RESEARCH ARTICLE

Using the combined gene approach and multiple analytical methods to improve the phylogeny and classification of *Bombus* (Hymenoptera, Apidae) in China

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Abstract

Bumble bees are vital to our agro-ecological system, with approximately 250 species reported around the world in the single genus *Bombus*. However, the health of bumble bees is threatened by multiple factors: habitat loss, climate change, pesticide use, and disease caused by pathogens and parasites. It is therefore vitally important to have a fully developed phylogeny for bumble bee species as part of our conservation efforts. The purpose of this study was to explore the phylogenetic relationships of the dominant bumble bees on the Tibetan plateau and in northern China as well as their placement and classification within the genus *Bombus*. The study used combined gene analysis consisting of sequence fragments from six genes, 16S rRNA, COI, EF-1α, Argk, Opsin and PEPCK, and the phylogenetic relationships of 209 *Bombus* species were explored. Twenty-six species, including 152 gene sequences, were collected from different regions throughout China, and 1037 gene sequences representing 183 species were obtained from GenBank or BOLD. The results suggest that the 209 analyzed species belong to fifteen subgenera and that most of the subgenera in *Bombus* are monophyletic, which is in accordance with conventional morphology-based classifications. The phylogenetic trees also show that nearly all subgenera easily fall into two distinct clades: short-faced and long-faced. The study is the first to investigate the phylogenetic

placement of *Bombus turneri* (Richards), *Bombus opulentus* Smith, *Bombus pyrosoma* Morawitz, *Bombus longipennis* Friese, *Bombus minshanensis* Bischoff, and *Bombus lantschouensis* Vogt, all of which are widely distributed throughout different regions of China. The knowledge and understanding gained from the findings can provide a molecular basis to accurately classify *Bombus* in China and to define strategies to conserve biodiversity and promote pollinator populations.

Introduction

Bumble bees belong to the genus *Bombus*, which has been classified in the tribe Bombini of the subfamily Apinae of the family Apidae. Four sister tribes including Bombini, Apini (e.g., honey bees), Meliponini (e.g., stingless bees), and Euglossini (e.g., orchard bees) belong to the corbiculate clade within the family Apidae (Winston and Michener 1977). With other species of bees and pollinators, bumble bees provide pollination services to vegetable crops in large greenhouses and to a great diversity of plants in the wild, and contribute substantially to the agriculture economy (Velthuis and Van Doorrn 2006; Carolan et al. 2012; Williams et al. 2012a) and biological diversity of ecosystems (Wang and Li 1998; Sun et al. 2003; He and Liu 2004; Goulson et al. 2008; An et al. 2011; Vergara and Fonseca-Buendía 2012). To date, there are approximately 250 known species of bumble bees in the world, with approximately 125 species documented in China (Cameron et al. 2007; Williams et al. 2010; An et al. 2011). However, with degradation of the ecological environment, human activity, pathogen infection and exposure to pesticides, populations of bumble bees are declining in China, especially in northwest China (Yang 1999; Xie et al. 2008), with some species even becoming extinct in certain areas (Rasmont et al. 2005; Williams 2005; Colla and Packer 2008; Goulson et al. 2008). Therefore, it is very important to know the distribution and phylogenetic evolution of bumble bee species in these regions, to be able to design effective conservation strategies for their protection.

The taxonomic status of closely related bumble bee taxa is often unclear. In the early twentieth century, we relied on morphological characters to classify *Bombus*. However, because of highly variable color patterns and the presence of convergent evolution in morphology, it is difficult to accurately identify the species within *Bombus* based only on morphological features (Hines and Williams 2012). Subsequently, male genitalia have been used to distinguish the different subgenera; they are more reliable than color pattern in classifying subgenera, although they do not unambiguously distinguish between species under certain circumstances (Stephen 1957; Thorp et al. 1983). With the development of molecular techniques, identification based on molecular markers has become a powerful tool in the phylogenetic analysis and placement of species of *Bombus* (Michener 2007). However, it is critical to choose appropriate genetic markers in molecular phylogenetic reconstructions. Over the years, the genes

Cytochrome Oxidase subunit I (COI) and Cytochrome b (Cytb) have been used in phylogenetic studies on insects (Boehme et al. 2010; Wang and Qiao 2010). A specific COI fragment that is 648 bp long with enough genetic information and base variation has been used to effectively distinguish species (Hebert et al. 2003, 2004). COI has been widely applied to species identification and phylogenetics in insects (Pedersen 1996, 2002). Further, the mitochondrial gene 16S rRNA and nuclear genes elongation factor-1 alpha F2 copy (EF-1α), long-wavelength rhodopsin copy 1 (Opsin), arginine kinase (Argk), and phosphoenolpyruvate carboxykinase (PEPCK) have also been used for phylogenetic analysis (Kawakita et al. 2003, 2004; Cameron et al. 2007). The mitochondrial gene 16S rRNA is a useful marker for examining the phylogenetic position of some insects (Yoshizawa and Johnson 2003), and is the most informative for phylogenetic analysis of closely related species (Whitfield and Cameron 1998). EF-1α, which can promote aminoacyl-tRNA to combine with ribosomes, is often recognized as a good molecular marker to resolve the classification of insects at the phylogenetic level of family or genus (Cho et al. 1995; Mardulyn and Cameron 1999; Rokas et al. 2002; Danforth et al. 2004). The Opsin gene belongs to the family of the light absorption receptor proteins. It can distinguish evolutionary divergence in hymenopteran insects, including Cynipidae and Halictidae (Rokas et al. 2002; Danforth et al. 2004), and can be used to deduce the relationships between these and the corbiculate Apinae (Mardulyn and Cameron 1999; Cameron and Mardulyn 2001, 2003; Michel-Salzat and Whitfield 2004). ArgK is a kind of phosphate kinase which distributes broadly in the tissue of insects and is also a relatively conserved nuclear gene. The PEPCK sequence contains two parts, the high variation intron and the conserved exon, which are largely applied in the classification of the order Lepidoptera (Friedlander et al. 1996).

While advances in molecular marker techniques have led to significant improvements in population genetic analysis, the standard mitochondrial barcode fragment or nuclear genes are sometimes not informative enough to help understand the genetic variability of species. When multiple genes are combined for phylogenetic analysis, a much clearer view of the phylogeny among closely related species can be generated. Kawakita et al. (2004) elucidated the phylogeny of 65 species of bumble bees through the use of three nuclear genes, and analyzed their geographic distribution and character evolution. Later, Cameron et al. (2007) made a robust phylogeny with a comprehensive analysis of 219 species of bumble bees from all over the world, including some species from China, which utilized mitochondrial gene 16S rRNA and four nuclear gene sequences (Opsin, EF-1α, Argk, and PEPCK). Their results suggested that, overall, *Bombus* is monophyletic, with the subgenera grouped into two distinct clades, the short-faced and the long-faced, the first including a diverse New World clade. Their paper systematically analyzed the relationships among all subgenera and provided a foundation for the phylogeny of *Bombus*, although there remained some species that could not be included.

To improve our understanding of the phylogenetic relationships of *Bombus* in China, we conducted a phylogenetic analysis of 209 species by combining sequence fragments of two mitochondrial genes (COI and 16S rRNA) and four nuclear genes (EF-1α, Opsin, ArgK, and PEPCK). We obtained 152 gene sequences from 26 species recently collected
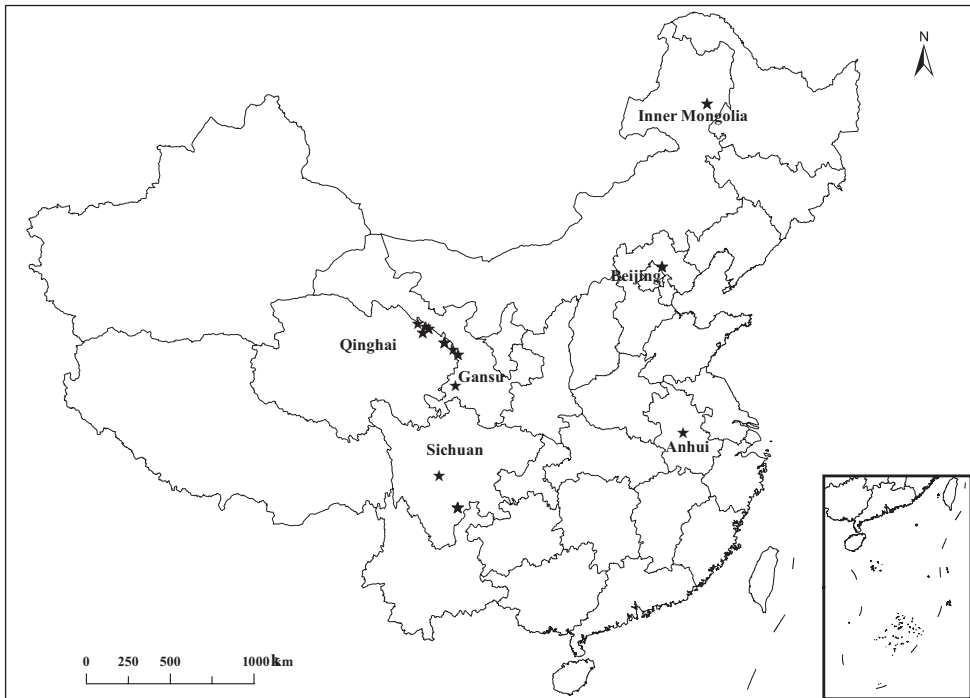
Figure 1. Sampling sites of *Bombus* spp. across different regions of China.

from different regions of China. An additional 1093 gene sequences representing 183 additional species of *Bombus* were obtained from GenBank or BOLD. Among the 26 recently-collected species, *B. pyrosoma* Morawitz and *B. lantschouensis* Vogt are two common native species and important pollinators, characterized by having more workers in the colony, by ease of rearing in an indoor environment, and by a widespread distribution in China (Peng et al. 2009). Through this study, we have gained an insight into Chinese bumble bee species distributions and phylogenetic relationships, which in turn could be applied to our efforts of biodiversity conservation to promote pollinator populations.

Materials and methods

Bumble bee collection

Bumble bee specimens were collected with nets and as a random sample at any given locality in the Sichuan, Inner Mongolia, Qinghai, Anhui, Gansu provinces and Beijing, China, between 2006 and 2012, and after capture were transferred directly into 100% ethanol. The samples were kept at -20 °C for subsequent analysis and voucher specimens are deposited at the Institute of Apicultural Research, Chinese Academy of Agricultural Science, Beijing, China. Exact collection localities are listed in Table 1 and shown in Fig. 1.

Table 1. *Bombus* samples collected during this study: classification, collectors, collection localities, voucher numbers, and GenBank accession numbers. "NA" indicates that the gene sequences could not be amplified by PCR.

Sample ID	Subgenus	Species	Province	Latitude and longitude	Altitude (m)	GenBank accession numbers					
						16S	COI	EF-1α	Opsin	Argk	PEPCK
BG104	Alpigenobombus Skorikov	kashmirensis Friese	Qinghai	37°59.967'N, 100°45.016'E	3214	KX791783	KX791757	KX791657	NA	KX791731	KX791707
BG017	Bombus s.s. Latreille	longipennis Friese	Qinghai	37°10.689'N, 102°03.091'E	2596	KX791770	KX791744	KX791644	KX791670	KX791720	KX791694
BG038		patagiatus Nylander	Inner Mongolia	48°41.426'N, 122°45.7'E	419	KX791787	KX791761	KX791661	KX791685	KX791735	KX791711
BG040		lucorum (Linnaeus)	Inner Mongolia	48°41.426'N, 122°45.7'E	419	KX791785	KX791759	KX791659	KX791683	KX791733	KX791709
BG054		lantschouensis Vogt	Gansu	36°34.728'N, 102°58.077'E	2096	KX791784	KX791758	KX791658	KX791682	KX791732	KX791708
BG177		ignitus Smith	Beijing	40°38.478'N, 117°15.188'E	314	KX791766	KX791740	KX791640	KX791666	KX791716	KX791690
BG191		minshanensis Bischoff	Gansu	34°54.161'N, 102°50.735'E	3138	KX791772	KX791746	KX791646	KX791672	KX791721	KX791696
BG133	Megabombus Dalla Torre	trifasciatus Smith	Sichuan	28°19.672'N, 103°07.999'E	2062	KX791778	KX791752	KX791652	KX791678	KX791727	KX791702
BG007	Melanobombus Dalla Torre	supremus Morawitz	Qinghai	37°41.413'N, 100°34.324'E	3146	KX791788	KX791762	KX791662	KX791686	KX791736	KX791712
BG029		sichelii Radoszkowski	Qinghai	37°10.689'N, 102°03.091'E	2596	KX791777	KX791751	KX791651	KX791677	KX791726	KX791701
BG078		rufofasciatus Smith	Qinghai	38°09.402'N, 100°11.705'E	2912	KX791776	KX791750	KX791650	KX791676	KX791725	KX791700
BG095		ladakhensis Richards	Qinghai	37°56.211'N, 100°57.941'E	3418	KX791768	KX791742	KX791642	KX791668	KX791718	KX791692
BG146		friseanus Skorikov	Sichuan	28°19.672'N, 103°07.999'E	2062	KX791765	KX791739	KX791639	KX791665	KX791715	KX791689
BG179		pyrosoma Morawitz	Beijing	40°38.478'N, 117°15.188'E	314	KX791774	KX791748	KX791648	KX791674	KX791723	KX791698
BG141		festivus Smith	Sichuan	28°19.672'N, 103°07.999'E	2062	KX791764	KX791738	KX791638	KX791664	KX791714	KX791688
BG003	Mendacibombus Skorikov	waltoni Cockerell	Qinghai	37°41.413'N, 100°34.324'E	3146	KX791789	KX791763	KX791663	KX791687	KX791737	KX791713
BG167	Psithyrus Lepeletier	turneri (Richards)	Anhui	31°49.021'N, 117°14.281'E	1700	KX791779	KX791753	KX791653	NA	KX791728	KX791703
BG025	Pyrobombus Dalla Torre	lepidus Skorikov	Qinghai	37°10.689'N, 102°03.091'E	2596	KX791769	KX791743	KX791643	KX791669	KX791719	KX791693
BG137		flavescens Smith	Sichuan	28°19.672'N, 103°07.999'E	2062	KX791782	KX791756	KX791656	KX791681	KX791730	KX791706
BG028	Subterraneobombus Vogt	personatus Smith	Qinghai	37°10.689'N, 102°03.091'E	2596	KX791773	KX791747	KX791647	KX791673	KX791722	KX791697
BG049		melanurus Lepeletier	Gansu	36°34.728'N, 102°58.077'E	2096	KX791771	KX791745	KX791645	KX791671	NA	KX791695
BG093		difficillimus Skorikov	Qinghai	37°56.211'N, 100°57.941'E	3418	KX791780	KX791754	KX791654	KX791679	NA	KX791704
BG060	Thoracobombus Dalla Torre	filchnerae Vogt	Gansu	36°49.855'N, 102°39.003'E	2210	KX791781	KX791755	KX791655	KX791680	KX791729	KX791705
BG153		impetuosus Smith s. l.	Sichuan	28°19.672'N, 103°07.999'E	2062	KX791767	KX791741	KX791641	KX791667	KX791717	KX791691
BG155		remotus (TKalcü)	Sichuan	30°02.905'N, 101°58.049'E	2833	KX791775	KX791749	KX791649	KX791675	KX791724	KX791699
BG172		opulentus Smith	Beijing	40°38.478'N, 117°15.188'E	314	KX791786	KX791760	KX791660	KX791684	KX791734	KX791710

Morphology

All species were identified according to the morphological characters of bumble bees as described by Williams (1998). Subgenera and species were authenticated by the characters of the genitalia and other key taxonomic characters such as body size, color pattern, and leg structure (Williams et al. 2009; An et al. 2014). The detailed morphological classification is presented in Suppl. material 1.

Genomic DNA extraction

For the extraction of nucleic acid, the muscle tissue of each individual bee's thorax was cleanly cut off with scissors, immediately put into an aseptic tube and ground in liquid nitrogen with a pestle. DNA was extracted from bee muscle tissue using a Wizard˚Genomic DNA Purification Kit (A1120, Promega). DNA extracts were kept at -20 °C until needed as a DNA template for the PCR.

PCR amplification and sequencing

The specific primers used to amplify the two mitochondrial genes (COI and 16S rRNA) and four nuclear genes (Opsin, EF-1α, Argk, and PEPCK) are shown in Table 2. PCR reactions were performed using a Mastercycler 5333 (Eppendorf) in 25 μL PCR Mix (2×), 2 μL template genomic DNA (about 50 ng), 1 μL of each primer (forward and reverse), 21 μL ddH$_2$O, with a final volume of 50 μL. PCR parameters for amplification were as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles denaturation at 94 °C for 1 min, annealing at 50–60 °C for 1 min, elongation at 72 °C for 1 min and final elongation at 72 °C for 10 min. The annealing temperatures for each gene were: 50 °C for PEPCK and Argk, 53 °C for EF-1α, 55 °C for 16S rRNA, 56 °C for COI, and 60 °C for Opsin. PCR products were electrophoresed in 1.2% agarose gel containing 0.5 μg/ml GoldView (GV) and visualized under UV light. PCR products were purified and then sent to Invitrogen for sequencing. After manual editing and error checking, we then performed a BLAST database search in GenBank to identify and include the closest matches of the same sequence for *Bombus* taxa. We obtained 152 valid sequences belonging to 26 *Bombus* species. The sequences used in this analysis have been deposited in GenBank. The list of sequences with their codes and the respective GenBank accession numbers can be found in Table 1.

Sequence analysis and construction of the phylogenetic tree

Altogether, 1245 gene sequences were used to conduct the phylogenetic analysis. One hundred and fifty-two (152) sequences from 26 bumble bee species collected during this study (Tab. 1) and 1037 sequences from 183 bumble bee species retrieved from GenBank or BOLD (see Cameron et al. 2007) were used to construct the phylogenetic tree. The Apini *Apis mellifera* Linnaeus and *Apis dorsata* Fabricius, the

Table 2. Primer information for the six genes used in this study.

Gene	Primer sequence (5'→3')	Reference
COI	ATTCAACCAATCATAAAGATATTGG (LepF)	Hebert et al. (2004)
	TAAACTTCTGGATGTCCAAAAAATCA (LepR)	
16S rRNA	CACCTGTTTATCAAAAACAT (16S Wb)	Williams et al. (2011, 2016)
	TATAGATAGAAACCAATCTG (16SIR)	
Opsin	AATTGCTATTAYGARACNTGGGT (Opsin-F)	Lin and Danforth (2004)
	ATATGGAGTCCANGCCATRAACCA (Opsin-R)	
EF-1α	GGRCAYAGAGATTTCATCAAGAAC (F2-ForH)	Franklin (1954)
	TTGCAAAGCTTCRKGATGCATTT (F2-RevH2)	
Argk	GTTGACCAAGCYGTYTTGGA (Argk1-F)	Kawakita et al. (2003)
	CATGGAAATAATACGRAGRTG (Argk1-R)	
	GACAGCAARTCTCTGCTGAAGAA (Argk2-F)	
	AGAACAATTATCTYAAATRCTAARCTTC (FHv5-F)	
	GGTYTTGGCATCGTTGTGGTAGATAC (Argk2-R)	
PEPCK	GTSTCTTATGGGAGSGGTTACGG (FH2-F)	Michel-Salzat and Whitfield
	TGTATRATAATTCGCAAYTTCAC (FHv4-F)	(2004)
	CTGCTGGRGTYCTAGATCC (RHv4-R)	

Meliponini *Liotrigona mahafalya* Brooks & Michener, *Heterotrigona itama* Cockerell, *Plebeia frontalis* Friese, *Trigona amazonensis* Ducke, *Geniotrigona thoracica* Smith and *Hypotrigona gribodoi* (Magretti) and the Euglossini *Eulaema boliviensis* (Friese) and *Euglossa imperialis* Cockerell were used as outgroups, as in Cameron et al. (2007).

The sequence data were aligned by ClustalX using default settings and visually checked using BioEdit (V7.0.9.0). We referred to Cameron et al. (2007), who had submitted the sequences to GenBank, and downloaded sequences of 16S rRNA, ArgK, EF-1α, Opsin, PEPCK and COI of bumble bees and outgroups from GenBank or BOLD. Phylogenetic analysis was conducted in MEGA 6.0 (Tamura et al. 2013).

Phylogenetic relationships were estimated by Bayesian analysis, maximum likelihood (ML) analysis, maximum parsimony (MP) analysis and Neighbor Joining (NJ) analysis, separately. Model selection for each gene was based on the Akaike Information Criterion (AIC) in Modeltest (Posada and Crandall 1998) and MrModeltest (Nylander 2004); the best model parameters for each gene partition were GTR+I+G. Bayesian analysis was performed using MrBayes v. 3.2.6 (Ronquist et al. 2012). Two independent Markov Chain Monte Carlo (MCMC) runs were conducted for 10 million generations, sampling every 1000 generations. Tracer v.1.6 was used to establish the convergence between two runs (Rambaut et al. 2014). Burn-in samples, which were the first 25% of the yielded trees, were discarded, then the remaining trees were used to generate a majority-rule consensus tree with posterior probabilities (PP). ML analysis were conducted using the GTRGAMMAI model of RAxML v.7.2.6. Node support was assessed via 1000 bootstrap replicates (Stamatakis 2006). MP analysis was performed using PAUP* v.4.0a165 (Swofford 2002). The tree bisection reconnection (TBR) branch swapping algorithm was used, and 100 random addition replicates were performed using heuristic strategy. Support values were assessed under the heuristic search with TBR and 100 jackknife replicates each with 100 random addition searches. NJ analysis was performed using MEGA v. 6.0 (Tamura et al. 2013). The phylogenetic trees were displayed and edited utilizing Figtree v.1.4.0.
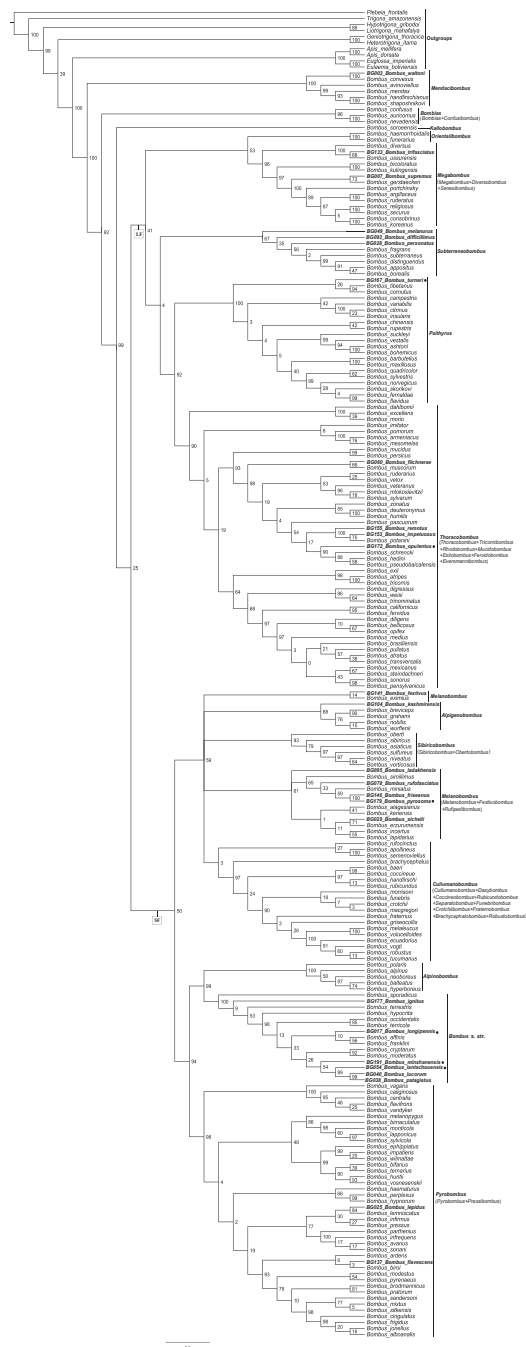
Results and discussion

Phylogenetic analysis

The results of the phylogenetic analysis of 209 *Bombus* species and ten outgroup species showed the same topology structure in two trees, which is similar to results in Cameron et al. (2007). The *Bombus* genus was divided into two distinct clades by Bayesian Inference (BI), ML and MP: the short-faced and the long-faced (Figs 2, 3). The morphological differences between the short-faced and long-faced clades are based on characters of the head including the length of the tongue, and on the presence/absence of a mid-basitarsal spine. The short-faced species are generally short-tongued without a mid-basitarsal spine, while the long-faced species are long-tongued with a mid-basitarsal spine. The subgenera *Mendacibombus*, *Confusibombus*, *Bombias*, and *Kallobombus* are separated into two clades, which is consistent with previous studies (Williams 1994; Pedersen 2002; Kawakita et al. 2003, 2004; Cameron et al. 2007) and well supported by posterior probability (PP) and bootstrap values. This is the first report on the phylogenetic evolution and classification status of *B. turneri* (Richards), *B. opulentus* Smith, *B. pyrosoma*, *B. longipennis* Friese, *B. minshanensis* Bischoff, and *B. lantschouensis*, which were collected from the Anhui, Qinghai, and Gansu provinces and Beijing in China, whereas *B. longipennis*, *B. minshanensis* and *B. lantschouensis* were revised by Williams et al. (2012b). Combining morphological data (Suppl. material 1) with the previous studies on *Bombus* taxonomy (Williams et al. 2009; An et al. 2014), we conclude that 1) *B. turneri* belongs to the subgenus *Psithyrus*, 2) *B. opulentus* is one of the species in the subgenus *Thoracobombus*, 3) *B. pyrosoma* belongs to *Melanobombus*, and 4) *B. longipennis*, *B. minshanensis*, and *B. lantschouensis* are grouped into *Bombus* s. str. These species are widely distributed throughout China. *Bombus longipennis* is distributed mainly in the medium elevation of mountains and on the Tibetan plateau in China, with a yellow-banded color pattern that is quite similar to that of *B. lucorum* (Linnaeus) and *B. cryptarum* (Fabricius). Therefore, *B. longipennis* has been confused with *B. lucorum* (An et al. 2014). *Bombus minshanensis* is primarily distributed in the medium to high elevations of the east Tibetan plateau meadows in China and has often been confused with *B. patagiatus* Nylander because of the similar white-banded color pattern in the females (An et al. 2014). *Bombus lantschouensis* is a common bumble bee species widely distributed in low, medium, and high elevation river valleys, mountains, and plateaus in China. *Bombus lantschouensis* has also been confused with *B. patagiatus*, as both species share the similar yellow-banded color pattern (An et al. 2014). *Bombus pyrosoma* is easily misidentified as *B. validus* Friese because of the similar white band (An et al. 2014). *Bombus turneri* is a parasitic bumble bee species and has a very small population, being found only in the medium elevations of the edge of the east Qinghai-Tibetan plateau and the loess plateau in China (An et al. 2014). *Bombus opulentus* has a dominant distribution in the low and medium elevations of mountains and plateaus in China. The brown and black color pattern of this species is similar to that of *B. longipes* Friese (An et al. 2014). These consistencies between molecular phylogeny and morphological classification have furthered knowledge of the distribution and evolutionary history of *Bombus* in China.
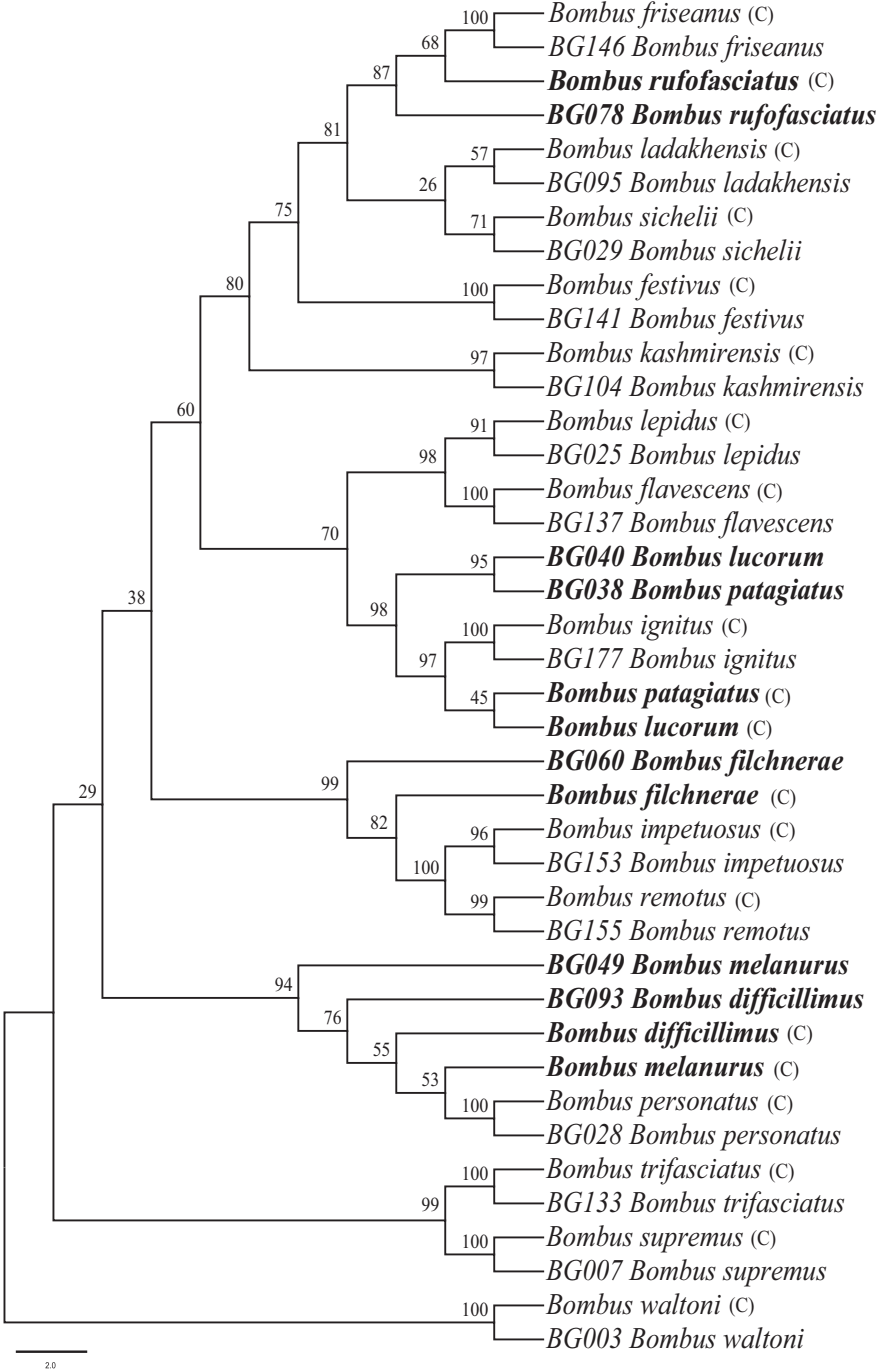
Figure 2. Estimated phylogeny of *Bombus* based on six combined gene sequences (mitochondrial genes 16S rRNA and COI, nuclear genes Opsin, ArgK, EF-1α, and PEPCK) analyzed by Bayesian Inference and Maximum Likelihood. Subgeneric clades are noted at the right of the figure, values above branches are posterior probabilities (BI), values below branches are bootstrap values (ML). Species in bold font were collected by the authors in China and a black spot indicates species that were not included in the previous phylogeny of *Bombus* of Cameron et al. (2007). The outgroups are at the top of the tree. Abbreviations: SF, short-faced clade; LF, long-faced clade. Subgenera that were synonymized are in parentheses.

Figure 3. Estimated phylogeny of *Bombus* based on six combined gene sequences (mitochondrial genes 16S rRNA and COI, nuclear genes Opsin, ArgK, EF-1α, and PEPCK) analyzed by Maximum Parsimony. Subgeneric clades are noted at the right of the figure and values on branches are the bootstrap values. Species in bold font were collected in China and a black spot indicates species that were not included in the phylogeny of *Bombus* of Cameron et al. (2007). The outgroups are at the top of the tree. Abbreviations: SF, short-faced clade; LF, long-faced clade. Subgenera that were synonymized are in parentheses.

Our phylogeny is consistent with the studies reported by Cameron et al. (2007) in terms of the number and variety of subgenera in the short-faced and long-faced clades and the relationships among the different subgenera. We added six new species of *Bombus* from China into the trees. As a result, there were some differences in the subgenera *Psithyrus*, *Thoracobombus*, *Melanobombus* and *Bombus* s. str.; *B. lantschouensis* is sister to *B. lucorum* and *B. patagiatus*; *B. longipennis* and *B. affinis* Cresson were not well supported by BI and ML methods (PP = 0.49; bootstrap values = 33) in the *Bombus* s. str. clade, and *B. minshanensis* together with *B. lantschouensis* and *B. lucorum* + *B. patagiatus* formed a branch in the *Bombus* s. str. clade (Fig. 2).

Besides 20 species of bumble bees in our samples which were also included in the phylogenetic trees of Cameron et al. (2007), we replaced the original sequences in Cameron et al.'s study with new sequences generated from this study and reconstructed the phylogenetic trees (Figs 2, 3). The results were consistent with Cameron et al.'s phylogeny for most of the species, but there were some variations in the placements of *B. kashmirensis* Friese, *B. rufofasciatus* Smith, *B. friseanus* Skorikov, *B. lucorum*, and *B. patagiatus*. For example, *B. kashmirensis* was sister to *B. nobilis* Friese in Cameron et al.'s analysis, while it was placed in a single clade and grouped with four other species, *B. breviceps* Smith, *B. grahami* (Frison), *B. nobilis*, and *B. wurflenii* Radoszkowski, in our study. Also, *B. rufofasciatus* was sister to *B. miniatus* Bingham and grouped with *B. friseanus* + *B. formosellus* (Frison) in Cameron et al.'s study, but in our study it grouped with *B. miniatus* Bingham, *B. friseanus*, and *B. pyrosoma*. In *Bombus* s. str., *B. lucorum* is sister to *B. franklini* (Frison) and *B. patagiatus*, whereas it was sister to *B. cryptarum* (Fabricius) in Cameron et al.'s phylogeny. Furthermore, *B. lucorum* was sister to *B. patagiatus* in Cameron et al.'s study, but grouped with *B. lantschouensis* in our study. These differences may be due to the combined gene approach or to the addition of new species sequences in our study, and further research is needed to clarify this problem.

Based on the sequences of five genes (16S rRNA, Argk, EF-1α, Opsin, and PEPCK), we analyzed the relationships between the same 20 species and built one phylogenetic tree using the ML analysis (Fig. 4). In general, the results suggested that most species of *Bombus* were stable in genetic evolution and that their taxonomic positions showed no significant change with the variation of distribution areas. The results showed that nearly all specimens of the same species formed one clade in the phylogenetic tree (Fig. 4). We compared all gene sequences of the six species from Cameron et al. (2007) to our samples and found that there were some sequence variations between both groups of samples, which may reflect the adaptation to different geographical environments and evolutionary pathways in certain bumble bee species.

To ensure the accuracy in the classification of species using the combined gene approach, we utilized six genes to analyze the relationships among species. In Cameron et al. (2007), *B. ruderarius* (Müller) and *B. velox* (Skorikov) were sister species supported by PP = 0.52, but in our analysis *B. ruderarius* and *B. velox* are sister species supported by PP = 0.99 and bootstrap values = 61 in the BI and ML analyses, respectively. In Cameron et al. (2007), *B. cryptarum* and *B. patagiatus* were sister species (PP = 0.52), and then they attached to *B. moderatus* Cresson (PP = 0.90). However, in our study, *B. crypatarum* and *B. moderatus* are sister species (PP = 1.00; bootstrap values = 97)

Figure 4. Estimated phylogeny of the same samples using both new sequences and sequences from Cameron et al. (2007), based on five combined gene sequences (mitochondrial gene 16S rRNA, nuclear genes Opsin, ArgK, EF-1α, and PEPCK) analyzed by Maximum Likelihood. Values on the branches are the bootstrap values, (C) represents Cameron et al.'s species, and "BG" represents our species. The bold font indicates species from both datasets that did not cluster together into monophyletic clades.

Figure 5. Estimated phylogeny of *Bombus* based on six combined gene sequences (mitochondrial genes 16S rRNA and COI, nuclear genes Opsin, ArgK, EF-1α, and PEPCK) analyzed by Neighbor Joining. Subgeneric clades are noted at the right of the Figure, and values on branches are the bootstrap values of NJ. Species in bold font were collected in China and a black spot indicates species that were not included in the phylogeny of *Bombus* of Cameron et al. (2007). The outgroups are at the bottom of the tree. Subgenera that were synonymized are in parentheses.

and their relationship with *B. patagiatus* is distant (Fig. 2). Although most species are strongly supported by bootstrap values in the BI and ML phylogenetic trees based on the six combined genes, there are certain species which are not well supported. For example, *B. longipennis* and *B. affinis* are sister species in the tree but the support values are low (PP = 0.49; bootstrap values = 33). *Bombus longipennis*, *B. affinis*, and *B. franklini* are sister clades, and their support values are also low (PP = 0.27; bootstrap values = 38) (Fig. 2). As shown in Fig. 3, the phylogenetic trees obtained by the MP method showed that there are two distinct clades (short-faced and long-faced) including nearly all subgenera of *Bombus*. This, in general, is consistent with the results obtained using the BI and ML methods, while there were still some variations among subgenera in the topology structure of the phylogenetic trees, and most of the support values on the branches were very low. The phylogenetic tree obtained by the NJ method is different from those resulting from the other three methods (Fig. 5). There are not two distinct clades, and the phylogenetic relationships of some species are not consistent with morphology (Figs 1–3). For instance, *B. kashmirensis*, *B. balteatus* Dahlbom, *B. hyperboreus* Schönherr, *B. neoboreus* Sladen, *B. alpinus* (Linnaeus) and *B. polaris* Curtis belong to the same subgenus, *Alpinobombus*. However, *B. kashmirensis* formed a single clade in the NJ phylogenetic tree. Likewise, *B. haematurus* Kriechbaumer was also removed from the subgenus *Pyrobombus* and formed a single branch in the NJ phylogeny (Fig. 5). The support values were also low for many bumble bee species in the NJ phylogenetic tree. These results suggest that the BI and ML methods were better than MP and NJ to analyze the phylogenetic relationships among a large number of samples.

Furthermore, based on the monophyletic groups of bumble bees in the phylogenetic trees of Cameron et al. (2007), morphology, and the important behavioral and ecological characters of bumble bees, Williams et al. (2008) simplified the subgeneric classification of bumble bees from 38 to 15 subgenera. The results of our BI, ML, and MP analyses are consistent with what Williams proposed (Figs 2, 6). Moreover, we constructed the phylogenetic tree by BI based on the combined six genes (Fig. 6), and most clades were well supported by posterior probabilities except the one formed by *Melanobombus* and its sister clade *Sivircobombus* + *Cullumanobombus* (PP = 0.44). It may be that these 15 subgenera are in close proximity in a molecular evolutionary sense, and we need to distinguish them using other information. These results suggest that molecular methods can determine the taxonomic status of the majority of species in *Bombus*, and that it is consistent with morphological identification, but there are a few species in the phylogenetic tree for which the posterior probability and bootstrap values are a little low, and their classification may need to be further supported by combining other criteria with morphology.

Distinguishing bumble bee species

There are many *Bombus* species distributed in diverse regions all over the world. Previous studies revealed that color pattern and the characters of the male genitalia could clearly distinguish the subgenera of *Bombus* (Franklin 1954; Hines et al. 2006). There have been some problems in some cryptic species complexes; for instance, according
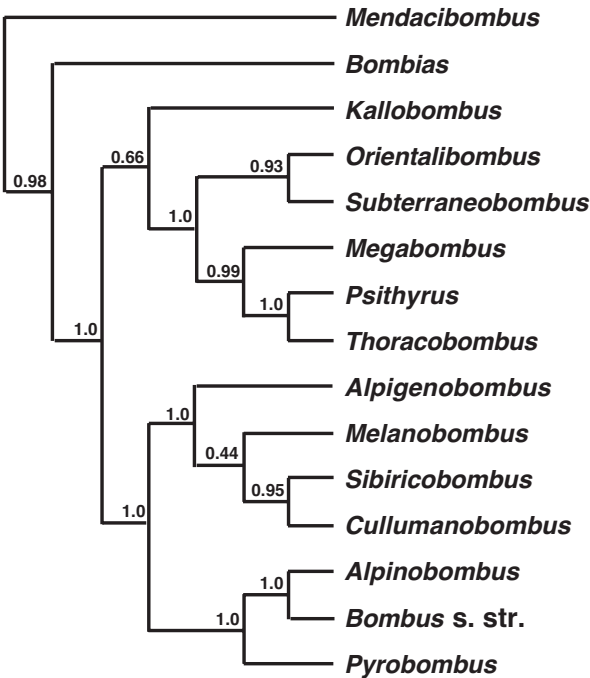
Figure 6. Phylogenetic relationships of the subgenera of *Bombus* from the Bayesian Inference tree (Fig. 2); nearly all of them are well supported by posterior probabilities.

to the color pattern, it is easy to consider *B. cryptarum*, *B. lucorum*, and *B. magnus* Vogt as one species, but the chemical and molecular evidence suggests that they are three distinct species (Carolan et al. 2012). Molecular methods are a powerful tool for inference of phylogenetic relationships (Ratnasingham and Hebert 2013). Because the evolutionary rates of single genes are different, each genetic marker has its advantages and disadvantages in phylogenetic analysis and a single gene cannot always clearly resolve the classification of species. Hines et al. (2006) found that combined genes can obtain stronger support values in some nodes compared to individual genes. In the present study, we also explored the power of combining multiple genetic markers which are conserved in evolution and accurately infer phylogenetic relationships of species (Cameron and Mardulyn 2003; Hebert et al. 2003; Magnacca and Brown 2012; Isaka and Sato 2014; Kjer et al. 2014; Schmidt et al. 2015). When multiple genes were combined, we could generate a clearer phylogeny to accurately determine the taxonomy of species by relying on the ability of the individual genes to reconstruct a known phylogeny and a set of genes to accurately infer the phylogenetic relationships of species. Although it is possible to resolve the taxonomy of species within *Pyrobombus* with combined multiple genes, it is still difficult to distinguish among all *Bombus* species.

China has the largest diversity of *Bombus* species in the world (Williams et al. 2010). However, it has been an increasing challenge to effectively protect and utilize the abundant resource of bumble bees in China. Our results significantly enhance our understanding of the taxonomic status and distribution of *Bombus* in China and provide

an important foundation in further revealing the evolutionary history of *Bombus* and strengthening the protection of bumble bees as a resource. Some species readily reproduce, so they have been successfully commercially reared for pollination in greenhouses. For example, *B. terrestris* (Linnaeus) (subgenus *Bombus* s. str.) has been reared commercially in Europe and *B. impatiens* Cresson (subgenus *Pyrobombus*) has been reared commercially in North America. Although we could introduce these *Bombus* species to our country for pollination of crops in large greenhouses, there is the potential that they could bring new pathogens that would threaten the native species. As a result, the most practical solution would be to identify native species that can be readily reared for large-scale production. Our results showed that *B. longipennis*, *B. minshanensis*, *B. lantschouensis*, and *B. terrestris* belong to the subgenus *Bombus* s. str. and have a close phylogenetic relationship within the subgenus. These species are widely distributed throughout China, so they may represent an ideal option for commercial rearing in China. Our future goal is to distinguish all species of *Bombus* completely and accurately using a combination of different methods, thereby leading to a better understanding of the distribution and evolutionary history of bumble bees in China and improving our strategies of biodiversity conservation to promote pollinator populations.

Acknowledgements

References

An JD, Huang JX, Shao YQ, Zhang SW, Wang B, Liu XY, Wu J, Williams PH (2014) The bumblebees of North China (Apidae, *Bombus* Latreille). Zootaxa 3830: 1–89. https://doi.org/10.11646/zootaxa.3830.1.1

An JD, Williams PH, Zhou BF, Miao XQ, Qi WZ (2011) The bumblebees of Gansu, Northwest China (Hymenoptera, Apidae). Zootaxa 2865: 1–36. https://doi.org/10.11646/zootaxa.2865.1.1

Boehme P, Amendt J, Disney RHL, Zehner R (2010) Molecular identification of carrion-breeding scuttle flies (Diptera: Phoridae) using COI barcodes. International Journal of Legal Medicine 124: 577–581. https://doi.org/10.1007/s00414-010-0429-5

Cameron SA, Hines HM, Williams PH (2007) A comprehensive phylogeny of the bumble bees (*Bombus*). Biological Journal of The Linnean Society 91: 161–188. https://doi.org/10.1111/j.1095-8312.2007.00784.x

Cameron SA, Mardulyn P (2001) Multiple molecular data sets suggest independent origins of highly eusocial behavior in bees (Hymenoptera: Apinae). Systematic Biology 50: 194–214. https://doi.org/10.1080/10635150151125851

Cameron SA, Mardulyn P (2003) The major opsin gene is useful for inferring higher level phylogenetic relationships of the corbiculate bees. Molecular Phylogenetics and Evolution 28(3): 610–613. https://doi.org/10.1016/S1055-7903(03)00055-1

Carolan JC, Murray TE, Fitzpatrick U, Crossley J, Schmidt H, Cederberg B, McNally L, Paxton RJ, Williams PH, Brown MJ (2012) Colour patterns do not diagnose species: quantitative evaluation of a DNA barcoded cryptic bumblebee complex. PloS ONE 7: e29251. https://doi.org/10.1371/journal.pone.0029251

Cho S, Mitchell A, Regier JC, Mitter C, Poole RW, Friedlander TP, Zhao SW (1995) A highly conserved nuclear gene for low-level phylogenetics: Elongation factor-1α recovers morphology-based tree for heliothine moths. Molecular Biology and Evolution 12: 650–656.

Colla SR, Packer L (2008) Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis* Cresson. Biodiversity and Conservation 17: 1379–1391. https://doi.org/10.1007/s10531-008-9340-5

Danforth BN, Brady SG, Sipes SD, Pearson A (2004) Single-copy nuclear genes recover cretaceous-age divergences in bees. Systematic Biology 53: 309–326. https://doi.org/10.1080/10635150490423737

Franklin HJ (1954) The evolution and distribution of American bumblebee kinds. Transactions of the American Entomological Society 80: 43–51. https://www.jstor.org/stable/25077681

Friedlander TP, Regier JC, Mitter C, Wagner DL (1996) A nuclear gene for higher level phylogenetics: phosphoenolpyruvate carboxykinase tracks mesozoic-age divergences within Lepidoptera (Insecta). Molecular Biology and Evolution 13: 594–604. https://doi.org/10.1093/oxfordjournals.molbev.a025619

Goulson D, Lye GC, Darvill B (2008) Decline and conservation of bumble bees. Annual Review of Entomology 53: 191–208.

He YP, Liu JQ (2004) Pollination ecology of *Gentiana straminea* Maxim. (Gentianaceae), an alpine perennial in the Qinghai-Tibet Plateau. Acta Ecologica Sinica 24: 215–220.

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society B-Biological Sciences 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of the United States of America 101: 14812–14817. https://doi.org/10.1073/pnas.0406166101

Hines HM, Cameron AC, Williams PH (2006) Molecular phylogeny of the bumble bee subgenus *Pyrobombus* (Hymenoptera: Apidae: *Bombus*) with insights into gene utility for lower-level analysis. Invertebrate Systematics 20: 289–303. https://doi.org/10.1071/IS05028

Hines HM, Williams PH (2012) Mimetic colour pattern evolution in the highly polymorphic *Bombus trifasciatus* (Hymenoptera: Apidae) species complex and its comimics. Zoological Journal of the Linnean Society 166: 805–826. https://doi.org/10.1111/j.1096-3642.2012.00861.x

Isaka Y, Sato T (2014) Molecular phylogenetic and divergence time estimation analyses of the sawfly subfamily Selandriinae (Hymenoptera: Tenthredinidae). Entomological Science 17: 435–439. https://doi.org/10.1111/ens.12080

Kawakita A, Sota T, Ascher JS, Ito M, Tanaka H, Kato M (2003) Evolution and phylogenetic utility of alignment gaps within intron sequences of three nuclear genes in bumble bees (*Bombus*). Molecular Biology and Evolution 20: 87–92. https://doi.org/10.1093/molbev/msg007

Kawakita A, Sota T, Ito M, Ascher JS, Tanaka H, Kato M, Roubik DW (2004) Phylogeny, historical biogeography, and character evolution in bumble bees (Bombus: Apidae) based on simultaneous analysis of three nuclear gene sequences. Molecular Phylogenetics and Evolution 31: 799–804. https://doi.org/10.1016/j.ympev.2003.12.003

Kjer KM, Zhou X, Frandsen PB, Thomas JA, Blahnik RJ (2014) Moving toward species-level phylogeny using ribosomal DNA and COI barcodes: an example from the diverse caddisfly genus *Chimarra* (Trichoptera: Philopotamidae). Arthropod Systematics and Phylogeny 72: 345–354.

Lin CP, Danforth BN (2004) How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets. Molecular Phylogenetics and Evolution 30: 686–702. https://doi.org/10.1016/S1055-7903(03)00241-0

Magnacca KN, Brown MJF (2012) DNA barcoding a regional fauna: Irish solitary bees. Molecular Ecology Resources 12: 990–998. https://doi.org/10.1111/1755-0998.12001

Mardulyn P, Cameron SA (1999) The major Opsin in bees (Insecta: Hymenoptera): a promising nuclear gene for higher level phylogenetics. Molecular Phylogenetics and Evolution 12: 168–176. https://doi.org/10.1006/mpev.1998.0606

Michel-Salzat A, Whitfield JB (2004) Preliminary evolutionary relationships within the parasitoid wasp genus *Cotesia* (Hymenoptera: Braconidae: Microgastrinae): combined analysis of four genes. Systematic Entomology 29: 371–382. https://doi.org/10.1111/j.0307-6970.2004.00246.x

Michener CD (2007) The bees of the World. 2nd edition. Johns Hopkins University Press, Baltimore, 992 pp.

Nylander JAA (2004) MrModeltest, version 2. http://www.abc.se/~nylander/

Pedersen BV (1996) A phylogenetic analysis of cuckoo bumblebees (*Psithyrus*, Lepeletier) and bumblebees (*Bombus*, Latreille) inferred from sequences of the mitochondrial gene cytochrome oxidase I. Molecular Phylogenetics and Evolution 5: 289–297. https://doi.org/10.1006/mpev.1996.0024

Pedersen BV (2002) European bumblebees (Hymenoptera: Bombini) phylogenetic relationships inferred from DNA sequences. Insect Systematics and Evolution 33: 361–386. https://doi.org/10.1163/187631202X00208

Peng WJ, Huang JX, Wu J, An JD (2009) Geographic distribution and bionomics of six bumblebee species in North China. Chinese Bulletin of Entomology 46: 115–120.

Posada P, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. https://github.com/beast-dev/tracer/releases [Accessed 13 January 2018]

Rasmont P, Pauly A, Terzo M, Patiny S, Michez D, Iserbyt S, Barbier Y, Haubruge E (2005) The survey of wild bees (Hymenoptera, Apoidea) in Belgium and France. FAO, Rome, 18 pp.

Ratnasingham R, Hebert PDN (2013) A DNA-based registry for all animal species: the barcode index number (BIN) System. PLoS ONE 8: e66213. https://doi.org/10.1371/journal.pone.0066213

Rokas A, Nylander JAA, Ronquist F, Stone GN (2002) A maximum-likelihood analysis of eight phylogenetic markers in gallwasps (Hymenoptera: Cynipidae): implications for insect phylogenetic studies. Molecular Phylogenetics and Evolution 22: 206–219. https://doi.org/10.1006/mpev.2001.1032

Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Schmidt S, Schmid-Egger S, Morinière J, Haszprunar H, Hebert PDN (2015) DNA barcoding largely supports 250 years of classical taxonomy: identifications for Central European bees (Hymenoptera, Apoidea partim). Molecular Ecology Resources 15: 985–1000. https://doi.org/10.1111/1755-0998.12363

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Stephen WP (1957) Bumble bees of western America (Hymenoptera: Apoidea). Technical Bulletin, Agricultural Experimental Station, Oregon State University 40: 2–163.

Sun HQ, Luo YB, Song G (2003) A preliminary study on pollination biology of an endangered Orchid, Changnienia amoena, in Shennongjia. Acta Botanica Sinica 45: 1019–1023.

Swofford DL (2002) PAUP* Phylogenetic analysis using parsimony (*and other methods) v.4.0a165. Sinauer Associates, Sunderland.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Thorp RW, Horning DS, Dunning LL (1983) Bumblebees and cuckoo bumble bees of California (Hymenoptera: Apidae). Bulletin of the California Insect Survey 23: 1–79.

Velthuis HH, Van Doorrn A (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie 37: 421–451. https://doi.org/10.1051/apido:2006019

Vergara CH, Fonseca-Buendía P (2012) Pollination of greenhouse tomatoes by the Mexican bumblebee Bombus ephippiatus (Hymenoptera: Apidae). Journal of Pollination Ecology 7: 27–30. https://doi.org/10.26786/1920-7603(2012)1

Wang H, Li DZ (1998) A preliminary study of pollination biology of *Pedicularis* (Scrophulariaceae) in Northwest Yunnan, China. Acta Botanica Sinica 40: 204–210.

Wang JF, Qiao GX (2010) DNA barcoding of genus *Toxoptera* Koch (Hemiptera: Aphididae): Identification and molecular phylogeny inferred from mitochondrial COI sequences. Insect Science 16: 475–484. https://doi.org/10.1111/j.1744-7917.2009.01270.x

Whitfield JB, Cameron SA (1998) Hierarchical analysis of variation in the mitochondrial 16S rRNA gene among Hymenoptera. Molecular Biology and Evolution 15: 1728–1743. https://doi.org/10.1093/oxfordjournals.molbev.a025899

Williams PH (1994) Phylogenetic relationships among bumble bees (*Bombus* Latr.): a reappraisal of morphological evidence. Systematic Entomology 19: 327–344. https://doi.org/10.1111/j.1365-3113.1994.tb00594.x

Williams PH (1998) An annotated checklist of bumble bees with an analysis of patterns of description (Hymenoptera: Apidae, Bombini). Bulletin of the British Museum (Natural History) Entomology 67: 79–152.

Williams PH (2005) Does specialization explain rarity and decline among British bumblebees? A response to Goulson et al. Biological Conservation 122: 33–43. https://doi.org/10.1016/j.biocon.2004.06.019

Williams PH, An JD, Brown MJ, Carolan JC, Goulson D, Huang JX, Ito M (2012a) Cryptic bumblebee species: consequences for conservation and the trade in greenhouse pollinators. PloS ONE 7: e32992. https://doi.org/10.1371/journal.pone.0032992

Williams PH, An JD, Huang JX (2011) The bumblebees of the subgenus *Subterraneobombus*: integrating evidence from morphology and DNA barcodes (Hymenoptera, Apidae, *Bombus*). Zoological Journal of the Linnean Society 163: 813–862. https://doi.org/10.1111/j.1096-3642.2011.00729.x

Williams PH, An JD, Huang JX, Yao J (2010) BBCI: A new initiative to document Chinese bumble bees for pollination research. Journal of Apicultural Research 49: 221–222. https://doi.org/10.3896/IBRA.1.49.2.15

Williams PH, Brown MJF, Carolan JC, An JD, Goulson D, Aytekin AM, Best LR, Byvaltsev AM, Cederberg B, Dawson R, Huang JX, Ito M, Monfared A, Raina RH, Schmid-Hempel P, Sheffield CS, Šima P, Xie ZH (2012b) Unveiling cryptic species of the bumblebee subgenus *Bombus* s. str. world-wide with COI barcodes (Hymenoptera: Apidae). Systematics and Biodiversity 10: 21–56. https://doi.org/10.1080/14772000.2012.664574

Williams PH, Cameron SA, Hines HM, Cederberg B, Rasmont P (2008) A simplified subgeneric classification of the bumblebees (genus *Bombus*). Apidologie 39: 46–74. https://doi.org/10.1051/apido:2007052

Williams PH, Huang JX, Rasmont P, An JD (2016) Early-diverging bumblebees from across the roof of the world: the high-mountain subgenus *Mendacibombus* revised from species' gene coalescents and morphology (Hymenoptera, Apidae). Zootaxa 4204: 1–72. https://doi.org/10.11646/zootaxa.4204.1.1

Williams PH, Tang Y, Yao J, Cameron S (2009) The bumblebees of Sichuan (Hymenoptera: Apidae, Bombini). Systematics and Biodiversity 7: 101–189. https://doi.org/10.1017/S1477200008002843

Winston ML, Michener CD (1977) Dual origin of highly social behavior among bees. Proceedings of the National Academy of Sciences of the United States of America 74: 1135–1137. https://doi.org/10.1073/pnas.74.3.1135

Xie Z, Williams PH, Tang Y (2008) The effect of grazing on bumblebees in the high rangelands of the eastern Tibetan Plateau of Sichuan. Journal of Insect Conservation 12: 695–703. https://doi.org/10.1007/s10841-008-9180-3

Yang DR (1999) The status of species diversity and conservation strategy of bumble bees, a pollination insect in Lancang River Basin of Yunnan, China. Chinese Biodiversity 7: 1967–1973.

Yoshizawa K, Johnson KP (2003) Phylogenetic position of Phthiraptera (Insecta: Paraneoptera) and elevated rate of evolution in mitochondrial 12S and 16S rDNA. Molecular Phylogenetics and Evolution 29: 102–114. https://doi.org/10.1016/S1055-7903(03)00073-3

Supplementary material 1

Key to the 26 species of the genus *Bombus*
Authors: Liu-Hao Wang, Shan Liu, Yu-Jie Tang, Yan-Ping Chen, Jie Wu1, Ji-Lian Li
Data type: key
Explanation note: Key to the 26 species of the genus *Bombus* for males and females.
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Link: https://doi.org/10.3897/zookeys.1007.34105.suppl1

RESEARCH ARTICLE

Pimpla Fabricius, 1804 (Ichneumonidae, Pimplinae) from Uruguay: a replacement name, new records, and an identification key to the species

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Abstract

We report new faunistic records of *Pimpla* Fabricius, 1804 from Uruguay. The following species are reported from the country for the first time: *P. albomarginata* Cameron, 1846, *P. caerulea* Brullé, 1846, *P. perssoni* Gauld, 1991, and *P. semirufa* Brullé, 1846. In addition, we propose a replacement name for *Pimpla rufipes* Brullé, 1846 and provide diagnosis, digital images, and an identification key for all the *Pimpla* species known to occur in Uruguay.

Keywords

Faunistics, homonymy, neotropics, parasitoids, parasitoid wasps, South America, taxonomy

Introduction

The parasitoid wasp family Ichneumonidae (Darwin wasps) is among the largest animal families anywhere on Earth (Klopfstein et al. 2019). It is taxonomically challenging, and many species are either rare or at least rarely collected. One exception is the genus *Pimpla* Fabricius, 1804 (Pimplinae, Pimplini). It is composed of moderately large (in tropical regions), often colorful species which are abundant in many entomological collections (Townes 1969; Porter 1970).

With over 200 valid species (Yu et al. 2016; Watanabe and Matsumoto 2019), this genus is among the largest genera within the subfamily Pimplinae. The species of *Pimpla* are known to be idiobiont endoparasitoids of prepupae and pupae of Lepidoptera (Gauld 1991). The genus is characterized by simple and large tarsal claws (females), the straight apex of the ovipositor, a weakly concave internal margin of the compound eye in front of the antennal insertion (Gauld et al. 1998), and mid tarsomere IV medioventrally with a longitudinal band of fine hair (an autapomorphy) (Gauld et al. 2002).

On account of the taxonomical works of Charles C. Porter in South America (Porter 1970) and Ian D. Gauld in Central America (Gauld 1991; Gauld et al. 1998), the genus is one of the best-known Darwin wasp genera in the Neotropical region. Porter (1970) reported 35 (21 of them new) species from South America and Gauld (1991) and Gauld et al. (1998) found 17 (six of them new) species in Costa Rica.

The aim of this paper is to provide new records of *Pimpla* from Uruguay. In addition, we provide diagnosis, high-quality layer-stacked photographs, and an identification key for the species currently known from the country. This work is part of a series of articles reporting new *Pimpla* records from South America. This work was started by Pádua et al. (2019).

Material and methods

Study area

The field sampling was conducted in four locations in the municipality of Castillos, Rocha Department, Uruguay, between December 2014 and December 2016 (see Fernandes et al. 2019).

Specimens studied

Pimpla specimens were collected by Malaise trapping, and the specimens are deposited in the Invertebrate Collection of Instituto Nacional de Pesquisas da Amazônia (INPA; curator: Marcio L. Oliveira).

Morphology and distribution

General morphological terminology follows that of Gauld (1991). New distributional records are indicated with an asterisk (*).

Photographs

Digital images were taken using a Leica DMC4500 digital camera attached to a Leica M205A stereomicroscope and combined using the software Leica Application Suite V4.10.0. The final images were edited in Adobe Photoshop.

Abbreviations

BMNH Natural History Museum, London, UK;
IML Institute Miguel Lillo, Tucumám, Argentina;
MNCR Museo Nacional de Costa Rica, San José, Costa Rica;
MNHN Muséum national d'Histoire naturelle, Paris, France.

Distribution maps

The distribution maps were created using SimpleMappr online software (Shorthouse 2010).

Key to the Uruguayan species of *Pimpla* Fabricius, 1804

1   Female **2**
–   Male (the male of *P. cyanipennis* Brullé, 1846 is unknown) **9**
2   Mesosoma and metasoma metallic blue (Fig. 2A) ....*P. caerulea* **Brullé, 1846**
–   Mesosoma and metasoma black, brown, yellow, reddish, or a combination of these colours (Figs 1A, 3A, 5A, 6A, 8A, 9A, 10A) **3**
3   Fore wing hyaline, with an apical darkened area (Fig. 8A); malar space 0.3–0.4 times as long as basal width of mandibles......................*P. perssoni* **Gauld, 1991**
–   Fore wing without an apical darkened area (Figs 1A, 3A, 5A, 6A, 9A, 10A); malar space > 0.6 times as long as basal width of mandibles..**4**
4   Laterotergite V < 1.7 times as long as wide (Figs 5E, 6E, 9E) **5**
–   Laterotergite V > 2.1 times as long as wide (Figs 1E, 8E, 10E) **8**
5   Metasoma entirely reddish (Fig. 5A) ....*P. golbachi* **(Porter, 1970)**
–   Metasoma entirely black or reddish with tergites VI+ black (Figs 3A, 9A) **6**
6   Ovipositor > 1.7 times as long as hind tibia; meso- and metacoxa black (Figs 3A, C, 4A, C) ....*P. cyanipennis* **Brullé, 1846**
–   Ovipositor < 1.6 times as long as hind tibia; meso- and metacoxa reddish brown (Figs 6A, 9A)..**7**
7   Metasoma black (Fig. 6A) ....*P. patirrufa* **nom. nov.**
–   Metasoma reddish with tergites V+ or VI+ black (Fig. 9A) ....
      ....*P. semirufa* **Brullé, 1846**
8   Dorsal valve of ovipositor apically with teeth (Fig. 10F) ....
      .... *P. tomyris* **Schrottky, 1902**
–   Dorsal valve of ovipositor apically without teeth (Fig. 1F)....................................
      ....*P. albomarginata* **Cameron, 1886**
9   Mesosoma and metasoma with a metallic blue (Fig. 2B)....*P. caerulea* **Brullé, 1846**
–   Mesosoma and metasoma black, brown, yellow, reddish, or a combination of these colours (Figs 1B, 5B, 6B, 8B, 9B, 10B) **10**
10   Fore wing hyaline with an apical darkened area (Fig. 8B) ....
      ....*P. perssoni* **Gauld, 1991**
–   Fore wing without an apical dark area (Figs 1B, 5B, 6B, 9B, 10B) **11**

11   Metasomal tergites with fine punctures (Figs 1D, 10D) **12**
–   Metasomal tergites with strong punctures (Figs 5D, 6D, 9D) **13**
12   Mesosoma reddish with profuse white marks (Fig. 1B); metasoma black and white banded (Fig. 1B) ....***P. albomarginata* Cameron, 1886**
–   Mesosoma shining black with variable patterning of yellow markings on pronotum, tegula, scutellum, postscutellum and propodeum (a pair of elliptic blotches) (Fig. 10B); metasoma reddish brown, with a pair of large yellow blotches laterally on tergites I–IV (Fig. 10B) ....***P. tomyris* Schrottky, 1902**
13   Mesosoma entirely shining black (Fig. 6B) ....***P. patirrufa* nom. nov.**
–   Mesosoma black with hind corners of meso- and metapleuron brown and tegula white or shining black with lower hind corner of mesopleuron brown, and metapleuron red with a little black staining along front margin (Figs 5B, 9B)..............**14**
14   Metasoma reddish with tergite VI+ black (Fig. 9B) ....***P. semirufa* Brullé, 1846**
–   Metasoma entirely reddish (Fig. 5B)...........................***P. golbachi* (Porter, 1970)**

Faunistics and taxonomy

Pimpla Fabricius, 1804

Pimpla Fabricius, 1804: 112. Type species: *Ichneumon instigator* Fabricius (= *Ichneumon hypochondriaca* Retzius), by subsequent designation (Opinion 159, International Commission on Zoological Nomenclature 1945: 282).

Coccygomimus Saussure, 1892: pl. 14, fig. 12. Type species: *Coccygomimus madecassus* Saussure, by monotypy.

Habropimpla Cameron, 1900: 96. Type species: *Habropimpla bilineata* Cameron, by monotypy.

Lissotheronia Cameron, 1905: 139. Type species: *Lissotheronia flavipes* Cameron, by monotypy.

Phytodiaetoides Morley, 1913: 221. Type species: *Phytodiaetoides megaera* Morley = *Pimpla flavipalpis*, by original designation.

Pimplidea Viereck, 1914: 117. Type species: *Pimpla pedalis* Cresson, by original designation.

Coelopimpla Brèthes, 1916: 402. Type species: *Coelopimpla amadei* Brèthes, by original designation.

Liotheronia Enderlein, 1919: 147. Type species: *Liotheronia kriegeri* Enderlein, by original designation.

Dihyboplax Enderlein, 1919: 148. Type species: *Dihyboplax flavipennis* Enderlein, by original designation.

Neogabunia Brèthes, 1927: 322. Type species: *Neogabunia paulistana* Brèthes = *Pimpla tomyris* Schrottky, by monotypy.

Opodactyla Seyrig, 1932: 60. Type species: *Pimpla* (*Opodactyla*) *waterloti* Seyrig, by original designation.

Oxypimpla Noskiewicz & Chudoba, 1951: 42, 56. Type species: *Pimpla turionellae* Linnaeus, by monotypy.

Jamaicapimpla Mason, 1975. Type species: *Ephialtes nigroaeneus* Cushman, by original designation.

Diagnosis. *Pimpla* can be distinguished from other genera of Pimplini (*sensu* Porter 1970 as *Coccygomimus*) by the combination of the following character states: 1) inner margin of eye weakly to rather strongly concave above antennal socket; 2) clypeus not divided by a transverse suture; 3) malar space 0.35–1.4 times as long as basal width of mandible; 4) mandible broad and with upper tooth approximately as long as the lower tooth; 5) notaulus weak or absent, without a distinct frontal crest; 6) propodeum with median longitudinal carinae varying from absent to sometimes weakly traceable throughout; 7) pleural carina usually present but sometimes absent; 8) length of fore wing 2.7–18.0 mm; 9) hind femur without a ventral tooth; 10) tarsal claws large and simple, without a basal lobe or an enlarged hair with a flattened tip; 11) metasoma varying from closely punctured to sometimes almost impunctate; 12) females with ovipositor approximately straight, ovipositor tip never sharply decurved.

Gauld et al. (2002) found a single autapomorphy for the genus: mid tarsomere IV medioventrally with a longitudinal band of fine hairs.

Pimpla albomarginata Cameron, 1886
Figure 1A–F

Pimpla albo-marginata Cameron, 1886: 267. Holotype ♀, Mexico (BMNH).
Coccygomimus albomarginatus; Townes and Townes 1966: 24.

Diagnosis. This species can be distinguished from the other Uruguayan species of the genus by the combination of the following character states: 1) wings hyaline; 2) clypeus with apex deeply bilobed; 3) malar space wide, longer than basal mandibular width, that males less than 0.6 times basal mandibular width; 4) mesoscutum entirely black; 5) postscutellum black; 6) mesopleural suture weakly faveolated; 7) propodeum with conspicuous posterolateral tubercles; 8) fore wing Rs more or less straight and cu-a slightly distal to the base of Rs&M; 9) coxae without black markings and fore coxa with markings; 10) metasoma black and white banded; 11) laterotergites V broad, more than 0.5 times as broad as long; 12) tergite I of female short and broad, in profile strongly convex, in profile with moderately high blunt hump; 13) sternite I with strongly produced swelling; 14) apex of ovipositor with dorsal valve of ovipositor apically without teeth.

Biological notes. Nothing is known about the host preferences of this species.

Distribution. Colombia, Costa Rica, Mexico, Panama, Venezuela, and Uruguay* (Fig. 11A).

Figure 1. *Pimpla albomarginata* Cameron, 1886 **A** ♀, habitus, lateral view **B** ♂, habitus, lateral view **C** ♀, face, frontal view **D** ♀, metasoma, dorsal view **E** ♀, metasoma, ventral view (arrow pointing to laterotergite V) **F** ♀, ovipositor apex.

Material examined. URUGUAY, Rocha, Don Bosco, Bosque-Campo, 34°05'02.6"S, 53°45'44.5"W, 10.VI.2015, Malaise trap I (E. Castiglioni and team leg.), 1♀, INPA; Cardoso, Campo Natural, 34°05'28.0"S, 53°52'11.4"W, 10.VI.2015, Malaise trap II (E. Castiglioni and team leg.), 1♀, INPA; Don Bosco, Bosque-Campo, 34°05'02.6"S, 53°45'44.5"W, 12.I.2015, Malaise trap I (E. Castiglioni and team leg.), 1♂, INPA.

Pimpla caerulea Brullé, 1846
Figure 2A–F

Pimpla caerulea Brullé, 1846: 101. Type: ♀, Brazil (MNHN).
Coccygomimus caeruleus caeruleus; Townes and Townes 1966: 24.
Coccygomimus caeruleus glaucus; Townes and Townes 1966: 25.

Diagnosis. This species can be distinguished from the other Uruguayan species of the genus by the combination of the following character states: 1) wings more or less blackish; 2) body metallic blue (male with fore coxae white marked); 3) laterotergite V narrow, less than 0.3 times as long as wide.

 Biological notes. Parasitoid of *Alabama argillacea* (Hübner, 1818) (Noctuidae) (Porter 1970).

 Distribution. Argentina, Bolivia, Brazil, Ecuador, Guatemala, Mexico, Peru, Paraguay, Uruguay* (Fig. 11B), and Venezuela.

 Material examined. Uruguay, Rocha, Don Bosco, Bosque-Campo, 34°05'02.6"S, 53°45'44.5"W, 29.XII.2014, Malaise trap II (E. Castiglioni and team leg.), 1♀, INPA; idem, but 12.I.2015, Malaise trap II, 1♀ and 3♂♂, INPA; idem, but 12.III.2015, Malaise trap II, 1♂, INPA; idem, but 26.II.2015, Malaise trap II, 1♀, INPA; idem, but 28.I.2015, Malaise trap I, 1♂, INPA; idem, but 28.I.2015, Malaise trap II, 1♂, INPA; idem, but 29.XII.2014, Malaise trap I, 1♂, INPA; idem, but 29.XII.2014, Malaise trap II, 2♀♀ and 2♂♂, INPA.

Pimpla cyanipennis Brullé, 1846
Figures 3A–C, 4A–C

Pimpla cyanipennis Brullé, 1846: 101. Syntype: ♀, Uruguay (MNHN).
Coccygomimus cyanipennis; Townes and Townes 1960: 328.

Diagnosis. This species can be distinguished from the other Uruguayan species of the genus by the combination of the following character states: 1) wings darkened; 2) mesosoma and metasoma black; 3) laterotergite V 1.6–1.7 times as long as wide; 4) legs orange, except coxa, trochanter, trochantellus, apex of hind tibia and tarsus black; 5) tergite II silky shining, slightly coriaceous and mostly (except of apical rim), with almost uniformly distributed, large, strong, from more or less adjacent to confluent punctures; 6) malar space 1.0–1.2 times as long as basal width of mandibles; 7) ovipositor approx. 1.75 times as long as hind tibia; 8) ovipositor cylindric, with apex of dorsal valve without teeth and ventral valve with gently convex teeth on tip.

 Biological notes. Nothing is known about the host preferences of this species.

 **Distribution.** Argentina and Uruguay (Fig. 11C).

 Material examined. Syntype, Chile (♀, EY9374), examined by photo (Fig. 3A–C). Syntype, Chile (sex undetermined, EY9375), examined by photo (Fig. 4A–C).
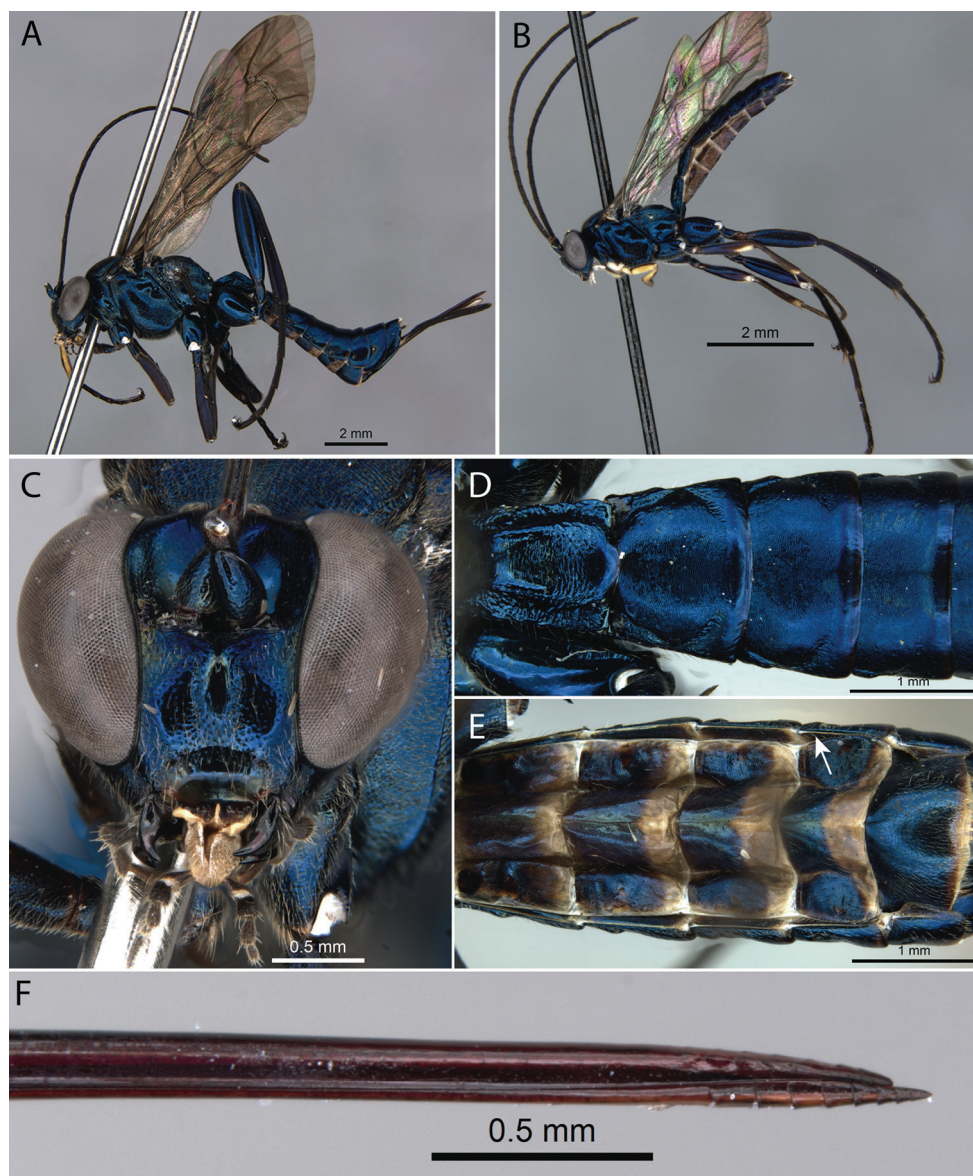
Figure 2. *Pimpla caerulea* Brullé, 1846 **A** ♀, habitus, lateral view **B** ♂, habitus, lateral view **C** ♀, face, frontal view **D** ♀, metasoma, dorsal view **E** ♀, metasoma, ventral view (arrow pointing to laterotergite V) **F** ♀, ovipositor apex.

Remarks. Brullé (1846) described *P. cyanipennis* based on specimens from Montevideo (Uruguay; C. Gaudichaud collector). Later, Porter (1970) expanded the distribution of the species to Argentina. However, Porter did not study the type specimens of this species, deposited at MNHN. We analyzed the syntypes (EY9374 and EY9375), and verified that the type locality on the label is in Chile (C. Gay collector). The French botanist and naturalist Claude Gay carried out several expeditions in the Andes, es-
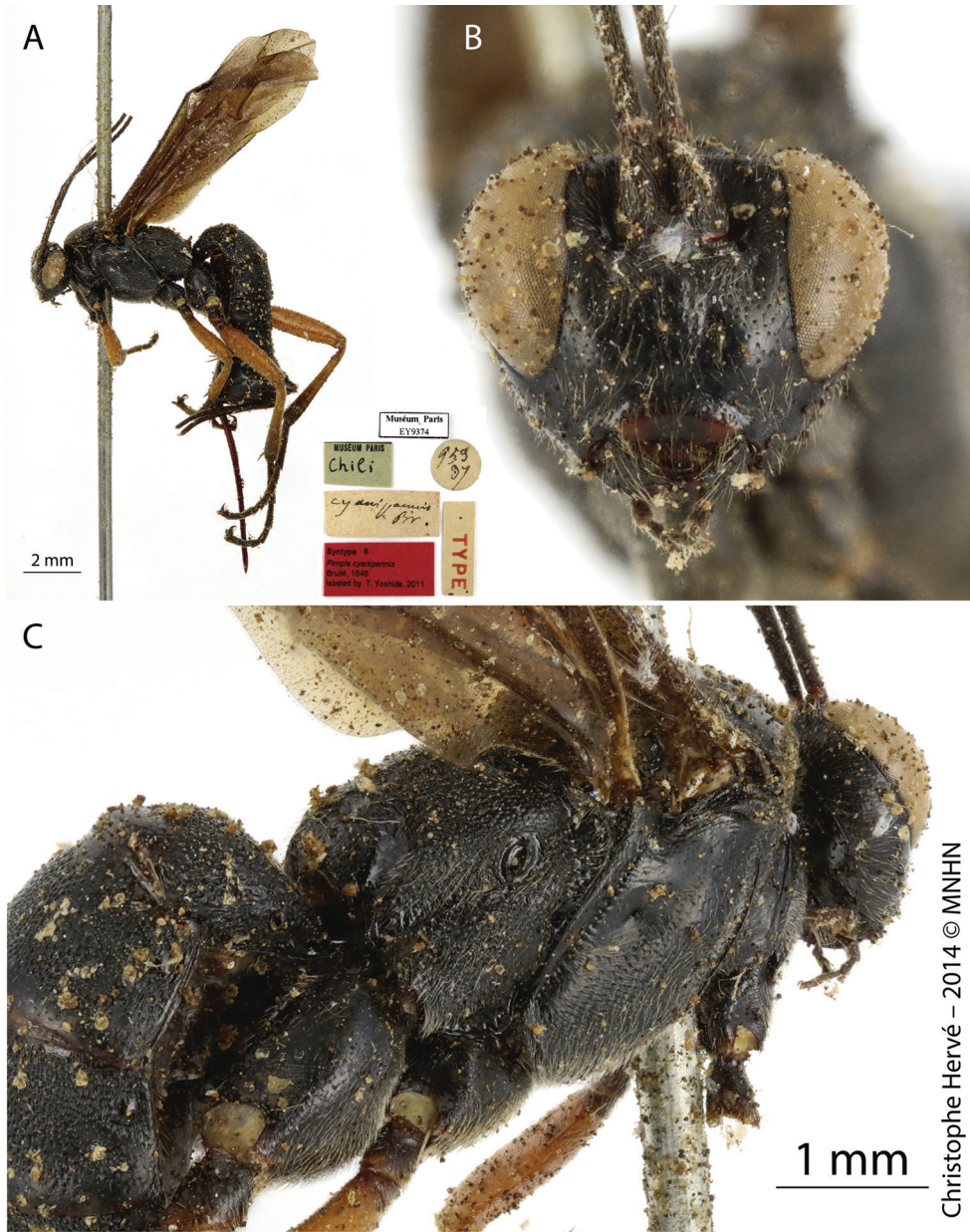
Figure 3. *Pimpla cyanipennis* Brullé, 1846 (Syntype, ♀) **A** habitus, lateral view **B** face, frontal view **C** mesosoma and part of metasoma, dorsolateral view. Figures by Christophe Hervé, MNHN.

pecially in Chile and Peru. A large part of the material deposited by him in MNHN originated from these countries. Furthermore, Gaudichaud, who was appointed by Brullé as a collector of types, made several expeditions in Uruguay and Brazil (materials also deposited in MNHN). Thus, we hypothesize that: 1) the labels may have been un-intentionally replaced in specimens, 2) the photos of the labels may have been added to

Figure 4. *Pimpla cyanipennis* Brullé, 1846 (Syntype, sex?) **A** habitus, lateral view **B** face, frontal view **C** mesosoma, dorsal view. Figures by Christophe Hervé, MNHN.

the specimens in a wrong way in the MNHN database, or 3) Brullé may have confused the type locality when describing this species. Townes (1961) corrected inconsistencies in type localities in some species described by Brullé in MNHN, but he did not men-

tion this species. In fact, we have studied the type specimens by using only photos, and we believe that only an *in situ* specimen examination can solve this inconsistency. Thus, we have decided to report this species only from Argentina and Uruguay.

Pimpla golbachi (Porter, 1970)
Figure 5A–F

Ephialtes kreibohmi Blanchard, 1942; *nomen nudum* according to Townes and Townes 1966: 29.
Coccygomimus golbachi Porter, 1970: 153. Holotype ♀, Argentina (IML).

Diagnosis. This species can be distinguished from the other Uruguayan species of the genus by the combination of the following character states: 1) wings hyaline; 2) mesosoma black with hind corners of meso- and metapleuron brown and tegula white; 3) metasoma reddish; 4) laterotergite V 1.3 times as long as wide; 5) legs reddish, except of fore coxa often more or less broadly blackish basally, hind tibia sometimes slightly dusky, especially near apex, and tarsi usually duller often slightly dusky on apical segment; 6) tergite II shiny and with almost uniformly distributed large, deep, adjacent to reticulately confluent punctures, except narrowly smooth on apex; 7) malar space 0.8–1.0 (0.6–0.9 in male) times as long as basal width of mandibles; 8) ovipositor approx. 1.45–1.7 times as long as hind tibia; 9) ovipositor cylindric, dorsal valve with apex without teeth and ventral valve with gently convex teeth on tip.

Biological notes. Parasitoid of Gelechiidae: *Pectinophora gossypiella* (Saunders, 1844); Noctuidae: *Alabama argillacea* (Hübner, 1818) (Porter 1970); Pieridae: *Colias lesbia* (Fabricius, 1775) (Avalos et al. 2011); Pyralidae: *Diaphania hyalinata* (Linnaeus, 1767); Tortricidae: *Rhyacionia buoliana* (Denis & Schiffermüller, 1775) (Porter 1970). Based on the material collected in our samples in Uruguay, we verified that the peak of occurrence of this species in the sampled locations was between November and January.

Distribution. Argentina, Bolivia, Brazil, Colombia, Paraguay, and Uruguay (Fig. 11D).

Material examined. Uruguay, Rocha, Castillos, Branaa, Agricultura, 34°03'31.8"S, 53°50'05.2"W, 30.XI.2015, Malaise trap II (E. Castiglioni and team leg.), 2♂♂, INPA; Castillos, Llambi, Pasto-agricultura, 34°24'7.04"S, 54°08'1.48"W, 12.II.2016, Malaise trap II (E. Castiglioni and team leg.), 1♀, INPA; idem, but 15.III.2016, Malaise trap II, 2♂♂, INPA; idem, but 28.I.2016, Malaise trap II, 1♀ and 1♂, INPA; Castillos, Cardoso, Campo Natural, 34°05'26.8"S, 53°52'14.4"W, 14.I.2016, Malaise trap I (E. Castiglioni and team leg.), 1♂, INPA; idem, but 15.III.2016, Malaise trap II, 1♀, INPA; idem, but 21.XII.2016, Malaise trap II, 1♂, INPA; idem, but 29.III.2016, Malaise trap I, 1♂, INPA; idem, but 29.III.2016, Malaise trap II, 1♂, INPA; idem, but 10.IV.2015, Malaise trap I, 1♀ and 1♂, INPA; idem, but 11.II.2015, Malaise trap II, 1♂, INPA; idem, but 12.I.2015, Malaise trap II, 1♂, INPA; idem, but 13.XI.2015, Malaise trap I, 4♂♂, INPA; idem, but 15.XII.2015, Malaise trap I, 1♂, INPA; idem,
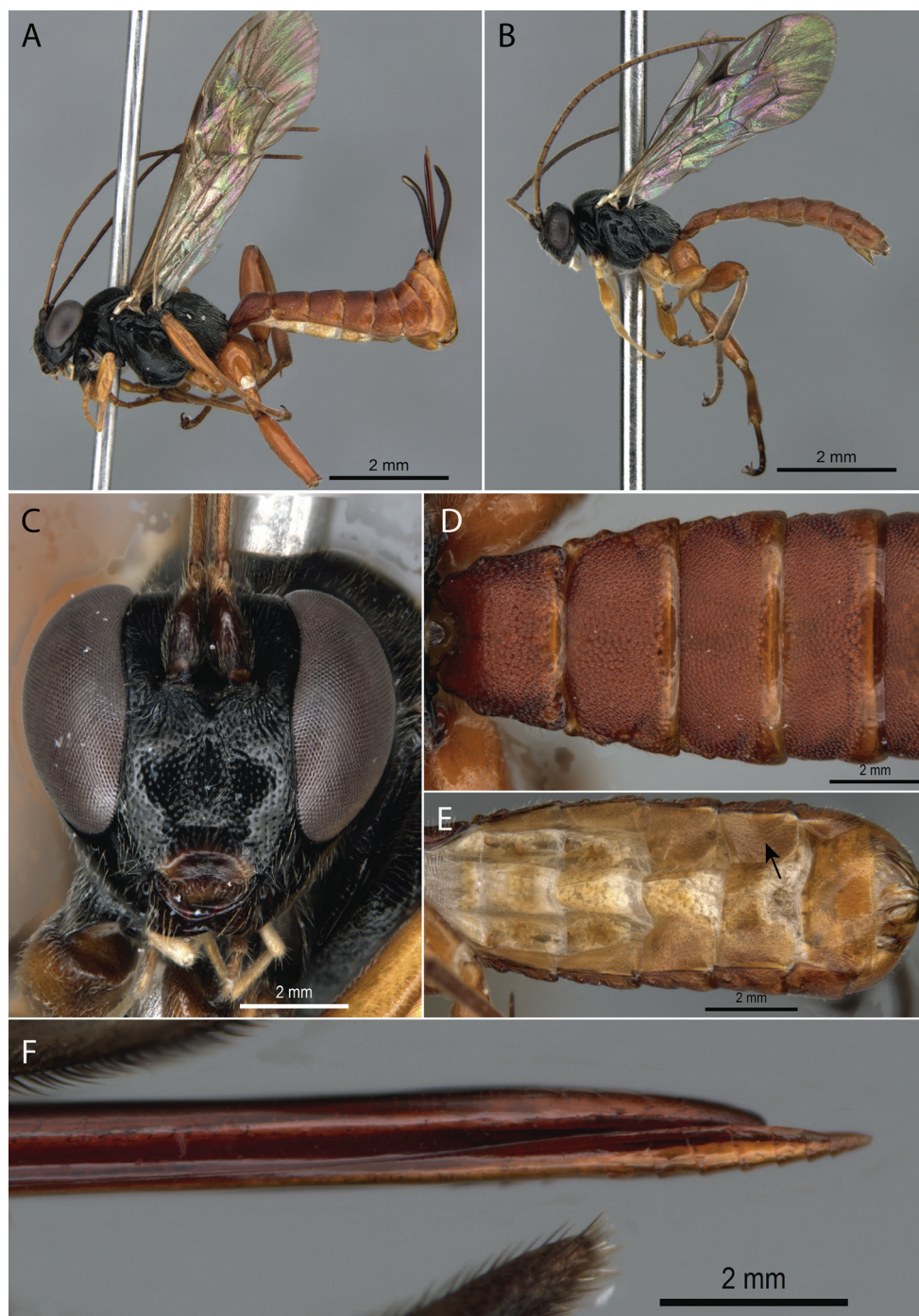
Figure 5. *Pimpla golbachi* (Porter, 1970) **A** ♀, habitus, lateral view **B** ♂, habitus, lateral view **C** ♀, face, frontal view **D** ♀, metasoma, dorsal view **E** ♀, metasoma, ventral view (arrow pointing to laterotergite V) **F** ♀, ovipositor apex.

but 15.XII.2015, Malaise trap II, 1♂, INPA; idem, but 26.II.2015, Malaise trap II, 1♂, INPA; idem, but 26.V.2015, Malaise trap II, 1♂, INPA; idem, but 27.IV.2015, Malaise trap I, 1♀, INPA; idem, but 27.VII.2015, Malaise trap I, 1♀, INPA; idem, but 28.I.2015, Malaise trap II, 1♀, INPA; idem, but 29.XII.2015, Malaise trap I, 1♂, INPA; Castillos, Don Bosco, Bosque-Campo, 34°05'1.07"S, 53°45'43.08"W, 14.I.2016, Malaise trap I (E. Castiglioni and team leg.), 2♂♂, INPA; idem, but 14.I.2016, Malaise trap II, 1♀, INPA; idem, but 29.XII.2015, Malaise trap I, 1♀ and 2♂♂, INPA; idem, but 11.IX.2015, Malaise trap I, 1♀ and 1♂, INPA; idem, but 12.I.2015, Malaise trap I, 1♀ and 1♂, INPA; idem, but 12.I.2015, Malaise trap II, 1♂, INPA; idem, but 12.III.2015, Malaise trap I, 1♂, INPA; idem, but 13.XI.2015, Malaise trap I, 1♂, INPA; idem, but 13.XI.2015, Malaise trap II, 2♂♂, INPA; idem, but 15.XII.2015, Malaise trap I, 1♂, INPA; idem, but 15.XII.2015, Malaise trap II, 1♂, INPA; idem, but 27.X.2015, Malaise trap I, 1♂, INPA; idem, but 27.X.2015, Malaise trap II, 1♀, INPA; idem, but 28.I.2015, Malaise trap I, 2♂♂, INPA; idem, but 28.I.2015, Malaise trap II, 1♂, INPA; idem, but 28.IX.2015, Malaise trap I, 1♀, INPA; idem, but 29.XII.2014, Malaise trap I, 1♀ and 1♂, INPA; idem, but 29.XII.2014, Malaise trap II, 3♂♂, INPA; idem, but 30.XI.2015, Malaise trap I, 3♀♀, INPA.

Remarks. Townes and Townes (1966) reported a new species of *Coccygomimus* as "*Coccygomimus* n. sp." from Argentina and considered *Ephialtes kreibohmi* Blanchard, 1942 to be *nomen nudum* of it. Later, Porter (1970) described the species mentioned by Townes and Townes (1966) as *Coccygomimus golbachi*.

Pimpla patirrufa nom. nov.
Figures 6A–F, 7A–C

Pimpla rufipes Brullé, 1846: 102. Lectotype: ♀, Uruguay (MNHN). Non *Pimpla rufipes* (Miller, 1759).
Coccygomimus rufipes; Townes and Townes 1960: 338.
Coccygomimus rufipes; Townes 1961: 173.
Coccygomimus rufipes; Townes and Townes 1966: 27.

Diagnosis. This species can be distinguished from the other Uruguayan species of the genus by the combination of the following character states: 1) wings hyaline with weak brownish staining; 2) mesosoma shining black; 3) metasoma black with more or less brown staining on apical rims; 4) laterotergite V 1.4–1.6 times as long as wide; 5) legs orange with fore coxae orange or black, fore and mid tarsi slightly duller orange to slightly dusky, hind tibia duller orange with rather weak blackish staining on apex, hind tarsus extensively blackish to black; 6) tergite II with larger and stronger punctures; 7) malar space 1.0–1.1 (0.85–1.0 in male) times as long as basal width of mandibles; 8) ovipositor 1.3–1.6 times as long as hind tibia; 9) ovipositor cylindric, apex of dorsal valve without teeth and ventral valve with gently convex teeth on tip.

**Biological notes.** Parasitoid of *Plusia* sp. (Noctuidae) (Porter 1970).

Figure 6. *Pimpla patirrufa* nom. nov. **A** ♀, habitus, lateral view **B** ♂, habitus, lateral view **C** ♀, face, frontal view **D** ♀, metasoma, dorsal view **E** ♀, metasoma, ventral view (arrow pointing to laterotergite V) **F** ♀, ovipositor apex.

Distribution. Argentina, Brazil, and Uruguay (Fig. 11F).

Material examined. *Lectotype*, Uruguay, Montevideo (♀, EY9414), examined by photo (Fig. 8A–C). URUGUAY, Rocha, Branaa, Agricultura, 34°02'33.7"S, 53°50'03.1"W, 11.II.2015, Malaise trap II (E. Castiglioni and team leg.), 1♂, INPA; idem, but 12.I.2015, Malaise trap I, 3♂♂, INPA; idem, but Malaise trap II, 1♀ and
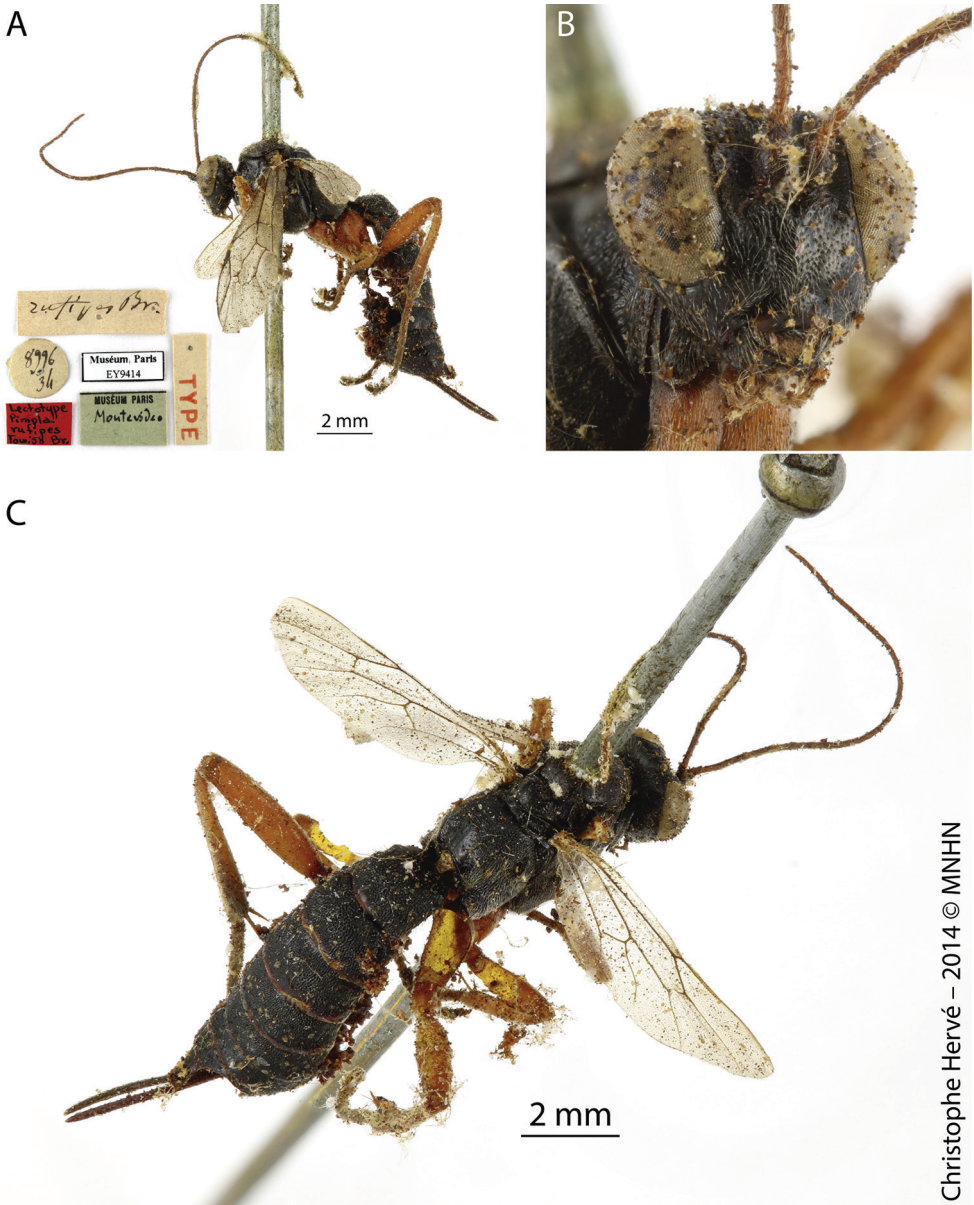
Figure 7. *Pimpla patirrufa* nom. nov. (Lectotype of *Pimpla rufipes* Brullé, 1846, ♀) **A** habitus, lateral view **B** face, frontal view **C** mesosoma and metasoma, dorsal view. Figures by Christophe Hervé, MNHN.

1♂, INPA; idem, but 12.III.2015, Malaise trap II, 1♂, INPA; idem, but 27.IV.2015, Malaise trap I, 1♂, INPA; idem, but 28.I.2015, Malaise trap II, 2♂♂, INPA; idem, but 29.XII.2014, Malaise trap II, 1♀, INPA; idem, but 30.XI.2015, Malaise I, 1♂, INPA; Cardoso, Campo Natural, 34°05'26.8"S, 53°52'14.4"W, 12.I.2015, Malaise trap I (E. Castiglioni and team leg.), 1♂, INPA; idem, but 26.II.2015, Malaise trap I, 1♂, INPA; idem, but 28.I.2015, Malaise trap I, 1♂, INPA; idem, but

29.XII.2014, Malaise trap I, 1♂, INPA; idem, but 29.XII.2014, Malaise trap II, 1♂, INPA; idem but 21.XII.2016, Malaise trap II, 1♂, INPA; Castillos, Llambi, Pasto-agricultura, 34°24'7.04"S, 54°08'1.48"W, 08.XII.2016, Malaise trap I (E. Castiglioni and team leg.), 1♂, INPA; idem, but 21.XII.2016, Malaise trap I, 1♀, INPA; idem, but 26.II.2015, Malaise trap II, 1♂, INPA; Don Bosco, Bosque-Campo, 34°05'02.6"S, 53°45'44.5"W, 10.VI.2015, Malaise trap I (E. Castiglioni and team leg.), 1♂, INPA; idem, but 11.II.2015, Malaise trap I, 1♂, INPA; idem, but 26.II.2015, Malaise trap I, 1♂, INPA; idem, but 28.I.2015, Malaise trap II, 1♂, INPA; idem, but 29.XII.2014, Malaise trap I, 2♂♂, INPA; idem, but 29.XII.2014, Malaise trap II, 1♂, INPA.

Etymology. The new specific name "patirrufa" is derived from the Spanish words "patas rufas", and refers to the Spanish transliteration of "rufipes", the original name proposed by Brullé. The name is to be treated as a noun in apposition.

Remarks. *Pimpla patirrufa* nom. nov. is a replacement name for *P. rufipes* Brullé, 1846. The name "rufipes" was already occupied by *Pimpla rufipes* (Miller, 1759). According to the International Code Zoological Nomenclature, Article 57 (ICZN 1999), we propose a replacement name for this primary junior homonym. This homonymy may have caused some confusion in the literature. Çoruh and Kesdek (2008), Özbek and Çoruh (2012), and Çoruh et al. (2014) cited *P. rufipes* Brullé, 1846 from Turkey and Horstmann (2001) cited this species from Germany, but these authors most probably wanted to refer to *P. rufipes* (Miller, 1759). *Pimpla rufipes* (Miller, 1759) is a widespread Old World species (Yu et al. 2016). However, as *Coccygomimus instigator* (Fabricius, 1793) (currently junior synonym of *P. rufipes* (Miller, 1759), it was introduced at least five times between 1972 and 1978 to USA from Morocco, Yugoslavia, Iran, Poland and Romania (Coulson et al. 1986). Zwakhals (2005) listed some morphological differences of *P. rufipes* (Miller, 1759) in comparsion with other European species of *Pimpla*. Some of these characteristics assure us that this species is not closely related to *P. rufipes* Brullé, 1846, as pubescence is whitish and the coxae and trochanter are reddish in Brullé's species and pubescence is fuscous and the coxae and trochanter are black in Miller's species. In addition, *P. rufipes* Brullé, 1846 has a distribution restricted to South America (Argentina, Brazil, and Uruguay).

Pimpla perssoni Gauld, 1991
Figure 8A–F

Pimpla perssoni Gauld, 1991: 508. Holotype ♀, Costa Rica (MNCR).

Diagnosis. This species can be distinguished from the other Uruguayan species of the genus by the combination of the following character states: 1) wings yellowish with distal margin of the fore wing blackish; 2) mesosoma yellow with black marks on mesoscutum (three stripes), hind part of tegula, hind margin of scutellum, anterior margin of mesopleuron, 7-shaped mark on upper hind part of mesopleuron, a continuous anterior band along the anterior margin of metapleura, and propodeum and
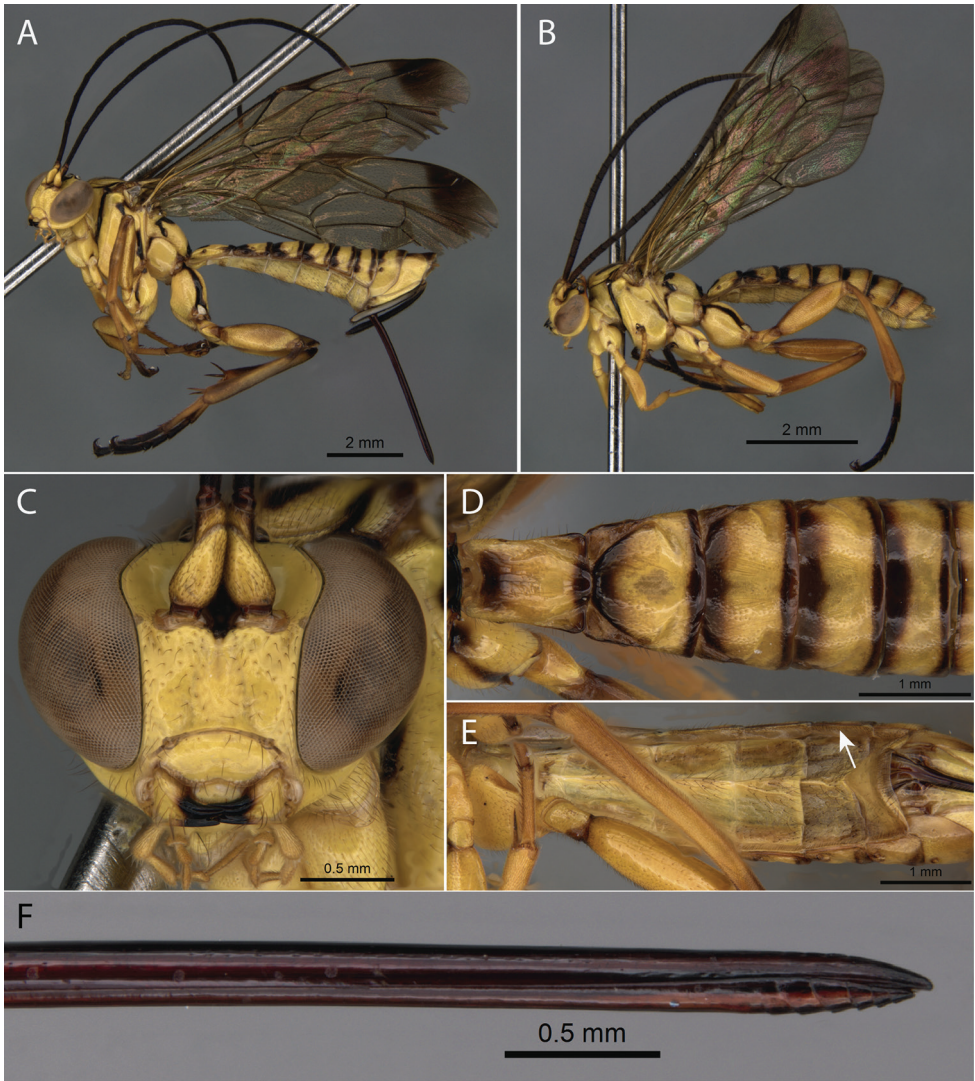
Figure 8. *Pimpla perssoni* Gauld, 1991 **A** ♀, habitus, lateral view **B** ♂, habitus, lateral view **C** ♀, face, frontal view **D** ♀, metasoma, dorsal view **E** ♀, metasoma, ventral view (arrow pointing to laterotergite V) **F** ♀, ovipositor apex.

hind rim of propodeum; 3) metasoma yellow with tergites I–IV anteriorly broadly and posteriorly narrowly black and with posterior tergites anteriorly black; 4) laterotergite V 2.7–3.4 times as long as wide; 5) legs yellow with dorsal longitudinal black band on med and hind coxa, fore, mid and hind (except the first tarsomere) tarsi strongly infuscate, femur darkened dorsally and ventrally, and tibia infuscate proximally, tibia with close and dark pubescence, giving them a dirty yellow appearance; 6) tergite II highly polished, with very fine sparse punctures, and with anterolateral corners separated by deep oblique grooves; 7) malar space 0.3–0.4 times as long as basal width of mandi-

bles; 8) ovipositor 1.25–1.3 times as long as hind tibia; 9) apex of ovipositor slightly compressed, with weak denticles arranged in a median row on dorsal valve, and with ventral valve not expanded laterally, with a few weak teeth.

Biological notes. Nothing is known about the host preferences of this species.

Distribution. Brazil, Costa Rica, Mexico, and Uruguay* (Fig. 11E).

Material examined. Uruguay, Rocha, Don Bosco, Bosque-Campo, 34°05'02.6"S, 53°45'44.5"W, 27.III.2015, Malaise trap II (E. Castiglioni and team leg.), 1♀, INPA; idem, but 28.I.2015, Malaise trap II, 1♂, INPA; idem, but 12.I.2015, Malaise trap II, 1♀, INPA; idem, but 29.XII.2014, Malaise trap I, 1♂, INPA; idem, but 12.I.2015, Malaise trap I, 1♂, INPA; idem, but 28.I.2015, Malaise trap I, 1♂, INPA.

Pimpla semirufa Brullé, 1846
Figure 9A–F

Pimpla semirufa Brullé, 1846: 103. Type: ♀, Brazil (MNHN).
Coccygomimus semirufus; Townes and Townes 1966: 28.

Diagnosis. This species can be distinguished from the other Uruguayan species of the genus by the combination of the following character states: 1) wings hyaline; 2) mesosoma shining black, lower hind corner of mesopleuron slightly brown, metapleuron red with a little black staining along front margin and sometimes also along dorsal margin and propodeum red with slight to extensive black staining basad and in spiracular area; 3) metasoma reddish with tergite V often with a little blackish staining apico-laterally, tergite VI broadly to almost wholly black and tergites VII+ completely black; 4) laterotergite V 1.6 times as long as wide; 5) legs red, fore coxa black except becoming more or less broadly reddish toward apex below, fore and mid tarsi little duller reddish with slight dusky staining on apical segment, hind femur often with slight dusky tinge above on apex, hind tibia dull red basad and blackish on about apical half, hind tarsus dull red with much dusky staining; 6) tergite II shining with abundant, large, strong, mostly adjacent to confluent punctures, except on the narrow smooth apical rim; 7) malar space 0.8–1.0 times as long as basal width of mandibles; 8) ovipositor 1.3–1.6 times as long as hind tibia; 9) ovipositor cylindric, apex of dorsal valve without teeth and ventral valve with gently convex teeth on tip.

Biological notes. Nothing is known about the host preferences of this species.

Distribution. Argentina, Brazil, and Uruguay*(Fig. 11G).

Material examined. Uruguay, Rocha, Cardoso, Campo Natural, 34°05'26.8"S, 53°52'14.4"W, 10.VI.2015, Malaise trap II (E. Castiglioni and team leg.), 1♀, INPA; idem, but 15.XII.2015, Malaise trap I, 1♂, INPA; idem, but 24.VI.2015, Malaise trap II, 1♂, INPA; Castillos, Don Bosco, Bosque-Campo, 34°05'1.07"S, 53°45'43.08"W, 21.XII.2016, Malaise trap I (E. Castiglioni and team leg.), 1♂, INPA; idem, but 21.XII.2016, Malaise trap I, 1♂, INPA; Castillos, Don Bosco, Bosque-Campo, 34°05'1.07"S, 53°45'43.08"W, 21.XII.2016, Malaise trap I (E.
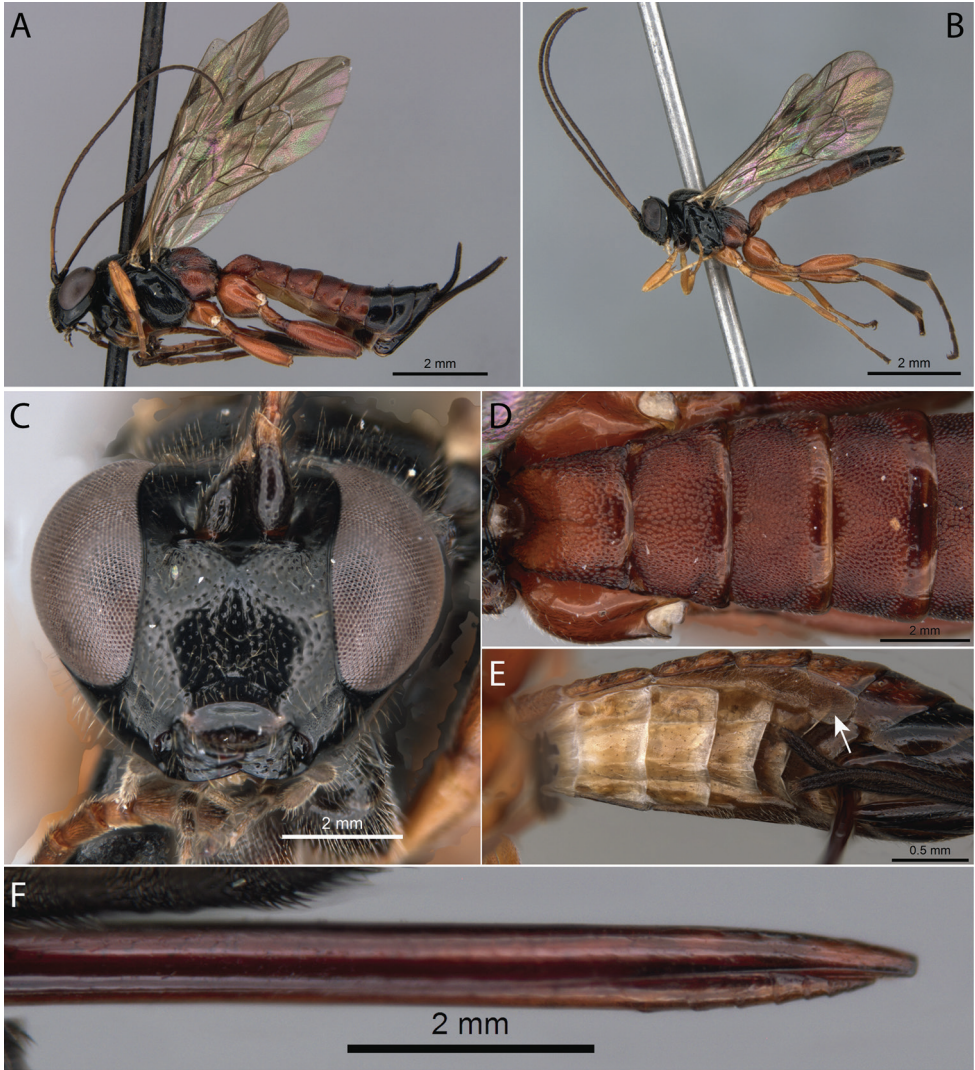
Figure 9. *Pimpla semirufa* Brullé, 1846 **A** ♀, habitus, lateral view **B** ♂, habitus, lateral view **C** ♀, face, frontal view **D** ♀, metasoma, dorsal view **E** ♀, metasoma, ventral view (arrow pointing to laterotergite V) **F** ♀, ovipositor apex.

Castiglioni and team leg.), 2♂♂, INPA; Castillos, Llambi, Pasto-agricultura, 34°24'7.04"S, 54°08'1.48"W, 08.XII.2016, Malaise trap I (E. Castiglioni and team leg.), 1♂, INPA; idem, but 15.III.2016, Malaise trap II, 1♀, INPA; Don Bosco, Bosque-Campo, 34°05'02.6"S, 53°45'44.5"W, 10.VI.2015, Malaise trap I (Castiglioni and team leg.), 1♂, INPA; idem, but 12.I.2015, Malaise trap II, 2♂♂, INPA; idem, but 13.X.2015, Malaise trap II, 1♂, INPA; idem, but 14.I.2016, Malaise trap I, 2♂♂, INPA; idem, but 28.I.2015, Malaise trap I, 2♀♀, INPA; idem, but 29.XII.2014, Malaise trap II, 1♀, INPA.

Pimpla tomyris Schrottky, 1902
Figure 10A–F

Pimpla tomyris Schrottky, 1902: 95. Types: ♂, ♀, Argentina (lost).
Pimpla videonis; Townes and Townes 1966: 28.
Neogabunia paulistana; Townes and Townes 1966: 29.
Coccygomimus tomyris; Townes and Townes 1966: 28.

Diagnosis. This species can be distinguished from the other Uruguayan species of the genus by the combination of the following character states: 1) wings hyaline with pale yellow staining; 2) mesosoma shining black with variable yellow markings on pronotum, tegula, scutellum, postscutellum, and propodeum (a pair of elliptic blotches); 3) metasoma reddish brown with a pair of large yellow blotches laterally in tergites I–II (tergites I–IV in males); 4) laterotergite V 2.1–2.2 times as long as wide; 5) legs yellow, except for fore and mid coxa black (sometimes) and hind coxa with a black mark, femur and basal half of tibia reddish brown and last tarsus blackish; 6) tergite II rather dully to brightly shining with moderately strong to fine or very fine micro-reticulation and mostly sparse, irregularly spaced, small to large, obscure to well-defined punctures; 7) malar space 0.6–1.0 (0.4–0.7 in male) times as long as basal width of mandibles; 8) ovipositor 1.45 times as long as hind tibia; 9) ovipositor moderately depressed, apex of dorsal and ventral valves apically with teeth, the apical ridge-bearing portion not unusually flattened and in profile slightly convex.

Biological notes. Parasitoid of Erebidae: *Hypercompe indecisa* (Walker, 1855), *Hyposcrisias fuscipennis* (Burmeister, 1878); Limacodidae: *Phobetron hipparchia* (Crammer, 1777); Papilionidae: *Papilio thoas thoantiades* (Burmeister, 1878); Psychidae: *Oiketicus kirbyi* (Guilding, 1927), *O. platensis* (Berg, 1883); Saturniidae: *Eudyaria venata* (Butler, 1871), *Hylesia nigricans* (Berg, 1875); Tortricidae: *Rhyacionia buoliana* (Denis & Schiffermüller, 1775) (Yu et al. 2016).

Distribution. Argentina, Bolivia, Brazil, Paraguay, Peru, Uruguay, (Fig. 11H) and Venezuela.

Material examined. Uruguay, Rocha, Castillos, Cardoso, Campo Natural, 34°05'26.8"S, 53°52'14.4"W, 28.XI.2016, Malaise trap I (E. Castiglioni and team leg.), 1♀, INPA; idem, but Don Bosco, Bosque-Campo, 34°05'02.6"S, 53°45'44.5"W, 12.I.2015, Malaise trap II, 1♂, INPA; idem, but except 28.I.2015, Malaise trap II, 1♂, INPA.

Discussion

During the last 30 years, the Darwin wasp fauna of some Neotropical countries (i.e. Brazil, Costa Rica, and Peru) have been sampled in more detail. These studies have revealed a very high species richness and a plethora of new taxa from many parts of the region (e.g. Gauld 1991; Sääksjärvi et al. 2004; Veijalainen et al. 2012). However, most parts of the Neotropical region have remained understudied.

Figure 10. *Pimpla tomyris* Schrottky, 1902 **A** ♀, habitus, lateral view **B** ♂, habitus, lateral view **C** ♀, face, frontal view **D** ♀, metasoma, dorsal view **E** ♀, metasoma, ventral view (arrow pointing to laterotergite V) **F** ♀, ovipositor apex.

Uruguay's biodiversity knowledge is still very fragmentary (Aldabe et al. 2008) and this is also shown by the genus *Pimpla*. Before of our study, only four species of *Pimpla* were known from the country: *P. cyanipennis* Brullé, 1846; *P. golbachi* (Porter, 1970); *P. patirrufa* nom. nov.; and *P. tomyris* Schrottky, 1902 (Yu et al. 2016). Here, we have doubled the species richness of *Pimpla* in Uruguay. All known Uruguayan species are also widely distributed in South America or the Neotropical region in general. *Pimpla*
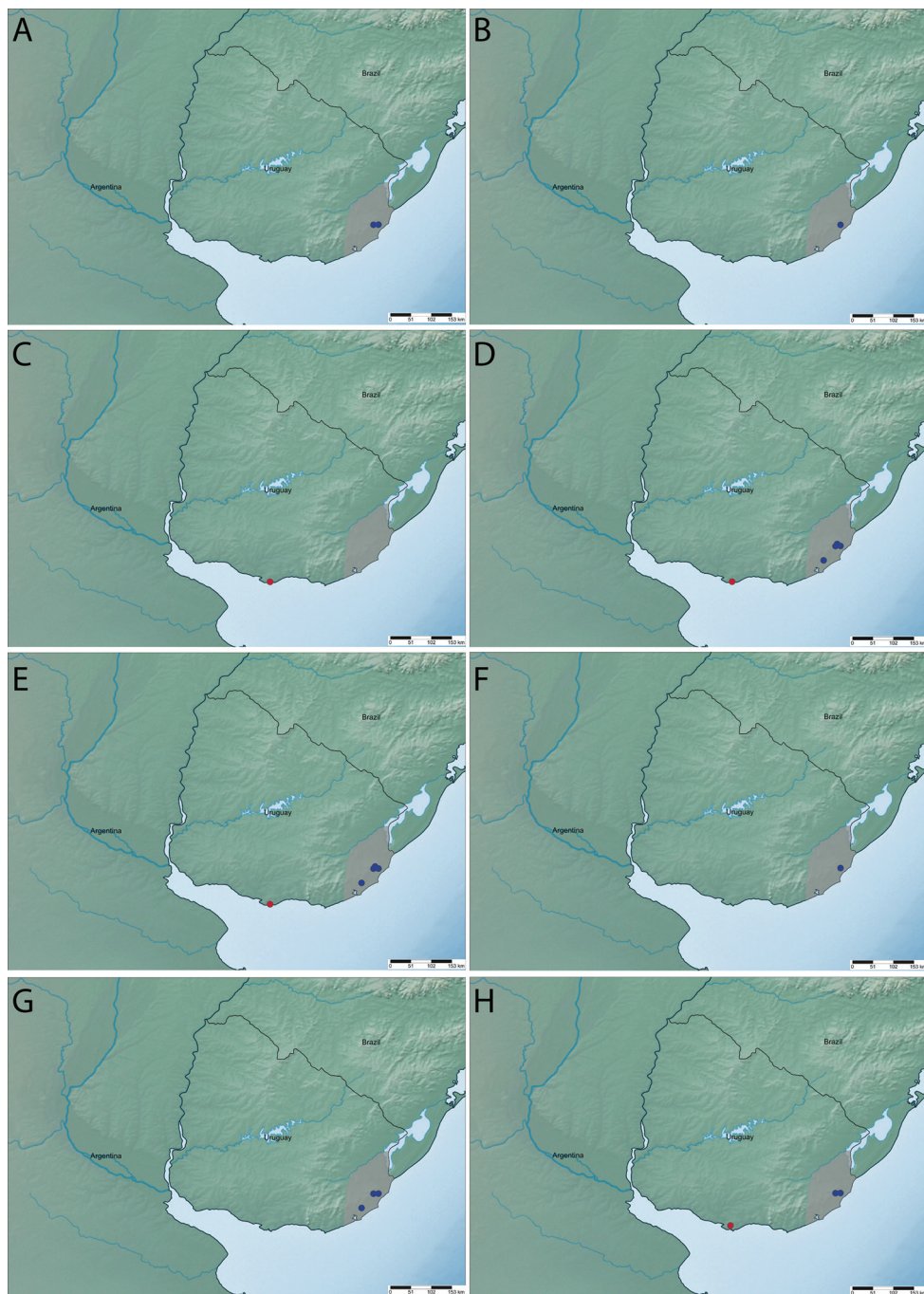
Figure 11. Distribution of *Pimpla* spp. in Uruguay **A** *P. albomarginata* Cameron, 1886 **B** *P. caerulea* Brullé, 1846 **C** *P. cyanipennis* Brullé, 1846 **D** *P. golbachi* (Porter, 1970) **E** *P. perssoni* Gauld, 1991 **F** *P. patirrufa* nom. nov. **G** *P. semirufa* Brullé, 1846 **H** *P. tomyris* Schrottky, 1902. Gray area = Rocha Department. Red circle = previous record. Blue circle = new record.

species are moderately large and strong-flying insects, which explains their wide distribution over vast regions.

We hope that this study draws more attention to Uruguay's apparently rich, but very little-known, Darwin wasp fauna.

Acknowledgements

References

Aldabe J, Bartesaghi L, Blanco D, Brazeiro A, Calvar M, García G, Tagliani LG, González EM, Rivas M, Scarlato G, Soutullo A (2008) Capítulo 4: Biodiversidad. In: CLAES PNUMA, DINAMA (Eds). GEO Uruguay: informe del estado del ambiente. Gráfica Mosca, Montevideo, 178–239.

Avalos S, Mazzuferi V, Berta C, La Porta N, Serra G (2011) Structure of the Parasitic Complex of *Colias lesbia* (Lepidoptera: Pieridae) on Lucerne Crop, in Córdoba, Argentina. Revista Chilena de Entomología 36: 15–24.

Brullé MA (1846) Tome Quatrième. Des hyménoptères. Les ichneumonides. In: Lepeletier de Saint-Fargeau A (Ed.) Histoire Naturelles des Insectes. IV. Paris, 56–324.

Coulson JR, Fuester RW, Schaefer PW, Ertle LR, Kelleher JS, Rhoads LD (1986) Exploration for and importation of natural enemies of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), in North America: an update. Proceedings of the Entomological Society of Washington 88: 461–475.

Fernandes DRR, Pádua DG, Lara RIR, Perioto NW, Burla JP, Castiglioni E (2019) Subfamily composition of Ichneumonidae (Hymenoptera: Ichneumonoidea) from eastern Uruguay. Entomological Communications 1: ec01016. https://doi.org/10.37486/2675-1305.ec01016

Gauld ID (1991) The Ichneumonidae of Costa Rica I. Memoirs of the American Entomological Institute 47: 1–589.

Gauld ID, Wahl DB, Broad GR (2002) The suprageneric groups of the Pimplinae (Hyme-noptera: Ichneumonidae): a cladistic re-evaluation and evolutionary biological study. Zoological Journal of the Linnean Society 136: 421–485. https://doi.org/10.1046/j.1096-3642.2002.00031.x

Gauld ID, Gómez JAU, Hanson PS (1998) Guía de los Pimplinae de Costa Rica (Hymenop-tera: Ichneumonidae). Revista de Biología Tropical 46: 1–189.

Horstmann K (2001) Ichneumonidae. In: Dathe HH, Taeger A, Blank SM (Eds) Verzeichnis der Hautflügler Deutschlands (Entomofauna Germanica 4). Entomolische Nachrichten und Berichte (Dresden), Bieheft 7: 69–103.

International Commission on Zoological Nomeclature (1945) International Commission on Zoological Nomeclature. Nature 155: 751–752. https://doi.org/10.1038/155751c0

International Commission on Zoological Nomeclature (1999) International Code of Zoologi-cal Nomeclature. London, International Trust for Zoological Nomeclature. Fourth Edi-tion. The International Trust for Zoological Nomenclature, London, 306 pp.

Klopfstein S, Santos BF, Shaw MR, Alvarado M, Bennett AM, Pos DD, Giannotta M, Herrera-Florez AF, Karlsson D, Khalaim AI, Lima AR, Mikó I, Sääksjärvi IE, Shimizu S, Spasojevic T, van Noort S, Vilhelmsen L, Broad GR (2019) Darwin wasps: a new name heralds re-newed efforts to unravel the evolutionary history of Ichneumonidae. Entomological Com-munications 1: ec01006. https://doi.org/10.37486/2675-1305.ec01006

Özebek H, Çoruh S (2012) Larval parasitoids and larval diseases of *Melacosoma neustria* L. (Lepidoptera: Lasiocampidae) detected in Erzurum Province, Turkey. Turkish Journal of Zoology 36(4): 447–459. https://doi.org/10.3906/zoo-1104-12

Pádua DG, Araujo RO, Mazariegos LA (2019) *Pimpla* Fabricius (Hymenoptera: Ichneumoni-dae: Pimplinae) from Colombia. Zootaxa 4683(3): 439–446. https://doi.org/10.11646/zootaxa.4683.3.8

Sääksjärvi IE, Haataja S, Neuvonen S, Gauld ID, Jussila R, Salo J, Burgos AM (2004) High local species richness of parasitic wasps (Hymenoptera: Ichneumonidae; Pimplinae and Rhyssinae) from the lowland rainforests of Peruvian Amazonia. Ecological Entomology 29(6): 735–743. https://doi.org/10.1111/j.0307-6946.2004.00656.x

Porter CC (1970) A revisión of the South American species of *Coccygomimus* (Hymenoptera, Ichneumonidae). Studia Entomologica 13: 1–192.

Quicke DLJ (2015) The Braconid and Ichneumonid Parasitoid Wasps: Biology, Sys-tematics, Evolution and Ecology. Wiley Blackwell, Chichester, 688 pp. https://doi.org/10.1002/9781118907085

Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. http://www.simplemappr.net/ [Accessed on: 2020-7-6]

Townes H (1961) Some ichneumonid types in European museums that were described from no locality or from incorrect localities (Hymenoptera). Proceedings of the Entomological Society of Washington 63: 165–178.

Townes H (1969) The genera of Ichneumonidae, part 1. Memoirs of the American Entomo-logical Institute 11: 1–305. https://doi.org/10.1007/BF02027741

Townes H, Townes M (1966) A catalogue and reclassification of the Neotropic Ichneumonidae. Memoirs of the American Entomological Institute 8: 1–367.

Veijalainen A, Wahlberg N, Broad GR, Erwin TL, Longino JT, Sääksjärvi IE (2012) Unprecedented ichneumonid parasitoid wasp diversity in tropical forests. Proceedings of the Royal Society B 279: 4694–4698. https://doi.org/10.1098/rspb.2012.1664

Watanabe K, Matsumoto R (2019) Review of the Genus *Pimpla* Fabricius, 1804 (Hymenoptera: Ichneumonidae, Pimplinae) from Japan. Japanese Journal of Systematic Entomology 25(2): 217–224.

Yu DS, van Achterberg C, Horstmann K (2016) World Ichneumonoidea 2015: Taxonomy, Biology, Morphology and Distribution. Taxapad 2016. Database on flash-drive.

Zwakhals CJ (2005) *Pimpla processioneae* and *P. rufipes*: specialist versus generalist (Hymenoptera: Ichneumonidae, Pimplinae). Entomologische Berichten 65(1): 14–16.

RESEARCH ARTICLE

Revision of the endemic Afrotropical genus *Tetractenion* (Hymenoptera, Ichneumonidae) with an identification key to genera of Banchinae for the region

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Abstract

The Afrotropical banchine fauna comprises 12 genera: *Apophua* Morley, *Atropha* Kriechbaumer, *Cryptopimpla* Taschenberg, *Exetastes* Gravenhorst, *Glyptopimpla* Morley, *Himertosoma* Schmiedeknecht, *Lissonota* Gravenhorst, *Sjostedtiella* Szépligeti, *Spilopimpla* Cameron, *Syzeuctus* Förster, *Tetractenion* Seyrig, and *Tossinola* Viktorov. A well-illustrated revised key to the genera using high definition images is provided, and the endemic Afrotropical genus *Tetractenion* is revised, previously represented by two described species. Four new species are described: *T. ibayaensis* **sp. nov.**, *T. pascali* **sp. nov.**, *T. pseudolutea* **sp. nov.**, and *T. rosei* **sp. nov.** The first species-level identification key is provided for this rare genus. Based on morphological attributes the hypothesis is presented that the species in this genus are probably nocturnal. All images and online interactive Lucid keys are available at: www.waspweb.org and the associated underlying data is made available as Suppl. materials 1, 2 LIF3 files to this paper for inter-exchange with other key production software.

Keywords

Introduction

Banchinae is a cosmopolitan group of moderately small to large-sized parasitoid wasps (Gauld et al. 2002). The group is usually well represented in all faunas and amongst the most commonly collected of all ichneumonids (Gauld et al. 2002; Broad et al. 2011). There are roughly 1800 described species and 66 genera of Banchinae currently recognized (Khalaim and Ruíz-Cancino 2012; Watanabe and Maeto 2012, 2014; Broad 2014; Choi et al. 2015; Reynolds Berry and van Noort 2016; Herrera-Florez 2017; Vas 2017; Watanabe 2017, 2018, 2020; Kasparyan and Kulitzky 2018; Li et al. 2018; Sheng et al. 2018; Watanabe and Sheng 2018; Kang et al. 2019, 2020; Yu et al. 2020). With the banchine fauna poorly known for many areas of the world, and a number of undescribed genera and species from tropical regions preserved in museum collections, the number of species is certainly far greater (Broad et al. 2011).

Most Banchinae can be readily diagnosed by the following characters: 1) a submetapleural carina anteriorly generally expanded into a lobe; 2) an arched posterior transverse carina of the propodeum; and 3) a dorsal apical notch on the ovipositor (Wahl and Sharkey 1993; Broad et al. 2011). "However, some or all of these characters do not apply to aberrant genera and species" (Broad et al. 2011) and therefore these morphological characters cannot be classified as synapomorphies for the Banchinae. An additional two apomorphies were later proposed (Gauld and Wahl 2000; Broad et al. 2011): firstly, the subapical flagellomeres of female antennae possess elongate placoid sensilla only on the dorsal surface, with smaller, rounded sensilla on the ventral surface; and secondly, the posterior corner of the pronotum is rounded, slightly twisted and flattened. While within the Ophioniformes group (Ophioninae, Ctenopelmatinae, Banchinae, Mesochorinae, Nesomesochorinae, Metopiinae, Campopleginae, Tatogastrinae, Cremastinae, Tersilochinae, Anomaloninae, Neorhacodinae, Oxytorinae, Stilbopinae, Sisyrostolinae, and Lycorinae; Gauld 1985; Wahl 1991, 1993; Quicke et al. 2009) these two characters are phylogenetically informative, it has been established that they are not synapomorphic for the subfamily Banchinae (Broad et al. 2011).

Morphologically, Banchinae can be subdivided into three tribes namely Banchini, Glyptini, and Atrophini (Townes and Townes 1973; Yu et al. 2020). In addition to having very short ovipositors, Banchini differs from the other tribes by having eight or more sensilla on the larval prelabium (Quicke 2015). Atrophini possess a reduced hypostoma (Quicke 2015) and Glyptini share similar modifications on the metasoma with *Lycorina* and some Pimplinae taxa, in that taxa within Glyptini typically possess triangular areas on tergites II–IV, delimited by paired, lateromedian grooves (Shimizu 2019), though the precise pattern of grooves differs in the former taxa. Given that Pimplinae and Lycorininae are distantly related subfamilies to Banchinae (Bennett et al. 2019), the similarity of these structures is likely to be the result of analogous character states that appear similar as a result of convergence.

Within the Afrotropical region, the subfamily comprises 12 genera and 187 described species: *Apophua* Morley, *Atropha* Kriechbaumer, *Cryptopimpla* Taschenberg, *Exetastes*

Gravenhorst, *Glyptopimpla* Morley, *Himertosoma* Schmiedeknecht, *Lissonota* Gravenhorst, *Sjostedtiella* Szépligeti, *Syzeuctus* Förster, *Spilopimpla* Cameron, *Tetractenion* Seyrig, and *Tossinola* Viktorov. A dichotomous identification key to banchine genera within the Afrotropical region was last produced by Townes and Townes (1973), providing the most comprehensive taxonomic treatment to date. Nevertheless, the generic key is outdated and not supported with applicable illustrations of character states. Subsequent to this treatment, the genus *Glyptopimpla* was removed from synonymy with *Teleutaea* Förster and re-instated as a valid genus and as a senior synonym of *Zygoglypta* Momoi and *Orientoglypta* Kuslitzky (Gupta 2002).

Tetractenion, placed in the tribe Banchini, is a very rare genus restricted to the Afrotropical region (Townes 1969; Yu et al. 2020). Until now, *Tetractenion* included only two species, *T. luteum* Seyrig, recorded from continental central Africa (Democratic Republic of Congo and Kenya) and *T. acaule* Seyrig, recorded from Madagascar (Seyrig 1932, 1935). The purpose of this paper is to revise the genus *Tetractenion*, and to provide well-illustrated updated keys to the genera of Banchinae in the Afrotropical region, and to the species of *Tetractenion*. Online Lucid keys are available on www.waspweb.org and the associated underlying data is made available as Suppl. materials 1, 2: LIF3 files to this paper for inter-exchange with other key production software.

Materials and methods

Photographs

Specimens were either pinned or point mounted on black, acid-free cards for examination (using a Leica M205C stereomicroscope with LED light source), photography, and long-term preservation. Images were taken using the Leica LAS 4.4 system which comprised a Leica Z16 microscope with a Leica DFC450 Camera with a 0.63× video objective attached. The imaging process, using an automated Z-stepper, was managed using the Leica Application Suite V 4.4 software installed on a desktop computer. Diffused lighting was achieved using a Leica Dome. Images of the types held in Musée Royal de l'Afrique Centrale, Tervuren (**RMCA**) were kindly made available by Stéphane Hanot and Arnaud Henrard and those in the Muséum national d'Historie naturelle, Paris (**MNHN**) were kindly made available by Agnièle Touret-Alby. All images presented in this paper are available at www.waspweb.org (van Noort 2020).

Depositories

Codens follow Arnett et al. (1993), and updated according to the online version http://hbs.bishopmuseum.org/codens/

NHMUK The Natural History Museum, London, England (Gavin Broad);
MNHN   Muséum national d'Historie naturelle, Paris (Agnièle Touret-Alby);

RMCA   Musée Royal de l'Afrique Centrale, Tervuren (Stéphane Hanot);
SAMC   Iziko South African Museum, Cape Town, South Africa (Simon van Noort);
CASC   California Academy of Sciences, San Francisco, United States of America
            (Robert Zuparko).

Nomenclature and abbreviations

The morphological terminology follows Wahl and Sharkey (1993), but the wing venation nomenclature follows Gauld (1991). Most morphological terms are also defined on the HymAToL website (http://www.hymatol.org) and HAO website (http://portal.hymao.org/projects/32/public/ontology/). The following morphometric abbreviations are used (in order of appearance in the descriptions):

A   antenna length, from base of scape to flagellar apex (mm);
B   body length, from toruli to metasomal apex (mm);
CT   (clypeus transversality index): maximum width of clypeus: median height;
F   fore wing length, from tegula to wing apex (mm);
Fl$_n$ (length index of flagellomere n): length: width of flagellomere n;
IO   (inter-ocellar index): shortest distance between posterior ocelli: ocellus diameter;
ML   (malar space length index): malar space (shortest distance between mandible base and compound eye): basal mandibular width;
OO   (oculo-ocellar index): shortest distance between eye and posterior ocellus: ocellus diameter;
OT   (ovipositor sheath-tibia index): length of ovipositor sheath: length of hind tibia.

   The first three measurements (absolute measures) were measured on all specimens in the type series, with measurements from the primary type reported separately in brackets if necessary.

Identification keys

Identification keys were produced in two formats to facilitate accessibility by a range of end-users and to meet the requirements of publishing both static and dynamic interactive keys under an open access model (Penev et al. 2009; Sharkey et al. 2009): 1. Traditional dichotomous keys these are published below and also made available as interactive keys supported by links to species pages on www.waspweb.org; 2. Online interactive Lucid matrix keys were produced, hosted on www.waspweb.org, and made available as Suppl. materials 1, 2: LIF3 files to this paper for import to, and interexchange with other key production software. The LIF3 file is an XML-based file that stores all the Lucid4 key data, allowing exchange of the key with other key developers (Penev et al. 2009; Sharkey et al. 2009). In contrast to dichotomous keys where a choice needs to be made at each key couplet to continue, Lucid matrix keys use a different approach where relevant states from multiple character features can be selected

independently until identification is achieved (www.lucidcentral.org). All keys were produced using high quality annotated images, highlighting diagnostic characters that are integrated into the key above each couplet. This is a user-friendly output making the keys readily accessible to a wide range of users with diverse expertise. This key format circumvents the requirement of familiarity with morphological terminology associated with the particular group, because the characters are visually illustrated making the keys usable by the lay person (van Noort et al. 2015).

Results

Key to Banchinae genera of the Afrotropical region
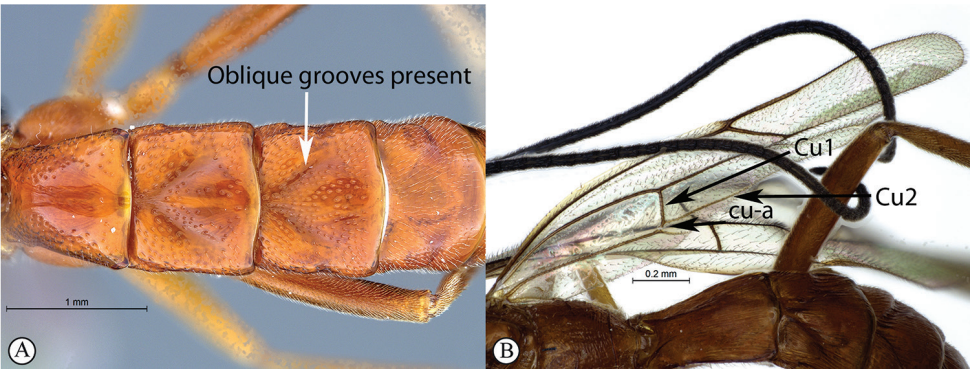
1   Tergites II–IV with a median pair of (usually) deep oblique grooves that converge anteriorly and diverge posteriorly (A); Cu1 longer than cu-a, such that Cu2 arises below middle of these combined veins (nervellus of Townes) (B) ... **2**
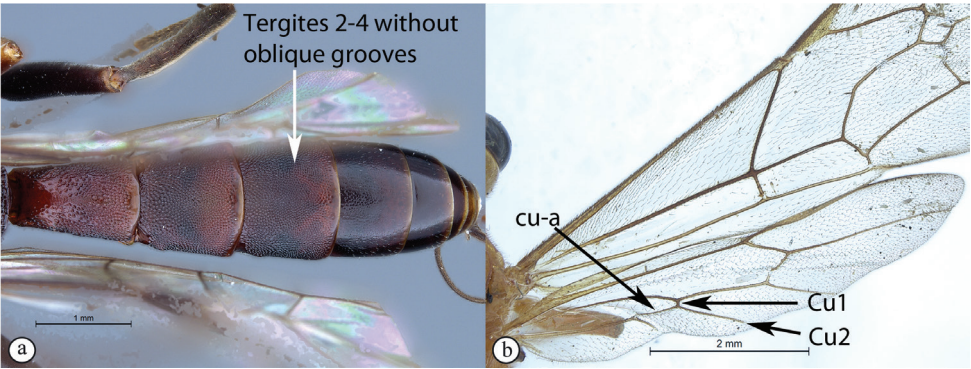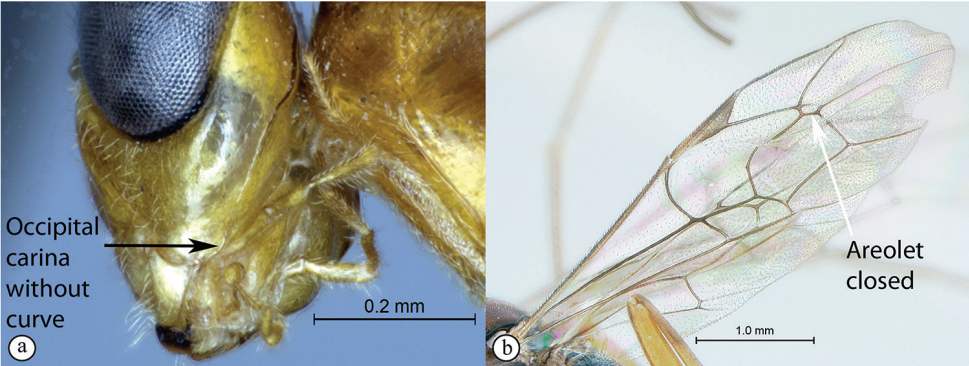

–   Tergites II–IV without a median pair of oblique grooves (a); Cu1 often longer than cu-a, but may be shorter (b)...**4**

Occipital carina strongly curved

Areolet open

A   0.2 mm

B   1 mm

2   Occipital carina strongly curved before junction with hypostomal carina (A); areolet open, i.e., vein 3rs-m absent (B) .... ***Apophua***


Occipital carina without curve

Areolet closed

a   0.2 mm

b   1.0 mm

–   Occipital carina without a strong curve before junction with hypostomal carina (a); areolet closed (b)...**3**

Malar space 0.5-0.8x as long as basal mandibular width

Epomia long and strong

A   B

3   Malar space 0.5–0.8× as long as basal width of mandible (A); epomia long and strong (B)...***Glyptopimpla***
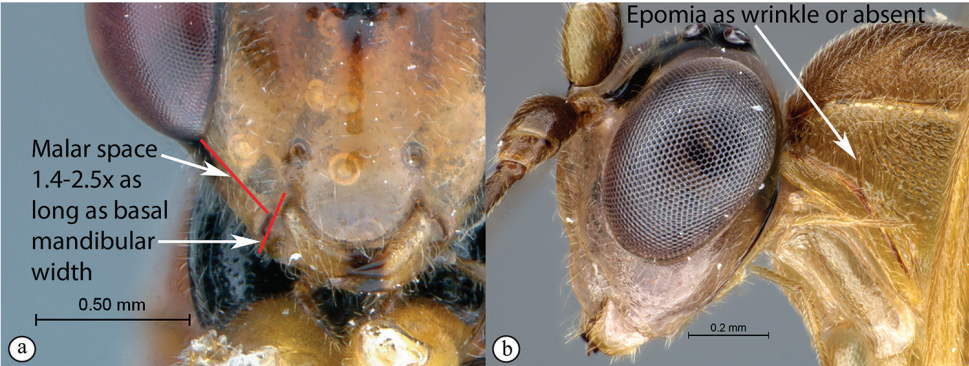
–   Malar space 1.4–2.5× as long as basal width of mandible (a); epomia usually absent or indistinct, only represented as a short wrinkle (b) ...... ***Sjostedtiella***
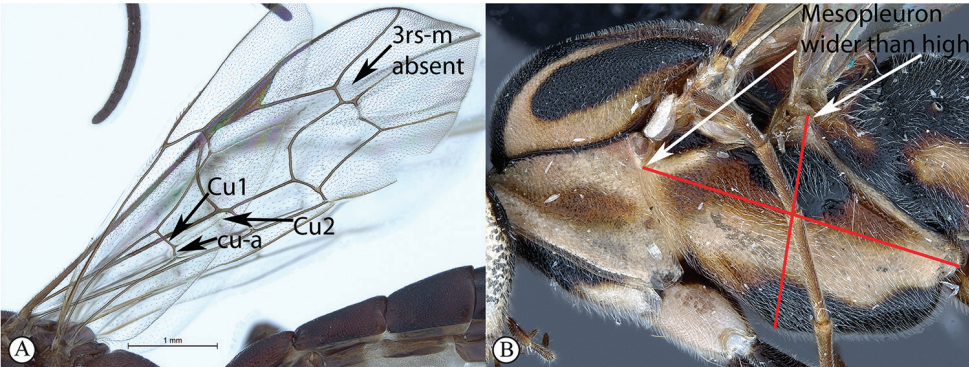
4   Hind wing with Cu1 longer than cu-a such that Cu2 arises below the middle of these combined veins (nervellus of Townes), Cu2 rarely absent (A); fore wing with 3rs-m sometimes lacking, shape of areolet when closed various (A); mesopleuron usually wider than high (B) **5**
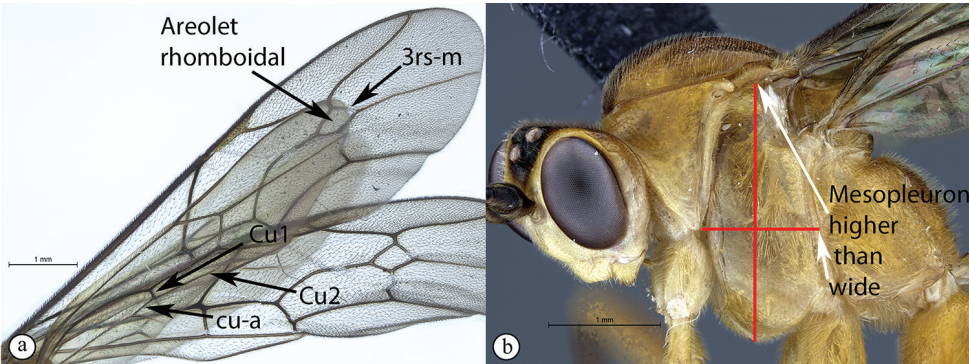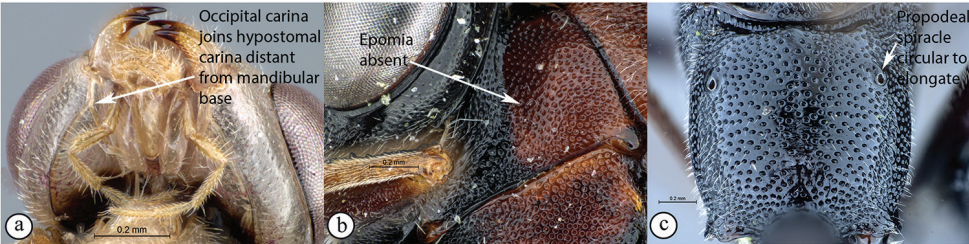

–   Hind wing with Cu1 shorter than cu-a such that Cu2 arises above the middle of these combined veins (nervellus of Townes) (a); fore wing with 3rs-m always present, areolet rhomboidal (a); mesopleuron usually higher than wide (b)...**11**
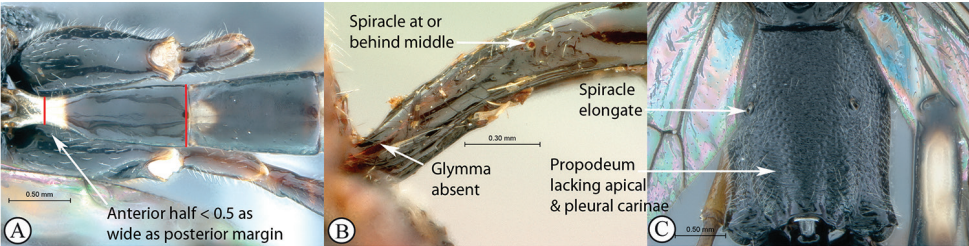
5   Occipital carina joining hypostomal carina at base of mandible (A); epomia usually present (B); propodeal spiracle elliptic (C) .... *Syzeuctus*

–   Occipital carina joining hypostomal carina distant from base of mandible (a); epomia usually absent (b); propodeal spiracle circular to elongate (c).........**6**

6   Tergite I with anterior half slender, less than half as wide as posterior margin (A); glymma absent (B); with its spiracle at or behind middle (A, B); propodeum lacking carinae (C); propodeal spiracle elongate (C)...............***Atropha***
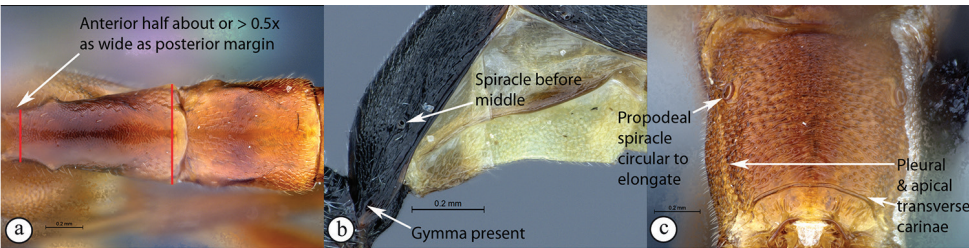
–   Tergite I with anterior half about or more than half as wide as posterior margin (a); glymma present (a); with its spiracle in front of middle (a, b); propodeum usually with either an apical transverse carina or pleural carina, or both (c); propodeal spiracle usually circular to elongate (c) **7**

7   Apex of submetapleural lobe tooth-like (A); tarsal claws simple with a single basal tooth above (B); areolet open (C); occipital carina broadly interrupted above ....***Tossinola***
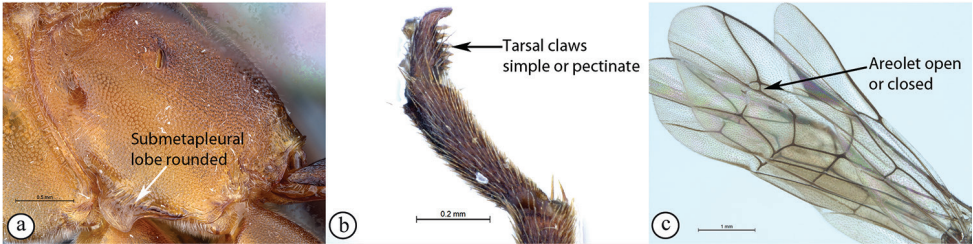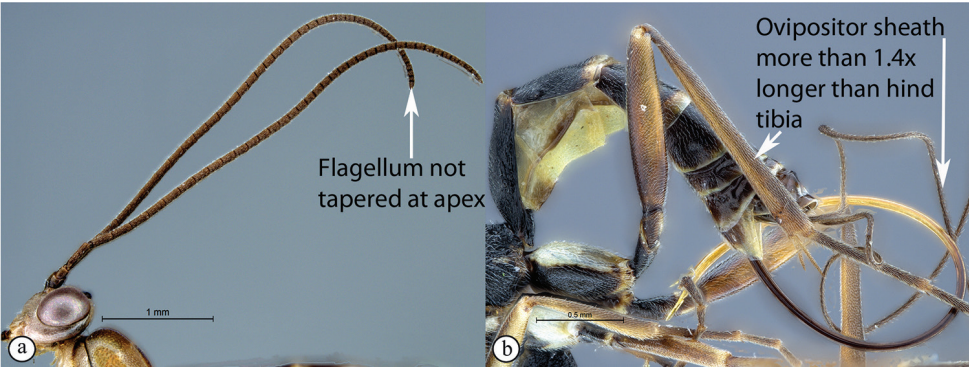

–   Apex of submetapleural lobe rounded (a); tarsal claws simple or pectinate (b); areolet open or closed (c); occipital carina complete...................................**8**
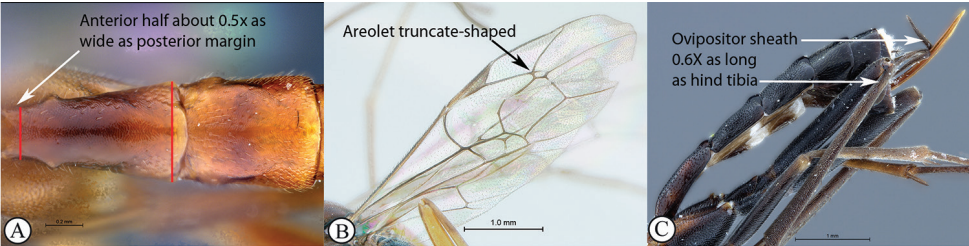

8   Apical 0.3–0.4 of flagellum tapered to a slender apex (A); ovipositor sheath 0.6–1.2× as long as hind tibia (B) **9**

–   Flagellum not tapered at the apex (a); ovipositor sheath usually more than 1.4× as long as hind tibia (b)...**10**

9   First tergite evenly and rather strongly narrowed anteriorly , about half as wide as posterior margin (A); areolet always truncate-shaped (B); ovipositor 0.6× as long as hind tibia (C) .... *Cryptopimpla*
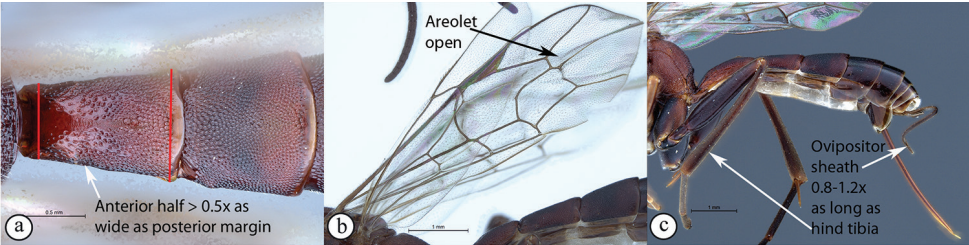
–   First tergite stout, only moderately narrowed anteriorly, more than half as wide as posterior margin (a); areolet always open (b); ovipositor 0.8–1.2× as long as hind tibia (c) .... *Spilopimpla*
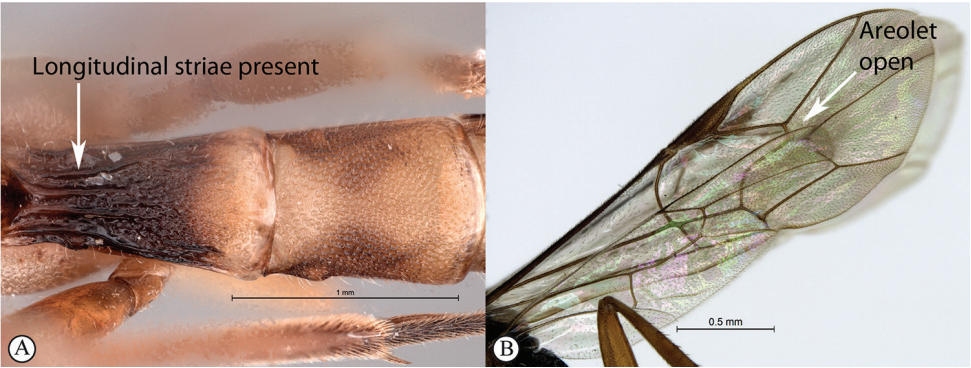
10 First tergite nearly always with longitudinal striae (A); areolet open (B) ....
....***Himertosoma***
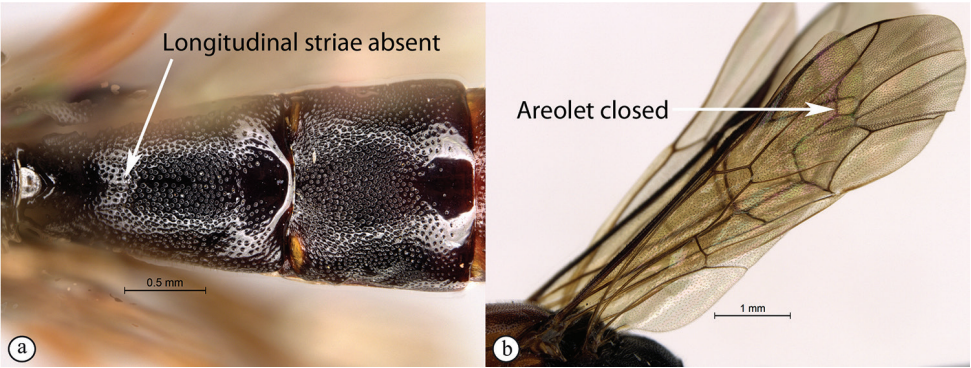
– First tergite rarely covered with longitudinal striae (a); areolet closed or some-
times lacking (b) ....***Lissonota***

11 Occipital carina joining hypostomal carina above base of mandible (A); oviposi-
tor sheath short to long, 0.14–1.8× as long as hind tibia (B); mandibular teeth
usually subequal in length (C); apical clypeal margin normal (C).......***Exetastes***
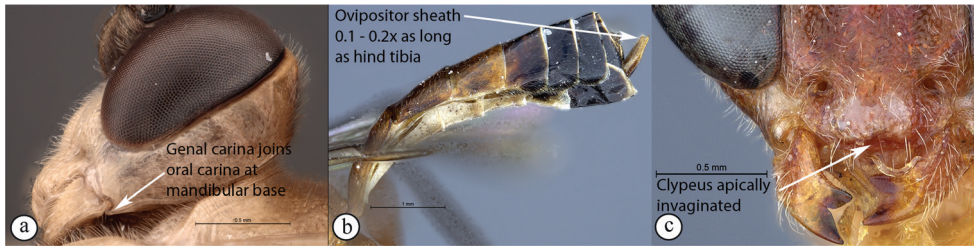
– Occipital carina joining hypostomal carina at base of mandible (a); ovipositor sheath always short, 0.1–0.2× as long as hind tibia (b); lower tooth of mandible always longer than upper tooth (c); clypeus apically invaginated (c) .. **Tetractenion**

Details of morphological characters that were used to update the key to Afrotropical banchine genera

1. Occipital carina with strong curve prior to meeting hypostomal carina: this character (couplet 1A), although noted in the global generic key by Townes (1969), was not included in the previously published generic key to Afrotropical Banchinae (Townes and Townes 1973). This is a strong and reliable character distinguishing *Apophua* from the remaining Glyptini genera, *Sjostedtiella* and *Glyptopimpla*.

2. Shape of the areolet when closed: the shape of the areolet has been found to be a useful character to separate the tribes/genera. When distinguishing the tribes Atrophini and Banchini, the areolet in *Exetastes* and *Tetractenion* is always large and rhomboidal with a very short stalk, whereas in those Atrophini that possess an areolet it is always small, but variably shaped (couplet 4A, a). An anteriorly truncate areolet (couplet 9B, veins 2rs-m and 3rs-m meeting RS separately) present in many *Cryptopimpla* species has been reported (Townes 1969; Sheng 2011; Takasuka et al. 2011) to be a character state that is constant for all Afrotropical *Cryptopimpla* species (Reynolds Berry and van Noort 2016). In *Syzeuctus* and *Atropha*, the areolet is triangular with a long stalk and in *Lissonota* the areolet, when closed, is nearly always petiolate (i.e., dorsal aspect pointed, veins 2rs-m and 3rs-m meet before RS, couplet 10c; Townes 1969).

3. Mesopleuron compressed in *Exetastes* Group: *Tetractenion* and *Exetastes* species have stocky bodies with the mesopleuron laterally compressed (higher than wide) and often flat whereas in Atrophini the mesopleuron is usually wider than high (dorsoventrally compressed, couplet 4B, b).

4. Distinguishing *Tossinola*: the length of the ovipositor sheath relative to the hind tibia has previously been used as an additional character to separate *Tossinola* from the other Afrotropical genera in the tribe Atrophini where the areolet is open (Townes and Townes 1973). However, the relative lengths overlap across *Lissonota*, *Cryptopimpla* and *Tossinola* species, making it an unreliable character to separate these genera. While a medially, broadly interrupted occipital carina is still the most diagnostic character for the genus *Tossinola*, another useful character is the state of the apex of the submetapleural carinae: tooth-like in *Tossinola* (Townes 1969) but rounded in the other Afrotropical banchine genera.

5. Flagellum apically tapered: as observed by Townes (1969), the flagella of the genera *Lissonota* and *Himertosoma* are not, or may only be weakly, apically tapered. For *Cryptopimpla* and *Spilopimpla* species, the flagella are tapered to a slender apex (couplet 8 A, a; Townes 1969; Reynolds Berry and van Noort 2016).

6. Distinguishing *Cryptopimpla*: all Afrotropical *Cryptopimpla* possess a first tergite that is evenly and rather strongly narrowed toward the base (couplet 9A; Reynolds Berry and van Noort 2016). In addition, the ovipositor sheath ca. 0.6× as long as the hind tibia is diagnostic of *Cryptopimpla* species (couplet 9C; Reynolds Berry and van Noort 2016).

7. Distinguishing *Himertosoma* from *Lissonota*: the absence of a crease separating the fifth laterotergite from the fifth metasomal tergite has been suggested as the single defining character that separates *Himertosoma* from *Lissonota* (couplet 10A, a; Watanabe and Maeto 2012). However, this does not appear to be a defining character for Afrotropical *Lissonota* species as the absence/presence of the crease varies within and among species. Given that the areolet can also sometimes be open in *Lissonota* species, assessment of the sculpture of the first metasomal tergite is required to separate *Lissonota* from *Himertosoma*. *Himertosoma* species nearly always have longitudinal striae present whereas *Lissonota* species rarely possess either strong punctures or longitudinal striae (couplet 10B, b; Townes 1969).

8. Length of the mandibular teeth: in the global description of the genus *Exetastes* by Townes (1969), he noted that the length of the lower mandibular tooth relative to the upper could be either equal or slightly longer/shorter. "Slightly" is a poor character description, especially concerning mandibular teeth, which wear out throughout the wasp's life. In the description of the genus, based on Costa Rican species, by Gauld et al. (2002), all species had equal mandibular teeth. Relative length of the mandibular teeth is a more reliable character, if one of the teeth is markedly longer or shorter. For example, in Afrotropical *Cryptopimpla* the upper tooth is distinctly longer than the lower tooth (Reynolds Berry and van Noort 2016). Similarly, it has been previously noted (Townes 1969), and further corroborated during this revision of *Tetractenion*, that the upper mandibular tooth is distinctly shorter in all species, making it a diagnostic feature for the genus. While most Afrotropical *Exetastes* have equal mandibular teeth, *Exetastes discretus* (Morley 1917) and an undescribed species in SAMC has mandibles with the lower tooth distinctly longer than the upper. This warrants further investigation, because these two genera are closely related, as they both form part of the *Exetastes* group. This character may represent a transition between the two genera.

9. Clypeus apically invaginated: this is a diagnostic feature of *Tetractenion*, while in Afrotropical *Exetastes*, as has been observed in other species, the clypeal edge is convex or straight, without a median indentation (Gauld et al. 2002).

Tetractenion Seyrig, 1932

Tetractenion Seyrig, 1932, Mém. Acad. Malgache 11: 167. Type: *Tetractenion acaule* Seyrig. Monobasic.

Diagnosis (updated from Townes 1969). Fore wing 6.4–10 mm long. Body of moderate proportions, the hind legs long. Frons unarmed. Head with three lobes on the face, tentorial pits deep; clypeus small, laterally convex with declivity, apically invaginated, with clypeal edge convex. Antennae long and slender, apically tapered. Teeth of mandible both triangular, the lower tooth longer than the upper tooth. Labium not elongate. Occipital carina joining hypostomal carina at the base of mandible. Epicnemial carina present and ending at anterior edge of mesopleuron. Apex of scutellum rounded, notauli present. Propodeum weakly convex, often with transverse wrinkling and with a posterior transverse carina and lateral longitudinal carinae present, but faint or reduced. Pro-and meso-tarsal claws pectinate to apex, meta-tarsal claws pectinate or simple. Areolet is often large and rhomboidal with a short stalk, receiving 2m-cu at center. Fore wing with cu-a opposite 1A or a little distad, ramellus present or absent on 1m-cu. Hind wing with Cu1 shorter than cu-a such that Cu2 arises above the middle of these combined veins. Metasomal tergite I without dorsolateral carinae. Epipleura of tergites II and III ca. 0.15× as wide as long. Posterior third of metasoma moderately laterally compressed. Ovipositor sheath ca. 0.1–0.2× as long as hind tibia.

Biology. Unknown.

Distribution. Angola, Cameroon, Democratic Republic of Congo, Kenya, Madagascar, Namibia, Nigeria, South Africa.

Species richness

T. acaule Seyrig, 1932
T. ibayaensis sp. nov.
T. luteum Seyrig, 1932
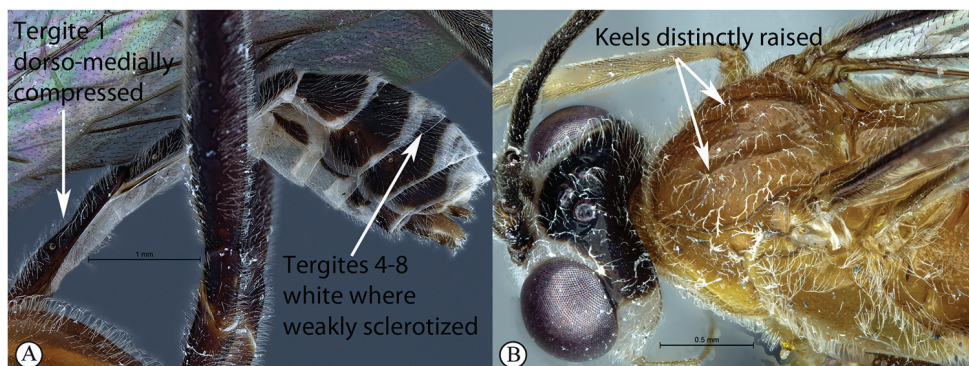*T. pascali* sp. nov.
T. pseudolutea sp. nov.
T. rosei sp. nov.

Key to Afrotropical species of the genus *Tetractenion*

1   Metasomal tergite I distinctly dorso-medially compressed, tergites IV–VIII white where weakly sclerotized (A); keels distinctly raised on mesoscutal lobes (B); notauli abbreviated, not reaching the scutellum .... ***T. acaule***
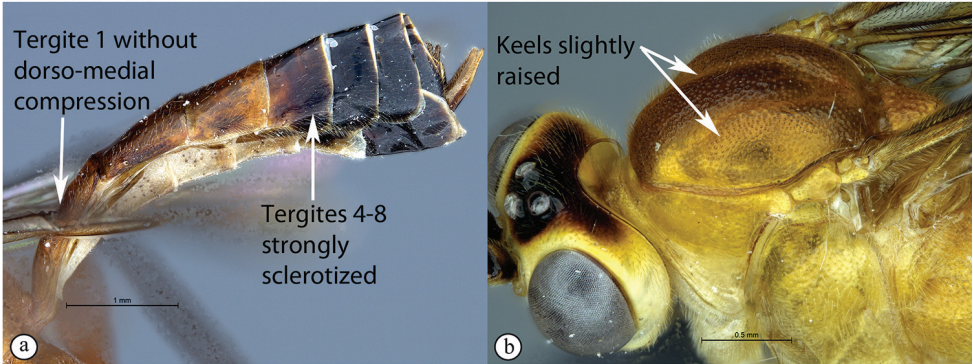
– Metasomal tergite I with dorso-medial compression weak or absent, tergites IV–VIII strongly sclerotized (a); keels only slightly raised on mesoscutal lobes (b); notauli present, posteriorly meeting before reaching the scutellum **2**
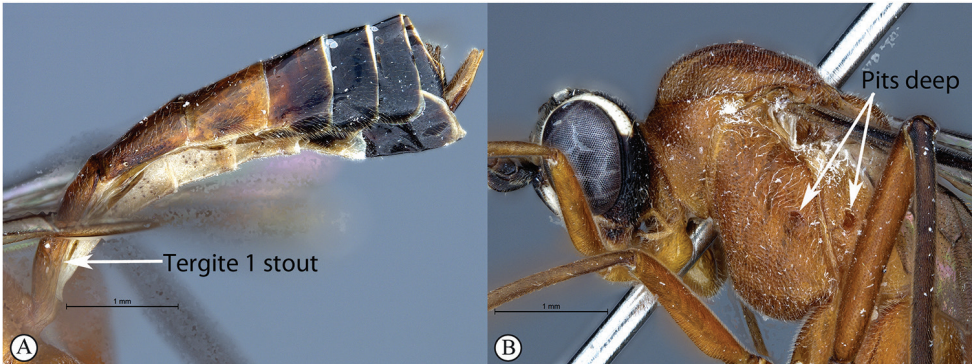
2 Metasomal tergite I stout, ca. as long as wide in dorsal view (A); pits on the mesopleuron and propodeum are large and deep (B)....***T. ibayaensis* sp. nov.**
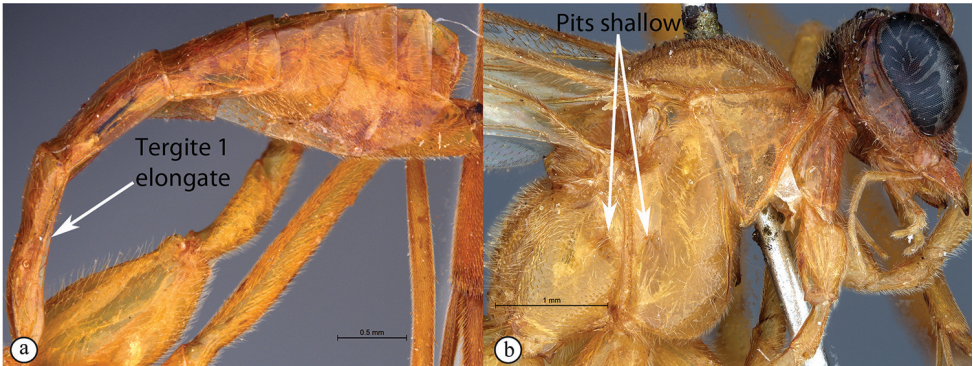
– Metasomal tergite I elongate, ca. 2× as long as wide in dorsal view (a); pits on the mesopleuron and propodeum shallow (b) **3**
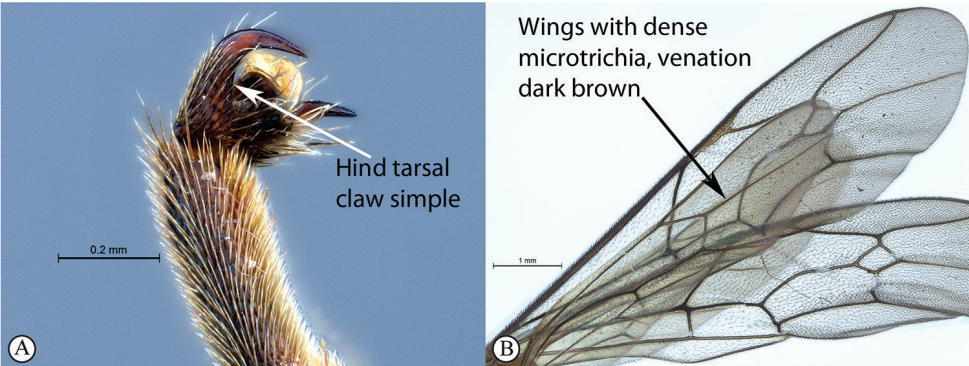
3   Hind tarsal claw simple (A); wings with dense microtrichia, venation dark (B) .... *T. luteum*
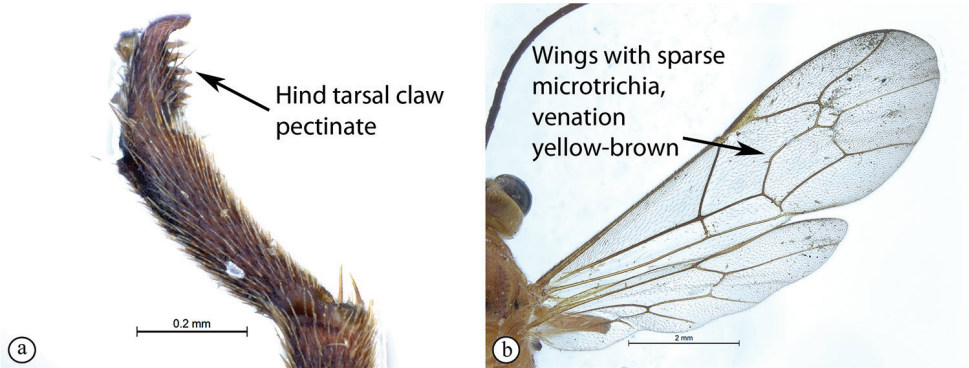
–   Hind tarsal claw pectinate (a); wings usually with sparser microtrichia, venation usually yellowish-brown (b) **4**
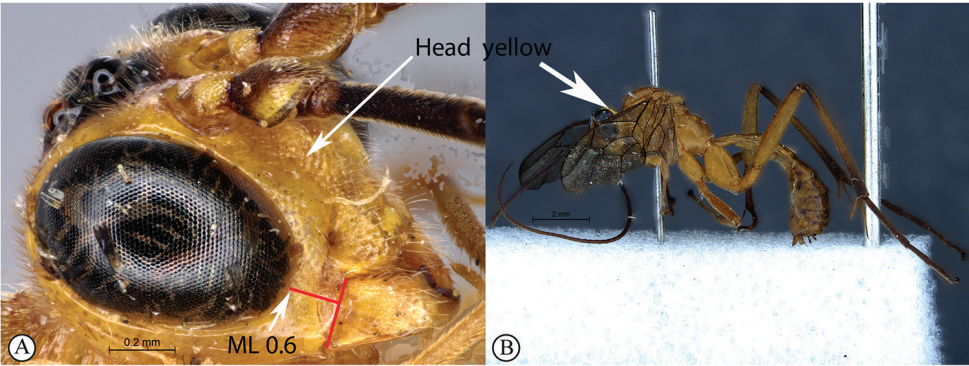
4   ML 0.6 (A); head yellow, congruent with yellow body (B) .... *T. pseudolutea* **sp. nov.**
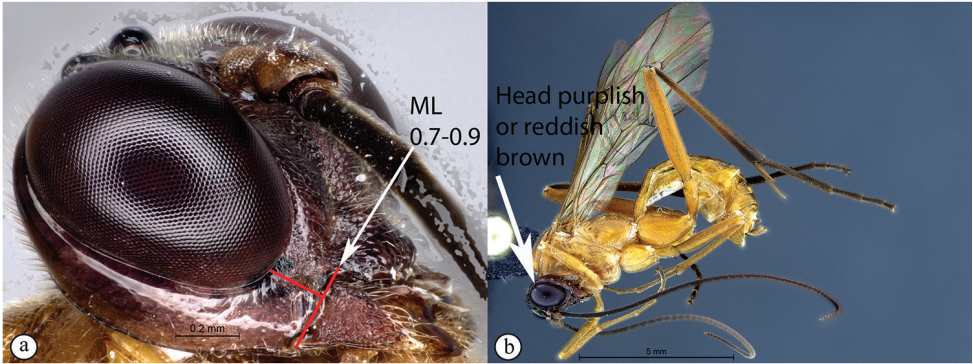
– ML 0.7–0.9 (a); head dark purplish-brown or reddish, contrasting with yellow body (b) **5**
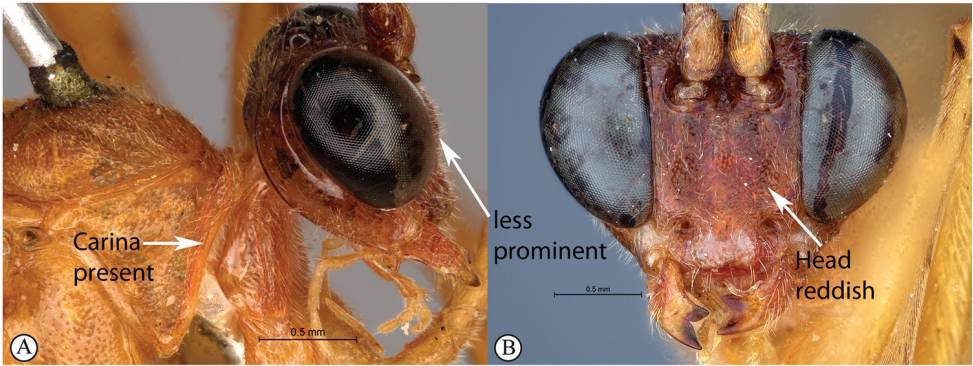
5 Pronotal collar with strong carina present (A); head reddish and less robust, face weakly three lobed (B) .... ***T. rosei* sp. nov.**
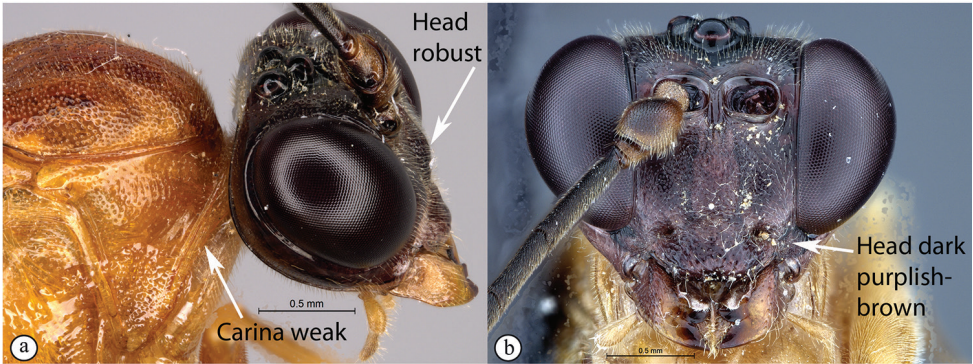
– Pronotal collar weakly wrinkled (a); head dark purplish-brown and more robust, face strongly three-lobed .... ***T. pascali* sp. nov.**

Species descriptions

Tetractenion acaule Seyrig, 1932
Fig. 1

Type material. *Lectotype* ♀: Madagascar, Rogez, Forêt Cote Est, Muséum Paris, 1.31. A. Seyrig, EY9333, [White label with TYPE written in red] [Red type label]: Lectotype ♀ *Tectractenion acaule*, Seyrig, 1932, designated by Townes and Townes (1973), labeled by T. Yoshida, 2011 (MNHN) (photos of Lectotype examined: http://coldb. mnhn.fr/catalognumber/mnhn/ey/ey9333). *Non-type* ♀ (examined): Madagascar, Bekily, Reg. Sud. de L'ile, Feb 1930 and Jan–Feb 1931, Coll. Mus. Congo, Col. P.L.G. Benoit, Tectractenion acaule, det. P.L.G. Benoit, 1953 (RMNH). *Additional material.* ♀: Madagascar: Majunga Prov., Besalampy District, Marofototra dry forest, 17 km W of Besalampy, 4–11 February 2008, 16°43.30'S, 44°25.42'E, coll. M. Irwin, R. Harin'Hala, Malaise, dry wash in forest, elev. 170 ft MG-42A-20 (CASC).

Differential diagnosis. *Tetractenion acaule* is immediately distinguishable from all other *Tetractenion* species by its unique color combination of a red mesosoma and a mostly black metasoma; distinct keels are present on outer mesoscutal lobes, the notauli do not reach the scutellum; metasomal tergite I has a distinct medial compression in the dorso-ventral view, tergite II have distinct gastrocoeli, and tergites IV–VIII are dorso-posteriorly weakly sclerotized, appearing as large membranous white areas on the dorsal surface. *Tetractenion acaule* closely resembles *T. ibayaensis* as both species are similar in color, having largely fulvous bodies with a white face and the hind femur infuscate, whereas the remaining *Tetractenion* species are largely yellow in color with yellow hind femora. *Tetractenion acaule* can easily be distinguished from *T. ibayaensis* by having a white gena and weakly sclerotized metasomal tergites IV–VIII; the head is narrow, straight behind the eyes; a distinct carina is present on the pronotal collar; distinct keels are present on the outer mesoscutal lobes, with the notauli not reaching the scutellum; pits on the mesopleuron and propodeum are shallow; metasomal tergite I is distinctly dorso-medially compressed; gastrocoeli on tergite II are distinct; tergites IV–VIII are postero-dorsally weakly sclerotized and white; and tarsal claws on the hind leg are simple. In *T. ibayaensis* the gena is brown and tergites IV–VIII are strongly sclerotized; the head is rounded behind the eyes; with no more than a wrinkle present on the pronotal collar; the mesoscutal lobes are hardly present, the notauli reach the scutellum; pits on the mesopleuron and propodeum are deep; metasomal tergite I is stout and indistinctly dorso-ventrally compressed in the medial region; gastrocoeli on tergite II are indistinct; and tarsal claws on the hind legs are pectinate.

Description (updated from Seyrig 1932). Size 9–11 mm. *Color*: head white with a large black central area on occiput, reaching eyes on vertex and pointed on frons; antenna black, without pale ring; mesosoma red; metasomal tergites I and II red, though tergite II sometimes brownish, following tergites black with large membranous white areas from tergite IV; legs red, hind femur, tibia and tarsus infuscate; wings with sparse microtrichia, venation brown, pterostigma brown and centrally translucent reddish.
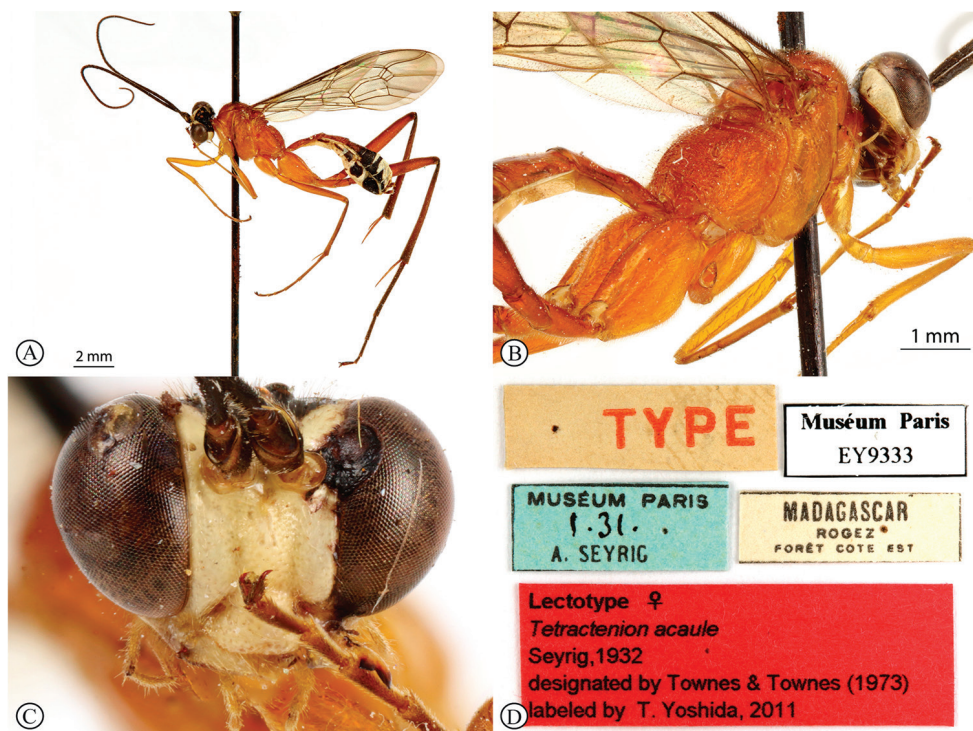
Figure 1. *Tetractenion acaule* Lectotype (MNHN) **A** habitus, lateral view **B** habitus, dorsal view **C** head, anterior view **D** data labels. Photographs of lectotype RECOLNAT (ANR-11-INBS-0004) – Christophe Hervé – 2014. http://coldb.mnhn.fr/catalognumber/mnhn/ey/ey9333 (used with permission of Agnièle Touret-Alby – Curator of Hymenoptera MNHN).

Head narrow, straight behind eyes; occiput deeply and angularly excavated, occipital carina strong, extending to lower gena at base of mandible; eyes very large; malar space almost half as long as mandibular basal width; face and clypeus finely, evenly and rather sparsely punctate on a shiny background; face with three lobes, tentorial pits deep; clypeus small, laterally convex with declivity, apically invaginated, with clypeal edge convex; mandibular teeth triangular, lower tooth longer than upper tooth; antenna long, slender and apically tapered.

Mesosoma stout; mesonotum deeply punctate, inter-punctuate spaces about as wide as punctures, rather matt, but not coriaceous; keels distinctly raised on outer mesoscutal lobes of mesoscutum, notauli abbreviated, not reaching the scutellum; apex of scutellum rounded; pronotum shining with a distinct thickened carina on collar, sparsely and very finely punctate; mesopleuron higher than wide, sparsely but more deeply punctate, speculum similarly punctate, background hardly shining, epicnemial carina ending at anterior edge of mesopleuron; shallow pits on mesopleuron and propodeum; metapleuron matt and deeply punctate; propodeum weakly convex, roughly punctate dorsally, punctate posteriorly confluently grading into transverse wrinkles, posterior transverse carina reduced, lateral longitudinal carinae present but faint, spiracle roundish-elliptic and small.

Metasoma hardly punctate at base of tergite II, indistinctly punctate beyond base; tergite I elongate, more than twice as long as wide, tapered anteriorly, glymma present, spiracle positioned slightly in front of middle and protruding, especially dorsally, with a distinct medial depression dorso-ventrally; tergite II longer than wide or subquadrate with gastrocoeli distinct; tergite III quadrate to transverse; metasomal tergites IV–VIII moderately laterally compressed; ovipositor sheath concealed or hardly protruding.

Fore wing without ramellus on Rs-M vein; Rs hardly sinuate; areolet large and quadrate with a short stalk receiving 2m-cu at center. Hind wing with Cu1 shorter than cu-a such that Cu2 arises above the middle of these combined veins. Legs very long; hind femur reaching beyond metasomal apex; length of tibia III plus tarsus III as long as body; spurs of tibia III longer than half metatarsal length; tarsal claws on hind leg simple.

Male hardly different: temples a bit less narrowed behind eyes, metasomal tergite II entirely black.

Distribution. Madagascar.

Tetractenion ibayaensis **Reynolds Berry & van Noort, sp. nov.**
http://zoobank.org/07849542-A5AB-42C4-80D1-95229B303561
Fig. 2

Type material. *Holotype* ♀: Tanzania, Mkomazi Game Reserve, Ibaya Camp, north west side, 3°57.91'S, 37°48.09'E, 22–24 April 1996, S. van Noort, *Acacia/Commiphora/Combretum* bushland, Yellow P. Trap, SAM-HYM-P019172 (SAMC).

Differential diagnosis. *Tetractenion ibayaensis* is immediately distinguishable from other *Tetractenion* species by having a largely fulvous body and a white face, with the occiput, gena and metasomal tergites IV–VIII dark brown to black, and the hind tibia and tarsus infuscate. The clypeal and mandibular setae are long. The metasoma is hardly laterally compressed with metasomal tergite I stout, being about as long as wide. Pits on the mesopleuron and propodeum are visibly large and deep. In addition, the clypeus is hardly apically invaginated and the propodeal spiracle is distinctly circular and not circular-elliptical as in the other species.

The head is rounded behind the eyes distinguishing the species from *T. acaule* and *T. pascali*. The pronotal collar is no more than a wrinkle, separating the species from *T. acaule* and *T. rosei*. Pectinate tarsal claws on the hind legs separates the species from *T. acaule* and *T. luteum*. Metasomal tergites II and III are quadrate separating the species from all other *Tetractenion* species except *T. acaule* where tergites II and III are sometimes subquadrate and quadrate, respectively; and T. luteum where tergite III is quadrate. Sparse microtrichia on the wings distinguishes *T. ibayaensis* from *T. luteum* and *T. pascali*, and the pterostigma is brown separating the species from *T. luteum*, *T. pascali*, *T. rosei*, and *T. pseudolutea*.

Description. *Body* mostly fulvous; tibia and tarsus III brown; metasomal tergites IV–VIII brown to nearly black; head with face and area around eyes white; frons and
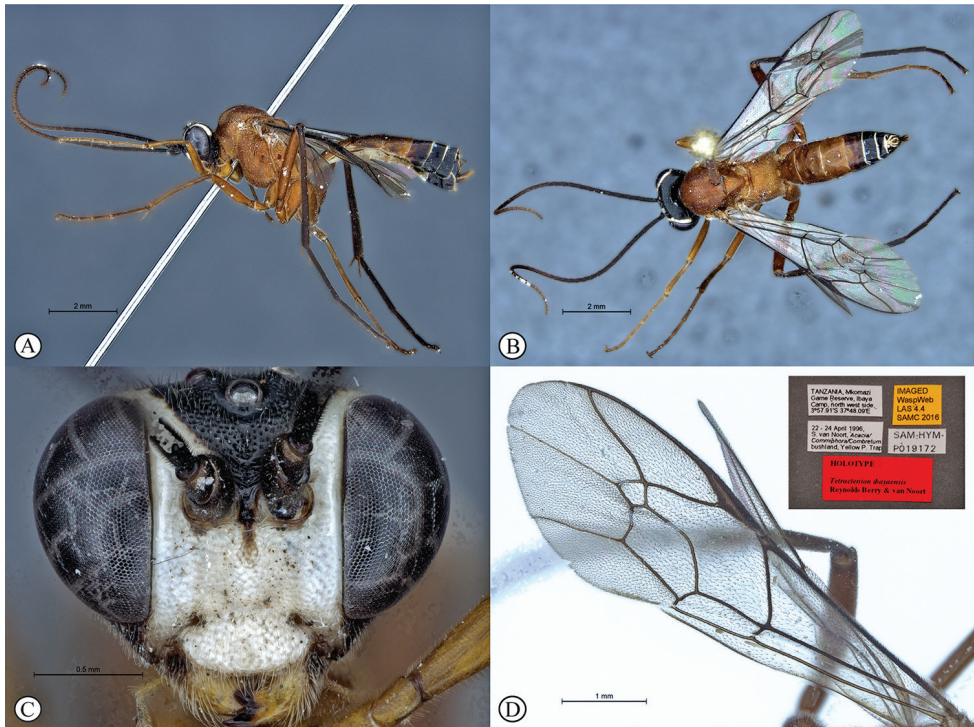
Figure 2. *Tetractenion ibayaensis* sp. nov. Holotype **A** habitus, lateral view **B** habitus, dorsal view **C** head, anterior view **D** wing (inset: data labels).

occiput dark brown to near black; mandibles yellow with base and tips brown. Sparse microtrichia on wings, venation and pterostigma brown.

Head rounded behind eyes; occiput deeply and angularly excavated, occipital carina strong, extending to lower gena at mandibular base; malar space half as long as mandibular basal width; eyes very large; face and clypeus finely and evenly punctate on a shiny background; face with three lobes, tentorial pits deep; clypeus small, laterally convex with declivity, apically invaginated, clypeal edge convex; mandibular teeth triangular, lower tooth longer than upper tooth; clypeal and mandibular setae long; antenna long, slender and apically tapered.

Mesosoma stout and deeply punctate on a shiny background; mesopleuron higher than wide, epicnemial carina ending at anterior edge of mesopleuron; deep pits on the mesopleuron and propodeum; pronotum moderately punctate on a shiny background with no more than a wrinkle on collar; mesososcutal lobes hardly present on mesoscutum, notauli posteriorly meeting before reaching the scutellum; propodeum weakly convex, posteriorly confluently grading into weak transverse wrinkles, posterior transverse carina indistinct, lateral longitudinal carinae reduced, spiracle small and circular.

Metasoma with tergite I stout, tapered anteriorly, not distinctly dorso-ventrally compressed in medial region, glymma present, spiracle positioned in front of middle and protruding, especially dorsally, hardly punctate dorso-laterally, metasoma

indistinctly punctate beyond and shining; gastrocoeli on tergite II indistinct; tergites II and III quadrate, tergites IV–VIII only slightly higher than wide.

Fore wing without ramellus on Rs-M vein; areolet large and quadrate with a short stalk receiving 2m-cu at center. Hind wing with Cu1 shorter than cu-a such that Cu2 arises above the middle of these combined veins. Legs very long; hind femur reaching beyond metasomal apex, length of tibia III plus tarsus III as long as body; spurs of tibia III longer than half metatarsal length; tarsal claws pectinate.

CT 2.1; ML 0.5; IO 1.6; OO 1.6; Fl$_1$ 4.3; OT 0.2; B 8.1 mm; A 8.1 mm; F 6.4 mm.

Etymology. Named after the type locality. Noun in apposition.

Distribution. Tanzania.

Tetractenion luteum Seyrig, 1935
Fig. 3

Type material. *Holotype* ♂: Nyeri, Kenya, June 1932 (MNHN). *Paratype* ♀: Elizabethville, Democratic Republic of Congo, 4 January 1921, M. Bequaert, Det. PLG Benoit, 1952 (RMCA). *Additional material.* ♀: South Africa, Eastern Cape, Pearston, Plains of Camdeboo Game Reserve, 32°32.033'S, 25°14.267'E, 969 m, 30.x.2009–22.ii.2010, S. van Noort, Malaise Trap, Camdeboo Escarpment Thicket, PCD09-ACA1-M02, SAM-HYM-P047483 (SAMC). ♂, ♀: South Africa, Eastern Cape, Asante Sana Game Reserve, 32°16.762'S, 24°57.309'E, 1186 m, 6.x.2010–17.i.2011, S. van Noort, Malaise Trap, Southern Karoo Riviere Riverine Woodland, ASA09-WOO1-M18, SAM-HYM-P047487 (SAMC, NHMUK). ♂: South Africa, Eastern Cape, Asante Sana Game Reserve, 32°16.762'S, 24°57.309'E, 1186 m, 7 Apr–28 July 2010, S. van Noort, Malaise Trap, Southern Karoo Riviere Riverine Woodland, ASA09-WOO1-M10, SAM-HYM-P047484 (SAMC). ♂: South Africa, Eastern Cape, Asante Sana Game Reserve, 32°15.841'S, 24°57.091'E, 1354 m, 6.x.2010–17.i.2011, S. van Noort, Malaise Trap, Camdeboo Escarpment Thicket, ASA09-BUS1-M17, SAM-HYM-P047485 (SAMC). Namibia, near Windhoek: a bush between kleine Kuppe and Aus Born Mountains, A. Gumovsky, 23–25.xii.2011, SAM-HYM-P047488 (SAMC). ♂: *Exetaste*s sp. indet. In B.M. G.J. Kerrich det. 1958. Pres by Com Inst Ent BM 1960-3. U.C. [Nigeria], Ibadan, 9.9.1953, Coll. G.H. Caswell, P49 (NHMUK).

Differential diagnosis. *Tetractenion luteum* is immediately distinguishable from the other species in the genus as this species is the only yellow-colored *Tetractenion* species to possess simple hind tarsal claws, and this character is consistent in both sexes. The head is rounded behind the eyes, distinguishing the species from *T. acaule* and *T. pascali*. The malar space nearly as long as the width of the base of the mandible separates *T. luteum* from *T. acaule*, *T. pseudolutea*, and *T. ibayaensis*. The pronotal collar is weakly wrinkled, separating the species from *T. acaule* and *T. rosei*. Metasomal tergite II is longer than wide and distinguishes the species from *T. ibayaensis*; and a quadrate tergite III separates *T. luteum* from *T. pseudolutea*, *T. pascali*, and *T. rosei*. Furthermore, *T. pascali* is the only other species that possess dense microtrichia on the wings.
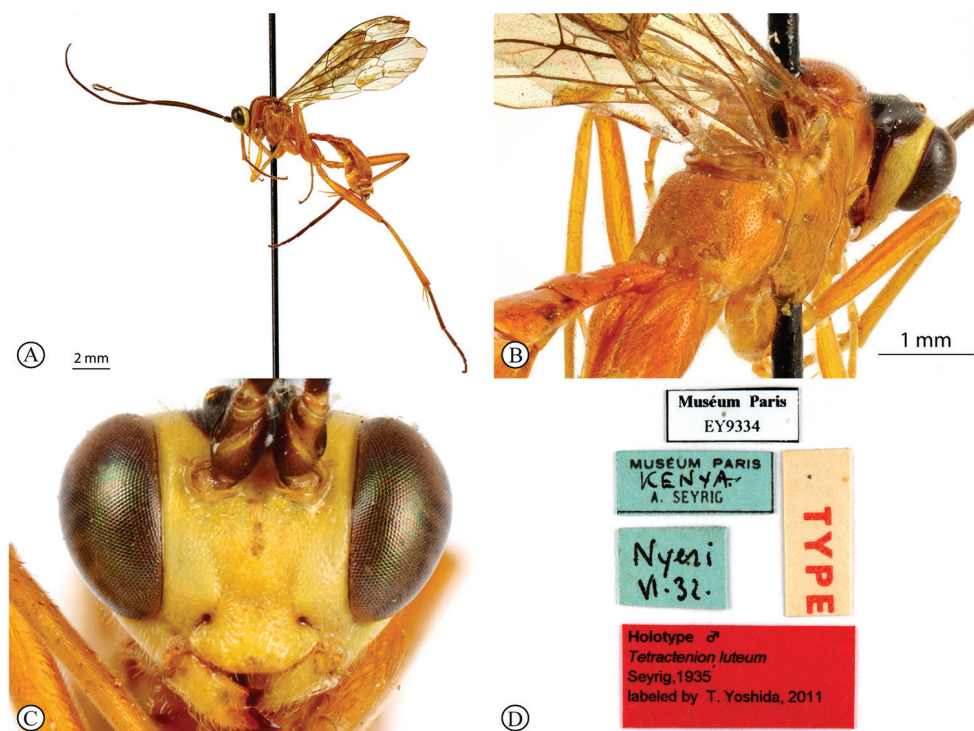
Figure 3. *Tetractenion luteum* Holotype (MNHN) **A** habitus, lateral view **B** habitus, dorsal view **C** head, anterior view **D** data labels. Photographs of holotype RECOLNAT (ANR-11-INBS-0004) – Christophe Hervé – 2014. http://coldb.mnhn.fr/catalognumber/mnhn/ey/ey9334 (used with permission of Agnièle Touret-Alby – Curator of Hymenoptera MNHN).

Description (updated from Seyrig 1935). Size 7.6–10.4 mm. ***Color***: head yellow with black marking on occiput to middle of frons, no contact with eyes on vertex; meso- and metasoma, fore and mid legs uniformly yellow, hind leg mostly yellow with shades of infuscation on tibia and tarsus infuscate; wings with dense microtrichia, venation brown, pterostigma yellow.

Head with temple short, rounded behind eyes; occiput deeply and angularly excavated, occipital carina strong, extending to lower gena at base of mandible; eyes very large, malar space a bit shorter than width of mandibular base; face and clypeus finely, evenly and rather sparsely punctate on a matt background; face with three lobes, tentorial pits deep; clypeus small, laterally convex with declivity, apically invaginated, with clypeus edge convex; mandibular teeth triangular, lower tooth longer than upper tooth; antenna about as long as body, slender and apically tapered.

Mesosoma stout, matt to sub-polished; pronotum finely punctate on a sub-polished background, no more than a wrinkle present on pronotal collar; mesoscutum moderately punctate, mesoscutal lobes hardly present, notauli posteriorly meeting before reaching the scutellum; mesonotum and mesopleuron finely punctate; mesopleuron higher than wide, epicnemial carina ending at anterior edge of mesopleuron;

shallow pits on mesopleuron and propodeum; propodeum weakly convex, matt to sub-polished, moderately punctate posteriorly confluently grading into transverse wrinkles, posterior transverse carina reduced, lateral longitudinal carinae present but faint, spiracle small and circular-elliptical.

Metasoma with a sub-polished background, anterior half of tergite I and dorso-lateral region of tergite II hardly punctate, indistinctly punctate beyond base; tergite I twice as long as wide, glymma present, tapered anteriorly, weak to indistinctly dorso-ventrally depressed in the medial region, spiracle positioned in front of middle and protruding, especially dorsally; tergite II longer than wide, gastrocoeli indistinct; tergite III quadrate.

Fore wing with ramellus absent on Rs-M vein; areolet large and quadrate with a short stalk receiving 2m-cu at center. Hind wing with Cu1 shorter than cu-a such that Cu2 arises above the middle of these combined veins. Legs very long; hind femur reaching beyond metasomal apex, length of tibia III plus tarsus III as long as body, spurs of tibia III longer than half metatarsal length; fore and mid tarsal claws pectinate, hind tarsal claws simple.

Distribution. Democratic Republic of Congo, Kenya, Namibia, Nigeria, and South Africa.

Tetractenion pascali **Reynolds Berry & van Noort, sp. nov.**
http://zoobank.org/026D51D5-49D1-485A-BF2C-784C6ED5F5F0
Fig. 4

Type material. *Holotype* ♀: Namibia, near Windhoek, between Mandume Ndemufayo Avenue and Western Bypass, 23.xii.2011, SAM-HYM-P047471 (SAMC). *Paratypes* ♂: South Africa, Eastern Cape, Asante Sana Game Reserve, 32°16.762'S, 24°57.309'E, 1186 m, 23 Feb–7 April 2010, S. van Noort, Malaise Trap, Southern Karoo Riviere, Riverine Woodland, ASA09-WOO1-M06, SAM-HYM-P044553 (SAMC). ♀: Namibia, near Windhoek: a bush between kleine Kuppe and Aus Born Mountains, A. Gumovsky, 23–25.xii.2011 (NHMUK).

Differential diagnosis. *Tetractenion pascali* is immediately distinguishable from all other *Tetractenion* species by having a color combination of a largely yellow body and a dark head. The facial features are more robust compared to the other species, with the three lobes on the face prominent and the mandibles larger, and the spiracle on the second tergite of the metasoma is hardly protruding. In addition, though the posterior transverse carina may be reduced or faint in the other species, it is distinct in *T. pascali*. The malar space nearly as long as the width of the mandibular base separates *T. pascali* from *T. acaule*, *T. pseudolutea*, and *T. ibayaensis*. Pectinate hind tarsal claws distinguish *T. pascali* from *T. luteum* and *T. acaule*; and a weakly wrinkled pronotal collar separates the species from *T. acaule* and *T. rosei*. Metasomal tergites II and III are longer than wide separating the species from *T. ibayaensis* and *T. acaule*, *T. luteum*, and *T. ibayaensis*, respectively. *Tetractenion luteum* is the only other species besides *T. pascali* that possess dense microtrichia on the wings.
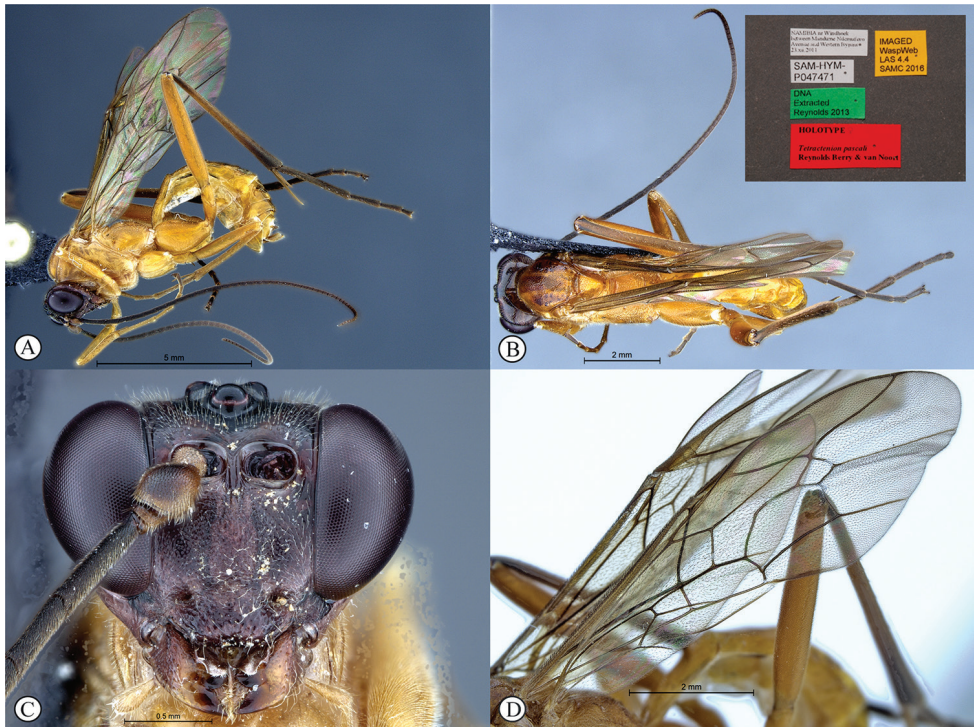
Figure 4. *Tetractenion pascali* sp. nov. Holotype **A** habitus, lateral view **B** habitus, dorsal view (inset: data labels) **C** head, anterior view **D** wings.

Description. *Color*: head brown, mandibles yellow from base to brown at apex. Antennae brown. Body yellow with red-brown areas on metanotum; tibia III with shades of infuscation, tarsus III infuscate. Wings with dense microtrichia, pterostigma yellow, venation brown.

Head narrowed straight behind eyes; occiput deeply and angularly excavated, occipital carina strong, extending to lower gena at mandibular base; malar space nearly as long as basal mandibular width; eyes very large; face and clypeus features robust, mandibles large; face three-lobed and punctate on a shiny background, punctures on second lobe and clypeus deeper than punctures on lobes flanking eyes, tentorial pits deep; clypeus small, laterally convex with declivity, apically invaginated, clypeal edge convex; mandibular teeth triangular, lower tooth longer than upper tooth; antenna long, slender and apically tapered.

Mesosoma stout and moderately punctate on a shiny background; pronotum with no more than a wrinkle on collar; mesoscutal lobes present on mesoscutum, notauli posteriorly meeting before reaching the scutellum; mesopleuron higher than wide, epicnemial carina present at ending at anterior edge of mesopleuron; shallow pits on mesopleuron and propodeum. Propodeum weakly convex, punctate and posteriorly confluently grading into transverse wrinkles, posterior transverse carina present and distinct, lateral longitudinal carinae present but faint, spiracle small and circular-elliptical.

Metasoma indistinctly punctate on a shiny background; tergite I elongate, twice as long as wide, tapered anteriorly, dorso-ventrally compressed in the medial region, glymma present, spiracle positioned in front of middle and hardly protruding; tergite II longer than wide, gastrocoeli indistinct; tergite III longer than wide; tergites IV–VIII higher than wide.

Fore wing without ramellus on Rs-M vein; areolet large and quadrate with a short stalk receiving 2m-cu at center. Hind wing with Cu1 shorter than cu-a such that Cu2 arises above the middle of these combined veins. Legs very long, hind femur reaching beyond metasomal apex, length of tibia III plus tarsus III as long as body; spurs of tibia III longer than half metatarsal length; tarsal claws pectinate.

Males: similar to females; ramellus present.

CT 2–2.4; ML 0.7–0.9; IO 1.2–1.3; OO 1.6–2.1; Fl$_1$ 4.5–4.8; OT 0.2; B 7.7–11.5 mm; A 11–14 mm; F 9.2–10 mm.

Etymology. Named after our colleague, Pascal Rousse, who first noted this to be a new species.

Distribution. Namibia and South Africa.

Comments. In males, the ramellus on the fore wing is present, distinguishing the species from *T. acaule* and *T. luteum*. The wings of *T. rosei* are inter-locked; this character could not be compared.

Tetractenion pseudolutea Reynolds Berry & van Noort, sp. nov.
http://zoobank.org/687360CC-2EEA-4136-9B8A-E202F638CC25
Fig. 5

Type material. *Holotype* ♀: Angola (A11), Bruco, 26.ii–2.iii.1972, Southern African Exp. B.M. 1972-1 (NHMUK). *Paratypes* 2♀: Angola (A11), Bruco, 26.ii–2.iii.1972, Southern African Exp. B.M. 1972-1 (NHMUK). ♀: Umbilo, Durban, Natal, 26.10.19, A.L. Bevis, Imp. Inst. Ent. Brit. Mus., 1933-190 (NHMUK). ♂: Cameroon, Ahal, 28.ix.1953. C.I.E. Coll. 15098. Pres. by Com. Inst. Ent. B.M. 1962-1. *Exetastes* sp. det. J.F. Perkins (NHMUK). ♀: Namibia, near Windhoek, between Mandume Ndemufayo Avenue and Western Bypass, 23.xii.2011 [collector not named], SAM-HYM-P047486 (SAMC).

Differential diagnosis. While the color pattern of *Tetractenion pseudolutea* is identical to *T. luteum*, it is distinguishable from *T. luteum* by having pectinate tarsal claw on the hind leg. The head is rounded behind the eyes, separating the species from *T. acaule* and *T. pascali*. The pronotal collar with no more than a wrinkle present distinguishes the species from *T. acaule* and *T. rosei*. Pectinate tarsal claws on the hind leg separates *T. pseudolutea* from *T. acaule* and *T. luteum*. Metasomal tergites II and III are longer than wide distinguishing *T. pseudolutea* from *T. ibayaensis*; and *T. acaule*, *T. luteum*, and *T. ibayaensis*, respectively. Sparse microtrichia on the wings distinguishes the species from *T. luteum* and *T. pascali*; yellowish-brown venation separates the species from *T. acaule*, *T. luteum*, *T. ibayaensis* and *T. pascali*; and a yellow pterostigma distinguishes the species from *T. acaule* and *T. ibayaensis*.

Figure 5. *Tetractenion pseudolutea* sp. nov. Holotype **A** habitus, lateral view **B** habitus, dorsal view **C** head, anterior view **D** wings (inset: data labels).
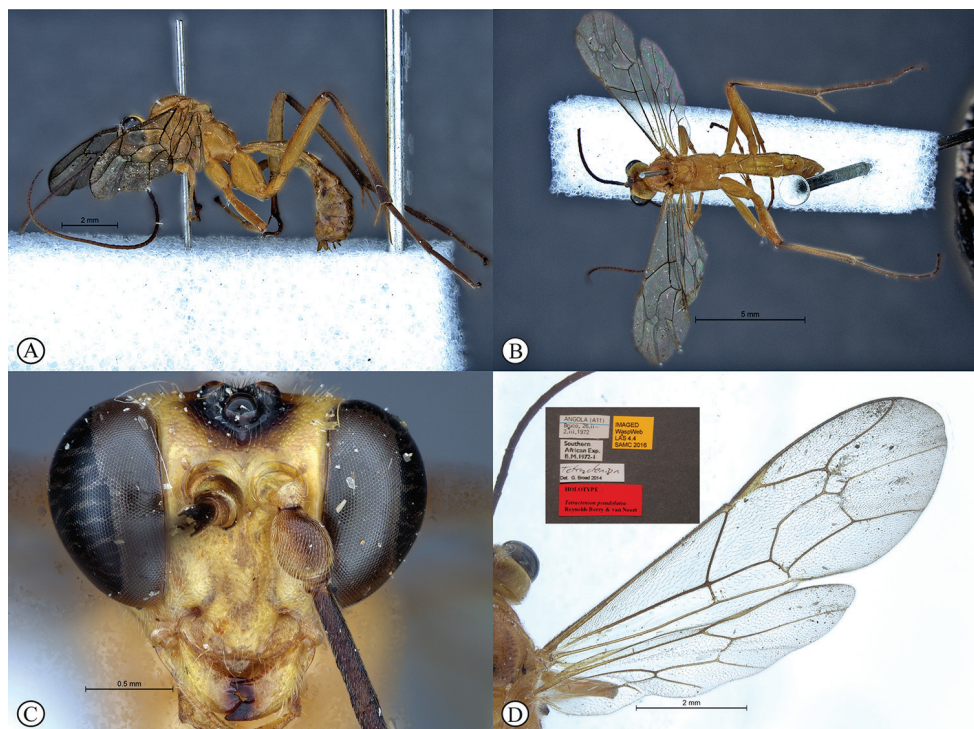
Description. The ***body color*** is the same as in *Tetractenion luteum*, except for density of microtrichia on the wings. *Tetractenion pseudolutea* has sparse microtrichia on the wings with yellow-brown venation, and the pterostigma is yellow.

Head is rounded behind eyes; occiput deeply and angularly excavated, occipital carina strong, extending to lower gena at base of mandible; eyes very large, malar space more than half as long as wide as base of mandible; face and clypeus finely and evenly punctate, background hardly shining; face with three lobes, tentorial pits deep; clypeus small, laterally convex with declivity, apically invaginated, clypeal edge convex; mandibular teeth triangular, lower tooth longer than upper tooth; antennae long, slender and apically tapered.

Mesosoma stout; mesoscutum deeply punctate, mesoscutal lobes hardly present, notauli posteriorly meeting before reaching the scutellum; pronotum finely punctate on a shiny background, no more than a wrinkle present on collar; mesopleuron and mesonotum finely punctate; mesopleuron higher than wide, epicnemial carina ending at anterior edge of mesopleuron; pits on mesopleuron and propodeum are shallow; propodeum weakly convex, finely punctate, posteriorly confluently grading into transverse wrinkles, posterior transverse carina reduced, lateral longitudinal carinae present but faint, spiracle small and circular-elliptical.

Metasoma indistinctly punctate on a shiny background; tergite I twice as long as wide, tapered anteriorly, sometimes weakly dorso-ventrally depressed in the medial region, glymma present, spiracle positioned in front of middle and protruding, especially dorsally; tergite II longer than wide, gastrocoeli indistinct; tergite III longer than wide; tergites IV–VIII moderately laterally compressed.

Fore wing with ramellus rarely present on Rs-M vein; areolet large, quadrate, with a short stalk receiving 2m-cu at the center. Hind wing with Cu1 shorter than cu-a such that Cu2 arises above the middle of these combined veins. Legs very long, hind femur reaching beyond metasomal apex, length of tibia III plus tarsus III as long as body, spurs of tibia III longer than half metatarsal length; tarsal claws pectinate.

CT 2.3; ML 0.6; IO 0.9–1.0; OO 1.7; Fl$_1$ 4.6–5.6; OT 0.1; B 9.1–10.7 mm; A 11.3–11.8 mm; F 8.6–9.8 mm.

Etymology. This species at first glance appears to be identical in coloration to *T. luteum* but has morphological differences.

Distribution. Angola, Cameroon, Namibia, and South Africa.

Tetractenion rosei Reynolds Berry & van Noort, sp. nov.

http://zoobank.org/A83D4196-5E8B-4D2D-AB29-7F0BDF15C377
Fig. 6

Type material. *Holotype* ♂: Cameroon, Yaoundé, 1953, C.I.E. Coll. 15098. Pres. by Com. Inst. Ent., B. M. 1962-1. *Exetastes* sp. ♀ det. J. F. Perkins (NHMUK).

Differential diagnosis. *Tetractenion rosei* is immediately distinguishable from other *Tetractenion* species by the reddish color of the head and pronotum in combination with a yellow body, completely yellow legs with venation on the wings also yellow. The head is not narrowed straight behind the eyes but rather rounded, distinguishing the species from *T. acaule* and *T. pascali*. The malar space nearly as long as the basal mandible width separates *T. rosei* from *T. acaule*, *T. pseudolutea*, and *T. ibayaensis*. *Tetractenion acaule* is the only other species besides *T. rosei* possessing a thickened and well-defined carina on the pronotal collar.

Pectinate hind tarsal claws separate the species from *T. acaule* and *T. luteum*. Sparse microtrichia on the wings distinguishes the species from *T. luteum* and *T. pascali*, and the pterostigma is yellow distinguishing the species from *T. acaule* and *T. ibayaensis*. Metasomal tergites II and III are longer than wide separating *T. rosei* from *T. ibayaensis*; and *T. acaule*, *T. luteum*, and *T. ibayaensis*, respectively.

Description. *Color:* head and pronotum reddish, black area restricted to region of ocelli. Body, legs, antennae yellow. Wings with sparse microtrichia, venation yellow, pterostigma yellow.

Head rounded behind eyes; occiput deeply and angularly excavated, occipital carina strong, extending to lower gena at mandibular base; malar space nearly as long as basal mandibular width; eyes very large; face and clypeus moderately and evenly punctate on a shiny background; face with three lobes, tentorial pits deep; clypeus small, laterally

Figure 6. *Tetractenion rosei* sp. nov. Holotype **A** habitus, lateral view **B** habitus, dorsal view **C** head, anterior view **D** wings (inset: data labels).

convex with declivity, apically invaginated, clypeal edge convex; mandibular teeth triangular, lower tooth longer than upper tooth; antenna long, slender and apically tapered.

Mesosoma stout with a shiny background; mesopleuron moderately punctate, epicnemial carina ending at anterior edge of mesopleuron; pits on the mesopleuron and propodeum shallow; mesonotum moderately punctate; pronotum sparsely and finely punctate on a shiny background with a well-defined carina on collar; mesoscutum deeply punctate, mesoscutal lobes hardly present, notauli posteriorly meeting before reaching the scutellum; propodeum weakly convex, deeply punctate posteriorly confluently grading into transverse wrinkles, posterior transverse carina indistinct, lateral longitudinal carinae present, spiracle small and round.

Metasoma indistinctly punctate on a shiny background; tergite I more than twice as long as wide, tapered anteriorly, slight dorso-ventral depression in medial region, glymma present, spiracle in front of middle and protruding; tergites II and III longer than wide; gastrocoeli on tergite II indistinct; tergites IV–VIII higher than wide.

Hind wing with Cu1 shorter than cu-a such that Cu2 arises above the middle of these combined veins. Legs very long, hind femur reaching beyond metasomal apex, length of tibia III plus tarsus III as long as body, spurs of tibia III longer than half metatarsal length; tarsal claws pectinate.

CT 1.6; ML 0.9; IO 1.4; OO 2.2; Fl$_1$ 3.5; B 9.3 mm; F 8.6 mm.

Etymology. Named because of the reddish color of the head and pronotal collar. Noun in apposition.

Distribution. Cameroon.

Comments. This is a rare species known only from one female specimen. Sampling in other areas of the Afrotropical region has so far not produced any further specimens. The wings are inter-locked in such a way that a useful diagnostic character of the wings cannot be seen, i.e., whether the ramellus is present or not.

Discussion

Since publication of the first key to genera of Banchinae in the Afrotropical region (Townes and Townes 1973), new knowledge on the subfamily has been acquired and there have been recent technological advances allowing for production of good quality images to illustrate relevant diagnostic character states. The revised and updated key is now more user-friendly. The genus *Tetractenion* was previously represented by two species and the present study has yielded an additional four species restricted to the Afrotropical region. Representative specimens in world collections remain rare and apart from those that are housed in the NHMUK and the CASC only; to our knowledge, there are no additional historical specimens present in world collections.

The general habitus and coloration of *Tetractenion* species suggest that this is possibly a nocturnal genus. A list of characters associated with being nocturnal or crepuscular includes a general brown-yellow color; long antennae; large eyes and large ocelli (Gauld and Huddleston 1976; Warrant et al. 2004; Greiner 2006). In addition, most species with this suite of characters are koinobionts (Quicke 2015). The benefit of being a koinobiont nocturnal species is that these parasitoid wasps are able to access hosts that are hidden during the day; many caterpillars conceal themselves during the day and come out to feed at night (Quicke 2015). Diurnal wasps, on the other hand, are faced with the pressures of predation and competition for limited resources (Warrant 2008). Where known, all Banchinae are koinobiont endoparasitoids of Lepidopteran caterpillars (Gauld and Mitchell 1978; Gauld et al. 2002; Fernandes et al. 2010; Broad et al. 2011; Tschopp et al. 2013). *Tetractenion* species possess very large eyes and long antennae relative to body size (A = 8.1–14 mm, B = 7.6–11.5 mm). However, the ocelli were not found to be particularly large (IO = 0.9–1.6, OO = 1.6–2.2); i.e., an ocellus with a large diameter would result in IO and OO indices with values less than one. Like other members of the Banchinae tribe Banchini, *Tetractenion* have very short ovipositors to allow for attack on exposed caterpillars (Fitton 1985, 1987). Members of the tribes Glyptini and Atrophini have ovipositors about as long as or longer than the metasoma and exploit semi- to -concealed hosts in leaf rolls, tunnels, buds, etc. (Townes 1969; Quicke 2015). This provides further support that the genus may have evolved to utilize host resources not readily available during the day. Given the endemicity to the Afrotropical region, *Tetractenion* is also predicted to be a more derived genus within the subfamily Banchinae. Phylogenetic analyses of the

subfamily within the Afrotropical region established that the tribe Banchini (only the *Exetastes* group is present in the Afrotropical region), represented by *T. pascali* and two *Exetastes* species, to be the most derived of the three tribes present in the region (Reynolds Berry 2019). Dating of the genus *Tetractenion* could not be determined based on a single species, but given that *Exetastes* has a cosmopolitan distribution and *Tetractenion* is an African endemic it is likely that *Tetractenion* is the more derived of the two genera.

Although there have been recent comprehensive long-term inventory surveys conducted across many parts of Africa with many rich, recently collected bulk samples that still need to be sorted, in reality, comprehensive sampling of Ichneumonidae in the region has been relatively limited to specific areas (Hopkins et al. 2019a, 2019b; Klopfstein et al. 2019; van Noort 2019). The perceived rarity (two species represented by a single specimen) of *Tetractenion* suggests that, with implementation of further rigorous inventory surveys across the many inadequately sampled areas of Africa, there are still more *Tetractenion* species to be discovered. Due to the relatively limited availability of specimens within this genus, any assessments of the distribution and diversification of the different species are still likely to be biased. This is corroborated by new locality records presented in this paper demonstrating bias in previously recorded distributional data: *T. luteum* was previously recorded from the Democratic Republic of Congo and Kenya (Seyrig 1935; Yu et al. 2020), but additional records from southern Africa suggest that this species probably has a more widespread distribution across Africa.

Most of the species have the pro-, meso-, and meta-tarsal claws pectinate to the apex. *Tetractenion luteum* and *T. acaule* (a Madagascan endemic) are the only two species that possess simple tarsal claws on the hind leg. While the overall color patterns of *T. pseudolutea* are identical to *T. luteum*, it is readily distinguishable from *T. luteum* by having a pectinate tarsal claw on the hind leg. With most *Tetractenion* species having pectinate tarsal claws on the hind leg, it is plausible that this character state is the plesiomorphic condition. Based on the assumption that it is more parsimonious for evolutionary trajectories to proceed via the reduction of morphological characters, rather than evolution of more complex character states, *T. luteum* and *T. acaule* are most probably the more derived species within the genus, but this hypothesis requires corroboration with the addition of genetic evidence, and a thorough phylogenetic analyses based on both morphological and molecular characters.

The current revision has increased the species richness of the genus threefold. Further comprehensive sampling will undoubtedly uncover additional *Tetractenion* species in the Afrotropical region.

Acknowledgements

References

Arnett RH, Samuelson GA, Nishida GM (1993) The Insect and Spider Collections of the World. Sandhill Crane Press, Gainsville, 316 pp.

Bennett AMR, Cardinal S, Gauld ID, Wahl DB (2019) Phylogeny of the subfamilies of Ichneumonidae. Journal of Hymenoptera Research 71: 1–156. https://doi.org/10.3897/jhr.71.32375

Broad GR (2014) A revision of *Sachtlebenia* Townes, with notes on the species of Townesion Kasparyan (Hymenoptera: Ichneumonidae: Banchinae). Proceedings of the Russian Entomological Society 85(1): 63–76. https://doi.org/10.47640/1605-7678_2014_85_1_63

Broad GR, Sääksjärvi IE, Veijalainen A, Notton DG (2011) Three new genera of Banchinae (Hymenoptera: Ichneumonidae) from Central and South America. Journal of Natural History 45(21–22): 1311–1329. https://doi.org/10.1080/00222933.2011.552809

Choi JK, Kang GW, Lee JW (2015) Two new species of *Leptobatopsis* Ashmead (Hymenoptera: Ichneumonidae: Banchinae) from South Korea and gynandromorphy in *L. nigricapitis*. Zootaxa 3964(2): 275–287. https://doi.org/10.11646/zootaxa.3964.2.7

Fernandes LBDR, Dias Filho MM, Fernandes MA, Penteado-Dias AM (2010) Ichneumonidae (Hymenoptera) parasitoids of Lepidoptera caterpillars feeding on *Croton floribundus* Spreng (Euphorbiaceae). Revista Brasileira de Entomologia 54(2): 263–269. https://doi.org/10.1590/S0085-56262010000200009

Fitton MG (1985) The ichneumon-fly genus *Banchus* (Hymenoptera) in the Old World. Bulletin of the British Museum (Natural History) (Entomology) 51: 1–60. https://www.biodiversitylibrary.org/part/14889#/summary

Fitton MG (1987) A review of the *Banchus* group of ichneumon-flies, with a revision of the Australian genus *Philogalleria* (Hymenoptera: Ichneumonidae). Systematic Entomology 12(1): 33–45. https://doi.org/10.1111/j.1365-3113.1987.tb00545.x

Gauld ID (1985) The phylogeny, classification and evolution of parasitic wasps of the subfamily Ophioninae (ichneumonidae). Bulletin of the British Museum (Natural History) 51: 1–185. https://www.biodiversitylibrary.org/part/14890#/summary

Gauld ID (1991) The Ichneumonidae of Costa Rica 1. Memoirs of the American Entomological Institute 47: 1–589.

Gauld ID, Huddleston T (1976) The nocturnal Ichneumonoidea of the British Isles. Including a key to the genera. Entomologist'S, Gazette 27(1): 35–49. https://www.cabdirect.org/cabdirect/abstract/19760533821

Gauld ID, Gomez J, Godoy C (2002) Subfamily Banchinae. In: Gauld ID (Ed.) The Ichneumonidae of Costa Rica, 4. Memoirs of the American Entomological Institute 66: 263–746.

Gauld ID, Mitchell P (1978) The Taxonomy, Distribution and Host Preferences of African Parasitic Wasps of the Subfamily Ophioninae (Hymenoptera: Ichneumonidae). Commonwealth Agricultural Bureau, Slough, 287 pp.

Gauld ID, Wahl DB (2000) The Townesioninae: a distinct subfamily of Ichneumonidae (Hymenoptera) or a clade of the Banchinae? Transactions of the American Entomological Society 126(3): 279–292. https://www.jstor.org/stable/25078717?seq=1

Gupta VK (2002) Glyptopimpla Morley (Hymenoptera: Ichneumonidae: Banchinae) – a valid genus with descriptions of new species from the Orient. Oriental Insects 36(1): 221–237. https://doi.org/10.1080/00305316.2002.10417332

Greiner B (2006) Visual adaptations in the night active wasp *Apoica pallens*. Journal of Comparative Neurology 495(3): 255–262. https://doi.org/10.1002/cne.20882

Herrera-Florez AF (2017) A new species of *Sphelodon* Townes (Hymenoptera: Ichneumonidae: Banchinae) from Colombia. Zootaxa 4277(2): 289–294. https://doi.org/10.11646/zootaxa.4277.2.11

Hopkins T, Roininen H, van Noort S, Broad GR, Kaunisto K, Sääksjärvi IE (2019a) Extensive sampling and thorough taxonomic assessment of Afrotropical Rhyssinae (Hymenoptera, Ichneumonidae) reveals two new species and demonstrates the limitations of previous sampling efforts. ZooKeys 878: 33–71. https://doi.org/10.3897/zookeys.878.37845

Hopkins T, Roininen H, Sääksjärvi IE (2019b) Extensive sampling reveals the phenology and habitat use of Afrotropical parasitoid wasps (Hymenoptera: Ichneumonidae: Rhyssinae). Royal Society Open Science 6: 190913. https://doi.org/10.1098/rsos.190913

Kang GW, Kolarov J, Lee JW (2019) *Cryptopimpla* (Hymenoptera, Ichneumonidae, Banchinae) of South Korea, with description of two new species. ZooKeys 830: 99–109. https://doi.org/10.3897/zookeys.830.31974

Kang GW, Kolarov J, Lee JW (2020) A review of South Korean *Alloplasta* Förster (Hymenoptera: Ichneumonidae:Banchinae) with description of a new species. Zootaxa 4763(2): 270–280. https://doi.org/10.11646/zootaxa.4763.2.9

Kasparyan DR, Kuslitzky WS (2018) Contribution to the fauna of the ichneumon-wasp genus *Rhynchobanchus* Kriechbauer, 1894 (Hymenoptera, Ichneumonidae: Banchinae) in the Russian Far East. Entomological Review 98(6): 748–752. https://doi.org/10.1134/S001387381806012X

Khalaim AI, Ruíz-Cancino E (2012) Mexican species of *Exetastes* (Hymenoptera: Ichneumonidae: Banchinae), with description of three new species. Revista Mexicana de Biodiversidad 83: 370–379. https://doi.org/10.22201/ib.20078706e.2012.2.953

Klopfstein S, Santos BF, Shaw MR, Alvarado M, Bennett AM, Dal Pos D, Giannotta M, Herrera Florez AF, Karlsson D, Khalaim AI, Lima AR, Mikó I, Sääksjärvi IE, Shimizu S, Spasojevic T, van Noort S, Vilhelmsen L, Broad GR (2019) Darwin wasps: a new name heralds renewed efforts to unravel the evolutionary history of Ichneumonidae. Entomological Communications 1: ec01006. https://doi.org/10.37486/2675-1305.ec01006

Li ZJ, Li T, Yan J, Sheng ML (2018) The genus *Rhynchobanchus* Kriechbaumer in China, with descriptions of a new species and first record of the genus from Oriental region (Hymenoptera, Ichneumonidae, Banchinae). ZooKeys 752: 125–136. https://doi.org/10.3897/zookeys.752.23884

Morley C (1917) On some South African Ichneumonidae in the collection of the South African Museum. Annals of the South African Museum 17: 191–229.

Penev L, Sharkey M, Erwin T, van Noort S, Buffington M, Seltmann K, Johnson N, Taylor M, Thompson C, Dallwitz M (2009) Data publication and dissemination of interactive keys under the open access model. ZooKeys 21: 1–17. https://doi.org/10.3897/zookeys.21.274

Quicke DLJ (2015) The Braconid and Ichneumonid Parasitoid Wasps. Biology, Systematics, Evolution and Ecology. Wiley Blackwell, Oxford, 704 pp. https://doi.org/10.1002/9781118907085

Reynolds Berry T (2019) Systematics of the parasitoid wasp subfamily Banchinae (Hymenoptera; Ichneumonidae) in the Afrotropical region, PhD thesis, Stellenbosch University, South Africa.

Reynolds Berry T, van Noort S (2016) Review of Afrotropical *Cryptopimpla* Taschenberg (Hymenoptera, Ichneumonidae, Banchinae), with description of nine new species. ZooKeys 640: 103–137. https://doi.org/10.3897/zookeys.640.10334

Seyrig A (1932) Les Ichneumonides de Madagascar. I Ichneumonidae Pimplinae. Mémoires de l'Académie Malgache. Fascicule 11, 183 pp.

Seyrig A (1935) Mission scientifique de l'Omo. Tome III. Fascicule 18. Hymenoptera, II. Ichneumonidae: Cryptinae, Pimplinae, Tryphoninae et Ophioninae. Mémoires du Muséum National d'Histoire Naturelle, Paris 4: 1–100.

Sharkey M, Yu D, van Noort S, Seltmann K, Penev L (2009) Revision of the Oriental genera of Agathidinae (Hymenoptera, Braconidae) with an emphasis on Thailand and interactive keys to genera published in three different formats. ZooKeys 21: 19–54. https://doi.org/10.3897/zookeys.21.271

Sheng ML (2011) Five new species of the genus *Cryptopimpla* Taschenberg (Hymenoptera, Ichneumonidae) with a key to species known from China. ZooKeys 117: 9–49. https://doi.org/10.3897/zookeys.117.1302

Sheng ML, Sun SP, Wang XN, Wu HW (2018) A new genus and species of subfamily Banchinae (Hymenoptera, Ichneumonidae) from China. Zootaxa 4413(3): 541–550. https://doi.org/10.11646/zootaxa.4413.3.8

Shimizu S, Ogawa R (2019) Discovery of the subfamily Lycorininae Cushman and Rohwer, 1920 (Hymenoptera: Ichneumonidae) from Indonesia, based on *Lycorina longicauda* Shimizu, sp. nov., with a key to the Oriental *Lycorina* species. Austral Entomology 58(1): 148–155. https://doi.org/10.1111/aen.12347

Takasuka K, Watanabe K, Konishi K (2011) Genus *Cryptopimpla* Taschenberg new to Sulawesi, Indonesia, with description of a new species (Hymenoptera, Ichneumonidae, Banchinae). Journal of Hymenoptera Research 23: 65–75. https://doi.org/10.3897/jhr.23.1595

Townes HK (1969) Genera of Ichneumonidae, Part 3 (Lycorininae, Banchinae, Scolobatinae, Porizontinae). Memoirs of the American Entomological Institute 13: 1–307.

Townes HK, Townes M (1973) A catalogue and reclassification of the Ethiopian Ichneumonidae. Memoirs of the American Entomological Institute 19: 1–416.

Tschopp S, Riedel A, Kropf C, Nentwig W, Klopfstein S (2013) The evolution of host associations in the parasitic wasp genus *Ichneumon* (Hymenoptera: Ichneumonidae): convergent adaptations to host pupation sites. BMC Evolutionary Biology 13(1): 1–74. https://doi.org/10.1186/1471-2148-13-74

van Noort S (2019) Assessing the status quo of Afrotropical ichneumonid knowledge. Zenodo. http://doi.org/10.5281/zenodo.3395821

van Noort S (2020) WaspWeb: Hymenoptera of the Afrotropical region. www.waspweb.org [accessed on 20 Feb 2020]

van Noort S, Buffington ML, Forshage M (2015) Afrotropical Cynipoidea (Hymenoptera). ZooKeys 493: 1–176. https://doi.org/10.3897/zookeys.493.6353

Vas Z (2017) Data to the Vietnamese ichneumon wasp fauna with description of a new *Teleutaea* species (Hymenoptera: Ichneumonidae). Folio Entomologica Hungarica 78: 101–110. https://doi.org/10.17112/FoliaEntHung.2017.78.101

Wahl DB (1988) A review of the mature larvae of the Banchini and their phylogenetic significance, with comments on the Stilbopinae (Hymenoptera: Ichneumonidae). In: Gupta VK (Ed.) Advances in Parasitic Hymenoptera Research. Brill, Leiden, 147–161.

Wahl DB (1991) The status of *Rhimphoctona*, with special reference to the higher categories within Campopleginae and the relationships of the subfamily (Hymenoptera: Ichneumonidae). Society 117: 193–213.

Wahl DB, Sharkey MJ (1993) Chapter 10. Superfamily Ichneumonoidea. In: Goulet H, Huber JT (Eds) Hymenoptera of the World: An Identification Guide to Families. Agriculture Canada, Ottawa, 358–509.

Warrant EJ (2008) Seeing in the dark: vision and visual behaviour in nocturnal bees and wasps. Journal of Experimental Biology 211: 1737–1746. https://doi.org/10.1242/jeb.015396

Warrant EJ, Kelber A, Gislén A, Greiner B, Ribi W, Wcislo WT (2004) Nocturnal vision and landmark orientation in a tropical halictid bee. Current Biology 14(15): 1309–1318. https://doi.org/10.1016/j.cub.2004.07.057

Watanabe K (2017) Revision of the genus *Amphirhachis* Townes, 1970 (Hymenoptera, Ichneumonidae, Banchinae) from Japan. ZooKeys 685: 49–64. https://doi.org/10.3897/zookeys.685.13552

Watanabe K (2020) Taxonomic study of the tribe Banchini (Hymenoptera: Ichneumonidae: Banchinae) from Japan. Japanese Journal of Systematic Entomology, Supplementary Series 2: 1–58.

Watanabe K, Maeto K (2012) A new species of the genus *Himertosoma* from the Ryukyus, Japan, with a key to species from the Palaearctic and Oriental regions (Hymenoptera, Ichneumonidae, Banchinae). ZooKeys 234: 59–66. https://doi.org/10.3897/zookeys.234.3794

Watanabe K, Maeto K (2014) Revision of the genus *Apophua* Morley, 1913, from Japan (Hymenoptera, Ichneumonidae, Banchinae). Zootaxa 3784(5): 501–527. https://doi.org/10.11646/zootaxa.3784.5.1

Watanabe K, Sheng ML (2018) Taxonomic notes on *Exetastes fukuchiyamanus* Uchida, 1928 (Hymenoptera, Ichneumonidae, Banchinae), with description of a new species from japan and China. Zootaxa 4399(2): 281–288. https://doi.org/10.11646/zootaxa.4399.2.11

Yu DSK, van Achterberg C, Hortsmann K (2020) Taxapad 2012, Ichneumonoidea 2011. www.taxapad.com [accessed on 20 Aug 2020]

Supplementary material 1

Lucid Interchange Format version 3 (LIF3) for the key to the genera of Afrotropical Banchinae (Hymenoptera, Ichneumonidae)

Authors: Terry Reynolds Berry, Simon van Noort

Data type: Lucid Interchange Format version 3

Explanation note: The LIF3 file is an XML-based file that stores all the Lucid4 key data, allowing exchange of the key with other key developers.

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Link: https://doi.org/10.3897/zookeys.1007.55543.suppl1

Supplementary material 2

Lucid Interchange Format version 3 (LIF3) and Lucid SDD files for the key to *Tetractenion* species (Hymenoptera, Ichneumonidae, Banchinae)

Authors: Terry Reynolds Berry, Simon van Noort

Data type: Lucid Interchange Format version 3 (LIF3) and Lucid SDD files

Explanation note: The LIF3 file is an XML-based file that stores all the Lucid4 key data, allowing exchange of the key with other key developers.

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Link: https://doi.org/10.3897/zookeys.1007.55543.suppl2

RESEARCH ARTICLE

Shards, sequences, and shorelines: two new species of *Bembidion* from North America (Coleoptera, Carabidae)

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Abstract

Two new species of *Bembidion* are described from river shores in North America. One, *Bembidion mimbres* **sp. nov.**, from the Gila River watershed in the lands of the Mimbres culture in New Mexico and Arizona, is closely related to the widespread *Bembidion levigatum*. DNA sequences from several linkage groups and morphology provide evidence of the distinctiveness of *B. mimbres*. The second, *Bembidion corgenoma* **sp. nov.**, has been the subject of recent genomic and transcriptomic studies. It belongs in the *Bembidion transversale* subgroup, and occurs from California north to British Columbia, east to Montana and Nevada. The *B. transversale* subgroup as a whole is reviewed, and morphological characters that distinguish *B. corgenoma* from the similar and sympatric *B. transversale* and *B. erosum* are described and illustrated. DNA sequences of these three species show no consistent differences in 28S, COI, CAD, and Topoisomerase, and a coalescent species delimitation analysis reveals no notable structure within the complex. This is the first known trio of species within *Bembidion* for which those genes provide no clear signal of species boundaries. A neotype is designated for the one name in the group that lacks a primary type, *Bembidium haplogonum* Chaudoir. Chromosomes of the new species and their relatives are as is typical for *Bembidion*, with eleven pairs of autosomes and an XY/XX sex chromosome system.

Keywords

Bembidiini, ground beetle, molecular systematics, species delimitation, taxonomy, Trechinae

Introduction

Bembidion is a very large genus of small beetles with more than 1,200 species worldwide (Lorenz 2005). Most species of these small predators live along the edges of bodies of water and can be abundant in their habitats.

In the course of an ongoing project revising the bembidiine carabids of America north of Mexico, a number of undescribed species have been discovered. Most of these will be described in due course within complete revisions of subgenera or species groups. However, two of these new species are or will be soon discussed in the scientific literature, and warrant description more quickly, in order to provide them with names. These two are also especially significant, as they have cultural connections to humans, implicit or explicit, of very different sorts.

One of them is a member of the subgenus *Hydrium*, a group of relatively large *Bembidion* that is widespread in the Northern Hemisphere. The new species (Fig. 1) is only known from the Gila River watershed of southeastern Arizona and southwestern New Mexico, where it lives along the banks of rivers and creeks, on the ground a few meters away from the shoreline (Fig. 2), most commonly under willows (*Salix*). The distribution of this new species is within that of the Mimbres culture, which flourished in that area one thousand years ago. This culture is perhaps best known for black-on-white Mimbres pottery, the designs of which depicted people, cultural icons, and organisms (Hegmon et al. 2018). The people of the Mimbres culture were deeply aware of the arthropods in their environment, as indicated by the astonishing array of images on their pottery of insects, including among others geometrid larvae (https://core.tdar. org/image/383483/1452-style-iii-bowl-from-cameron-creek; https://doi.org/10.6067/ XCV8Z60P2N), Orthoptera (https://core.tdar.org/image/383111/2685-style-iii-bowl-from-swarts; https://doi.org/10.6067/XCV80Z7364), dragonflies, and ant lions (Hegmon, et al. 2018). In honor of these peoples who were so connected to the small organisms in their midst, this elegant beetle species is given the name *Bembidion mimbres*.

The second species described here is connected to humans via modern biological research: it is becoming the first model species of *Bembidion* for genomic and transcriptomic studies. It is member of the *B. transversale* species group of the *Ocydromus* complex of *Bembidion*, containing some of the largest *Bembidion* in North America (Lindroth 1963; Maddison and Swanson 2010). The *B. transversale* group consists of two subgroups, the *B. transversale* subgroup and the *B. mexicanum* subgroup (Maddison 2012; Maddison and Swanson 2010). Maddison and Swanson (2010) considered the *B. transversale* subgroup to contain three species (*B. transversale* Dejean, *B. perspicuum* (LeConte), and *B. sarpedon* Casey), although they noted that "*B. transversale*" showed enough morphological variation to suggest that it may contain multiple species. Because of the especially complex pattern of variation, it only recently become clear that the genomic and transcriptomic model species was undescribed. The new species (Fig. 3) is common in Oregon and California, with some populations in neighboring regions, living along cobble and gravel shores of rivers and creeks (Fig. 4). It is the best sequenced *Bembidion* genomically and transcriptomically (Gustafson et al. 2019; Gustafson et al. 2020; Pflug

Figure 1. Paratype male of *Bembidion mimbres* (voucher number V100327) from the type locality.

Figure 2. Habitat of *Bembidion mimbres* at USA: New Mexico: Grant Co., Gila River, Billings Vista, 1320 m, 32.8163°N, 108.6032°W (type locality). Arrow indicates approximate location of most specimens. Found in the same habitat were *Bembidion aratum* (LeConte), *B. impotens* Casey, *B. scintillans* Bates, *B. horni* Hayward, *B. rupicola* (Kirby), *B. clemens* Casey, *B rapidum* (LeConte), and *Omophron obliteratum* Horn.

Figure 3. Paratype male of *Bembidion corgenoma* (voucher number V101452) from the type locality. Scale bar: 1 mm.

et al. 2020), and has been used as one of the models for developing a UCE probe set for adephagan beetles (Gustafson, et al. 2019; as *B. haplogonum* Chaudoir). It is a center-piece of ongoing and future studies of genome size in carabids (e.g., Pflug, et al. 2020). In this paper, it is described as *Bembidion corgenoma*.

An important step enabling future research about these beetles is providing the species with stable names. Although we now know the two new species in many ways un-imaginable to those who lived a thousand years ago, including detailed aspects of their

Figure 4. Habitats of *Bembidion corgenoma* **A** USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m, 44.5491°N, 123.2449°W (type locality). *B. corgenoma* and *B. recticolle* are both common in this habitat. *B. corgenoma* is more common near the water in areas with vegetation growing nearby among the gravel and cobbles **B** USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W, habitat of *B. corgenoma* and *B. erosum*, as well as *Bembidion haruspex* Casey, *B. vandykei* Blaisdell, *B. curtulatum* Casey, *B. platynoides* Hayward, and *B incrematum* LeConte.

DNA and genomes, we know very little about these two species in nature. With the decreasing emphasis on natural history in modern biology, it is possible that a person of the Mimbres culture knew aspects of the daily life of *B. mimbres* much better than we ever will. However, perhaps this paper, in giving names to the two species and presenting identification tools allowing them and their near relatives to be distinguished, will inspire research about these beetles, including into their way of life along river shores.

Materials and methods

Members of *Bembidion* were examined from or will be deposited in the collections listed below. Each collection's listing begins with the code used in the text.

CAS	California Academy of Sciences, San Francisco, USA;
CNC	Canadian National Collection, Ottawa, Canada;
CSCA	California State Collection of Arthropods, Sacramento, USA;
EMEC	Essig Museum Entomology Collection, University of California, Berkeley, USA;
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, USA;
NHMUK	The Natural History Museum, London, UK;
MNHN	Muséum National d'Histoire Naturelle, Paris, France;
MSBA	Museum of Southwestern Biology, University of New Mexico, Albuquerque, USA;
OSAC	Oregon State Arthropod Collection, Oregon State University, Corvallis, USA;
UAIC	University of Arizona Insect Collection, Tucson, USA;
UASM	University of Alberta Strickland Museum, Edmonton, Canada;
UBCZ	Spencer Entomological Museum, University of British Columbia, Vancouver, Canada;
USNM	National Museum of Natural History, Smithsonian Institution, Washington, DC, USA;
ZMUM	Zoological Museum, Moscow State University, Moscow, Russia.

Collecting methods

Specimens were collected by hand or using an aspirator; specimens were found during the day in their habitat, or with the aid of a headlamp at night, when the beetles are more actively moving on the surface. Specimens for morphological studies were killed and preserved in maple (*Acer*) sawdust to which ethyl acetate was added. Specimens for DNA sequencing were collected into 95% or 100% ethanol. For chromosomal studies, live specimens were placed in simple Carnoy's solution (three parts 100% ethanol : one part glacial acetic acid), and the abdomens were opened up shortly after death to allow better penetration of the fixative; the specimens were stored in Carnoy's in a -20 °C freezer, with vials contained within multiple layers of plastic to prevent the release of acid fumes.

Morphological methods

General methods of specimen preparation for morphological work, and terms used, follow Maddison (1993; 2008). Genitalia were prepared, after dissection from the body, by treatment in 10% KOH at 65 °C for 10 minutes followed by a series of multi-hour baths of distilled water, 5% glacial acetic acid, distilled water, and then ethanol. Male genitalia were then mounted in Euparal between two small coverslips attached to archival-quality heavyweight watercolor paper, and, once dried, pinned beneath the specimen. Male genitalia were examined for 30–60 specimens each of *B. transversale*, *B. erosum*, and *B. corgenoma*, and four or five specimens each of *B. levigatum* and *B. mimbres*.

Photographs of entire beetles and antennae were taken with a Leica M165C dissecting scope and a Sony NEX-7 camera, and of male genitalia with either a Leica Z6 Apo lens and DMC4500 camera or a Leica DM5500B compound microscope and DMC425C camera. A stack of images from different focal positions was then merged using the PMax procedure in Zerene Systems's Zerene Stacker; the final images thus potentially have some artifacts caused by the merging algorithm. Measurements were made using Leica Application Suite v4.9 from images acquired using these imaging systems.

Cytogenetic methods

Twenty-two males were examined for chromosome number and sex-chromosome system. Methods used were as outlined by Maddison (1985; 2008). Males examined were: 1 *Bembidion mimbres* from USA: New Mexico: Grant Co., Billings Vista, Gila River, 1310 m, 32.8137°N, 108.6031°W; 2 *B. transversale* from USA: Colorado: Fremont Co., Arkansas River at Texas Creek 1880 m, 38.4100°N, 105.5854°W; 3 *B. erosum* from USA: California: Del Norte Co., Wilson Creek , 3 m, 41.6051°N, 124.1005°W; 3 *B. perspicuum* from USA: Arizona: Cochise Co., San Pedro R at Charleston, 31.6239°N, 110.1722°W; 3 *B. sarpedon* from USA: Colorado: Las Animas Co., Purgatoire R., 2.7 km W Cokedale 1910 m, 37.1295°N, 104.6390°W; 4 *B. pernotum* from USA: Colorado: Las Animas Co., Purgatoire R., 2.7 km W Cokedale 1910 m, 37.1292°N, 104.6398°W; 3 *B. mexicanum* from USA: Arizona: Pima Co., Santa Rita Mtns, Box Canyon, 1455 m 31.7981°N, 110.7767°W; 3 *B. lugubre* from USA: Arizona: Pima Co., Santa Rita Mtns, Box Canyon, 1455 m, 31.7981°N, 110.7767°W. In addition, the vouchers of the *B. transversale* group studied in Maddison (1985) were re-examined and re-identified.

Molecular methods

DNA extraction and sequencing

Genes studied, and abbreviations used in this paper, are: 28S: 28S ribosomal DNA (D1-D3 domains); 18S: 18S ribosomal DNA; COI: cytochrome *c* oxidase I; *wg*: *wingless*; CAD: carbamoyl phosphate synthetase domain of the *rudimentary* gene; ArgK: arginine kinase; Topo: topoisomerase I.

DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit. Fragments for the seven genes were amplified using the polymerase chain reaction (PCR) on an Eppendorf Mastercycler ProS Thermal Cycler, using TaKaRa Ex Taq and the basic protocols recommended by the manufacturers. Primers and details of the cycling reactions used are given in Maddison (2012) and Maddison and Cooper (2014). The amplified products were then cleaned, quantified, and sequenced at the University of Arizona's Genomic and Technology Core Facility using a 3730 XL Applied Biosystems automatic sequencer. Assembly of multiple chromatograms for each gene fragment and initial base calls were made with Phred (Green and Ewing 2002) and Phrap (Green 1999) as orchestrated by Mesquite's Chromaseq package (Maddison and Maddison 2018a, c) with subsequent modifications by Chromaseq and manual inspection. Multiple peaks at a single position in multiple reads were coded using IUPAC ambiguity codes.

Taxon sampling for DNA studies

For the phylogenetic study of *Bembidion* (*Hydrium*), 19 specimens of the subgenus *Hydrium*, as well as five species serving as outgroups (Table 1) were used. Of the 152 sequences examined, 97 were newly acquired, with 55 being from previous publications (Maddison 2012; Maddison and Cooper 2014; Maddison et al. 2019; Maddison and Maruyama 2019; Maddison and Ober 2011). For the phylogenetic study of the *B. transversale* group, I sampled 56 specimens of the *B. transversale* subgroup, as well as three species of the *B. mexicanum* subgroup (Table 2). Of the 237 sequences examined, 109 were newly sequenced, with 128 from previous publications (Kanda et al. 2015; Maddison 2012; Maddison and Swanson 2010; Wild and Maddison 2008). Sequences of the two holotypes listed in Tables 1 and 2 are "genseq-1", of paratypes "genseq-2", and the remainder are all "genseq-4" (Chakrabarty et al. 2013). In addition, sequences of the newly designated neotype of *Bembidium haplogonum* Chaudoir were acquired and deposited in GenBank (accession numbers MW151478, MW151506, and MW151563), and are "genseq-1". Localities of the sequenced specimens of *B. levigatum*, *B. mimbres*, *B. transversale*, *B. erosum*, and *B. corgenoma* are given in Table 3.

Sequence alignment

Alignment was not difficult for any of the protein-coding genes. There were no insertion or deletions (indels) evident in the sampled CAD, ArgK, Topo, *wg*, or COI sequences. An alignment of 28S was conducted in MAFFT version 7.130b (Katoh and Standley 2013), using the L-INS-i search option and otherwise default parameter values.

Molecular phylogenetic analysis

Maximum likelihood analysis was conducted for each gene individually using IQ-TREE version 1.6.7.1 (Nguyen et al. 2015), as orchestrated by Mesquite's Zephyr package (Maddison and Maddison 2018b, c). The ModelFinder feature within IQ-TREE

Table 1. Sampling of members of *Bembidion* (*Hydrium*) and related subgenera for DNA-based study. Four-digit numbers under "#" are D.R. Maddison DNA voucher numbers; the specimen marked with an * is the holotype of *B. mimbres*. All other specimens listed of *B. mimbres* are paratypes. An abbreviation for state or province of capture is given under "Loc"; further information on *B. levigatum* and *B. mimbres* specimens is given in Table 3. The last eight columns contain GenBank accession numbers.

	#	Loc	28S	COIBC	COIPJ	COI-like	wg	CAD	ArgK	Topo
Outgroups										
B. incisum Andrewes			JN170312	JN171013			JN171381	MK118269	JN170532	JN171195
B. dyschirinum LeConte	0896		JN170332	JN171029	MW151370		GU556029	JN170799	JN170553	JN171210
B. lampros (Herbst)	1727		JN170370	MF616889	MW151371		JN171439	JN170838	JN170593	MF616755
B. properans (Stephens)	1279		JN170408	JN171088	MW151372		JN171482	JN170883	JN170640	JN171270
B. luridicorne Solsky	1122		JN170377	JN171064	MW151373		JN171447	JN170847	JN170602	JN171245
Bembidion (Hydrium)										
B. interventor Lindroth	1131		JN170365	JN171052	MW151374		JN171434	JN170833	JN170588	JN171232
B. nitidum (Kirby)	1941		JN170392	MF616897	MW151375		JN171464	JN170864	JN170620	JN171257
B. obliquulum LeConte	1299		JN170395	KJ624355	MW151376		JN171467	MK118221	JN170623	KJ624308
B. levigatum Say	1693	VA	MW151391	JN171059	JN171059		JN171441	JN170841	JN170596	JN171240
	1255	IN	MW151392	MW151377	MW151377			MW151435		MW151406
	1694	IA	MW151393	MW151378	MW151378		MW151428	MW151436	MW151420	MW151407
	0763	NE	GU556083	MW151379	MW151379		GU556032			
	1256	TX	MW151394	MW151380	MW151380			MW151437	MW151421	MW151408
	2343	TX	MW151395	MW151381	MW151381		MW151429	MW151438	MW151422	MW151409
B. mimbres sp. nov.	0280	NM	MW151396	MW151382	MW151382		MW151430	MW151439	MW151423	MW151410
	1220	NM	MW151404			MW151366		MW151447		MW151418
	1267	NM	MW151405			MW151367		MW151448		MW151419
	1944	NM	MW151403	MW151389	MW151383	MW151368	MW151434	MW151446	MW151427	MW151417
	2117	NM	MW151397	MW151383	MW151384	MW151369	MW151431	MW151440		MW151411
	2118	NM	MW151398	MW151384	MW151385			MW151441	MW151424	MW151412
	2119	NM	MW151399	MW151385	MW151386			MW151442		MW151413
	2131*	NM	MW151400	MW151386			MW151432	MW151443	MW151425	MW151414
	2134	NM	MW151401	MW151387	MW151387			MW151444		MW151415
	2135	NM	MW151402	MW151388	MW151388		MW151433	MW151445	MW151426	MW151416

Table 2. Sampling of members of *Bembidion transversale* group for DNA-based study. Four-digit numbers under "#" are D.R. Maddison DNA voucher numbers. Under "T", the holotype of *B. corgenoma* is indicated by "H", and paratypes by "P". An abbreviation for state or province of capture is given under "Loc"; further information on specimens of *B. transversale*, *B. erosum*, and *B. corgenoma* is given in Table 3. The last four columns contain GenBank accession numbers.

	#	T	Loc	28S	COI	CAD	Topo
B. mexicanum subgroup							
Bembidion lugubre LeConte	1712		AZ	JN170375	JN171062	JN170845	JN171243
Bembidion mexicanum Dejean	2192		NM	GU454739	GU454769	JN170854	JN171250
Bembidion pernotum Casey	2483		CO	JN170403	JN171082	JN170875	JN171263
B. transversale subgroup							
Bembidion sarpedon Casey	2484		CO	JN170432	JN171106	JN170908	JN171285
	2514		CO	KU233761	KU233815	KU233926	KU234052
	3009		CO	KU233764	KU233818	KU233929	KU234055
	3761		UT	KU233771	KU233823	KU233933	KU234059
	3776		UT	KU233775	KU233827	KU233937	KU234063
Bembidion perspicuum (LeConte)	1120		AZ	GU454740	GU454770	JN170877	JN171265
	2318		CA	GU454743	KU233812	KU233923	KU234049
	3774		CA	KU233773	KU233825	KU233935	KU234061
	3775		CA	KU233774	KU233826	KU233936	KU234062
	2485		CO	GU454748	GU454778	KU233924	KU234050
	2182		NM	GU454749	KU233810	KU233922	KU234047
Bembidion erosum Motschulsky	2596		CA	MW151550	MW151493	MW151522	MW151465
	2607		CA	MW151551	MW151494	MW151523	MW151466
	3561		CA	MW151552	MW151495	MW151524	MW151467
	3562		CA	MW151553	MW151496	MW151525	MW151468
	3584		CA	MW151554	MW151497	MW151526	MW151469
	4050		CA	MW151549	MW151492	MW151521	MW151464
	4212		CA	MW151555	MW151498	MW151527	MW151470
	4033		OR	MW151556	MW151499	MW151528	MW151471
	2162		BC	KU233749	KU233803	KU233915	KU234040
	2194		BC	KU233757	KU233811	MW151529	KU234040
Bembidion transversale Dejean	2160		NS	GU454762	KU233802	KU233914	KU234048
	2486		CO	GU454755	GU454785	KU233925	KU234039
	2157		WY	EU677688	GU454797	EU677541	KU234051
	4690		MT	MW151561	MW151504	MW151534	MW151476
	4927		MT	MW151562	MW151505	MW151535	MW151477
	5064		WA	MW151560	MW151503	MW151533	MW151475
	5613		OR	MW151557	MW151500	MW151530	MW151472
	4219		OR	MW151558	MW151501	MW151531	MW151473
	5612		OR	MW151559	MW151502	MW151532	MW151474
Bembidion corgenoma sp. nov.	4052		CA	KU233782	KU233831	KU233941	KU234067
	2181		CA	KU233755	KU233809	KU233921	KU234046
	4054		CA	KU233783	KU233832	KU233942	KU234068
	2180		CA	KU233754	KU233808	KU233920	KU234045
	3772	P	CA	KU233772	KU233824	KU233934	KU234060
	4961	P	CA	MW151536	MW151479	MW151508	MW151451
	4962	P	CA	MW151537	MW151480	MW151509	MW151452
	4218		CA	MW151544	MW151487	MW151516	MW151459
	2597		CA	MW151538	MW151481	MW151510	MW151453
	2608		CA	MW151539	MW151482	MW151511	MW151454
	3559		CA	KU233769	KU233821	KU233931	KU234057
	3560		CA	KU233770	KU233822	KU233932	KU234058
	3583		CA	MW151540	MW151483	MW151512	MW151455
	4959		NV	MW151541	MW151484	MW151513	MW151456
	2346		NV	GU454763	GU454793	MW151507	MW151450
	5670	P	OR	MW151545	MW151488	MW151517	MW151460

	#	T	Loc	28S	COI	CAD	Topo
Bembidion corgenoma sp. nov.	5671	P	OR	MW151546	MW151489	MW151518	MW151461
	5672	P	OR	MW151547	MW151490	MW151519	MW151462
	5673	H	OR	MW151548	MW151491	MW151520	MW151463
	4032		OR	KU233780	KU233829	KU233939	KU234065
	2973		OR	KU233763	KU233817	KU233928	KU234054
	3205	P	OR	KU233791	KU233841	KU233979	KU234056
	4034		OR	KU233781	KU233830	KU233940	KU234066
	3021		ID	KU233790	KU233840	KU233973	KU234070
	2165		WA	KU233750	KU233804	KU233916	KU234041
	5065	P	OR	MW151542	MW151485	MW151514	MW151457
	2190		BC	MW151543	MW151486	MW151515	MW151458

Table 3. Localities of capture of *Bembidion* specimens of *B. levigatum*, *B. mimbres*, and the *B. transversale* subgroup whose DNA was sequenced. Four-digit numbers at the start of each row are D.R. Maddison DNA voucher numbers.

Bembidion levigatum Say

0763 USA: Nebraska: Lancaster Co., Lincoln, Wilderness Park along Salt Creek, 360 m 40.6983°N, 96.6837°W

1255 USA: Indiana: Crawford Co., Ohio River near Schooner's Point, 120 m 38.1571°N, 86.3379°W

1256 USA: Texas: Somervell Co., Brazos River and Route 67, 175 m 32.2694°N, 97.6637°W

1693 USA: Virginia: Danville City Co., Danville, Dan River, 36.5828°N, 79.4246°W

1694 USA: Iowa: Hamilton Co., Boone River near Stratford, 275 m, 42.3123°N, 93.9327°W

2343 USA: Texas: Bastrop Co., Colorado River near Utley, 115 m, 30.1853°N, 97.4496°W

Bembidion mimbres sp. nov.

0280 USA: New Mexico: Grant Co., Gila River near Gila, 1370 m 32.969°N, 108.587°W

1220 USA: New Mexico: Grant Co., Billings Vista, Gila River, 1310 m 32.8137°N, 108.6031°W

1267 USA: New Mexico: Grant Co., Billings Vista, Gila River, 1310 m 32.8137°N, 108.6031°W

1944 USA: New Mexico: Grant Co., Billings Vista, Gila River, 1310 m, 32.8137°N, 108.6031°W

2117 USA: New Mexico: Grant Co., Gila River, Billings Vista, 1320 m, 32.8163°N, 108.6032°W

2118 USA: New Mexico: Grant Co., Gila River, Billings Vista, 1320 m, 32.8163°N, 108.6032°W

2119 USA: New Mexico: Grant Co., Gila River, Billings Vista, 1320 m, 32.8163°N, 108.6032°W

2131 USA: New Mexico: Grant Co., Gila River, Billings Vista, 1320 m, 32.8163°N, 108.6032°W

2134 USA: New Mexico: Grant Co., Gila River, Billings Vista, 1320 m, 32.8163°N, 108.6032°W

2135 USA: New Mexico: Grant Co., Gila River, Billings Vista, 1320 m, 32.8163°N, 108.6032°W

Bembidion transversale Dejean

2157 USA: Wyoming: Albany Co., Laramie River, Laramie, 2175 m, 41.2897°N, 105.6224°W

2160 Canada: Nova Scotia: Hantsport, Halfway River, 45.0487°N, 64.1835°W

2486 USA: Colorado: Fremont Co., Arkansas River at Texas Creek, 1880 m, 38.4106°N, 105.5844°W

4219 USA: Oregon: Harney County, Banks of Silver Creek, 1379 m, 43.7278°N, 119.6256°W

4690 USA: Montana: Gallatin Co., Beaver Creek along Hwy 287, 1969 m, 44.8633°N, 111.3679°W

4927 USA: Montana: Gallatin Co., Beaver Creek along Hwy 287, 1969 m, 44.8633°N, 111.3679°W

5064 USA: Washington: Whitman Co., Palouse River 6 mi NE Colfax, 600 m 46.9259°N, 117.3037°W

5612 USA: Oregon: Wallowa Co., Wallowa Lake State Park, 1334 m 45.2841°N, 117.2075°W

5613 USA: Oregon: Harney Co., Banks of Donner und Blitzen River 1296 m, N 42.8002, W 118.8682

Bembidion erosum Motschulsky

2162 Canada: British Columbia: Hope, Fraser River near mouth of Coquihalla River, 49.3961°N, 121.4351°W

2194 Canada: British Columbia: Hope, Fraser River near mouth of Coquihalla River, 49.3961°N, 121.4351°W

2596 USA: California: Del Norte Co. rt 101 @ Wilson Creek, 41.60530°N, 124.10060°W

2607 USA: California: Del Norte Co. rt 101 @ Wilson Creek, 41.60530°N, 124.10060°W

3561 USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W

3562 USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W

3584 USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W

4033 USA: Oregon: Curry Co., Floras Creek at route 124 SE Langlois, 21 m, 42.9132°N, 124.4251°W

4050 USA: California: Monterey Co., Big Sur River, Andrew Molera State Park, 7 m, 36.285°N, 121.8544°W

4212 USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W

| Bembidion corgenoma sp. nov.	
2165	USA: Washington: Whatcom Co., Nooksack River 1.4 mi S of Deming, 70 m, 48.808°N, 122.2019°W
2180	USA: California: Sonoma Co., Russian River, 3 mi NE Healdsburg
2181	USA: California: Marin Co., Nicasio Reservoir, 70 m, 38.088°N, 122.7383°W
2190	Canada: British Columbia: Clearwater, N. Thompson River, 440 m, 51.6395°N, 120.0294°W
2346	USA: Nevada: Eureka Co., I-80W bridge 1.6 mi E exit 254 (Dunphy), Humbolt R., 1408 m 40°42.31'N, 116°31.87'W
2597	USA: California: Del Norte Co. rt 101 @ Wilson Creek, 41.60530°N, 124.10060°W
2608	USA: California: Del Norte Co. rt 101 @ Wilson Creek, 41.60530°N, 124.10060°W
2973	USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m, 44.5491°N, 123.2451°W
3021	USA: Idaho: Cassia Co., Sublett Res.
3205	USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m, 44.5491°N, 123.2451°W
3559	USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W
3560	USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W
3583	USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W
3772	USA: California: Tehama Co., Red Bluff, Sacramento River, 73 m, 40.1759°N, 122.229°W
4032	USA: Oregon: Coos Co., Crooked Creek S of Bandon, 7 m, 43.0814°N, 124.4335°W
4034	USA: Oregon: Curry Co., Floras Creek at route 124 SE Langlois, 21 m, 42.9132°N, 124.4251°W
4052	USA: California: Monterey Co., Big Sur River, Andrew Molera State Park, 7 m, 36.285°N, 121.8544°W
4054	USA: California: San Luis Obispo Co., Pismo State Beach, 4 m, 35.0999°N, 120.6267°W, 29.iv.2014
4218	USA: California: San Luis Obispo Co., San Simeon State Park, San Simeon Creek, 4 m, 35.5955°N, 121.1233°W
4959	USA: Nevada: Carson City Co., Carson River at Silver Saddle Ranch, SE Carson City, 1405 m 39.1315°N, 119.706°W
4961	USA: California: Tehama Co., Red Bluff, Sacramento River, 73 m 40.1759°N, 122.229°W
4962	USA: California: Tehama Co., Red Bluff, Sacramento River, 73 m 40.1759°N, 122.229°W
5065	USA: Oregon: Linn Co., Willamette River, Truax Island, 60 m 44.5853°N, 123.1913°W
5670	USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m 44.5491°N, 123.2449°W
5671	USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m 44.5491°N, 123.2449°W
5672	USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m 44.5491°N, 123.2449°W
5673	USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m 44.5491°N, 123.2449°W

(Kalyaanamoorthy et al. 2017) was used to find the optimal character evolution models. The MFP model option was used for 28S, and the TESTMERGE option for the protein-coding genes. The TESTMERGE option sought the optimal partition of sites, beginning with the codon positions in different parts. Twenty searches were conducted for the maximum-likelihood tree for each matrix.

For the *B. transversale* group, a multi-species coalescent approach was conducted with the 28S, COI, CAD, and Topo data to provide an algorithmic analysis of species boundaries. STACEY version 1.2.5 (Jones 2017) was used as implemented in BEAST version 2.6.2 (Bouckaert et al. 2014), with the epsilon value set to $1*10-4$, CollapseWeight parameters to 0.5 and 10, and with a Beta prior. I evaluated sampling sufficiency using ESS values in Tracer version 1.7.1 (Rambaut et al. 2018); after four independent runs of 1E9 generations each, all ESS values exceeded 200, except for mutationRate.s:Topo, whose ESS value was 191. As I saved trees every 100,000 generations, with the first 10% of the trees discarded as the burn-in period, this yielded a sample of 72,000 trees.

Data resources

Sequences have been deposited in GenBank with accession numbers MW151366–MW151563. Aligned data for each specimen as well as files containing inferred trees for each gene are available in Supplementary material S1 and S2, and have been deposited in the Dryad Digital Repository, https://doi.org/10.5061/dryad.18931zcw1.
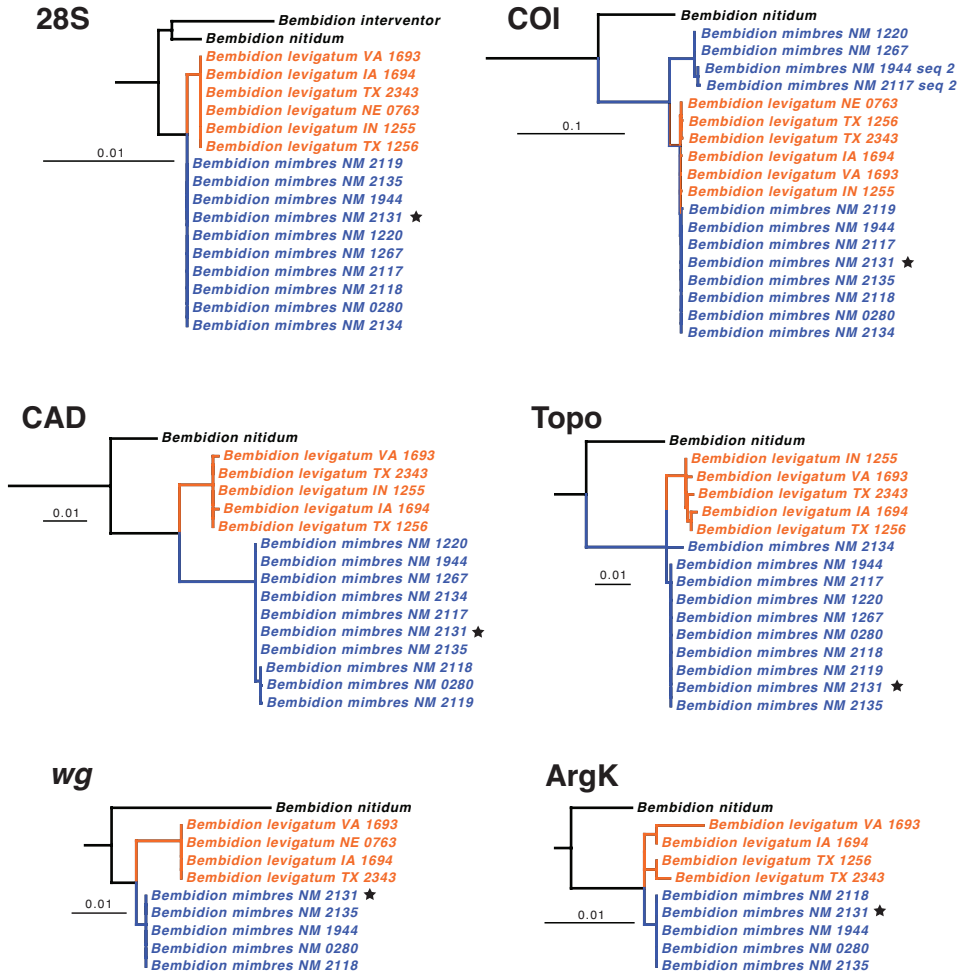
Figure 5. Maximum likelihood gene trees of subgenus *Hydrium*. Only *B. levigatum* + *B. mimbres* + its sister group shown (other taxa were present in the analysis, and reconstructed outside this clade, but were removed after the analysis to simplify this figure). Holotype of *B. mimbres* indicated by a star. Scale bar 0.1 units, as reconstructed by IQ-TREE.

Results

Molecular results

In the analysis of DNA data for the subgenus *Hydrium*, *B. levigatum* and *B. mimbres* sp. nov. differed in all genes except COI (Fig. 5), providing evidence that they are two separate species. In all gene trees except 28S, *B. nitidum* was the sister group of *B. levigatum* + *B. mimbres* (Suppl. materials 1).

The majority of specimens of *B. levigatum* and *B. mimbres* were indistinguishable in COI, but there were four sequences of *B. mimbres* that formed a separate clade (for

specimens 1220, 1267, as well as the second sequences of 1944 and 2117). These four sequences have 29 sites at which they differ from all other sampled *B. levigatum* + *B. mimbres*, at 20 of which these four sequences have the same base as in at least one other sampled *Hydrium* species. These four sequences might be nuclear copies or numts (Thalmann et al. 2004), or they could represent the effects of *Wolbachia* infections (Smith et al. 2012). Although it is possible these are the true mitochondrial copies of COI, and that the other sequences are numts, the evidence points to the four unusual sequences being something other than true mitochondrial copies: the chromatograms for these four unusual sequences have several double peaks, indicating polymorphism within the PCR products for non-synonymous differences. These four sequences have been deposited in GenBank as "COI-like" sequences.

In each of the four genes studied in the *B. transversale* group, the maximum likelihood tree showed a monophyletic *B. transversale* subgroup (Suppl. materials 2), with *B. perspicuum* and *B. transversale* s. l. (= *B. transversale* + *B. erosum* + *B. corgenoma*) forming a clade, the sister of which is *B. sarpedon*. None of the three species within *B. transversale* s. l. form a clade in any of the four genes studied (Fig. 6). The multi-species coalescent STACEY tree also showed no distinction between these species based upon the combined analysis of 28S+COI+CAD+Topo (Fig. 7).

Cytogenetic results

All males examined are inferred to have 22 autosomes (i.e., 11 pairs of autosomes) and an XY/XX sex chromosome system (Table 4).

Re-examination of voucher specimens identified as *B. transversale* in Maddison (1985) showed that they belong to three species. The specimens from Alberta and near Fernie, BC, are *B. transversale*; the specimen from near Cache Creek, BC, is *B. corgenoma*; the specimen from Salmon Valley, BC, is *B. erosum*. The specimens called "*B.* sp nr *transversale*-Nr 1" are *B. pernotum*, and the specimens called "*B.* sp nr *transversale*-Nr 2" are *B. lugubre*. The specimens reported as belonging to "*B.* sp.nr. *transversale*" in Pflug et al. (2020) are all *B. corgenoma*. These new identifications are incorporated into the summary shown in Table 4.

Morphological results

Morphological results for *Bembidion* (*Hydrium*) are presented in the taxonomic section below.

Members of the *B. transversale* subgroup are very similar morphologically. DNA sequence data of 28S, COI, CAD, and Topo do not reveal any consistent phylogenetic structure within *B. transversale* s. l. (Figs 6, 7), suggesting that it is perhaps a single species. In all other bembidiines investigated to date (e.g., Maddison 2008; Maddison and Cooper 2014; Maddison and Sproul 2020; Sproul and Maddison 2017), every form judged by morphological evidence as a distinct species is revealed as a clade in the tree of at least one of these four genes. I detected no variation in chromosomes within

Table 4. Chromosome numbers and sex chromosomes of *Bembidion* (*Hydrium*) and *Bembidion transversale* group males. The Sample column indicates the total number of specimens examined in this paper and in previous papers. "1" in Reference indicates Maddison (1985); "2" indicates Pflug et al. (2020).

|	2n male	Sample	Locality	Reference
B. levigatum	22+XY	2♂	TX	1
B. mimbres	22+XY	1♂	NM	this paper
B. transversale	22+XY	5♂2♀	AB, BC, CO	1, this paper
B. erosum	22+XY	4♂	BC, OR	1, this paper
B. corgenoma	22+XY	15♂	OR, BC	1, 2
B. perspicuum	22+XY	4♂	CO, AZ	1, this paper
B. sarpedon	22+XY	3♂	CO	this paper
B. pernotum	22+XY	6♂	CO	1, this paper
B. mexicanum	22+XY	5♂	CO, AZ	1, this paper
B. lugubre	22+XY	11♂	AZ, CA, Mexico	1, this paper

the group (Table 4); although *Bembidion* species typically have similar chromosomes (Maddison 1985), some subgenera have species that differ by chromosome number (Maddison 2008). However, in spite of the lack of genetic evidence supporting multiple species, the morphological results reveal that this complex consists of at least three distinct but very similar species.

Examination of primary types (documented in the Taxonomic Treatment section, below) indicates that two of the species have names (*Bembidion transversale* and *B. erosum*), and the third is described here as *B. corgenoma*; these names will be used in advance of the Taxonomic Treatment to simplify the text.

The morphological evidence indicating that there are three species includes color (Figs 11A–C, 12), mentum shape (Fig. 13), and male genitalic structure (Figs 14–17), with the patterns summarized below and graphically in Fig. 18.

The dark and pale western species (*B. erosum* and *B. corgenoma*) are broadly sympatric from southern California through British Columbia (Figs 19, 20), and are found microsympatrically (on the same gravel bank) at Wilson Creek, Del Norte County, California (41.6051°N, 124.1005°W), as well as along Floras Creek, Curry County, Oregon (42.9132°N, 124.4251°W), and the Siletz River E of Kernville, Oregon (44.8720°N, 123.9223°W). They differ consistently in size of a sclerotized lobe of the internal sac (Fig. 15B vs. C); the thickness of the tip of the flagellar sheath, with *B. corgenoma* having a somewhat triangular sclerotized region of the tip (arrow in Fig. 16E), as opposed to a thin dark line in *B. erosum* (Fig. 16C, D); and color, with *B. erosum* being generally darker (Fig. 11B) than *B. corgenoma* (Fig. 11C), especially the appendages (Fig. 12B vs C). In addition, most males of *B. erosum* have the ventral surface of the apex of the aedeagus more strongly curved downward (Fig. 14C, D). Although there are no universal distinctions between the two species in the genes sequenced, the six *B. erosum* and five *B. corgenoma* sequenced from the gravel bank shown in Fig. 4B consistently differ in one base in Topoisomerase, suggesting, combined with differences in genitalia and color, that there is no or extremely limited gene flow at that locality.

The ranges of the two generally paler species (*B. transversale* and *B. corgenoma*) overlap in Nevada, Washington, Idaho, and Montana (Fig. 19), and there are three localities
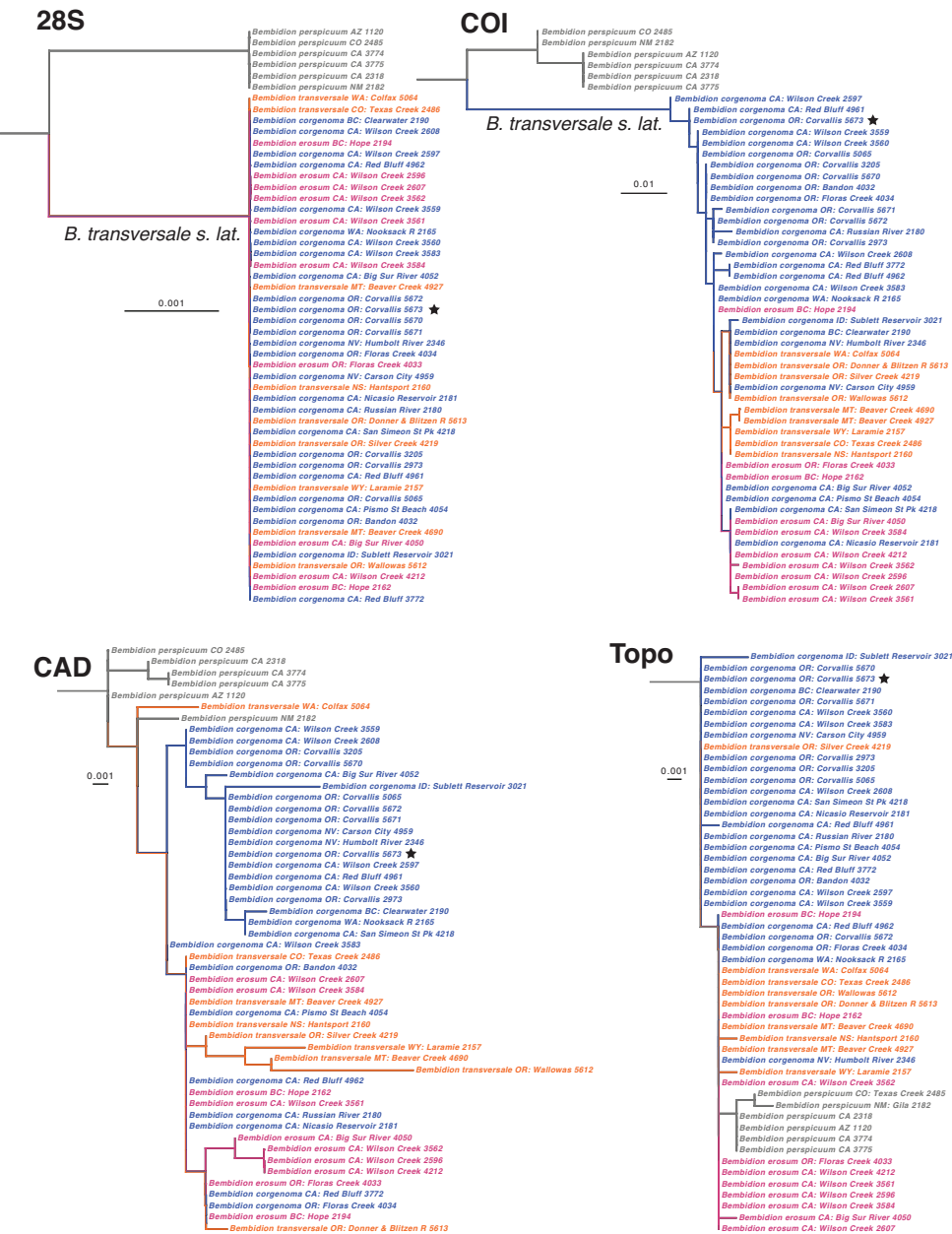
Figure 6. Maximum likelihood gene trees of the *Bembidion transversale* subgroup. *B. mexicanum* subgroup and *B. sarpedon* were present in the analysis, and reconstructed outside this clade, but were graphically removed to simplify this figure. Holotype of *B. corgenoma* indicated by a star. Scale bar as reconstructed by IQ-TREE.

Figure 7. Majority rule consensus tree of trees found from a STACEY analysis. Numbers on branches are estimates of the Bayesian posterior probability of a clade, expressed as a percentage.

at which they co-occur (16 mi W Lolo Pass, Ravalli Co., Montana; Walla Walla, Washington; Spokane, Washington; all in OSAC). There are subtle but consistent differences in the male genitalia, as well as striking differences in the mentum, and I am convinced any gene exchange in the region of overlap is minimal. In the overlap region there are a very few specimens of *B. transversale* with paler antennae (similar to typical *B. corgenoma*), and there is one population at Hayden Lake, Idaho (CAS), which contains *B. transversale* typical in all regards except for one specimen that has the anterior margin of the mentum somewhat intermediate between the two species (Fig. 13B). With these minor exceptions, the differences in mentum, genitalia, and color are consistent throughout the overlap range among the many males whose genitalia were dissected.

Bembidion erosum and *B. transversale* are the two most similar species within the trio, differing most notably in the anterior lateral region of the mentum: in *B. erosum* this region is large and triangular, similar to that standard in *Bembidion* (i.e., like those shown in Fig. 13A), in contrast to the modified mentum of *B. transversale* (Fig. 13B–D), in which the anterior lateral region is much reduced. The male genitalia are very similar, both having a larger lobe on the basal sclerite (Fig. 15A, B), and a thin, non-triangular apex to the flagellar sheath (Fig. 16A–D). However, the flagellar sheath is more dorso-ventrally compressed in *B. transversale* (Fig. 16A, B), and the flagellar complex is thinner (arrow in Fig. 17A, B). With one exception, the known ranges of *B. erosum* and *B. transversale* do not overlap, with *B. erosum* in the United States being restricted to the Cascades and west, and *B. transversale* only known from east of the Cascades (Figs 19, 20). The one exception is in eastern Washington: there is a series of seven specimens of *B. erosum* labeled "Colbert, Wash / V 30 1937 / Dan Bonnell". The two males from this series have typical *B. erosum* genitalia, and the mentum has the anterior lateral region large and triangular. Colbert is less than 20 km NNE of Spokane, where both *B. transversale* and *B. corgenoma* have been collected. As the only record of *B. erosum* east of the Cascades, some doubt is cast upon its authenticity, but there is no reason otherwise to question the locality data.

In addition, there is a form in the Sierra Nevada of California that requires further examination. My limited study of it indicates that it has all of the morphological features of *B. transversale*, except that it has a normal mentum shape, with large and triangular anterior lateral regions.

Taxonomic Treatment

Subgenus *Hydrium*

The subgenus *Hydrium*, as defined by Maddison (2012), contains seven species in the Palaearctic region (Marggi et al. 2017) as well as five species in the Nearctic region (Lindroth 1963): *Bembidion nitidum*, *B. interventor*, *B. obliquulum*, *B. levigatum*, and the new species described here.

The species key in Lindroth (1963) can be modified as follows to take into account the new species.

19   More than two setae on the clypeus, and at least one long seta on the front angle of the prothorax. Elytra without the typical pair of distinguishable dorsal punctures but most intervals with a row of small punctures, each carrying a long seta **19A**

–   Clypeus with only two setae. Elytra with two dorsal punctures on third interval, otherwise without setigerous punctures **20**

19A   Elytra with a row of long setae on all intervals; prothorax wide, sides very rounded (Fig. 8A) .... ***B. levigatum***

–   Elytra with a row of long setae on most intervals, but lacking on intervals 2 and 4. Prothorax narrower, with straighter sides (Fig. 8B) ....***B. mimbres***

Bembidion mimbres sp. nov.

http://zoobank.org/5F95B4F6-9FFD-4841-8E69-794A258381A2

Holotype. Male, in OSAC, labeled: "USA: New Mexico: Grant Co., Gila River, Billings Vista, 1320 m, 32.8163°N, 108.6032°W, 11.viii.2005. DRM 05.043. D.R. & J.H.A. Maddison", "David R. Maddison DNA2131 DNA Voucher" [pale green paper], "HOLOTYPE Bembidion mimbres David R. Maddison" [partly handwritten, on red paper], "Oregon State Arthropod Collection OSAC_0002000007 [matrix code]" [printed on both sides of white paper]. Genitalia in glycerin vial pinned beneath specimen; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the holotype are MW151386, MW151400, MW151414, MW151425, MW151432, and MW151443.

    Paratypes (116). USA: New Mexico: Grant Co., Gila River, Billings Vista, 1320 m, 32.8163°N, 108.6032°W [Type locality] (44: OSAC, USNM, MCZ, NHMUK, MNHM, MSBA), USA: New Mexico: Grant Co., Billings Vista, Gila River, 1310 m, 32.8137°N, 108.6031°W (28: OSAC, CAS, UAIC, EMEC); USA: New Mexico: Grant Co., Gila River near Cliff, 1350 m, 32.9124°N, 108.5897°W (12: OSAC); USA: New Mexico: Grant Co., Gila River near Gila, 1370 m, 32.9692°N, 108.5868°W (3: OSAC); USA: New Mexico: Grant Co., Gila River near Gila, 1370 m, 32.969°N, 108.587°W (4: OSAC); USA: New Mexico: Grant Co., Gila River, Gila National Forest, 1315 m, 32.8167°N, 108.6035°W (14: OSAC); USA: New Mexico: Gila R., jct US 260, nr. Gila (1: UASM); USA: Arizona: Navajo Co., Carrizo Ck nr. Carrizo (10: UASM).

    Type locality. USA: New Mexico: Grant Co., Gila River, Billings Vista, 32.8163°N, 108.6032°W.

    Derivation of specific epithet. *Bembidion mimbres* is named in honor of the people of the Mimbres culture, who lived alongside this species, including at the type locality, and who illustrated the insects in their world on their pottery (Hegmon, et al. 2018). The name is to be treated as a noun in apposition.

    Diagnosis and description. Adults of this species are relatively large *Bembidion* (5.3–6.3 mm in length), with a striking appearance because of the smooth and shiny dorsal surface with its metallic reflections (Figs 1, 8B). Body piceous, with an aeneous,
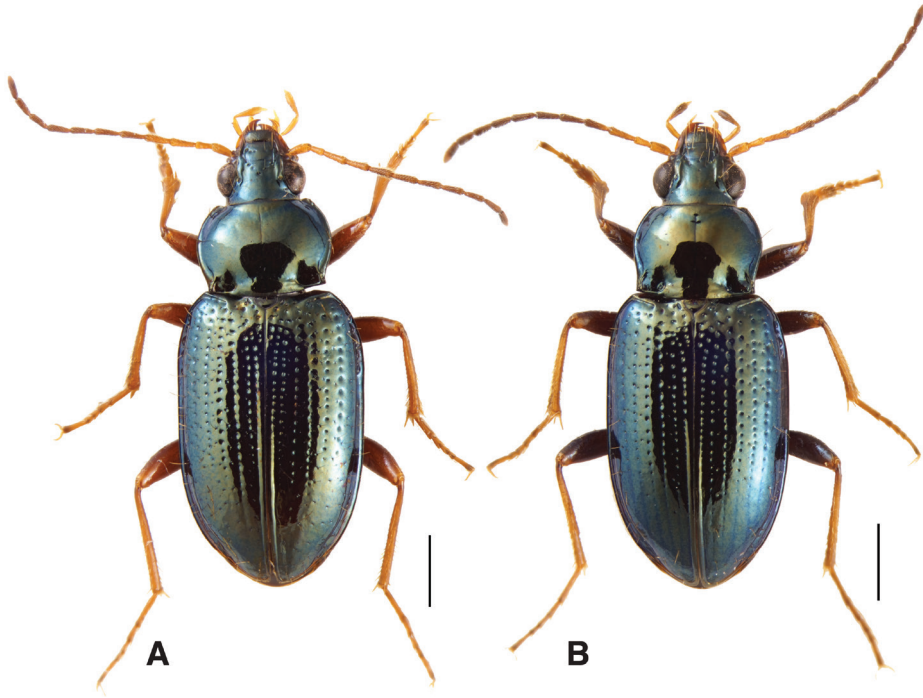
Figure 8. Adult males of subgenus *Hydrium* **A** *Bembidion levigatum* (voucher V100820, from USA: Utah: Grand Co., Moab, Colorado River, 1190 m, 38.5739°N, 109.5769°W) and **B** *B. mimbres* (a paratype from the type locality, voucher V101458). Scale bars: 1 mm.

green, or blue metallic reflection. Legs with tarsi and tibiae testaceous, femora infuscated. First three antennomeres testaceous, with the tip of the third infuscated in some specimens; fourth basally testaceous. Palps testaceous except for the penultimate maxillary article, which is infuscated. Mentum with anterior lateral regions large and triangular as typical for a *Bembidion*; mentum tooth incised at tip, and thus bifid (similar to *B. levigatum*). Prothorax with relatively straight sides, especially posteriorly (Fig. 8B); with distinct posterolateral carina close to the lateral margin; posterior region of pronotum smooth, impunctate. Lateral bead of elytra extending inside shoulder well toward the midline, sharply angulate. Striae consisting of rows of distinct punctures, without an associated groove; on stria 1 complete; striae 2–6 absent in the posterior third; stria 7 virtually absent, represented by at most minute punctulae. Dorsal surface lacking microsculpture, and thus very shiny. More than two setae on the clypeus, and extra setae on the frons, at least anteriorly; at least one long seta near the front angle of the prothorax; prosternum with at most two setae. Elytra without the typical pair of distinguishable setose punctures associated with interval 3, but with a row of long setae on all intervals except 2 and 4. Aedeagus (Fig. 9C,D) with ventral margin thinner, and with internal sac sclerites very similar to those of *B. levigatum*, but with slight differences, especially basally.
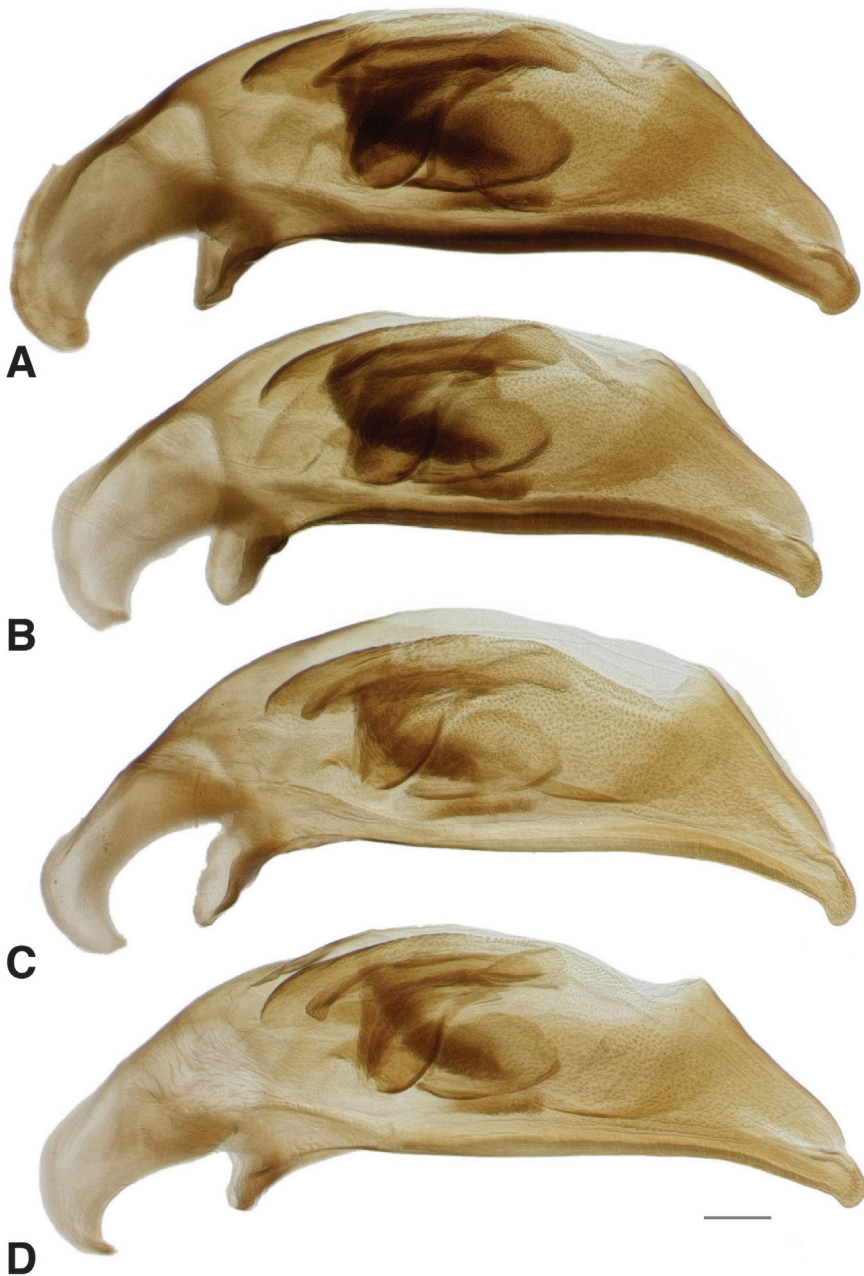
Figure 9. Male genitalia of subgenus *Hydrium* **A** *B. levigatum* (voucher DNA1693, Virginia: Danville City Co., Danville) **B** *B. levigatum* (voucher DNA2343, Texas: Bastrop Co., Colorado River near Utley) **C** *B. mimbres* (voucher DNA2134, USA: New Mexico: Grant Co., Gila River, Billings Vista) **D** *B. mimbres* (voucher DNA2135, USA: New Mexico: Grant Co., Gila River, Billings Vista). Scale bar: 0.1 mm.
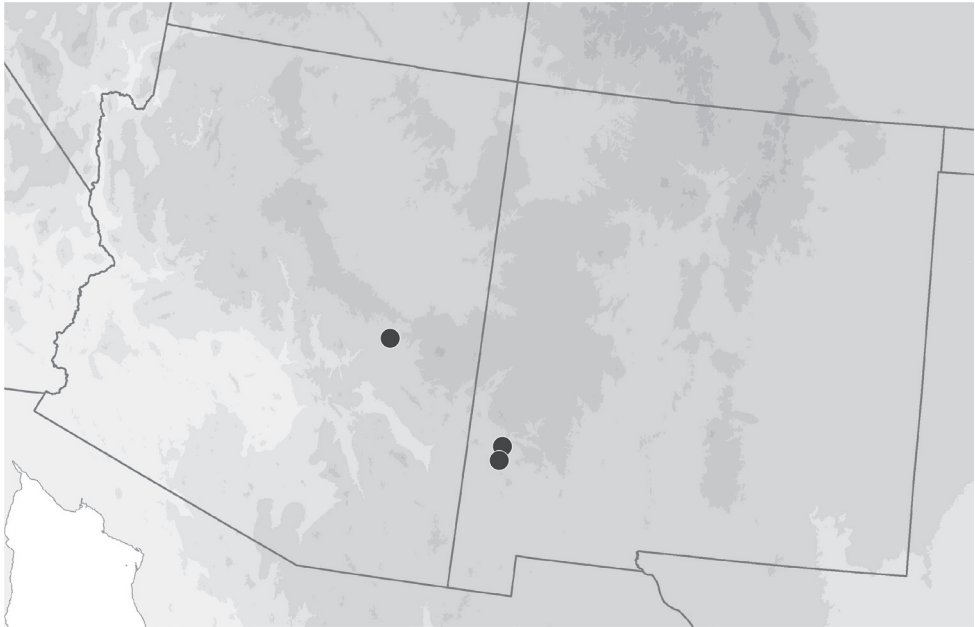
Figure 10. Geographic distribution of *B. mimbres* in Arizona and New Mexico.

Most easily distinguished from *B. levigatum* by the narrower prothorax with straighter sides (Fig. 8B), the lack of setae on elytral intervals 2 and 4, and having at most one or two setae on the prosternum.

Additional characteristics. Diploid chromosome number 24, with 11 pairs of autosomes and an XY/XX sex chromosome system.

Geographic distribution. Known from the Gila River watershed in Arizona and New Mexico (Fig. 10).

Habitat. At the type locality, found at night on damp sandy soil about 2–4 meters from the river shore, in the shade of a large willow (Fig. 2). At a site a few meters away, 28 specimens were found at night on damp clay/sand soil among small *Salix* and *Populus* saplings 2–8 meters from the water's edge; in spite of extensive searching in the same area, only one specimen was found during the day. At other sites along the Gila River, found in habitats similar to those in which *B. levigatum* is found, on damp sand and silt on the steep upper bank of the river, mostly at night.

Bembidion levigatum Say

Bembidium levigatum Say, 1823: 84. Lectotype female in MNHN, designated by Lindroth and Freitag (1969). Type locality Missouri.

Bembidion laevigatum delawarense Casey, 1924: 24. Holotype male in USNM (type number 36814), examined. Type locality Pennsylvania.

Diagnosis and geographic distribution. Adults of this species are large and distinctively wide, with a broad, rounded prothorax (Fig. 8A), and with a very shiny dorsal surface, with a green or bluish metallic reflection. The clypeus, frons, anterior corner of the prothorax, and all elytral intervals have long setae in addition to the standard set in *Bembidion*; the prosternum has four or more setae. Aedeagus as in Fig. 9A, B. A very widespread species, found throughout most of the eastern United States and a small region of southern Canada, from Maine to Florida, north and west to Alberta and Montana, south to Utah, New Mexico, Texas, and Mexico (Bousquet 2012). I have also seen specimens from the Grand Canyon in northern Arizona (two specimens in MSBA labeled "USA AZ Coconino Co Grand Canyon Nat. Park, N36.77 W111.655 RMBL 29-30 August 2002 coll. Cobb, Brantley, Lightfoot").

Bembidion transversale species group

The *Bembidion transversale* group contains large *Bembidion* found primarily on river shores of cobble, gravel, and sand from Canada to Guatemala. Members of the group are characterized by large size (5.8–8 mm); posterolateral carina of pronotum lacking or indistinct and somewhat oblique; lateral bead of elytra not prolonged onto shoulder; crista clavicularis absent; elytral striae distinct and mostly complete; elytral microsculpture transverse; two discal setae of elytra in or close to third stria. It belongs to what has been called the Nearctic *Ocydromus* Clade (Maddison 2012), although that group is not closely related to subgenus *Ocydromus*. The only subgeneric name available for the Nearctic *Ocydromus* Clade is *Leuchydrium* Casey, although the type species (*Bembidion tigrinum* LeConte) is quite distant from the *B. transversale* group (Maddison 2012).

There are now eight recognized species in the *B. transversale* group in the United States and Canada:

Bembidion transversale subgroup
    Bembidion transversale Dejean, 1831
    Bembidion erosum (Motschulsky, 1850)
    Bembidion corgenoma Maddison, sp. nov.
    Bembidion perspicuum (LeConte, 1848)
    Bembidion sarpedon Casey, 1918
Bembidion mexicanum subgroup
    Bembidion mexicanum Dejean, 1831
    Bembidion lugubre LeConte, 1857
    Bembidion pernotum Casey, 1918

There is a total of 20 species-group names that have been applied to members of the *B. transversale* group (for details beyond those provided below, see Maddison and Swanson (2010)). I have examined detailed photographs of the primary type of one (the holotype of *Bembidium transversale* Dejean, in the MNHN), and the

primary types themselves of 18. The twentieth lacked a type series, and a neotype is designated below.

The species key in Lindroth (1963) can be modified as follows to take into account species in the *B. transversale* subgroup. Specimens from this group are not easy to identify.

145 Prothorax (Lindroth 1963: figs 168a–b) without or with very faint, oblique latero-basal carina **145A**

– Prothorax with latero-basal carina well developed, less oblique................**146**

145A Mentum with anterior lateral region reduced, not triangular, each consisting of a mesal denticle and a more lateral rounded bump (Fig. 13B–D). Antenna with at least the second and third antennomeres apically infuscated. Tip of aedeagus not abruptly curved downward (Fig. 14A, B); basal sclerotized lobe large (Fig. 15A); apex of flagellar sheath with long, thin dark line (Fig. 16A, B)..***B. transversale***

– Mentum with anterior lateral region as typical for a *Bembidion*: triangular, large, and with anterior margin significantly anteriad of the central tooth (Fig. 13A). Other characteristics either as mentioned above or not **145B**

145B Paler, with antennae gradually becoming slightly darker toward the apex; pronotum in most specimens dark rufous. Prothorax with lateral margins more rounded, very shiny, with weaker microsculpture and less punctuation. Relatively flat elytral intervals with small punctures in striae. Aedeagus with ventral margin having a slight downward bulge. Internal sac sclerite complex of male genitalia narrower in side view, with relatively long and thin flagellar complex. Known from NM, CO, WY, UT, AZ ....***B. sarpedon***

– Darker, with at least antennomeres 4–11 infuscated; pronotum rufous or piceous. Prothorax with lateral margins less rounded, less shiny, and in most specimens with more punctures. Aedeagus without ventral bulge. Internal sac sclerite complex less narrow, with a dorso-ventrally wider flagellar complex..........**145C**

145C Prothorax with later margins less sinuate, with more notable punctures in the basal region (Maddison and Swanson 2010: fig. 4B); aedeagus with ostial flag more dorsal, and with a more abrupt curve at its anterior end (Maddison and Swanson 2010: fig. 6B)..***B. perspicuum***

– Prothorax with lateral margins more sinuate, flatter, with a smoother basal region (Maddison and Swanson 2010: fig. 4A); aedeagus with ostial flag extending further ventrally, and with gentler curvature (Maddison and Swanson 2010: fig. 6A) **145D**

145D Legs and basal three antennomeres pale, testaceous or rufo-testaceous. Elytral striae deeper. Tip of aedeagus not abruptly curved downward (Fig. 14E, F); basal sclerotized lobe small (Fig. 15C); apex of flagellar sheath with dark area triangular (Fig. 16E, F) ....***B. corgenoma***

– Legs in most specimens darker (in southern specimens infuscated); second and third antennomeres infuscated, at least apically. Elytral striae shallower.

Tip of aedeagus abruptly curved downward (Fig. 14C, D); basal sclerotized lobe large (Fig. 15B); apex of flagellar sheath with long, thin dark line (Fig. 16C, D)...***B. erosum***

Bembidion transversale Dejean, 1831

Bembidium transversale Dejean, 1831:110. Holotype female, in MNHN, examined by Kipling Will, who provided photographs that confirmed the identification. Type locality restricted to Nipigon, Ontario, by Lindroth (1963).

Diagnosis and geographic distribution. Adults of this species (Fig. 11A) are characterized by the reduced anterior lateral regions of the mentum (Fig. 13B–D), and the antenna with at least the second and third antennomeres apically infuscated (Fig. 12A). The prothorax is more cordate than in other species. The aedeagus has its ventral surface relatively straight, with the tip not abruptly curved downward (Fig. 14A, B); the basal sclerotized lobe is large (Fig. 15A), and the apex of the flagellar sheath has a long, thin dark line (Fig. 16A, B). This is the easternmost species, found from Newfoundland and Nova Scotia west through Ontario to southeastern British Columbia, central Oregon, northeastern Nevada, northern Utah, and Colorado (western portion of distribution shown in Fig. 19).

Bembidion erosum (Motschulsky, 1850)

Peryphus erosus Motschulsky, 1850:10. Lectotype female, in ZMUM, examined, designated by Bousquet and Larochelle (1993), labeled "type" [handwritten], "California" [handwritten on red paper], "Peryphus erosus Mots California" [handwritten on green paper], [a rectangle of blank red paper], "LECTOTYPE Peryphus erosus Motschulsky Des. by Y. Bousquet '91" [partly handwritten on red paper]. Type locality California.

Bembidion marinicum Casey, 1918:57. Holotype female in USNM (type number 36919), examined. Type locality Marin County, California.

Diagnosis and geographic distribution. Most adults of this species are the darkest members of this group (Fig. 11B), with the second and third antennomeres infuscated (Fig. 12B), at least apically, and with dark femora, although the more northern populations (e.g., from mainland British Columbia) have paler legs and paler ground color of the body. Prothorax moderately cordate, with a smooth basal region with few punctures. Tip of aedeagus abruptly curved downward (Fig. 14C, D); basal sclerotized lobe large (Fig. 15B); apex of flagellar sheath with long, thin dark line (Fig. 16C, D). This species is coastal, occurring from Haida Gwaii in British Columbia south along the coast to Big Sur and neighboring areas of California, with only one record from east of the Cascades (Fig. 20).
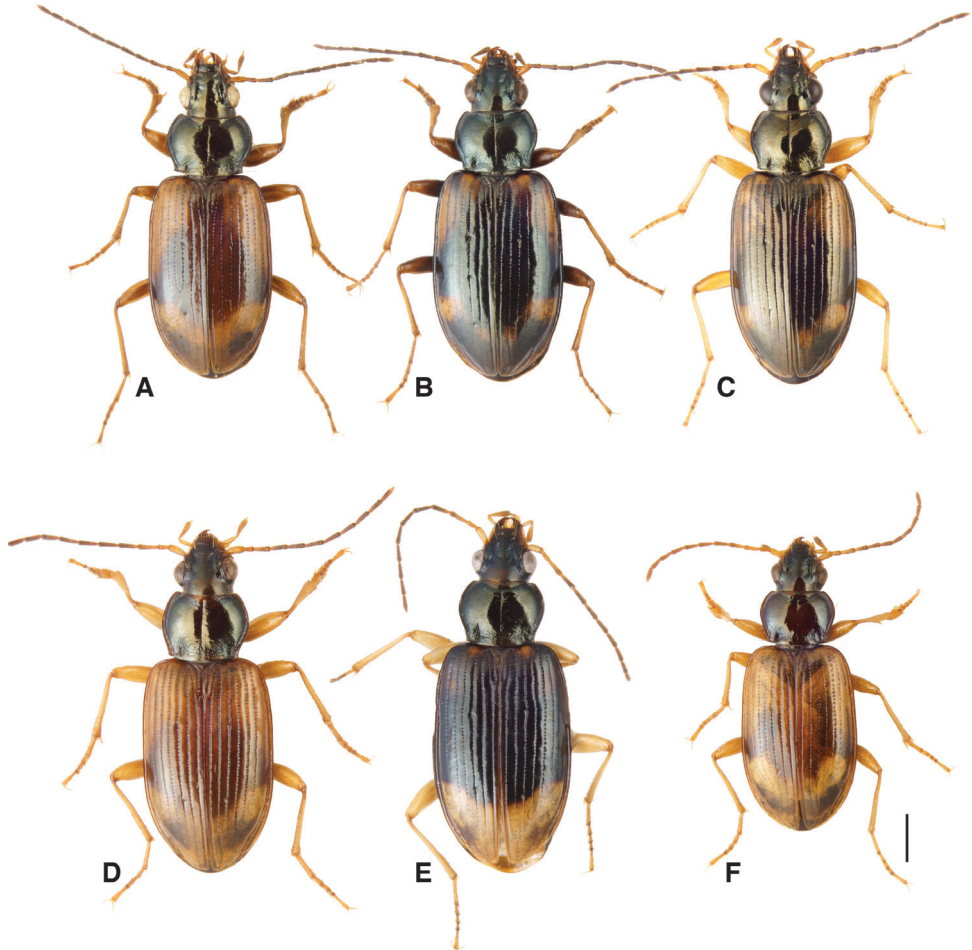
Figure 11. Adult males of *Bembidion transversale* subgroup members **A** *B. transversale* (voucher V101454, Canada: Alberta: Burbank, junction of Red Deer and Blindman Rivers, 52.3542°N, 113.7556°W) **B** *B. erosum* (voucher V101453, USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W). **C** *B. corgenoma* (voucher V101452, from type locality) **D** *B. perspicuum*, light form (voucher V101461, USA: Arizona: Cochise Co., San Pedro R at Charleston, 31.6239°N, 110.1722°W) **E** *B. perspicuum*, dark form (neotype of *Bembidium haplogonum* Chaudoir, USA: California: Lake Co., North Branch Cache Creek at hwy 20, 305 m 38.9881°N, 122.5400°W) **F** *B. sarpedon* (voucher V101459, USA: Colorado: Las Animas Co., Cokedale, Reilly Canyon, 37.1346°N, 104.6114°W). Scale bar 1.0 mm.

Bembidion corgenoma sp. nov.

http://zoobank.org/BF5E001D-F543-4149-8081-0BF7B99A8484

Holotype. Male, in OSAC, labeled: "USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m, 44.5491°N, 123.2449°W, 7.x.2019. DRM 19.210. D.R. Maddison", "David R. Maddison DNA5673 DNA Voucher" [pale green paper], "HOLOTYPE

Figure 12. Antennae of *B. transversale* subgroup **A** *B. transversale* (voucher V101457, Canada: Ontario: Thunder Bay Dist., Rossport) **B** *B. erosum* (voucher V101456, USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W) **C** *B. corgenoma* (voucher V101455, USA: Oregon: Coos Co., Crooked Creek S of Bandon, 43.0814°N, 124.4335°W). Scale bar 0.1 mm.

Bembidion corgenoma David R. Maddison" [partly handwritten, on red paper], "Oregon State Arthropod Collection OSAC_0002000008 [matrix code]" [printed on both sides of white paper]. Genitalia mounted in Euparal in between coverslips pinned with specimen; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the holotype are MW151449, MW151463, MW151491, MW151520, and MW151548.

Paratypes (193). USA: Oregon: Benton Co., Corvallis, Willamette River, 44.5491°N, 123.2449°W, 60 m [type locality] (78: OSAC, CNC, CAS, UAIC, UASM, MCZ, EMEC, CSCA); USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m, 44.5491°N, 123.2451°W (10: OSAC); USA: Oregon: Benton Co., Corvallis, Willamette River, 44.5475°N, 123.2428°W, 60 m (35: OSAC, USNM,

NHMUK, MNHM, UBCZ); USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m, 44.5478°N, 123.2430°W (2: OSAC); USA: Oregon: Benton Co., Corvallis, 62 m, 44.5491°N, 123.2449°W (6: OSAC); USA: Oregon: Linn Co., Willamette River, Truax Island, 44.5853°N, 123.1913°W, 60 m (12: OSAC); USA: Oregon: Lane Co., Goodman Creek, Willamette NF, 43.8441°N, 122.6736°W, 290 m (2: OSAC); USA: Oregon: Coos Co., Crooked Creek S of Bandon, 43.0814°N, 124.4335°W, 7 m, 24.iii.2014 (26: OSAC); USA: California: Tehama Co., Red Bluff, Sacramento River, 40.1759°N, 122.229°W, 73 m (22: OSAC, CAS, EMEC).

Type locality. USA: Oregon: Benton Co., Corvallis, Willamette River, 44.5491°N, 123.2449°W.

Derivation of specific epithet. The specific epithet is formed from the Latin word *cor*, meaning heart, and *genoma*, a modification (for easier pronunciation) of the coined word "genome". *Corgenoma* refers to this species being the heart or current focus of genomic studies in small carabid beetles. *Cor-* also alludes to the type locality of Corvallis, Oregon, whose name is derived from Latin, and means "heart of the valley". It is to be treated as a noun in apposition.

Diagnosis and description. Length (5.8–7.4). Relatively light in color compared to *B. transversale* and *B. erosum*, with legs and basal three antennomeres pale, testaceous or rufo-testaceous. Head and prothorax piceous, with metallic reflections, on pronotum green or aeneous, on head bluish or green. Elytra paler, with shoulders and most of the anterior half testaceous with an orange tint, bordered posteriad by a dark band (with intervals 1–3 in this region dark rufous), followed by a pale testaceous band that either extends to the apex or that is bounded posteriad by dark lateral spots which in the darkest individuals merge in the middle. Mentum with anterior lateral region as typical for a *Bembidion*: triangular, large, and with anterior margin significantly anteriad of the central tooth (Fig. 13A); central tooth trapezoidal, rounded. Prothorax cordate, with more sinuate margins than *B. perspicuum*, with a relatively smooth basal region, with minute punctures; pronotum without or with very faint, oblique posterolateral carina as in other members of this group. Elytral striae 1–5 complete; stria 6 distinct and strong through much of its length; stria 7 shallower, less impressed than 6, but distinct. Microsculpture of elytra very transverse, with little tendency to form meshes. Two discal setae on each elytron, close to third stria. Tip of aedeagus not abruptly curved downward (Fig. 14E, F); basal sclerotized lobe small (Fig. 15C); apex of flagellar sheath with dark area triangular (Fig. 16E, F).

Additional characteristics. Diploid chromosome number 24, with 11 pairs of autosomes and an XY/XX sex chromosome system (Pflug, et al. 2020). Genome size (1C), as measured by flow cytometry, 2118 Mb in males and 2193 Mb in females (Pflug, et al. 2020). Most specimens with singleton (non-conjugated) sperm (Gómez and Maddison 2020).

Available genomic and transcriptomic data. Transcriptomic data for one specimen is available on NCBI's Sequence Read Archive at accession SRR8801541, and genomic data of four specimens at accessions SRR8518612, SRR8518625, SRR8518626, and SRR8518631 (Pflug, et al. 2020).

Bembidion corgenoma

A

Corvallis, OR

Bembidion transversale

B

Hayden Lake, ID

C

Wallowa State Park, OR

D

Michigan
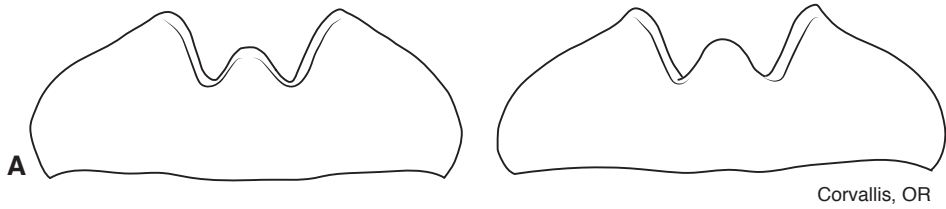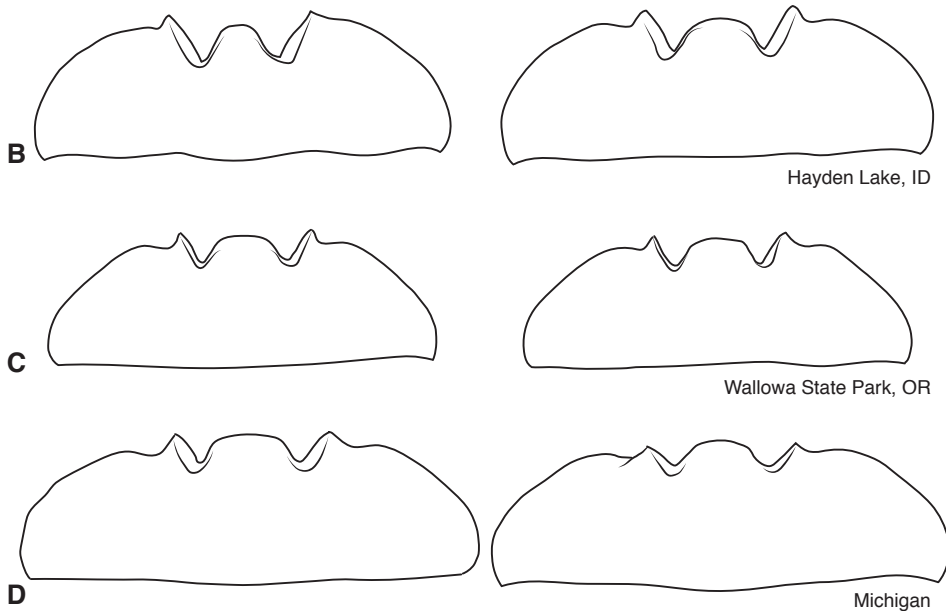
Figure 13. Mentum of *B. corgenoma* and *B. transversale* **A** Two specimens of *B. corgenoma* from Corvallis, Oregon **B** Two specimens of *B. transversale* from Hayden Lake, Idaho **C** Two specimens of *B. transversale* from Wallowa State Park, Oregon **D** Two specimens of *B. transversale* from Point aux Pins, Michigan.

Notes. This species was called *Bembidion haplogonum* Chaudoir in Gustafson et al. (2019; 2020), and *B.* sp. nr. *transversale* in some other publications (Gómez and Maddison 2020; Kanda, et al. 2015; Pflug, et al. 2020).

Geographic distribution. This species occurs from central British Columbia south through the Willamette Valley of Oregon, the Central Valley of California, with some records in Nevada, Idaho, eastern Washington, and Montana (Fig. 19), thus overlapping with the range of *B. transversale*.

Geographic variation. The specimens on or close to the beaches of the Pacific Ocean appear on average slightly paler than more inland specimens.
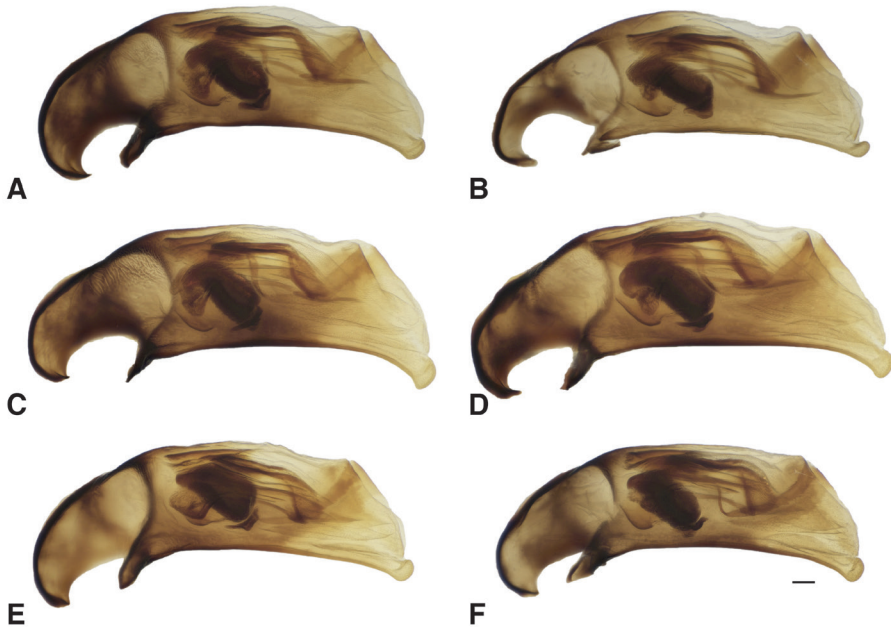
Figure 14. Male genitalia of *B. transversale* subgroup **A** *B. transversale* (voucher DNA4219, USA: Oregon: Harney County, Banks of Silver Creek, 1379 m, 43.7278°N, 119.6256°W) **B** *B. transversale* (voucher DNA2161, Canada: Alberta: Lethbridge, Oldman River, 800 m, 49.7043°N, 112.866°W) **C** *B. erosum* (voucher DNA4033, USA: Oregon: Curry Co., Floras Creek at route 124 SE Langlois, 21 m, 42.9132°N, 124.4251°W) **D** *B. erosum* (voucher DNA3562, USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W) **E** *B. corgenoma* (voucher DNA2180, USA: California: Sonoma Co., Russian River, 3 mi NE Healdsburg) **F** *B. corgenoma* (holotype, voucher DNA5673, USA: Oregon: Benton Co., Corvallis, Willamette River, 60 m, 44.5491°N, 123.2449°W). Scale bar: 0.1 mm.
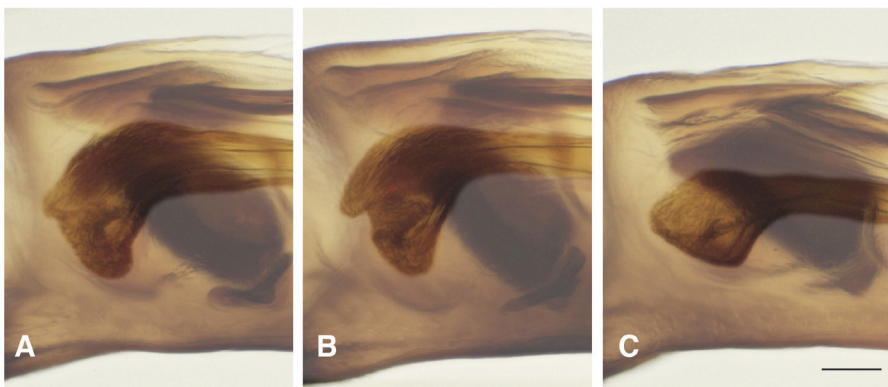
Figure 15. Basal sclerotized lobe of internal sac of male *Bembidion transversale* group members **A** *B. transversale* (voucher DNA4219, USA: Oregon: Harney County, Banks of Silver Creek, 1379 m, 43.7278°N, 119.6256°W) **B** *B. erosum* (voucher DNA4033, USA: Oregon: Curry Co., Floras Creek at route 124 SE Langlois, 21 m, 42.9132°N, 124.4251°W) **C** *B. corgenoma* (voucher DNA2180, USA: California: Sonoma Co., Russian River, 3 mi NE Healdsburg). Scale bar: 0.1 mm.
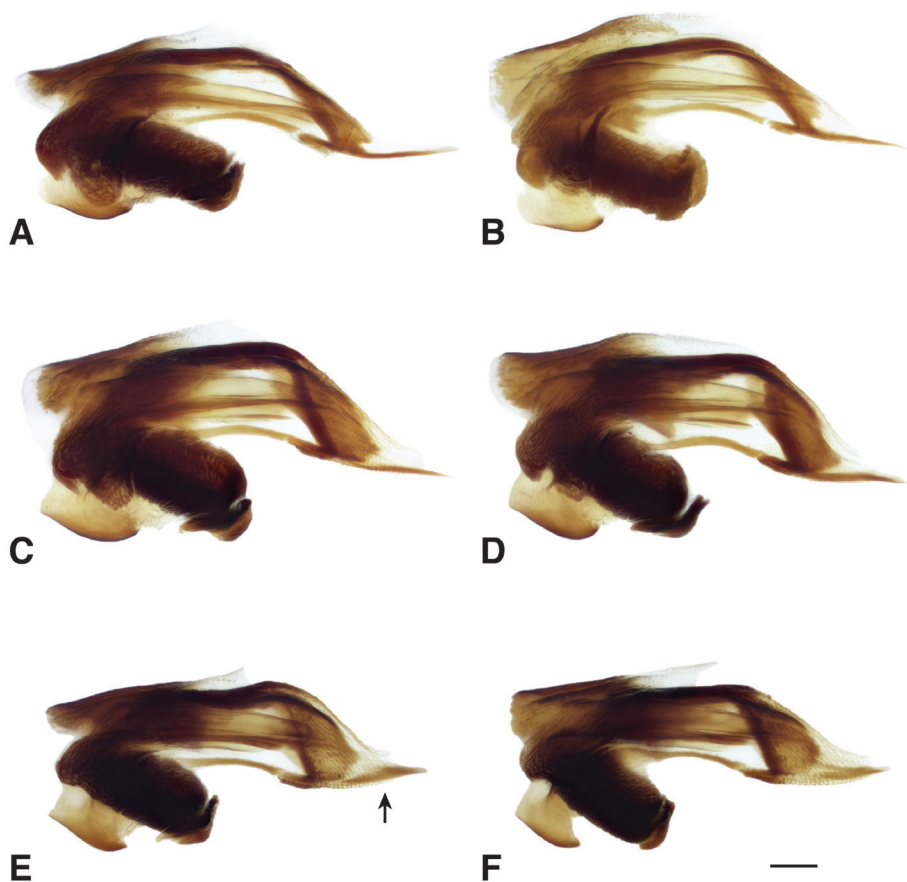
Figure 16. Central sclerite complex of *B. transversale* subgroup **A, B** *B. transversale* (vouchers V101437 and V101436, USA: Michigan: Port aux Pins, Bois Blanc Isl.) **C, D** *B. erosum* (vouchers V101440 and V101439, USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W) **E, F** *B. corgenoma* (vouchers V101428 and V101430, USA: Oregon: Benton Co., Corvallis, Willamette River, 44.5475°N, 123.2428°W). Scale bar: 0.1 mm.

Habitat. This species occurs on gravel or cobble shores of the rivers and creeks (Fig. 4), more often where the bank is relatively flat and has small amount of clay and silt mixed in with sand and gravel under the rocks. They also can be common under cobbles on the shores of small creeks on the upper portions of beaches of the Pacific Ocean.

Bembidion perspicuum (LeConte, 1848)

Ochthedromus perspicuus LeConte, 1848: 466. Holotype male, in MCZ (type number 5510), external structure and aedeagus examined. Type locality "Rocky Mountains".

Ochthedromus mannerheimii LeConte, 1852:190. Lectotype female, designated by Maddison and Swanson (2010), in MCZ (type number 35571). Type locality San Diego, California. Although a male in the same unit tray as the lectotype is, by genitalic characters, a member of *Bembidion corgenoma*, I am uncertain about the lectotype. The almost complete absence of the seventh stria and the pronotal shape suggests *Bembidion perspicuum*, but the base of the pronotum is not as punctured as typical for that species. I tentatively place it as a synonym of *Bembidion perspicuum*. However, as *Bembidion mannerheimii* LeConte, 1852, is a junior homonym of *Bembidion mannerheimii* Sahlberg, 1827, this name is unavailable in any event.

Bembidium haplogonum Chaudoir, 1868: 241. Neotype male, in MNHN, here designated, labeled "USA: California: Lake Co., North Branch Cache Creek at hwy 20, 305 m 38.9881°N, 122.54°W, 5.viii.2010. DRM 10.090. K.W. Will & D.R. Maddison", "David R. Maddison DNA5681 DNA Voucher" [pale green paper], "NEOTYPE Bembidium haplogonum Chaudoir designated D.R. Maddison" [partly handwritten, on white paper, bordered by red lines]. Genitalia mounted in Euparal in between coverslips pinned with specimen; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the neotype are MW151478, MW151506, and MW151563. Details about the choice of neotype are provided below.

Bembidion acomanum Casey, 1918: 59. Lectotype female, designated by Lindroth (1975), in USNM (type number 36916), examined. Type locality Jemez Springs, New Mexico (Lindroth 1975).

Bembidion excursum Casey, 1918: 59. Holotype female, in USNM (type number 36915), examined. Type locality Tucson, Arizona.

Bembidion tuolumne Casey, 1924:30. Lectotype male, designated by Lindroth (1975), in USNM (type number 36917), external structure and aedeagus examined. In Maddison and Swanson (2010), this was treated as tentatively a synonym of *B. transversale*. Further examination of the lectotype, including of its genitalia, reveal that this is a specimen of *B. perspicuum*. Type locality Tuolumne, California.

Designation of a neotype for *Bembidium haplogonum* Chaudoir. Lindroth (1963: 341) could not find the original type series for *Bembidium haplogonum* Chaudoir in the MNHN. Thierry Deuve and David Kavanaugh have both searched for it, and could not find it in the Chaudoir collection, although other specimens were found that had been collected in California by Pierre Joseph Michel Lorquin. As the original type series is presumed lost, I here designate a neotype.

Chaudoir's (1868) description of *Bembidium haplogonum* is detailed enough to make determination of the species he was describing clear. In his description, the large size (8 mm) and absence of a carina near the hind angle of the prothorax could only apply (within California, the type locality) to a member of the *B. transversale* group. The color pattern of the elytra ("*sur les élytres, une petite tache au milieu du bord antérieur de chacune, et une bande transversale un peu arquée aux trois quarts, d'un jaune testacé pâle, peu distinctes, surtout la tache basale*", which translates to "on the elytra, a small spot on the front
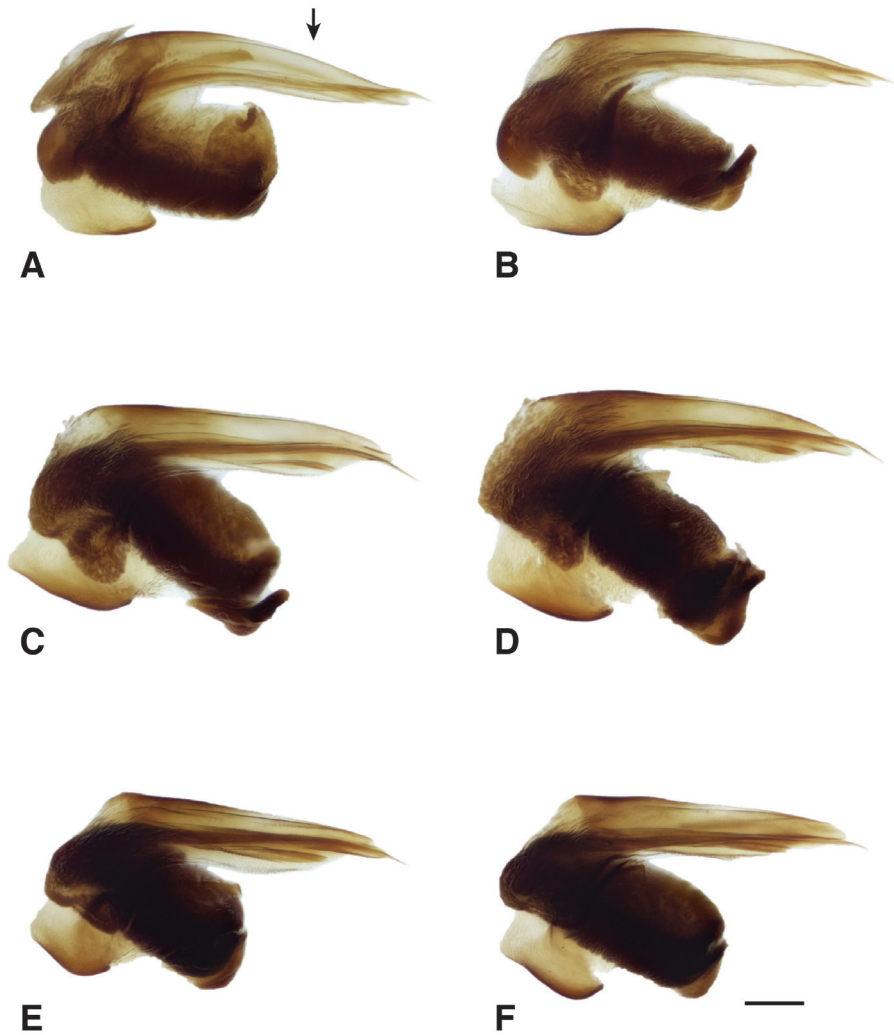
Figure 17. Flagella of *B. transversale* subgroup **A, B** *B. transversale* (vouchers V101438 and V101435, USA: Michigan: Port aux Pins, Bois Blanc Isl.) **C, D** *B. erosum* (vouchers V101442 and V101441, USA: California: Del Norte Co., Wilson Creek, 3 m, 41.6051°N, 124.1005°W) **E, F** *B. corgenoma* (vouchers V101434 and V101431, USA: Oregon: Benton Co., Corvallis, Willamette River, 44.5475°N, 123.2428°W). Scale bar: 0.1 mm.

edge of each, and a slightly curved transverse band at three-quarters, of a pale testaceous yellow, indistinct, especially the basal spot") can only apply to some specimens of *B. erosum*, *B. lugubre*, or the northern, dark form of *B. perspicuum* (Fig. 11E), as the other species in California have the entire basal third to half of the elytra pale. The description of the appendages as having the first 3.5 antennomeres, palps, and legs all pale yellow
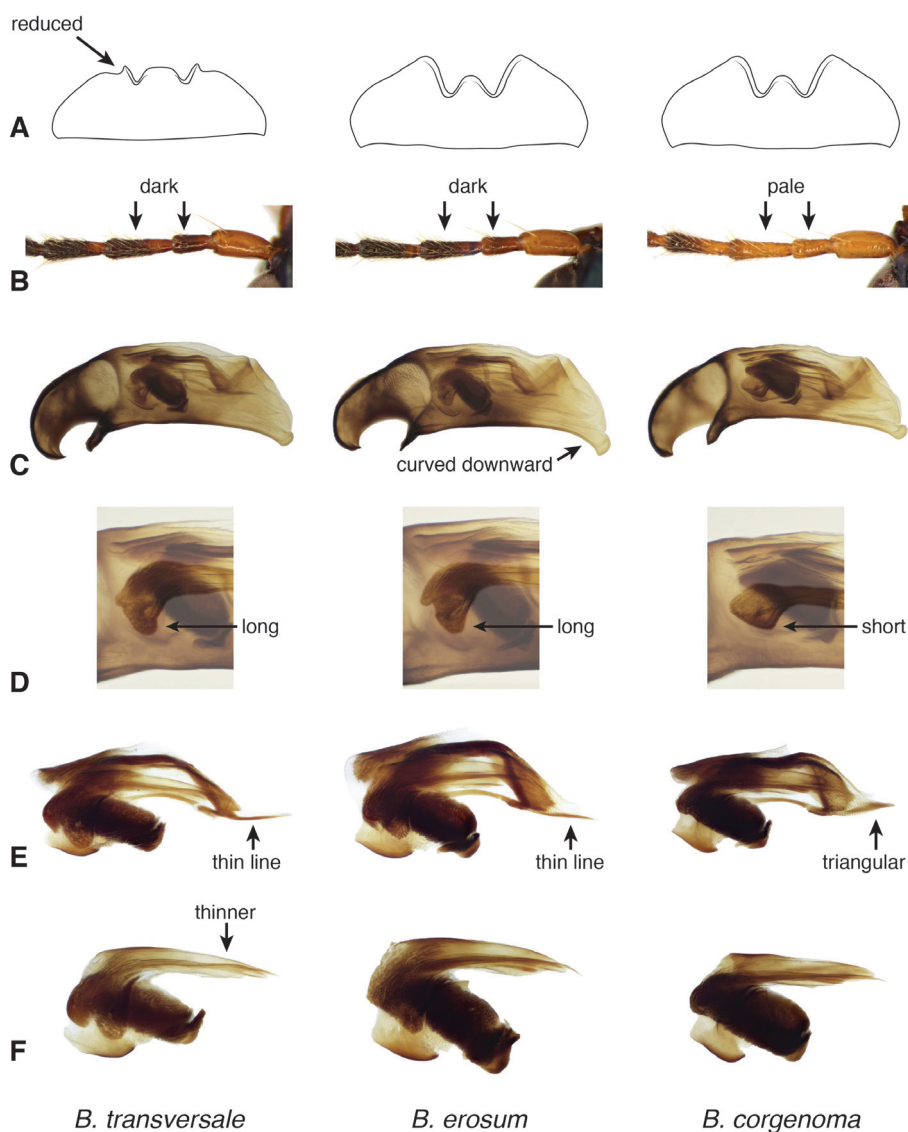
Figure 18. Summary of morphological differences between three species in the *B. transversale* subgroup in **A** mentum **B** antennal color **C** curvature of ventral margin of the aedeagus **D** basal sclerotizes lobe size **E** apex of flagellar sheath, **F** flagellum.

eliminates *B. erosum*, as the palps, femur, and antennomeres 2–11 are dark in California specimens of that species. I have seen no specimens of *B. lugubre* with an isolated small spot on the front edge of each elytron; the only specimens that have a pale area in the basal half of the elytra have the entire sides and basal regions of the elytra a pale rufous,
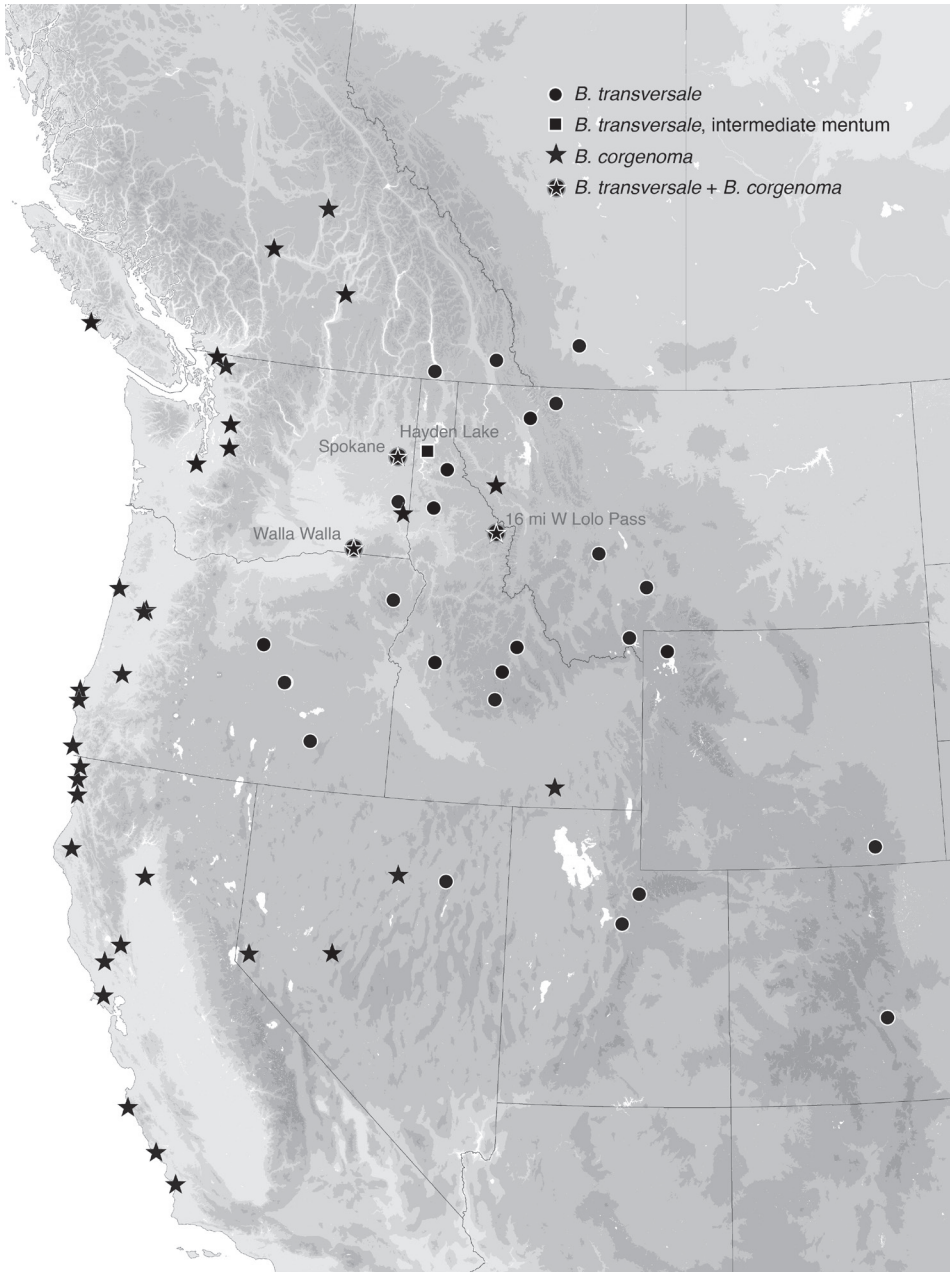
Figure 19. Geographic distribution of *B. transversale* and *B. corgenoma* (eastern portion of distribution of *B. transversale* not shown).

with a darker disc; this paler form of *B. lugubre* occurs north of Los Angeles in California. The metallic coloration of the pronotum ("*Le dessus d'un vert brillant un peu cuivreux*") is characteristic of *B. perspicuum*, but not *B. lugubre*; the latter has no metallic sheen in
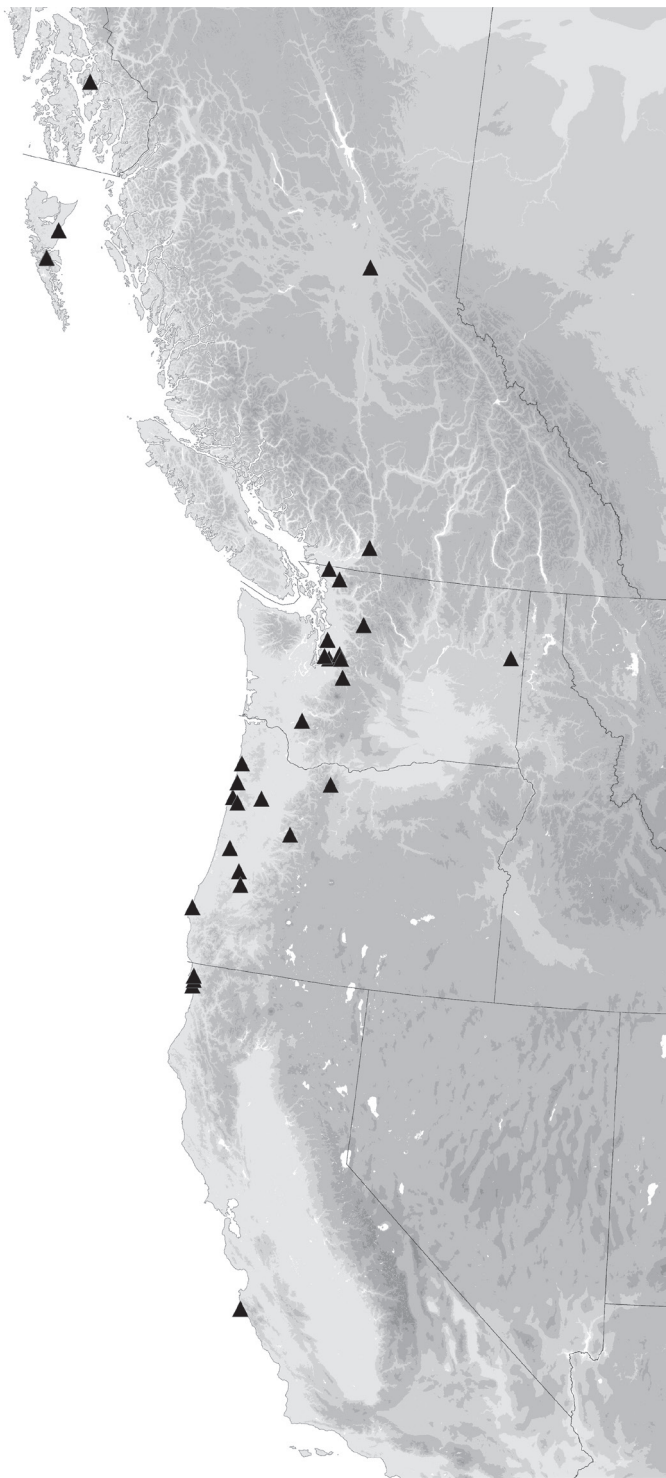
Figure 20. Geographic distribution of *B. erosum*.

any specimens I have seen from California, and in only very few individuals elsewhere. The flatter prothorax with less rounded sides and a wider lateral gutter is also distinctive of *B. perspicuum* relative to all three other species from California (*B. erosum*, *B. lugubre*, and *B. corgenoma*), as is the distinctly punctured pronotal base ("*distinctement ponctué tout le long de la base*"). The large size (8 mm) is more characteristic of *B. perspicuum*; I have seen no specimens of *B. corgenoma* longer than 7.5 mm, but have seen specimens of *B. perspicuum* that are 7.9 mm in length, and specimens of *B. perspicuum* are, in general, larger than those of *B. corgenoma*. Finally, the virtual absence of the seventh elytral stria is characteristic of *B. perspicuum* relative to *B. corgenoma*. As Lorquin travelled extensively in areas where the dark form of *B. perspicuum* occurs (Grinnell 1904), it is certainly reasonable that a specimen of that form could have been seen by Chaudoir.

My early interpretations of Chaudoir's descriptions were in error, and led me to believe that Chaudoir's specimen was a member of what I here call *Bembidion corgenoma*; that mistake led me to call the species studied in Gustafson et al. (2019) *Bembidion haplogonum*. As a correct reading of the original description shows that *Bembidium haplogonum* refers to the dark form of *B. perspicuum*, I have designated a specimen from northern California with a color pattern matching Chaudoir's description as the neotype (shown in Fig. 11E).

Diagnosis and geographic distribution. Adults of this species are large, and have a pronotum that is flatter than in other members of the group, with less rounded sides, and with the basal region more evidently punctate (Maddison and Swanson 2010: fig. 4). At least antennomeres 4–11 infuscated. Specimens from most areas are relatively pale (Fig. 11D), with the front half of the elytra pale, but specimens from northern California and Oregon are much darker (Fig. 11E), with only elytral apices being pale. Aedeagal characterc are described in Maddison and Swanson (2010). This species is known from Texas, Kansas, Colorado, New Mexico, Arizona, Utah, Nevada, California, and Oregon.

Bembidion sarpedon Casey, 1918

Bembidion sarpedon Casey, 1918: 58. Lectotype male, designated by Lindroth (1975), in USNM (type number 36914); external structure and aedeagus examined. Type locality Cañon City, Colorado.

Bembidion animatum Casey, 1918: 62. Lectotype female, designated by Lindroth (1975), in USNM (type number 36918), examined. Type locality Jemez Springs, New Mexico (Lindroth 1975).

Diagnosis and geographic distribution. Adults of this species (Fig. 11F) are the palest members of this group, with legs entirely testaceous or rufo-testaceous, with antennae gradually becoming slightly darker toward the apex, and pronotum in most specimens dark rufous as opposed to the piceous or black of other species. The dorsal surface is shinier than in other species, especially the pronotum, because of the nearly effaced microsculpture. The prothorax is moderately cordate; the elytral intervals are

flatter than in related species, with small punctures in the striae. The ventral margin of the aedeagus has a slight downward bulge, and the internal sac sclerite complex of male genitalia is narrow in lateral view, with a long and thin flagellar complex. Known from New Mexico and Colorado west to Arizona and Utah, and north to Mammoth Hot Springs, Wyoming (OSAC).

Concluding remarks

The pathways that led to the recognition of the two species described here were very different. When I encountered *Bembidion mimbres* for the first time, as pinned specimens at the University of Alberta's Strickland Museum in 1981–1982, I immediately recognized them as an undescribed species. They shared the large size, setose elytra, shiny surface, and striking color of the distinctive *Bembidion levigatum*, but did not share *B. levigatum*'s unusual prothorax shape and width.

In contrast, it took at least 12 years of study for me to become confident that *B. corgenoma* was a new species, and that the *B. transversale* subgroup consisted of at least five species (*B. sarpedon*, *B. perspicuum*, *B. transversale*, *B. erosum*, and *B. corgenoma*). The distinctiveness of *B. sarpedon* and *B. perspicuum* was recognized many years ago. The specimens that remained (*B. transversale* s. l.), however, were so complex in their variation patterns, so lacking in a differentiating signal in DNA sequences, and with such similar genitalia, that at times I thought there was just one species in *Bembidion transversale* s. l., and at other times more.

I had become so accustomed to the clarity provided by DNA sequences in my other taxonomic projects on bembidiines that I had become somewhat skeptical of the value of traditional taxonomic methods utilizing only patterns of morphological variation. Two events changed my mind, as they caused the patterns to become evident at last. The first event was Kip Will's collecting of both dark and light specimens from the shores of Wilson Creek in north coastal California. They were so obviously different in color that I expected them to have clearly different genitalia, and different DNA sequences. My cursory inspection revealed only the slightest difference in the overall shape of the aedeagus (I had not yet noticed the differences in the structures of the internal sac), not notable enough to be significant in itself. In addition, all six sequenced dark specimens from that gravel bar differed from all five sequenced light specimens in one base in Topoisomerase, but they did not differ in 28S, COI, and CAD. The correlation between color, aedeagal shape, and that single base in Topoisomerase convinced me that there were likely two species at that site in northern California, although if so they would be much more similar than are most other pairs of closely related, sympatric *Bembidion* species. Examination of Motschulsky's specimens eventually revealed that the dark species had a name, *Bembidion erosum*, but the pale species at Wilson Creek and elsewhere continued to trouble me: other than the normal mentum, I saw no consistent differences from the more eastern *Bembidion transversale*. The distinctiveness of the pale western form (here called *B. corgenoma*) did not become evident until

the basic morphological work was done: thorough examination of the genitalia of 63 *B. corgenoma* males and 33 *B. transversale* males, focused on the area of geographic overlap, revealed the consistent differences shown in Fig. 18, especially the shape of the basal sclerotized lobe (Fig. 18D) and the tip of the flagellar sheath (Fig. 18E). This confirmed that sequences of four genes will not necessarily reveal the presence or absence of gene flow, and that even in *Bembidion*, a group in which DNA sequences often work very well for species delimitation, species boundaries are sometimes more quickly uncovered by traditional morphological methods.

The lack of observed differentiation in DNA sequences between *Bembidion transversale*, *B. erosum*, and *B. corgenoma* suggests that these are young, recently differentiated species. The contrast is striking between this trio and other bembidiines; in most bembidiines, sequences in at least one of the handful of standard genes provides a clear signal of lack of gene flow between species (e.g., Maddison 2008; Maddison and Cooper 2014; Maddison and Sproul 2020; Sproul and Maddison 2017). Why is the signal of species boundaries so clear in most bembidiine groups, but not *B. transversale* s. l.? Given genomic resources now available for this group, one fruitful and available avenue of future research would be comparison of coalescent patterns of thousands of regions of the genome within both this trio of *Bembidion transversale* group species and other groups of bembidiines with similar levels of morphological divergence.

References

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Computational Biology 10: e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Bousquet Y (2012) Catalogue of Geadephaga (Coleoptera, Adephaga) of America, north of Mexico. ZooKeys 245: 1–1722. https://doi.org/10.3897/zookeys.245.3416

Bousquet Y, Larochelle A (1993) Catalogue of the Geadephaga (Coleoptera: Trachypachidae, Rhysodidae, Carabidae including Cicindelini) of America north of Mexico. Memoirs of the Entomological Society of Canada 167: 1–397. https://doi.org/10.4039/entm125167fv

Casey TL (1918) A review of the North American Bembidiinae. Memoirs on the Coleoptera 8: 1–223.

Casey TL (1924) Additions to the known Coleoptera of North America. Memoirs on the Coleoptera 11: 1–347. https://doi.org/10.5962/bhl.title.48776

Chakrabarty P, Warren M, Page LM, Baldwin CC (2013) GenSeq: An updated nomenclature and ranking for genetic sequences from type and non-type sources. ZooKeys 346: 29–41. https://doi.org/10.3897/zookeys.346.5753

Chaudoir M, de (1868) Observations synonymiques sur les Carabiques de l'Amérique septentrionale et descriptions d'espèces nouvelles de ce pays. Revue et Magazin de Zoologie, Series 2, 20: 239–245.

Dejean PFMA (1831) Spécies général des Coléoptères de la collection de M. le Comte Dejean. Paris, I–VIII, 384 pp.

Gómez RA, Maddison DR (2020) Novelty and emergent patterns in sperm: Morphological diversity and evolution of spermatozoa and sperm conjugation in ground beetles (Coleoptera: Carabidae). Journal of Morphology 281: 862–892. https://doi.org/10.1002/jmor.21144

Green P (1999) Phrap. Version 0.990329. http://phrap.org

Green P, Ewing B (2002) Phred. Version 0.020425c. http://phrap.org

Grinnell F (1904) An early collector in California. Entomological News 15: 202–204.

Gustafson GT, Alexander A, Sproul JS, Pflug JM, Maddison DR, Short AEZ (2019) Ultraconserved element (UCE) probe set design: Base genome and initial design parameters critical for optimization. Ecology and evolution 9: 6933–6948. https://doi.org/10.1002/ece3.5260

Gustafson GT, Baca SM, Alexander AM, Short AEZ (2020) Phylogenomic analysis of the beetle suborder Adephaga with comparison of tailored and generalized ultraconserved element probe performance. Systematic Entomology 45: 552–570. https://doi.org/10.1111/syen.12413

Hegmon M, McGrath JR, O'Hara III FM, Russell WG (2018) Mimbres pottery designs in their social contexts. In: Roth BJ, Gilman PA, Anyon R (Eds) New Perspectives on Mimbres Archaeology: Three Millenia of Human Occupation in the North America Southwest. The University of Arizona Press, Tucson, 149–168. https://doi.org/10.2307/j.ctv5cg8bb.10

Jones G (2017) Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. Journal of Mathematical Biology 74: 447–467. https://doi.org/10.1007/s00285-016-1034-0

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Kanda K, Pflug JM, Sproul JS, Dasenko MA, Maddison DR (2015) Successful recovery of nuclear protein-coding genes from small insects in museums using Illumina sequencing. PLoS ONE 10: e0143929. https://doi.org/10.1371/journal.pone.0143929

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

LeConte JL (1848) A descriptive catalogue of the geodephagous Coleoptera inhabiting the United States east of the Rocky Mountains. Annals of the Lyceum of Natural History of New York 4: 173–233, 334–474. https://doi.org/10.1111/j.1749-6632.1848.tb00277.x

LeConte JL (1852) Descriptions of new species of Coleoptera, from California. Annals of the Lyceum of Natural History of New York 5: 185–216. https://doi.org/10.1111/j.1749-6632.1852.tb00123.x

Lindroth CH (1963) The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska. Part 3. Opuscula Entomologica Supplementum XXIV: 201–408.

Lindroth CH (1975) Designation of holotypes and lectotypes among ground beetles (Coleoptera, Carabidae) described by Thomas L. Casey. The Coleopterists Bulletin 29: 109–147.

Lindroth CH, Freitag R (1969) North American ground-beetles (Coleoptera, Carabidae, excluding Cicindelinae) described by Thomas Say: designation of lectotypes and neotypes. Psyche 76: 326–361. https://doi.org/10.1155/1969/90590

Lorenz W (2005) Systematic list of extant ground beetles of the world (Insecta Coleoptera "Geadephaga": Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae). Second edition. Published by the author [Hörmannstrasse 4, D-82327], Tutzing, [i–iii,] 530 pp.

Maddison DR (1985) Chromosomal diversity and evolution in the ground beetle genus *Bembidion* and related taxa (Coleoptera: Carabidae: Trechitae). Genetica 66: 93–114. https://doi.org/10.1007/BF00139715

Maddison DR (1993) Systematics of the Holarctic beetle subgenus *Bracteon* and related *Bembidion* (Coleoptera: Carabidae). Bulletin of the Museum of Comparative Zoology 153: 143–299.

Maddison DR (2008) Systematics of the North American beetle subgenus *Pseudoperyphus* (Coleoptera: Carabidae: *Bembidion*) based upon morphological, chromosomal, and molecular data. Annals of Carnegie Museum 77: 147–193. https://doi.org/10.2992/0097-4463-77.1.147

Maddison DR (2012) Phylogeny of *Bembidion* and related ground beetles (Coleoptera: Carabidae: Trechinae: Bembidiini: Bembidiina). Molecular Phylogenetics and Evolution 63: 533–576. https://doi.org/10.1016/j.ympev.2012.01.015

Maddison DR, Cooper KW (2014) Species delimitation in the ground beetle subgenus *Liocosmius* (Coleoptera: Carabidae: *Bembidion*), including standard and next-generation sequencing of museum specimens. Zoological Journal of the Linnean Society 172: 741–770. https://doi.org/10.1111/zoj.12188

Maddison DR, Kanda K, Boyd OF, Faille A, Porch N, Erwin TL, Roig-Juñent S (2019) Phylogeny of the beetle supertribe Trechitae (Coleoptera: Carabidae): Unexpected clades, isolated lineages, and morphological convergence. Molecular Phylogenetics and Evolution 132: 151–176. https://doi.org/10.1016/j.ympev.2018.11.006

Maddison DR, Maddison WP (2018a) Chromaseq: a Mesquite package for analyzing sequence chromatograms. Version 1.31. http://chromaseq.mesquiteproject.org

Maddison DR, Maddison WP (2018b) Zephyr: a Mesquite package for interacting with external phylogeny inference programs. Version 3.0. http://zephyr.mesquiteproject.org

Maddison DR, Maruyama M (2019) Phylogenetic relationships and convergent evolution of ocean-shore ground beetles (Coleoptera: Carabidae: Trechinae: *Bembidion* and relatives). Systematic Entomology 44: 39–60. https://doi.org/10.1111/syen.12307

Maddison DR, Ober KA (2011) Phylogeny of minute carabid beetles and their relatives based upon DNA sequence data (Coleoptera, Carabidae, Trechitae). ZooKeys 147: 229–260. https://doi.org/10.3897/zookeys.147.1871

Maddison DR, Sproul JS (2020) Species delimitation, classical taxonomy and genome skimming: a review of the ground beetle genus *Lionepha* (Coleoptera: Carabidae). Zoological Journal of the Linnean Society 189: 1313–1358. https://doi.org/10.1093/zoolinnean/zlz167

Maddison DR, Swanson AP (2010) A preliminary characterization of *Bembidion perspicuum* LeConte, with a reclassification of related species (Coleoptera, Carabidae) north of México. ZooKeys 43: 15–31. https://doi.org/10.3897/zookeys.43.390

Maddison WP, Maddison DR (2018c) Mesquite: a modular system for evolutionary analysis. Version 3.51. http://mesquiteproject.org

Marggi W, Toledano L, Neri P (2017) Tribe Bembidiini Stephens, 1827, subtribe Bembidiina Stephens, 1827. In: Löbl I, Löbl D (Eds) Catalogue of Palearctic Coleoptera. Brill, Leiden, Boston, 294–342.

Motschulsky TV de (1850) Die Kaefer Russlands. Gautier, Moscau, [iv + xi +] 91 pp.

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi.org/10.1093/molbev/msu300

Pflug JM, Holmes VR, Burrus C, Johnston JS, Maddison DR (2020) Measuring genome sizes using read-depth, k-mers, and flow cytometry: methodological comparisons in beetles (Coleoptera). G3: Genes, Genomes, Genetics 10: 3047–3060. https://doi.org/10.1534/g3.120.401028

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67: 901–904. https://doi.org/10.1093/sysbio/syy032

Smith MA, Bertrand C, Crosby K, Eveleigh ES, Fernandez-Triana J, Fisher BL, Gibbs J, Hajibabaei M, Hallwachs W, Hind K, Hrcek J, Huang D-W, Janda M, Janzen DH, Li Y, Miller SE, Packer L, Quicke D, Ratnasingham S, Rodriguez J, Rougerie R, Shaw MR, Sheffield C, Stahlhut JK, Steinke D, Whitfield J, Wood M, Zhou X (2012) *Wolbachia* and DNA barcoding insects: patterns, potential, and problems. PLoS ONE 7: e36514. https://doi.org/10.1371/journal.pone.0036514

Sproul JS, Maddison DR (2017) Cryptic species in the mountaintops: species delimitation and taxonomy of the *Bembidion breve* species group (Carabidae: Coleoptera) aided by genomic architecture of a century-old type specimen. Zoological Journal of the Linnean Society 183(3): 556–583. https://doi.org/10.1093/zoolinnean/zlx076

Thalmann O, Hebler J, Poinar HN, Pääbo S, Vigilant L (2004) Unreliable mtDNA data due to nuclear insertions: a cautionary tale from analysis of humans and other great apes. Molecular Ecology 13: 321–335. https://doi.org/10.1046/j.1365-294X.2003.02070.x

Wild AL, Maddison DR (2008) Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. Molecular Phylogenetics and Evolution 48: 877–891. https://doi.org/10.1016/j.ympev.2008.05.023

Supplementary material 1

Mesquite NEXUS file containing the DNA sequence data and resulting phylogenetic trees from maximum likelihood analyses for the subgenus *Hydrium*
Authors: David R. Maddison
Data type: NEXUS file (DNA sequences, phylogenetic trees)
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/zookeys.1007.60012.suppl1

Supplementary material 2

Mesquite NEXUS file containing the DNA sequence data and resulting phylogenetic trees from maximum likelihood analyses for the *Bembidion transversale* group
Authors: David R. Maddison
Data type: NEXUS file (DNA sequences, phylogenetic trees)
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/zookeys.1007.60012.suppl2

RESEARCH ARTICLE

Three new species of *Exocelina* Broun, 1886 from the southern slopes of the New Guinea central range, with introduction of the *Exocelina skalei* group (Coleoptera, Dytiscidae, Copelatinae)

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Abstract

Three new species of the genus *Exocelina* Broun, 1886: *E. brazza* **sp. nov.**, *E. amabilis* **sp. nov.** and *E. mimika* **sp. nov.** are described from New Guinea. The former two species are placed into the *E. ekari* group, while the latter is suggested to be a member of a separate lineage, the newly introduced *E. skalei* group. The only other species of that group is *E. skalei* Shaverdo & Balke, 2014. The group is phylogenetically sister of the *E. ekari* group. All species have been collected on the southern slopes of the New Guinea central range, a region that remains mostly unsampled.

Introduction

Three new species of the genus *Exocelina* Broun, 1886 were discovered on the southern slopes of the New Guinea central range. Two of them, *E. brazza* sp. nov. and *E. amabilis* sp. nov., belong to the largest *Exocelina* species group, the *E. ekari* group. To date, this group contains 56 species (including the two new species) endemic to New Guinea (Balke 1998; Shaverdo et al. 2005, 2012, 2014, 2016, 2020; Shaverdo and Balke 2019). The third species, *E. mimika* sp. nov., shares the same combination of characters including reduced setation of the paramere with *E. skalei* Shaverdo & Balke, 2014. For these two species, the *E. skalei* group, which is the sister group of the *E. ekari* group, is proposed. *Exocelina skalei* is only known from Kaimana in the Bird's Neck region of New Guinea, which is geographically in principle a westward extension of the central highlands, with Kaimana situated at the south coast.

Including the results of this work, 145 species of *Exocelina* are now described from New Guinea and 202 species worldwide (Nilsson and Hájek 2020; Shaverdo et al. 2020). As in most of our previous papers on the genus, all species data will be presented on the species-id.net portal automatically created by ZooKeys with the publication of this paper.

Materials and methods

The present work is based on material from the following collections:

KSP Koleksi Serangga Papua, at the Biology Department of Universitas Cenderawasih (UNCEN), Waena, Papua, Indonesia;
MZB Museum Zoologicum Bogoriense, Cibinong, Indonesia.

Our methods follow those described in detail in our previous articles (Shaverdo et al. 2012, 2014; Shaverdo and Balke 2014). The terminology to denote the orientation of the genitalia follows Miller and Nilsson (2003). All specimen data are quoted as they appear on the labels attached to the specimens. Label text is cited using quotation marks; comments in square brackets are ours. The following abbreviations were used: TL (total body length), TL-H (total body length without head), MW (maximum body width).

Species descriptions and taxonomy notes

Exocelina brazza Shaverdo & Balke, sp. nov.
http://zoobank.org/5114E4B1-7F5D-4ED4-B74A-6B4A485FDF55
Figs 1–5

Type locality. Indonesia: Papua Province, Yahukimo Regency, Dekai, upper Brazza River, near 04°44'27.9"S, 139°39'15.2"E, 300 m a.s.l.

Figure 1. Habitus and colouration of *Exocelina brazza* sp. nov., holotype.

Type material. *Holotype*: male "Indonesia: Papua, Dekai, upper Brazza, 300 m, 2/3.vi.2015, near -4,741084724 139,654211075976, Sumoked (Pap045)" (MZB). ***Paratypes*:** 2 males, 10 females with the same label as the holotype, one male and one female additionally with green text labels "6991" and "6990", respectively (KSP, MZB).

Description. *Body size and form*: Beetle small: TL-H 3.05–3.3 mm, TL 3.45–3.7 mm, MW 1.65–1.8 mm (holotype: TL-H 3.3 mm, TL 3.7 mm, MW 1.8 mm), with oblong-oval habitus (Fig. 1).

Colouration: Dorsally brown, with reddish pronotal sides (Fig. 1). Head reddish in anterior half (in front of eyes) and reddish-brown to brown in posterior half (at eye level and behind); pronotum reddish-brown on disc and with broad reddish sides, sometimes also reddish along anterior and posterior margins; elytron reddish-brown to dark brown, with reddish sutural lines; head appendages yellow to yellowish-red, legs reddish. Teneral specimens paler.

Surface sculpture: Shiny dorsally, with fine punctation and microreticulation. Head with dense punctation (spaces between punctures 1–3 times size of punctures), distinctly finer and sparser anteriorly and posteriorly; diameter of punctures smaller than diameter of cells of microreticulation. Pronotum with distinctly finer and relatively sparser punctation than on head. Elytra with very sparse and fine punctation, almost invisible. Pronotum and elytra with weakly impressed microreticulation; head with stronger microreticulation. Metaventrite and metacoxa distinctly but weakly microreticulate, metacoxal plates with longitudinal strioles and transverse wrinkles. Abdominal ventrites with weak microreticulation, strioles, and fine sparse punctation, coarser and denser on two last abdominal ventrites.

Structures: Pronotum with distinct lateral bead. Base of prosternum and neck of prosternal process with ridge, slightly rounded anteriorly. Blade of prosternal process broadly lanceolate, relatively broad and short, slightly convex medially, with distinct bead and few setae. Abdominal ventrite 6 broadly rounded apically.

Male: Antenna modified: antennomeres 3–4 strongly enlarged, antennomere 5 distinctly enlarged and antennomeres 6–10 stout (Fig. 1). Pro- and mesotarsomeres 1–3 not dilated. Protarsomere 4 cylindrical, narrow, with large, thick, strongly curved anterolateral hook-like seta. Protarsomere 5 ventrally with anterior row of 12 and posterior row of 5 short setae (Fig. 2D). Median lobe with discontinuous outline and distinct submedian constriction in ventral view; apex elongate in lateral view (Fig. 2A, B). Paramere without distinct dorsal notch; subdistal part with relatively long, dense setae; proximal setae inconspicuous (Fig. 2C). Abdominal ventrite 6 broadly rounded, with 10–14 lateral striae on each side.

Female: Pro- and mesotarsi not modified. Abdominal ventrite 6 without striae.

Habitat. The specimens were collected from a small creek on an almost flat primary forest floor, about 100 m from the upper Brazza River (Figs 3, 4).

Distribution. Indonesia: Papua Province. This species is known only from the type locality (Fig. 5).

Etymology. The species is named after the Brazza River. The name is a noun in the nominative singular standing in apposition.

Affinities. The species evidently belongs to the *E. ekari* group due to the discontinuous outline of its median lobe. Within the group, it can be placed close to the shiny species with antennomeres 3 and 4 larger than other antennomeres (including the recently described *E. athesphatos* Shaverdo et al., 2020 and *E. tsinga* Shaverdo et al., 2020), to which it is assumed to be closely related. However, *E. brazza* sp. nov. can be easily distinguished from all of them by its small size, shape of the male antennae, median lobe and paramere.
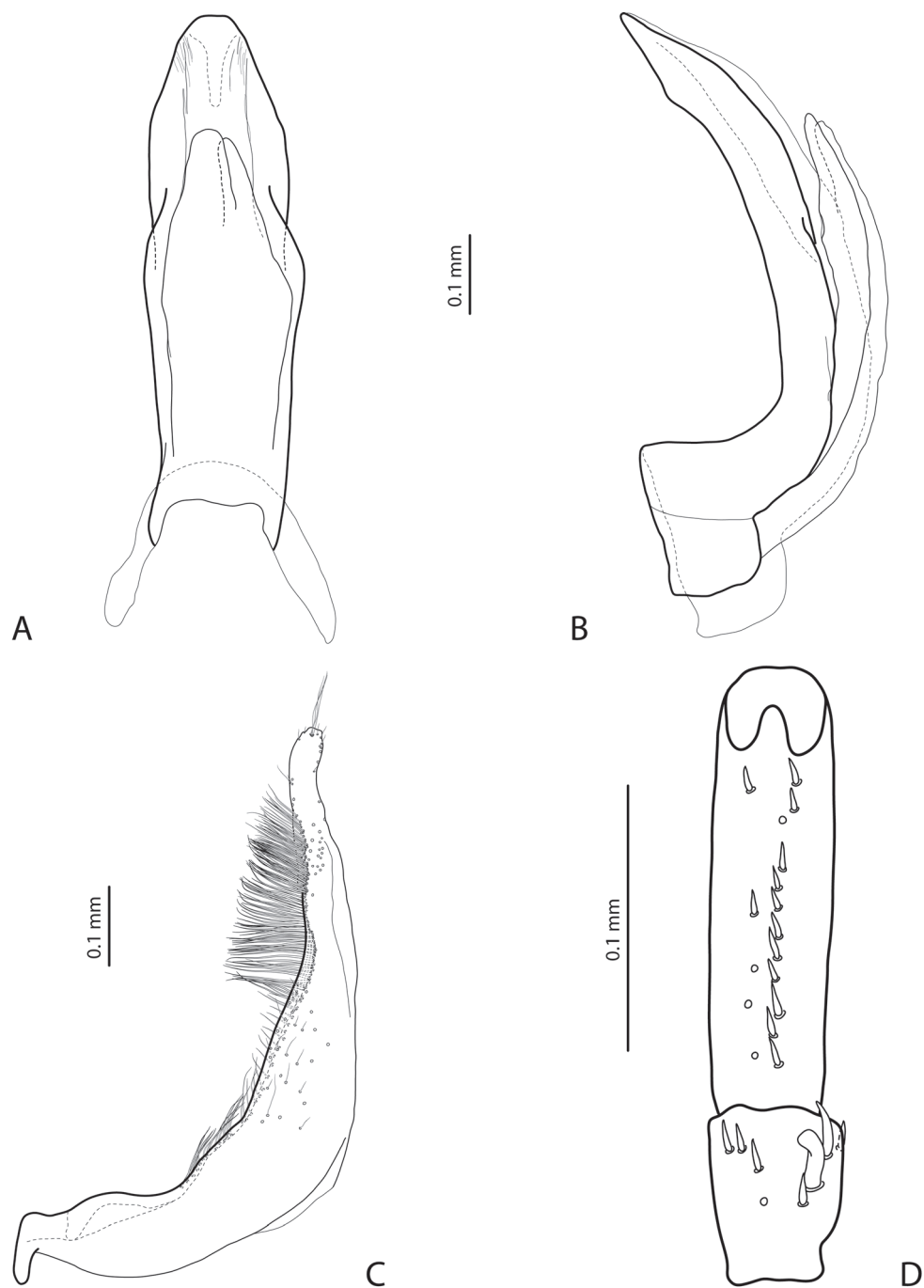
Figure 2. *Exocelina brazza* sp. nov., paratype **A** median lobe in ventral view **B** median lobe in lateral view **C** right paramere in external view **D** right male protarsomeres 4–5 in ventral view.

Figure 3. Habitat area of *Exocelina brazza* sp. nov., a view from the forest down into Brazza River.

Figure 4. Habitat of *Exocelina brazza* sp. nov., a small creek in the primary forest, ca 100 m from the Brazza River.
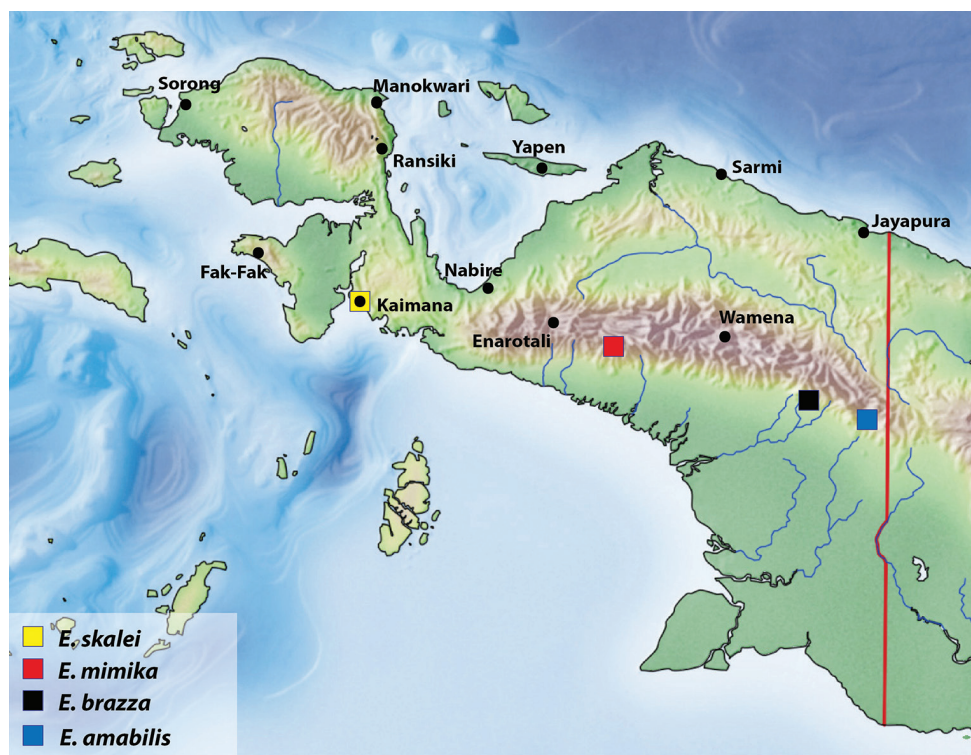
Figure 5. Map of the western part of New Guinea showing the species distribution.

Exocelina amabilis Shaverdo & Balke, sp. nov.

http://zoobank.org/C7F77D9E-27BD-446D-9E9E-415D6B6D143C
Figs 5–7

Type locality. Indonesia: Papua Province, Pegunungan Bintang Regency, south from Ok Sibil, tributary Digul River, 05°03'25.9"S, 140°43'21.1"E, 359 m a.s.l.

Type material. *Holotype*: male "Indonesia: Papua, S Ok Sibil, tributary Digul Riv [River], 359m, 9.vi.2015, -5,05718389 140,722535848617, Sumoked (Pap051)" (MZB). *Paratypes*: 3 males, 8 females with the same label as the holotype, 2 males additionally with green text label "6997" and "6998" (KSP, MZB).

Description. *Body size and form*: Beetle small: TL-H 2.65–3.45 mm, TL 3.55–3.85 mm, MW 1.75–1.9 mm (holotype: TL-H 3.25 mm, TL 3.65 mm, MW 1.8 mm), with oblong-oval habitus (Fig. 6).

Colouration: Dorsally piceous (Fig. 6), with dark brown posterior part of head and lateral parts of pronotum, and sometimes with middle part of pronotum and elytral sutural lines; head appendages and legs yellowish-red, metathoracic legs darker distally.

Surface sculpture: Relatively shiny dorsally, with very fine, sparse punctation and distinctly impressed microreticulation. Head with dense punctation (spaces between punctures 1–3 times size of punctures), distinctly finer and sparser anteriorly and posteriorly;

Figure 6. Habitus and colouration of *Exocelina amabilis* sp. nov., holotype.

diameter of punctures almost equal to diameter of cells of microreticulation. Pronotum with much sparser and finer punctation than head. Elytra with very sparse and fine punctation, often inconspicuous. Pronotum and elytra with distinctly impressed microreticulation; head with microreticulation stronger. Metaventrite and metacoxae distinctly microreticulate, metacoxal plates with longitudinal strioles and transverse wrinkles. Abdominal ventrites with distinct microreticulation, strioles, and very fine and sparse punctation.
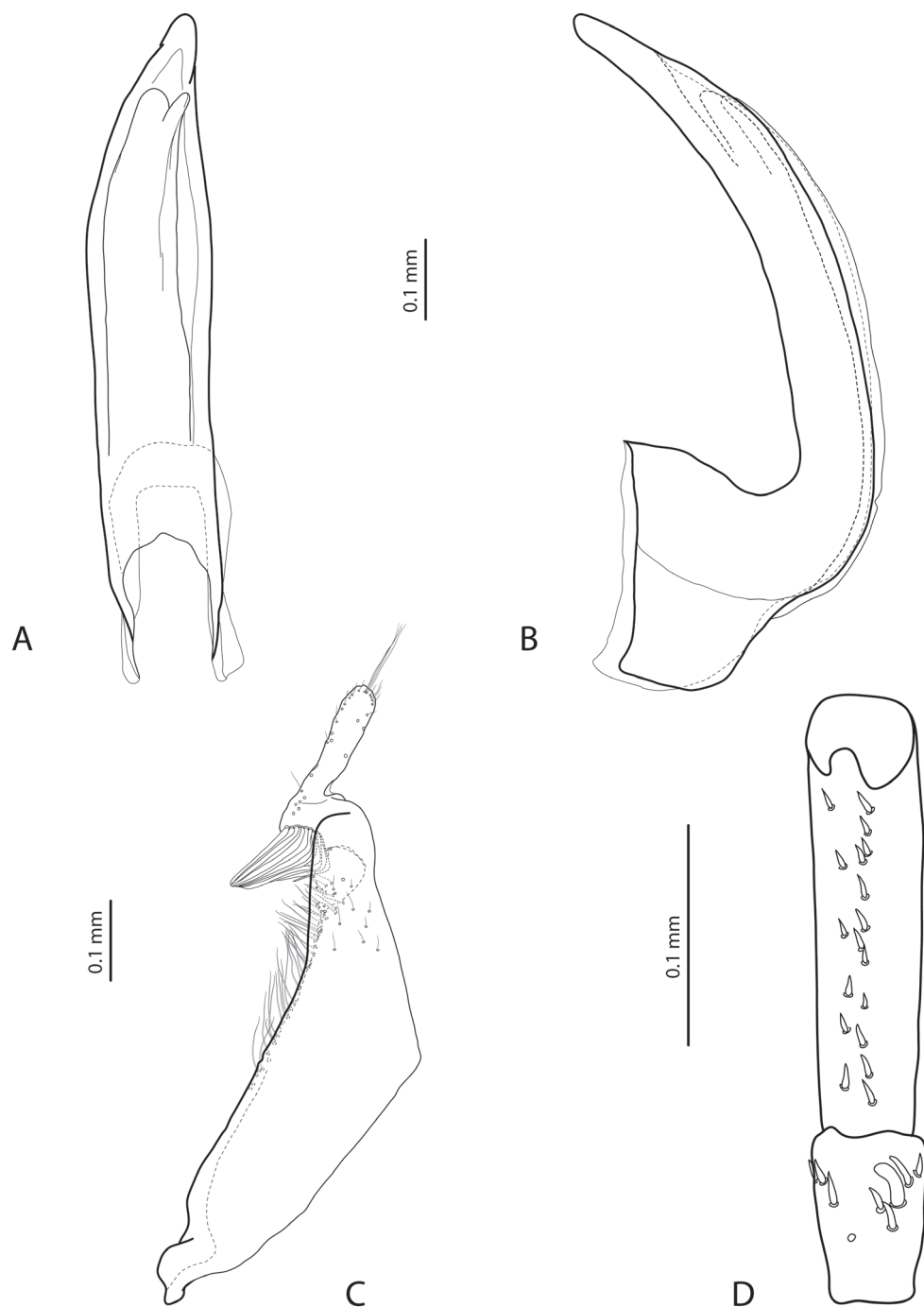
Figure 7. *Exocelina amabilis* sp. nov., paratype **A** median lobe in ventral view **B** median lobe in lateral view **C** right paramere in external view **D** right male protarsomeres 4–5 in ventral view.

Structures: Pronotum with narrow lateral bead. Base of prosternum and neck of prosternal process with distinct ridge, slightly rounded anteriorly. Blade of prosternal process lanceolate, relatively broad, slightly convex, with distinct lateral bead and few setae.

Male: Antenna simple. Pro- and mesotarsomeres 1–3 not dilated. Protarsomere 4 cylindrical, narrow, with medium-sized, thick, slightly curved anterolateral hook-like seta. Protarsomere 5 ventrally with anterior row of 12 and posterior row of 6 short, thick, pointed setae (Fig. 7D). Median lobe with slightly discontinuous outline (see apically); in lateral view, more or less evenly curved, with broadly pointed apex; in ventral view, tapering to broadly pointed apex (Fig. 7A, B). Paramere with very deep dorsal notch, separating subdistal part; subdistal part broad, transverse, situated under apex of proximal part, with large tuft of dense, thick, flattened setae; proximal setae sparse, thin, much more inconspicuous than subdistal (Fig. 7C). Abdominal ventrite 6 with extremely weak, small medial impression, visible only apically, concave apically, with 12–16 lateral striae on each side.

*Female*: Pro- and mesotarsi not modified. Abdominal ventrite 6 slightly truncate or very slightly concave apically, without medial impression and lateral striae.

Habitat. The specimens were collected from the gravel banks of a primary forest stream. The beetles were mainly hidden in the coarse gravel and were only obtained after some digging.

Distribution. Indonesia: Papua Province. The species is known only from the type locality (Fig. 5).

Etymology. The species name is a Latin adjective and means "loveable".

Affinities. The species evidently belongs to the *E. ekari* group due to the discontinuous outline of its median lobe. It is similar to *E. utowaensis* Shaverdo, Hendrich & Balke, 2012 in modification of the abdominal ventrite 6, body size, colouration and shape but distinctly differs from it in having a pronotal bead (absent in *E. utowaensis*) and different shape of the median lobe, paramere, and anterolateral hook-like seta of the male protarsomere 4 (it is also larger in *E. utowaensis*). The species is also similar to *E. athesphatos* in modification of the abdominal ventrite 6 and paramere, but distinctly differs from it in smaller size and having simple male antennae.

Exocelina mimika Shaverdo & Balke, sp. nov.
http://zoobank.org/368BA68B-265B-4FEC-BCF0-080373747401
Figs 5, 8, 9

Type locality. Indonesia: Papua Province, Mimika Regency, Tsinga Village, Beanekogom River, 04°11.629'S, 137°13.756'E, 1690 m a.s.l.

Type material. *Holotype*: male "Indonesia: Papua, Kabupaten [Regency] Mimika, Desa [Village] Tsinga, Sungai [River] Beanekogom,", "1690m, 25–30.v.2017, 04°11.629'S, 137°13.756'E, B. Sumoked (Pap66-Bob04)" (MZB). *Paratypes*: 3 males with the same label as the holotype (KSP, MZB).

Figure 8. Habitus and colouration of *Exocelina mimika* sp. nov., holotype.

Description. *Body size and form***:** Beetle medium-sized: TL-H 4.8–5.0 mm, TL 5.2–5.4 mm, MW 2.5–2.6 mm (holotype: TL-H 4.8 mm, TL 5 mm, MW 2.5 mm), with oblong-oval habitus (Fig. 8).

*Colouration***:** Dorsally piceous (Fig. 8), sometimes with dark brown posterior part of head, lateral sides of pronotum, and dark brown elytral sutural lines; head append-ages yellowish brown, legs yellowish brown to reddish-brown. Teneral specimens paler.
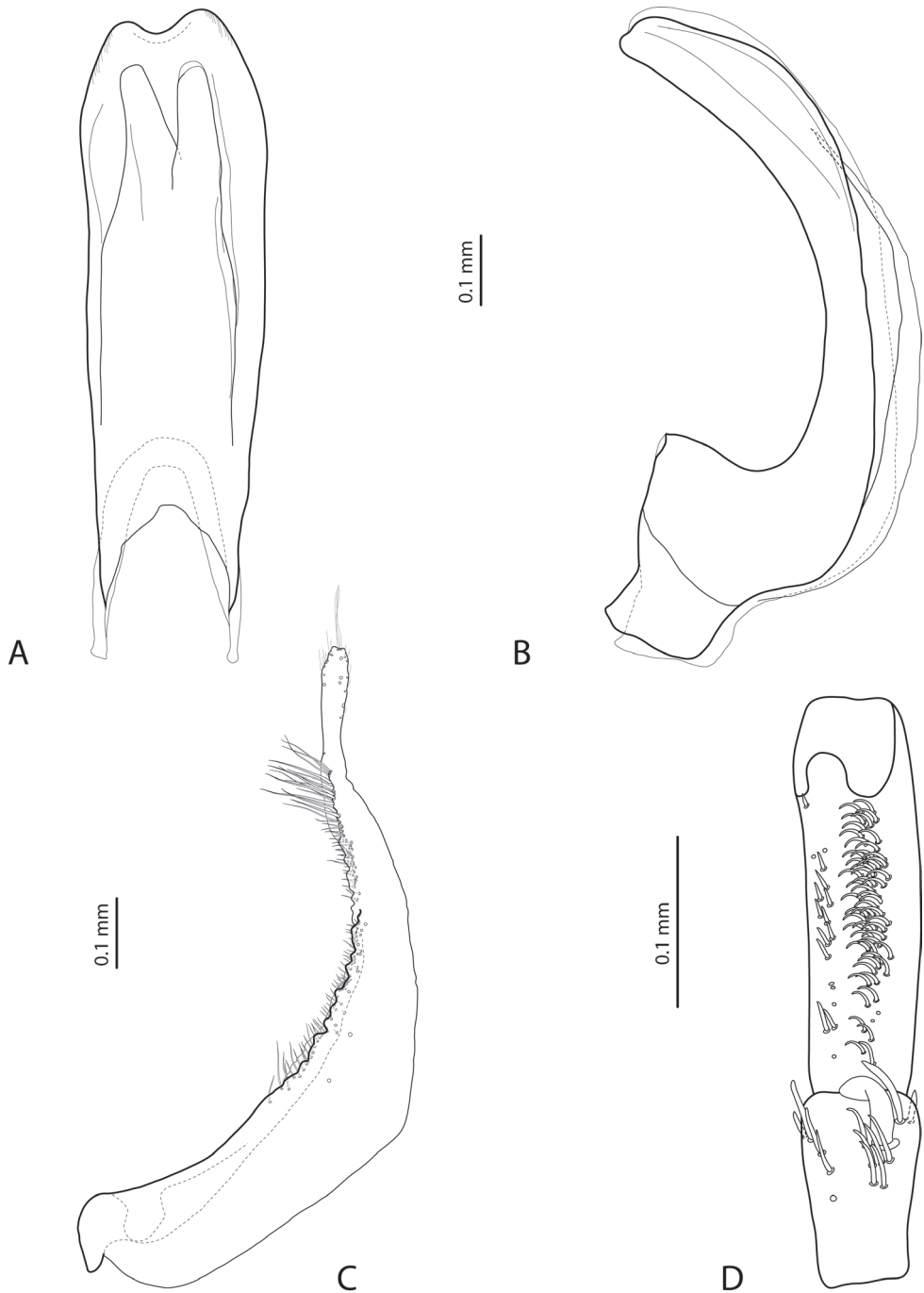
Figure 9. *Exocelina mimika* sp. nov., paratype **A** median lobe in ventral view **B** median lobe in lateral view **C** right paramere in external view **D** right male protarsomeres 4–5 in ventral view.

Surface sculpture: Shiny dorsally, with distinct punctation and weakly impressed microreticulation. Head with relatively dense, unevenly distributed punctation (spaces between punctures 1–4 times size of punctures), distinctly finer and sparser anteriorly and posteriorly; diameter of punctures equal to diameter of cells of microreticulation. Pronotum and elytra with punctation sparser and finer than on head and with distinct but weakly impressed microreticulation; head with stronger microreticulation. Metaventrite and metacoxae distinctly microreticulate, metacoxal plates with longitudinal strioles and transverse wrinkles. Abdominal ventrites with distinct microreticulation, strioles, and fine but distinct punctation.

Structures: Pronotum with distinct lateral bead. Base of prosternum and neck of prosternal process with distinct ridge, very slightly rounded anteriorly. Blade of prosternal process lanceolate, narrow, slightly convex, with distinct lateral bead and few setae. Abdominal ventrite 6 broadly rounded.

Male: Antenna simple. Pro- and mesotarsomeres 1–3 very slightly dilated. Protarsomere 4 simple, narrow, with large, thick, strongly curved anterolateral hook-like seta. Protarsomere 5 ventrally with anterior band of more than 50 and posterior row of 20 relatively long setae (Fig. 9D). Median lobe robust, with continuous outline and without setation; lateral margins thick in apical half; apex with very short blunt prolongation in lateral view and deeply concave in ventral view (Fig. 9A, B). Paramere without dorsal notch and with reduced setation: setae very thin, inconspicuous, sparse, longer subdistally and proximally and very short medially (Fig. 9C). Abdominal ventrite 6 broadly rounded, with 2–7 lateral striae on each side.

Female: Unknown.

Habitat. The specimens were collected from rock pools at the edge of a fast-flowing mountain stream.

Distribution. Indonesia: Papua Province. The species is known only from the type locality (Fig. 5).

Etymology. The species is named after Mimika Regency, where it was collected. The name is a noun in the nominative singular standing in apposition.

Affinities and introduction of the *E. skalei* group. The other *Exocelina* species known from the Tsinga area is *E. tsinga* Shaverdo et al., 2020, which is very similar to the new species in size, body shape, colouration, and surface sculpture. The males of these species can be easily distinguished due to the modified antennae of *E. tsinga* and different shape and setation of their genitalia; female identification, however, could be problematic.

Based on morphological characters, we cannot place the new species in any known species group. The new species resembles representatives of the *E. jaseminae* group in the shape of the median lobe and *E. mekilensis* Shaverdo & Balke, 2019 and species of the *E. ullrichi* group (Shaverdo and Balke 2014) in the reduced setation of the paramere. However, in the shape of the median lobe and setation of the paramere, the new species is the most similar to *E. skalei*. Thus, we assume that these two species might be closely related. *Exocelina skalei* has been so far treated as a member of the *E. ekari* group but according to the molecular analyses, it is the sister clade of the *E. ekari* group (Toussaint et al. 2014, 2015).

Therefore, for *E. skalei* and *E. mimika* sp. nov., herein we introduce a new species group, the *E. skalei* group, with the following diagnostic characters:

– beetle small or medium-sized (TL-H 2.9–5.0 mm), with continuous body outline of broadly oval or oblong-oval shape;
– elytra without striae/strioles, with distinct punctation, beetles shiny or submatt;
– pronotum with distinct lateral bead;
– antennomeres not modified;
– male protarsomere 4 simple, with medium-sized or large, slightly or strongly curved anterolateral hook-like seta;
– median lobe of aedeagus without setation, with continuous or slightly discontinuous apically outline;
– paramere without dorsal notch and with strongly reduced setation: setae very thin and sparse, some longer setae distinct subdistally; setae tiny, inconspicuous medially and proximally.

Since the southern slopes of the central range is a poorly studied area, we assume that more species of the *E. skalei* group await discovery. The new material, including its molecular analysis, would help to confirm group delimitation.

Acknowledgements

References

Balke M (1998) Revision of New Guinea *Copelatus* Erichson, 1832 (Insecta: Coleoptera: Dytiscidae): The running water species, Part I. Annalen des Naturhistorischen Museum Wien 100B: 301–341.

Broun T (1886) Manual of the New Zealand Coleoptera. Parts III and IV. Wellington, Government Printer, 817–973.

Miller KB, Nilsson AN (2003) Homology and terminology: communicating information about rotated structures in water beetles. Latissimus 17: 1–4.

Nilsson AN, Hájek J (2020) A World Catalogue of the Family Dytiscidae, or the Diving Beetles (Coleoptera, Adephaga). Internet version, 2020-01-01. http://www.waterbeetles.eu

Shaverdo HV, Sagata K, Balke M (2005) Five new species of the genus *Papuadytes* Balke, 1998 from New Guinea (Coleoptera: Dytiscidae). Aquatic Insects 27(4): 269–280. https://doi.org/10.1080/01650420500290169

Shaverdo HV, Surbakti S, Hendrich L, Balke M (2012) Introduction of the *Exocelina ekari*-group with descriptions of 22 new species from New Guinea (Coleoptera, Dytiscidae, Copelatinae). ZooKeys 250: 1–76. https://doi.org/10.3897/zookeys.250.3715

Shaverdo HV, Balke M (2014) *Exocelina kinibeli* sp.n. from Papua New Guinea, a new species of the *E. ullrichi*-group (Coleoptera: Dytiscidae). Koleopterologische Rundschau 84: 31–40.

Shaverdo H, Sagata K, Panjaitan R, Menufandu H, Balke M (2014) Description of 23 new species of the *Exocelina ekari*-group from New Guinea, with a key to all representatives of the group (Coleoptera, Dytiscidae, Copelatinae). ZooKeys 468: 1–83. https://doi.org/10.3897/zookeys.468.8506

Shaverdo H, Panjaitan R, Balke M (2016) A new, widely distributed species of the *Exocelina ekari*-group from West Papua (Coleoptera, Dytiscidae, Copelatinae). ZooKeys 554: 69–85. https://doi.org/10.3897/zookeys.554.6065

Shaverdo HV, Balke M (2019) A new species of the *Exocelina ekari* group and new faunistic data on 12 species of *Exocelina* BROUN, 1886 from New Guinea (Coleoptera: Dytiscidae). Koleopterologische Rundschau 89: 1–10.

Shaverdo H, Surbakti S, Sumoked B, Balke M (2020) Two new species of the *Exocelina ekari* group from New Guinea with strongly modified male antennae (Coleoptera, Dytiscidae, Copelatinae). ZooKeys 960: 63–78. https://doi.org/10.3897/zookeys.960.55007

Toussaint EFA, Hall R, Monaghan MT, Sagata K, Ibalim S, Shaverdo HV, Vogler AP, Pons J, Balke M (2014) The towering orogeny of New Guinea as a trigger for arthropod megadiversity. Nature Communications 5: e4001. https://doi.org/10.1038/ncomms5001

Toussaint EFA, Hendrich L, Shaverdo H, Balke M (2015) Mosaic patterns of diversification dynamics following the colonization of Melanesian islands. Scientific Reports 5: e16016. https://doi.org/10.1038/srep16016

RESEARCH ARTICLE

The marine fishes of St Eustatius Island, northeastern Caribbean: an annotated, photographic catalog

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Abstract

Sint Eustatius (Statia) is a 21 km² island situated in the northeastern Caribbean Sea. The most recent published sources of information on that island's marine fish fauna is in two non-governmental organization reports from 2015–17 related to the formation of a marine reserve. The species-list in the 2017 report was based on field research in 2013–15 using SCUBA diving surveys, shallow "baited underwater video surveys" (BRUVS), and data from fishery surveys and scientific collections over the preceding century. That checklist comprised 304 species of shallow (mostly) and deep-water fishes. In 2017 the Smithsonian Deep Reef Observation Project surveyed deep-reef fishes at Statia using the crewed submersible Curasub. That effort recorded 120 species, including 59 new occurrences records. In March-May 2020, two experienced citizen scientists completed 62 SCUBA dives there and recorded 244 shallow species, 40 of them new records for Statia. The 2017–2020 research effort increased the number of species known from the island by 33.6% to 406. Here we present an updated catalog of that marine fish fauna, including voucher photographs of 280 species recorded there in 2017 and 2020. The Statia reef-fish fauna likely is incompletely documented as it has few small, shallow, cryptobenthic species, which are a major component of the regional fauna. A lack of targeted sampling is probably the major factor explaining that deficit, although a limited range of benthic marine habitats may also be contributing.

Introduction

Sint Eustatius island, known locally as Statia, is a 21 km² island in the northeastern Caribbean, and is one of the Leeward Islands in the Lesser Antilles. Until recently there were very few published accounts relating to the marine-fish fauna of Statia. The most comprehensive are represented by two non-governmental organization (NGO) environmental reports to the Statia government by van Kuijk et al. (2015) and Davies and Piontek (2016, 2017). Those two reports referred to only one older scientific publication, by Metzelaar (1919), relating to the fish fauna of that island, among other islands of the Dutch Caribbean. Davies and Piontek (2017) combined their own results from visual surveys with information from BRUV (Baited Remote Underwater Video) surveys by van Kuijk et al. (2015), and a variety of historical scientific collections and fisheries surveys to produce a general list of 307 species (modified to 304, see below), which included both deep- and shallow-water species. In this paper we use the results of deep-reef research using a crewed submersible in 2017 and shallow SCUBA surveys in 2020 to add to the checklist of the island's marine fish fauna. We also include voucher photographs of most of the species observed and collected during those two surveys. In addition to representing vouchers for the species records, the photographs are intended for use by managers, citizen scientists, recreational divers and fishers who want to identify fishes they see and catch at Statia. Hopefully they will also stimulate future documentation of previously unreported species there. Finally, we compare aspects of the ecological structure of the Statia fauna to that of the regional, Greater Caribbean fauna to assess how complete the faunal inventory is for Statia.

Materials and methods

Study area

As one of the Dutch Caribbean islands, Statia sits among Saba, Sint Marten and St Kitts and Nevis (Figure 1) and shares a 200-m insular shelf with the last two islands (Suppl. material 1: Figure S1). Statia is surrounded by a narrow 200-m shelf, which is most extensive on the leeward, western side (Figure 2). The island has a limited diversity of marine habitats. It lacks large, deep embayments, particularly on the western side, that would otherwise provide sheltered locations for development of fringing and back-reef areas. Statia has little well-developed coral reef and most reef areas are of relatively low relief. Due to the general degree of exposure of the entire island to ocean swells it lacks
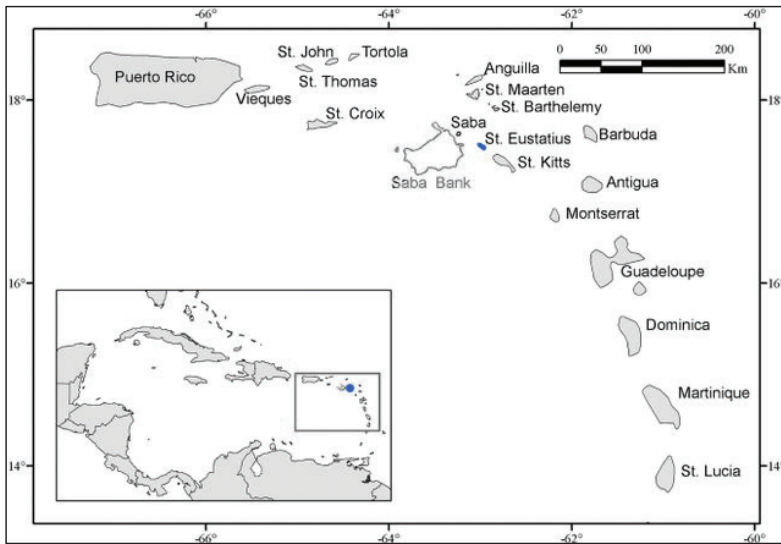
Figure 1. Location of Sint Eustatius. The Caribbean Sea, with the location of Sint Eustatius island indicated in the inset. Source: Hoetjes and Carpenter (2010: fig. 1).

Figure 2. Study sites at Sint Eustatius Island. Location of dive sites during 2017 and 2020: Black stars indicate submersible dives, blue stars 2017 SCUBA dives, red stars 2020 SCUBA dives (some individual stars indicate multiple dives in very close proximity), purple star an intertidal snorkeling site, and the red outline shows limits of the shore-diving area in 2020. See Suppl. material 2: Table S1 for georeferenced date on dive sites. Generalized 20 m, 30 m, 200 m and 500 m isobaths in blue; other lines indicate marine and terrestrial reserve areas. (Base map from Statiaparks, openstreemap.org, CC-BY-SA 2.0 with bathymetry data corrected from CARMABI/WWF/E.Imms (https://www.dcbd.nl/document/bathymetry-map-seas-surrounding-st-eustatius-saba-and-st-maarten, accessed 10 July 2020)

any mangroves and has little in the way of seagrass beds, which are now dominated by a non-native species of *Halodule* (van Kuijk et al. 2015; Hoeksema 2016).

The Caribbean Sea, with the location of Sint Eustatius island indicated in the inset. Source: Hoetjes and Carpenter (2010: fig. 1).

Data sources

Published species lists

A comprehensive set of species records came from two NGO studies, which were included in a report by Hoeksema (2016). van Kuijk et al. (2015) recorded 107 species during "baited underwater video surveys" (BRUVs) at 104 sites in shallow water (<30 m deep) scattered around all sides of the island in 2013. Davies and Piontek (2016, 2017) recorded 206 species during 38 of their own shallow, roving-diver surveys in 2015, and augmented that list with a list of species they extracted from historical literature, museum records (from major online aggregators, see below), photographs of fishes caught at the island that they obtained from various sources, and fisheries surveys. They added the species recorded by van Kuijk et al. (2015) to those they had seen and extracted from other sources to produce a combined list of 307 species.

Research in 2017 and 2020

In 2017 the Smithsonian Institution's Deep Reef Observation Project (DROP) worked with the crewed submersible Curasub to make collections and observations on deep-reef fishes at Statia, to complement similar prior work at the Antillean islands of Dominica and Curaçao (e.g., Baldwin et al. 2018). The submersible was launched close to shore from the tender vessel R/V Chapman and towed by a surface boat to locations along the outer reef slope off the southwest coast where the shallow reef flat transitioned to the slope (~ 40–50 m). Eleven submersible dives were made off the southwestern edge of the island's 200 m platform (see Figure 2, and Suppl. material 2: Table S1). Each dive lasted approximately five hours and reached a maximum depth of 143–305 m, depending on the habitat at that particular site. Submersible surveys follow the methods used by Baldwin et al. (2018). Dives were roving surveys with the submersible facing the reef and moving laterally while slowly descending the slope. Periodically, stops were made to collect specimens using an anesthetic (quinaldine in ethanol) ejection system attached to the sub's manipulator arms, coupled with a suction pump attached to one arm that emptied into a holding chamber. On five of the eleven dives visual records of fishes were obtained by CB and LT, who were seated in the front of the submersible and linked their sightings of identifiable fishes to depth measurements recorded from a digital depth gauge inside the submersible. High-definition video was also recorded on five dives from a camera mounted on the front of the sub. Five scuba-based collection dives to a maximum depth of 20 m were also made by LT and CB, who were targeting sponge-associated gobies. A total of 210 specimens was collected, and 6475 individu-

als were recorded from visual observations during the SCUBA and submersible dives by DROP. Some of those specimens represent undescribed species or belong to groups with uncertain taxonomy.

Two of the authors, CJE and AME, are citizen scientists with extensive experience photographing reef fishes at various sites in the Greater Caribbean. In 2020 they spent two months (mid-March to mid-May) living at Statia and SCUBA diving daily to obtain photographic vouchers of the fishes they observed. They made 62 dives, each of approximately one-hour duration, at depths between 1–30 m on both hard-reef, sand, rubble and seagrass habitats, as well as on sunken wrecked ships. Half of those dives were nearshore in a restricted area, as, during the second half of their stay at the island, they lacked dive-boat support and were able to dive only from the shoreline (see Figure 2, and Suppl. material 2: Table S1). During those dives CJE and AME accumulated photographs of the great majority of fish species they saw. They also obtained recent photographs of a few species taken by local divers and fishers at Statia that they did not see or photograph themselves.

Online aggregators

In addition, we also assessed information provided by three major aggregators of online georeferenced location data on marine fishes (GBIF https://www.gbif.org/, OBIS https://obis.org/, and FishNet2 http://www.fishnet2.net/search.aspx, all accessed on 7 May 2020), searching for records in ~ 120-km^2 quadrat based on Admiralty Chart 487G that encompassed Statia and the surrounding shelf area: the area bounded by 17.433°N to 17.533°N and – 62.933°W to – 63.033°W. That quadrat contained almost 100 km^2 of marine habitat. That area is a little larger than and centered on the area shown in Figure 2. Those sites regularly update the information they contain and might have had additional records to those found by Davies and Piontek (2017).

Location of dive sites during 2017 and 2020: Black stars indicate submersible dives, blue stars 2017 SCUBA dives, red stars 2020 SCUBA dives (some individual stars indicate multiple dives in very close proximity), purple star an intertidal snorkeling site, and the red outline shows limits of the shore-diving area in 2020. See Suppl. material 2: Table S1 for georeferenced date on dive sites. Generalized 20 m, 30 m, 200 m and 500 m isobaths in blue; other lines indicate marine and terrestrial reserve areas. (Base map from Statiaparks, openstreemap.org, CC-BY-SA 2.0 with bathymetry data corrected from CARMABI/WWF/E.Imms (https://www.dcbd.nl/document/bathymetry-map-seas-surrounding-st-eustatius-saba-and-st-maarten, accessed 10 July 2020)

The structure of the Statia reef-fish fauna

Zoogeography

Members of the entire Statia fauna as currently known (Table 1; hereafter Statia20) were assessed in terms of their global and local geographical ranges, as follows: (a) Endemism

Table 1. Updated checklist of marine fishes from Sint Eustatius Island, 2020. Key to column headings and entries: **DROP** – CP = collected and photographed; C collected only; V = visual observation only; **Estapé** – P = photographed by CJE and AME; (P) photographed by 3rd parties; V = visual observation only by CJE and AME. **New** – species is a new record resulting from 2017–20 research, and its source. Other sources of species records are van Kuijk et al. 2015 (**vK15**), Davies and Piontek 2017 (**DP17**), **GBIF**, and **OBIS**. DROP in GBIF indicates record in GBIF is derived from 2017 DROP collection specimens deposited in the fish collection of the US National Museum of Natural History. FishNet 2 records are not indicated separately because all such records are included by GBIF. **NA**- not applicable to non-native *Pterois volitans*. **Plate** – number indicates supplemental plate containing the voucher photograph of that species. **Zoogeography (Zoo)**-Global geographic range of species; GC = Greater Caribbean endemic; NWA = GC plus temperate eastern USA; WA = GC plus Brazil; TA = WA plus central or East Atlantic; PAC = Pacific; EP = East Pacific; IWP = Indo-west Pacific; PAN = Pantropical or Circumglobal. **Range** – extent of geographic range – L = range limited, not more than one third of the Greater Caribbean; remainder are more widely distributed in that region. **Deep** – species entirely or largely restricted to depths below 40 m. **Yes** indicates a species conforms to the heading of the column; ? indicates insufficient data.

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
ACANTHURIDAE												
Acanthurus chirurgus (Bloch, 1787)	Doctorfish		V	P	Yes	Yes	Yes	Yes	1	GC		
Acanthurus coeruleus Bloch & Schneider, 1801	Blue Tang		V	P	Yes	Yes	Yes	Yes	1	GC		
Acanthurus tractus Poey, 1860	Northern Ocean Surgeonfish		V	P	Yes	Yes	Yes	Yes	1	GC		
ACHIRIDAE												
Gymnachirus nudus Kaup, 1858	Flabby Sole	Estapé		P						GC		
ACROPOMATIDAE												
Synagrops bellus (Goode & Bean, 1896)	Blackmouth Bass					Yes				WA		Yes
AETOBATIDAE												
Aetobatus narinari (Euphrasen, 1790)	Spotted Eagle Ray			(P)	Yes	Yes			1	WA		
ANTENNARIIDAE												
Antennarius multiocellatus (Valenciennes, 1837)	Longlure Frogfish			P		Yes			1	WA		
Histrio histrio (Linnaeus, 1758)	Sargassumfish			(P)		Yes			1	PAN		
APOGONIDAE												
Apogon aurolineatus (Mowbray, 1927)	Bridle Cardinalfish					Yes				GC		
Apogon maculatus (Poey, 1860)	Flamefish			P		Yes			1	GC		
Apogon pillionatus Bohlke & Randall, 1968	Broadsaddle Cardinalfish	DROP	V							GC		
Apogon planifrons Longley & Hildebrand, 1940	Pale Cardinalfish	Estapé		P						WA		
Apogon pseudomaculatus Longley, 1932	Twospot Cardinalfish	DROP	C				DROP			WA		
Apogon quadrisquamatus Longley, 1934	Sawcheek Cardinalfish			P		Yes			1	WA		
Apogon townsendi (Breder, 1927)	Belted Cardinalfish			P		Yes			1	WA		
Astrapogon puncticulatus (Poey, 1867)	Blackfin Cardinalfish	Estapé		V						WA		
Astrapogon stellatus (Cope, 1867)	Conchfish					Yes				WA		
Paroncheilus affinis (Poey, 1875)	Bigtooth Cardinalfish		V	P		Yes			1	TA		

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Phaeoptyx conklini (Silvester, 1915)	Freckled Cardinalfish	Estapé		P					1	GC		
Phaeoptyx pigmentaria (Poey, 1860)	Dusky Cardinalfish					Yes			1	TA		
ARGENTINIDAE												
Argentina steuarti Cohen & Atsaides, 1969						Yes				GC		Yes
Glossanodon pygmaeus Cohen, 1958	Pygmy Argentine	DROP	CP						1	WA		Yes
ATHERINIDAE												
Atherina harringtonensis Goode, 1877	Reef Silverside					Yes				GC		
Atherinomorus stipes (Müller & Troschel, 1848)	Hardhead Silverside	Estapé		P					1	WA		
AULOSTOMIDAE												
Aulostomus maculatus Valenciennes, 1841	Atlantic Trumpetfish				Yes	Yes	Yes		1	GC		
BALISTIDAE												
Balistes capriscus Gmelin, 1789	Gray Triggerfish			P		Yes			1	TA		
Balistes vetula Linnaeus, 1758	Queen Triggerfish			P	Yes	Yes		Yes	1	TA		
Canthidermis sufflamen (Mitchill, 1815)	Ocean Triggerfish		V			Yes				WA		
Melichthys niger (Bloch, 1786)	Black Durgon			P	Yes	Yes		Yes	1	PAN		
Xanthichthys ringens (Linnaeus, 1758)	Sargassum Triggerfish	DROP	V							WA		
BELONIDAE												
Platybelone argalus argalus (Lesueur, 1821)	Keeltail Needlefish					Yes				WA		
Tylosurus crocodilus (Péron & Lesueur, 1821)	Houndfish			P		Yes			1	PAN		
BLENNIIDAE												
Entomacrodus nigricans Gill, 1859	Pearl Blenny			P		Yes			1	GC		
Hypleurochilus pseudoaequipinnis Bath, 1994	Oyster Blenny	Estapé		P					1	WA		
Hypleurochilus springeri Randall, 1966	Orangespotted Blenny	Estapé		P					1	GC		
Hypsoblennius exstochilus Bohlke, 1959	Longhorn Blenny			(P)		Yes			2	GC		
Ophioblennius macclurei (Silvester, 1915)	Redlip Blenny			P		Yes			2	GC		
Parablennius marmoreus (Poey, 1876)	Seaweed Blenny			P		Yes			2	WA		
BOTHIDAE												
Bothus lunatus (Linnaeus, 1758)	Peacock Flounder			P	Yes	Yes			2	TA		
Bothus ocellatus (Agassiz, 1831)	Eyed Flounder			P		Yes			2	WA		
Chascanopsetta lugubris Alcock, 1894	Pelican Flounder					Yes				TA,IWP		Yes
CALLIONYMIDAE												
Callionymus bairdi (Jordan, 1888)	Lancer Dragonet			P		Yes			2	WA		
Foetorepus species		DROP	CP						13	WA?	?	Yes
CAPROIDAE												
Antigonia capros Lowe, 1843	Deepbody Boarfish	DROP	V							TA,IWP		Yes
CARANGIDAE												
Alectis ciliaris (Bloch, 1787)	African Pompano					Yes				PAN		

Species in families	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Caranx bartholomaei (Cuvier, 1833)　Yellow Jack			P		Yes			2	TA		
Caranx crysos (Mitchill, 1815)　Blue Runner			P		Yes			2	TA		
Caranx hippos (Linnaeus, 1766)　Crevalle Jack					Yes				WA		
Caranx latus Agassiz, 1831　Horse-eye Jack			P	Yes	Yes			2	TA		
Caranx lugubris Poey, 1860　Black Jack		V		Yes	Yes	Yes			PAN		
Caranx ruber (Bloch, 1793)　Bar Jack		V	P	Yes	Yes		Yes	2	WA		
Decapterus macarellus (Cuvier, 1833)　Mackerel Scad			P		Yes			2	PAN		
Decapterus punctatus (Cuvier, 1829)　Round Scad			P		Yes			2	TA		
Elagatis bipinnulata (Quoy & Gaimard, 1825)　Rainbow Runner			P		Yes			2	PAN		
Selar crumenophthalmus (Bloch, 1793)　Bigeye Scad			P		Yes			2	PAN		
Seriola rivoliana Valenciennes, 1833　Almaco Jack			P	Yes	Yes			2	PAN		
Trachinotus falcatus (Linnaeus, 1758)　Permit			P		Yes			2	WA		
Trachinotus goodei Jordan & Evermann, 1896　Palometa			P		Yes			2	WA		
CARCHARHINIDAE											
Carcharhinus leucas (Müller & Henle, 1839)　Bull Shark					Yes				PAN		
Carcharhinus limbatus (Müller & Henle, 1839)　Blacktip Shark				Yes	Yes				PAN		
Carcharhinus perezii (Poey, 1876)　Reef Shark			V	Yes	Yes				WA		
Galeocerdo cuvier (Peron & Lesueur, 1822)　Tiger Shark					Yes				PAN		
Negaprion brevirostris (Poey, 1868)　Lemon Shark			P		Yes			2	TA,EP		
CENTROPHORIDAE											
Centrophorus granulosus (Bloch & Schneider, 1801)　Large Gulper Shark					Yes				TA,IWP		Yes
CHAENOPSIDAE											
Acanthemblemaria aspera (Longley, 1927)　Roughhead Blenny			P		Yes			2	GC		
Acanthemblemaria maria Böhlke, 1961　Secretary Blenny			P	Yes	Yes	Yes		2	GC		
Acanthemblemaria spinosa Metzelaar, 1919　Spinyhead Blenny			P	Yes	Yes	Yes		2	GC		
Chaenopsis limbaughi Robins & Randall, 1965　Yellowface Pikeblenny			P		Yes			2	GC		
Emblemaria pandionis Evermann & Marsh, 1900　Sailfin Blenny			P	Yes	Yes			2	GC		
Emblemaria vitta Williams, 2002　Ribbon Blenny	Estapé		(P)					2	GC		
Emblemariopsis bahamensis Stephens, 1961　Blackhead Blenny	Estapé		P					3	GC	L	
Emblemariopsis carib Victor, 2010　Carib Blenny	Estapé		P					3	GC	L	
CHAETODONTIDAE											
Chaetodon capistratus Linnaeus, 1758　Foureye Butterflyfish		V	P	Yes	Yes	Yes	Yes	3	GC		
Chaetodon ocellatus Bloch, 1787　Spotfin Butterflyfish			P	Yes	Yes		Yes	3	WA		
Chaetodon sedentarius Poey, 1860　Reef Butterflyfish		V		Yes	Yes		Yes		WA		
Chaetodon striatus Linnaeus, 1758　Banded Butterflyfish		V	P	Yes	Yes	Yes	Yes	3	WA		
Prognathodes aculeatus (Poey, 1860)　Longsnout Butterflyfish		C	P	Yes	Yes	Yes	Yes	3	WA		

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Prognathodes guyanensis (Durand, 1960)	Guyana Butterflyfish	DROP	V							GC		Yes
CHAUNACIDAE												
Chaunax suttkusi Caruso, 1989	Pale-cavity Gaper					Yes				TA		Yes
CHIMAERIDAE												
Chimaera cubana Howell Rivero, 1936	Cuban Chimaera					Yes				GC		Yes
Hydrolagus alberti Bigelow & Schroeder, 1951	Gulf Chimaera					Yes				GC		Yes
CHLOPSIDAE												
Chilorhinus suensonii Lutken, 1852	Seagrass Eel					Yes				WA		
CHLOROPHTHALMIDAE												
Chlorophthalmus agassizi Bonaparte, 1840	Shortnose Greeneye					Yes				TA		Yes
Parasudis truculenta (Goode & Bean, 1895)	Longnose Greeneye					Yes				WA		Yes
CIRRHITIDAE												
Amblycirrhitus pinos (Mowbray, 1927)	Redspotted Hawkfish			P		Yes	Yes		3	WA		
CLUPEIDAE												
Harengula clupeola (Cuvier, 1829)	False Pilchard					Yes				WA		
Harengula humeralis (Cuvier, 1829)	Redear Sardine					Yes				GC		
Jenkinsia lamprotaenia (Gosse, 1851)	Dwarf Herring					Yes				GC		
Opisthonema oglinum (Lesueur, 1818)	Atlantic Thread Herring					Yes				WA		
Sardinella aurita Valenciennes, 1847	Spanish Sardine					Yes				TA		
CONGRIDAE												
Ariosoma balearicum (Delaroche, 1809)	Bandtooth Conger	Estapé		(P)		Yes			3	TA		
Heteroconger longissimus Gunther, 1870	Brown Garden Eel			P	Yes	Yes	Yes		3	WA		
Xenomystax bidentatus (Reid, 1940)	Twopatched-teeth Conger					Yes				TA		Yes
CORYPHAENIDAE												
Coryphaena hippurus Linnaeus, 1758	Dolphinfish							Yes		PAN		
CRURIRAJIDAE												
Cruriraja rugosa Bigelow & Schroeder, 1958	Rough Leg Skate					Yes				GC		Yes
CYNOGLOSSIDAE												
Symphurus marginatus (Goode & Bean, 1886)	Margined Tonguefish					Yes				WA		Yes
DACTYLOPTERIDAE												
Dactylopterus volitans (Linnaeus, 1758)	Flying Gurnard			P	Yes	Yes	Yes		3	TA		
DASYATIDAE												
Hypanus americanus Hildebrand & Schroeder, 1928	Southern Stingray			P	Yes	Yes	Yes		3	WA		
DIODONTIDAE												
Chilomycterus antillarum Jordan & Rutter, 1897	Web Burrfish			P	Yes	Yes	Yes		3	WA		
Chilomycterus schoepfii (Walbaum, 1792)	Striped Burrfish				Yes					NWA		

Species in families	English common name	New	DROP	vK15	Estapé	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Diodon holocanthus Linnaeus, 1758	Balloonfish				P	Yes			3	PAN		
Diodon hystrix Linnaeus, 1758	Porcupinefish			Yes	P	Yes	Yes		3	PAN		
DIRETMIDAE												
Diretmus argenteus Johnson, 1864	Silver Spinyfish					Yes				PAN		Yes
ECHENEIDAE												
Echeneis naucrates Linnaeus, 1758	Sharksucker			Yes	P	Yes	Yes		3	PAN		
Echeneis neucratoides Zuiew, 1786	Whitefin Sharksucker	Estapé			P				3	NWA		
Remora remora (Linnaeus, 1758)	Remora					Yes				PAN		
EPHIPPIDAE												
Chaetodipterus faber (Broussonet, 1782)	Atlantic Spadefish					Yes				WA		
ETMOPTERIDAE												
Etmopterus hillianus (Poey, 1861)	Caribbean Lantern Shark					Yes				NWA		Yes
Etmopterus robinsi Schofield & Burgess, 1997	West Indian Lantern Shark					Yes				GC		Yes
FISTULARIIDAE												
Fistularia tabacaria Linnaeus, 1758	Bluespotted Cornetfish			Yes	P	Yes			3	TA		
GERREIDAE												
Eucinostomus jonesii (Gunther, 1879)	Slender Mojarra					Yes				WA		
Eucinostomus lefroyi (Goode, 1874)	Mottled Mojarra				P	Yes			3	WA		
Gerres cinereus (Walbaum, 1792)	Yellowfin Mojarra					Yes				WA		
GINGLYMOSTOMATIDAE												
Ginglymostoma cirratum (Bonnaterre, 1788)	Nurse Shark			Yes	(P)	Yes	Yes		3	TA		
GOBIESOCIDAE												
Derilissus lombardii Sparks & Gruber, 2012	Tailspot Clingfish	DROP	CP						3	GC		Yes
GOBIIDAE												
Antilligobius nikkiae Van Tassell & Colin, 2012	Sabre Goby	DROP	CP				DROP		3	GC		Yes
Bathygobius antilliensis Tornabene, Baldwin & Pezold, 2010	Antilles Frillfin	Estapé			P				3	GC		
Coryphopterus dicrus Bohlke & Robins, 1960	Colon Goby				P	Yes			3	WA		
Coryphopterus eidolon Bohlke & Robins, 1960	Pallid Goby				P	Yes			3	GC		
Coryphopterus glaucofraenum Gill, 1863	Bridled Goby				P	Yes			3	WA		
Coryphopterus hyalinus Bohlke & Robins, 1962	Glass Goby				P	Yes			4	GC		
Coryphopterus kuna Victor, 2007	Kuna Goby	Estapé			P				4	GC		
Coryphopterus lipernes Bohlke & Robins, 1962	Peppermint Goby				P	Yes			4	GC		
Coryphopterus personatus (Jordan & Thompson, 1905)	Masked Goby		V		P	Yes			4	GC		
Coryphopterus thrix Bohlke & Robins, 1960	Bartail Goby				P	Yes			4	WA		
Coryphopterus tortugae (Jordan, 1904)	Sand Goby				P	Yes			4	GC		
Coryphopterus venezuelae Cervigon, 1966	Sand-Canyon Goby	Estapé			P				4	GC		

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Ctenogobius saepepallens (Gilbert & Randall, 1968)	Dash Goby	Estapé		P					4	GC		
Elacatinus chancei (Beebe & Hollister, 1933)	Shortstripe Goby		C	P		Yes	Yes		4	GC	L	
Elacatinus evelynae (Böhlke & Robins, 1968)	Sharknose Goby			P		Yes	Yes		4	GC		
Genus 1 species 5		DROP	CP						13	GC?	?	Yes
Genus 1 species 6		DROP	CP						13	GC?	?	Yes
Genus 2 species 1		DROP	CP						13	GC?	?	Yes
Ginsburgellus novemlineatus (Fowler, 1950)	Ninelined Goby	Estapé		P					4	GC		
Gnatholepis thompsoni Jordan, 1904	Goldspot Goby		V	P		Yes	Yes		4	TA		
Lythrypnus elasson Böhlke & Robins, 1960	Dwarf Goby	DROP/ Estapé	C	P					4	GC		
Micvogobius carri Fowler, 1945	Seminole Goby	Estapé		P					4	WA		
Nes longus (Nichols, 1914)	Orangespotted Goby	Estapé		P		Yes			4	GC		
Palatogobius grandoculus Greenfield, 2002	Bigeye Goby	DROP	CP				DROP		4	GC		Yes
Palatogobius incendius Tornabene, Robertson & Baldwin, 2017	Ember Goby	DROP	C				DROP			GC		Yes
Pinnichthys aimoriensis Van Tassell & Tornabene, 2016	Thiony's Goby	DROP	CP						4	GC		Yes
Priolepis hipoliti (Metzelaar, 1922)	Rusty Goby			P		Yes			4	WA		
Psilotris belenae (Randall, 1968)	Hovering Dartfish		V	P		Yes	Yes		4	GC		
Risor ruber (Rosen, 1911)	Tusked Goby		C	P		Yes			4	WA		
Tigrigobius dilepis (Robins & Böhlke, 1964)	Orangesided Goby			P		Yes			4	GC		
Tigrigobius multifasciatus (Steindachner, 1876)	Greenbanded Goby	Estapé		P					4	GC	L	
Varicus cephalocellatus Gilmore, Van Tassell & Baldwin, 2016	Ocellated Split-Fin Goby	DROP	CP				DROP		4	GC	L	Yes
Varicus veliguttatus Van Tassell, Baldwin & Gilmore, 2016	Spotted-Sail Goby	DROP	CP				DROP		4	GC		Yes
GRAMMATIDAE												
Gramma linki Starck & Colin, 1978	Yellowcheek Basslet	DROP	CP				DROP		5	GC		
Gramma loreto Poey, 1868	Fairy Basslet			P	Yes	Yes			5	GC		
Lipogramma evides Robins & Colin, 1979	Banded Basslet	DROP	CP				DROP		5	GC		Yes
Lipogramma klayi Randall, 1963	Bicolor Basslet	DROP	CP						5	GC		Yes
Lipogramma levinsoni Baldwin, Nonaka & Robertson, 2016	Hourglass Basslet	DROP	CP						5	GC		Yes
Lipogramma regia Robins & Colin, 1979	Royal Basslet	DROP	CP				DROP		5	GC		Yes
Lipogramma trilineata Randall, 1963	Threeline Basslet	DROP	CP				DROP		5	GC		Yes
GRAMMICOLEPIDIDAE												
Grammicolepis brachiusculus Poey, 1873	Thorny Tinselfish					Yes				PAN		Yes
HAEMULIDAE												
Anisotremus surinamensis (Bloch, 1791)	Black Margate			P	Yes	Yes			5	WA		
Brachygenys chrysargyreum (Gunther, 1859)	Smallmouth Grunt			P		Yes	Yes	Yes	5	GC		
Haemulon album Cuvier, 1830	Margate			P	Yes	Yes			5	WA		

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Haemulon aurolineatum Cuvier, 1830	Tomtate			P	Yes	Yes	Yes		5	WA		
Haemulon carbonarium Poey, 1860	Caesar Grunt			P	Yes	Yes	Yes	Yes	5	GC		
Haemulon flavolineatum (Desmarest, 1823)	French Grunt			P	Yes	Yes	Yes	Yes	5	GC		
Haemulon macrostomum Gunther, 1859	Spanish Grunt					Yes				GC		
Haemulon melanurum (Linnaeus, 1758)	Cottonwick			P		Yes			5	WA		
Haemulon parra (Desmarest, 1823)	Sailors Choice					Yes				WA		
Haemulon plumierii (Lacepede, 1801)	White Grunt			P		Yes			5	WA		
Haemulon sciurus (Shaw, 1803)	Bluestriped Grunt			(P)		Yes	Yes		5	GC		
Haemulon striatum (Linnaeus, 1758)	Striped Grunt		V	V		Yes				WA		
Haemulon vittatum (Poey, 1860)	Boga			P		Yes			5	GC		
HALOSAURIDAE												
Halosaurus ovenii Johnson, 1864	Stripejaw Halosaur					Yes				TA/IWP		Yes
HEMIRAMPHIDAE												
Hemiramphus brasiliensis (Linnaeus, 1758)	Ballyhoo			P		Yes			5	WA		
HOLOCENTRIDAE												
Corniger spinosus Agassiz, 1831	Spinycheek Soldierfish	DROP	V							TA		Yes
Holocentrus adscensionis (Osbeck, 1765)	Squirrelfish		V	P	Yes	Yes	Yes		5	TA		
Holocentrus rufus (Walbaum, 1792)	Longspine Squirrelfish		V	P	Yes	Yes	Yes		5	GC		
Myripristis jacobus Cuvier, 1829	Blackbar Soldierfish		V	P		Yes	Yes		5	TA		
Neoniphon coruscum (Poey, 1860)	Reef Squirrelfish			P		Yes			5	GC		
Neoniphon marianus (Cuvier, 1829)	Longjaw Squirrelfish		C	P		Yes	Yes		5	GC		
Neoniphon vexillarium (Poey, 1860)	Dusky Squirrelfish			P		Yes			5	GC		
Ostichthys trachypoma (Gunther, 1859)	Bigeye Soldierfish	DROP	CP				DROP		6	WA		Yes
Plectrypops retrospinis (Guichenot, 1853)	Cardinal Soldierfish	Estapé		P					6	WA		
ISTIOPHORIDAE												
Istiophorus platypterus (Shaw, 1792)	Sailfish					Yes				TA		
Makaira nigricans Lacepede, 1802	Blue Marlin					Yes				PAN		
KYPHOSIDAE												
Kyphosus bigibbus Lacepede, 1801	Gray Seachub	Estapé		P					6	TA/IWP		
Kyphosus cinerascens (Forsskal, 1775)	Topsail Seachub			P		Yes			6	PAN		
Kyphosus sectatrix (Linnaeus, 1766)	Bermuda Chub			P		Yes			6	PAN		
Kyphosus vaigiensis (Quoy & Gaimard, 1825)	Yellow Chub			V		Yes				PAN		
LABRIDAE												
Labrinae												
Bodianus rufus (Linnaeus, 1758)	Spanish Hogfish		V	P	Yes	Yes		Yes	6	WA		
Clepticus parrae (Bloch & Schneider, 1801)	Creole Wrasse		V	P	Yes	Yes	Yes		6	GC		

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Decodon puellaris (Poey, 1860)	Red Hogfish	DROP	CP				DROP		6	WA		Yes
Decodon species 2		DROP	CP						13	GC		Yes
Halichoeres bathyphilus (Beebe & Tee-Van,1932)	Greenband Wrasse	DROP	V							GC		Yes
Halichoeres bivittatus (Bloch, 1791)	Slippery Dick			P	Yes	Yes			6	WA		
Halichoeres cyanocephalus (Bloch, 1791)	Yellowcheek Wrasse			P	Yes	Yes			6	GC		
Halichoeres garnoti (Valenciennes, 1839)	Yellowhead Wrasse		V	P	Yes	Yes	Yes		6	GC		
Halichoeres maculipinna (Müller & Troschel, 1848)	Clown Wrasse			P	Yes	Yes			6	GC		
Halichoeres pictus (Poey, 1860)	Rainbow Wrasse			P		Yes			6	GC		
Halichoeres poeyi (Steindachner, 1867)	Blackear Wrasse			P	Yes	Yes			6	WA		
Halichoeres radiatus (Linnaeus, 1758)	Puddingwife			P	Yes	Yes			6	WA		
Thalassoma bifasciatum (Bloch, 1791)	Bluehead		V	P	Yes	Yes	Yes		6	GC		
Xyrichtys martinicensis Valenciennes, 1840	Rosy Razorfish			P	Yes	Yes			6	GC		
Xyrichtys novacula (Linnaeus, 1758)	Pearly Razorfish			P		Yes			6	WA		
Xyrichtys splendens Castelnau, 1855	Green Razorfish			P	Yes	Yes	Yes		6	GC		
Scarinae												
Cryptotomus roseus Cope, 1871	Bluelip Parrotfish			P		Yes			6	WA		
Scarus coeruleus (Bloch, 1786)	Blue Parrotfish				Yes					GC		
Scarus guacamaia Cuvier, 1829	Rainbow Parrotfish					Yes				GC		
Scarus iseri (Bloch, 1789)	Striped Parrotfish			P	Yes	Yes		Yes	6	GC		
Scarus taeniopterus Desmarest, 1831	Princess Parrotfish		V	P	Yes	Yes	Yes	Yes	6	GC		
Scarus vetula Bloch & Schneider, 1801	Queen Parrotfish			P	Yes	Yes	Yes	Yes	6	GC		
Sparisoma atomarium (Poey, 1861)	Greenblotch Parrotfish			P		Yes			6	GC		
Sparisoma aurofrenatum (Valenciennes, 1840)	Redband Parrotfish		V	P	Yes	Yes	Yes	Yes	7	GC		
Sparisoma chrysopterum (Bloch & Schneider, 1801)	Redtail Parrotfish			P	Yes	Yes		Yes	7	GC		
Sparisoma radians (Valenciennes, 1840)	Bucktooth Parrotfish			P		Yes			7	WA		
Sparisoma rubripinne (Valenciennes, 1840)	Yellowtail Parrotfish			P		Yes		Yes	7	GC		
Sparisoma viride (Bonnaterre, 1788)	Stoplight Parrotfish		V	P	Yes	Yes		Yes	7	GC		
LABRISOMIDAE												
Brockius nigricinctus Howell Rivero, 1936	Spotcheek Blenny	Estapé		P					7	GC		
Gobioclinus bucciferus Poey, 1868	Puffcheek Blenny	Estapé		P					7	GC		
Gobioclinus gobio (Valenciennes, 1836)	Palehead Blenny	Estapé		P					7	WA		
Gobioclinus guppyi (Norman, 1922)	Mimic Blenny	Estapé		P					7	WA		
Labrisomus nuchipinnis (Quoy & Gaimard, 1824)	Hairy Blenny			P	Yes	Yes			7	TA		
Malacoctenus aurolineatus Smith, 1957	Goldline Blenny			P		Yes			7	GC		
Malacoctenus boehlkei Springer, 1959	Diamond Blenny					Yes				GC		
Malacoctenus erdmani Smith, 1957	Imitator Blenny	Estapé		P					7	GC		

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Malacoctenus macropus (Poey, 1868)	Rosy Blenny	Estapé		P					7	GC		
Malacoctenus triangulatus Springer, 1959	Saddled Blenny			P		Yes			7	GC		Yes
LOBOTIDAE												
Lobotes surinamensis (Bloch, 1790)	Atlantic Tripletail									TA/IWP		Yes
LOPHIIDAE												
Lophiodes monodi Le Danois, 1971	Club-bait Goosefish					Yes				GC		Yes
LUTJANIDAE												
Apsilus dentatus Guichenot, 1853	Black Snapper					Yes				GC		Yes
Etelis oculatus (Valenciennes, 1828)	Queen Snapper					Yes				WA		Yes
Lutjanus analis (Cuvier, 1828)	Mutton Snapper			P	Yes	Yes			7	WA		
Lutjanus apodus (Walbaum, 1792)	Schoolmaster		V	P	Yes	Yes	Yes		7	GC		
Lutjanus buccanella (Cuvier, 1828)	Blackfin Snapper		V	P		Yes		Yes	7	WA		
Lutjanus cyanopterus (Cuvier, 1828)	Cubera Snapper			P		Yes			7	WA		
Lutjanus griseus (Linnaeus, 1758)	Gray Snapper			(P)	Yes	Yes			7	TA		
Lutjanus jocu (Bloch & Schneider, 1801)	Dog Snapper			P	Yes	Yes			7	TA		
Lutjanus mahogoni (Cuvier, 1828)	Mahogany Snapper		V	P	Yes	Yes	Yes	Yes	7	GC		
Lutjanus purpureus (Poey, 1866)	Caribbean Red Snapper					Yes				TA		
Lutjanus synagris (Linnaeus, 1758)	Lane Snapper			P	Yes	Yes	Yes		7	TA		
Lutjanus vivanus (Cuvier, 1828)	Silk Snapper					Yes				TA		Yes
Ocyurus chrysurus (Bloch, 1791)	Yellowtail Snapper			P	Yes	Yes		Yes	7	TA		
Pristipomoides sp.[1]			V							WA?	?	Yes
MACROURIDAE												
Gadomus arcuatus (Goode & Bean, 1886)	Doublethread Grenadier					Yes				TA		Yes
Gadomus dispar (Vaillant, 1888)	Onelong Grenadier					Yes				TA		Yes
Hymenocephalus aterrimus Gilbert, 1905	Nobeard Grenadier					Yes				WA/PAC		Yes
Hymenocephalus billsam Marshall & Iwamoto, 1973	Bigeye Grenadier					Yes				WA		Yes
Malacocephalus laevis (Lowe, 1843)	Velvet Grenadier					Yes				PAN		Yes
Nezumia aequalis (Günther, 1878)	Atlantic Blacktip Grenadier					Yes				TA		Yes
Ventrifossa macropogon Marshall, 1973	Longbeard Grenadier					Yes				WA/WPAC		Yes
MALACANTHIDAE												
Malacanthus plumieri (Bloch, 1786)	Sand Tilefish		V	P	Yes	Yes	Yes		7	WA		
MEGALOPIDAE												
Megalops atlanticus Valenciennes, 1847	Tarpon			P		Yes			8	TA		
MERLUCCIIDAE												
Steindachneria argentea Goode & Bean, 1896	Luminous Hake					Yes				GC		Yes

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
MONACANTHIDAE												
Aluterus scriptus (Osbeck, 1765)	Scrawled Filefish			P	Yes	Yes			8	PAN		
Cantherhines macrocerus (Hollard, 1853)	Whitespotted Filefish			P	Yes	Yes	Yes	Yes	8	WA		
Cantherhines pullus (Ranzani, 1842)	Orangespotted Filefish			P	Yes	Yes	Yes	Yes	8	TA		
Monacanthus ciliatus (Mitchill, 1818)	Fringed Filefish			P	Yes	Yes			8	TA		
Monacanthus tuckeri Bean, 1906	Slender Filefish			P	Yes	Yes			8	GC		
Stephanolepis setifer (Bennett, 1831)	Pygmy Filefish			P	Yes	Yes			8	WA		
MUGILIDAE												
Mugil curema Valenciennes, 1836	White Mullet					Yes				TA		
MULLIDAE												
Mulloidichthys martinicus (Cuvier, 1829)	Yellow Goatfish		V	P	Yes	Yes	Yes		8	TA		
Pseudupeneus maculatus (Bloch, 1793)	Spotted Goatfish		V	P	Yes	Yes	Yes		8	WA		
MURAENIDAE												
Echidna catenata (Bloch, 1795)	Chain Moray			P		Yes			8	WA		
Enchelycore carychroa Bohlke & Bohlke, 1976	Chestnut Moray	Estapé		(P)					8	TA		
Enchelycore nigricans (Bonnaterre, 1788)	Viper Moray	Estapé		(P)					8	TA		
Gymnothorax funebris Ranzani, 1839	Green Moray			P	Yes	Yes			8	TA		
Gymnothorax miliaris (Kaup, 1856)	Goldentail Moray			P	Yes	Yes	Yes		8	TA		
Gymnothorax moringa (Cuvier, 1829)	Spotted Moray			P	Yes	Yes	Yes		8	TA		
Gymnothorax vicinus (Castelnau, 1855)	Purplemouth Moray			(P)		Yes			8	TA		
NARCINIDAE												
Narcine bancroftii (Griffith & Smith, 1834)	Lesser Electric Ray					Yes				GC		
OGCOCEPHALIDAE												
Dibranchus atlanticus Peters, 1876	Atlantic Batfish	DROP				Yes				TA		Yes
Ogcocephalus corniger Bradbury, 1980	Longnose Batfish	DROP	CP						8	GC		
Zalieutes mcginyi (Fowler, 1952)	Tricorn Batfish	DROP	CP						8	GC		Yes
OPHICHTHIDAE												
Myrichthys breviceps (Richardson, 1848)	Sharptail Eel				Yes	Yes				WA		
Myrichthys ocellatus (Lesueur, 1825)	Goldspotted Eel	Estapé		P					8	WA		
Ophichthus ophis (Linnaeus, 1758)	Spotted Snake Eel				Yes	Yes				WA		
OPHIDIIDAE												
Brotula barbata (Bloch & Schneider, 1801)	Atlantic Bearded Brotula	DROP	CP				DROP		8	TA		
Neobythites elongatus Nielsen & Retzler, 1994	Elongate Cusk-eel				Yes	Yes				GC		Yes
Parophidion schmidti (Woods & Kanazawa, 1951)	Dusky Cusk-eel	Estapé		P					8	GC		
OPISTOGNATHIDAE												
Opistognathus aurifrons (Jordan & Thompson, 1905)	Yellowhead Jawfish			P	Yes	Yes	Yes		8	WA		
Opistognathus macrognathus Poey, 1860	Banded Jawfish				Yes	Yes				GC		

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Opisthognathus maxillosus Poey, 1860	Mottled Jawfish	Estapé		P					8	GC		
OSTRACIIDAE												
Acanthostracion polygonius Poey, 1876	Honeycomb Cowfish		V	P	Yes	Yes	Yes		8	WA		Yes
Acanthostracion quadricornis (Linnaeus, 1758)	Scrawled Cowfish		V	P		Yes	Yes		9	TA		
Lactophrys bicaudalis (Linnaeus, 1758)	Spotted Trunkfish			P		Yes			9	TA		
Lactophrys trigonus (Linnaeus, 1758)	Trunkfish			P	Yes	Yes			9	TA		
Lactophrys triqueter (Linnaeus, 1758)	Smooth Trunkfish			P	Yes	Yes	Yes		9	WA		
PARALICHTHYIDAE												
Citharichthys cornutus (Gunther, 1880)	Horned Whiff	DROP				Yes				WA		Yes
Gastropsetta frontalis Bean, 1895	Shrimp Flounder		CP				DROP		9	GC		
Syacium micrurum Ranzani, 1842	Channel Flounder			P		Yes			9	WA		
PARAZENIDAE												
Cyttopsis rosea (Lowe, 1843)	Red Dory					Yes				TA/IWP		Yes
PEMPHERIDAE												
Pempheris schomburgkii Müller & Troschel, 1848	Glassy Sweeper			P		Yes			9	WA		
PENTANCHIDAE												
Apristurus canutus Springer & Heemstra, 1979	Hoary Cat Shark					Yes				GC		Yes
Galeus antillensis Springer, 1979	Antilles Sawtail Catshark					Yes				GC	L	Yes
PERCOPHIDAE												
Bembrops ocellatus Thompson & Suttkus, 1998	Ocellate Duckbill					Yes				GC		Yes
Bembrops quadrisella Thompson & Suttkus, 1998	Saddleback Duckbill					Yes				GC		Yes
Chrionema squamentum (Ginsburg, 1955)	Scalychin Flathead	DROP	CP				DROP		9	GC		Yes
PERISTEDIIDAE												
Peristedion truncatum (Gunther, 1880)	Black Armored Searobin					Yes				WA		Yes
POLYMIXIIDAE												
Polymixia lowei Gunther, 1859	Beardfish					Yes				WA		Yes
POMACANTHIDAE												
Centropyge argi Woods & Kanazawa, 1951	Cherubfish		V	P		Yes			9	GC		
Holacanthus ciliaris (Linnaeus, 1758)	Queen Angelfish		V	P	Yes	Yes		Yes	9	WA		
Holacanthus tricolor (Bloch, 1795)	Rock Beauty		V	P	Yes	Yes	Yes	Yes	9	WA		
Pomacanthus arcuatus (Linnaeus, 1758)	Gray Angelfish				Yes	Yes		Yes		WA		
Pomacanthus paru (Bloch, 1787)	French Angelfish		V	P	Yes	Yes	Yes	Yes	9	WA		
POMACENTRIDAE												
Abudefduf saxatilis (Linnaeus, 1758)	Sergeant Major			P		Yes			9	TA		
Abudefduf taurus (Müller & Troschel, 1848)	Night Sergeant			P		Yes			9	TA		
Chromis cf. *enchrysura*[2]		DROP	CP				DROP		13	WA		Yes

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Chromis cyanea (Poey, 1860)	Blue Chromis		V	P	Yes	Yes	Yes		9	GC		
Chromis insolata (Cuvier, 1830)	Sunshinefish		V	P	Yes	Yes	Yes		9	GC		
Chromis multilineata (Guichenot, 1853)	Brown Chromis		V	P	Yes	Yes	Yes		9	TA		
Chromis scotti Emery, 1968	Purple Reeffish	DROP	V							WA		
Microspathodon chrysurus (Cuvier, 1830)	Yellowtail Damselfish			P	Yes	Yes	Yes	Yes	9	WA		
Stegastes adustus (Troschel, 1865)	Dusky Damselfish			P	Yes	Yes			9	GC		
Stegastes diencaeus (Jordan & Rutter, 1897)	Longfin Damselfish			P	Yes	Yes			9	GC		
Stegastes leucostictus (Müller & Troschel, 1848)	Beaugregory				Yes	Yes				GC		
Stegastes partitus (Poey, 1868)	Bicolor Damselfish		V	P	Yes	Yes	Yes		9	GC		
Stegastes planifrons (Cuvier, 1830)	Threespot Damselfish			P		Yes			9	GC		
Stegastes xanthurus (Poey, 1860)	Cocoa Damselfish			P		Yes			9	GC		
PRIACANTHIDAE												
Heteropriacanthus cruentatus (Lacepède, 1801)	Glasseye Snapper			P		Yes	Yes		9	TA		
Priacanthus arenatus Cuvier, 1829	Bigeye		V			Yes				TA		
Pristigenys alta (Gill, 1862)	Short Bigeye	DROP	V							WA		
RHINCODONTIDAE												
Rhincodon typus Smith, 1828	Whale Shark					Yes				PAN		
SCIAENIDAE												
Equetus lanceolatus (Linnaeus, 1758)	Jackknife-fish		V	P	Yes	Yes			10	WA		
Eqetus punctatus (Bloch & Schneider, 1801)	Spotted Drum			P		Yes	Yes		10	WA		
Pareques acuminatus (Bloch & Schneider, 1801)	High-hat			P		Yes			10	WA		
Umbrina coroides Cuvier, 1830	Sand Drum					Yes				WA		
SCOMBRIDAE												
Acanthocybium solandri (Cuvier, 1832)	Wahoo					Yes				PAN		
Euthynnus alletteratus (Rafinesque, 1810)	Little Tunny			P		Yes			10	TA		
Katsuwonus pelamis (Linnaeus, 1758)	Skipjack Tuna					Yes				PAN		
Scomberomorus cavalla (Cuvier, 1829)	King Mackerel			P		Yes			10	WA		
Scomberomorus regalis (Bloch, 1793)	Cero		V	P	Yes	Yes			10	WA		
Thunnus atlanticus (Lesson, 1831)	Blackfin Tuna			P		Yes			10	WA		
SCORPAENIDAE												
Pontinus castor Poey, 1860	Longsnout Scorpionfish	DROP	CP				DROP		10	GC		Yes
Pontinus nematophthalmus (Gunther, 1860)	Spinythroat Scorpionfish	DROP	CP						10	WA		Yes
Pterois volitans (Linnaeus, 1758)	Red Lionfish		V	P			Yes		10	NA	NA	NA
Scorpaena plumieri Bloch, 1789	Spotted Scorpionfish			P		Yes	Yes		10	TA		
Scorpaenodes caribbaeus Meek & Hildebrand, 1928	Reef Scorpionfish	Estapé		P	Yes	Yes			10	WA		
SERRANIDAE												
Alphestes afer (Bloch, 1793)	Mutton Hamlet			(P)		Yes	Yes		10	TA		

Species in families[3]	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Balduinella vivanus (Jordan & Swain, 1885)[3]	Red Barbier	DROP	V							WA		Yes
Bathyanthias species A		DROP	CP						13	GC	L	Yes
Bullisichthys caribbaeus Rivas, 1971	Pugnose Bass	DROP	CP				DROP		10	GC		Yes
Cephalopholis cruentata (Lacepède, 1802)	Graysby		V	P	Yes	Yes	Yes	Yes	10	GC		
Cephalopholis fulva (Linnaeus, 1758)	Coney		V	P	Yes	Yes	Yes	Yes	10	WA		
Diplectrum bivittatum (Valenciennes, 1828)	Dwarf Sand Perch	Estapé		P					10	WA		
Epinephelus adscensionis (Osbeck, 1765)	Rock Hind		V	(P)		Yes			10	TA		
Epinephelus guttatus (Linnaeus, 1758)	Red Hind		V	P	Yes	Yes	Yes	Yes	10	WA		
Epinephelus striatus (Bloch, 1792)	Nassau Grouper					Yes				GC		
Gonioplectrus hispanus (Cuvier, 1828)	Spanish Flag	DROP	V		Yes	Yes				WA		Yes
Hypoplectrus chlorurus (Cuvier, 1828)	Yellowtail Hamlet			P		Yes			10	GC		
Hypoplectrus guttavarius (Poey, 1852)	Shy Hamlet			P		Yes			10	GC		
Hypoplectrus indigo (Poey, 1851)	Indigo Hamlet	DROP	V			Yes				GC		
Hypoplectrus nigricans (Poey, 1852)	Black Hamlet					Yes				GC		
Hypoplectrus puella (Cuvier, 1828)	Barred Hamlet		V	P	Yes	Yes	Yes		10	GC		
Hypoplectrus species 1	Bluelip Hamlet	Estapé		P					13	GC		
Hypoplectrus unicolor (Walbaum, 1792)	Butter Hamlet					Yes				GC		
Liopropoma carmabi (Randall, 1963)	Candy Basslet	DROP	CP				DROP		10	WA		Yes
Liopropoma mowbrayi Woods & Kanazawa, 1951	Cave Basslet	DROP	CP				DROP		10	GC		
Liopropoma olneyi Baldwin & Johnson, 2014	Yellow-Spotted Basslet	DROP	CP				DROP		10	GC	L	Yes
Liopropoma rubre Poey, 1861	Peppermint Basslet			P		Yes			11	GC		
Mycteroperca interstitialis (Poey, 1860)	Yellowmouth Grouper					Yes				WA		
Mycteroperca tigris (Valenciennes, 1833)	Tiger Grouper					Yes				WA		
Mycteroperca venenosa (Linnaeus, 1758)	Yellowfin Grouper			P	Yes	Yes			11	WA		
Paranthias furcifer (Valenciennes, 1828)	Atlantic Creolefish		V	P	Yes	Yes			11	TA		
Plectranthias species A		DROP	CP						13	GC	L	Yes
Pronotogrammus martinicensis (Guichenot, 1868)	Roughtongue Bass	DROP	CP				DROP		11	WA		Yes
Rypticus bistrispinus (Mitchill, 1818)	Freckled Soapfish					Yes				WA		
Rypticus saponaceus (Bloch & Schneider, 1801)	Greater Soapfish		V	P	Yes	Yes	Yes		11	TA		
Serranus annularis (Gunther, 1880)	Orangeback Bass	DROP	V							WA		
Serranus baldwini (Evermann & Marsh, 1899)	Lantern Bass			P	Yes	Yes	Yes		11	WA		
Serranus flaviventris (Cuvier, 1829)	Twinspot Bass					Yes				WA		
Serranus fuscula (Poey, 1861)	Twospot Sea Bass	DROP	CP				DROP		11	WA		Yes
Serranus luciopercanus Poey, 1852	Crosshatch Bass	DROP	V							GC		Yes
Serranus notospilus Longley, 1935	Saddle Bass	DROP	V							GC		
Serranus phoebe Poey, 1851	Tattler		V			Yes				WA		Yes

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Serranus tabacarius (Cuvier, 1829)	Tobaccofish		V	P	Yes	Yes	Yes		11	WA		
Serranus tigrinus (Bloch, 1790)	Harlequin Bass		V	P	Yes	Yes	Yes		11	GC		
Serranus tortugarum Longley, 1935	Chalk Bass		V	P	Yes	Yes	Yes		11	GC		
SETARCHIDAE												
Setarches guentheri Johnson, 1862	Deepwater Scorpionfish					Yes				TA/IWP		Yes
SPARIDAE												
Calamus bajonado (Bloch & Schneider, 1801)	Jolthead Porgy				Yes	Yes				WA		
Calamus calamus (Valenciennes, 1830)	Saucereye Porgy			P	Yes	Yes			11	WA		
Calamus pennatula Guichenot, 1868	Pluma Porgy			P	Yes	Yes			11	WA		
SPHYRAENIDAE												
Sphyraena barracuda (Edwards, 1771)	Great Barracuda		V	P	Yes	Yes	Yes	Yes	11	PAN		
Sphyraena borealis DeKay, 1842	Sennet			P		Yes			11	WA		
SPHYRNIDAE												
Sphyrna mokarran (Rüppell, 1837)	Great Hammerhead					Yes				PAN		
SQUALIDAE												
Squalus clarkae Pfleger, Grubbs, Cotton & Daly-Engel, 2018	Gulf Dogfish					Yes				GC		Yes
SYMPHYSANODONTIDAE												
Symphysanodon berryi Anderson, 1970	Slope Bass	DROP	CP				DROP		11	TA		Yes
Symphysanodon octoactinus Anderson, 1970	Insular Bunquelovely	DROP	CP				DROP		11	GC		Yes
SYNGNATHIDAE												
Amphelikturus dendriticus (Barbour, 1905)	Seahorse Pipefish	Estapé		P					11	WA		
Bryx dunckeri (Metzelaar, 1919)	Pugnose Pipefish					Yes				WA		
Cosmocampus albirostris (Kaup, 1856)	Whitenose Pipefish					Yes				WA		
Halicampus crinitus (Jenyns, 1842)	Banded Pipefish	Estapé		V						WA		
Hippocampus erectus Perry, 1810	Lined Seahorse			P			Yes		11	WA		
Hippocampus reidi Ginsburg, 1933	Longsnout Seahorse			P		Yes			11	GC		
SYNODONTIDAE												
Synodus foetens (Linnaeus, 1766)	Inshore Lizardfish					Yes			11	NWA		
Synodus intermedius (Agassiz, 1829)	Sand Diver			P	Yes	Yes	Yes		11	WA		
Synodus synodus (Linnaeus, 1758)	Red Lizardfish			P		Yes			11	TA		
Trachinocephalus myops (Forster, 1801)	Snakefish			P		Yes			11	TA		
TETRAODONTIDAE												
Canthigaster jamestyleri Moura & Castro, 2002	Goldface Toby	DROP	CP				DROP		11	GC		
Canthigaster rostrata (Bloch, 1786)	Sharpnose Puffer		V	P	Yes	Yes	Yes		11	GC		
Sphoeroides dorsalis Longley, 1934	Marbled Puffer	DROP/Estapé	CP	P			DROP		12	GC		
Sphoeroides nephelus (Goode & Bean, 1882)	Southern Puffer					Yes	Yes			GC		

Species in families	English common name	New	DROP	Estapé	vK15	DP17	GBIF	OBIS	Plate	Zoo	Range	Deep
Sphoeroides spengleri (Bloch, 1785)	Bandtail Puffer			P	Yes	Yes			12	WA		
TRACHICHTHYIDAE												
Hoplostethus occidentalis Woods, 1973	Western Roughy					Yes				WA		Yes
TRIACANTHODIDAE												
Hollardia hollardi Poey, 1861	Reticulate Spikefish					Yes			12	GC		Yes
TRIGLIDAE												
Bellator egretta (Goode & Bean, 1896)	Streamer Searobin	DROP	CP						12	GC		Yes
TRIPTERYGIIDAE												
Enneanectes altivelis Rosenblatt, 1960	Lofty Triplefin	Estapé		P					12	GC		
Enneanectes boehlkei Rosenblatt, 1960	Roughhead Triplefin	Estapé		P					12	GC		
Enneanectes jordani (Evermann & Marsh, 1899)	Mimic Triplefin			P		Yes			12	GC		
Enneanectes matador Victor, 2013	Matador Triplefin	Estapé		P					12	GC		

Notes:

1. *Pristipomoides*. This is *P. aquilonaris* and/or *P. macrophthalmus*. Statia is within the geographical range of both species.

2. *Chromis* cf. *enchrysura* is an undescribed species recorded as *C. enchrysura* in the GBIF database, where it is a DROP entry.

3. The *Balduinella* "*vivanus*" population from the Caribbean likely is a separate species from *B. vivanus*, which was described from specimens collected on the north coast of Cuba.

Photograph credits: B Brown: *A. nikkiae*, *B. barbata*, *C. jamestyleri*, *D. puellaris*, *D. lombardii*, *Foeteropus* sp, *G. linkii*, *L. moubrayi*, *L. klayi*, *L. regia*, *P. grandoculus*, *S. fuscula*, *V. cephalocellatus*, *Z. mcgyntii*; M and R Bentley: *A. narinari*, *E. carychroa*, *E. adscensionis*, *G. cirratum*, *L. griseus*, *H. sciurus*; M Harterink: *A. balearicum*, *E. carib*, *E. vitta*, *E. nigricans*, *G. vicinus*, *H. exstochilus*; M Pistor (STENAPA): *A. afer*, *H. histrio*; all other photographs are by the two sets of coauthors during their respective expeditions in 2017 and 2020.

– we noted whether each is a Greater Caribbean endemic, or is distributed more widely in the tropical western Atlantic (i.e., to the north and south of the Greater Caribbean, or on both sides of the Atlantic, or in the Indo-Pacific as well as the Atlantic). (b) Geographical range size – we noted which species have small geographical ranges within the Greater Caribbean, which we defined as ranges that span no more than one third of the area of that region (based on maps of their ranges in Robertson and Van Tassell 2019).

Ecological structure

The research during 2017–2020 was aimed at documenting the reef-associated bony fishes of Statia. For analyses of the structure of the Statia20 fauna we assigned those species to the following ecological groups (following Robertson and Tornabene 2020): Reef-associated fishes include demersal and benthic species that use hard substrata (coral- and rock reefs), and soft bottoms (sand, gravel, mud, seagrass and macroalgal beds growing on sediment, estuaries and mangroves) immediately adjacent to or within the matrices of reefs. Benthic species are restricted to living on and in the bottom, while demersal species use both the bottom and the near-bottom water column. Cryptobenthic fishes are visually and/or behaviorally cryptic due to their form and coloration, and to their maintaining a close association with the benthos, directly on or within it. Small size (here maximum total length (TL) ≤10 cm) also is thought to be important for crypsis among such species. Core families of cryptobenthic reef fishes (Core CRFs) (see Brandl et al. 2018, 2019) found in the western Atlantic include the Apogonidae, Blenniidae, Bythitidae, Callionymidae, Chaenopsidae, Dactyloscopidae, Gobiesocidae, Gobiidae, Grammatidae, Labrisomidae, Opistognathidae, Syngnathidae, Tripterygiidae. To these families we added the Dinematichthyidae, which was split from the Bythitidae by Møller et al. (2016) shortly before Brandl et al. (2018) assembled their list of Core CRF families, and contains many shallow, reef-associated species. Species in the list are divided into two depth classes, based on their depth ranges: shallow species are those commonly found above 40 m depth, and deep species are those entirely or largely restricted to depths below 40 m.

In the Greater Caribbean region reef-associated bony fishes comprise ~ 900 species from 304 genera in 76 families (Robertson and Tornabene 2020). Reef-fish faunas of deep reefs down to ~ 250 m are dominated by the same set of families that are common on shallow reefs (Baldwin et al. 2018). At the regional level ~ 95% of those reef-associated species are non-pelagic, demersal and benthic forms, which were the focus of the 2017–2020 research at Statia. The relative abundance of the different ecological groups in the Statia20 fauna was compared to: (a) that of the regional fauna to assess similarities and differences; (b) that of the Statia fauna of Davies and Piontek (2017) (hereafter Statia17) to assess any changes; and (c) that of the Saba Exclusive Economic Zone (EEZ) (which includes Statia) (hereafter Saba17) prior to the 2017–2020 research to assess the identity and ecotypes of species that, although they are not on the Statia20 list, do occur very near Statia. Finally, we compare the relative abundances of the different ecogroups in the Statia20 fauna to those at one of the best sampled reefs

in the Greater Caribbean, which has the largest published fauna: Alligator Reef in the Florida Keys (see Williams et al. 2010). The Alligator reef faunal checklist was recently updated and expanded (Starck et al. 2017; Estapé et al. 2020; hereafter Alligator20), and, hence, should provide a useful comparison.

A list of reef-associated fishes known from Alligator Reef was extracted from the list in Starck et al. (2017), and Estapé et al (2020) by comparing it to the checklist of regional reef-associated fishes of Robertson and Tornabene (2020). A faunal list for the Saba EEZ (see Suppl. material 1: Figure S1) was obtained by using the "Species List Assembly" tool in Robertson and Van Tassell (2019) (https://biogeodb.stri.si.edu/caribbean/en/research/index/list), as follows: within the tool the following combination of factors was selected – all species/ political area/ Saba EEZ. The confirmed species on the list generated (those with actual records within that EEZ) were then used here. A few species represented solely by data from the 2017–20 research at Statia that were on the Saba EEZ list generated by that tool were excluded from that list for the present comparisons.

Results

Modifications to the list of Davies and Piontek (2017)

We reduced the number of species on the list of Davies and Piontek (2017) (which is unchanged from that of Davies and Piontek 2016) from 307 to 304 through three deletions. Those included *Emblemariopsis occidentalis* Stephens, 1970, *Pterois miles* (Bennett, 1828) and *Enneanectes pectoralis* (Evermann & Marsh, 1899). Those authors recorded *E. occidentalis* and provided a photograph (on p 75 of Davies and Piontek 2016) of the fish they gave this name. However, *E. occidentalis* is now known to be restricted to the Bahamas (B Victor pers. comm., 26 May 2020). Authors CJE and AME photographed two species of this genus at Statia, *E. bahamensis* and *E. carib*. While *E. carib* (and *E. occidentalis*) has a simple ocular cirrus, *E. bahamensis* lacks such a cirrus. As the fish in Davies and Piontek's (2016) photograph clearly has an ocular cirrus it cannot be *E. bahamensis*. B Victor (pers. comm., 26 May 2020) examined that photograph and concluded it is of either *E. carib* or possibly *E leptocirris* Stephens 1970, which has an ocular cirrus and is known from the Puerto Rican plateau, 185 km from Statia. Hence, we deleted *E. occidentalis* from the list but did not include *E. leptocirris* due to the uncertain identification of that photograph. The Indo-west Pacific lionfish *P. volitans* apparently is a hybrid of two Indo-west Pacific species, and the West Atlantic population of this lionfish appears to be composed almost entirely of *P. volitans* (Wilcox et al. 2018). Hence, we excluded *P. miles* from the list as it is unlikely to be present at Statia and any such an occurrence has not been confirmed genetically. Davies and Piontek (2017) included both *Enneanectes pectoralis* and *E. jordani* on the list. However, we excluded *E. pectoralis* as it recently has been shown to be a synonym of *E. jordani* (see Victor 2017). In addition, we changed the names for two of Davies and Piontek's (2017) species: Davies and Piontek (2017) recorded *L. campechanus* (Poey, 1860), which is now known to be restricted to the Gulf of Mexico and US area. The

taxonomic separation of *L. purpureus*, which ranges from the Caribbean to Brazil, from *L. campechanus* was recently confirmed by da Silva et al. (2020). Davies and Piontek (2017) recorded *S mitsukurii* Jordan & Snyder, 1903. However, the Greater Caribbean population was recently renamed *S. clarkae* (see Ehemann et al. 2019) and *S. mitsukurii* is now regarded as restricted to the Eastern Atlantic and Indo-west Pacific. Those changes reduced the Statia17 list from 307 to 304 species.

Additions from other sources

The Van Kuijk et al. (2015) list of 106 species contained one species (*Chilomycterus schoepfi*) not included by Davies and Piontek (2017) in their list. FishNet2 supplies data based on museum records to GBIF and all 34 species records from FishNet2 were also in the GBIF list and are not separately indicated in Table 1. The GBIF list included 103 species, and, after discounting the 27 DROP2017 collection records included therein, none of the 76 remaining species represented "new" records that are not on the Davies and Piontek (2017) list. OBIS, which also supplies data to GBIF, produced 37 records, 13 of which (all common, widely distributed species) were not in the GBIF list, but only one of which (*Coryphaena hippurus*) was not in any other database.

DROP recorded a total of 120 species, 59 of which were not in any other list, except for two new records it shared with the Estapé 2020 list. Eight of those 59 records are of species that have yet to be described and named. The Estapé 2020 list includes 244 records, 40 of them new, plus two other new additions they share with DROP. Summing the deletions and additions from various sources produced a total of 406 species for the Statia20 checklist (see Table 1).

Photographic plates

The 13 photographic plates (Suppl. materials 4–16: Plates S1–S13) include images of 280 species, 69% of those on the Statia20 list. In addition, Davies and Piontek (2017) provided images of *Chimaera cubana*, which are not included in the supplemental plates. Of the plate images, 40 species come from DROP collections, 226 were taken by CJE and AME and 14 were provided to them by local divers and fishers at Statia (Table 1). Images are available from other sources for all remaining species listed in Table 1 (except the seven species of macrourids), on their individual species pages at https://biogeodb.stri.si.edu/caribbean/en/pages.

Structure of the Statia20 reef-associated bony fish fauna

Global geographical ranges

Greater Caribbean endemics represent the largest group of species in the Statia fauna, and, together with more widely ranging western Atlantic endemics, constitute almost three quarters of the species. Trans-Atlantic species and species found outside as well as
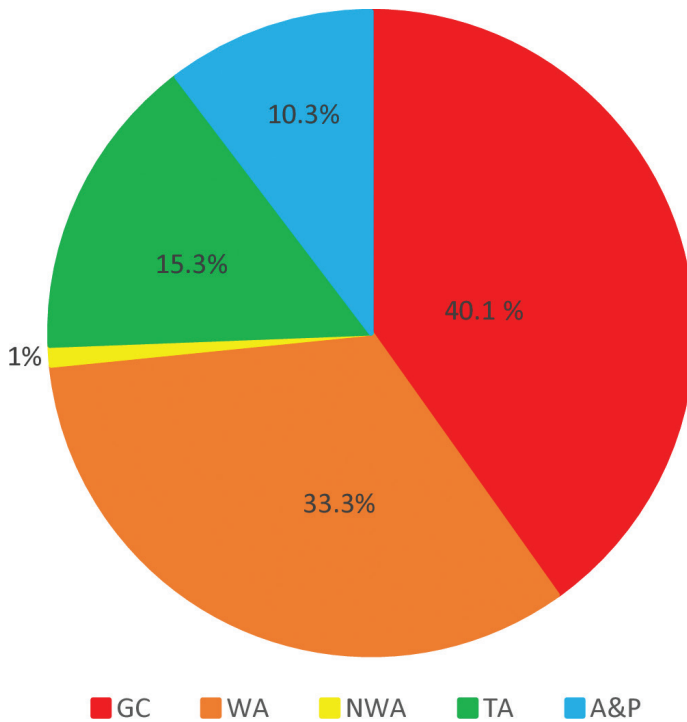
Figure 3. Percentages of the Sint Eustatius marine fish fauna represented by groups of species with different global geographical ranges. GC = Greater Caribbean endemics; NWA = GC plus temperate eastern USA; WA = GC plus Brazil; TA = WA plus central or East Atlantic; and A&P = species found in both the Atlantic and various parts of the Indo-Pacific.

inside the Atlantic represented only a quarter of the fauna (Table 1, Figure 3). The relative abundances of species with different types of large-scale geographic ranges are very similar to those of species in the well documented fauna of nearby St. Croix (Smith-Vaniz and Jelks 2014). Species found in Brazil constituted one third of the Statia fauna, while those extending northwards from the Greater Caribbean represented only 1%, a reflection of the greater effects of temperature limitation on northward extension of ranges as compared to effects of the Amazon-Orinoco outflow on limitation of range extension much further south of the Greater Caribbean.

Extent of geographical ranges within the Greater Caribbean

The vast majority of species are widely distributed within the Greater Caribbean, with only nine (2.25%) of them having ranges limited to a restricted part of the Caribbean. Among those nine, five are deep-living species, and five belong to Core CRF families (Table 1). The four shallow species with restricted ranges are all Core CRFs. None of the species were micro-endemics, restricted to Statia or that island plus immediately surrounding islands, and no micro-endemics are known to exist in that general area.

Ecology – Depth

The number of deep species increased from 44 on the Statia17 list to 86 in the Statia20 fauna (Table 1), representing an increase from 14.5% in the former to 21.2% in the latter. Among the reef-associated bony fishes (Table 2) the number of deep species increased from 6 (2.7%) to 39 (11.7%) in those two lists.

Ecology – Reef-associated bony fishes

The Statia20 fauna of such species is 38.3% larger than the Statia17 fauna, with numbers of shallow species increasing by 24.8% (from 214 to 267) and of deep species increasing 6.2-fold (from 6 to 39). This led to substantial increases in the relative abundance of deep-reef species, and of benthic, cryptobenthic, small cryptobenthic and core CRFs on both shallow and deep reefs. The Saba17 fauna was 71% larger than that of Statia17, with greater percentages of deep-reef, benthic, cryptobenthic, small cryptobenthic and Core CRFs. The Saba17 fauna was 23% larger than the Statia20 fauna and had a greater proportion of shallow species and fewer deep species, and higher proportions of shallow members of cryptobenthic, small cryptobenthic and Core CRF groups. Thirty-two percent of the Saba17 species were not in the Statia20

Table 2. Characteristics of assemblages of reef fishes at different locations in the Greater Caribbean region. Percentages of ecotypes in the entire regional fauna, the entire faunas from each local area, and within each of two depth subgroups refer to number of species as a % of the entire fauna and of each depth subgroup. Assemblages include those at Statia in 2017 and 2020 (Statia17 and Statia20), in the Saba EEZ in 2017 (Saba17), of species in the Saba17 fauna that are not currently known to occur at Statia (Saba17-Statia20), of the Saba EEZ in 2020 (Statia20 + Saba17), and of Alligator reef in 2020 (Alligator20). Small species are those with ≤ 10 cm maximum total length. Percentage values for individual sites that are greater than the regional value are shown in red, those below the regional value are in blue.

	Region	Statia20	Statia17	Saba17	Saba20	Alligator20	Saba17 – Statia20
ALL SPECIES (n)	903	306	220	377	427	427	121
Demersal species%	35.0	55.1	66.8	47.5	46.1	49.4	19.0
Benthic species%	65.0	43.1	33.2	52.5	53.9	50.6	81.0
Cryptobenthic species%	59.2	40.8	30.9	49.1	50.1	46.4	73.6
Small cryptobenthic species%	41.6	24.8	15.5	30.2	31.9	24.8	49.6
Core CRF species%	45.8	28.8	20.5	33.4	35.1	27.6	48.8
SHALLOW SPECIES%	**85.1**	**87.3**	**97.3**	**93.4**	**88.0**	**95.3**	**90.1**
Non-cryptic species%	40.8	59.6	68.1	50.3	47.6	58.6	23.9
Cryptobenthic species%	59.2	40.4	31.3	49.7	52.4	41.1	76.1
Small cryptobenthic species%	41.3	23.2	15.4	30.7	31.9	25.8	53.2
Core CRF species%	46.2	28.5	21.0	34.1	35.4	29.0	52.3
DEEP SPECIES%	**14.9**	**12.7**	**2.7**	**6.6**	**12.0**	**4.7**	**9.9**
Non-cryptic species%	40.3	56.5	83.3	60.0	54.9	75	50.0
Cryptobenthic species%	59.7	43.5	16.7	40.0	45.1	25	50.0
Small cryptobenthic species%	43.3	35.6	16.7	24.0	31.4	5	16.7
Core CRF species%	44.0	38.5	0	24.0	33.3	0	16.7

Notes: see methods for classification of ecotypes. For lists of species in the Saba EEZ and Statia2020, and their ecotypic classifications see Suppl. material 2: Table S1. *Pterois volitans* and *Pristipomoides spp* are excluded from Suppl. material 2: Table S1 and the calculations in Table 2. The former is non-native and the specific identity of *Pristipomoides* at Statia is uncertain.

fauna. Those 121 species comprised mainly shallow cryptobenthic types, including small-cryptobenthic and Core-CRF species. When those are combined with the Statia20 fauna the resultant Saba20 fauna has substantial increases in the proportions of shallow cryptobenthic, small cryptobenthic and core CRF species compared to the Statia20 fauna. Relative to the regional fauna, however, the faunas of Statia17, Statia20, Saba17, and Saba20 all had deficits of deep species of all types and of shallow cryptobenthic species, including small- and Core-CRF species. The Alligator20 fauna of reef-associated species is the same size as the Saba20 fauna. It has the same characteristics as the Statia17 and Saba17 faunas: a large deficit of deep-reef fishes and deficits of shallow cryptobenthic species, including small- and Core-CRF species. Although there has been some collecting at Alligator reef of shallow cryptobenthic species there has been no submersible-based collecting there.

Discussion

The efforts of van Kuijk et al. (2015) and Davies and Piontek (2017) substantially increased our knowledge of the known ichthyofauna of Statia, from 215 to 304 species. The information added through the research in 2017 and 2020 has produced a further significant increase, by 33.6%, to 406 species. While the size of the Statia17 fauna was similar to that known for other islands in the Caribbean (Williams et al. 2010; Davies and Piontek 2017) the Statia20 fauna is distinctly larger. That can be attributed to the combination of research on deep-reef fishes by DROP in 2017 and on shallow species by CJE and AME in 2020. Williams et al. (2010) compared the size of the Saba Bank fauna to the faunas of various Caribbean sites and two in the Florida Keys. The size of the large known fauna at one of those Florida sites, Alligator Reef, has increased by ~ 20% since the Williams et al. (2010) study (see Starck et al. 2017; Estapé et al. 2020). However, the current state of knowledge for the other Caribbean sites referred to by Davies and Piontek (2017) and Williams et al. (2010) is unclear.

Zoogeographically the two largest groups of species in the Statia20 fauna are Greater Caribbean endemics and western Atlantic endemics, and the smallest group is of species found in the Indo-Pacific as well as the Atlantic. This mixture is fairly representative of the Greater Caribbean fish fauna as a whole (Robertson and Cramer 2014), and similar to that of nearby St. Croix (Smith-Vaniz and Jelks 2014). The vast majority of the species in the Statia20 fauna are widely distributed in the Greater Caribbean. Among the very few (2.25%) with restricted ranges most information on range-size is available for the shallow species, which belong to two of the most speciose Core CRF families in the Greater Caribbean, the Gobiidae and Chaenopsidae. High levels of local endemism is a feature of some CRF taxa (Brandl et al. 2018) and regionally those two families have substantial proportions of species with restricted ranges, as defined here: 78.7% of 47 chaenopsids and 42.4% of 139 reef-associated gobies (see species maps in Robertson and Van Tassell 2019).

Most species recorded in the Statia17 fauna are readily visible reef fishes, demersal and non-cryptic benthic species commonly found on wider Caribbean reefs, and the proportions of cryptobenthic (particularly small ones) and deep-reef species were relatively low. Davies and Piontek (2017) recognized that both those groups were probably underrepresented in their checklist due to inadequate sampling. Aspects of data collection that affect the adequacy of sampling at a location include its spatial distribution, techniques used, and the depth of sampled habitats. Of all research efforts to date at Statia only the shallow BRUV sampling by van Kuijk et al. (2015) can be regarded as spatially representative, as it was well dispersed around both exposed and sheltered sides of the island. SCUBA-based sampling by Davies and Piontek (2017) and both DROP and the Estapés was largely limited to the more sheltered platform on the western side of the island, and the submersible sampling by DROP was restricted to one small area at the southwest corner of the island shelf. Hence, there are large areas of habitat on the seaward platform and on deep reefs around three quarters of the island that remain unsampled. Furthermore, roving SCUBA surveys are largely limited to providing information on larger, more readily visible demersal and pelagic species (Ackerman and Bellwood 2000; Smith-Vaniz et al. 2006; Alzate et al. 2014). BRUVs are similarly limited: only 10.3% of the 106 species recorded by van Kuijk et al. (2015) are cryptobenthic forms, and only 2.8% are small cryptobenthic species (see Suppl. materials 2, 3: Tables S1, S2).

Rotenone is an ichthyocide commonly used in small quantities by researchers to extract cryptobenthic fishes hiding within reef structures or buried in soft bottoms, and is an important tool for elucidating the contribution of such species to reef-fish faunal assessments (Ackerman and Bellwood 2000; Smith-Vaniz et al. 2006; Robertson and Smith-Vaniz 2008). Davies and Piontek (2017) indicated that sampling using ichthyocides to extract cryptobenthic species hiding within the matrix of the reef at Statia likely would increase the size of the fauna. Rotenone sampling has been employed on shallow reefs of Saba Bank by Williams et al. (2010), and can account for the large numbers of small cryptobenthic species encountered there that are not on the Statia20 checklist: 60% of the 142 species collected by Williams et al. (2010) at Saba bank using that ichthyocide are cryptobenthic forms. Given that that bank is very close to Statia (the two shallow platforms are < 20 km apart) and, since it lacks mangroves, seagrasses and intertidal habitats, the bank may have even lower habitat diversity than Statia. Hence, it seems quite likely that many of the cryptobenthic species, particularly the small ones, found on that bank will be encountered at Statia when appropriate sampling has been done. However, the increase in numbers of shallow cryptobenthic species at Statia from 2017 to 2020 does show that organized searching by skilled citizen scientists can contribute substantially to knowledge of cryptobenthic species. The activities of CJE and AME added 33 shallow cryptobenthic species to the checklist, 31% of the total and 85% of the new records for that ecogroup in the 2020 fauna, and equivalent to 49% of the number present in the Statia17 fauna (Tables 1, 2).

The DROP submersible-based sampling is the only organized research on deep-reef fishes conducted to date at Statia or in the Saba EEZ. It produced more than half

the new records in the Statia 2020 fauna, including records of eight recently discovered species that currently lack scientific names. It dramatically increased the numerical and proportional abundance of deep-reef species in the general fauna and in the reef-associated component. A lack of such research at Saba bank and Alligator Reef accounts for the very low abundance of deep-reef fishes at those sites.

The proportional abundances of shallow cryptobenthic species, including small species and core CRFs, are also distinctly lower in the Statia20 fauna than the regional fauna. Even if all 121 reef-associated species in the Saba EEZ that are not known from Statia are assumed to be at Statia those proportions still remain below the regional levels. Some of that difference is probably due to sampling artifacts. However, the proportional abundances of those ecotypes in a local fauna like that of Statia, or Alligator Reef, may always be lower than the regional level. In the Greater Caribbean small cryptobenthic species, particularly Core-CRF species such as blennioids and gobies, often have small geographical ranges (see above), which are scattered in different parts of the region (see Robertson and Van Tassell 2019). While the regional level of the proportional abundance of such taxa is based on an aggregate of many such species from a large area, only a subset of species in those taxa will be found at any single site and their proportional contribution to local faunal richness most likely will be lower than the regional level. The Statia20 fauna includes 33.9% of the Greater Caribbean's reef-associated fish fauna. That percentage rises to 47.3% in Saba20. Whether a tiny island with a small area of a limited range of habitats is likely to support many more species, and whether pelagic recruitment of reef fishes from nearby islands found around three sides of Statia helps sustain the Statia fauna are both debatable issues that bear on the size of its marine fish fauna.

Conclusions

The research reported in the present study substantially increased our knowledge of the size of the marine fish fauna of Statia and resulted in the discovery of a significant number of undescribed deep-reef species. Although that island fauna is now one of the best documented in the Greater Caribbean there is still much to do to provide a thorough assessment of its diversity. Collecting with ichthyocide (or anesthetics) is essential for effective sampling of the fauna of small, shallow cryptobenthic reef fishes present there, and sampling of both deep and shallow reef fishes needs to be more effectively distributed across the range of habitats present at the island. No single site in the Caribbean Sea has been subject to sufficiently thorough sampling to provide a clear understanding of the size of its entire marine fish fauna, the size of its reef-associated fish fauna, or even the size of its shallow, reef-associated fauna, let alone its deep-reef fish fauna.

Permits

Animal-Care Permission

Acknowledgements

References

Ackerman JL, Bellwood DR (2000) Reef fish assemblages: a re-evaluation using enclosed rotenone stations. Marine Ecology Progress Series 206: 227–237. https://doi.org/10.3354/meps206227

Alzate A, Zapata FA, Girald A (2014) A comparison of visual and collection-based methods for assessing community structure of coral reef fishes in the Tropical Eastern Pacific. Revista de Biología Tropical 62: 359–371. https://doi.org/10.15517/rbt.v62i0.16361

Baldwin CC, Tornabene L, Robertson DR (2018) Below the mesophotic. Scientific Reports 8 (4920): 1–13. https://doi.org/10.1038/s41598-018-23067-1

Brandl SJ, Goatley CHR, Bellwood DR, Tornabene L (2018) The hidden half: ecology and evolution of cryptobenthic fishes on coral reefs. Biological Reviews 93: 1846–1873. https://doi.org/10.1111/brv.12423

Brandl SJ, Tornabene L, Goatley CHR, Casey JM, Morais RA, Côté IM, Baldwin CC, Parravicini V, Schiettekatte MD, Bellwood DR (2019) Demographic dynamics of the smallest marine vertebrates fuel coral reef ecosystem functioning. Science 364: 1189–1192. https://doi.org/10.1126/science.aav3384

da Silva R, Pedraza-Marrón CR, Sampaio I, Betancur-R R, Gomes G, Schneider H (2020) New insights about species delimitation in red snappers (*Lutjanus purpureus* and *L. campechanus*) using multilocus data. Molecular Phylogenetics and Evolution 147: e106780. https://doi.org/10.1016/j.ympev.2020.106780

Davies M, Piontek S (2016) Marine fishes of St. Eustatius. In: Hoeksem BW (Ed.) Marine biodiversity survey of St. Eustatius, Dutch Caribbean, 2015. Naturalis Biodiversity Center, Leiden, and ANEMOON Foundation, Bennebroek, 73–82. https://www.persistent-identifier.nl/urn:nbn:nl:ui:19-616970

Davies M, Piontek S (2017) Marine fishes of St. Eustatius, Dutch Caribbean. Marine Biodiversity 47: 27–35. https://doi.org/10.1007/s12526-016-0575-1

Ehemann NR, González-González LDV, Tagliafico A, Weigmann S (2019) Updated taxonomic list and conservation status of chondrichthyans from the exclusive economic zone of Venezuela, with first generic and specific records. Journal of Fish Biology 95: 753–771. https://doi.org/10.1111/jfb.14061

Estapé CJ, Morgan Estapé A, Starck WA (2020) The fishes of Alligator Reef and environs in the Florida Keys: a 2020 update. Journal of the Ocean Science Foundation 36: 16–19. https://doi.org/10.5281/zenodo.4243097

Hoeksema B [Ed.] (2016) Marine biodiversity survey of St. Eustatius, Dutch Caribbean, 2015. Naturalis Biodiversity Center, Leiden, and ANEMOON Foundation, Bennebroek, 157 pp. https://www.persistent-identifier.nl/urn:nbn:nl:ui:19-616970

Hoetjes PC, Carpenter KE (2010) Saving Saba Bank: Policy Implications of Biodiversity Studies. PLoS ONE 5: e10769. https://doi.org/10.1371/journal.pone.0010769

Metzelaar J (1919) Report on the fishes, collected by Dr J Boeke in the Dutch West Indies 1904–1905, with comparative notes on marine fishes of tropical West Africa. F.J. Belanfante, 's-Gravenhage, 314 pp.

Møller PR, Knudsen W, Schwarzhans W, Nielsen JG (2016) A new classification of viviparous brotulas (Bythitidae) – with family status for Dinematichthyidae – based on molecular, morphological and fossil data. Molecular Phylogenetics and Evolution 100: 391–408. https://doi.org/10.1016/j.ympev.2016.04.008

Robertson DR, Cramer KL (2014) Defining and Dividing the Greater Caribbean: Insights from the biogeography of shorefishes. PLoS ONE 9: 1–16. https://doi.org/10.1371/journal.pone.0102918

Robertson DR, Smith-Vaniz WF (2008) Rotenone: An Essential but Demonized Tool for Assessing Marine Fish Diversity. BioScience 58: 165–170. https://doi.org/10.1641/B580211

Robertson DR, Tornabene L (2020) Reef-associated bony fishes of the Greater Caribbean: a checklist (Version 3). Zenodo. https://doi.org/10.5281/zenodo.4279301

Robertson DR, Van Tassell J (2019) Shorefishes of the Greater Caribbean: online information system. Version 2.0. Smithsonian Tropical Research Institute, Balboa, Panama. https://biogeodb.stri.si.edu/caribbean/en/pages

Smith-Vaniz WF, Jelks HL (2014) Marine and inland fishes of St. Croix, U. S. Virgin Islands: an annotated checklist. Zootaxa 3903: 1–120. https://doi.org/10.11646/zootaxa.3803.1.1

Smith-Vaniz WF, Jelks HL, Rocha LA (2006) Relevance of cryptic fishes in biodiversity assessments: A case study at Buck Island National Monument, St. Croix. Bulletin of Marine Science 79: 17–48.

Starck WA, Estapé CJ, Morgan Estapé A (2017) The fishes of Alligator Reef and environs in the Florida Keys: a half-century update. Journal of the Ocean Science Foundation 27: 74–117. https://doi.org/10.5281/zenodo.851651

van Kuijk T, de Graaf M, Nagelkerken L, Boman E, Debrot AO (2015) Baseline assessment of the coral reef fish assemblages of St. Eustatius. Technical Report C058/15. IMARES, Wageningen, 49 pp.

Victor BC (2017) The status of *Enneanectes jordani* and a new species of triplefin blenny from the Greater Caribbean (Teleostei: Tripterygiidae). Journal of the Ocean Science Foundation 27: 48–73.

Wilcox CL, Motomura H, Matsunuma M, Bowen BW (2018) Phylogeography of Lionfishes (*Pterois*) Indicate Taxonomic Over Splitting and Hybrid Origin of the Invasive *Pterois volitans*. Journal of Heredity 109: 162–175. https://doi.org/10.1093/jhered/esx056

Williams JT, Carpenter KE, Van Tassell JL, Hoetjes P, Toller W (2010) Biodiversity Assessment of the Fishes of Saba Bank Atoll, Netherlands Antilles. PLoS ONE 5: e10676. https://doi.org/10.1371/journal.pone.0010676

Supplementary material 1

Figure S1

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Map of EEZ

Explanation note: Map of Saba EEZ.

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Link: https://doi.org/10.3897/zookeys.1007.58515.suppl1

Supplementary material 2

Table S1

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Dive site list

Explanation note: List of dive sites with dates and georeferenced coordinates.

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Link: https://doi.org/10.3897/zookeys.1007.58515.suppl2

Supplementary material 3

Table S2

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Occurences

Explanation note: Fish species occurrences at Saba and Statia.

Link: https://doi.org/10.3897/zookeys.1007.58515.suppl3

Supplementary material 4

Plate S1

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Photographs

Explanation note: Voucher photographs of fishes.

Link: https://doi.org/10.3897/zookeys.1007.58515.suppl4

Supplementary material 5

Plate S2

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Photographs

Explanation note: Voucher photographs of fishes.

Link: https://doi.org/10.3897/zookeys.1007.58515.suppl5

Supplementary material 6

Plate S3

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Photographs

Explanation note: Voucher photographs of fishes.

Link: https://doi.org/10.3897/zookeys.1007.58515.suppl6

Supplementary material 7

Plate S4

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Photographs

Explanation note: Voucher photographs of fishes.

Link: https://doi.org/10.3897/zookeys.1007.58515.suppl7

Supplementary material 8

Plate S5

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Photographs

Explanation note: Voucher photographs of fishes.

Link: https://doi.org/10.3897/zookeys.1007.58515.suppl8

Supplementary material 9

Plate S6
Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin
Data type: Photographs
Explanation note: Voucher photographs of fishes.
Link: https://doi.org/10.3897/zookeys.1007.58515.suppl9

Supplementary material 10

Plate S7
Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin
Data type: Photographs
Explanation note: Voucher photographs of fishes.
Link: https://doi.org/10.3897/zookeys.1007.58515.suppl10

Supplementary material 11

Plate S8
Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin
Data type: Photographs
Explanation note: Voucher photographs of fishes.
Link: https://doi.org/10.3897/zookeys.1007.58515.suppl11

Supplementary material 12

Plate S9

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Photographs

Explanation note: Voucher photographs of fishes.

Link: https://doi.org/10.3897/zookeys.1007.58515.suppl12

Supplementary material 13

Plate S10

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Photographs

Explanation note: Voucher photographs of fishes.

Link: https://doi.org/10.3897/zookeys.1007.58515.suppl13

Supplementary material 14

Plate S11

Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin

Data type: Photographs

Explanation note: Voucher photographs of fishes.

Link: https://doi.org/10.3897/zookeys.1007.58515.suppl14

Supplementary material 15

Plate S12
Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin
Data type: Photographs
Explanation note: Voucher photographs of fishes.
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Link: https://doi.org/10.3897/zookeys.1007.58515.suppl15

Supplementary material 16

Plate S13
Authors: David Ross Robertson, Carlos J. Estapé, Allison M. Estapé, Ernesto Peña, Luke Tornabene, Carole C. Baldwin
Data type: Photographs
Explanation note: Voucher photographs of fishes.
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Link: https://doi.org/10.3897/zookeys.1007.58515.suppl16