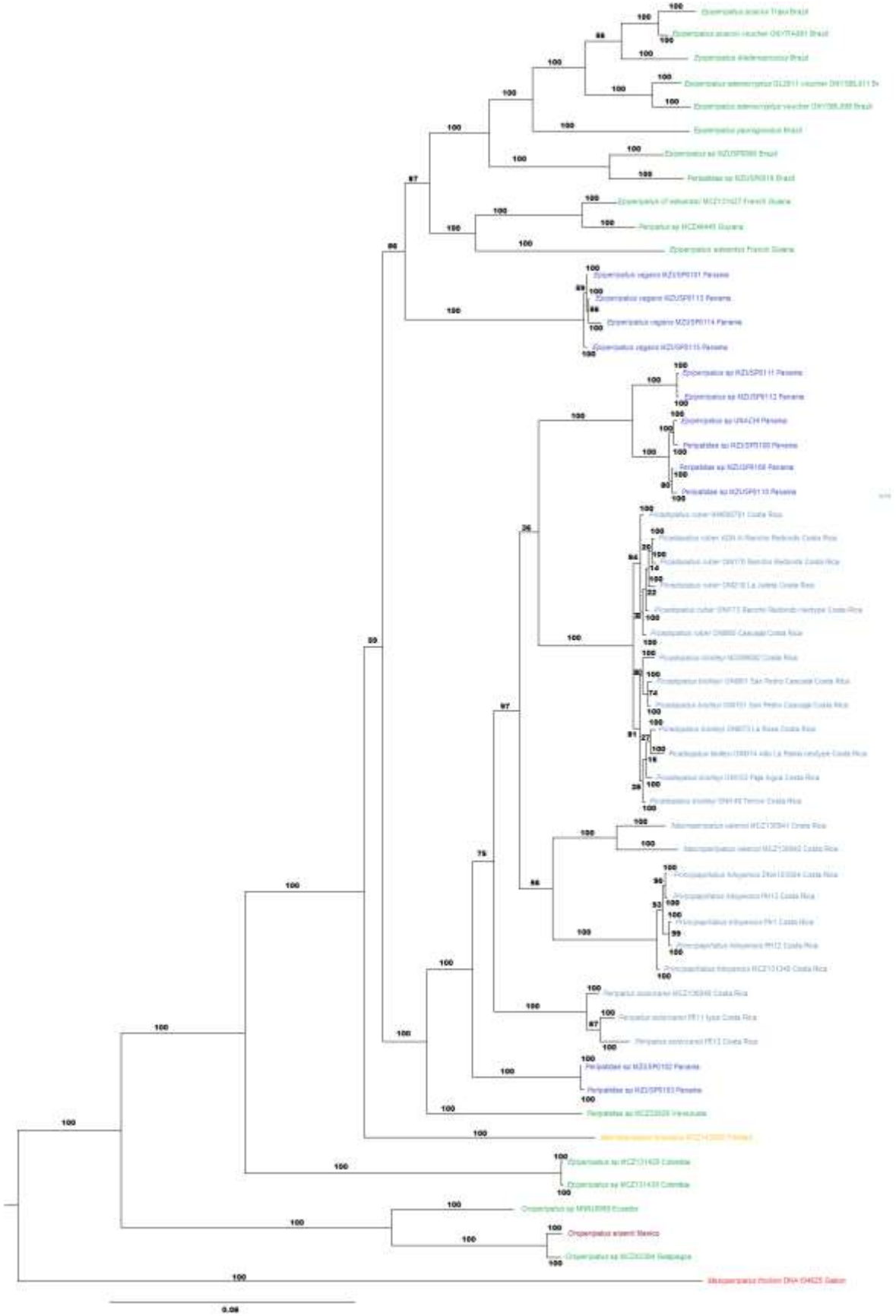

Fig. 4. Bayesian analysis of the Peripatidae dataset. Percentage of bayesian support is offered near every branch; note the long branches between the African *Mesoperipatus tholloni* used as outgroup and the rest of neopatida. Color code as in Fig 3.

TABLE 3
Autapomorphies from each genus compared to the analyzed species

Genus	**Distinctive character**	***P. biolleyi***	***P. ruber***
Cerradopatus	Rounded interpedal structures present in three separate pairs per segment, with variable size and shape, thus covered with a finely cuticle.	0	0
Eoperipatus	Males with a single and medial anal gland opening; four circular pits on males gonopore; a single complex with two different kinds of scales is formed by the crural tubercles united by a dermal fold.	0	0
Epiperipatus	Absent	-	-
Heteroperipatus	One posterior and three anterior distal foot papillae.	0	0
Macroperipatus	Dermal papillae with quadrangular bases, covered with flat scales; primary papillae with undeveloped apical pieces, only one collar of small scales.	0	0
Mesoperipatus	Male anal gland openings in a single medial groove before the anus, separated by a dermal fold; three spinous pads per leg.	0	0
Oroperipatus	Two or more distal foot papillae on anterior and posterior foot region.	0	0
Peripatus	More than two crural tubercles present in pregenital leg pairs in males; dorsal primary papillae apical piece larger than basal piece	0	0
Plicatoperipatus	Each segment with twenty-four dorsal plicae, "apical-most scales of basal piece thorn-shaped, as high as the apical piece and sticking out" (Oliveira et al. 2012a)	0	0
Principapillatus	Characteristic head pattern, largest and medium-sized primary papillae arranged in an alternated pattern	0	0

Speleoperipatus	Eyes not visible; body pigmentation absent	0	0
Typhloperipatus	Eyes not visible; uterine embryos of almost the same age	0	0

Emended Diagnosis

Pairs of legs range from 27-28 in males to 29-31 in females. Paired interpedal structures fused and bearing a small scales with a smooth texture (Fig. 9A). Type I crater shaped papillae absent (Fig. 8E); type II crater-shaped papillae without remnants of a rudimentary apical piece, composed of more-less flattened scales (Fig. 8F). Primary papillae scales with a flattened apex from which thin roots and with few ramifications emerge (Fig. 9E).

TABLE 4

Morphological characters evaluated.

Character	Species	
	P. biolleyi	*P. ruber*
No head pattern present	1	1
Frequently between 36-42 complete antennae rings	1	1
Antennae tip shows type I sensillum alternating with type II sensillum	1	1
One-two accessory tooth on the outer jaw	0	1
One-two accessory tooth on the inner jaw	0	1
A row of 8 to 13 denticles on inner jaw	1	1
No dorsal papillae obvious pattern	1	1
Dorsal midline asymmetrically flanked by accessory or primary papillae	1	1
Incomplete or bifurcated dorsal plicae are present above the legs	1	1

Primary papilla of sub-conical shape and roundish base	1	1
Primary papillae separated by three accessory papillae	1	1
Larger primary papillae with six-eight scale ranks, medium sized primary papillae with four-five scale ranks	1	1
Sub-conical apical piece with two-three scales	1	1
Primary papillae scales with a flattened apex from which thin roots and with few ramifications emerge	1	0
Primary papillae scales with a conical apex from which thick, long roots with numerous branches emerge	0	1
Hyaline organs present on each side of the dorsal midline	1	1
Four spinous pads	1	1
Spinous pads with large bristles with poorly developed rudimentary bases with prominent scales	1	1
Nephridial tubercle always indenting the lower part of the third and the upper of the fourth pad	1	1
27-28 pairs in males and 30-31 in females	0/1	0/1
27-28 pairs of legs in males and 29-31 in females	0/1	0/1
One-two bristles on the proximal and two bristles in the distal setiform ridges	1	1
Two posterior and one anterior foot papillae	1	1
A large bean-shaped papillae located in a pouch, present in the dorsal part at each leg above the foot	1	1
Enlarged nterpedal structures fused along the ventral midline, with smooth texture small scales	1	0
Paired rounded interpedal structures with irregular texture big scales	0	1
Type I crater shaped papillae with a rudimentary apical piece	0	1

Type II crater shaped papillae with a rudimentary apical piece	0	0
Ventral and smaller preventral organs appearing as a vertical slit surrounded of big smooth scales.	1	1
Crural glands with two separated tubercles covered with scales with smooth surface apices, they occur in the two pregenital pairs of legs of males	1	1
One pair of anal glands that open to the exteriorly via a pair of slits anteriorly to the anus	1	1
Male with a cruciform gonopore	1	1
Female with a slit like gonopore	1	1
Coloration ranging from bright red, brick color to reddish brown	1	1
A black mid-line easily seen in the dorsal integument	1	1
Ventral surface reddish	1	1
A white or reddish band near or in the tip of each black antenna on some specimens	1	1

Non-diagnostic features

Measurements. Maximum body size in 70% ethanol length of 1.6 to 6.4 cms.

Head. Antennal rings from 36-42 (Fig. 5A,C), tip of the antennae contains only type II sensillum (Fig. 5C,F), the next adjacent six antennal rings show only type II sensillum and chemoreceptors in the upper ring part, from the seven to the 17th antennal ring type I and type II sensillum and chemoreceptors are found in their upper part; from the second to the eight antennal ring narrow rings with only type II sensillum alternate with rings that contain both type of sensillum (Fig. 5C,D). From the 18th antennal ring to the base of the antennae only type I sensillum are found dorsally, whereas spindle-shaped sensilla appear on the ventral surface since the 22nd ring until the antennae base (Fig. 5D,E). Smooth textured eyes located laterally and behind the antennae base (Fig. 6A). External lips consist of 15 lobes and the internal lip of 7 lobes (Fig. 5A), as in all neotropical

species an unpaired lip lobe is present (Oliveira et al. 2012a); one accessory tooth on the outer jaw; one or two accessory tooth on the inner jaw with a row of 8 to 11 denticles (Fig. 11C,D).

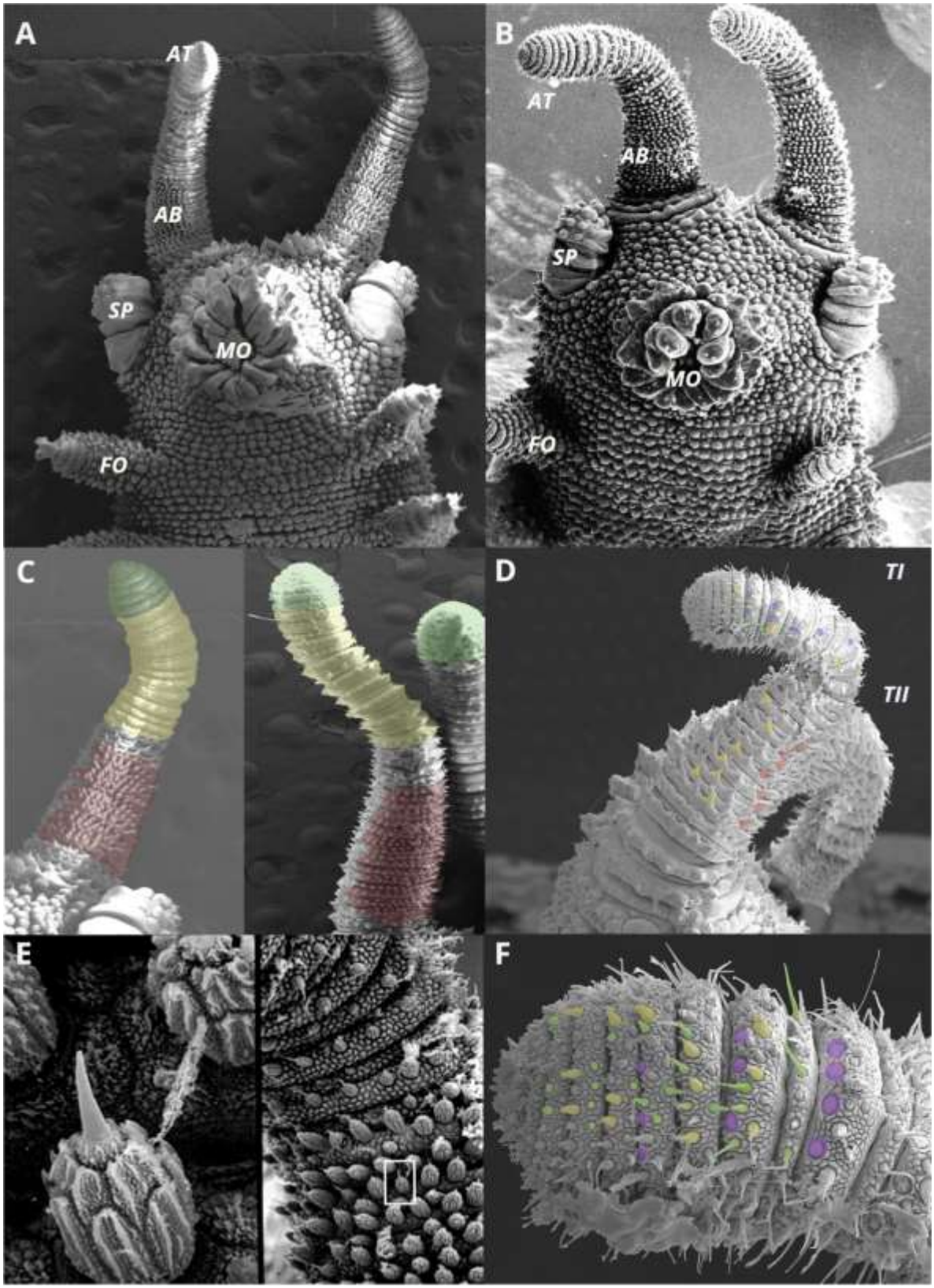

Fig. 5.A. *P. biolleyi* head ventral view, tip of the antennae (TA) and antennae's base (BA) are clearly distinguishable, a unpaired-lipped mouth (MO) is present, as well as slime papillae (SP), first foot (FO) presents four spinous pads; **B**. *P.ruber* displays the same disposition. **C**. Location of antennal structures on *P. biolleyi* (left) and *P. ruber* (right) note that each type of sensillum is more abundant at certain parts: type I sensillum (green) are abundant the tip; type II sensillum (yellow) are common in the middle (ventral and dorsal) and the base (dorsal); spindle-shaped sensillae (red) are exclusive from the ventral zone close to the base; **D**. Distribution of antennae sensillum in *P. ruber*, type I sensillum are found in the tip with chemoreceptors (purple), type II sensillum are rarely seen on the tip and more commonly in the mid antennal zone, whereas the base of the antennae is ventrally occupied by spindle-shaped sensillae (color code as above); **E**. Details on the spindle-shaped sensillae in *P. ruber*; **F**. Details on the disposition of sensillum in antennal tip in *P. ruber* (color code as above).

Dorsal integument. 12 dorsal plicae present per segment, only seven cross to the ventral side; incomplete or bifurcated dorsal plicae present above the legs; dorsal midline symmetrically flanked with one accessory papillae at each side, although in some plica small primary papilla and one accessory papilla flank the dorsal midline (Fig. 6C); hyaline organs present at each side of the dorsal midline, each hyaline organ between two adjacent plicae.

Ventral integument. Ventral and smaller preventral organs are found along the ventral midline between each leg pair and separated by one plica, each consists of a vertical slit surrounded of smooth scales (Fig. 8A). Interpedal structures are fused and bear a smooth texture (Fig. 9A). They are located in furrows between the fifth and sixth plicae of subsequent pairs of legs. While type I crater shaped papillae are absent, rudiments of a 6-8 scale "collar" persist (Fig. 8E). Type II crater shaped papillae present a central depressions surrounded by a 10 scales collar without a rudimentary apical piece (Fig. 8F).

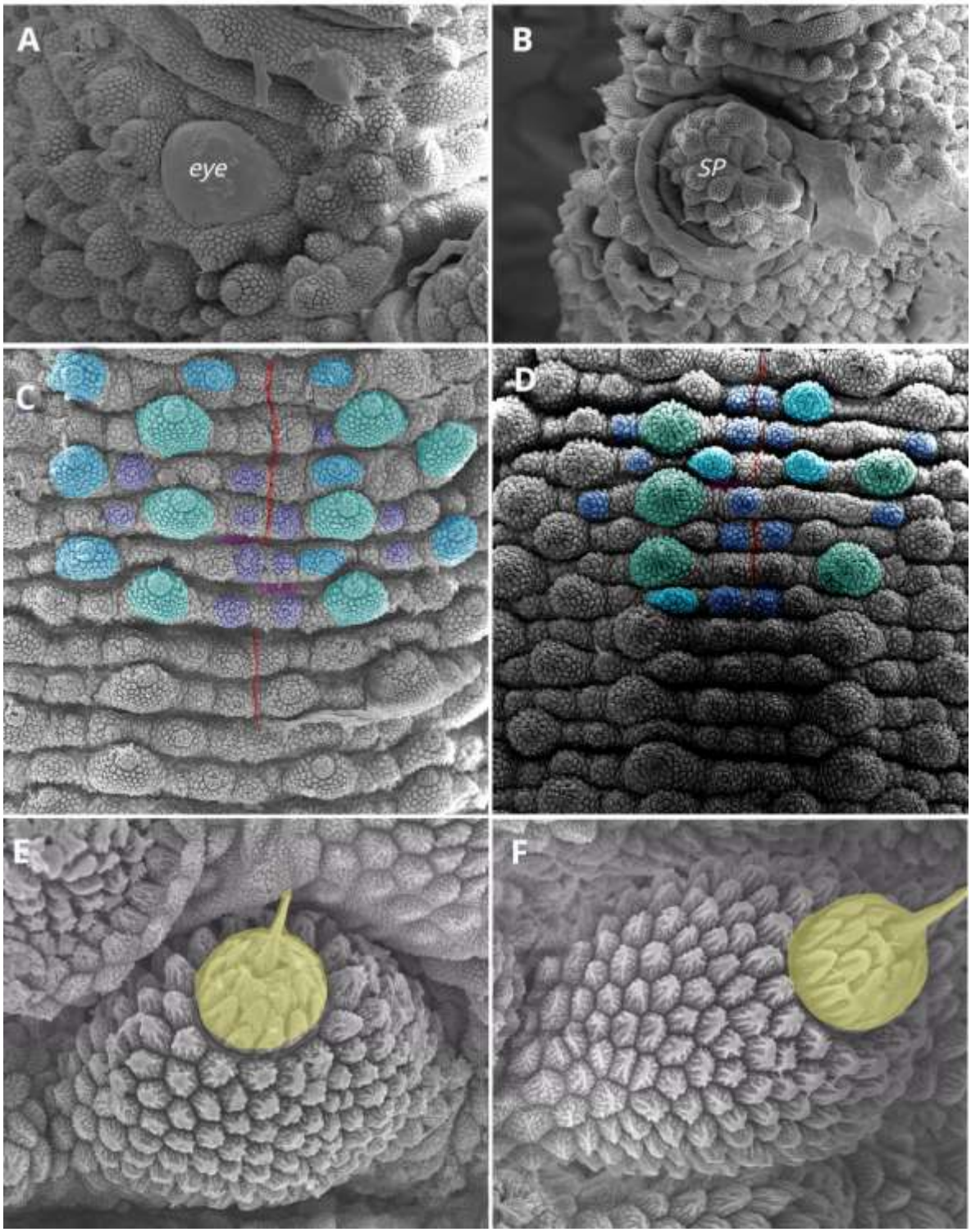

Fig. 6. A. Details on eye of *P. ruber*; **B**. Slime papillae (SP) morphology on *P. ruber*; **C**. dorsal integument of P. biolleyi present plicae with largest primary papillae (light blue) alternating with plicae with mid-sized primary papillae (blue), accessory papillae (purple) are abundant and flank the midline (red); **D**. *P.ruber* present the same arrangement, note the collapsed papillae due to fixation

artifacts; **E**. details on primary papillae of *P. biolleyi*, note the conical shape and the roundish apical piece with a straight bristle (yellow); **F**. primary papillae of *P. ruber* alike.

Legs. Four complete spinous pads, deprived of a fifth one (Fig. 7C); only two pads exist in the last pair of legs and three in the penultimate; two-three bristles on the proximal and two bristles in the distal setiform ridges (Fig. 7E).

Posterior region. Crural tubercles on the two pregenital pairs of legs of males (Fig. 10A,C). Male with a cruciform genital pad (Fig. 10C), female genital pad consists in a vertical slit (Fig. 10E); a pair of anal glands, found anteriorly to the male anus (Fig. 11A).

Remarks on behavior and habitat. Specimens were found living alone, rarely in pairs; did moss patches and soil walls seem to be their preferred habitats, they inhabited secondary forest, and even pastures near human settlements. When groups are kept in terrariums individuals frequently bite each other as we noted when new scars appeared in their skins, presumably this is a hierarchy establishment mechanism. A high mortality rate is shown by the species when taken outside their highland habitat to lower lands with higher temperatures, although it feds “normally” during captivity.

Colour pattern. Ranging from bright red, brick color to reddish brown (depends on the light), a marked black mid-line is easily seen in the dorsal integument, leg coloration reddish grey-black with yellowish spinous pads on the underside, ventral surface reddish (Fig. 2A). Some specimens show a white or reddish band near or in the tip of each black antenna. Neonates have an overall pinkish color.

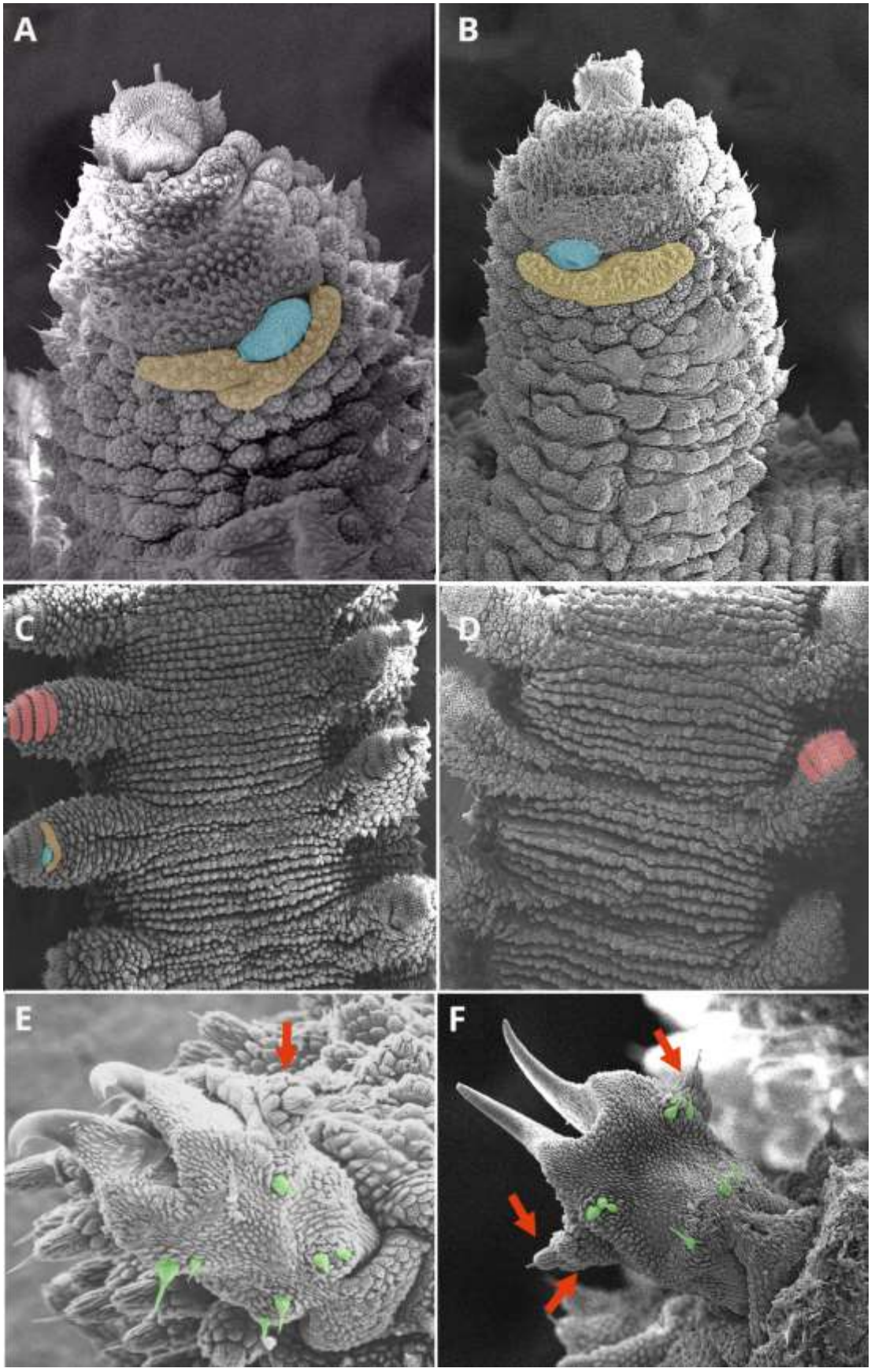
A
B
C
D
E
F

Fig. 7. A. Fourth leg of *P. biolleyi* contains the nephridial tubercle (light blue), which indents both third and fourth spinous pads, note that the latter is deformed in the examined specimen (orange); **B**. fourth leg of *P. ruber* shows the same arrangement, but in this case the fourth pad is normal; **C**. overall ventral view of *P.biolleyi* where the four spinous pads per leg are present (red); **D**. four spinous pads are found in *P. ruber* as well; **E**. Foot details of *P. biolleyi*, where two-three bristles occur on the proximal and two bristles in the distal setiform ridges are found (green), it also present two posterior and one anterior foot papillae signaled by red arrows.

Picadopatus ruber, comb. nov.

Synonyms. *Peripatus ruber* (Fuhrmann, 1913).

Neotype: MZUCR 68-01 one male and one female in 70% ethanol (Costa Rica, San José Province, cantón of Goicoechea, distrito Rancho Redondo, Premontane Wet Forest in Holdridge system, approximately 9°57'41.17" N - 83°56'55.96" W, approximately 2 000 m.a.s.l., J.P. Barquero-González, B. Morera-Brenes & J. Monge-Nájera col., i. 2017).

Neoparatypes: MZUCR 69-01 one female and one male, prepared for SEM [as for holotype].

Emended Diagnosis.

Pairs of legs 27 in males and 31 in females; separated interpedal structures with big scales (Fig. 9B). Type I crater shaped papillae with a 9 scale collar and a rudimentary apical piece (Fig. 8G); type II crater shaped papillae with big and rugous scales (Fig. 8C,D,H). Primary papilla scales with a conical apex from which thick, long roots with numerous branches appear (Fig. 9F).

Non-diagnosis features.

Measurements. Maximum body lenght in 70% ethanol 2.2-5.4cms.

Head. Antennal rings from 36-42 (Fig. 5B,C,D), sensillum and chemoreceptor distribution as in *P. biolleyi* (Fig. 5D,E,F). Smooth textured eyes behind the base of the antennae (Fig. 6A). External lips as in *P. biolleyi* (Fig. 5B); one accessory tooth on the outer jaw, one or two accessory tooth on the inner jaw with a row of 8 to 11 denticles (Fig. 11C,D).

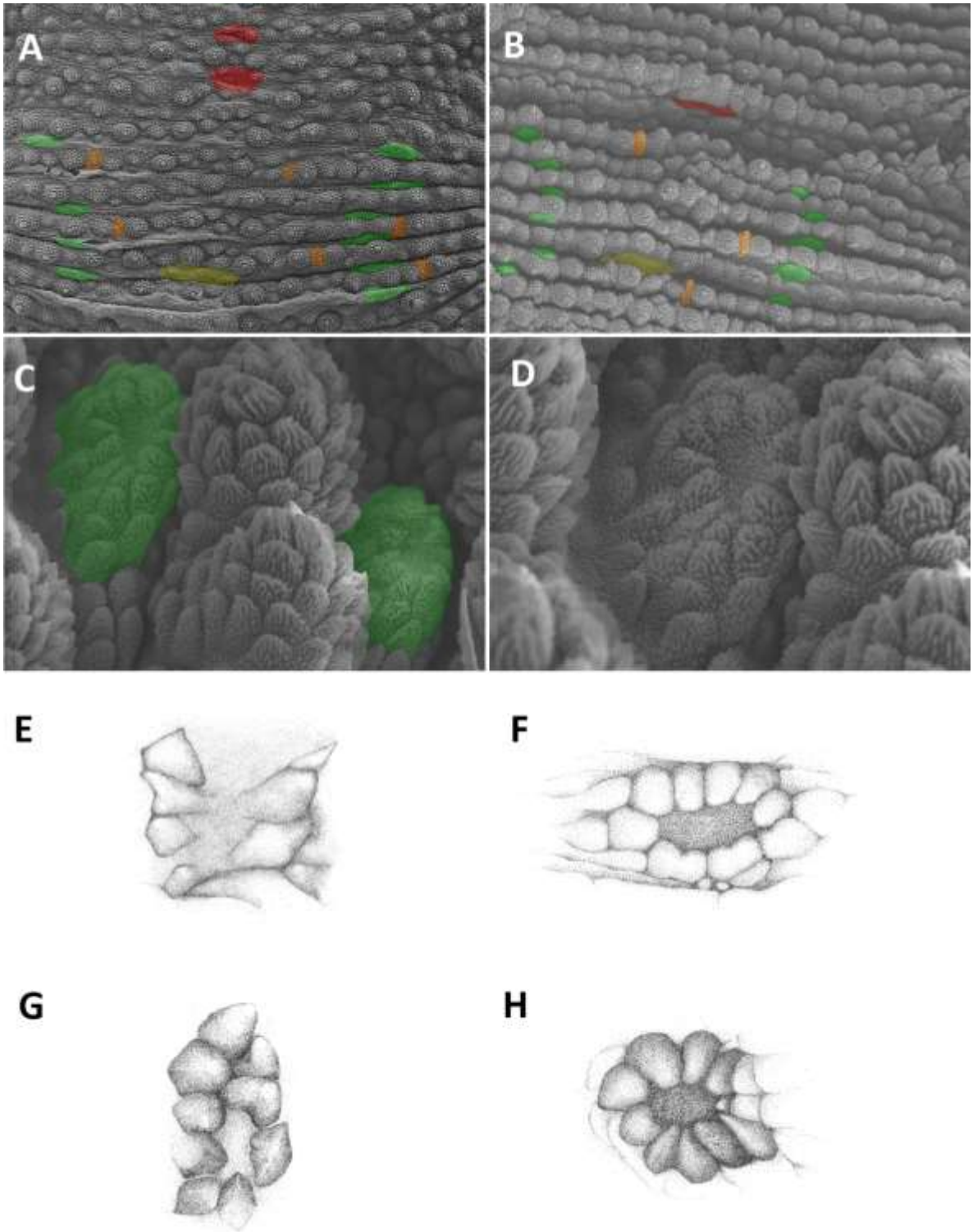

Fig. 8. A. Ventral integument of *P. biolleyi* showing several structures: pre-ventral and ventral organs (red), interpedal structures (yellow), vestigial papillae (orange), also type II crater shaped papillae (green) are present; **B**. the same display is showed on *P. ruber* although there are differences on each

structure and there is a type I crater shaped papillae (orange); **C**. type II crater shaped papillae on *P. ruber* bear no rudimentary apical piece; **D**. details on type II crater shaped papillae include a very rugous texture both in the scales and the crater; **E**. some vestigial papillae reminds of crater-shaped ones in *P. biolleyi*; **F**. *P. biolleyi* type II crater shaped papillae have smaller scales but also lacks the rudimentary apical piece; **G**. type I crater shaped papillae shows the remnants of a rudimentary apical piece and have a defined crater on *P. ruber*; **H**. type II crater shaped papillae have larger and fewer scales on *P.ruber*.

Dorsal integument. 12 plicae per segment, only seven cross to the ventral side; incomplete or bifurcated dorsal plicae present above the legs; dorsal midline flanked as in *P. biolleyi* (Fig. 6D).

Ventral integument. Ventral and smaller preventral organs hardly visible, each consists of a vertical slit surrounded of smooth scales (Fig. 8B). Type I crater shaped papillae show a 9 scale collar, a rudimentary apical piece present (Fig. 8G); type II crater shaped papillae with big and rugous scales (Fig. 8C,D,H) without apical piece.

Legs. Four complete spinous pads (Fig. 7D), other leg characters as in *P. biolleyi* (Fig. 7A,F)

Posterior region. As in *P. biolleyi* (Fig. 10B,D,F; Fig 11B).

Remarks on behavior and habitat. As in *P. biolleyi*.

Colour pattern. As in *P. biolleyi*.

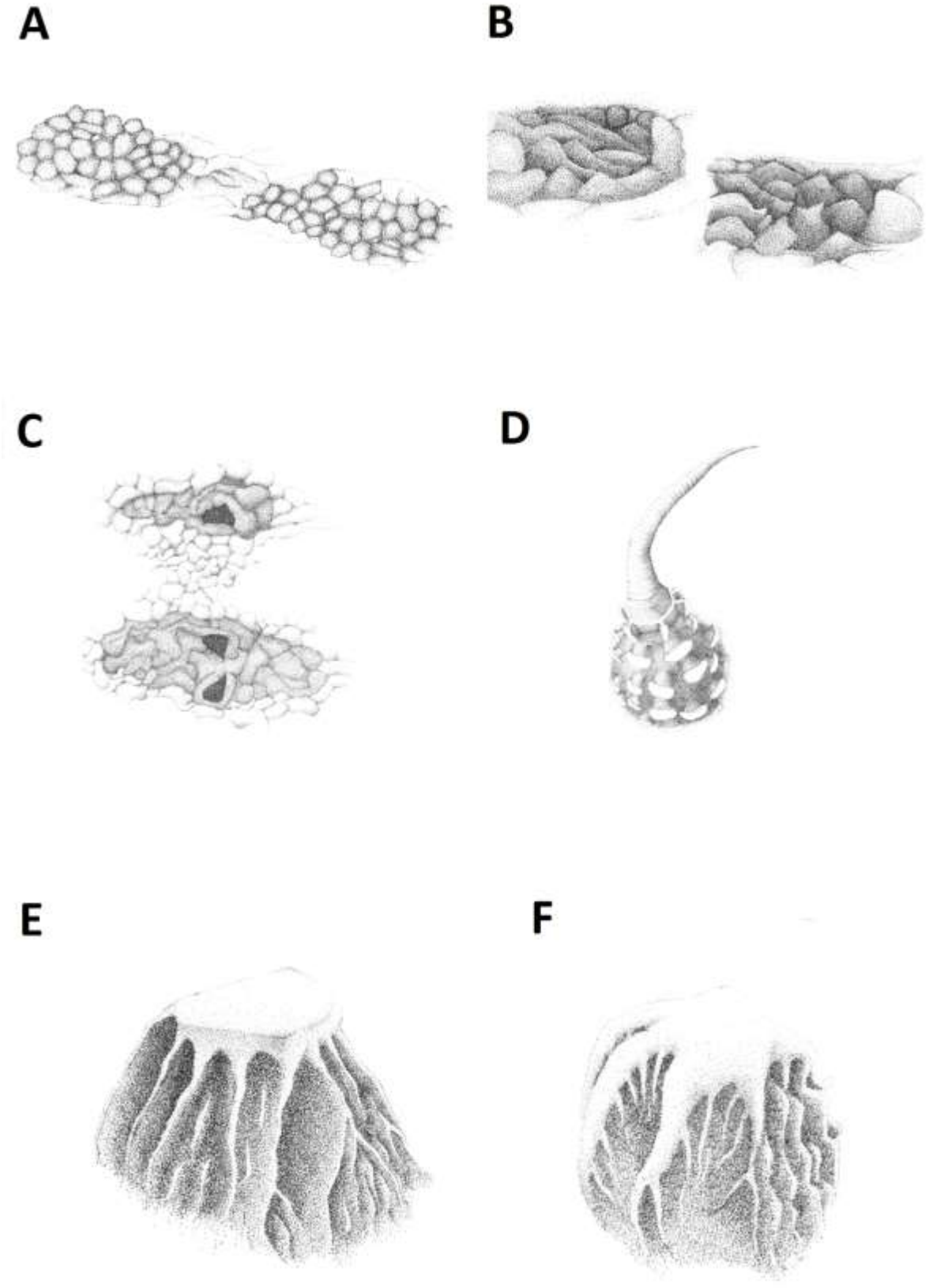

Fig. 9. A. Fused interpedal structures with smooth scales are found in *P. biolleyi*; **B**. interpedal structures with bigger rugged scales are separated in *P.ruber*; **C**. preventral and ventral organs alike in

both species; **D**. details on spinous pads bristle base, similar in both species; **E**. scales of the primary papillae have a flattened apex on *P. biolleyi*; F. on *P. ruber* the apex is conical.

Key to species of Onychophora from Costa Rica.

1. Males with 27-28 and females with 29-31 leg pairs, nephridial tubercle always indenting the lower part of the third and the upper of the fourth spinous pad; dorsal plicae do not show obvious papillae patterns, a pair of crural tubercles bases fused by a dermal fold covered with smooth scales on the surface of the apices and a single slit-like opening…….....*Picadopatus* 6
2. Males with 26–29 and females with 30–32 leg pairs; largest primary papillae organised in prominent rows parallel to dorsal midline; presence of a characteristic head pattern……..……...*Principapillatus*
3. Males with 34 and females with 39-41 leg pairs; nephridial tubercle free from third and fourth pads, in lateral posterior position; conical primary papillae in dorsal plicae, usually 5 to 12 accessory papillae between two of primary ones.…..…….....…”*Peripatus”*
4. Female with 34 leg pairs; no apparent frontal organs; primary and accessory papillae projecting from square or oblong bases; nephridial tubercle free……...…”*Macroperipatus”*
5. Males with 25-27 and females with 28-29 leg pairs; nephridial tubercle free form third spinous pad and only partly surrounded by the fourth one; no hyaline organs……...……”*Epiperipatus*”
6. Males with 27-28 and females with 29-31 leg pairs; paired interpedal structures fused and bearing a small scales with a smooth texture; type I crater shaped papillae absent; type II crater shaped papillae with no remnants of a rudimentary apical piece with small scales……...*P. biolleyi*

- Males with 27 and females with 31 leg pairs; a pair of separated interpedal structures with big scales; type I crater shaped papillae present, with a rudimentary apical piece; type II crater shaped papillae without remnants of a rudimentary apical piece and with rugous large scales….…….…….…*P. ruber*

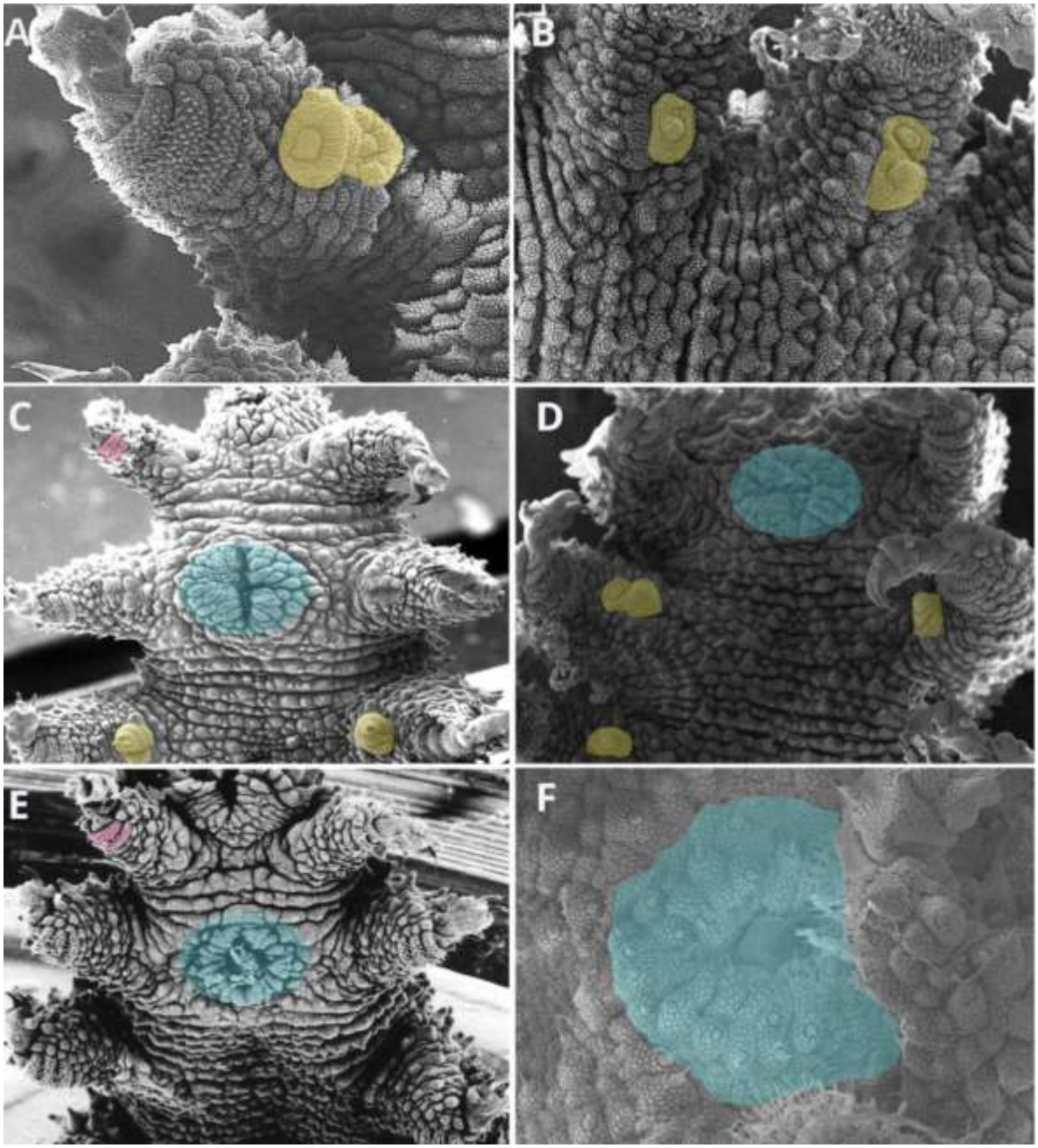

Fig. 10. A. Paired crural tubercles (yellow) are present in two pregenital leg pairs in males of *P. biolleyi*; **B**. the same arrangement is found in *P. ruber,* note that these tubercles can be embedded or exposed in the same specimen and their bases are fused by a dermal fold; **C**. a cruciform gonopore (light blue) is present in males of *P. biolleyi*, also the last pair of legs only have two spinous pads; **D**. *P. ruber* have an equal arrangement; **E**. a slit like gonopore is found on females of *P. biolleyi*; **F**. horizontal detail of female's *P. ruber* gonopore (the right part is the terminal end).

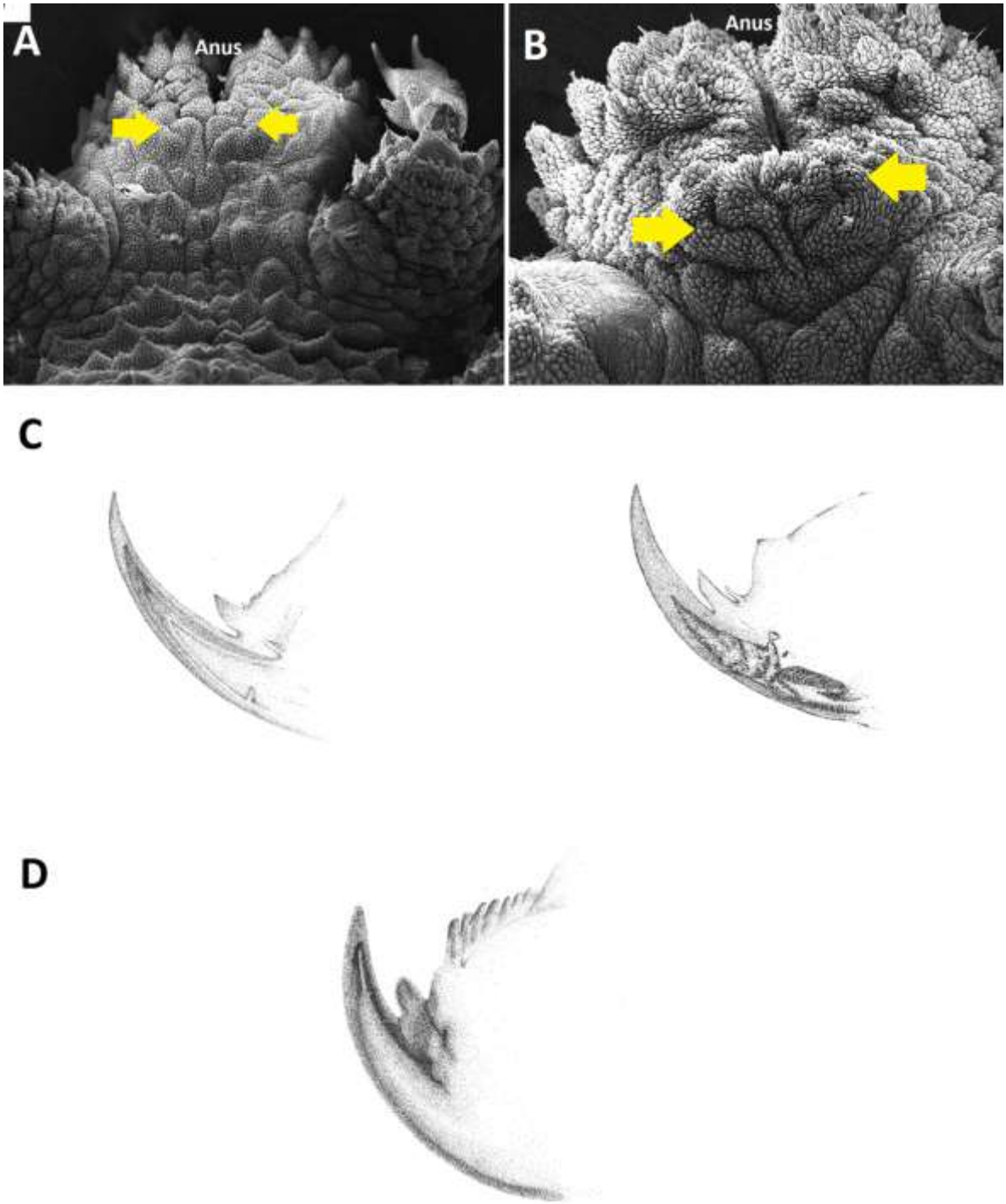

Fig. 11. A. Anal glands (yellow arrows) on *P. biolleyi* are close to the anus; **B**. the character is equal on *P. ruber***; C**. outer jaw tooth consist on a sharp principal tooth with one or two accessory tooth on

both species; **D**. a thick principal tooth with one or two accessory tooth is found on both species as well as a row of denticles.

Conservation:

We witness a considerably large forest coverture with additional large vegetation patches between urban settlements that serve as connectors for onychophoran populations, allowing the proper genetic flow necessary for allele interchange, vital to species survival; complementing these data, the constant presence and collection of both *Picadopatus* species for more than 30 years (Morera-Brenes & Monge Nájera pers. comm.) can be considered an additional indicator of stable populations. Our results highlight a speciation promoting landscape, as the localities of all the specimens of *P. biolleyi* and *P. ruber* clade are only separated by a maximum of 10 km, a small area containing such diversity of species has a biological significance and urge for conservation actions; moreover other saproxilic taxa sharing a similar life history and frequently found along with onychophorans are expected to show similar pattern in this landscape, hence onychophorans can serve as a flag species to protect other similar understudied invertebrate fauna with low dispersal ability, as invertebrates are historically ignored for conservation purposes (Ferrier et al. 1999; McKenzie et al. 2000).

DISCUSSION

The studied species were assigned to the genus *Picadopatus* gen. nov., because of their unique combination of characters and that autapomorphies of the described Peripatidae genera (Oliveira et al. 2012a, 2014) are absent. In contrast to representatives of *Eoperipatus*, males do not have a single and medial anal gland opening located in a pad anterior to the anus, four circular pits on male's genital pad nor the crural tubercles linked by a dermal fold. Also are not *Heteroperipatus* because the absence of one posterior and three anterior foot papillae. Do not belong to *Macroperipatus* as they lack the characteristic shape of dermal papillae. Are not *Mesoperipatus* as they do not have the male's anal gland openings together in a single medial groove anterior to the anus and it present four rather than three spinous pads. Because they possess one anterior and two

posterior foot papillae are not representative of *Oroperipatus*. The occurrence of 12 dorsal plicae instead of 24, the nonexistence of apical-most scales of basal piece thorn-shaped, as high as the apical piece and sticking out, is inconsistent with *Plicatoperipatus*. The reddish pigmentation and external functional eyes avoids them to be *Speleoperipatus*, this last feature also prevents them to fit in *Typhloperipatus*. Finally the differ from *Principapillatus* because the lack of a characteristic head pattern and of largest primary papillae organized in prominent rows parallel to dorsal midline; or to *Cerradopatus* as they do not poses dorsal large primary papillae appearing as bright spots and three separate pairs of interpedal structures.

Four genera of Peripatidae are described for Costa Rica: *Epiperipatus*, *Macroperipatus*, *Peripatus* and *Principapillatus*; but only the last genus have been meticulously studied (Oliveira et al. 2012a; Giribet et al. 2018). *P. biolleyi* and *P. ruber* were classified as *Peripatus* by Bouvier (1902) and by Furhmann (1913) respectively, although if we consider that the two species have an apical piece of small size with less than four scales rank and that males bear crural tubercles only in two pregenital leg pairs, their classification within *Peripatus* is erroneous.

P. biolleyi was moved to *Epiperipatus* (Clark, 1913; Peck, 1975); however this genus is problematic (Oliveira et al. 2012a,; Giribet et al. 2018), it contains specimens from locations as distant as the Antilles, Central and South America, consequently the morphological characters defining the genus –dorsal primary papillae of only one type; primary papillae intergrade through all sizes in medium to large specimens, in small individuals some are larger; accessory papillae found occasionally between close primary ones; males present a pair of crural tubercles in two pre-genital pairs of legs; primary papillae with a low number of scale ranks in basal and apical pieces– overlap with other genera (Sampaio-Costa et al. 2018). We interpreted this high variability as an artificial grouping of different genera in one genus.

P. ruber was described based on a single specimen, since then it have not been studied again, this could have prevent it to be included in *Epiperipatus* by Clark (1913) or Peck (1975); the number

of accessory tooth on both jaws, the inner jaw denticles number and the position of the nephridial tubercle served Fürhmann, (1913) to consider it as a different species; nevertheless we found that these characters overlap with those of *P. biolleyi*. Tooth bear a significant intraspecific variation (Oliveira et al. 2010) in principal and accessory tooth as in denticles number (from 8 to 11), and in their shape, as is the case for the position of the nephridial tubercle; in the case of leg count we must highlight that the number of *P. ruber* sampled were lower than those of *P. biolleyi*, this could explain the differences found, more sampling could probe an overlap in leg number as often is the case with cryptic species (Daniels et al. 2008) or confirm our findings, for the above reasons we excluded these characters. We found that the novel features proposed by Oliveira et al. (2012a) helped to distinguish between species better than traditional ones from Bouvier (1902; 1905) and still used (Contreras-Félix et al. 2018; Sampaio-Costa et al. 2018). Nevertheless Fürhmann (1913) correctly differentiate *P. ruber* as another species, our phylogenetic and morphological analysis back this up: *P. biolleyi* and *P. ruber* differ in the shape of interpedal structures; the absence of type I crater shaped papillae on the former; type II crater-shaped papillae shape; the morphology of primary papillae scales. Individuals of a "*E. biolleyi*" from the locality of Los Juncos, Cascajal de Coronado, San José, Costa Rica were studied by Oliveira et al (2012a), most of its characters are consistent with *P. biolleyi* and *P. ruber*, but it have some differences like the number of scales on the collar of the type II crater shape papillae and the rudimentary apical piece present on both crater shaped papillae, both topologies group it within the *P. biolleyi* clade, but fails resolve its status with the analyzed genes; it could be either a different species or a case of intraspecific variation; we did not include crater shaped papillae morphology as diagnostic for the genus nor the primary scale shape, as they seem only useful at species level. Other specimens of close localities fall within one of both species complex and could constitute cryptic species, but this need to be tested with the use of more genes especially the more conserved 18S, and morphology. Anyway we can state that *P. biolleyi* and *P. rube*r are different species.

Our phylogenetic analysis revealed congruent clades divided by geographic regions on both topologies, the only differences were in the position of the brazilian *E. diadenoproctus*, that were

grouped within *E. acacioi* on the Neopatida topology and with *Epiperipatus adenocryptus* on the Peripatidae one in both with low support; the other difference was the position of *E. vagans*, in the Neopatida topology it grouped within the Costa Rican-Panamanian clade with a low support, while on the Peripatidae tree it almost reaching the monophyly threshold joining it with the clade of species from Brazil, French Guiana and Guyana. Giribet et al. (2018) discovered this grouping variation when they analyzed their trimmed Peripatidae dataset (that includes specimens of *Eoperipatus* and *M. tholloni*); as we also experienced this grouping of *E. vagans* when we included the african species, hence differences on both topologies could be caused by the long branches between *M. tholloni* and neopatids (reason why it was excluded on our dataset too as the previous authors did). Our trees showed that species classified as *Epiperipatus* and *Peripatus* are grouped with other genera in clades divided by countries instead of by genera as Giribet et al. 2018 found; in the case of the former Costa Rican-Panamanian species are unrelated to the type species of the genus (*E. edwardsii*); the problem with *Peripatus* is that DNA of the type species (*P. juliformis*) is unavailable because specimens have not been found despite collecting efforts, but it is likely that this species is grouped within the Caribbean Islands clade (Giribet et al. 2018) which in turn is nested outside the Costa Rican-Panamanian clade –our representative from the islands is separated with a maximum support from them–. We considered these facts as a probe that both *P. biolleyi* and *P. ruber* are a new genus rather than a representative from *Epiperipatus* or *Peripatus*, thus it is improbable that both genera inhabit these countries.

Giribet et al. (2018) proposed two solutions to the problematic taxonomy of peripatids: re-adopt Bouvier's system with only *Oroperipatus* and *Peripatus* genera dividing Neopatida; or continuing erecting numerous genera; although they prefered to leave the imperfect system intact while specimens of *Heteroperipatus*, *Speleoperipatus* and *Typhloperipatu*s are collected and got their DNA sequenced; nonetheless we favored none of these options. We considered that because species group by geographic zone it will be more appropriate to erect genera by geographic location, as it is urgent to appropriately describe the unknown species and grouping them within the extant genera would be erroneous, senseless and subjected to a future rearrangement; this have the advantage of

making communication easier with governmental agencies, environmental managers and decision takers on conservation measures.

The high complexity presented on Onychophoran morphology can be only explored using costly technology like SEM; however when appropriately used it allows proper taxonomic identifications (Read, 1988b). The utility of color patterns for identification of neopatids is considered as a temporal solution (Barquero-González et al. 2016b; Sosa-Bartuano, Monge-Nájera & Morera-Brenes, 2018), that demand a posterior taxonomic backup and is constrained by the occurrence of cryptic species or species with a wide arrange of color patterns (Daniels et al. 2009; Oliveira et al. 2012a). In this study, color distinction was useful, as *Picadopatus* is the only known Costa Rican genera with a red dorsal color and greyish legs, the only similar species is *P. solorzanoi* but it presents yellow legs and is much more robust and big, in addition its only found on Caribbean lowlands while *Picadopatus* is found on Central valley highlands.

This highland habitats are vulnerable to destruction and alteration mainly due to urbanization, a known threat to onychophoran because their lack of resilience to severe environmental changes and their small populations (New, 1995; Mesibov & Ruhberg, 1991); although some species can thrive in such environments, they are conditioned by the occurrence of appropriate patches (Cupul-Magaña & Navarrete-Heredia, 2008; Barrett et al. 2016; Toledo-Matus et al. 2018; Monje-Nájera, 2018). In the case of *Picadopatus*, both species subsist since their discovery and are regularly found. Collection localities have retained similar forest patches since 35 years ago (Monge-Nájera & Morera-Brenes pers.comm.), and are capable of allowing individual exchanges between populations. The conserved morphology suggests a recent species divergence event. The localities of Goicochea and Vázquez de Coronado shelter two onychophoran species within a small area (10 km), a pattern observed in Drake Bay at the South (Barquero-Gonzalez, Monge-Nájera & Morera-Brenes, 2018) and in Guayacan of Siquirres on the Caribbean zone of the country (Kubicki pers. comm.); this exemplifies the tendency of onychophorans towards endemism and the high species diversity within Neopatida (Sampaio-Costa et al. 2009; Oliveira et al 2010). Hence conservation programs must target the maintenance of

landscapes that promotes speciation. Invertebrates have been excluded from conservation policies (Ferrier et al. 1999; Dunn, 2005), but they can elucidate these kinds of processes, which are also congruent with diversity and endemism in other phylums (Sato et al. 2018), here we showed that this region favors speciation and have to be protected.

Both species experience threats that include direct collection by locals to be sold or of the moss they live in (used for Christmas decorations), those activities are in need for a better regulation. Nevertheless we considered that while the accelerated urbanization and logging practices are controlled, there is no reason why *Picadopatus* species cannot thrive another 100 years or more. Finally the genetic Bayesian inference and geographic distance between the type species of the genus *Epiperipatus* and the species from Costa Rica, show that this genus does not occurs in the country and instead masks a high diversity including cryptic species. Costa Rica has a remarkably rich endemic undescribed Onychophoran fauna in need for urgent studies like the present.

Capítulo 3.

COMPORTAMIENTO ALIMENTICIO DE LOS ONICÓFOROS DE COSTA RICA

Feeding behavior of Costa Rican velvet worms: food hiding, parental feeding investment and ontogenetic diet shift (Onychophora: Peripatidae)

The basic feeding behavior of onychophorans has been known for over a century: they capture prey with aid of an adhesive ejected by the slime papillae located near the mouth, by using their teeth to penetrate the prey carcass they ingest the partially digested tissues (Moseley, 1874; Bouvier, 1905; Manton, 1937; Manton & Heatley, 1937; Lavallard & Campiglia, 1971; Ruhberg &Storch 1977; Read & Hughes 1987; Baer & Mayer 2012). The adhesive is metabolically valuable (glue reserves take 19 days to be replenished after exhaustion) and remains adhering to the prey are re-ingested (Read & Hughes, 1987). In the species *Euperipatoides rowelli* Reid, 1996 prey are hunted collectively and consumed in hierarchical order according to size. Nevertheless, feeding behavior has only been studied in adults few of over 200 named species (Read & Hughes, 1987; Reinhard & Rowell, 2005; Mayer et al. 2015). Here we report, for the first time in the phylum Onychophora, food hiding behavior, parental feeding investment and an ontogenetic shift in feeding habits diets of juveniles.

From December 2016 to January 2018, individuals from eight morph-species of onychophorans from the sub-family Neopatida were housed in separate plastic containers terraria (collection permit codes: SINAC-SE-CUSBSE-PI-R-133-2016 and SINAC-SE-CUSBSE-PI-R-015-2017). The full names and geographic origin of species are detailed in table 5 (see Barquero-González et al. 2016a; Sosa et al. 2018 for details). Terraria used consisted on plastic containers (size 33 x 20 x 12 cm for seven species) containing 2 cm dirt, mosses, twigs and bark; for the Gandoca onychophoran (Sosa et al. 2018) a larger terrarium (size 45 x 29 x 39 cm with 3 cms of dirt) was used. Each terrarium contained from one to three females (identified by leg counting and observed parturitions) and various newborns, rarely one male, and were kept with natural light and constant humidity of

99% and temperature of 25-27°C at day and 22-23°C at night (Inkbird Thermometer & Hygrometer ITH-10). Morph-species were fed once a week with live or freshly killed crickets and took descriptive notes on their feeding behavior (Table 5).

Fig. 12. Quesada Burgundy Brown Onychophoran morph-species feeding sequence includes the use of adhesive to secure prey (A: adult female inspecting a dead cricket that we placed in the terrarium; before eating, she applied adhesive nonetheless), and hiding unfinished prey (she used her mandibles to drag it under a piece of wood, B-C). Adults can feed simultaneously, without aggression, on different prey parts (D).

The first author observed feeding behavior of all morph-species. Food was introduced from 19:00 to 20:00 h. Prey not consumed within this interval was left there for 12 hours more, and then removed to prevent contamination. Small prey were dragged, or pieces of larger prey were carried in the mouth, to be hidden in burrows, under moss or objects (San Carlos (N=3), Drake (N=5) and Fortuna (N=2), Fig. 12B, C), food hiding has evolved independently in some invertebrates, including Silphid beetles (Lawrence, J. F. & Newton, A. F., Jr. 1995), and invertebrates (e.g. lions, see Estes, 1991). Hiding unfinished prey must allow onychophorans, which hunt only a few times per month and are slow to process food (Read & Hughes, 1987), to protect this resource from scavengers and predators.

TABLE 5

Details on individuals of each species kept in terraria for the studies with their feeding preferences.

Species	**Time kept**	**Adult individuals kept**	**Head first feeding**	**Thorax first feeding**	**Abdomen first feeding**
Agujas Purple Brown Onychophoran	5 months	2 (plus 1 offspring)	2	2	6
Gandoca Blue Onychophoran*	5 months	1 (plus 7 offspring)	3	5	0
Quesada Burgundy Brown Onychophoran	5 months	3 (plus 2 offspring)	8	1	0
Sarapiquí Yellow Brown Onychophoran	1 month	2 (plus 1 offspring)	2	0	0
San Vito Collared Onychophoran*	2 months	2 (plus 3 offspring)	1	0	4
Fortuna Burgundy Brown Onychophoran*	2 months	2 (plus 1 offspring)	4	2	0
Batán Burgundy Brown Onychophoran*	1 month	3 (plus 1 offspring)	3	0	0
Epiperipatus biolleyi	1 month	5 (plus 7 offspring)	4	0	0
Peripatus solorzanoi	5 months	3 (plus 5 offspring)	6	2	0

During the feeding process, adults could also feed on different prey parts without aggression (Fig. 12D), additional studies about their genetic relationship could yield interesting results (Monge-Nájera, 1995), which would explain why they shared prey. Specimens of the kept morph-species from Gandoca started the feeding process on the thorax (Fig. 13A); while Batán, Quesada, Sarapiquí and Fortuna, as well as *Epiperipatus biolleyi* and *Peripatus solorzanoi* started feeding by the prey head (Fig. 13B); Agujas and San Vito species preferred the abdominal region (Fig. 13C).

During their first two weeks of life, the young only fed on the adhesive threads used to capture prey by the mother, rather than on the prey itself; and after those two weeks, adult females of the Gandocan morph-species and *P. solorzanoi* shared the prey with their offspring or the young captured prey by themselves. The consumption of the slime from their mother may perhaps represent parental feeding investment, solitary adult onychophorans are used to consume their own slime after catching and feeding on a prey (Read & Hughes, 1987) and can be aggressive at feeding in group (Reinhard & Rowell, 2005). If indirect fitness could have played a role at shaping this trait in onychophoran evolutionary history have to be considered for future studies.

An ontological diet shift is present in a variety of organisms; from zooplankton to butterflies and turtles. The shift can be either continuous as in scorpions (Polis, 1984) or discrete as in butterflies and reptiles (Werner & Gilliam, 1984). In the case of onychophorans, by weight their slime consists of 90 % water and 10 % proteins, carbohydrates and lipids (Corrales-Ureña et al., 2017), Read & Hughes (1987) considered in their studies that glue and prey flesh had similar energetic values, which can be enough for the requirements of a newborn onychophoran, however this have not tested.

We suggest two hypotheses for the emergence of the diet shift, based on the fact that we have observed the young feeding on slime although the prey carcass is available to fed on: (1) feeding on the slime might be an adaptation to an immature digestive system. The digestive enzymes may not be developed enough to digest prey in a practical time, or more moultings could be needed for mandibles to become hard enough to cut the prey's cuticles. (2) Energetically, the maternal adhesive slime might

be a better option than the prey itself, if its peptides are recycled. This might be similar to web consumption in orb-weaver spiders (Opell, 1998), but have to be tested.

Fig. 13. Some onychophorans start feeding by removing the head (e.g. *P. solorzanoi*, A), others by opening the abdomen (San Vito Onychophoran, B), and others by separating the thorax (Fortuna Onychophoran, C).

CONCLUSIONES

El presente trabajo demuestra que existen bastantes incógnitas respecto a la biología de los onicóforos, muchas de ellas precisan de estudios urgentes ya que son fundamentales para ejercer una conservación exitosa del filo. Hemos evaluado el caso de dos especies de una pequeña área de Costa Rica, y descubrimos que aspectos tan básicos como establecer cuáles y cuántas especies habitan una zona tan delimitada son cuestiones más complejas de lo que originalmente se pensaba, esto debido a la existencia de especies crípticas.

Mostramos que el grado alto de endemismo reportado para el filo en otros países también está presente en Costa Rica, más aun probamos que en distancias tan pequeñas como la muestreada (10 kilómetros), podemos hallar varios complejos de especies compartiendo hábitats, por lo que debe considerarse a esta como una localidad que ha promovido la especiación de onicóforos.

Los resultados del presente trabajo señalan que para distinguir a nivel de especie, los caracteres propuestos por Oliveira et al. (2012b) son más apropiados para la identificación, que los caracteres usados tradicionalmente por Bouvier (1902,1905) o Read (1988a); sin embargo la identificación morfológica a nivel de género parece ser aun elusiva, ya que son pocos los caracteres definitorios que se pueden usar, esto es causado por la alta variabilidad interespecífica y por las identificaciones erróneas que se han hecho tradicionalmente.

Otro hallazgo relevante fue la confirmación de que las muestras de todos los onicóforos Neotropicales se agrupan por región geográfica en lugar de por género, esto debilita inmensamente la validez de los géneros actualmente aceptados; sugiriendo que aquellos que tienen una amplia distribución, constituyen en realidad complejos diversos de géneros agrupados artificialmente dentro de unos pocos. En este caso las especies pertenecientes a *Epiperipatus, Macroperipatus* y *Peripatus* estarían restringidas a sus localidades tipo y cerca de estas, y el resto constituirían géneros nuevos aun sin describir, esto incluye a las especies de estos tres géneros reportadas en Costa Rica.

Finalmente podemos asegurar que el país alberga una rica fauna endémica de onicóforos aun sin describir, la cual no ha recibido la atención que merece y podría estar en potencial peligro, en especial si tomamos en cuenta que la biología de estos animales limita su capacidad de dispersión. Aunque en el caso de las especies estudiadas, la proximidad de asentamientos humanos y las consecuentes alteraciones, incluyendo ganadería y agricultura, no parecen haber diezmado a las poblaciones locales, sería recomendable darle una importancia especial al lugar, ya que como se pudo probar es un sitio que promueve la especiación de invertebrados que poseen este estilo de vida.

RECOMENDACIONES

Se debe dar mayor importancia a los invertebrados al establecer áreas de interés de conservación, ya que pueden elucidar sitos que favorecen procesos de especiación de fauna con limitada capacidad de desplazamiento y con requerimientos muy específicos.

Es necesario retomar la taxonomía de los onicóforos, utilizando estudios morfológicos, genéticos y de comportamiento, para esclarecer la diversidad de este filo, y comprender como protegerla de manera más eficiente.

Los resultados de investigaciones de este calibre deben traducirse y comunicarse de forma que el público general pueda conocerlos y saber su importancia para el país, y no solamente restringirse a publicaciones científicas.

Es necesario estudiar los sitios que se han reportado que podrían albergan varias especies simpátricas de onicóforos para confirmar o rechazar dicha afirmación, y proponer las acciones pertinentes para protegerlas.

Los sitios que se eligen para protección de la diversidad deberían ser los sitios que se demuestra que poseen mayor potencial para promover fenómenos de especiación.

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ANEXOS.

MATERIAL SUPLEMENTARIO

TABLE S1.

Partition for each studied gene.

Gene	Partition
12S	GTR + I + G
16S	GTR + I + G
18S	GTR + I + G
COI	GTR + I + G

Partitions found for the four studied genes using Jmodeltest 1.8.

TABLE S2.

Definition of the morphological terms used for Onychophora species description following Reid (1996) and Oliveira et al. (2010, 2014).

Term	Definition
Accessory papilla	Rudimentary dermal papillae an apical piece, generally smaller and located between two primary papillae.
Anal glands	Paired genital glands present only in the males, their exterior openings are via two separate openings in the case of most peripatids.
Bean-shaped papilla	A large bean-shaped papillae is located in a pouch in the dorsal part at each leg above the foot, it occurs only in the neotropical species of Peripatidae.
Crater shaped papilla	Dermal papillae that show a central depression which is surrounded by a collar of scales, some of them present rudimentary apical pieces, there are two types: type I crater-shaped papillae and type II crater-shaped papillae.
Crural tubercle	Structures which resemble a papillae, they contain the crural gland openings, occurring in males on the family Peripatidae.

Dorsomedian furrow	A cuticular furrow along the dorsal midline.
Foot papilla	Refers to the papillae on each foot, they are present on the distal or basal region.
Genital pad	A circular pad composed of papillae, that enclose the genital opening; happening between the penultimate pair of legs in the family Peripatidae.
Hyaline organ	Paired repeated structures at each side of the dorsomedian furrow, each of them lies between two adjacent plicae.
Interpedal structures	Paired segmental structures covered with a granulated cuticle; they can be found between the 5th and 6th ventral plicae along the midline.
Nephridial tubercle	Structure resembling a papillae were the nephridial opening occur; on the fourth and fifth leg pairs it happens as a tubercle placed distally on or between the spinous pads.
Preventral organs	Smaller segmental organs along ventral midline anterior to the ventral organs.
Primary papilla	Dermal papillae with a sensory bristle, usually larger than accessory papillae; it consists of an apical piece and a basal piece.
Setiform ridges	Paired regions of the ventral foot surface with various sensory bristles; there are two paired setiform ridges on each foot, the distal and the proximal one.
Spinous pads	Pads situated distally on the ventral part of the legs, they bear an spinous texture with many bristles occurring on each pad, they are usually arc-shaped and decrease in size distally.
Type I sensillum	Antennal sensillum with a prominent apical piece covered with scales and a textured bristle.
Type II sensillum	Antennal sensillum only composed of a texture bristle.
Ventral organs	Structure of a small size and a bright appearance situated ventrally between each leg pair.

TABLE S3.

Number of collected specimens of the type localities analyzed by each method.

Methodology	**Type specimens analyzed**	
	Picadopatus biolleyi	***Picadopatus ruber***
Colour pattern analysis	5	4
Light microscopy	5	4
Scanning electron microscopy	3	4

Methodologies and number of the studied specimens from the new genus.

TABLE S4.

Pairs of legs count of the studied specimens.

	Males		**Females**		
Species complex	27 pairs	28 pairs	29 pairs	30 pairs	31 pairs
P. biolleyi	3	4	2	10	5
P.ruber	3	-	-	-	6

Details of the number of appendices by sex and species are provided, males present less pairs of legs than females, no overlap occurred on the sampled specimens.

TABLE S5.

Antennal ring count of the studied individuals of the genus

	Antennal rings						
Species complex	36	37	38	39	40	41	42
P. biolleyi	2	4	6	2	3	5	2
P.ruber	-	2	1	1	3	-	2

Details of the number of antennal ring for each species of the genera.

TABLE S6

95% HPD Interval for Neopatida

Parameter	**Mean**	**Variance**	**Lower**	**Upper**	**Median**	**min ESS***	**avg ESS**	**PSRF+**
TL{all}	2.017.866	0.010336	1.823.228	2.220.936	2.015.036	3021.17	3158.97	1.000
r(A<->C){1}	0.160711	0.000722	0.109657	0.214140	0.159517	2159.98	2511.51	1.000
r(A<->G){1}	0.151090	0.001217	0.089109	0.222634	0.147386	1674.80	1928.24	1.000
r(A<->T){1}	0.145097	0.000362	0.108234	0.182608	0.144205	3372.20	3522.31	1.000
r(C<->G){1}	0.015298	0.000211	0.000001	0.044119	0.010984	3950.85	4113.03	1.000
r(C<->T){1}	0.512427	0.001794	0.430648	0.596359	0.512652	2037.67	2162.16	1.000
r(G<->T){1}	0.015378	0.000086	0.000168	0.032965	0.013887	4819.78	4910.03	1.000
r(A<->C){2}	0.014232	0.000103	0.000015	0.033623	0.012285	4305.67	5023.89	1.000
r(A<->G){2}	0.467754	0.003424	0.349741	0.577066	0.469137	1373.50	1435.44	1.000
r(A<->T){2}	0.144083	0.000722	0.093914	0.196735	0.142639	1591.76	1703.46	1.000
r(C<->G){2}	0.030834	0.000541	0.000013	0.076309	0.025382	3768.56	3775.88	1.000
r(C<->T){2}	0.237666	0.002998	0.138721	0.348288	0.233075	1440.18	1449.61	1.000
r(G<->T){2}	0.105431	0.000738	0.054447	0.158723	0.103569	2227.17	2331.49	1.000
r(A<->C){3}	0.022013	0.000063	0.007757	0.037442	0.021284	4114.72	4192.74	1.000
r(A<->G){3}	0.070182	0.000129	0.048964	0.092789	0.069319	3351.18	3360.74	1.000
r(A<->T){3}	0.093398	0.000290	0.061542	0.127138	0.092459	2605.05	3043.64	1.000
r(C<->G){3}	0.008615	0.000013	0.002024	0.015557	0.008253	5913.71	6348.48	1.000
r(C<->T){3}	0.754734	0.000763	0.697128	0.805115	0.756298	1951.78	2329.46	1.000

r(G<->T){3}	0.051058	0.000067	0.035591	0.067389	0.050524	4981.58	5252.53	1.000
r(A<->C){4}	0.001959	0.000004	0.000001	0.005909	0.001344	4498.94	4757.48	1.000
r(A<->G){4}	0.632815	0.001145	0.563788	0.696821	0.634433	1135.92	1274.67	1.000
r(A<->T){4}	0.078910	0.000077	0.061839	0.095900	0.078549	2944.76	3091.03	1.000
r(C<->G){4}	0.032714	0.000166	0.010407	0.058697	0.031146	2616.67	2848.89	1.000
r(C<->T){4}	0.175210	0.000878	0.121205	0.235504	0.172477	1101.54	1118.47	1.000
r(G<->T){4}	0.078392	0.000173	0.053812	0.105055	0.078015	3175.52	3264.70	1.000
pi(A){1}	0.448602	0.000539	0.403691	0.494322	0.448463	4184.51	4382.98	1.000
pi(C){1}	0.098198	0.000134	0.075528	0.120555	0.097630	3139.92	3268.38	1.000
pi(G){1}	0.084747	0.000238	0.055703	0.115690	0.084055	2407.04	2612.78	1.000
pi(T){1}	0.368454	0.000490	0.326394	0.412347	0.367931	3601.20	3725.40	1.000
pi(A){2}	0.423412	0.000560	0.378026	0.470573	0.423560	3638.51	4036.47	1.000
pi(C){2}	0.083786	0.000220	0.054980	0.112655	0.083117	2480.43	2526.18	1.000
pi(G){2}	0.127587	0.000375	0.091859	0.165768	0.126291	1439.65	1459.32	1.000
pi(T){2}	0.365215	0.000584	0.317101	0.411453	0.364842	3267.06	3464.48	1.000
pi(A){3}	0.180796	0.000091	0.163016	0.200146	0.180668	5872.38	6244.48	1.000
pi(C){3}	0.277810	0.000104	0.258100	0.298053	0.277747	6463.22	6685.83	1.000
pi(G){3}	0.305655	0.000111	0.285031	0.326410	0.305598	7782.17	8006.13	1.000
pi(T){3}	0.235739	0.000101	0.215868	0.255347	0.235439	5886.71	6212.02	1.000
pi(A){4}	0.321165	0.000227	0.291876	0.350559	0.321011	2471.90	2944.61	1.000
pi(C){4}	0.117506	0.000200	0.090562	0.145140	0.117088	1377.30	1390.10	1.000
pi(G){4}	0.111661	0.000056	0.097279	0.126524	0.111404	3858.89	3900.65	1.000
pi(T){4}	0.449667	0.000318	0.415232	0.485327	0.449817	3211.74	3505.04	1.000
alpha{1}	0.442661	0.003027	0.338728	0.550963	0.438448	15070.90	15096.26	1.000
alpha{2}	0.282185	0.000979	0.221874	0.343396	0.279996	8581.08	8690.29	1.000
alpha{3}	0.152411	0.000059	0.137483	0.167583	0.152096	8302.86	8883.70	1.000
alpha{4}	0.185165	0.000289	0.153060	0.218912	0.184370	9932.12	10267.86	1.000
pinvar{1}	0.059304	0.000410	0.023130	0.099766	0.057135	10938.19	12046.20	1.000
pinvar{2}	0.054686	0.000215	0.027918	0.083948	0.053452	17081.13	17438.55	1.000
pinvar{3}	0.071965	0.000202	0.044643	0.099690	0.071118	10406.57	11521.04	1.000
pinvar{4}	0.126128	0.000405	0.087499	0.165838	0.125465	12368.66	13083.02	1.000

Convergence diagnostic minimum and average values for parameter sampled in both runs for the Neopatida dataset with 95% confidence interval.

TABLE S7

95% HPD Interval for Peripatidae

Parameter	Mean	Variance	Lower	Upper	Median	min ESS*	avg ESS	PSRF+
TL{all}	3.453.869	0.055334	3.059.078	3.971.167	3.437.512	151.33	162.70	0.999
r(A<->C){1}	0.143670	0.000220	0.112027	0.174209	0.144139	230.88	265.08	1.000
r(A<->G){1}	0.146939	0.000336	0.120530	0.189396	0.145167	284.32	324.66	0.999
r(A<->T){1}	0.145793	0.000192	0.120245	0.172679	0.145167	189.17	277.08	1.001
r(C<->G){1}	0.013378	0.000118	0.000062	0.031224	0.011238	310.50	332.43	1.001
r(C<->T){1}	0.536348	0.001452	0.461305	0.606849	0.537986	242.40	303.70	0.999
r(G<->T){1}	0.013871	0.000064	0.000348	0.028258	0.012560	249.53	307.26	1.004
k_revmat{1}	3.816.438	0.553365	3.000.000	5.000.000	4.000.000	321.58	343.29	1.001
r(A<->C){2}	0.014417	0.000157	0.000072	0.036588	0.011873	240.32	256.42	0.999
r(A<->G){2}	0.388183	0.002853	0.327872	0.553022	0.373838	73.73	82.76	1.000
r(A<->T){2}	0.098517	0.000309	0.070622	0.136718	0.095639	110.26	131.48	1.000
r(C<->G){2}	0.060593	0.001185	0.003685	0.104293	0.072013	128.38	133.37	1.005
r(C<->T){2}	0.347763	0.003635	0.178209	0.427852	0.365721	68.09	81.19	0.999
r(G<->T){2}	0.090527	0.000449	0.048578	0.134173	0.089349	138.67	161.52	0.999
k_revmat{2}	3.838.356	0.574658	3.000.000	5.000.000	4.000.000	190.32	226.88	1.000
r(A<->C){3}	0.032272	0.000134	0.014353	0.054231	0.029922	298.24	331.62	1.008
r(A<->G){3}	0.515567	0.002896	0.418899	0.623750	0.515679	140.86	220.34	0.999
r(A<->T){3}	0.123256	0.000606	0.079131	0.179698	0.119671	224.22	275.40	1.000
r(C<->G){3}	0.028352	0.000050	0.015116	0.042277	0.028177	278.64	307.13	0.999
r(C<->T){3}	0.181032	0.001758	0.109790	0.258276	0.177993	263.81	284.32	0.999
r(G<->T){3}	0.119520	0.000498	0.075043	0.168800	0.118419	171.63	268.32	1.000
k_revmat{3}	4.331.507	0.499006	3.000.000	6.000.000	4.000.000	365.00	365.00	0.999
r(A<->C){4}	0.002679	0.000009	0.000001	0.008485	0.001655	338.06	351.53	0.999
r(A<->G){4}	0.481453	0.003430	0.398674	0.582934	0.488040	38.44	58.20	0.999
r(A<->T){4}	0.066094	0.000061	0.053981	0.083621	0.065224	126.64	164.62	1.003
r(C<->G){4}	0.056034	0.000221	0.019239	0.077406	0.060143	163.90	193.45	0.999
r(C<->T){4}	0.330186	0.004178	0.229358	0.425855	0.318409	38.01	58.77	0.999
r(G<->T){4}	0.063554	0.000074	0.047815	0.081747	0.063640	177.95	182.84	1.000
k_revmat{4}	4.258.904	0.592685	3.000.000	5.000.000	4.000.000	124.83	162.01	0.999
pi(A){1}	0.433616	0.000395	0.396638	0.471546	0.434000	247.17	301.47	1.001
pi(C){1}	0.098804	0.000083	0.080225	0.114631	0.098503	365.00	365.00	0.999
pi(G){1}	0.088313	0.000125	0.065760	0.108287	0.087785	270.36	303.90	1.000

pi(T){1}	0.379268	0.000310	0.345252	0.413807	0.378502	306.96	330.78	0.999
pi(A){2}	0.455955	0.000486	0.412595	0.497505	0.457298	160.35	170.43	0.999
pi(C){2}	0.061415	0.000203	0.040598	0.093629	0.058730	72.08	84.25	1.001
pi(G){2}	0.119388	0.000134	0.098497	0.142482	0.118956	365.00	365.00	0.999
pi(T){2}	0.363243	0.000495	0.316998	0.401351	0.362645	271.24	281.08	1.000
pi(A){3}	0.142985	0.000154	0.118681	0.166433	0.143124	338.06	351.53	0.999
pi(C){3}	0.424520	0.000221	0.394846	0.452623	0.425506	284.64	290.24	1.000
pi(G){3}	0.141961	0.000137	0.120285	0.164742	0.141596	323.42	344.21	1.000
pi(T){3}	0.290534	0.000176	0.265856	0.316214	0.290557	302.04	333.52	0.999
pi(A){4}	0.351795	0.000218	0.320868	0.377792	0.352075	98.81	114.34	0.999
pi(C){4}	0.078224	0.000127	0.058878	0.099679	0.078142	62.39	86.35	0.999
pi(G){4}	0.134744	0.000059	0.118891	0.148208	0.134596	208.73	232.59	1.002
pi(T){4}	0.435238	0.000241	0.406486	0.466182	0.435510	230.67	297.84	1.000
alpha{1}	0.473108	0.002909	0.378385	0.584651	0.469402	258.54	311.77	0.999
alpha{2}	0.284130	0.000798	0.227932	0.336888	0.282413	270.65	299.23	0.999
alpha{3}	0.191174	0.000177	0.170594	0.222865	0.190116	261.43	310.75	1.000
alpha{4}	0.156581	0.000145	0.134105	0.180479	0.156815	331.55	348.28	0.999
pinvar{1}	0.043424	0.000185	0.018948	0.068776	0.042385	364.23	364.62	0.999
pinvar{2}	0.055920	0.000279	0.026249	0.087701	0.053775	365.00	365.00	0.999
pinvar{3}	0.394517	0.000304	0.362099	0.429034	0.394668	365.00	365.00	0.999
pinvar{4}	0.061552	0.000127	0.039766	0.081770	0.060958	270.16	317.58	1.000

Convergence diagnostic minimum and average values for parameter sampled in both runs for the Peripatidae dataset with 95% confidence interval.

TABLE S8

Summary statistics of partitions

Statistics	Neopatida	Peripatidae
Average standard deviation of split frequencies	0.008872	0.010259
Maximum standard deviation of split frequencies	0.101521	0.048432
Average PSRF for parameter values (excluding NA and >10.0)	1.001	1.003
Maximum PSRF for parameter values	1.040	1.206

Summary statistics in combined chains of both datasets for partitions with frequency > = 0.05 in at least one run.

TABLE S9

Summary statistics

Statistics	Neopatida	Peripatidae
Mean	-16862.2967	-18260.9804
Standard error of mean	0.0929	0.0864
Standard deviation	9.8431	9.9772
Variance	96.8857	99.5435
Median	-16861.9543	-18260.5889
Value range	[-16912.0199, -16816.2136]	[-18317.3808, -18226.1897]
Geometric mean	n/a	n/a
95% HPD interval	[-16881.7343, -16843.3819]	[-18281.2059, -18242,1747]
Auto-correlation time (ACT)	1603.3804	1348.8003
Effective sample size (ESS)	11226.4	13345.3
Number of samples	1.8E5	1.8E5

Bayesian analysis summary statistics in combined chains of both datasets.

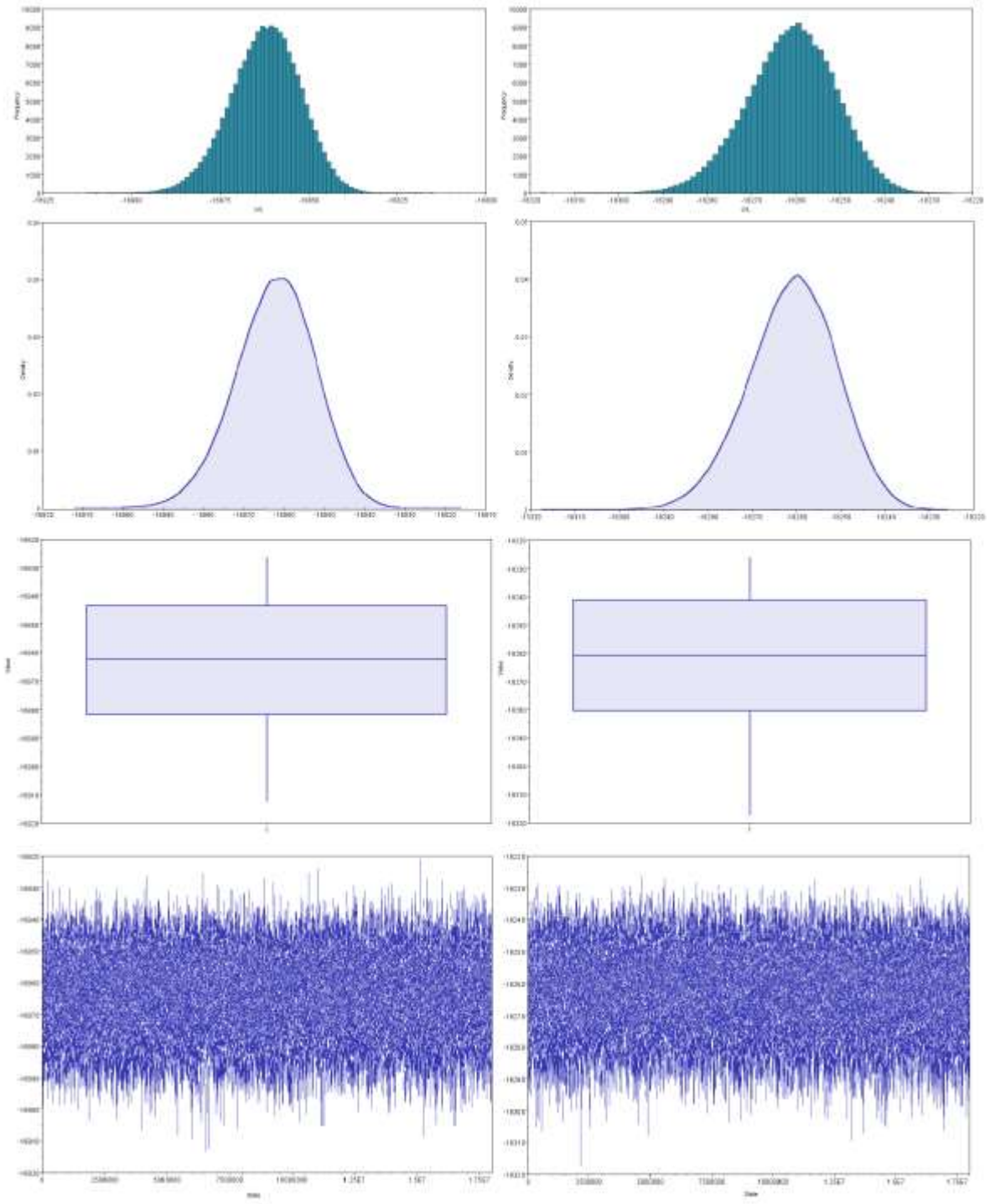

FIG S1. Convergence graphic representation of combined runs results: estimates parameters (a); marginal density's KDE (b) and box and whisker plot (c); trace (d). Right side: Neopatida dataset results; left side Peripatidae.