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# Molecular and morphological description of four new Ethmostigmus Pocock, 1898 (Chilopoda, Scolopendromorpha, Scolopendridae) species from China, with a key to Chinese species 

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Molecular and morphological descriptions of four new species of Ethmostigmus Pocock, 1898 (Chilopoda, Scolopendromorpha, Scolopendridae) from China, with a key to the Chinese species

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## Running head

New Ethmostigmus species from China


#### Abstract

In this study, we used a combination of morphological and DNA data to delineate Ethmostigmus centipedes in China. Two mitochondrial DNA loci (COI and 16S rRNA) and a nuclear DNA locus ( 28 S rRNA) were used to determine the phylogenetic positions of potential species using maximum likelihood and Bayesian inference approaches. On the basis of these analyses, five species of Chinese Ethmostigmus are newly recognized: Ethmostigmus pygomenasoides Lewis, 1992 stat. nov., and the four new species E. flavescens sp. nov., E. austroyunnanensis sp. nov., Ethmostigmus biocolor sp. nov., and E. motuoensis sp. nov. These species are described and keyed, and we map their distributions.


Keywords DNA, COI, Taxonomy, Yunnan, Xizang

## Introduction

Ethmostigmus Pocock, 1898 is a taxon comprising the largest Otostigminae centipedes, of which 20 valid Ethmostigmus species are listed in the Chilobase 2.0 database (Bonato et al. 2016), with distribution ranges extending throughout Africa, India, Southeast Asia, and Australia, including neighbouring islands (Bonato et al. 2016; Joshi \& Edgecombe 2019a; Koch \& Colless 1986; Lewis 1968; Schileyko \& Stoev 2016). Since Attems' global revision of the scolopendromorph centipedes almost 100 years ago (Attems 1930), there have been two large-scale taxonomic studies of this genus and a number of new species have been discovered, Koch (1983) described five novel Ethmostigmus species from Australia, and on the basis of an integration of molecular data, morphological, and geographical information, Joshi \& Edgecombe (2019a) discovered three new species, namely, E. agasthyamalaiensis Joshi \& Edgecombe, 2019, E. sahyadrensis Joshi \& Edgecombe, 2019, and E. praveeni Joshi \& Edgecombe, 2019 from the Western Ghats of India. Existing literature indicates only a nominate Ethmostigmus species and a subspecies of E. rubripes (Brandt, 1840) have been recorded to date in China: Scolopendra rapax Gervais, 1847 from unspecified localities in China, which is considered synonymous to E. rubripes rubripes (Brandt, 1840) by Kraepelin (1903), and E. rubripes platycephalus (Newport 1845), which has been noted from the Spratly inseln (=Nansha Islands) (Schileyko \& Stagl 2004; Schileyko \& Stoev 2016). Apart from these taxa, however, little is known regarding Ethmostigmus centipedes inhabiting mainland China.

In this study, we examined some newly discovered Ethmostigmus centipedes from Yunnan Province and the Xizang Autonomous Region of mainland China based on a combination of external morphological characters and molecular phylogeny [the mitochondrial DNA loci cytochrome $c$ oxidase subunit I (COI) and 16S rRNA (16S) and the nuclear locus 28 S rRNA (28S)]. Phylogenetic analysis of the Otostigminae subfamily, performed using DNA sequence data gained in the present and published sequence data retrieved from the GenBank database revealed that at least five Ethmostigmus centipede species are distributed throughout mainland China, four of which appear to be new species. A key to the Ethmostigmus species found in China is provided.

## Material and methods

Specimen collection and identification. Centipede specimens were collected in Yunnan Province and the Xizang Autonomous Region of China during the period from May 2018 to October 2021. During the course of field investigations, we photographed representative individuals using an Olympus E-M10 II camera to document the colouration of living specimens (Fig. 1). Upon collection, all specimens were deposited in $75 \%$ ethanol solution. Holotype and paratype specimens have been retained at the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, China (CMMI).

The morphological terminology used in this paper partially follows the standardized terminology (Bonato et al. 2010). Multi-focused montage photographs were obtained using Helicon Focus 6.7.1 software from a sequence of source images
acquired using an Olympus SZ16 stereo microscope. Distribution maps were generated using ArcMap 10.7.1 (Fig. 2).

Abbreviations: $\mathrm{VL}=$ ventrolateral, $\mathrm{VM}=$ ventromedial, $\mathrm{M}=$ medial, $\mathrm{DM}=$ dorsomedial, $\mathrm{CS}=$ corner spine, $\mathrm{T}=$ tergite, $\mathrm{UL}=$ ultimate leg, and ULBS $=$ ultimate leg-bearing segment.

Phylogenetic sampling and DNA sequence analyses. For the purposes of determining phylogenetic relationships, we selected two mitochondrial loci (16S and COI) and a single nuclear locus (28S). Previously determined Ethmostigmus sequences were retrieved from the GenBank database (Table 1). These sequences were augmented by those obtained from the Ethmostigmus specimens collected in this study. Genomic DNA was extracted from the legs of these specimens using DNeasy Blood \& Tissue Kits (Qiagen, Hilden, Germany), with the isolated DNA being resuspended in $100 \mu \mathrm{~L}$ of buffer and stored at $-20^{\circ} \mathrm{C}$ for subsequent analysis.

The 28S rRNA fragment was amplified using the primer pair 28SF4/28SR5 or Chilo28SF1/Chilo28SR1 (Jiang et al. 2022; Morgan et al. 2002); the 16S rRNA fragment was amplified using the primer pair 16Sar/16Sb (Palumbi et al. 1991; Xiong \& Kocher 1991); and COI was amplified using the primer pair LCO1490/HCOoutout or Chilo887F/Chilo887R (Folmer et al., 1994; Jiang et al., 2022; Schwendinger \& Giribet 2005). PCR reaction mixtures (total volume $25 \mu \mathrm{~L}$ ) contained $12.5 \mu \mathrm{~L}$ of $2 \times \mathrm{M} 5$ Mix (Transgen, Beijing, China), $0.4 \mu \mathrm{~L}$ each of the forward and reverse primers ( 10 $\mu \mathrm{mol} \cdot \mathrm{L}^{-1}$, Sangon, Shanghai, China), and $1 \mu \mathrm{~L}$ (approximately 20 ng ) of genomic DNA. Amplification was performed in a Veriti ${ }^{\mathrm{TM}}$ thermal cycler (Applied Biosystems, Foster City, CA, USA) using previously described cycling conditions (Jiang et al 2022). For phylogenetic analyses, we adopted Maximum likelihood and Bayesian inference approaches using PhyloSuite (Zhang et al. 2018). Standard statistical tests were applied to evaluate branch support (bootstrap support and posterior probability), and the bestfit substitution models and partitioning strategies were inferred for the combined COI, 16 S and 28S dataset using ModelFinder (Kalyaanamoorthy et al. 2017). Based on Akaike Information Criterion assessments, GTR $+\mathrm{I}+\mathrm{G} 4$ and $\mathrm{GTR}+\mathrm{G}+\mathrm{I}$ were selected as the best-fitting models for maximum likelihood and MrBayes analyses, respectively. Trees for combined dataset were constructed with IQ-TREE (Nguyen et al. 2015) using a maximum likelihood algorithm with 500,000 ultra-fast bootstrap replicates (Minh et al. 2013). Bayesian analyses were performed using MrBayes 3.2.6 (Ronquist et al. 2012) integrated into PhyloSuite with the default settings, and 20,000,000 Markov chain Monte Carlo generations, sampling every 1000 generations. Sequences have been deposited in the GenBank databases with the accessions numbers shown in Table 1.

## Results

## Taxonomic accounts

Order Scolopendromorpha Pocock, 1895
Family Scolopendridae Newport, 1844

## Genus Ethmostigmus Pocock， 1898

Diagnosis．Twenty antennal articles，basal four glabrous dorsally．Leg－bearing segment 7 with pair of spiracles．Spiracle on leg－bearing segment 3 enlarged．Forcipular trochanteroprefemoral process absent（or small，without teeth）．Coxopleural process bearing apical and lateral spines．Corner spine on ultimate leg prefemur simple．

## Ethmostigmus flavescens Jiang \＆Huang sp．nov．黄筛孔蜈蚣（Fig．1A，Fig．3）

 Material examined．Holotype．CHINA：Yunnan Province：Mengla county（21．72N，101．38E），CMMI 20191212003， 12 Dec．2019，leg．MX．Shi．
Paratypes．CHINA：Yunnan Province：Menghai county，one spm，CMMI 20200628001， 28 Jun．2020，leg．MX．Shi；Mengla county：one spm，Xishuangbanna Tropical Rainforest National Park：Mengyuanxianjing Scenic，21．7192N，101．3826E， 680 m a．s．l．CMMI 20200610005， 10 Jun．2020，leg．JD．Zhang，one spm，CMMI 20190507004， 7 May．2019，unspecified locality of Mengla county，leg．QY．Ji， 1 spm， CMMI 20191212010，data as for holotype．
Type locality．Yunnan Province：Mengla county．
Etymology．The specific name flavescens refers to the yellowish body colour of the new species．
Diagnosis．Forcipular coxosternal tooth－plates with four well－defined teeth on each side，coxopleural process 2．3－2．8 times length of ultimate sternite；with 1－2 apical spines， $0-1$ subapical spine， $0-1$ dorsal spine，and 1 lateral spine．Ultimate leg prefemur with $2 \mathrm{VL}, 2 \mathrm{VM}, 1 \mathrm{M}$ ，and 2 DM spines．
Holotype description．The colour description is based on photographs of a living specimen（Fig．1A）．Cephalic plate dark yellow，tergites yellow to dark yellow，posterior border of tergites with dark stripes．Antennae and legs pale yellow．Prefemur and femur of ultimate leg dark yellow，whereas tibia and tarsus pale yellow with black stripe on tibia and tarsus 1.

Body length 90 mm ．Antennae with 20 articles，basal four articles glabrous dorsally and three ventrally（Fig．3A，B）．Cephalic plate and tergites smooth，with large punctates．Forcipular coxosternal tooth－plates slightly longer than wide，with width－to－ length ratio of approximately 1．7：1．Tooth－plates with four well－defined main teeth on each side，the inner two teeth sharing a common base（Fig．3C）．Each tooth with three small lobes；innermost tooth has four or five accessory teeth．The base of tooth－plates defined by oblique sutures diverging at $100^{\circ}$ ．

Tergites smooth，without hairs or setae．Tergites with complete paramedian sutures from T3 to T20（Fig．3E）．Complete clearly visible margination beginning from T6．Tergite 21 with parallel lateral margins and width－to－length ratio of 1．5：1（Fig．3G）． Sternites with complete paramedian sutures starting from S3（Fig．3F）．The ultimate sternite surface without longitudinal depression，incurved posteriorly，forms a triangle diverging at $115^{\circ}$ ．

Coxopleural process 2．4－2．8 times length of sternite 21 （Fig．3H），with one robust apical spine，one subapical dorsal spine distinctly separated from apical spine，one small dorsal spine，and one lateral spine on left（right coxopleural process wounded and
showing regeneration，without apical spine and dorsal subapical spine）．Pores dense and pore－free area short，extending $40 \%-45 \%$ of length from distal part of coxopleural process to ultimate sternite margin．

All legs with very sparse short setae；first two pairs with two tarsal spurs，and legs 3－20 with one tarsal spur．Leg 1 with one tibial and one femoral spur．Legs $1-20$ with two claw spurs．Ultimate legs thick and moderately long（Fig．3O），with prefemur and femur length ratios of 1．44：1，femur and tibia of 1．3：1，tibia and tarsus 1 of 1．3：1，and tarsus 1 and tarsus 2 of 2．5：1．Ultimate leg without claw spur（Fig．3N），prefemur with large spines，as follows： $2 \mathrm{VL}, 2 \mathrm{VM}, 1 \mathrm{M}, 2 \mathrm{DM}$ ，and 1 corner spine（Fig．3D，I）．
Variation．Details are shown in Table 2．The studied material shows certain variation in the morphology and size of spiracles；in specimen CMMI 20200628001，the spiracle on leg－bearing segment 3 occupies nearly the entire surface of the respective pleuron， whereas the spiracle on leg－bearing segment 7 is large and round．The ultimate legs typically lack claw spurs，although specimens CMMI 20200628001 and CMMI 20190507004 have a single spur on the left leg．Spines on ultimate leg prefemur often have the pattern $2 \mathrm{VL}, 2 \mathrm{VM}, 1 \mathrm{M}, 2 \mathrm{DM}$ ，although some specimens were found to have $2 \mathrm{VL}, 1 \mathrm{VM}, 2 \mathrm{M}$ ，and 2 DM spines on one side．The shape and location of the coxopleural process spine are variable．Paratypes CMMI 20200610005 and CMMI 20200628001 have two equal－sized apical spines（Fig．3P），whereas the holotype has a large apical spine and a smaller subapical spine that is distinctly separated from the apical spine in the process（Fig．3H）．
Distribution．Yunnan（Mengla county，Menghai county）．Fig． 2.
Remarks．Scolopendra rapax Gervais， 1847 was originally described from a specimen collected in China，the tooth－plates of which are more or less square，with three teeth of which the inner one bears a cusp and the middle one of the three teeth is the largest． S．rapax is considered synonymous to E．rubripes spinosus（Newport，1845）（type location：Sri Lanka）．Ethmostigmus flavescens sp．nov．is very similar to E．rubripes spinosus with respect body colour and ultimate leg prefemur spine arrangement， although can be distinguished in that the tooth－plates of the former have four distinct teeth on each side，and legs 1－3 to 4 have two tarsal spurs，whereas the latter has three teeth on each side of the tooth－plate，and the two anterior pairs of legs have two tarsal spurs．Joshi and Edgecombe（2018）considered H．longicauda（Pocock，1891）from India to be a synonym of E．rubripes spinosus，with the former having forcipular coxosternal tooth－plates bearing 3.5 teeth，the innermost being small，and the ultimate leg prefemur bearing 2VL， $1 \mathrm{VM}, 1 \mathrm{M}$ ，and 2DM spines．

Ethmostigmus austroyunnanensis Jiang \＆Huang sp．nov．滇南筛孔蜈蚣（Fig．1B， Fig．4A－R）

## Material examined．

Holotype．${ }^{\lambda}$ ，CHINA：Yunnan Province：Mangshi：Menghuan Gold Tower scenic spot：Mts．Leiyarang，24．4279N，98．5959E， 1020 m a．s．l．，CMMI 20200608081， 08 Jun． 2020，leg．C．Jiang．

Paratypes. CHINA: Yunnan Province: Yingjiang county: 1才, CMMI 20200610001, Jun. 2020, leg. JD. Zhang, 1 q, CMMI 20190702003, 2 Jul. 2019, leg. JD. Zhang; 1q, CMMI 20210401105, 5 Apr. 2021, leg. JD. Zhang 1 ; Taiping town, CMMI 20201209104, 9 Dec. 2020, leg. MX. Shi.
Other materials: CHINA: Yunnan Province: Yingjiang county: $3 q$ ㅇ, Mangyun village, $24.5911 \mathrm{~N}, 97.7723 \mathrm{E}$, CMMI 20190507001-003, 07 May. 2019, leg. QY. Ji; $2 \widehat{J}^{\top}$ \& 2 ใ ㅇ, unspecified locality in Yingjiang county, CMMI 20201214112-115, 14 Dec. 2020, leg. QY. Ji; 1q, unspecified locality in Yingjiang county, CMMI 20190609029, 09 Jun. 2019, leg. JD. Zhang; Dehong Dai \& Jingpo autonomous prefecture: 1 juvenile, unspecified locality, CMMI 20200319004, 19 Mar. 2020, leg. JZ. Lu.
Type locality. Yunnan Province: Mangshi: Menghuan Gold Tower scenic spots.
Etymology. The specific name austroyunnanensis refers to the southern region of Yunnan, the locality in which the new species occurs.
Diagnosis. Forcipular coxosternal tooth-plates with four well-defined teeth on each side, coxopleural process short, approximately 1.6-1.9 times length of sternite 21, with two distinctly separated terminal spines and a lateral spine. Ultimate legs thick and moderately long, with prefemur and femur length ratios of approximately 1.1:1, femur and tibia of 1.3:1, tibia and tarsus 1 of 1.3:1, and tarsus 1 and tarsus 2 of 2.1:1. Ultimate legs prefemur with $3 \mathrm{VL}, 2 \mathrm{VM}, 1 \mathrm{M}$, and 2 DM spines. Ultimate leg tibia and tarsus 1 of males swollen.
Holotype description. The colour description is based on photographs of a living specimen (Fig. 1B). Cephalic plate and T1 reddish brown in coloration. Tergites 2 to 21 monochromatic brown. Antennae yellowish. Sternites and legs reddish orange. Leg colour gradually deepens from anterior to posterior legs. Prefemur and femur of ultimate leg jujube red, whereas tibia and tarsus are yellowish. Ultimate leg and leg 20 with black stripe on tibia and tarsus 1 .

Body length 93 mm . Posterior margin of cephalic plate underlying T1 (Fig. 4A). Antennae with 20 articles, basal four articles glabrous dorsally and three ventrally (Fig. 4B). Cephalic plate smooth, with large punctates. Forcipular coxosternal tooth-plates slightly longer than wide, with width-to-length ratio of approximately 1.7:1. Toothplates with four main teeth on each side (Fig. 4C), which can be divided into two groups, the outermost being smaller than other large teeth, the inner two and outer two teeth sharing respective common bases. Base of tooth-plates defined by oblique sutures diverging at $110^{\circ}$. Article 2 of second maxillary telopodite with spur.

Tergites smooth, without hairs or setae. Tergites with complete paramedian sutures from T3 to T20 (Fig. 4E). Complete clearly visible margination beginning from T6. ULBS tergite slightly curved posteriorly, without median furrow or depression. Tergite 21 with parallel lateral margins and width-to-length ratio of 1.5:1 (Fig. 4I).

Surface of sternite 21 with longitudinal depression, strongly incurved posteriorly, forming a triangle diverging at $120^{\circ}$ (Fig. 4J). Coxopleural process 1.8 times length of Sternite 21 , with two distinctly separated terminal spines and one lateral spine (Fig. 4J, K). Pores dense and pore-free area narrow, extending $66 \%$ of length from distal part of coxopleural process to margin of sternite 21 .

Legs 2－20 with very sparse short setae．First two pairs of legs with two tarsal spurs， legs 3－20 with one tarsal spine．Leg 1 with one tibial and one femoral spur．Ultimate legs without setae．Ultimate legs thick and long in male，with prefemur and femur length ratios of 1．1：1，femur and tibia length of 1．34：1，tibia and tarsus 1 length of 1．26：1， and tarsus 1 and tarsus 2 length of 2．05：1．Ultimate leg with two claw spurs（Fig．4P）， prefemur with large spines，as follows： $3 \mathrm{VL}, 2 \mathrm{VM}, 1 \mathrm{M}, 2 \mathrm{DM}$ spines，and 1 corner spine（Fig．4D，H）．

Variation．Details are shown in Table 2．Body length up to 120 mm （paratype CMMI 20210401105）．The ultimate leg shows sexual dimorphism，with the tibia and tarsus 1 prominently swollen in males（holotype，Fig．4Q）and slender in females（paratype CMMI 20201209104，Fig．4R）．

Distribution．Yunnan（Dehong Dai and Jingpo Autonomous Prefecture）．Fig． 2.
Remarks：Ethmostigmus austroyunnanensis sp．nov．shows pronounced sexual dimorphism in the ultimate leg，with the tibia and tarsus 1 being more prominently swollen in males than in females．Mating observations have indicated that specimens with swollen tarsi can spin webs and produce spermatophores and are accordingly sexed as male，whereas specimens lacking swollen tarsi and receiving spermatophores can be sexed as female．To the best of our knowledge，sexual dimorphism in the genus Ethmostigmus Pocock， 1898 is described here for the first time．

Ethmostigmus biocolor Jiang \＆Huang sp．nov．二色筛孔蜈蚣（Fig．1C，Fig．5） Material examined．
Holotype．CHINA：Yunnan Province：Yingjiang county（24．59N，97．77E），CMMI 20200610002，Jun．2020，leg．JD．Zhang．
Paratypes．CHINA：Yunnan Province：Yingjiang county：one spm，Mangyun village， 24．5911N，97．7723E，CMMI 20191212007， 12 Dec．2019，leg．MX．Shi；two spms， unspecified locality in Yingjiang county，CMMI 20201209102－103， 09 Dec．2020，leg． MX．Shi．
Other materials：CHINA：Yunnan Province：Yingjiang county：one spm，CMMI 20200610003，Jun．2020，leg．JD．Zhang；two spms，CMMI 20190712109－110，Jul． 2019，leg．MX．Shi；one spm，CMMI 20201209101， 9 Dec．2020，leg．MX．Shi．
Type locality．Yunnan Province：Yingjiang county．
Etymology．The specific name biocolor refers to the strong contrast in colouration of the body and locomotory legs．
Diagnosis．Forcipular coxosternal tooth－plates with four well－defined teeth on each side．Forcipule bearing small rudimentary forcipular trochanteroprefemoral process． with low median keel from T6 to T20．Coxopleural process approximately 2.5 times length of sternite 21 ，with one apical spine，one subapical spine，and one lateral spine． Ultimate leg prefemur with $3 \mathrm{VL}, 1-2 \mathrm{VM}, 1-2 \mathrm{M}$ ，and 2DM spines．
Holotype description．The colour description is based on photographs of a living specimen．Cephalic plate，antennae，tergites，and ultimate leg monochromatic blue－ violet．Sternites orange．Legs，except ultimate leg prefemur，orange－red；femur，tibia，
and tarsus 1 blue; tarsus 2 pale yellow.
Body length 90 mm . Posterior margin of cephalic plate underlying T1 (Fig. 5A). Antennae with 20 articles, basal four articles glabrous dorsally and three ventrally (Fig. 5B). Cephalic plate smooth, with large punctates. Forcipular coxosternal tooth-plates slightly longer than wide, with four well-defined main teeth on each side, and width-to-length ratio of approximately 1.36:1. Each tooth bearing small lobes, except outermost. Tooth-plate base defined by oblique sutures diverging at $100^{\circ}$. Article 2 of second maxillary telopodite with spur. Forcipule base with toothless rudimentary trochanteroprefemoral process (Fig. 5C).

Tergites weakly corrugated, with complete low rounded median keel from T6 to T20 (Fig. 5E), and incomplete in T21 (Fig. 5G). Tergites without hairs or setae. Complete clearly visible margination beginning from T6. ULBS tergite slightly curved posteriorly, without median furrow or depression. ULBS width-to-length ratio of 1.6:1 (Fig. 5G).

Surface of sternites smooth. Sternites with complete paramedian sutures starting from S3 (Fig. 5F). Sternite 21 surface with longitudinal depression that is strongly incurved posteriorly and forms a triangle that diverges at $95^{\circ}$ (Fig. 5H). Coxopleural process 2.5 times length of sternite 21 , with one apical spine, one dorsal subapical spine distinctly separated from apical spine, and one lateral spine (Fig. 5P). Pores dense and pore-free area narrow, extending $75 \%-80 \%$ of length from distal part of coxopleural process to margin of sternite 21.

All legs have very sparse short setae. All legs, including the ultimate leg, with two claw spurs. Leg 1 with two tarsal spurs, legs $2-20$ with one tarsal spur. Leg 1 with one tibial and one femoral spur. Ultimate legs long and slender, with prefemur and femur length ratio of $1.05: 1$, femur and tibia of $1.25: 1$, tibia and tarsus 1 of $1.20: 1$, and tarsus 1 and tarsus 2 of 2.05:1. Ultimate leg prefemur with large spines, as follows: $3 \mathrm{VL}, 1$ VM, 2 M, 2 DM spines, and 1 corner spine on left prefemur (Fig. 5D, I), and 3 VL, 1 VM, $1 \mathrm{M}, 1 \mathrm{DM}$ spines, and 1 corner spine on right prefemur (to be interpreted as regeneration).
Variation. Details are shown in Table 2. Body length up to 108 mm (paratype CMMI 20201209102). The spine on the ultimate leg prefemur often with 3 VL, 2 VM, 2 M, and 2 DM spines, but specimen CMMI 20200712109 with 3 VL, 1 VM, 2 M, and 2 DM spines and specimen CMMI 20200712109 with 4 VL spines on left leg prefemur. Distribution. Yunnan (Yingjiang county). Fig. 2.
Remarks. The trochanteroprefemoral process is deemed to be a plesiomorphic character that is shared within Rhysida, and considered the major characteristic distinguishing Rhysida from Ethmostigmus. Pocock (1891) described the amonotypic species Ethmophorus monticola as having four glabrous antennal articles and the presence of a forcipular trochanteroprefemoral process. Kraepelin (1903) transferred this species to Rhysida, which has a number of characters in common with Ethmostigmus, including a large spiracle on segment 3, robust forcipules, an elongated coxopleural process, and four glabrous basal antennal articles (Joshi et al. 2019). The presence of a trochanteroprefemoral process-like structure in Ethmostigmus biocolor sp. nov. limits its utility as a critical character separating Rhysida and Ethmostigmus.

Ethmostigmus motuoensis Jiang \＆Huang sp．nov．墨脱筛孔蜈蚣（Fig．1D，Fig．6） Material examined．
Holotype．CHINA：Xizang Autonomous Region：Motuo county（29．48N，96．09E）， CMMI 20211008105， 08 Oct．2021，leg．SY．Zhao．
Paratypes．CHINA：Xizang Autonomous Region：Motuo county，one spm，CMMI 20200511001， 11 May．2020，leg．MX．Shi；two spms，CMMI 20210316107－108，Mar． 2021，leg．MX．Shi；one spm，CMMI 20190712112，Jul．2019，leg．MX．Shi．
Type locality．Xizang Autonomous Region：Motuo county．
Etymology．The specific name motuonesis refers to Motuo county，the locality in which the new species occurs．
Diagnosis．Forcipular coxosternal tooth－plates with four well－defined teeth on each side．Forcipules with small rudimentary forcipular trochanteroprefemoral process． Tergite with low median keel from T6 to T20．Coxopleural process approximately three times length of sternite 21 ，with one apical spine，one subapical spine，one dorsal spine， and one lateral spine．Ultimate leg prefemur with $3 \mathrm{VL}, 2 \mathrm{VM}, 2 \mathrm{M}$ ，and 2DM spines．
Description．The colour description is based on photographs of a living specimen． Colouration of cephalic plate and T1 jujube red．Antennae and T2 to T21 reddish－brown in colour．Prefemur and femur of legs 1 to 19 orange red；tibia and tarsus 1 blue， gradually deepening in colour from anterior to posterior legs，tarsus 2 pale yellow．Leg 20 prefemur orange red；femur，tibia and tarsus 1 blue，tarsus 2 pale yellow．Ultimate leg prefemur jujube red；femur，tibia，and tarsus deep blue．

Body length 90 mm ．Posterior margin of cephalic plate underlying T1（Fig．6A）． Antennae with 20 articles，basal four articles glabrous dorsally and three ventrally（Fig． 6B）．Cephalic plate smooth with large punctates．Forcipular coxosternal tooth－plates slightly longer than wide，with four well－defined main teeth on each side，and width－ to－length ratio of approximately 1.46 ：1．Each tooth bearing indistinct small lobes， except outermost．Tooth－plate bases defined by oblique sutures diverging at $110^{\circ}$ ． Article 2 of second maxillary telopodite with spur．Forcipule base with toothless rudimentary trochanteroprefemoral process（Fig．6C）．

Tergites weakly corrugated，with complete low rounded median keel from T 7 to T20（Fig．6E），although incomplete in T21（Fig．6G）．Tergites without hairs or setae； complete paramedian sutures from T4 to T20．Complete clearly visible margination beginning from T4．ULBS tergites slightly curved posteriorly，without median furrow or depression．ULBS tergite width－to－length ratio of 1．6：1．

Surface of sternites smooth．Sternite paramedian sutures incomplete，covering $50 \%-80 \%$ of sternite length，and beginning at sternite 3 （Fig．6F）．Sternite 21 surface with deep longitudinal depression that is incurved posteriorly and forms a triangle that diverges at $125^{\circ}$ ．Coxopleural process incurved posteriorly，three times length of sternite 21 （Fig．6H），with one robust apical spine，one dorsal subapical spine distinctly separated from apical spine，one small dorsal spine，and one lateral spine（Fig．6P）． Pores dense and pore－free area narrow，extending $85 \%-90 \%$ length from distal part of coxopleural process to margin of sternite 21 ．

All legs with very sparse short setae．Legs 1－20 have two claw spurs．Leg 1 with two tarsal spurs，legs $2-20$ with one tarsal spur．Leg 1 with one tibial and one femoral spur．Ultimate legs long and slender，with prefemur and femur ratio of 1．35：1，femur and tibia of 1．11：1，tibia and tarsus 1 of 1．30：1；tarsus 1 and tarsus 2 of 2．17：1．Ultimate leg without claw spur（Fig．6N），prefemur with large spines，as follows： 3 VL， 2 VM， 2 M， 2 DM spines，and 1 corner spine（Fig．6D，I）．
Variation．Details are shown in Table 2．Body length up to 110 mm （paratype CMMI 20200511001）．The spine on the ultimate leg prefemur often with 3 VL， 2 VM， 2 M， and 2 DM spines，but paratype CMMI 20210316108 with three VM spines．The spines of the coxopleural process are variable，with two lateral spines on paratype CMMI 20190712112 and no dorsal spine on the right process．The length of sternite 21 of paratype CMMI 20190712112 is noticeably longer than that of other specimens，giving a coxopleural process to sternite 21 length ratio of 2．7：1．
Distribution．Xizang（Motuo county）．Fig． 2.
Remarks．This species is similar to E．biocolor sp．nov．with respect to the complete median keels on tergites and forcipule bearing a small rudimentary trochanteroprefemoral process，although differs in terms of the coxopleural process， which is clearly longer（～three times the length of sternite 21）and bears a dorsal spine， whereas in E．biocolor sp．nov．，the coxopleural process is approximately 2.5 times the length of sternite 21，and lacks a dorsal spine．Haase（1887）described the species Heterostoma rapax silhetense from Silhet（India），which is also synonymous to $E$ ． pygomegas（Kohlrausch，1879）from the Himalayas，the coxopleural process of which lacks a dorsal spine and the prefemur of the ultimate leg often has $3 \mathrm{VL}, 2 \mathrm{VM}, 2 \mathrm{M}$ ， and 2 DM spines，similar to E．biocolor sp．nov．However，both E．pygomegas （Kohlrausch，1879）and $H$ ．rapax silhetens（Haase，1887）lack complete median keels and a rudimentary trochanteroprefemoral process．Consequently，we consider both $E$ ． biocolor sp．nov．and Ethmostigmus motuonesis sp．nov．to be new species．

Ethmostigmus pygomenasoides Lewis， 1992 stat．nov．斑足筛孔蜈蚣（Fig．1E，Fig． 7）
Ethmostigmus trigonopodus pygomenasoides Lewis，1992：449－450，Fig．45－51，53－54． Type locality．Nepal：Myagdi．
Holotype．Specimen 7 （SMF 6455）， 87 mm ，NEPAL：Myagdi Distr．：near Pelma， 2650－2700 m， 29 May 1973，leg．J．Martens．（based on Lewis，1992）
Material examined．CHINA：Xizang Autonomous Region：Jilong county： Jilonggou：one spm，CMMI 20200905127， 05 Sept．2020，leg．JD．Zhang．
Diagnosis．Forcipular coxosternal tooth－plates with four well－defined teeth on each side，coxopleural process approximately two times length of sternite 21 ，terminally two－ spined，one to four dorsal spines，and one lateral spine．Ultimate leg prefemur with $2-$ $3 \mathrm{VL}, 1-2 \mathrm{VM}, 1-2 \mathrm{M}$ ，and $1-2 \mathrm{DM}$ spines．
Description of Chinese specimen．The colour description is based on photographs of a living specimen（Fig．1E）．Cephalic plate，antennae，and tergites monochromatic olive yellow．Legs 1 to 20 pale yellow，with black stripes on femur，tibia，and tarsus，the size
and color of which gradually deepen from anterior to posterior legs. Ultimate leg prefemur orange; femur, tibia, and tarsus pale yellow with light black stripes.

Body length 73 mm . Posterior margin of the cephalic plate underlying T1 (Fig. 7A). Antennae with 20 articles, basal four articles glabrous dorsally and three ventrally (Fig. 7B). Cephalic plate smooth, with large punctures. Forcipular coxosternal toothplates slightly longer than wide, with four main teeth on each side; width-to-length ratio of left and right tooth-plates approximately $1.40: 1$ and $1.57: 1$, respectively (Fig. 7C). Innermost tooth has two or three accessory teeth. Base of tooth-plates defined by oblique sutures diverging at $110^{\circ}$. Second maxillary claw with two accessory spines. Article 2 of second maxillary telopodite with several long setae.

Surface of tergites smooth and sparsely punctate with visible setae. Tergites 3 to 20 with complete paramedian sutures (Fig. 7E). Complete clearly visible margination beginning from T5 or T6. Tergite of ULBS slightly curved posteriorly, without median furrow or depression (Fig. 7G). T21 width-to-length ratio of 1.32:1.

Sternites with complete paramedian sutures starting from S3. Sternite 21 incurved posteriorly, forming triangle diverging at $120^{\circ}$ with weak longitudinal depression (Fig. 7 H ). Coxopleural process 2.03 times length of sternite 21 , with two distinctly separated terminal spines, three dorsal spines distant from terminal spines, and one lateral spine (Fig. 7P). Pores dense and pore-free area narrow, extending approximately $80 \%$ of length from distal part of coxopleural process to margin of sternite 21.

Legs with very short setae. All legs, including the ultimate leg, with two claw spurs. Leg 1 with one tibial spur and two tarsal spurs, without prefemoral and femoral spurs, and legs 2-20 with one tarsal spur. Leg 1 with one tibial and one femoral spur. Ultimate legs moderately long, prefemur and femur length ratios of 1.29:1, femur and tibia of 1.17:1, tibia and tarsus 1 of 1.32:1, and tarsus 1 and tarsus 2 of 1.95:1. Prefemur with large spines, as follows: $3 \mathrm{VL}, 2 \mathrm{VM}, 2 \mathrm{M}, 2 \mathrm{DM}$ spines, and 1 corner spine (Fig. 7D, I).

Distribution. Xizang (Jilong county) (Fig. 2); Nepal (Myagdi, Lalirpur, Taplejung, Gorkha).
Remarks. In their examination of five specimens of E. trigonopodus from West Nepal and three specimens from Central Nepal, Schileyko and Stagl (2004) found that these showed no distinct differences from typical representatives of the nominative form. However, the findings of molecular phylogeny analyses indicated that $E$. pygomenasoides and E. austroyunnanensis sp. nov. are reciprocally monophyletic and grouped with E. trigonopodus from Africa with relatively moderate node support (See blew), and thereby elevating the former to full specific status as E. pygomenasoides Lewis, 1992 stat. nov. E. trigonopodus from West Nepal needs to be re-examined and compared with African specimens to ascertain whether these specimens are E. trigonopodus or E. pygomenasoides. The original description by Lewis (1992) states that E. pygomenasoides is characterized by an ultimate leg prefemur bearing three rows of spines of 2-3 VL, 1-2 VM, and 1-2 DM, and a simple corner spine. However, Fig. 54 shown in this paper clearly indicates four rows of spines of $3 \mathrm{VL}, 2 \mathrm{VM}, 2 \mathrm{M}$, and 1 DM spine, which resemble the pattern observed in Chinese specimens. Furthermore, the specimens of E. pygomenasoides from Nepal generally lack small tooth lobes,
whereas the Chinese specimens examined in the present study are characterized by inner teeth bearing accessory teeth. Accordingly, further data will be necessary to clarify the range of variation in this species and distinguish it from another Himalayan species, E. pygomegas (Kohlrausch, 1879).

Molecular phylogenetic analysis. The COI, 16 S rRNA, and 28 S rRNA loci of Ethmostigmus species analysed in this study were sequenced and aligned with sequences obtained for taxa in other major lineages of Otostigminae, including Alluropus, Alipes, Digitipes, Edentistoma, Otostigmus, and Rhysida, with Scolopendra mutilans Koch, 1878 being selected as an outgroup. We accordingly obtained a final alignment dataset of 1299 base pairs (bp) of sequencing data, comprising 531, 455, and 314 bp of COI, 16 S , and 28 S , respectively. Phylogenetic analysis was conducted based on maximum likelihood and Bayesian approaches, which indicated that Ethmostigmus is a monophyletic taxon with likelihood bootstrap (BS) support of $95 \%$ and posterior probability (PP) of 1.00 . The genus Ethmostigmus is divided into the following three species groups: the trigonopodus-pygomegas group (clade A) comprising the African species E. trigonopodus, and the five China Ethmostigmus species E. austroyunnanensis sp. nov., E. biocolor sp. nov., E. metuoensis sp. nov., E. flavescens sp. nov. and E. pygomenasoides Lewis, 1992, with a PP of 1.00 and BS of $87 \%$; the rubripes group (clade B) comprising the two Australian species E. rubripes and E. curtipes; and the tristis group (clade C) comprising the five Indian Peninsula species $E$. coonooranus Chamberlin, 1920, E. tristis (Meinert, 1886), E. agasthyamalaiensis Joshi \& Edgecombe, 2019, E. sahyadrensis Joshi \& Edgecombe, 2019, and E. praveeni Joshi \& Edgecombe, 2019a, and a Sri Lanka species (E. rubripes spinosus) with PP of 0.91 and BS of $88 \%$ (Fig. 8). The distinct status of these three groups is also supported morphologically with respect to tooth plate characteristics, with the for rubripes, tristis, and trigonopodus-pygomegas groups being distinguished by 3, 3.5 (the innermost bearing a cusp), and 4 main teeth on each tooth-plate, respectively.

All five Ethmostigmus species collected in China belong to the trigonopoduspygomegas group and comprise three clades in the molecular phylogeny. One has moderately high support ( $\mathrm{PP}=1.00, \mathrm{BS}=94 \%$ ) for the reciprocally monophyletic Xizang species pair $E$. biocolor sp. nov. and $E$. metuoensis sp. nov., the monophyly of each of which is also strongly supported ( $\mathrm{PP}=1.00, \mathrm{BS}=100 \%$ ). The remaining two clades include two Yunnan species and one Xizang species, E. flavescens sp. nov., is sister to E. austroyunnanensis sp. nov., and E. pygomenasoides ( $\mathrm{PP}=0.9, \mathrm{BS}=85 \%$ ). With moderate node support ( $\mathrm{PP}=0.9$ ), E. austroyunnanensis $\mathbf{~ s p}$. nov. is considered a sister to E. pygomenasoides and grouped to E. trigonopodus.

## Discussion

According to Conservation International (conservation.org), there are 36 biodiversity hotspots distributed worldwide (Kellee, 2016)., and notably all five Ethmostignus species identified on mainland China have been discovered in biodiversity hotspot regions: E. flavescens sp. nov., E. austroyunnanensis sp. nov., and E. biocolor sp. nov. from the Indo-Burma hotspot region, and E. pygomenasoides and
E. motuoensis sp. nov. from the Himalayas. These species belong to a monophyletic group that is sister to Australian and Peninsular Indian species. Since divergence estimates and biogeographic studies of Ethmostigmus, Joshi and Edgecombe (2019b) have proposed that the early evolutionary history of the genus Ethmostigmus was driven by the Gondwanan break-up. They suggest that Ethmostigmus has a Gondwanan origin and has subsequently undergone independent radiation in Peninsular India and Australia. For the Africa-wide species E. trigonopodus (Leach, 1817), which is nested in the trigonopodus-pygomegas group and is sister to E. austroyunnanensis sp. nov. from Southwest China, it is more likely that Ethmostigmus in China evolved via dispersal/vicariance from Australasia and radiative evolution in the Indo-Burma biodiversity hotspot. However, to evaluate the potential evolutionary scenarios for trigonopodus-pygomegas group species in these locations, extensive taxon sampling across Southeast Asia (e.g. Myanmar and Thailand), East India, the Andaman and Nicobar Islands, and Sri Lanka is required.

We found that several of the external morphological features often used to identify Ethmostigmus differ significantly among the Ethmostigmus specimens collected in China. Consequently, distinguishing inherent variance across specimens from variation attributable to damage or regeneration represents a notable challenge, particularly with respect to the form and arrangement of spines on the ultimate legs or the coxopleural process. Accordingly, it is difficult to eliminate cryptic Ethmostigmus species in China and neighbouring regions solely based on the morphological characters of Chinese Ethmostigmus dispersed in biodiversity hotspots. This problem is highlighted by the Nepalese specimens of E. pygomenasoides initially described by Lewis (1992), which were found to show high variability in the number of spines on the ultimate legs (totaling $7-10$ spines in 3 or 4 rows) and variable dorsal spines (1-4) on the coxopleural process. Moreover, these characteristics overlap to varying extents with those of the sympatric species, E. pygomegas, which frequently has four rows of spines arranged in rows of 3, 2, 1-2, 2 on the ultimate leg (Fig. 9), whereas a syntype (NHMW MY1561) has four rows spines arranged in rows of 2, 2, 1, 2 on the ultimate leg (Fig. 10). Given this ambiguity, further data are required to evaluate the genetic variation among specimens and to determine whether the current taxonomic designations are attributable to misidentification or whether cryptic Ethmostigmus species exist.

## Key to the Ethmostigmus species of China

1. With three main teeth on each side of tooth-plates...................................................... 2

- With four main teeth on each side of tooth-plates. .3

2. Coxopleural process 2.6-3.2 times length of sternite
$\qquad$

- Coxopleural process 1.6-2 times length of sternite 21................E. rubripes rubripes

3. Coxopleural process 1.5-2.3 times length of sternite 21, coxopleural process with 2
apical spines............................................................................................................. 4

- Coxopleural process 1.3-3.3 times length of sternite 21, coxopleural process with 1 apical spine .5

4. Coxopleural process without dorsal spine, male ultimate leg tibia and tarsus 1 more
swollen than in female .E. austroyunnanensis sp. nov.

- Coxopleural process without 1-4 dorsal spines..............E. pygomenasoides stat. nov.

5. Two VL spines on ultimate leg prefemur..................................E. flavescens sp. nov.

- Three VL spines on ultimate leg prefemur .6

6. Coxopleural process 2.2-2.7 times length of sternite 21, coxopleural process with dorsal spine. .E. motuoensis sp. nov.

- Coxopleural process 2.7-3.3 times length of sternite 21, coxopleural process without
$\qquad$


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Figure legends


Figure 1. Habitus of Ethmostigmus from China. (A) E. flavescens sp. nov.; (B) and (C) E. austroyunnanensis sp. nov.; (D) E. biocolor sp. nov.; (E) E. motuoensis sp. nov.; (F) E. pygomenasoides Lewis, 1992 stat. nov.


Figure 2. Distribution of Ethmostigmus in China.


Figure 3. Ethmostigmus flavescens sp. nov. (A)-(O) holotype CMMI 20200608081. (A) Cephalic plate, T1 tergite, and proximal part of antennae, dorsal view; (B) cephalic plate and proximal part of antennae, ventral view, (C) tooth-plates, ventral view; (D) ultimate leg prefemur, lateral view; (E) tergites 9-12; (F) sternites 9-12; (G) tergite of the ultimate leg-bearing segment (ULBS), dorsal view; (H) coxopleura and sternite of the ULBS, ventral view; (I) ultimate leg prefemur, dorsal view; (J) spiracle on segment 3 ; (K) spiracle on segment 5 ; (L) spiracle on segment 7 ; (M) spiracle on segment 8 ; (N): ultimate leg tarsus 2 and claw. (O) segment 21, coxopleura and ultimate leg, ventral lateral view. (P) paratype CMMI 20201209104, coxopleuron, lateral view. (A)-(P). Scale bar $=1 \mathrm{~mm}$.


Figure 4. Ethmostigmus austroyunnanensis sp. nov. (A)-(Q) holotype CMMI 20200608081. (A) Cephalic plate, tergite T1, and proximal part of antennae, dorsal view; (B) cephalic plate and proximal part of antennae, ventral view, (C) tooth-plates, ventral view; (D) ultimate leg prefemur, ventral lateral view; (E) tergites 9-12; (F) sternites 7-10; (G) ultimate leg prefemur, dorsal view; (H) ultimate leg prefemur, ventral medial view; (I) tergite of the ultimate leg-bearing segment (ULBS), dorsal view; (J) coxopleura and sternite of the ULBS, ventral view; (K) coxopleuron, lateral view, scale $=1 \mathrm{~mm} ;(\mathrm{L})$ spiracle on segment $3 ;(\mathrm{M})$ spiracle on segment $5 ;(\mathrm{N})$ spiracle on segment 7; (O) spiracle on segment 8; (P): ultimate leg tarsus 2 and claw. (Q) ultimate leg, ventral lateral view; (R) paratype CMMI 20201209104, ultimate leg, ventral lateral view. $(A)-(R)$. Scale bar $=1 \mathrm{~mm}$.


Figure 5. Ethmostigmus biocolor sp. nov. holotype CMMI 20200608081. (A) Cephalic plate, tergite T1, and proximal part of antennae, dorsal view; (B) cephalic plate and proximal part of antennae, ventral view, (C) tooth-plates, ventral view; (D) ultimate leg prefemur, dorsal view; (E) tergites 9-12, dorsal view; (F) sternites 6-10, ventral view; (G) tergite of the ultimate leg-bearing segment, dorsal view; (H) coxopleuron and ultimate sternite, ventral view; (I) ultimate leg prefemur, ventral view; (J) spiracle on segment 3 ; (K) spiracle on segment 5 ; (L) spiracle on segment 7; (M) spiracle on segment $8 ;(\mathrm{N})$ : ultimate leg tarsus 2 and claw. (O) ultimate leg, dorsal view; (P) coxopleuron, lateral view. rp, rudimentary forcipular trochanteroprefemoral process. mk , median keel. Scale bar $=1 \mathrm{~mm}$.


Figure 6. Ethmostigmus motuonesis sp. nov. (A) Cephalic plate, tergite T 1 , and proximal part of antennae, dorsal view; (B) cephalic plate, the first legs, and proximal part of antennae, ventral view, (C) tooth-plates, lateral view; (D) ultimate leg prefemur, ventral view; (E) tergites 9-12, dorsal view; (F) sternites 6-9, ventral view; (G) tergite of the ultimate leg-bearing segment, dorsal view; $(\mathrm{H})$ coxopleuron and ultimate sternite, ventral view; (I) ultimate leg prefemur, ventral view; (J) spiracle on segment 3; (K) spiracle on segment 5 ; (L) spiracle on segment 7 ; (M) spiracle on segment 8 ; (N): ultimate leg tarsus 2 and claw. (O) ultimate leg, dorsal view; (P) coxopleuron, lateral view. (A)-(C), (E)-(M), paratype CMMI 20210316107. (D), (I), (N), (O), (P), holotype CMMI 20211008105. rp, rudimentary forcipular trochanteroprefemoral process. mk, median keel. Scale bar $=1 \mathrm{~mm}$.


Figure 7. Ethmostigmus pygomenasoides Lewis, 1992 stat. nov. specimen CMMI 20200905127. (A) Cephalic plate, tergite T1, and proximal part of antennae, dorsal view; (B) cephalic plate, the first legs, and proximal part of antennae, ventral view, (C) tooth-plates, lateral view; (D) ultimate leg prefemur, dorsal view; (E) tergites 9-12, dorsal view; (F) sternites 5-8, ventral view; (G) tergite of the ultimate leg-bearing segment, dorsal view; (H) coxopleuron and ultimate sternite, ventral view; (I) ultimate leg prefemur, ventral view; (J) spiracle on segment 3; (K) spiracle on segment 5; (L) spiracle on segment $7 ;(\mathrm{M})$ spiracle on segment $8 ;(\mathrm{N})$ : ultimate leg tarsus 2 and claw. ( O ) coxopleuron, leg 20 and ultimate leg, lateral view; ( P ) coxopleuron, lateral view. Scale bar $=1 \mathrm{~mm}$.


Figure 8. Maximum likelihood phylogenetic tree based on combined data for Ethmostigmus with likelihood bootstrap ( $\mathrm{BS}>70 \%$ ) and Bayesian posterior probability ( $\mathrm{PP}>0.9$ ) values indicated for each node. Specimen codes in parentheses denote the localities listed in Table 1.


Figure 9. Syntypes of E. pygomegas (Kohlrausch, 1879), specimen ZMH-A0000640, 1 jar and 1 vial. Jar label: Heterostoma rapax Gerv. Original zu H. pygomela Kohlr. Himalaya (pencil). Vial label: Heterostoma pygomega n. sp. (pencil on red paper) [Godeffroy number: 31796]. (A) tooth-plates, ventral view; (B) right antennae, dorsal view; (C) proximal leg; (D) tergites, dorsal view; (E) sternite, ventral view; (F) tergite of the ultimate leg-bearing segment, dorsal view; (G) coxopleuron and ultimate sternite, ventral view; (H, I) ultimate leg prefemur, ventral view; (J) coxopleuron, lateral view. DP, dorsal spine. Credit: Nadine Dupérré.


Figure 10. Syntype of E. pygomegas (Kohlrausch, 1879), specimen NHMW MY1561, 1 Ex., Originally labelled as Heterostoma rapax (pencil), Himalaya, [1882.I.14], [Godeffroy number: 14946]. (A) Cephalic plate, the first legs, and proximal part of antennae, ventral view; (B) tooth-plates, ventral view; (C) coxopleuron, lateral view; (D) tergite of the ultimate leg-bearing segment, dorsal view; (E) coxopleuron and ultimate sternite, ventral view; (F) ultimate leg prefemur, ventral lateral view; (G) ultimate leg prefemur, ventral medial view; (H) ultimate leg tarsus and claw; (I) ultimate leg, ventral medial view. Scale bar $=1 \mathrm{~mm}$. Credit: Oliver Macek.

Table 1 Samples used for phylogenetic analyses

| No. | Species | Voucher number | Taxon locality | GenBank accession number |  |  | Reference |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | COI | 16S | 28S |  |
| 1 | Ethmostigmus flavescens | CMMI 20191212010 | China: Yunnan: Mengla | ON231521 | ON232473 | ON232460 | This study |
| 2 | Ethmostigmus flavescens | CMMI 20190507004 | China: Yunnan: Mengla | ON231522 | ON232474 | ON232461 | This study |
| 3 | Ethmostigmus austroyunnanensis | CMMI 20201214112 | China: Yunnan: Yingjiang | ON231523 | ON232475 | ON232462 | This study |
| 4 | Ethmostigmus austroyunnanensis | CMMI 20190702003 | China: Yunnan: Yingjiang | ON231524 | ON232476 | ON232463 | This study |
| 5 | Ethmostigmus austroyunnanensis | CMMI 20200606081 | China: Yunnan: Mangshi | ON231525 | ON232477 | ON232464 | This study |
| 6 | Ethmostigmus motuoensis | CMMI 20210316107 | China: Xizang: Motuo | ON231526 | ON232478 | ON232465 | This study |
| 7 | Ethmostigmus motuoensis | CMMI 20190712112 | China: Xizang: Motuo | ON231527 | ON232479 | ON232466 | This study |
| 8 | Ethmostigmus bicolor | CMMI 20191212007 | China: Yunnan: Yingjiang | ON231519 | ON232471 | ON232458 | This study |
| 9 | Ethmostigmus bicolor | CMMI 20191212012 | China: Yunnan: Yingjiang | ON231520 | ON232472 | ON232459 | This study |
| 10 | Ethmostigmus pygomenasoides | CMMI 20200905127 | China: Xizang: Jilong | - | ON232480 | - | This study |
| 11 | Ethmostigmus rubripes spinosus | CMMI 20211117116 | Sri Lanka | ON231528 | ON232481 | ON232467 | This study |
| 12 | Ethmostigmus trigonopodus | CMMI 20200712003 | Ghana | ON209601 | ON209448 | ON212678 | This study |
| 13 | Ethmostigmus trigonopodus | CMMI 20211117117 | Cameroun | ON209602 | ON209449 | ON212679 | This study |
| 14 | Ethmostigmus agasthyamalaiensis | CES091065 | India: Kerala: Parambikulam Tiger Reserve | MH908726 | MH908695 | MH908710 | 1 |
| 15 | Ethmostigmus agasthyamalaiensis | CES091099 | India: Shendurney Wildlife Sanctuary: Pandimatte | MH908728 | MH908696 | MH908711 | 1 |
| 16 | Ethmostigmus agasthyamalaiensis | CES091335 | India: Kerala: Kottavasal Reserve Forest | MH908729 | MH908697 | MH908712 | 1 |
| 17 | Ethmostigmus coonooranus | CES091067 | India: Kerala: Parambikulam Tiger Reserve | MH908727 | MH908693 | - | 1 |
| 18 | Ethmostigmus coonooranus | CES091063 | India: Kerala: Parambikulam Tiger Reserve | MH908725 | MH908692 | - | 1 |
| 19 | Ethmostigmus coonooranus | CES091399 | India: Karnataka: Bramhagiri Wildlife Sanctuary | - | MH908694 | MH908709 | 1 |
| 20 | Ethmostigmus coonooranus | CES07286 | India: Karnataka: Coorg | JN004031 | JN003920 | - | 1 |
| 21 | Ethmostigmus praveeni | CES091011 | India: Karnataka: Kudremukh National Park | MH908722 | MH908686 | MH908703 | 1 |
| 22 | Ethmostigmus praveevni | CES091012 | India: Karnataka: Kudremukh National Park | MH908723 | MH908687 | MH908704 | 1 |


| 23 | Ethmostigmus praveeni | CES091025 | India: Karnataka: Shimoga |
| :---: | :---: | :---: | :---: |
| 24 | Ethmostigmus praveeni | CES091391 | India: Karnataka: Sakaleshapur Reserve Forest |
| 25 | Ethmostigmus sahyadrensis | CES08931 | India: Maharashtra: Mahabaleshwar Reserve Forest |
| 26 | Ethmostigmus sahyadrensis | CES08947 | India: Maharashtra: Sindhudurg |
| 27 | Ethmostigmus sahyadrensis | CES07279 | India: Maharashtra: Sindhudurg |
| 28 | Ethmostigmus trigonopodus | IZC 00146501 | Malawi: Southern Region: Zonba |
| 29 | Ethmostigmus tristis | CES08291 | India: Tamil Nadu: Namakkal |
| 30 | Ethmostigmus tristis | CES08292 | India: Tamil Nadu: Namakkal |
| 31 | Ethmostigmus tristis | CES08293 | India: Tamil Nadu: Namakkal |
| 32 | Ethmostigmus tristis | CES091373 | India: Tamil Nadu: Namakkal |
| 33 | Ethmostigmus tristis | CES091371 | India: Tamil Nadu: Namakkal |
| 34 | Ethmostigmus rubripes | gvc13592-1L | - |
| 35 | Ethmostigmus rubripes | gvc12159-1L | - |
| 36 | Ethmostigmus rubripes | AM KS 58455 | Australia: Queensland: Lizard Island |
| 37 | Ethmostigmus rubripes rubripes | IZ130653 | Australia |
| 38 | Ethmostigmus curtipes | WAM115537 | Australia |
| 39 | Alipes grandidieri | IZ130616 | Tanzania |
| 40 | Alipes crotalus | MCZ DNA100454 | Swaziland |
| 41 | Alluropus calcaratus | CUMZ 00470 | Laos: Ban NamHom: Xieang Khuang |
| 42 | Alluropus calcaratus | CUMZ 00484 | Thailand: Surat Thani: Donsak |
| 43 | Digitipes barnabasi | CES07244 | India: GoaKarnataka border: Dughsagar Water Falls |
| 44 | Digitipes coonoorensis | CES07132 | India: Kerala: New Amarambalam Wildlife Sanctuary |
| 45 | Digitipes jangii | CES07288 | India: Karnataka: Kodagu |
| 46 | Digitipes jonesii | CES07162 | India: Kerala: Thiruvananthapuram |
| 47 | Digitipes kalewaensis | CUMZ 00234 | Myanmar: Sagaing Division: Kale |
| 48 | Digitipes nudus | CES08961 | India: Kerala: Idukki |


| MH908724 | MH908688 | MH908705 | 1 |
| :---: | :---: | :---: | :---: |
| MH908731 | MH908689 | MH908706 | 1 |
| MH908720 | MH908690 | MH908707 | 1 |
| MH908721 | MH908691 | MH908708 | 1 |
| JN004030 | JN003919 | JN003975 | 1 |
| HQ402547 | HQ402495 | - | 2 |
| MH908717 | MH908698 | MH908713 | 1 |
| MH908718 | MH908699 | MH908714 | 1 |
| MH908719 | MH908700 | - | 1 |
| - | MH908702 | MH908716 | 1 |
| MH908730 | MH908701 | MH908715 | 1 |
| HQ986501 | - | - | Genbank |
| HQ986492 | - | - | Genbank |
| AF370836 | AY288721 | AF173281 | 2 |
| KF676542 | KF676475 | KF676373 | 3 |
| KF676515 | KF676474 | KF676372 | 3 |
| KF676514 | KF676473 | KF676371 | 3 |
| AY288742 | AY288720 | HM453273 | 2 |
| MF167838 | MF167771 | MF167905 | 5 |
| MF167821 | MF167754 | MF167888 | 5 |
| JX531837 | JX531707 | JX531784 | 6 |
| JN004035 | JN003924 | JN003981 | 7 |
| JX531839 | JX531709 | JX531786 | 6 |
| JN004042 | JN003931 | JN003986 | 7 |
| KP204116 | KP204112 | - | 5 |
| MK273245 | MK273357 | MK273467 | 1 |


| 49 | Edentistoma octosulcatum | ZMUTChi01 | Sarawak, Malaysia |
| :---: | :---: | :---: | :---: |
| 50 | Otostigmus aculeatus | CUMZ 00519 | Laos: Huaphan: Mueang Yommarat |
| 51 | Otostigmus angusticeps | MCZ DNA106500 | Papua New Guinea: Finisterre Mountains |
| 52 | Otostigmus astenus | MCZ DNA102463 | Fiji/Vanuatu |
| 53 | Otostigmus multidens | CUMZ 00527 | Thailand: Chiang Mai: Mae Taeng |
| 54 | Otostigmus politus politus | MCZ DNA106768 | China: Henan: Dengfeng |
| 55 | Otostigmus ruficeps | CES091342 | India: Kerala: Kollam |
| 56 | Otostigmus rugulosus | CUMZ 00537 | Thailand: Nan: Phu Phiang |
| 57 | Otostigmus scaber | CUMZ 00530 | Laos: Luang Phrabang: Wat Ban Hu |
| 58 | Otostigmus spinicaudus | MCZ DNA104645 | Tunisia: Kasserine |
| 59 | Otostigmus spinosus | CUMZ 00553 | Thailand: Nakhon Si Thammarat: Khanom |
| 60 | Otostigmus sulcipes | CUMZ 00532 | Thailand: Chiang Mai: Huai Hong Khrai |
| 61 | Rhysida afra | MCZ DNA104695 | South Africa: Eastern Cape |
| 62 | Rhysida aspinosa | CES07159 | India: Kerala: Thiruvananthapuram |
| 63 | Rhysida carinulata | IZ-130667 | Papua New Guinea: Western Province |
| 64 | Rhysida crassispina | CES091396 | India: Maharashtra: Matheran |
| 65 | Rhysida immarginata | CUMZ 00436 | Thailand: Ranong: Kra Buri |
| 66 | Rhysida konda | CES091394 | India: Odisha: Koraput |
| 67 | Rhysida lewisi | CES07238 | India: Karnataka: Uttara Kannada |
| 68 | Rhysida longipes | CUMZ 00432 | Myanmar: Bahin village: PK2 |
| 69 | Rhysida nuda | AM KS 58456, IZ130678 | Australia: New South Wales |
| 70 | Rhysida pazhuthara | CES091092 | India: Kerala: Kollam district |
| 71 | Rhysida polyacantha | IZ130665 | Australia: Northern Territory |
| 72 | Rhysida sada | CES091361 | India: Maharashtra: Raigad |
| 73 | Rhysida singaporensis | CUMZ 00446.3 | Singapore: Bukit Timah |
| 74 | Rhysida trispinosa | CES07217 | India: Karnatka: Uttara Kannada |


| KM492931 | KM492929 | KM492928 | 8 |
| :---: | :---: | :---: | :---: |
| MF167797 | MF167730 | MF167864 | 5 |
| KF676509 | - | KF676365 | 3 |
| HM453312 | HM453221 | HQ402532 | 2 |
| MF167795 | MF167728 | MF167862 | 5 |
| KF676512 | KF676470 | KF676368 | 3 |
| JX531901 | JX531771 | JX531822 | 6 |
| MF167787 | MF167720 | MF167854 | 5 |
| MF167802 | MF167735 | MF167869 | 5 |
| - | KF676472 | KF676370 | 3 |
| MF167785 | MF167718 | MF167852 | 5 |
| MF167803 | MF167736 | MF167870 | 5 |
| HQ402552 | HQ402500 | HQ402536 | 2 |
| MK273209 | MK273316 | MK273437 | 6 |
| KF676516 | - | KF676374 | 3 |
| MK273288 | MK273412 | MK273508 | 6 |
| MF167826 | MF167759 | MF167893 | 5 |
| MK273286 | MK273410 | MK273506 | 6 |
| JN004024 | JN003913 | JN003969 | 6 |
| MF167833 | MF167766 | MF167900 | 5 |
| DQ201432 | AY288722 | AF173282 | 2 |
| MK273268 | MK273386 | MK273488 | 6 |
| KF676518 | KF676476 | KF676376 | 3 |
| MK273279 | MK273402 | MK273499 | 6 |
| MF167844 | MF167777 | MF167911 | 5 |
| MK273214 | MK273322 | MK273441 | 6 |

[^0]Table 2. Comparison of the morphologies of Ethmostigmus centipedes from China. Prefemoral spine formula on ultimate legs: VL: ventrolateral spine, VM: ventromedial spine, M: median spine, DM: dorsomedial spine, SP: spine on prefemoral process/prefemoral corner process. UL: ultimate leg. Reference: $1=$ this study, $2=$ lewis $1992,3=$ Thofern et al. 2021, $4=$ Kohlrausch 1879.

| Characters | E. <br> flavescen $s$ | E. <br> austroyunna nensis | E. biocolor | E. motuoensis |  | nasoides | E. pygomegas |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Ref | 1 | 1 | 1 | 1 | 1 | 2 | 1,3,4 |
| Locality | Yunnan | Yunnan | Yunnan | Xizang | Xizang | Nepal | Himalaya, Bhutan; India; Myanmar |
| Body length (mm) | 75-90 | 81-101 | 76-108 | 87-110 | 73 | 28-100 | reach 120 |
| Number of antennal articles | 20 | 10 | 20 | 20 | 20 | ? | 20 |
| Number of glabrous articles (dorsal/ventral) | 4/3 | 4/3 | 4/3 | 4/3 | 4/3 | 4/3 | 4/3 |
| Main teeth on tooth plate Rudimentary forcipular trochanteroprefemoral process | 4 absent | 4 absent | 4 present | 4 present | $\begin{gathered} 4 \\ \text { absent } \end{gathered}$ | $\begin{gathered} 4 \\ \text { absent } \end{gathered}$ | $\begin{gathered} 4 \\ \text { absent } \end{gathered}$ |
| Length: wide of tooth plate | $\begin{aligned} & 1.68- \\ & 2.02: 1 \end{aligned}$ | 1.66-1.83:1 | $\begin{aligned} & 1.32- \\ & 1.38: 1 \end{aligned}$ | 1.37-1.46:1 | $\begin{gathered} \text { 1.27(R), } \\ 1.48(\mathrm{~L}) \end{gathered}$ | 1.40-1.57 | 1.52-1.68 |
| First tergite with complete paramedian sutures | 4-5 | 3-4 | 2-3(4) | 4-5(8) | 3 | 3-4 | ? |
| First tergite margin complete | 5-6 | 4-6 | 4-6 | 4-5 | 3 | 5-6 | ? |


| Tergites with median <br> keel | absent | absent | $6(8)-21$ | $7(8)-21$ | absent | absent | incomplete or absent |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :---: |
| Paramedian sutures on <br> sternites complete | $2-3$ | $3-5$ | $2-3$ | incomplete | 2 | ranging from short <br> anterior to virtually <br> complete | incomplete |


| Legs with femoral spur | 1 | 1 | $1(0)$ | $0(1)$ | 0 | $?$ | 1 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Legs with tibial spur | 1 | 1 | $1(0)$ | $1(0)$ | 1 | $?$ | 1 |
| Claw spines on UL | $0(1)$ | 2 | $2(1)$ | $0(1)$ | 2 | 2 | 0 |


[^0]:    $\begin{array}{lcc}75 & \text { Scolopendra mutilans } & \text { CMMI 20190702006 }\end{array} \quad$ China: Taiwan: Kenting $\quad$ MT093846 $\quad$ MT084409 $\quad$ MT084375 $\quad 4$ $7=$ Joshi \& Karanth 2011; $8=$ Vahtera \& Edgecombe 2014;

