RESEARCH ARTICLE

Six new species of the spider genus *Spiricoelotes* species (Araneae, Agelenidae) from caves in Jiangxi, China

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Abstract

Six new species of the spider genus *Spiricoelotes* Wang, 2002 are described, *S. anshiensis* Chen & Li, **sp. n.** (♂♀), *S. chufengensis* Chen & Li, **sp. n.** (♂♀), *S. nansheensis* Chen & Li, **sp. n.** (♂♀), *S. taipingensis* Chen & Li, **sp. n.** (♂♀), *S. xianheensis* Chen & Li, **sp. n.** (♂♀) and *S. xiongxinensis* Chen & Li, **sp. n.** (♀). All new species were collected from caves in Jiangxi Province, China.

Keywords

Introduction

The spider genus *Spiricoelotes* was established by Wang (2002) for one coelotine species from China: *Coelotes zonatus* Peng & Wang, 1997. Three valid *Spiricoelotes* species were known before the current study: *S. urumensis* (Shimojana, 1989) from the Ryukyu Islands, *S. zonatus* (Peng & Wang, 1997) and *S. pseudozonatus* Wang, 2003 from China (World Spider Catalog 2015). In this paper, six new *Spiricoelotes* species are described. All new species were collected from caves in the Jiangxi Province of China.

Material and methods

Specimens were examined with a LEICA M205C stereomicroscope. Images were captured with an Olympus C7070 wide zoom digital camera (7.1 megapixels) mounted on an Olympus SZX12 dissecting microscope. Epigynes and male palps were examined after dissection from the spiders' bodies.

All measurements were obtained using a LEICA M205C stereomicroscope and are given in millimeters. Leg measurements are shown as: total length (femur, patella + tibia, metatarsus, tarsus). Only structures (palp and legs) on the left side of the body were described and measured. The abbreviations and terminology used in the text follows Wang (2002). Abbreviations used in this paper and in the figure legends: A = epigynal atrium; ALE = anterior lateral eye; AME = anterior median eye; AME-ALE = distance between AME and ALE; AME-AME = distance between AME and AME; ALE-PLE = distance between ALE and PLE; CD = copulatory duct; CDA = dorsal conductor apophysis; CF = cymbial furrow; E = embolus; EB = embolic base; ET = epigynal teeth; FD = fertilization duct; H = epigynal hood; LTA = dorso-retrolateral tibial apophysis; OC = outgrowth of conductor; PA = patellar apophysis; PLE = posterior lateral eye; PME = posterior median eye; PME-PLE = distance between PME and PLE; PME-PME = distance between PME and PME; RTA = retrolateral tibial apophysis; S = spermatheca; SH = spermathecal head; SST = spermathecal stalk; ST = subtegulum; T = tegulum.

A partial fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified and sequenced for *S. anshiensis* sp. n., *S. chufengensis* sp. n., *S. nansheensis* sp. n., *S. taipingensis* sp. n., *S. xianheensis* sp. n. and *S. xiongxinensis* sp. n. following the protocol in Miller et al. (2009). Primers used in this study are: LCO1490 (5'-CWACAAAYCATARRGATATTGG-3') (Folmer et al. 1994) and HCO2198zz (5'-TAAACTTCCAGGTGACCAAAAAATCA-3') (this study). All sequences were blasted in GenBank and the accession numbers are provided in Table 1.

All specimens (including molecular vouchers) are deposited in the Institute of Zoology, Chinese Academy of Sciences in Beijing (IZCAS).

Table 1. Voucher specimen information.

Species	GenBank accession number	Sequence length	Collection localities
S. anshiensis sp. n.	KT896546	1196 bp	Chayuan Village, Shacun Town, Ji'an City, Jiangxi Province, China
S. chufengensis sp. n.	KT896541	1205 bp	Huangguan Village, Ningdu County, Ganzhou City, Jiangxi Province, China
S. nansheensis sp. n.	KT896544	1232 bp	Shuangqiao Town, Wanzai County, Yichun City, Jiangxi Province, China
S. taipingensis sp. n.	KT896542	1029 bp	Huangguan Village, Ningdu County, Ganzhou City, Jiangxi Province, China
S. xianheensis sp. n.	KT896543	1208 bp	Niedu Village, Chongyi County, Ganzhou City, Jiangxi Province, China
S. xiongxinensis sp. n.	KT896545	1232 bp	Zhanshan Village, Shangli County, Pingxiang City, Jiangxi Province, China

Taxonomy

Family Agelenidae C.L. Koch, 1837
Subfamily Coelotinae F.O.P.-Cambridge, 1893

Genus *Spiricoelotes* Wang, 2002

Spiricoelotes Wang, 2002: 129. Type species *Coelotes zonatus* Peng & Wang, 1997, from China.

Diagnosis. The males can be easily recognized from other coelotines by the strongly curved patellar apophyses, the absence of a dorsal apophysis of the conductor, and the slender, anteriorly extending conductor. (Fig. 1A–C). The females can be distinguished from other coelotines by the absence of epigynal teeth, the well-developed epigynal hoods and the long, strongly convoluted spermathecae (Fig. 2A–B).

Spiricoelotes anshiensis Chen & Li, sp. n.
http://zoobank.org/50A54C0B-F2F3-4663-AFC2-A8923FA64660
Figs 1–2, 12

Type material. Holotype ♂: China: Jiangxi: Ji'an City: Shacun Town, Chayuan Village, Anshi Cave, N26°31'24", E115°06'48", elevation: 332 m, 3.V.2013, Y.F. Luo and J.C. Liu. **Paratypes:** 5♀, same data as holotype.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male of the new species can be easily distinguished from all other *Spiricoelotes* by the long conductor (more than 1/2 length of cymbium, in previously known species subequal to or less than 1/2 length of cymbium) and looped conductor apex (with one loop, conductor of previously known species spiralled, rather than with looped apex), the long and broad outgrowth of the conductor (conductor of previously known species without outgrowth) (Fig. 1A–C). The female of the new species can be easily distinguished from all of the other *Spiricoelotes* by the bean-shaped atria (approximately 1/3 of epigynal plate, atria of previously known species obviously less than 1/3 of epigynal plate), the funnel-shaped copulatory ducts, the short spermathecae and the epigynal hoods close to each other (Fig. 2A–B).

Description. Male (holotype): Total length 5.59. Carapace 2.78 long, 1.94 wide. Abdomen 2.81 long, 1.63 wide. Eye sizes and interdistances: AME 0.14, ALE 0.15, PME 0.13, PLE 0.11; AME-AME 0.04, AME-ALE 0.02, PME-PME 0.06, PME-PLE 0.08. Leg measurements: I 11.50 (3.35, 3.45, 2.75, 1.95); II 12.24 (3.20, 4.16, 3.12, 1.76); III 10.84 (2.88 3.28, 2.88, 1.80); IV 14.57 (3.92, 4.10, 4.23, 2.32). Chelicerae with 3 promarginal and 4 retromarginal teeth. Palp: patellar apophysis longer than patella, with pointed tip, strongly curved; RTA with pointed tip, extending beyond the tibia; LTA short, approximately 1/4 length
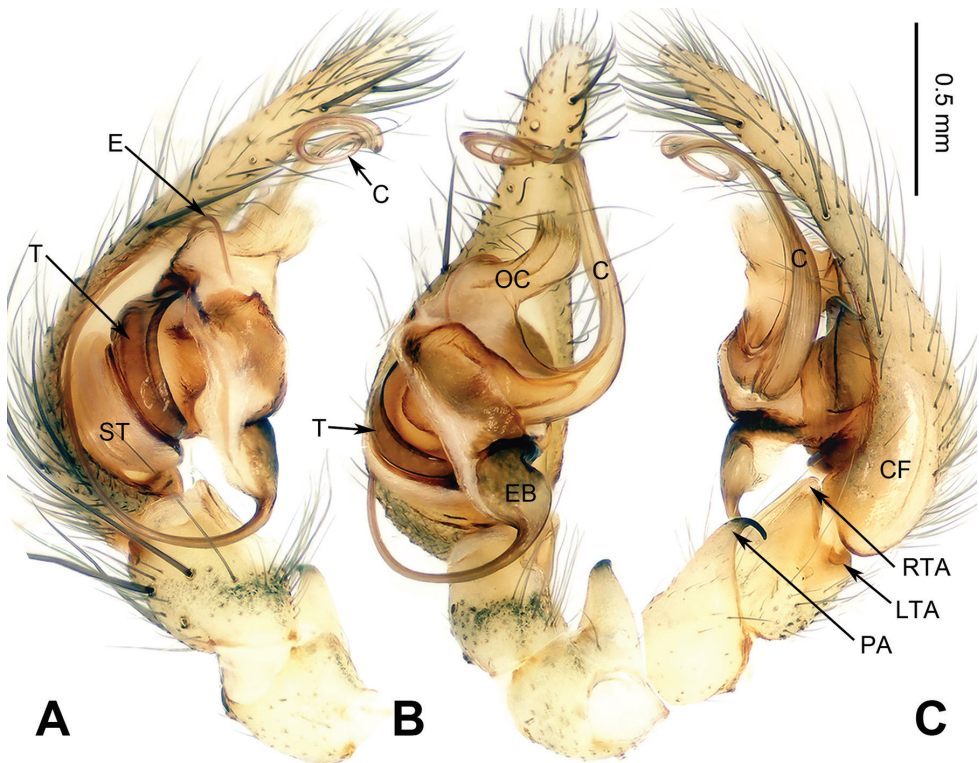
Figure 1. Left palp of *Spiricoelotes anshiensis* sp. n., holotype. **A** Prolateral **B** Ventral **C** Retrolateral. Scale bar equal for **A, B** and **C**.

of RTA; cymbial furrow long, approximately 1/3 length of cymbium; conductor long, slender, anteriorly extending, and apex looped; with outgrowth, located at the base of conductor; embolus beginning at 5:30 to 6 o'clock position (Fig. 1A–C).

Female (one of the paratypes): Total length 7.68. Carapace 3.28 long, 2.50 wide. Abdomen 4.40 long, 3.00 wide. Eye sizes and interdistances: AME 0.17, ALE 0.25, PME 0.19, PLE 0.17; AME-AME 0.04, AME-ALE 0.04, PME-PME 0.09, PME-PLE 0.08. Leg measurements: I 13.21 (3.60, 4.45, 3.12, 2.04); II 11.82 (3.50, 3.96, 2.60, 1.76); III 10.68 (3.04, 3.28, 2.76, 1.60); IV 14.88 (3.96, 4.85, 3.95, 2.12). Chelicerae as in male. Epigyne: atria bean-shaped, approximately 1/3 of epigynal plate, situated anteriorly and separated by septum (narrower than atria); hoods distinct, situated anteriorly, close to each other; spermathecae long and convoluted; copulatory ducts short, funnel-shaped (Fig. 2A–B).

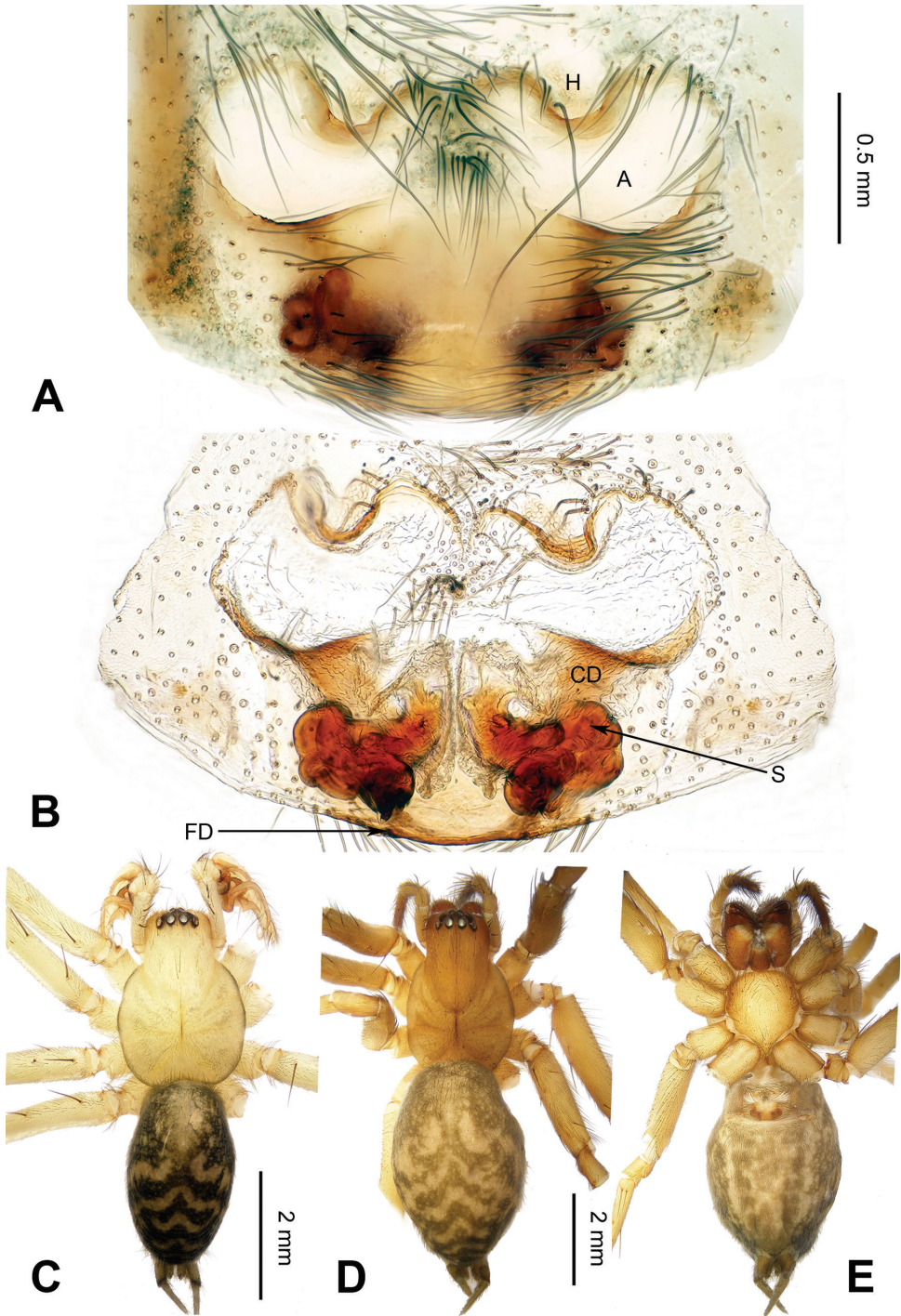**Distribution.** Known only from the type locality (Fig. 12).

Figure 2. Epigyne and habitus of *Spiricoelotes anshiensis* sp. n., **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars equal for **A** and **B**, equal for **D** and **E**.

Spiricoelotes chufengensis Chen & Li, sp. n.
http://zoobank.org/158012CD-B021-4A80-8A0C-F440F17E7D04
Figs 3–4, 12

Type material. Holotype ♂: China: Jiangxi: Ganzhou City: Ningdu County: Huangguan Village, Chufeng Cave, N26°29'35", E115°55'45", elevation: 395 m, 29.IV.2013, Y.F. Luo and J. Liu. **Paratypes:** 6♀, same data as holotype.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male of the new species has a uniquely shaped male palp and can be easily distinguished from all other *Spiricoelotes* by the more slender, needle-like conductor (conductor spiralled or with looped apex in other species) and the shorter cymbial furrow (approximately 1/4 length of cymbium, in other species approximately 1/2 length of cymbium) (Fig. 3A–C). The female of the new species can be easily
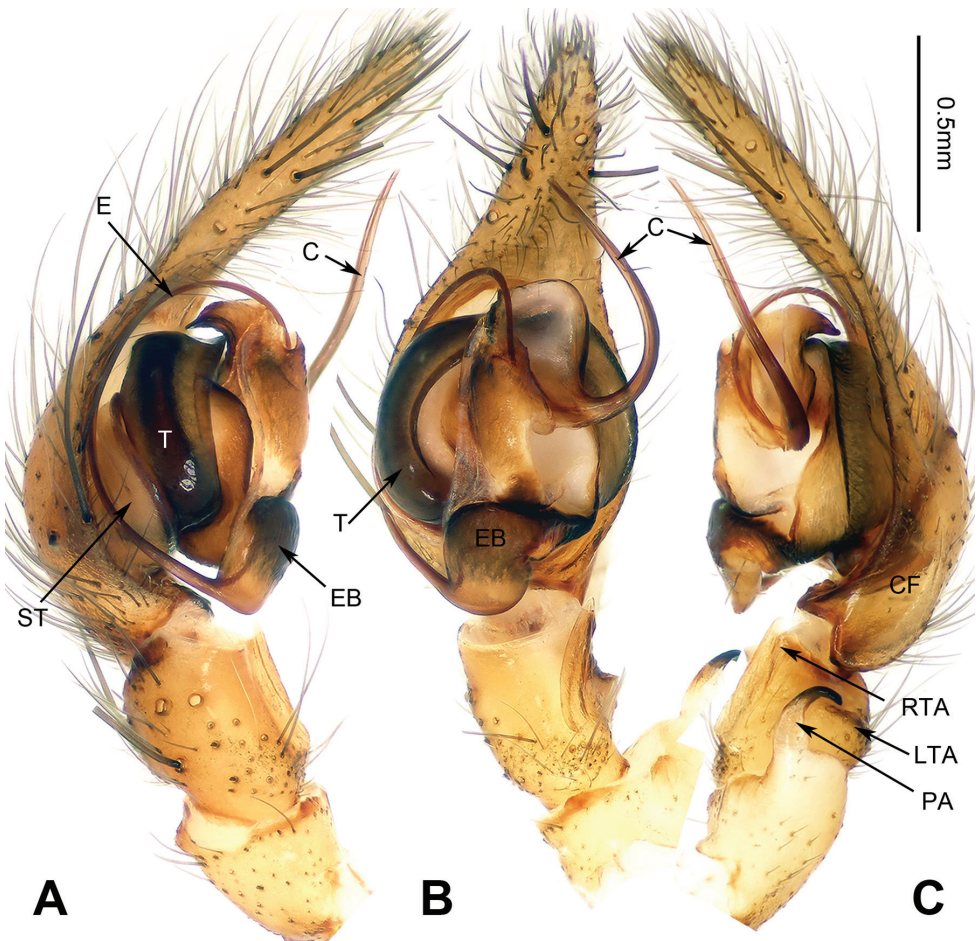

Figure 3. Left palp of *Spiricoelotes chufengensis* sp. n., holotype. **A** Prolateral **B** Ventral **C** Retrolateral. Scale bar equal for **A, B** and **C**.
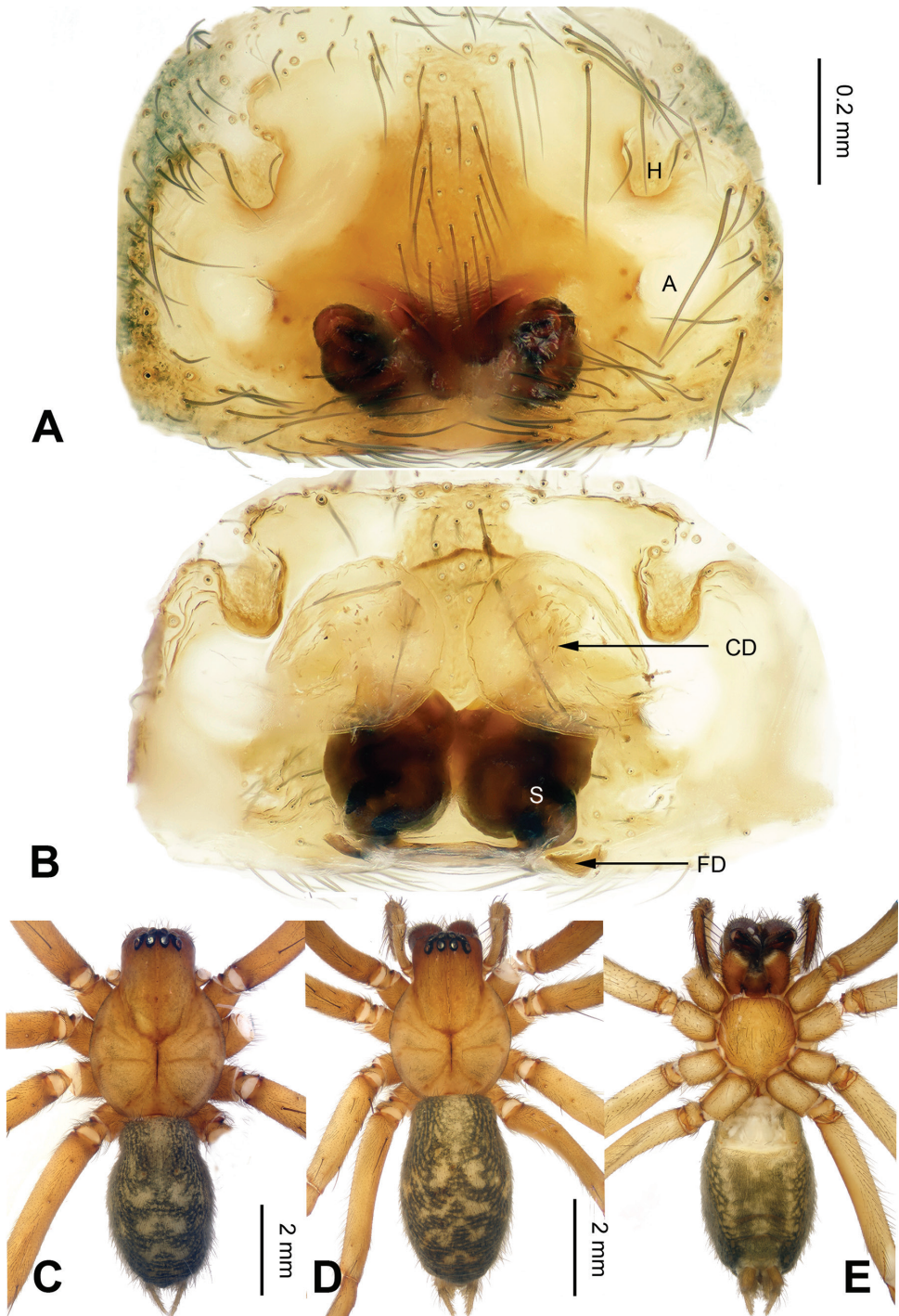
Figure 4. Epigyne and habitus of *Spiricoelotes chufengensis* sp. n., **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars equal for **A** and **B**, equal for **D** and **E**.

distinguished from all of the other *Spiricoelotes* by the smaller (subequal to hoods) and posterolaterally situated epigynal atria, the bulb-shaped copulatory ducts and the broader spermathecae (Fig. 4A–B).

Description. Male (holotype): Total length 8.05. Carapace 4.25 long, 3.00 wide. Abdomen 3.80 long, 2.25 wide. Eye sizes and interdistances: AME 0.20, ALE 0.14, PME 0.18, PLE 0.18; AME-AME 0.08, AME-ALE 0.04, PME-PME 0.14, PME-PLE 0.13. Leg measurements: I 15.05 (4.23, 5.12, 3.45, 2.25); II 13.55 (3.85, 4.30, 3.35, 2.05); III 12.85 (3.75, 4.05, 3.25, 1.80); IV 16.80 (4.65, 4.95, 4.75, 2.45). Chelicerae with three promarginal and five retromarginal teeth. Palp: patellar apophysis long, subequal to width of patella, with pointed tip, strongly curved; RTA with pointed tip, extending slightly beyond the tibia; LTA short; cymbial furrow short, approximately 1/4 length of cymbium; conductor long, needle-like, anteriorly extending; embolus, beginning at 6 o'clock to 6:30 position (Fig. 3A–C).

Female (one of the paratypes): Total length 7.52. Carapace 3.60 long, 2.48 wide. Abdomen 3.92 long, 2.36 wide. Eye sizes and interdistances: AME 0.15, ALE 0.19, PME 0.14, PLE 0.18; AME-AME 0.06, AME-ALE 0.03, PME-PME 0.11, PME-PLE 0.09. Leg measurements: I 13.15 (3.75, 4.45, 3.03, 1.92); II 11.24 (3.23, 3.80, 2.49, 1.72); III 10.62 (2.85, 3.32, 2.60, 1.85); IV 14.05 (3.80, 4.54 , 3.80, 2.00). Chelicerae as in male. Epigyne: atria small, located posterolaterally; hoods distinct, located antero-laterally; spermathecae broad, convoluted; copulatory ducts bulb-shaped (Fig. 4A–B).

Distribution. Known only from the type locality (Fig. 12).

Spiricoelotes nansheensis Chen & Li, sp. n.
http://zoobank.org/FD982663-7E2B-4493-A395-4A6E5FD4694E
Figs 5–6, 12

Type material. Holotype ♂: China: Jiangxi: Yichun City: Wanzai County: Shuang-qiao Town, Nanshe Cave, N28°10'8", E114°17'16", elevation: 195 m, 15.V.2013, Y.F. Luo and J.C. Liu. **Paratypes:** 1♂10♀, same data as holotype.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male of the new species has uniquely shaped palps and can be easily recognized from all of the other *Spiricoelotes* species by the extremely modified conductor (with two outgrowths at the base, only one in *S. anshiensis* sp. n., and none in other species), the looped apex (Fig. 5A–C). The female of the new species is similar to *S. chufengensis* sp. n. but can be distinguished from it by the larger and anteriorly situated atria, the more slender and longer copulatory ducts, the longer, more slender spermathecae that are separated from each other by copulatory ducts (Fig. 6A–B).

Description. Male (holotype): Total length 7.00. Carapace 3.40 long, 2.36 wide. Abdomen 3.60 long, 2.16 wide. Eye sizes and interdistances: AME 0.13, ALE 0.16, PME 0.15, PLE 0.08; AME-AME 0.05, AME-ALE 0.04, PME-PME 0.09, PME-PLE 0.06. Leg measurements: I 14.05 (3.60, 4.60, 3.55, 2.30); II 13.06 (3.50, 4.00, 3.35, 2.21); III 12.30 (3.44, 3.56, 3.33, 1.97); IV 16.05 (4.20, 5.00, 4.85, 2.00). Chelicerae
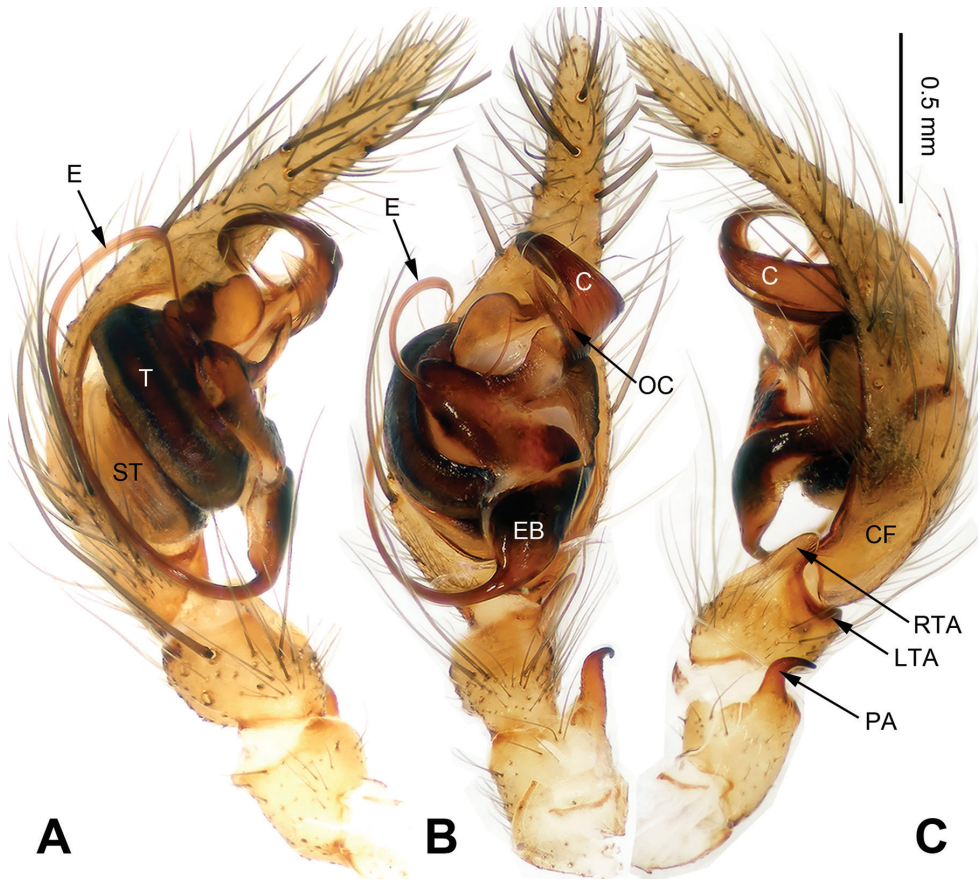
Figure 5. Left palp of *Spiricoelotes nansheensis* sp. n., holotype. **A** Prolateral **B** Ventral **C** Retrolateral. Scale bar equal for **A, B** and **C**.

with three promarginal and four retromarginal teeth. Palp: patellar apophysis long, subequal to patellar width, with pointed tip, strongly curved; RTA with blunt tip, extending beyond the tibia; LTA long, approximately 1/3 length of RTA; cymbial furrow short, approximately 1/5 length of cymbium; conductor broad, with looped apex, with two outgrowths at the base; embolus originates at 6 o'clock position (Fig. 5A–C).

Female (one of paratypes): Total length 7.16. Carapace 3.84 long, 2.48 wide. Abdomen 3.32 long, 2.08 wide. Eye sizes and interdistances: AME 0.10, ALE 0.15, PME 0.14, PLE 0.16; AME-AME 0.01, AME-ALE 0.04, PME-PME 0.09, PME-PLE 0.13. Leg measurements: I 13.80 (3.85, 4.10, 3.75, 2.10); II 13.08 (3.60, 4.08, 3.20, 2.20; III 12.68 (3.27, 3.96, 3.40, 2.05); IV 16.45 (4.15, 5.05, 4.80, 2.45); IV 16.66 (4.68, 5.00, 4.61, 2.37). Chelicerae as in male. Epigyne: atria small, located anteriorly, close to each other; hoods located anterolaterally; spermathecae long, convoluted; copulatory ducts slender, looped, located at center of vulva (Fig. 6A–B).

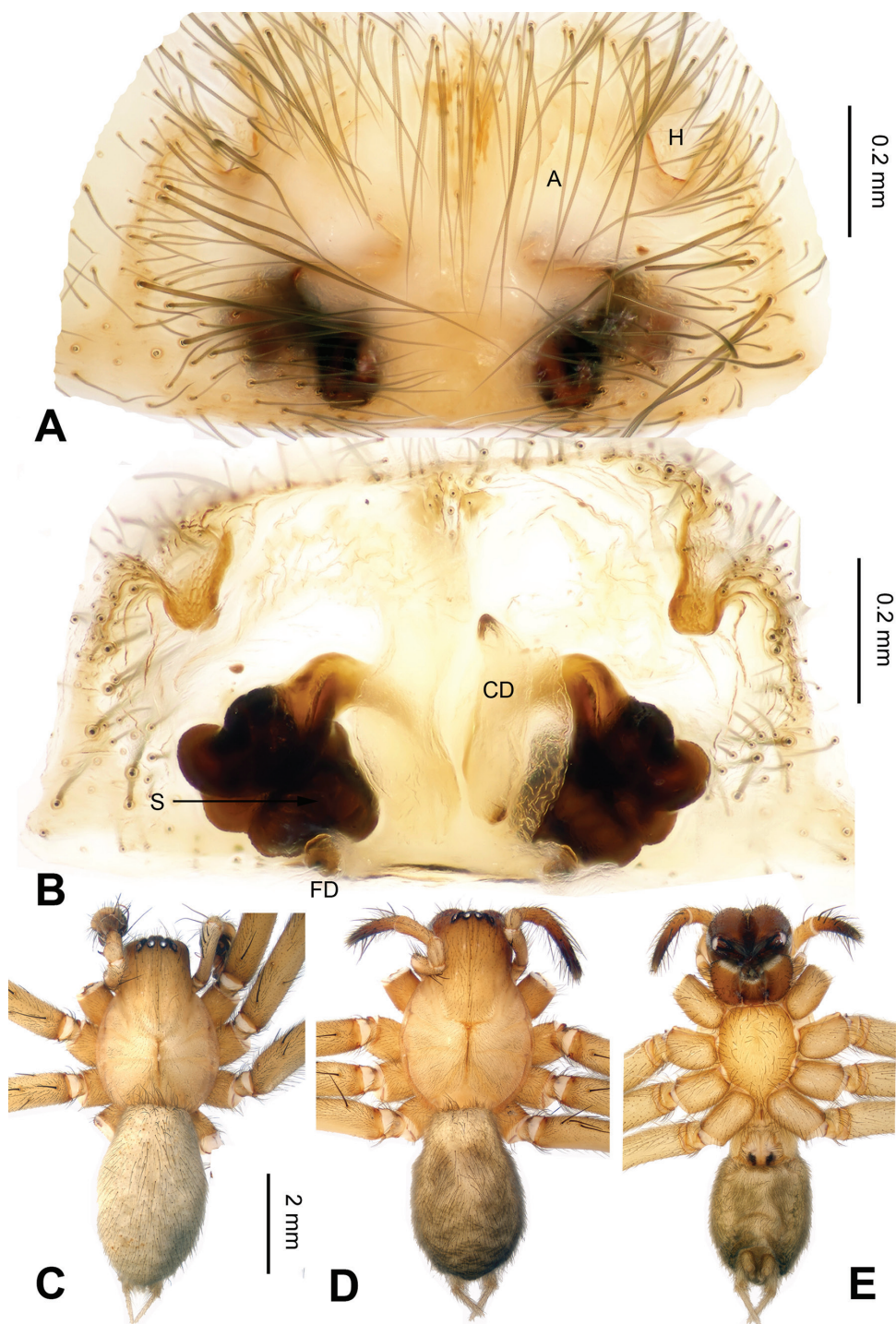**Distribution.** Known only from the type locality (Fig. 12).

Figure 6. Epigyne and habitus of *Spiricoelotes nansheensis* sp. n., **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars equal for **C, D** and **E**.

Spiricoelotes taipingensis Chen & Li, sp. n.

http://zoobank.org/D21CD9B1-63DC-4A86-9F9D-4DF0CBB8622F

Figs 7–8, 12

Type material. Holotype ♂: China: Jiangxi: Ganzhou City: Ningdu County: Huang-guan Village, Taiping Cave, N25°28'53", E115°54'35", elevation: 420 m, 29.IV.2013, Y.F. Luo and J.C. Liu. **Paratypes:** 10♀, same data as holotype.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male of the new species can be easily recognized by the short, broad conductor without a looped apex, the short cymbial furrow (approximately 1/5 length of cymbium, approximately 1/2, 1/3 or 1/4 length of cymbium in other species) and the strong patellar apophysis (twice as broad as in other species) (Fig. 7A–C). The female of the new species is similar to *S. anshiensis* sp. n. but can be distinguished by the larger atria (approximately 1/2 of epignal plate), the epignal hoods that are

Figure 7. Left palp of *Spiricoelotes taipingensis* sp. n., holotype. **A** Prolateral **B** Ventral **C** Retrolateral. Scale bar equal for **A, B** and **C**.
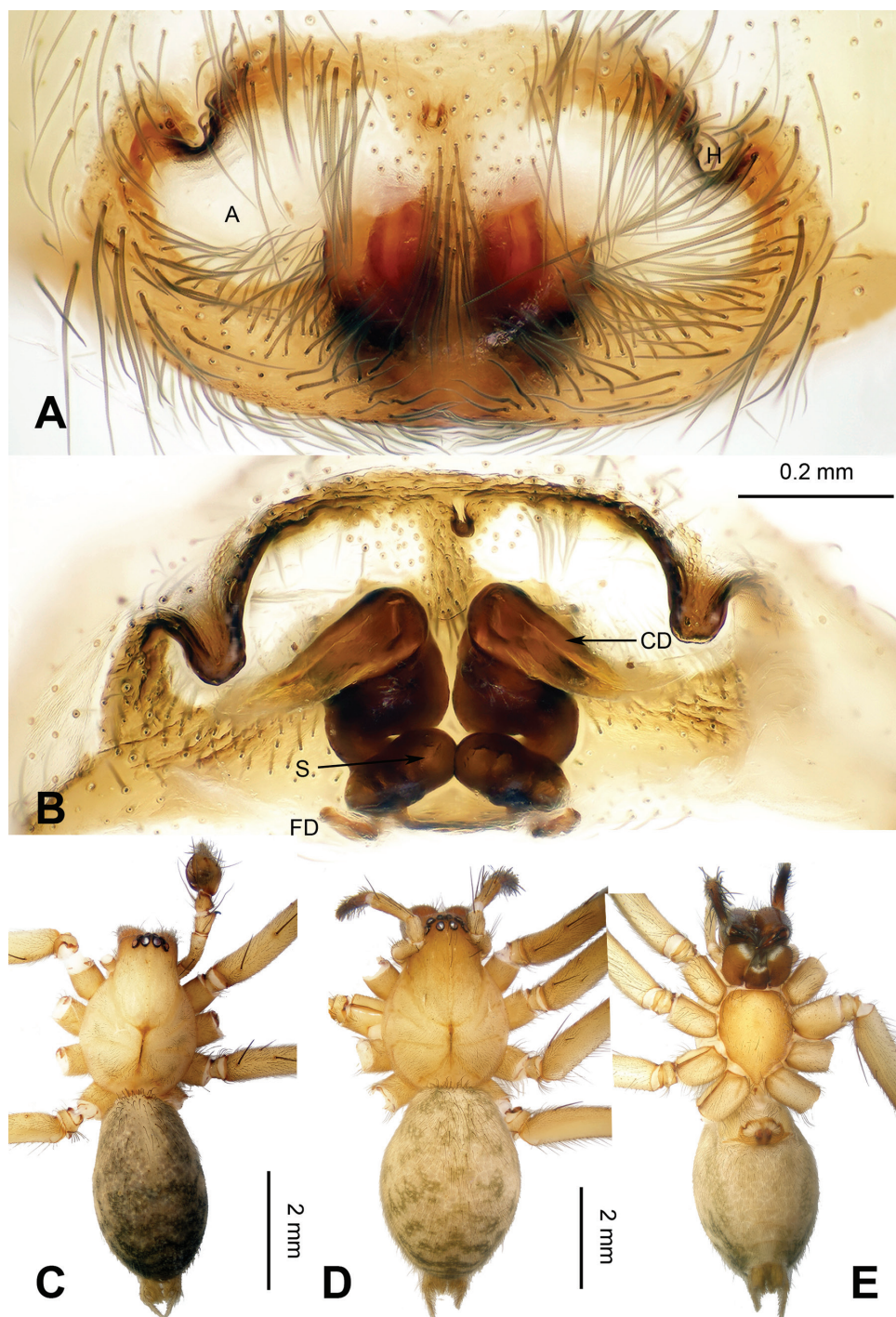
Figure 8. Epigyne and habitus of *Spiricoelotes taipingensis* sp. n., **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars equal for **A** and **B**, equal for **D** and **E**.

separated by twice their width, the slender and horizontally stretched copulatory ducts, the longer and helical spermathecae (Fig. 8A–B).

Description. Male (holotype): Total length 5.80. Carapace 2.60 long, 1.68 wide. Abdomen 3.20 long, 2.00 wide. Eye sizes and interdistances: AME 0.13, ALE 0.15, PME 0.16, PLE 0.15; AME-AME 0.02, AME-ALE 0.02, PME-PME 0.06, PME-PLE 0.04. Leg measurements: I 14.81 (3.76, 4.81, 3.84, 2.40); II 12.10 (2.82, 3.80, 3.52, 1.96); IV 16.81 (4.25, 5.00, 5.06, 2.50). Chelicerae with three promarginal and four retromarginal teeth. Palp: patellar apophysis subequal to patellar width, strongly curved; RTA with blunt tip, extending slightly beyond distal margin of tibia; LTA long, about 1/2 length of RTA; cymbial furrow short, approximately 1/5 length of cymbium; conductor short, anteriorly extending; embolus beginning at 7 o'clock position; base of embolus wider than tibia, triangular, horizontally directed (Fig. 7A–C).

Female (one of the paratypes): Total length 7.16. Carapace 3.36 long, 2.56 wide. Abdomen 3.80 long, 2.80 wide. Eye sizes and interdistances: AME 0.14, ALE 0.19, PME 0.16, PLE 0.19; AME-AME 0.06, AME-ALE 0.06, PME-PME 0.10, PME-PLE 0.09. Leg measurements: I 15.12 (4.17, 4.87, 3.20, 2.34); II 13.37 (3.72, 4.25, 3.25, 2.15); III 12.76 (3.44, 3.92, 3.4, 2.00); IV 16.66 (4.68, 5.00, 4.61, 2.37). Chelicerae as in male. Epigyne: atria large, occupying 1/2 of epigynal plate, located anteromedially; hoods distinct, located anterolaterally; spermathecae spiraled; copulatory ducts long and almost horizontally stretched (Fig. 8A–B).

Distribution. Known only from the type locality (Fig. 12).

***Spiricoelotes xianheensis* Chen & Li, sp. n.**
http://zoobank.org/43D32E4A-EEA8-4015-AEF2-8F43A9C3EF79
Figs 9–10, 12

Type material. Holotype ♂: China: Jiangxi: Ganzhou City: Chongyi County: Niedu Village, Xianhe Cave, N25°28'40", E114°06'56", elevation: 402 m, 23.IV.2013, Y.F. Luo and J.C. Liu. **Paratypes:** 1♂1♀, same data as holotype.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male of the new species is similar to *S. anshiensis* sp. n. and *S. nansheensis* sp. n. but can be distinguished from them by the less modified conductor (conductor without outgrowth at base in this species, with one outgrowth in *S. anshiensis* sp. n. and 2 outgrowths in *S. nansheensis* sp. n.) and having more loops in conductor (2 loops in this species, 1 loop in *S. anshiensis* sp. n. and *S. nansheensis* sp. n.) (Fig. 9A–C). The female of the new species has a uniquely shaped epigyne and can be distinguished from all of the other *Spiricoelotes* species by the medially situated and U-shaped atrium and the copulatory ducts that are looped around the spermathecal heads (Fig. 10A–B).

Description. Male (holotype): Total length 6.90. Carapace 3.20 long, 2.25 wide. Abdomen 3.70 long, 2.13 wide. Eye sizes and interdistances: AME 0.13, ALE 0.14, PME 0.15, PLE 0.17; AME-AME 0.05, AME-ALE 0.02, PME-PME 0.08, PME-PLE 0.06. Leg measurements: I 14.76 (3.96, 4.95, 3.55, 2.30); II 14.23 (3.88,
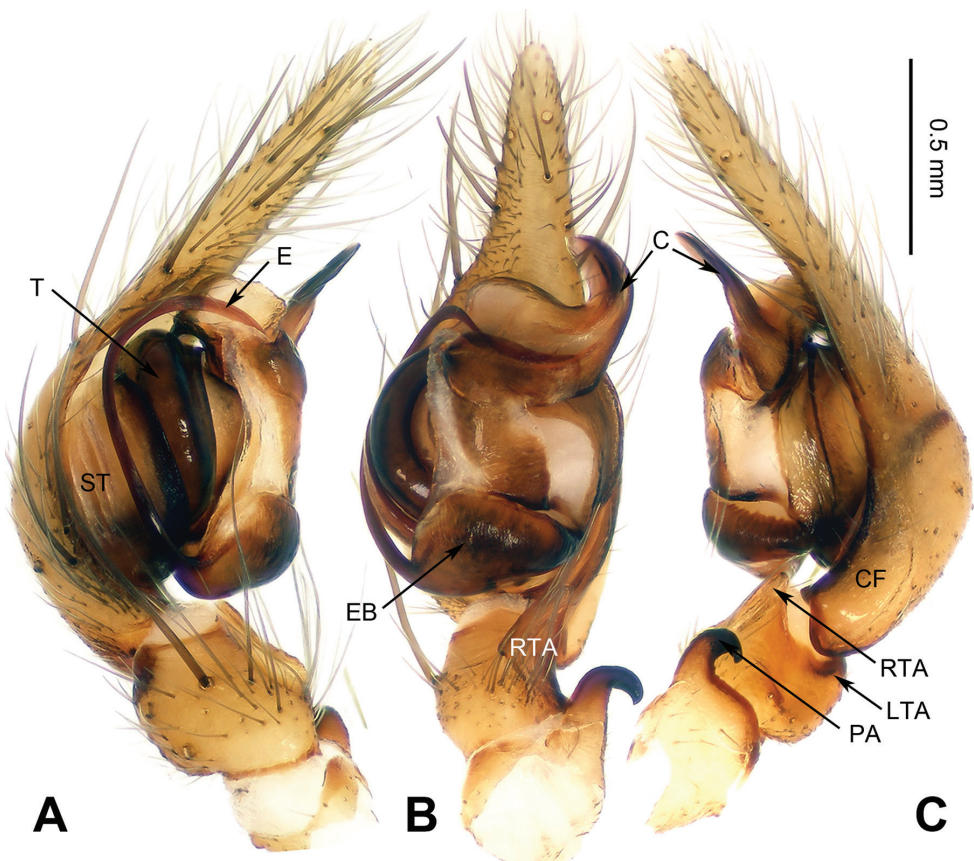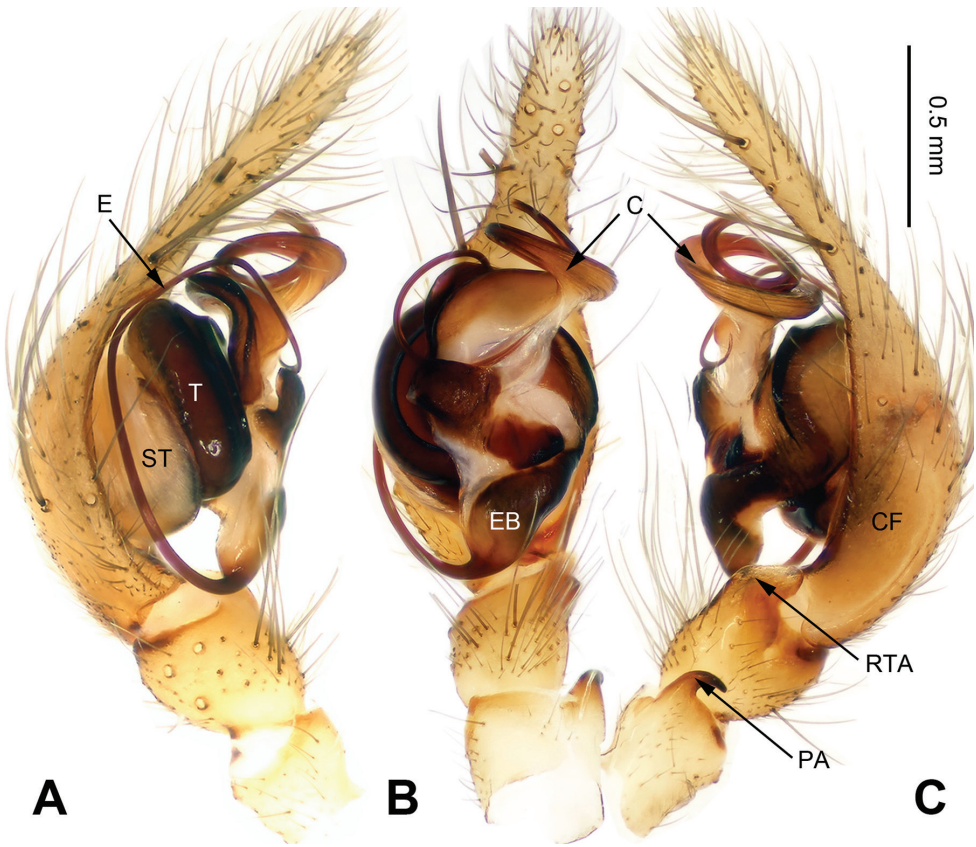
Figure 9. Left palp of *Spiricoelotes xianheensis* sp. n., holotype. **A** Prolateral **B** Ventral **C** Retrolateral. Scale bar equal for **A, B** and **C**.

4.80, 3.40, 2.15); III 13.08 (3.60, 3.80, 3.40, 2.28); IV 17.32 (4.50, 5.13, 5.13, 2.56). Chelicerae with 3 promarginal and 4 retromarginal teeth. Palp: patellar apophysis shorter than patellar width, strongly curved; RTA with blunt tip, extending beyond tibia; LTA short, about 1/4 length of RTA; cymbial furrow long, approximately 1/3 length of cymbium; conductor long, anteriorly extending, with looped apex; embolus beginning at 6 o'clock position; base of embolus rather small, ovoid, almost vertically directed (Fig. 9A–C).

Female (one of paratypes): Total length 7.87. Carapace 3.32 long, 2.28 wide. Abdomen 4.55 long, 3.00 wide. Eye sizes and interdistances: AME 0.13, ALE 0.14, PME 0.13, PLE 0.12; AME-AME 0.05, AME-ALE 0.04, PME-PME 0.09, PME-PLE 0.11. Leg measurements: I 14.12 (3.60, 4.60, 3.68, 2.24); II 12.32 (3.40, 3.92, 3.00, 2.00); III 12.38 (3.32, 3.76, 3.20, 2.10); IV 15.32 (4.17, 4.68, 4.17, 2.30). Chelicerae as in male. Epigyne: atrium small, situated medially, U-shaped; hoods distinct, located anterolaterally; spermathecae long, convoluted; spermathecal heads small; copulatory ducts broad, looped around spermathecal heads (Fig. 10A–B).

Distribution. Known only from the type locality (Fig. 12).

Figure 10. Epigyne and habitus of *Spiricoelotes xianheensis* sp. n., **A** Epigyne, ventral **B** Vulva, dorsal **C** Male habitus, dorsal **D** Female habitus, dorsal **E** Female habitus, ventral. Scale bars equal for **A** and **B**, equal for **D** and **E**.
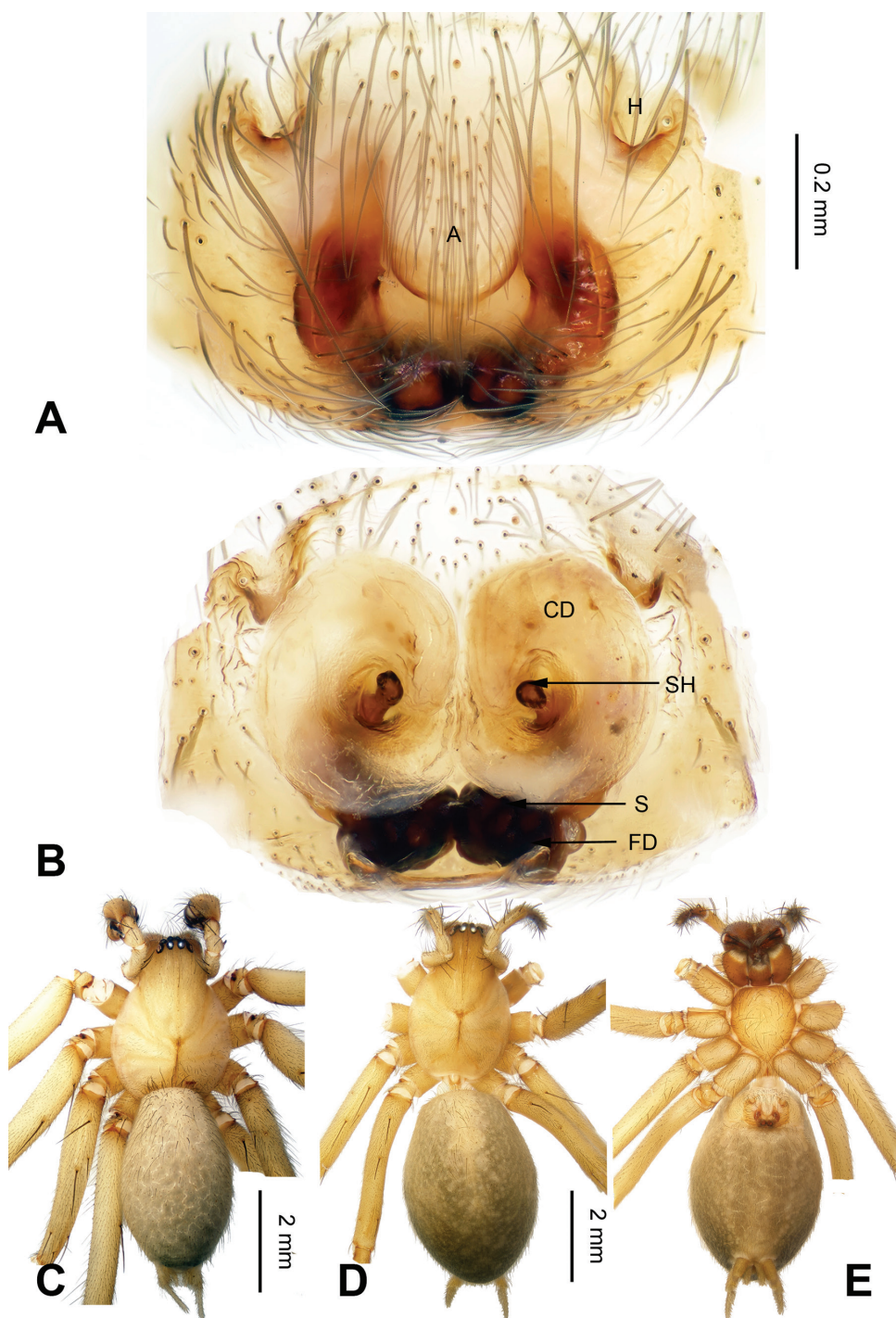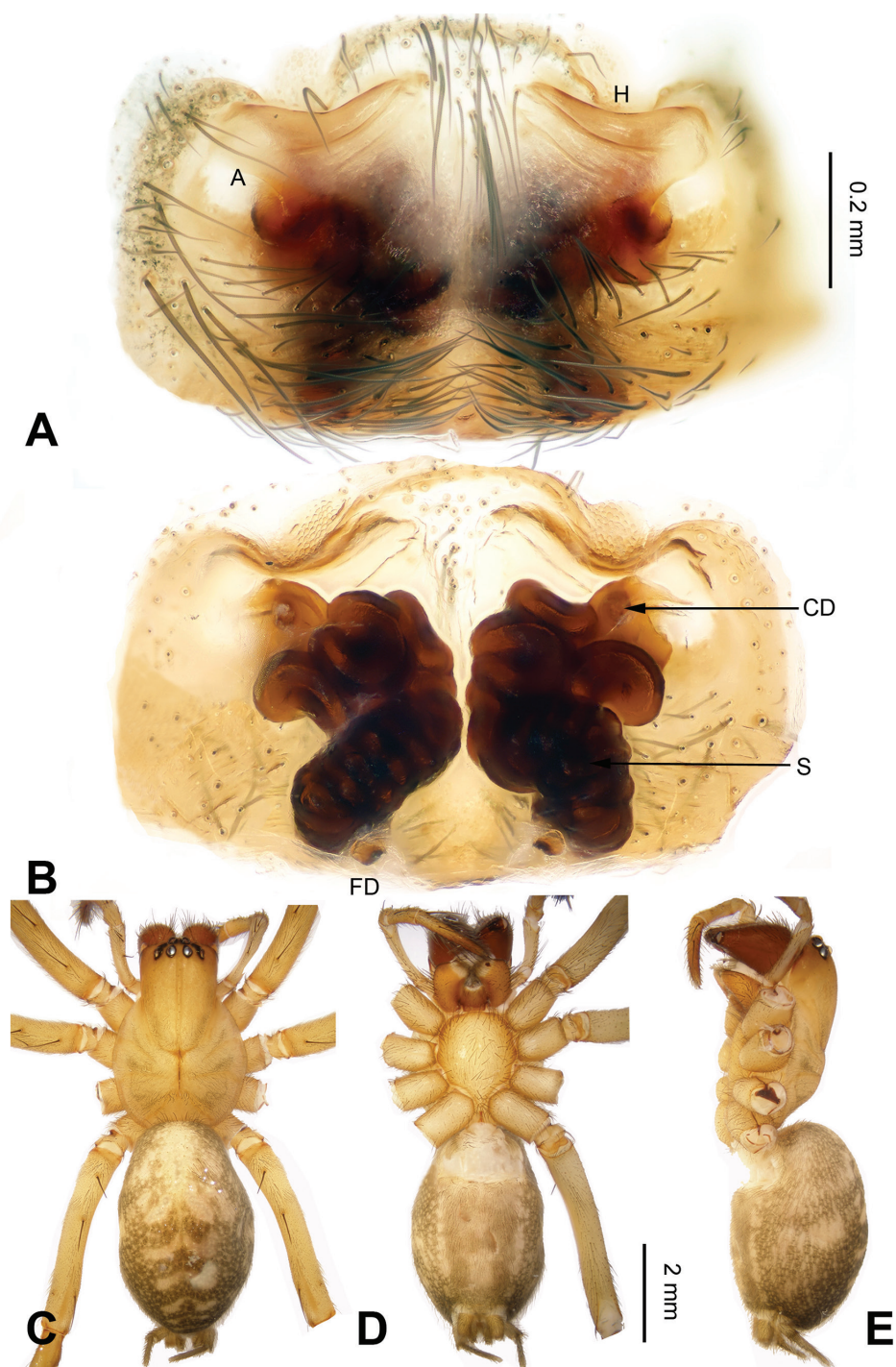
Figure 11. Epigyne and habitus of *Spiricoelotes xiongxinensis* sp. n., holotype. **A** Epigyne, ventral **B** Vulva, dorsal **C** Female habitus, dorsal **D** Female habitus, ventral **E** Female habitus, lateral. Scale bars: equal for **A** and **B**, equal for **C, D** and **E**.

Spiricoelotes xiongxinensis **Chen & Li, sp. n.**

http://zoobank.org/24ED870E-54BB-4D51-8B25-C6E30552F146
Figs 11, 12

Type material. Holotype ♀: China: Jiangxi: Pingxiang City: Shangli County: Futian Town, Zhanshan Village, Xiongxin Cave, N27°44'08", E113°49'10", elevation: 115 m, 9.V.2013, Y.F. Luo and J.C. Liu.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The female of the new species has a uniquely shaped epigyne and can be easily distinguished from all of the other *Spiricoelotes* species by the small and anteriorly situated epigynal atria, the broad epigynal hoods, the long and convoluted spermathecae and by the short copulatory ducts (Fig. 11A–B).

Description. Female (holotype): Total length 8.08. Carapace 3.85 long, 2.69 wide. Abdomen 4.23 long, 2.69 wide. Eye sizes and interdistances: AME 0.19, ALE 0.20, PME 0.19, PLE 0.18; AME-AME 0.11, AME-ALE 0.04, PME-PME 0.13, PME-PLE 0.14. Leg measurements: I 13.76 (3.72, 4.56, 3.36, 2.12); II 12.08 (3.44, 4.08, 3.00, 1.56); III 11.68 (3.20, 3.88, 3.04, 1.56); IV 15.66 (4.20, 4.75, 4.5, 2.21).
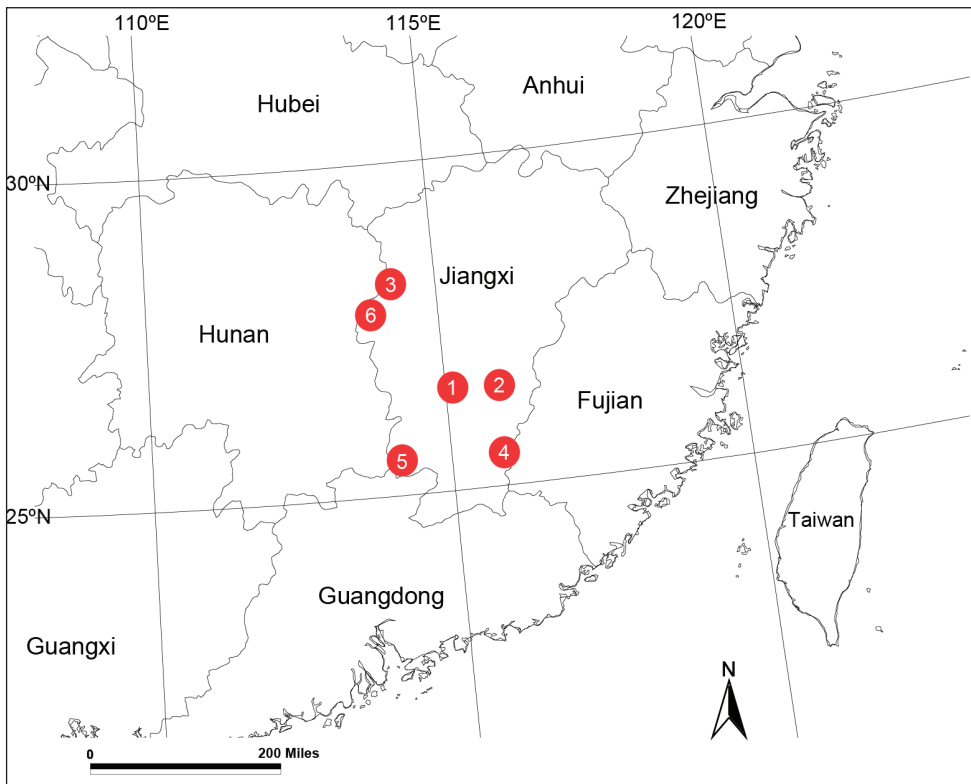

Figure 12. Localities of new *Spiricoelotes* species from China. **1** *S. anshiensis* sp. n. **2** *S. chufengensis* sp. n. **3** *S. nansheensis* sp. n. **4** *S. taipingensis* sp. n. **5** *S. xianheensis* sp. n. **6** *S. xiongxinensis* sp. n.

Chelicerae with five promarginal and five retromarginal teeth. Epigyne: atria small, located anterolaterally, distantly separated by septum; hoods distinct, located at anterior atrial margin; spermathecae long, convoluted; copulatory ducts broad and short, located anteriorly and horizontally directed (Fig. 11A–B).

Distribution. Known only from the type locality (Fig. 12).

Discussion

Currently nine species are known in the genus. Seven are known from both sexes and two from females only. The conductors of the five newly described species differ to a certain extent from the two previously known species. The tip of the conductor in *S. urumensis* and *S. zonatus* is spiralled, whereas in the newly described species, it has a looped apex or is unmodified, but never spiralled. All new species are assigned to this genus because they share a combination of characters with *S. zonatus*, the type species of *Spiricoelotes* that are lacking in other Coelotinae: strongly curved patellar apophysis, conductor anteriorly extending, median apophysis and dorsal apophysis of conductor absent (figs 1A–C, 3A–C, 5A–C, 7A–C, 9A–C; Wang 2002: figs 366–368).

Acknowledgements

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RESEARCH ARTICLE

A new sharpshooter genus for *Sphinctogonia lingula* Yang & Li (Hemiptera, Cicadellidae, Cicadellini) from China

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Abstract

A new genus, *Sphinctogoniella*, is described to accommodate *Sphinctogonia lingula* Yang & Li, 2002, its type species from China. *Sphinctogoniella lingula* (Yang & Li, 2002), **comb. n.** is re-described and illustrated. Differences between the new genus and *Sphinctogonia* Breddin, 1901 are tabulated.

Keywords

Auchenorrhyncha, leafhopper, Cicadellinae, new combination, taxonomy

Introduction

The Old World Cicadellinae genus *Sphinctogonia* Breddin, 1901 (type species: *S. lineolata* (Walker, 1857), Fig. 20) comprises currently nine species (Wilson et al. 2009) from Borneo, Laos, Malaysia, Indonesia and China (Young 1986; Zhang and Kuoh 1993; Yang and Li 2002).

The generic placement of *Sphinctogonia lingula* Yang & Li (2002) from China is re-accessed based on examination of its type series and more recently collected material. Its shorter body size and different coloration and male genitalia from other congeners indicate

that it is unsatisfactory to keep *S. lingula* in *Sphinctogonia*. In addition, we have found that *S. lingula* cannot be classified into any known cicadelline genus. Thus, the purpose of this paper is to erect a new genus to accommodate it together with its redescription.

Material and methods

The male and female genital structures were prepared according to the techniques described by Oman (1949) and Mejdalani (1998), respectively. The dissected parts are stored in small vials with glycerin and attached below the specimens. The morphological terminology adopted herein follows mainly Young (1986) and Dietrich (2005), except for the female genitalia (Nielson 1965; Davis 1975; Mejdalani 1998).

The type specimens and other specimens are deposited in the following institutions whose names are abbreviated in the text as follows:

GUGC	Institute of Entomology, Guizhou University, Guiyang, China;
BMNH	The Natural History Museum, London, UK;
FAFU	Fujian Agriculture and Forestry University, Fuzhou, China.

Results

Sphinctogoniella gen. n.
http://zoobank.org/5B4E6341-797A-4F8D-AD0D-FAAB2C46BF49
Figs 1–6, 13–18, 26–32

Type species. *Sphinctogonia lingula* Yang & Li, 2002.

Diagnosis. The new genus can be recognized by the following combination of features: (1) head anteriorly broadly rounded with ocelli located on imaginary line between anterior eye angles; (2) forewing with membrane distinct, veins obscure; (3) male pygofer without processes, surface with macrosetae near posterior margin; (4) subgenital plates slender, apex acute; (5) aedeagus slender, articulating sub-basally with unpaired paraphysis; (6) paraphysis with long spiniform processes; (7) style slender, extending posteriorly well beyond apex of connective, apex curved, hook-shaped; (8) female abdominal sternum VII well produced from ligulate base.

Description. Length. 8.7–9.8 mm.

Coloration. Head and thorax dorsum and forewings orange-red to red, with black markings.

External features. Head (Figs 1–6) with anterior margin broadly rounded, median length slightly shorter than interocular width; crown with surface slightly convex, with fovea between ocelli and anterior angles of eyes; ocelli located on imaginary line between anterior eye angles, lateral frontal sutures extending onto crown, attaining ocelli; ocellus closer to adjacent eye than to each other; frontoclypeus flattened medially, muscle
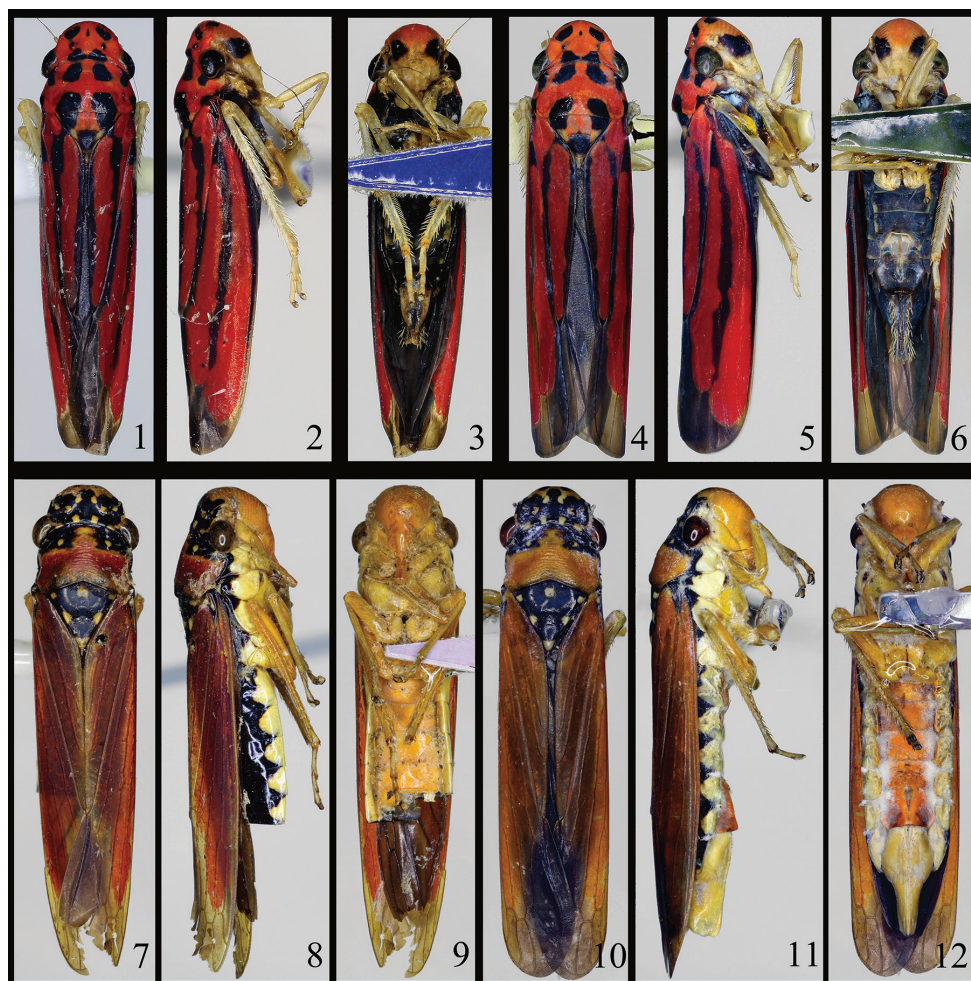
Figures 1–12. *Sphinctogoniella* and *Sphinctogonia* habitus. **1–6** *Sphinctogoniella lingula* (Yang & Li, 2002): **1–3** male (9.0 mm) **1** dorsal view **2** lateral view **3** ventral view **4–6** female (9.5 mm): **4** dorsal view **5** lateral view **6** ventral view **7–12** *Sphinctogonia lacta* Zhang & Kuoh, 1993: **7–9** male (15.9 mm) **7** dorsal view **8** lateral view **9** ventral view **10–12** female (16.7 mm) **10** dorsal view **11** lateral view **12** ventral view.

impressions distinct, anteclypeus convex longitudinally, apical margin sinuate, transclypeal suture indistinct medially. Pronotum (Figs 1, 4) slightly narrower than head, lateral margins divergent posteriorly, basal portion with transverse concavity, posterior margin slightly concave; mesonotum with surface of scutellum convex, transverse depression short and nearly straight; forewing with membrane distinct, veins obscure, base of second and third apical cells aligned transversely; hindleg with femoral setal formula 2:1:1.

Male genitalia. Male pygofer lobes tapered to apex (Fig. 13), without processes, with macrosetae near posterior margin. Subgenital plates (Fig. 14) tapered to acute apex, distal half with uniseriate macrosetae medially and some short microsetae laterally.
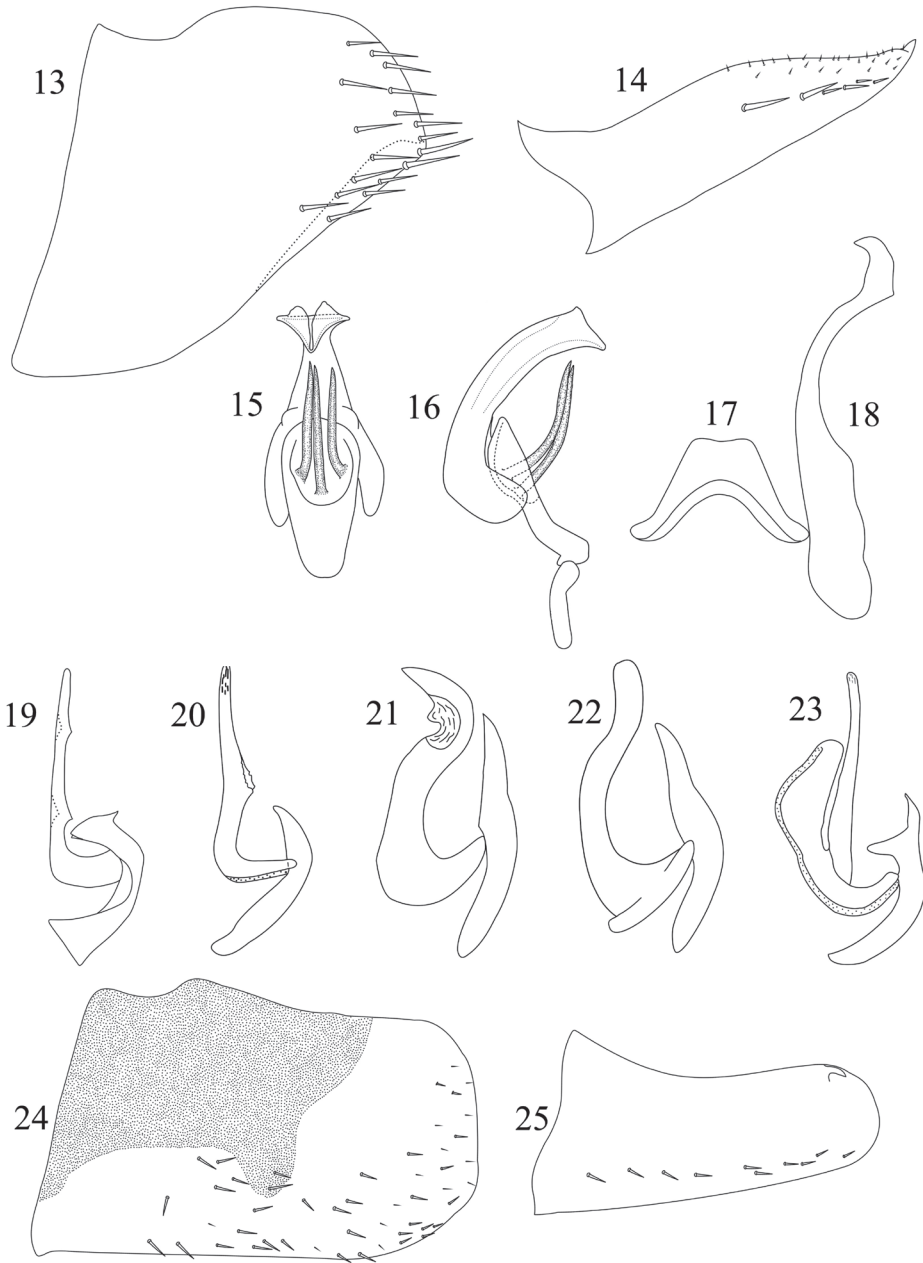
Figures 13–25. *Sphinctogoniella* and *Sphinctogonia* male genitalia. **13–18** *Sphinctogoniella lingula* (Yang & Li, 2002): **13** pygofer, lateral view **14** subgenital plate, ventral view **15** aedeagus and paraphysis, ventral view **16** connective, aedeagus and paraphysis, lateral view **17** connective, ventral view **18** style, ventral view **19–23** Lateral view of aedeagus and paraphysis: **19** *Sphinctogonia lacta* Zhang & Kuoh, 1993 **20** *S. lineolata* (Walker, 1857) **21** *S. comitatula* Melichar, 1926 **22** *S. servula* Breddin, 1901 **23** *S. avia* Young, 1986 **24–25** *Sphinctogonia lacta* Zhang & Kuoh, 1993: **24** pygofer, lateral view **25** subgenital plate, ventral view. **19–23** from Young (1986).

Aedeagus (Figs 15, 16) slender, articulating at its base with subapical part of paraphysis; gonopore apical on dorsal surface. Paraphysis (Figs 15, 16) unpaired with long spini-form processes. Connective (Fig. 17) broadly V-shaped. Style (Fig. 18) slender, extending posteriorly well beyond apex of connective, apex curved, hook-shaped.

Female genitalia. Sternite VII (Fig. 26) produced from ligulate base. Pygofer (Fig. 27), in lateral view, moderately produced; surface with macrosetae on posterior portion and ventral margin. Valvulae I (Figs 28, 29) of ovipositor, in lateral view, slightly expanded near apex; dorsal area with strigate sculpture in oblique lines extending from basal curvature to apex; ventral sculptured area restricted to apical portion, formed mostly by scale-like sculpture; apex of shaft acute. Valvulae II (Figs 30–32) of ovipositor, in lateral view, expanded beyond basal curvature; dorsal and ventral margins slightly convex; apex acute; preapical ventral prominence absent; 23 stout subtriangular teeth distributed from basal expanded portion to apical portion of shaft; teeth and apical portion of shaft bearing denticles. Gonoplacs, in lateral view, with basal half narrow and apical half distinctly expanded; apex rounded; surface with few setae on apical portion.

Distribution. China (Zhejiang, Fujian, Guangxi, Guizhou).

Etymology. The generic name is derived from *Sphinctogonia*. The gender of the genus is feminine.

Remarks. In Young's (1986) key to genera of the Old World Cicadellini the new genus runs to *Nanatka* Young, 1986, but differs from this genus in having a greater body size, the hindleg with femoral setal formula 2:1:1, the subgenital plates slender and acute apically (Fig. 14), and the paraphysis with long spiniform processes subapically (Figs 15, 16). Although the new genus also shares similarities with *Sphinctogonia* Breddin, 1901, such as the roundly produced head (Figs 1 and 4) and the male pygofer without processes (Fig. 13), it differs in the various features shown in Table 1.

Table 1. Differences between *Sphinctogoniella* **gen. n.** and *Sphinctogonia* Breddin, 1901.

	Sphinctogoniella gen. n.	*Sphinctogonia* Breddin, 1901
body size	8.7–9.8 mm	12.8–18.6 mm
pronotum	not constricted	clearly constricted
forewing	with distinct apical membrane, veins obscure	apical membrane absent, veins distinct
hindleg femoral setal formula	2:1:1	variable
male pygofer	slightly produced, with macrosetae on posterior area	moderately produced, with small weak setae on posterior and ventral area
subgenital plates	slender and acute apically; with uniseriate macrosetae medially and some short microsetae	often spine-like apically; with group of small weak setae, usually not arranged in rows
paraphysis	spoon-shaped in lateral view; with long spiniform processes subapically (Figs 15, 16)	usually boat-shaped in lateral view; without long processes, or with small teeth apically (Figs 19–23)
female pygofer	angular apically, with macrosetae on apical and ventral area (Fig. 27)	round apically, with small setae near apical and ventral area (Fig. 33)
valvula II	bearing less than 30 teeth on dorsal margin of blade (Fig. 30)	bearing many teeth (more than 50) on dorsal margin of blade (Fig. 34)
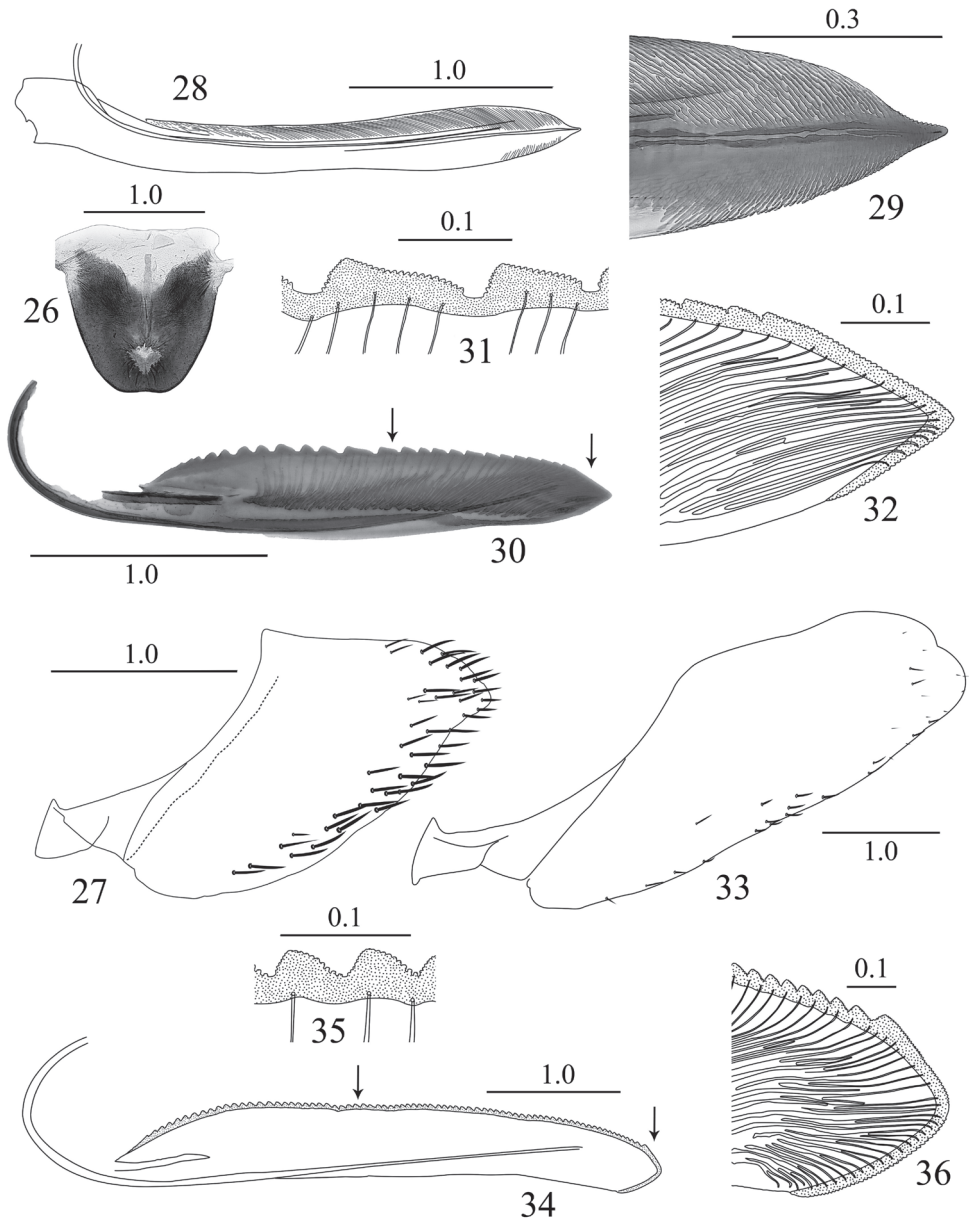
Figures 26–36. *Sphinctogoniella* and *Sphinctogonia* female genitalia. **26–32** *Sphinctogoniella lingula* (Yang & Li, 2002): **26** sternite VII, ventral view **27** pygofer, lateral view **28** valvula I, lateral view **29** apical portion of valvula I, lateral view **30** valvula II, lateral view **31** teeth of median portion of valvula II, lateral view **32** apical portion of valvula II, lateral view **33–36** *Sphinctogonia lacta* Zhang & Kuoh, 1993: **33** pygofer, lateral view **34** valvula II, lateral view **35** teeth of median portion of valvula II, lateral view **36** apical portion of valvula II, lateral view. Scale bars in millimeters.

***Sphinctogoniella lingula* (Yang & Li, 2002), comb. n.**
Figs 1–6, 13–18, 26–32

Sphinctogonia lingula Yang & Li *in* Li & Jin, 2002: 176.

Description. Length of males 8.7–9.5 mm, females 9.0–9.8 mm.

Coloration. Head and thorax dorsum and forewings orange-red to red, eyes and ocelli black. Head with two round black spots at apex; crown with anterior two black spots in front of ocelli, median portion with small black spot; basal margin with two connected triangular or trapeziform black spots behind ocelli. Pronotum with transverse anterior and posterior four black spots, anterior median two connecting with basal two black spots of crown, posterior median two connecting with black spots of basal angles of mesonotum; mesonotum with triangular black spots on basal angles, scutellum with large black spot; forewing with two longitudinal slender black stripes medially, basal angle black, apical membrane black brown, inner and outer margins black brown. Face orange yellow or off-white, apical portion of frontoclypeus with pair of lateral large black spots; anteclypeus with apico-median black marking in some specimens. Thoracic venter black brown to black, legs pale yellow brown. Abdominal venter black, sternites of posterior margin yellow white.

Male genitalia. Male pygofer lobes tapered to apex (Fig. 13) without process, with macrosetae near posterior margin. Subgenital plates (Fig. 14) tapered to acute apex, distal half with uniseriate macrosetae medially and some short microsetae laterally. Aedeagus (Figs 15, 16) slender, in lateral view curved ventrally, of similar width throughout length with apex truncate; in ventral view broad basally tapered to near apex then expanded apically with a pair of triangular flaps; articulating at its base with subapical part of paraphysis; gonopore apical on dorsal surface. Paraphysis (Figs 15, 16) un-paired, with ventral surface of distal half concave with three elongate spiniform processes, curved dorsally. Connective (Fig. 17) broadly V-shaped. Style (Fig. 18) slender, extending posteriorly well beyond apex of connective, apex curved, hook-shaped.

Female genitalia. Sternite VII (Fig. 26) ligulately produced, posterior margin with shallow concavity medially; internal sternite VIII membranous. Pygofer (Fig. 27), in lateral view, moderately produced; posterior margin with a subtriangular apical lobe; surface with macrosetae on posterior portion and extending anteriorly along ventral margin beyond its midlength. Valvifers I, in lateral view, nearly oval, bases slightly narrower. Valvifers II with small group of clustered setae near articulation point. Valvulae I (Figs 28, 29) of ovipositor, in lateral view, slightly expanded near apex; dorsal sculptured area extending from basal curvature to apex, broader near apex, formed by scale-like processes arranged in oblique lines; ventral sculptured area restricted to apical portion, formed mostly by scale-like processes; apex of shaft acute; dorsal margin forming denticles on apical portion of shaft. Valvulae II (Figs 30–32) of ovipositor, in lateral view, well expanded beyond basal curvature; dorsal and ventral margins slightly

convex; apex acute; preapical prominence absent; approximately 23 teeth distributed from basal expanded portion to apical portion of shaft; all teeth subtriangular, basal teeth with superior angle slightly rounded, later with flat posterior area; median teeth strongly produced; apical portion with dentate dorsal margin longer than ventral margin; ducts extending toward teeth and toward apical portion of shaft. Gonoplacs, in lateral view, with basal half narrow and apical half distinctly expanded; apex rounded; surface with few setae on apical portion.

Distribution. China (Zhejiang, Fujian, Guangxi, Guizhou).

Material examined. 1♂ (Holotype, FAFU), China, Fujian Province, Jianyang County, 7 April 1960, coll. Ma Cheng-lin; 1♀ (BMNH), China, Fujian Province, Yongan, Tianbaoshan, 17 May 2012, coll. Chang Zhi-min; 1♀ (GUGC), China, Fujian Province, Shilin County, 21 May 2012, coll. Long Jian-kun; 1♀ (Paratype, GUGC), China, Guangxi Province, Huaping, 5 June 1997, coll. Yang Mao-fa; 1♀ (GUGC), China, Guangxi Province, Huaping, 13 May 2014, coll. Qu Ling, Wu Yun-fei and Yang Hang; 1♂ (GUGC), China, Guangxi Province, Guilin, 26 April 2012, coll. Yang Zai-hua; 1♂ (BMNH), China, Guangxi Province, Guilin, 26 April 2012, coll. Zheng Wei-bin; 3♀♀ (Paratype, GUGC), China, Guizhou Province, Maolan, 26–30 May 1998, coll. Li Zi-zhong and Wang Lian-min; 3♂♂ (GUGC), 5♀♀ (GUGC), China, Guizhou Province, Daozhen County, Dashahe, 22–27 May 2004, coll. Song Qiong-zhang, Zhang Bin, Xu Fang-ling and Xu Pian; 1♀ (GUGC), China, Guizhou Province, Shibing County, Yuntaishan, 20 May 2009, coll. Yang Zai-hua.

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RESEARCH ARTICLE

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A new species of the genus *Anteon* Jurine (Hymenoptera, Dryinidae) from Laos

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Abstract

A new species of *Anteon* Jurine, 1807 is described from Laos, Houaphanh Province: *A. holzschuhi* **sp. n**. Morphologically the new species is similar to *A. semipolitum* Olmi, 2008, but it is distinguished by the sculpture of the face partly reticulate rugose and partly with deep punctures similar to areolae; in *A. semipolitum* the face is completely punctate and unsculptured among punctures. In addition, in the new species the distance from the outer edge of a lateral ocellus to the compound eye (OOL) is about 3.3 times as long as the distance between the inner edges of a lateral ocellus and the median ocellus (OL); in *A. semipolitum* OOL is less than twice as long as OL. Published identification keys to the Oriental species of *Anteon* are modified to include the new species.

Keywords

Taxonomy, *Anteon holzschuhi*, Oriental region, key, Houaphanh Province, Anteoninae

Introduction

Dryinidae (Hymenoptera, Chrysidoidea) are parasitoids of leafhoppers, planthoppers and treehoppers (Hemiptera, Auchenorrhyncha) (Carcupino et al. 1998; Guglielmino and Bückle 2003, 2010; Guglielmino et al. 2006, 2013, 2015; Guglielmino and Virla

1998). *Anteon* Jurine, 1807 is a genus that is present in all zoogeographical regions (Olmi 1984; Xu et al. 2013; Olmi and Virla 2014; Olmi and Xu 2015). In total 423 species have been described from all continents (Olmi and Xu 2015) and the genus was revised at the world level by Olmi (1984, 1991) and more recently in the Oriental, Neotropical and Eastern Palaearctic regions by Xu et al. (2013), Olmi and Virla (2014) and Olmi and Xu (2015) respectively.

The species of *Anteon* inhabiting the Oriental region were studied by Xu et al. (2013). More recently, Guglielmino and Olmi (2013) and Olmi et al. (2015) described further new species respectively from Indonesia (*Anteon seramense* Guglielmino & Olmi) and Thailand (*Anteon huettingeri* Olmi, Xu & Guglielmino). In total, 150 *Anteon* species have been described from the Oriental region (Xu et al. 2013; Guglielmino and Olmi 2013; Olmi et al. 2015).

Anteon species are parasitoids of leafhoppers belonging to the Cicadellidae (Guglielmino et al. 2013). As in almost all dryinids, females of *Anteon* have a chelate protarsus. Chelae are used to capture and restrain the host during oviposition and host-feeding (Olmi 1984, 1994).

In 2015 we examined additional specimens of *Anteon* from Laos and discovered a new species described in this paper.

Material and methods

The descriptions follow the terminology used by Olmi (1984) and Xu et al. (2013). The measurements reported are relative, except for the total length (head to abdominal tip, without antennae), which is expressed in millimetres. The following abbreviations are used in the descriptions: POL is the distance between the inner edges of the two lateral ocelli; OL is the distance between the inner edges of a lateral ocellus and the median ocellus; OOL is the distance from the outer edge of a lateral ocellus to the compound eye; OPL is the distance from the posterior edge of a lateral ocellus to the occipital carina; TL is the distance from the posterior edge of an eye to the occipital carina.

The types of all Oriental species of *Anteon* have been previously examined by the authors.

The type specimen described in this paper is deposited in the collection of the Oberösterreichisches Landesmuseum, Linz, Austria (OLL).

The description of the new species is based on the study of a single specimen. The authors are aware that descriptions of new taxa should normally be based on more individuals. However, Dryinidae are so rare that it is uncommon to collect more than one specimen of each species. In addition, on the basis of the experience and knowledge of the authors, the new species is sufficiently delimited by unique characters to justify its description.

Results

Genus *Anteon* Jurine, 1807

Anteon Jurine, 1807: 302. Type species: *Anteon jurineanum* Latreille, 1809, by subsequent monotypy.

Diagnosis. Female: Fully winged; rarely brachypterous; occipital carina complete; palpal formula 6/3; antenna without rhinaria; forewing with three cells enclosed by pigmented veins (costal, median and submedian); forewing with stigmal vein and pterostigma; distal part of stigmal vein much shorter than proximal part, occasionally slightly shorter, as long as, or longer than proximal part; propodeum usually with transverse keel between dorsal and posterior surface; protarsus chelate; inner side of enlarged claw with proximal prominence bearing one long bristle; tibial spurs 1/1/2. Male: Fully winged; rarely brachypterous; occipital carina complete; vertex of head usually without two oblique keels connecting posterior ocelli to occipital carina; palpal formula 6/3; forewing with three cells enclosed by pigmented veins (costal, median and submedian); forewing with stigmal vein and pterostigma; distal part of stigmal vein much shorter than proximal part, occasionally slightly shorter, as long as, or longer than proximal part; pterostigma less than four times as long as broad; propodeum usually with transverse keel between dorsal and posterior surface; paramere usually without inner branch wrapping penis; tibial spurs 1/1/2.

Anteon holzschuhi Olmi, Xu, Guglielmino & Speranza, sp. n.
http://zoobank.org/80257611-458A-45B3-B633-292B862C17A3

Diagnosis. Male with antenna filiform; face partly reticulate rugose and partly sculptured by deep punctures similar to areolae (Fig. 1B); OOL about 3.3 times as long as OL (Fig. 1A); notauli reaching about 0.8 length of scutum (Fig. 1A); posterior surface of propodeum with two complete longitudinal keels and median area unsculptured; distal part of stigmal vein much shorter than proximal part; paramere about as long as penis, without papillae on inner side, without distal inner process (Fig. 2); distivolsella not provided with two lateral processes (Fig. 2).

Description. Male. Fully winged (Fig. 1A). Length 4.5 mm. Head black, except mandible testaceous. Antenna brown-testaceous, except proximal half of segment 1 testaceous. Mesosoma black. Metasoma brown. Legs testaceous, except metacoxa basally brown. Antenna filiform. Antennal segments in following proportions: 17:10:16:15:14:14:14:14:13 (segment 10 missing in holotype). Head (Fig. 1A, B) shiny. Face partly rugose and partly strongly punctate, with deep punctures similar to areolae, unsculptured among punctures. Vertex and temple with deep punctures similar to areolae, unsculptured among punctures. Frontal line complete. Vertex with POL = 7; OL = 3; OOL = 10; OPL = 7; TL = 7; greatest breadth of posterior ocelli shorter
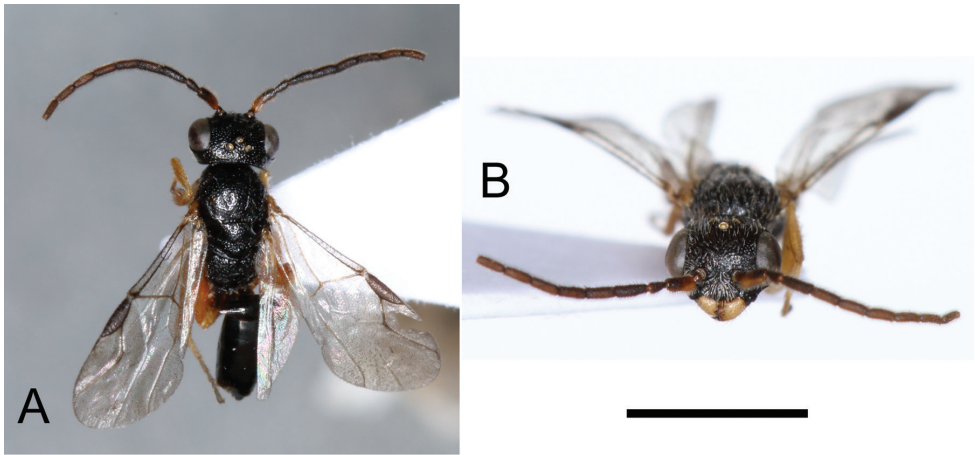
Figure 1. *Anteon holzschuhi* Olmi, Xu, Guglielmino & Speranza, sp. n., holotype: **A** habitus in dorsal view **B** head in frontal view. Scale bar: 2.77 mm (**A**), 2.20 mm (**B**).

than OPL (5:7). Occipital carina complete. Pronotum short and strongly punctate. Scutum and scutellum shiny, punctate, unsculptured among punctures. Notauli incomplete, reaching approximately 0.8 x length of scutum (Fig. 1A). Metanotum shiny, unsculptured. Propodeum with strong transverse keel between dorsal and posterior surface. Dorsal surface of propodeum reticulate rugose. Posterior surface of propodeum with two complete longitudinal keels, median area unsculptured, and lateral areas rugose. Forewing hyaline, without dark transverse bands. Distal part of stigmal vein much shorter than proximal part (7:16). Paramere (Fig. 2) about as long as penis, without distal inner pointed process and papillae, with long and broad dorsal proximal membranous process. Tibial spurs 1/1/2.

Female. Unknown.

Material examined. Holotype: male, Laos, Houaphanh Province, Phou Pan, Ort Ban Saleui environs, 20°13.30'N 103°59.26'E, 1350–1900 m, 6–11.iv.2014, C. Holzschuh and locals leg. (OLL).

Distribution. Laos.

Hosts. Unknown.

Etymology. The species is named after the collector, Mr Carolus Holzschuh (Villach, Austria).

Remarks. The new species is similar to *A. semipolitum* Olmi, 2008, by having the antenna filiform, notauli reaching about 0.8 × length of scutum (Fig. 1A), posterior surface of the propodeum with two complete longitudinal keels and unsculptured median area, distal part of stigmal vein much shorter than proximal part, paramere about as long as penis, without papillae on inner side, without distal inner process (Fig. 2) and distivolsella not provided with two lateral processes (Fig. 2). The main difference between the two species is in the facial sculpture (face partly reticulate rugose and partly sculptured by deep punctures similar to areolae in *A. holzschuhi* (Fig. 1B); face
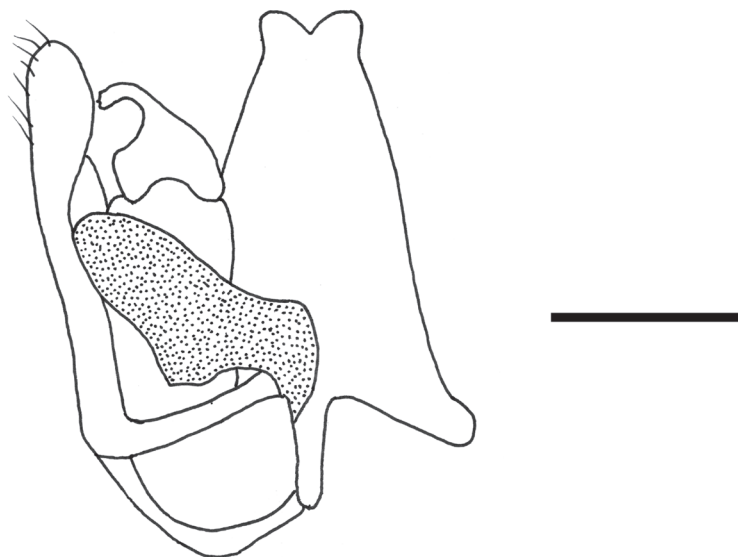
Figure 2. *Anteon holzschuhi* Olmi, Xu, Guglielmino & Speranza, sp. n., holotype: male genitalia (right half removed). Scale bar = 0.26 mm.

punctate and unsculptured among punctures in *A. semipolitum*). In addition, OOL is about three times as long as OL in *A. holzschuhi*, less than twice in *A. semipolitum*. In the key to the males of Oriental *Anteon* published by Xu et al. (2013), the new species can be included by replacing couplet 43 as follows:

43	Head partly or totally reticulate rugose (Fig. 1A, B)	**43'**
–	Head completely punctate and unsculptured among punctures	**44**
43'	Paramere with many papillae along inner side (Plate 38H in Xu et al. 2013)..***A. papillum* Xu, He & Olmi**
–	Paramere without papillae on inner side (Fig. 2; plate 42F in Xu et al. 2013)...**43''**
43''	Face punctate and unsculptured among punctures; OOL less than twice as long as OL...***A. semipolitum* Olmi**
–	Face partly reticulate rugose and partly sculptured by deep punctures similar to areolae (Fig. 1B); OOL about 3.3 times as long as OL (Fig. 1A) ....***A. holzschuhi* Olmi, Xu, Guglielmino & Speranza, sp. n.**

Conclusion

Mita and Okajima (2011), Xu et al. (2013) and Olmi et al. (2015) recorded from Laos 41 species of Dryinidae belonging to the following subfamilies and genera: Aphelopinae: *Aphelopus* Dalman, 1823 (five species); Anteoninae: *Anteon* Jurine, 1807

(nine species), *Deinodryinus* Perkins, 1907 (one species); Bocchinae: *Bocchus* Ashmead, 1893 (four species); Dryininae: *Dryinus* Latreille, 1804 (16 species); Gonatopodinae: *Neodryinus* Perkins, 1905 (four species), *Echthrodelphax* Perkins, 1903 (two species). With the description of the above new species the number of species now known in Laos is 42. No hosts of Laotian Dryinidae are known.

In comparison with the 77 species listed in the Chinese province of Guangdong (total area: 177900 km^2) (Xu et al. 2012c), the dryinid fauna of Laos (total area: 237800 km^2) is poorly known. Some common genera such as *Gonatopus* Ljungh, 1810 (no species listed in Laos) are clearly understudied. Further evidence of this is the fact that on the small island of Hainan (total area: 33210 km^2; 1/7 that of Laos) 56 species of Dryinidae are recorded (Xu et al. 2011a, 2011c). In contrast, in the more northern Chinese provinces of Shaanxi (total area: 205800 km^2) and Hunan (total area: 210000 km^2) only 36 (Xu et al. 2012a) and 17 (Xu et al. 2011b, 2012b, 2013) dryinid species have been recorded, respectively.

Acknowledgements

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RESEARCH ARTICLE

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Description of the female of *Haematonotus jenisi* Krajcik, 2006 and placement of the species within the genus *Atrichiana* Distant, 1911 (Coleoptera, Scarabaeidae, Cetoniinae)

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Abstract

The male of *Haematonotus jenisi* Krajcik, 2006 was described on the basis of a sole holotype specimen. A number of new specimens have recently been collected from the False Bay area of the iSimangaliso Wetland Park, in northeastern KwaZulu-Natal, including a number of females, thereby allowing the description of this sex for the first time. The new series also reveals that most morphological characters are actually typical for the genus *Atrichiana* Distant, 1911, rather than *Haematonotus* Kraatz, 1880. For this reason, it is here proposed that this species be placed within *Atrichiana*, thus becoming the second species of a genus that was previously regarded as monospecific, represented by the southern African endemic *A. placida* (Boheman, 1857). Like *A. placida*, *A. jenisi* exhibits a remarkable polymorphism, but also sexual dimorphism, particularly expressed at the level of the antennal clubs, which in the male are twice as long as in the female. Unfortunately, all specimens of the recent series were collected dead or drowning on the lake shores and therefore no further information on their life cycle and biology in general could be obtained. It seems that the species may represent a micro-endemism restricted to the Western Shores of Lake St Lucia, with habitat associated to Sand Forest and possibly also Northern Coastal Forest vegetation types.

Keywords

Scarabaeidae, Cetoniinae, genus *Atrichiana*, genus *Haematonotus*, revised status, female description, iSimangaliso Wetland Park, South Africa

Introduction

Krajcik (2006) described *Haematonotus jenisi* on the basis of the single male holotype available at the time, providing a clear set of diagnostic characters and outstanding illustrations of the general habitus and genitalia of this interesting species. Its discovery was unexpected, as the area where it was collected (Dukuduku Forest, near Mtubatuba) falls within the iSimangaliso Wetland Park, a UNESCO World Heritage Site, where intensive surveys had been conducted in the past with the aim of understanding its full biodiversity richness. The Cetoniinae were among the key invertebrate groups investigated at the onset of the Park's proclamation, within a dedicated project titled "Rare, threatened and endemic species project of the Greater St Lucia Wetland Park (Combrink and Kyle 2006). Krajcik's surprise description of this new species triggered an escalation in research in the broader area of the Dukuduku section of the Park and further north, along the entire Western Shores of Lake St Lucia.

Understanding that the emergence of the adults of this species was most likely linked to major rainfall events, dedicated searches were conducted throughout the area during the summer season, immediately after a rain of > 20 mm. It was, however, only in April 2010 that two old dead specimens were found along the drying shores of False Bay, in the northern reaches of Lake St Lucia. It was not clear how long the specimens had been drowned in the lake, as the water at the time was hypersaline (salinity >100) and thus capable of preserving the tissues in relatively good state for a long time. After this, three other specimens were collected between November 2010 and November 2014, all in the same area of False Bay and drowned on the shores of the lake. The specimen collected in November 2013 was still alive, but died within a period of 12 hours. It was only at the beginning of February 2015 that 11 specimens in various states of decomposition were again retrieved from the lake shores of False Bay, in the wake of a rain of 55 mm which fell on January 15. This time, the series exhibited four females and also specimens with different colour patterns, thereby revealing a remarkable variability previously undetected and allowing the full description of the species.

Krajcik (2006) had placed the species within the genus *Haematonotus* Kraatz, 1880, but did not provide justification for this in the form of a set of typical diagnostic characters. Virtually all characters identified in this study clearly show that the species is actually much closer to *Atrichiana* Distant, 1911 than *Haematonotus* and, thus, it is here proposed that it be moved to the former genus, as its second species along with *A. placida* (Boheman, 1857).

Methods

All specimens were collected drowned on the shores of False Bay, Lake St Lucia. Only one male specimen, collected in Nov 2013, was still alive while all other specimens were retrieved in various states of decomposition and immediately placed in ethyl acetate and later in a 10% formalin solution.

The description of morphological characters follows the terminology used by Krikken (1984) and Holm and Marais (1992). Specimen length was measured from the anterior margin of the clypeus to the apex of the pygidium. Specimen width represents the maximum width of the elytron. Photos of specimens dorsal and ventral habitus were taken with a Canon PowerShot G11 digital camera with macro setting. The background was removed from the photos using Microsoft Word 2010 (Picture Tools), in order to increase clarity of resolution. The Combine ZP Image Stacking Software by Alan Hadley (alan@micropics.org.uk) was used to obtain z-stacking composite images.

Repositories are abbreviated as follows: BMPC, Jonathan Ball and Andre Marais Private Collection, Cape Town, South Africa; DMSA, Durban Natural Science Museum, Durban, South Africa; RPPC, Renzo Perissinotto and Lynette Clennell Private Collection, Port Elizabeth, South Africa; TMSA, Ditsong National Museum of Natural History (formerly Transvaal Museum), Pretoria, South Africa.

Taxonomy

***Atrichiana jenisi* (Kajcik, 2006), comb. n.**
Figures 1, 2

Haematonotus jenisi Krajcik, 2006: 15.

Material examined. Sixteen male and four female specimens. 2 ♂: South Africa, Kwa-Zulu-Natal, False Bay, Lister's Point, 2 Apr 2010, found dead on lake shores, R. Perissinotto & L. Clennell leg. (RPPC); 2 ♂: same data as above but 3 Dec 2011 (DMSA, RPPC); 1 ♂: same data as above but Dukandlovu, 25 Nov 2013 found drowning on lake shores (RPPC); 7 ♂, 4 ♀: same data as above but 3 Feb 2015 (TMSA, BMPC, RPPC).

Genus placement. The summary of characters listed here below and in Table 1 supports the placement of the species within *Atrichiana*, rather than *Haematonotus*. With the exception of the pronotal tubercle, which is a feature more typical of *Haematonotus*, although also visible in *Atrichiana* as "pronotal elevation" (Holm and Marais 1992), all other characters fit well into the range reported for the latter genus, including the male genitalia (see figure 8, p. 14 in Krajcik 2006). Neither *Atrichiana* nor *Haematonotus* are known to exhibit much longer antennal clubs in male than in female or a sharp hemi-pronotal carina. However, these are characters encountered in various genera and may not reflect generic synapomorphies, but rather adaptive convergence.

Generic characters (Table 1):
1) Body dorsally velutinous with elytra asetose;
2) Extreme reduction and forward orientation of protibial denticles in male;
3) Tarsi generally elongate, particularly in male;
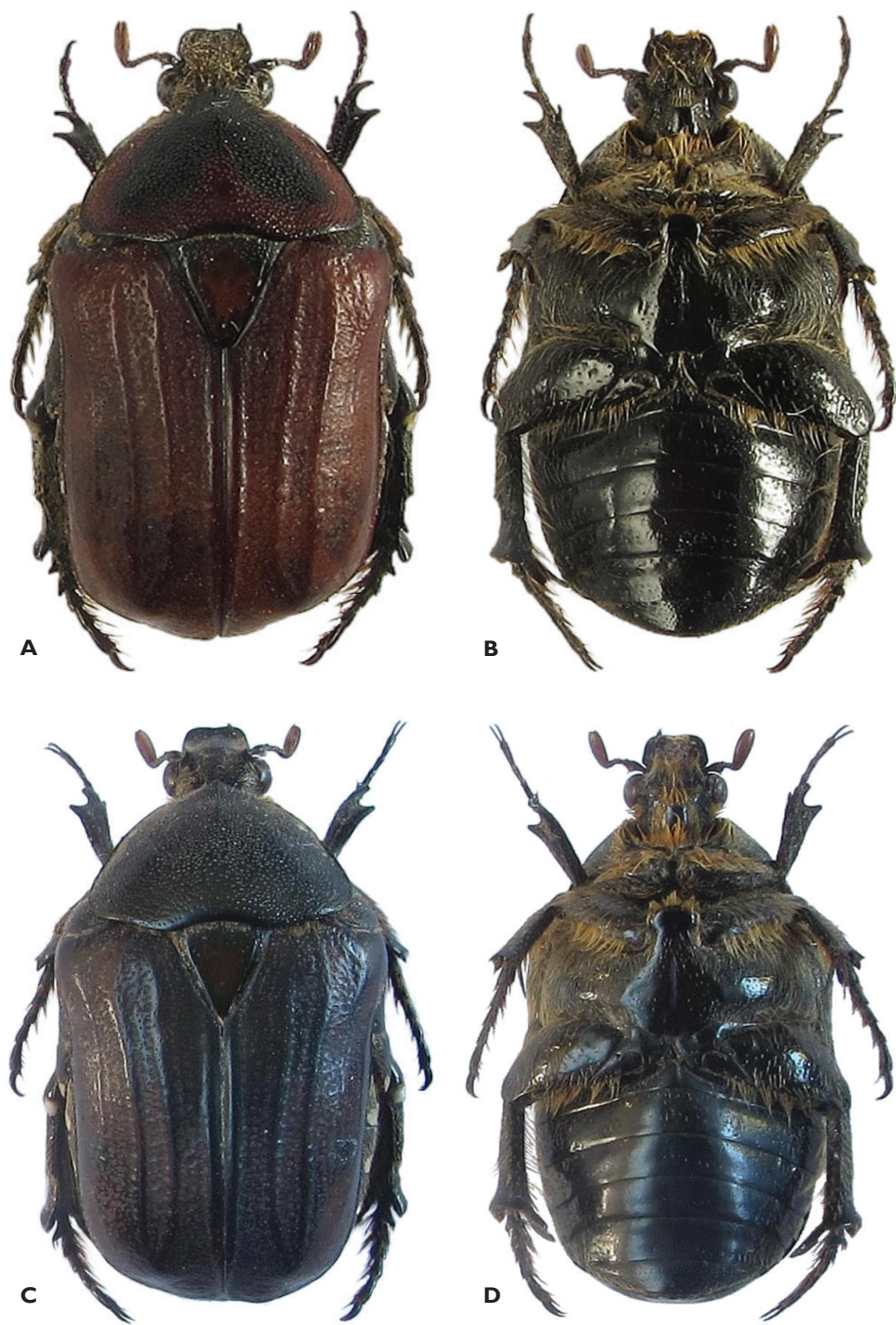4) Presence of anterior elevation on pronotum (forming carina and tubercle – closer to *Haematonotus*);

Figure 1. *Atrichiana jenisi*: **A** dorsal and **B** ventral habitus of light brown female specimen **C** dorsal and **D** ventral habitus of dark brown-green female specimen.

Table 1. Key generic characteristics of the genera *Haematonotus* Kraatz, 1880 and *Atrichiana* Distant, 1911, with closest matches in *A. jenisi* (Krajcik, 2006) highlighted in bold.

Haematonotus Kraatz, 1880	*Atrichiana* Distant, 1911
Body velutinous, setose and with cretaceous areas on both dorsum and venter (♂, except nigrito forms); or shiny, asetose and without cretaceous areas (♀)	Body dorsally velutinous and asetose; venter and pygidium shiny with cretaceous spots in both sexes
Clypeus strongly upturned apically	**Clypeus sharply upturned apically** (♂); or mildly upturned and bilobate (♀)
Pronotum trapezoid, with strong antescutellar arch **and prominent tubercle on anterior margin**	**Pronotum strongly attenuated forward, with base roundedly trisinuate** and anterior margin slightly protruding forward medially
Scutellum with round punctures denser in basal half and setiferous in ♂	**Scutellum flat and unsculptured**
Elytra with uneven costae raised and entire; with fine and dense sculpture	**Elytra with sutural costa roundedly raised, third and fifth less so; with diffuse sculpture**
Pygidium with basal and pre-apical lateral depressions; with bilateral cretaceous spots in ♂	**Pygidium with lateral depressions and cretaceous spots in both sexes (except darkest ♀)**
Mesometasternal process constrained and with protruding convexity	**Mesometasternal process laterally expanded and roundedly triangular**
Tarsi of average length	**Tarsi elongate, particularly in ♂**
Protibial denticle of ♂ well-developed, arched and produced forward	**Protibial denticle of ♂ extremely reduced and forwardly projected**
Meso- and metatibia with arched, uneven outer carina; weakly trilobed distally, with inner lobe more prominent	**Meso- and metatibia with no or poorly developed outer carina and spines**
Metatibial spur moderately ditated in ♀	**Metatibial spurs widely spatulate in ♀**
Aedeagus with ventral lobes of parameres distally slightly expanded, with small outer hooks and inconspicuous ventral setae	**Aedeagus with ventral lobes of parameres wider than dorsal lobes, with short setae ventro-apically**

5) Presence of white cretaceous spots on venter and pygidium;

6) Elytra with marked, uneven costae and diffuse sculpture;

7) Meso- and metatibia with no or poorly developed outer carina and spines, respectively;

8) Metatibial spurs widely spatulate in female;

9) Aedeagus with dorsal parameral lobes narrower than ventral and exhibiting short setae ventro-apically.

Species-specific characters (Figures 1A, C and 2A, C)

1) Antennal clubs twice as long in male compared to female;

2) Presence of cretaceous spot on each side of anterior pronotal declivity;

3) Presence of sharp vertical carina leading to tubercle on anterior half of pronotal midline;

4) Scutellum with narrow depression at middle of apical half.

Female description (Figures 1). *Size*. Length 17.3–19.6 mm; width 10.5–11.2 mm (n = 4).

Body: Generally shorter and wider than male; background colour from black or dark green to dark brown with or without obvious maculae or bands (Figure 1A, C),
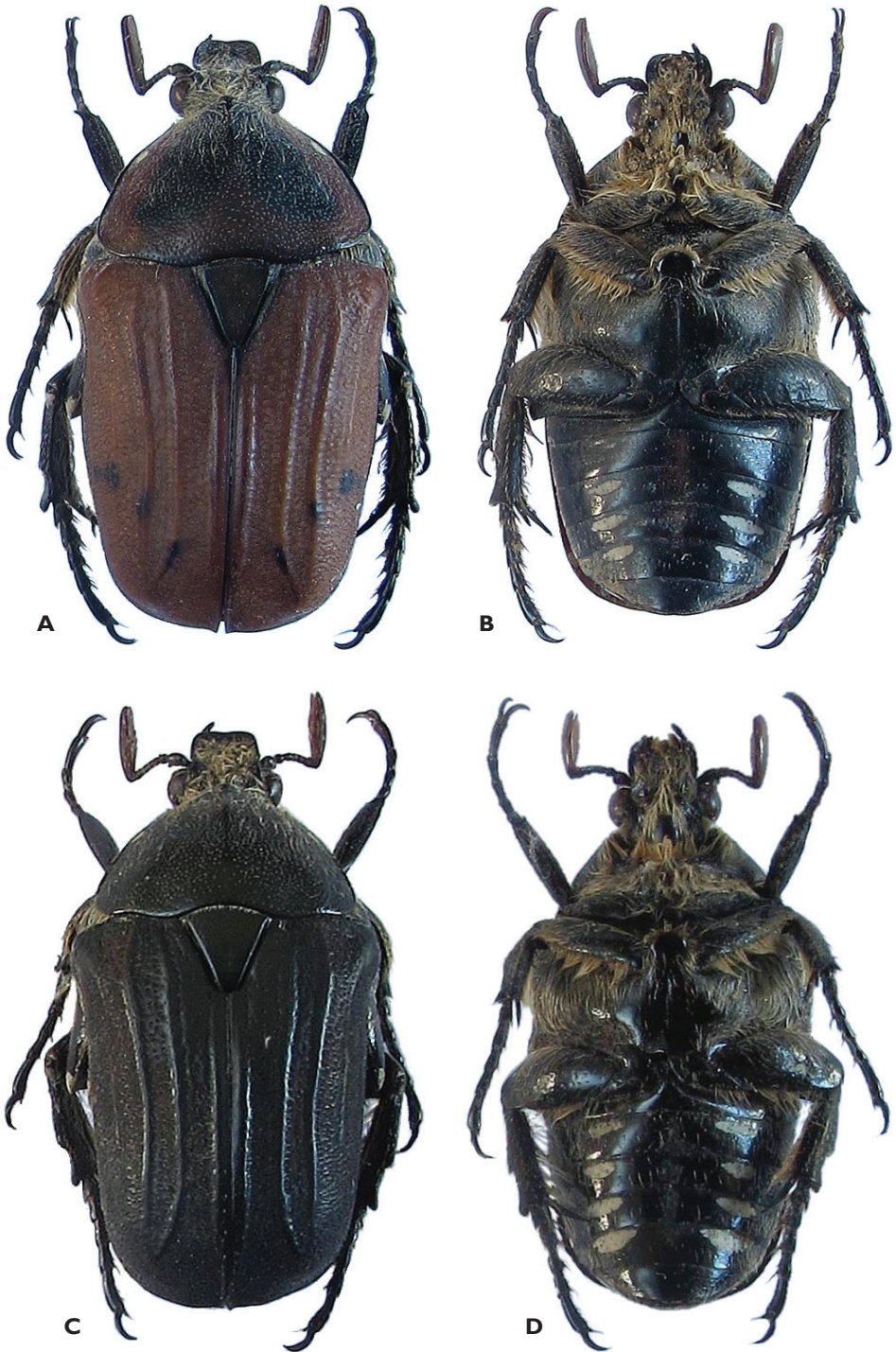
Figure 2. *Atrichiana jenisi*: **A** dorsal and **B** ventral habitus of light brown male specimen with black markings on apical half of elytra **C** dorsal and **D** ventral habitus of dark brown-green male specimen.

alternatively light brown with two black/green bands on pronotum and three maculae on apical half of elytra (Figure 2A); presence of cretaceous spots on antero-lateral margins of pronotum, pygidium and base of metatibia.

Head. Black with scattered whitish, long setae and dense round punctures; clypeus slightly upturned, but far less than in male, with depression at center forming two lateral lobes, which together with marked lateral ridges enclose frons into teaspoon-like concavity; frons exhibiting smooth convex ridge at middle, reaching anterior pronotal margin.

Pronotum. Black, brown or dark green, matt with dense round punctures becoming progressively larger from the anterior and lateral margins to the center and posterior margin; anterior and lateral margins bearing thin, long but flattened creamy setae; exhibiting bilateral macula of white tomentum on lateral declivity, approximately at mid length; anterior margin as wide as eyes, but sharply raised with tubercle at center reaching disc as sharp carina; posterolateral angle smoothly rounded, posterior margin markedly sinuate anteriad of scutellum.

Scutellum. Dark green to brown or black; perfectly triangular, with apex slightly rounded; without or with few scattered punctures, mainly along lateral margins; with visible mid-depression on apical half.

Elytron. Exhibiting prominent sutural, discal and lateral costae, with latter two arching markedly towards apical declivity; sparse short creamy setae occurring only on lateral and apical declivities; diffuse crescent to horseshoe sculpture throughout surface, except on costal ridges; colour varying from almost completely black to dark green and even light brown, in latter case with three dark maculae on apical half of elytra.

Pygidium. Dark green to black or brown, with bilateral cretaceous spots drastically reduced or absent; exhibiting short creamy setae near sides and apex; with fine, wrinkled sculpture throughout surface.

Appendages. Antennal clubs reddish brown, of normal length, shorter than pedicel and flagellum combined; pedicel and flagellum with scattered but long, erected creamy setae; protibia broadly expanded, with short and scattered creamy setae, bidentate with apical tooth hypertrophic and falciform; meso- and metatibia with long, dense creamy setae, with no or poorly developed outer carina and spines, respectively; metatibial spurs widely spatulate; profemur, mesepimeron, mesofemur and metafemur all covered with very long creamy setae.

Underside. Shiny black to dark green; creamy, long setae throughout surface, except at center of metasternum and abdominal sternites; mesosternal lobe flat and roundedly expanded; abdominal sternites with visible convexity at center and without any white tomentose markings between midline and lateral margins (Figure 1B, D).

Remarks. Atrichiana jenisi shows a marked sexual dimorphism, with the female exhibiting antennal clubs about half the length of that of its male counterpart and a shorter clypeus, with far less upturned margins than in the male (Figures 1 and 2). The female also has a shorter and broader body shape than the male and does not exhibit large white tomentose markings either on the pygidium or the abdominal sternites. Its protibial surface is far broader than in the male and its apical teeth are strongly developed, indicating an extraordinary adaptation to fossorial activity. It does, however,

possess fully developed and functional wings, which are not significantly different to those observed in the male. *Atrichiana jenisi* is closely related to the only other species currently known within the genus, *A. placida*. It differs from the latter in having a generally more oblong body, more marked elytral costae and a predominatly dark green to black, rather than light brown background colour. The most diagnostic characters separating the two species may, however, lie in *A. jenisi* exhibiting a carina in the mid anterior third of its pronotal disc, a bilateral white macula on the lateral declivity of the pronotum and a vertical depression in the apical half of its scutellum (Figures 1A, C and 2A, C). None of these characters are present in *A. placida*. Otherwise, the two species share a remarkable variability in background colouration, from black to dark or light brown, with various shades of dark maculation. A dark green ground colour is not yet known in *A. placida*, while this is predominant in *A. jenisi*. The two species are sympatric on the Western Shores of Lake St Lucia, where *A. placida* appears to be marginally present in low abundance while showing its preference for the Eastern Shores of the lake and the coastal dune area of the southern Indian Ocean. Conversely, *A. jenisi* is completely absent from the coastal area proper and appears to be restricted to the more inland forests, from Dukuduku in the south to False Bay in the north. No records beyond this narrow area are currently known for the species and it is therefore likely that it may be a micro-endemic of the Western Shores of Lake St Lucia.

Discussion

It is remarkable that *Atrichiana jenisi* was discovered only very recently, despite the intensive surveys that have been undertaken historically in the area of the iSimangaliso Park (Combrink and Kyle 2006), with its status of UNESCO World Heritage Site. This confirms the extraordinary value of this park, as hotspot of global biodiversity and center of endemism.

Virtually nothing is yet known about the biology of the species, as all specimens known so far have been retrieved dead or drowning on the shores of False Bay in Lake St Lucia (Figures 3 and 4). An exception is provided by the holotype described by Krajcik (2006), which was reportedly collected between Mtubatuba and Dukuduku in December 2007 (cf. specimen data label). This is most likely in the area of the old picnic site on the road to the St Lucia Estuary, which has now been enclosed within the game fence of the iSimangaliso Wetland Park and rehabilitated into the wilderness of the Dukuduku Forest section. At the time of the holotype collection, the picnic site represented the only resting point along the road, with access to the indigenous forest habitat.

Adults are most likely unable to feed, as no specimens have been collected either in fruit baited traps, on flowers or sap flows, despite the intense collections that were undertaken in the past 15 years, during the period leading to the proclamation of the Park as a World Heritage Site and afterwards (Combrink and Kyle 2006). What is even more extraordinary is that no specimens have so far been observed active in flight or on the ground during this period, apart from one male specimen collected in November 2013, which

Figure 3. Shoreline of False Bay near Lister's Point, where the first drowned specimens of *Atrichiana jenisi* were collected (Photo: Lynette Clennell, 22 Dec 2012).

Figure 4. Dukandlovu shoreline, southern False Bay, where several specimens of *Atrichiana jenisi* were retrieved dead in January/February 2015 (Photo: Lynette Clennell, 1 February 2015).

had fallen into the water at False Bay and was climbing up the stem of a sedge clump. All other specimens found in Lake St Lucia were already dead for some time, but most were still in a good state of preservation due to the high salinity of the lake waters at the time.

It appears that adults are active for a relatively short period of time at the onset of the rainy season, from November to February. Adult emergence is obviously linked to major rainfall events, with all fresh specimens collected immediately or shortly after substantial rain (> 20 mm). For instance, most specimens known so far were collected along the shores of False Bay on the 3rd of February 2015 (Figures 3 and 4). They had all died a while before, as tissue decomposition was already well underway, despite the high salinity of the water (around 30). The summer of 2014–2015 had been particularly dry in the region and the first substantial rain in the False Bay area fell only between the middle and the end of January (69 mm during 15–17 Jan 2015, 54 mm during 28–30 Jan 2015; False Bay Park Meteo Station). As neither adult activity nor freshly dead specimens were observed during the survey undertaken between January 31 and February 3, it seems most likely that all the dead specimens had emerged with the first rain event of 15–17 January.

Atrichiana jenisi appears to be an endemic of the Sand Forest, possibly extending into the Northern Coastal Forest vegetation unit (types FOz 8 and FOz 7, respectively, of Mucina and Rutherford 2006). Both vegetation units are seriously threatened in the Maputaland bioregion, with the Sand Forest actually regarded as critically endangered because of its vulnerability and the socio-economic pressure currently exerted on the region (Mucina and Rutherford 2006). However, the specific habitat of the larval stages of *A. jenisi* remains unknown. This, despite the many Cetoniinae collections and rearing of larvae that have taken place in the area over a relatively long period of time. It seems likely that its habitat may be either patchy and extremely restricted or otherwise unusual (e.g. aerial epiphytic deposits, bird nests etc.). Given the importance of this area and its unique biodiversity to the overall conservation and sustainability of the iSimangaliso Wetland Park, further investigations are required in order to unravel the enigmatic life cycle and ecology of this endemic species.

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RESEARCH ARTICLE

Morphology of juvenile stages of *Kuschelina bergi* (Harold) with biological information (Coleoptera, Chrysomelidae, Alticini)

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Abstract

Kuschelina bergi (Harold, 1881) is being studied to be evaluated as a natural enemy of *Phyla nodiflora* var. *minor* (Hook.) N. O'Leary & Múlgura (Verbenaceae), an invasive weed in Australia. Eggs, and 1st and 3rd instar larvae are described and illustrated for the first time. The following characters distinguish *Kuschelina bergi*: presence of two medial setae in prosternum, mesosternum and metasternum, absence of tubercle on sternum I and eight setae in abdominal segment IX. The 3rd instar larvae of *K. bergi* resemble *Kuschelina gibbitarsa* (Say) larvae: the body shape and details of mouthparts are similar, but the morphology of the mandible is different, as is the tarsungulus which has a single seta. Differences between *K. bergi* and other known larvae of Oedionychina are discussed. New biological data based on laboratory rearing and field observation are also presented and discussed.

Keywords

Introduction

Kuschelina Bechyné (1951) is a genus of Alticini leaf beetles, included in the subtribe Oedionychina (Scherer 1983, Duckett and Kjer 2003). It comprises approximately 30 species mainly distributed in temperate and subtropical areas of South America: Paraguay, Perú, Bolivia, Brazil, Chile, Uruguay and Argentina, and another seven species mentioned from Mexico and USA (Scherer 1983). Information on immature stages and on the biology and host–plants of *Kuschelina* is scarce. Some species were collected on Lamiaceae and Scrophulariaceae (Jolivet and Hawkeswood 1995).

Kuschelina bergi was originally described by Harold (1881) in the genus *Oedionychis* Latreille, 1829 from Buenos Aires (Argentina) and Montevideo (Uruguay). Since then, only one collection record of this species has been available (Bechyné 1957). This species is associated with the invasive weed, *Phyla nodiflora* var. *minor* (Hook.) N. O'Leary & Múlgura 2012 (= *P. canescens* (Kunth) Greene) (Verbenaceae). Preliminary observations suggest that this flea beetle could be used as a biological control agent of *Phyla nodiflora* in Australia (Julien et al 2012).

Larval morphology of oedionychines is poorly known. The only detailed study of members of this subtribe are those dealing with the larvae of *Alagoasa parana* Samuelson (1985), *Alagoasa januaria* Bechyné (Duckett and Swigonová 2002), and *Walterianella bucki* (Duckett and Cassari 2002). Mature larva of *K. gibbitarsa* (Say) were described briefly by Blake (1927), Böving and Craighead (1931), Lawson (1991).

The identification of K. bergi larvae is important to complete our knowledge of characters in addition to those of the adults and as a first step towards a better understanding of the insect-host plant relationships, needed to conduct studies for biocontrol of *Phyla nodiflora* var. *minor*. The purpose of this paper is to describe the immature stages of *K. bergi*. Biological notes, including field and laboratory host range, are presented as well as some comparative notes on larvae of other Oedionychina species.

Materials and methods

Insect collection and study material

Specimens utilized for the study came from adults and larvae of *K. bergi* which were collected on *P. nodiflora* from different locations in Argentina during field trips made from 2009 to 2013. A laboratory colony was set up to obtain sufficient specimens for morphological studies and laboratory tests. Specimens were preserved in 70% ethyl alcohol. The larvae were macerated in 10% KOH solution for several minutes and rinsed in water. The methods of dissection, preparation and examination of immature stages follow Goulet (1977) and May (1979).

Terminology for morphological features of immatures follows Le Sage (1986) and LeSage and Zmudzinska-Krzesinska (2004). Larvae were mounted on metal stubs and

coated with gold-palladium and examined with a scanning electron microscope (SEM) Jeol-JSM-T100. Drawings were made using a *camera lucida* on a Leitz compound microscope and a Wild dissecting microscope.

Biological studies

Biological observations were made on 50 eggs laid by adults collected near Tres Arroyos city (RN 3, Km 520, 25 km S Tres Arroyos S 38°32, W 60°31, Buenos Aires Province) in October 2009 and brought to FuEDEI facilities in Hurlingham, Buenos Aires. They were kept in Petri dishes (10 cm diameter, 2 cm high). The hatched larvae were fed fresh leaves of *P. nodiflora* var. *minor*. After about 50 days, when the larvae decreased their activity during the prepupal stage, they were transferred to another container (8 cm diameter, 5 cm high) with soil as a substrate for pupation.

Preliminary studies revealed that pupation is a vulnerable stage in the life cycle of *K. bergi*. Hence, we studied what role the type of substrate and humidity plays on the pupation success through a factorial design experiment. Third instar larvae (N = 180) were collected from the laboratory colony and individually placed in small plastic containers with three different substrates: sand, commercial soil and nothing (as a control) with two levels of humidity (with and without humidity). Treatment with humidity consisted of adding water to containers, at least twice a day, for a completely wet, but not soaking, substrate; treatment without humidity received no water. In the case of the no-substrate test, a piece of tissue paper soaked in water was added for the treatment with humidity. The successful pupation was estimated by registering the emergence of a live adult (yes or no). The data were analyzed using generalized linear modeling, a logistic regression method with over-dispersion accounted for through the use of quasi-binomial error variances. Analysis was performed using R version 3.1.2 (R Core team 2013).

Voucher specimens are deposited in the collections of the Entomology Department, Museo de La Plata (MLPA), Argentina and Fundación para el Estudio de Especies Invasivas Collection FuEDEI, Buenos Aires, Argentina.

Results

Description of immature stages of *Kuschelina bergi* (Harold, 1881) (Figures 2–12)

Eggs (Fig. 2) Shape cylindrical, symmetrical. Coloration bright yellow, surface with fine reticulate impressions. Eggs are laid in clusters or singly, standing on the substrate on one end.

Mature larvae (Figs. 3–4, 9–10). Length 3.4–3.9mm, body elongate, bright yellow, weakly convex in preserved specimens. Head capsule width 0.5-0.7mm, mouthparts tibiae and tarsi light brown. Thorax yellow tinged with brown.

Figure 1–2. **1** *Kuschelina bergi* (Harold), male, dorsal habitus **2** *Kuschelina bergi* (Harold), cluster of eggs deposited in soil.

Head (Figs 5–6). Hypognathous, rounded, well sclerotized. Epicranial suture distinct, Y-shaped; well developed, coronal suture short, extending less than one quarter of the length of the head, frontal arms pale, curved, extending to the antennal sockets. Each side of epicranial plate bearing three long setae fixed in the middle of the disc, two above antennal margin and another on the outer side of antennae; numerous micro-setae irregularly distributed. Endocarina present as a black line extending to fronto-clypeal suture. Frons bearing one pair of long setae inserted on the disc, two pairs fixed on anterior margin and another two pairs on clypeal area. Stemmata absent. Antennae short, 3-segmented; attached to head capsule by a large, translucent membrane; basal segment large, transverse; segment II, transverse bearing three setae, with a peg-like sensilla dorsally; segment III formed by a large conical sensory papilla at bearing three long and two sensilla with a large seta fixed on the outer side of antennae. Clypeus transverse, bearing four setae on each lateral margin. Labrum transverse, apical margin emarginated; bearing four large setae in a median row. Mandibles (Fig. 8) robust, slightly sclerotized at apex, 4-toothed; tooth I small, blunt; teeth II-IV subequal, sharply pointed, a long mandibular seta inserted dorso-laterally, penicillus consisting of two thick setae. Maxillae and cardo well-sclerotized, subtriangular, bearing two setae at outer side. Stipes quadrate, with a short, narrow inner seta and two long outer setae, mala densely setose, with numerous short setae. Maxillary palpi with palpiger bearing two long setae; segment I with two short setae; segment III conical. Labium (Fig. 7) with submentum not very sclerotized, widened at base, with a pair of long, filiform submental setae; prementum broad, with a pair of long setae between labial palpi; labial palpi 3-segmented, short. Hypopharynx densely setose.

Thorax. Pronotum transverse, bearing two transverse rows of nine anterior and six posterior unisetose tubercles, two fixed at the posterior outer corner; post epipleural tubercle bearing one seta; pre-hypopleural tubercle bearing 2 setae; prosternum with two pairs of median setae. Meso- metathorax subequal, each bearing two anterior and four posterior unisetose tubercles arranged in two rows; mesopleura with alar tubercle bearing two setae; spiracular sclerite bearing one large seta; metapleura with alar tubercle bearing two setae and anterior epipleural sclerite bearing a seta; meso-and metasterna each bearing two median bisetose tubercles. Spiracle annuliform situated in the mesothoracic region. Legs 5-segmented, slightly chitinized, equal in size; trochantin triangular, asetose; coxa trapezoidal bearing eight long setae, two club-like, the others simple, and two sensillae; trochanter, triangular, with four long simple setae; femur sub-rectangular bearing eight long setae; tibia bearing three setae; tarsungulus curved, bearing a setiform pulvillus with a short, thin seta.

Abdomen. Abdominal segments I-VIII with filiform setae, dorsally arranged into two rows, interior prescutal area bearing two setae, posteriorly interior scutoscutellar area with two setae, exterior scutoscutellar with one seta and posterior parascutal areas bearing one seta; epipleural area (Fig. 12) bearing two setae; ventrally segments I-VIII with a eusternellar area bearing two setae, interior sternellar areas each with two setae; exterior sternellar bearing two setae; segment IX (Fig. 11) forming a fleshy pygopod with eight pairs of long, filiform setae. Spiracles I-VIII annuliform, situated on the pleural tubercles.
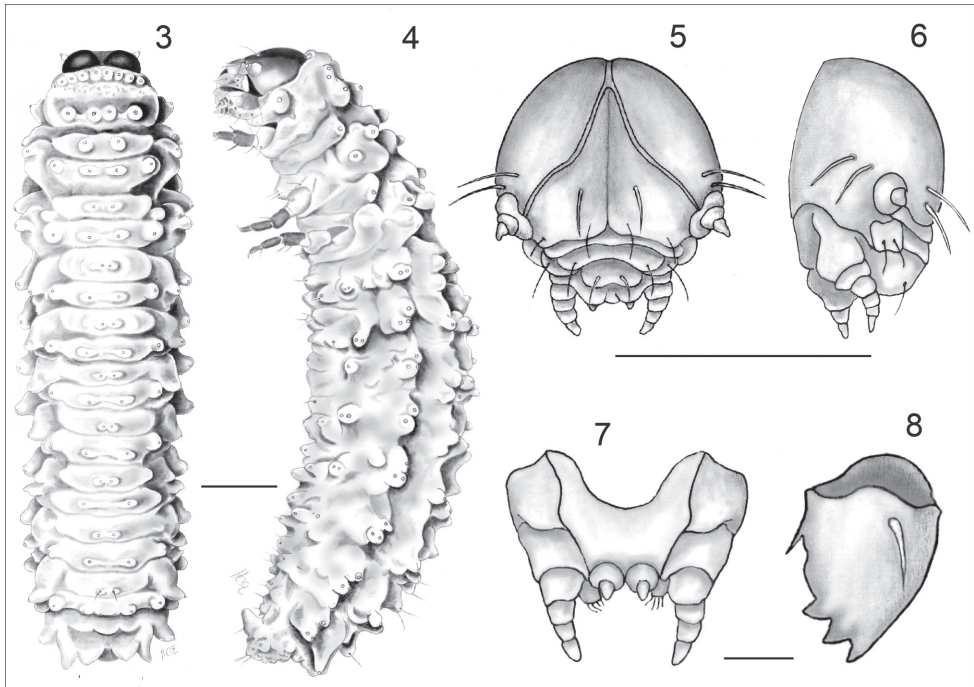
Figure 3–8. *Kuschelina bergi* (Harold) mature larvae **3** habitus, dorsal view **4** habitus, lateral view **5** cephalic capsule, frontal view **6** cephalic capsule, lateral view **7** labium and maxilla **8** mandible, dorsal view. Scale bars = 1 mm.

First instar larva. The first instar larva is very similar to the mature larva, but smaller in size, length 1.0–1.3mm, head capsule width 0.2–0.5mm. Recently emerged first instars are pale yellow. First instars can be distinguished from the mature larvae by the following characters: each side of epicranial plate with three long setae fixed in the middle of the disc, two above antennal margin and another on the outer side of antennae. Frons bearing one pair of long setae inserted on the disc, two pairs fixed on anterior margin and another two pairs on clypeal area. Egg bursters conical, sclerotized, situated on exterior scutoscutellar sclerites of meso-and metathorax.

Material examined. ARGENTINA: Buenos Aires: 13♂♂, RN 3, Km 520, 25 km S Tres Arroyos S 38°32, W 60°31, Sosa col.; 1♂, RP 11, Nueva Atlantis, S36°85 W56°69, Sosa col. (FUEDEI); 8♀♀, Rt. 226, 1 Km. E Bolívar, S36°26 W61°69, Sosa col. (FUEDEI).

Biological aspects. In the field, adults (Fig. 1) were found in leaf and stem litter on the ground. Adults and larvae feed on leaves; the former make circular holes, first instar larvae just scratch the epidermis and the final instars feed from the edge of the leaves towards the center.

Females dig a hole under the plants and lay eggs in masses of approximately ten eggs. Three larval instars were recorded, and the complete larval development took about two months. Pupation occurred in the soil and lasted over two weeks.
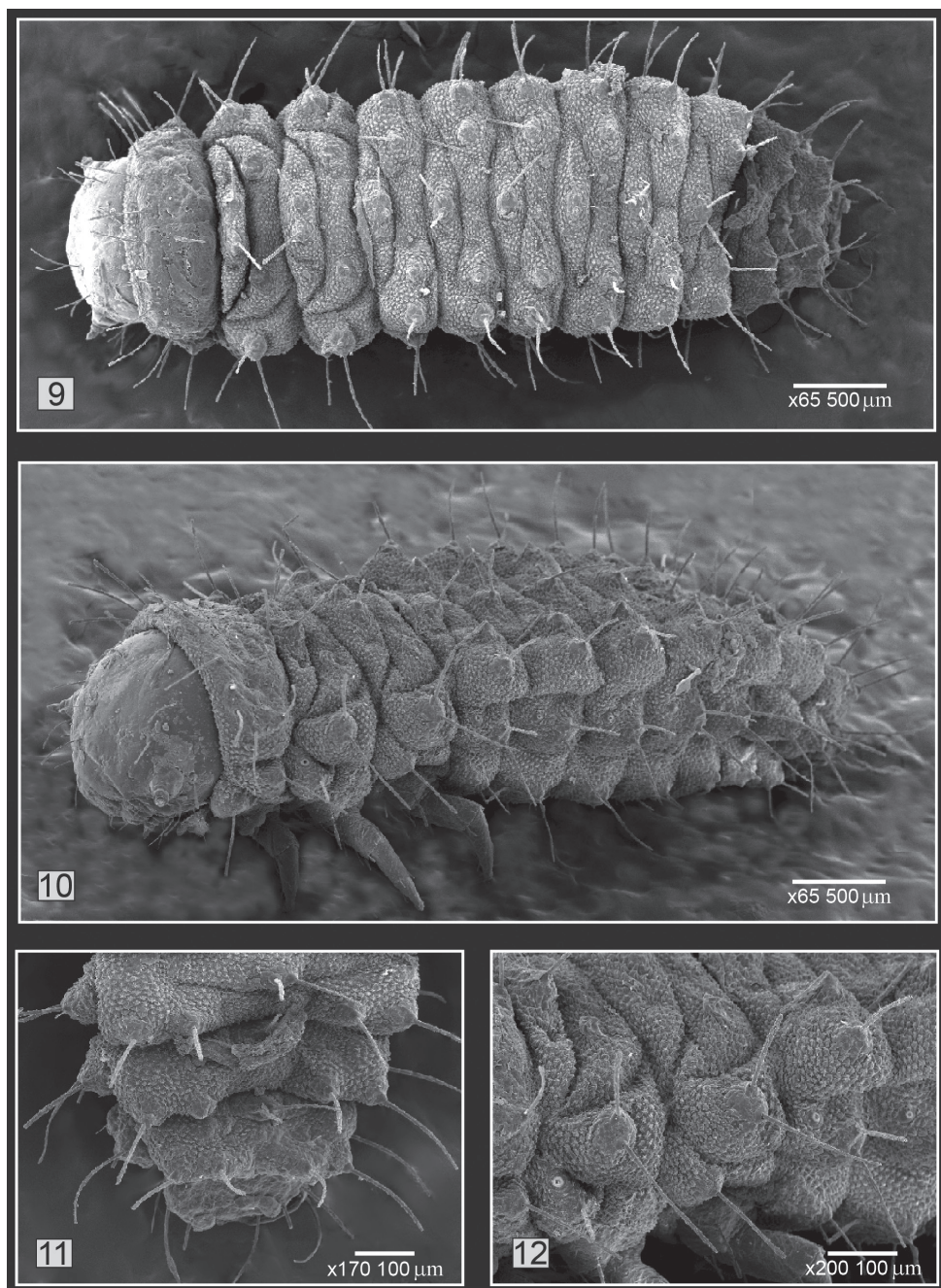
Figure 9–12. *Kuschelina bergi* (Harold) mature larvae **9** habitus, dorsal view **10** habitus, lateral view **11** abdomen: detail of segments 6-8, dorsal view **12** epipleural area, detail of seta. Scale bars = 100 μm.
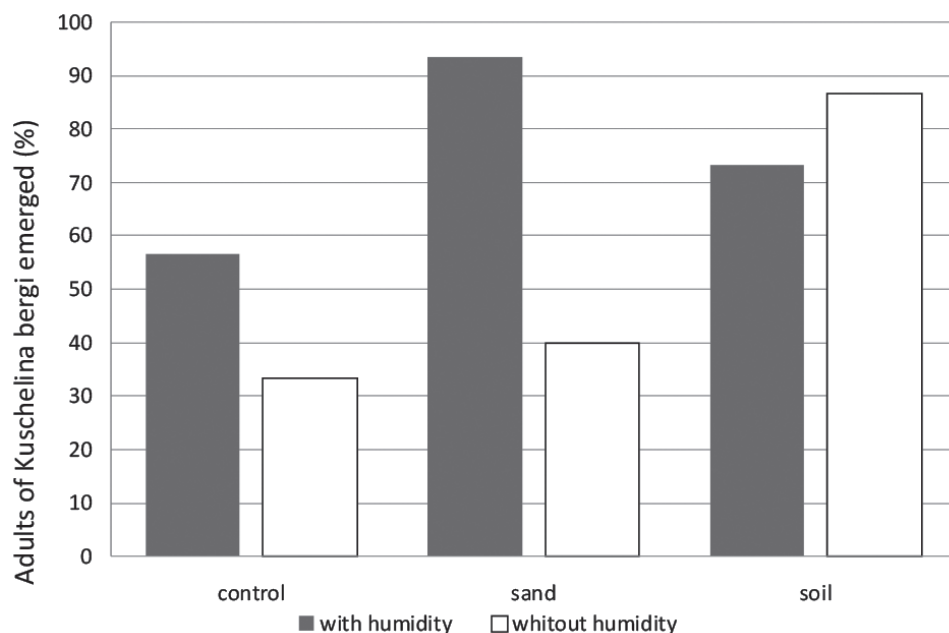
Figure 13. Adults of *Kuschelina bergi* emerged from three different kind of substrate in two conditions of humidity.

In the laboratory, the rate of emergence of adult *K. bergi* varied depending on the type of substrate and the humidity (Fig. 13). The pupation study revealed that *K. bergi* makes a "pupation chamber" with material from the substrate. In the dry sand containers, the larvae were covered in sand particles but they were not able to bury themselves nor were they able to build the chambers.

Pupation was more successful in moist sand, similar results were obtained in moist soil (t = -2.845, P = 0.00498), and dry soil showed slightly better results than moist soil.

In the "no substrate" treatment, where tissue paper was added for the tests with humidity, very few adults were able to emerge and survival was very low. Where there was no substrate and no moisture, again, a very low number of adults were able to emerge and the survival was extremely low. These results will strongly help to improve rearing methods.

Discussion

The brief description of *K. gibbitarsa* precludes detailed comparisons with *K. bergi* but both species share the following features: body shape, mouthparts with large mala carrying long setae along inner margin, submentum and mentum are fused. However, in contrast with the membranous pulvillus illustrated by Lawson (1991), we observed that *K. bergi* only have a single seta on the inner margin of the tarsungulus, and the

Table 1. Morphological comparison of third instar larvae of Walterianella bucki Bechyné, Alagoasa parana Samuelson, Alagoasa januaria Bechyné, Kuschelina gibbitarsa (Say), and Kuschelina bergi (Harold) (modified from Duckett and Swigoňová 2002).

Third instar	*Walterianella bucki*	*Alagoasa parana*	*Alagoasa januaria*	*Alagoasa gibbitarsa*	*Kuschelina bergi*
Club-like setae	present	absent	absent	absent	absent
Prominency of tubercles	dorso-lateral only	All dorsal and dorso-lateral	dorso-lateral only	dorso-lateral only	dorso-lateral only
Dorsolateral tubercles	Prominent and large	Less prominent, short and broad	Less prominent, long and thin	Less prominent, short and broad	Less prominent, short and broad
Median setae on prosternum	1 pair	1 pair	2 pairs	Not described	1 pair
Ventro-lateral tubercle of sg. I	present	Not present	present	Not described	Not present
Tubercles on meso-and metanotun	2 posterior unisetose	4 posterior unisetose	4 posterior unisetose	4 posterior unisetose	4 posterior unisetose
Dorsolateral tubercle sg. I-VIII	I-VII unisetose, VIII bisetose	bisetose	unisetose	Bi or trisetose	bisetose
Setation abd. sg. IX	8 dorsal, 6ventral setae	Not described	8dorsal,8 ventral setae	12 dorsal,10 ventral setae	8 dorsal setae
First instar					
Egg bursters	Absent	Present	Present	Present	Present

mandible has a pyramidal shape. *Kuschelina bergi* can be recognized by the presence of pro-meso-and metasterna with two medial setae, eight dorsal setae in abdominal segment 9 and the absence of tubercle on sternum I.

Duckett and Swigoňová (2002) listed morphological characters of the known Oedionychinae larvae (*W. bucki*, *A. parana*, *A. januaria*, and *K. gibbitarsa*) and mentioned that the prominent tubercles, chaetotaxy and the absence of stemmata are the main characters for recognizing larvae of the Oedionychina. Herein we summarized (Table 1) the diagnostic characters of *K. bergi* to facilitate comparison with the other larvae studied by Duckett and Swigoňová.

Final considerations

The status of *Kuschelina* as currently defined is somewhat unclear (Scherer 1983, Duckett 1998, Duckett and Swigonová 2002). Adults of some species are confused with mimetic forms of *Alagoasa*, a widely distributed genus in South and Central America. The first phylogenetic analysis of the subtribe Oedionychina (Duckett and Kjer 2003) suggested a close relationship between species of both genera.

As few larvae have been studied so far, we cannot make generalizations about not only the value of the characters to determine taxa but also for phylogenetic studies. Detailed descriptions (adult and immature stages) of *Kuschelina* are much needed and will contribute to resolving the taxonomic relationships among species and will provide biological knowledge on the Alticini, especially within the subtribe Oedionychina.

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RESEARCH ARTICLE

Salt lakes of La Mancha (Central Spain): A hot spot for tiger beetle (Carabidae, Cicindelinae) species diversity

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Abstract

The tiger beetle assemblage of the wetlands of La Mancha (central Spain) comprises nine species: *Calomera littoralis littoralis*, *Cephalota maura maura*, *Cephalota circumdata imperialis*, *Cephalota dulcinea*, *Cicindela campestris campestris*, *Cicindela maroccana*, *Cylindera paludosa*, *Lophyra flexuosa flexuosa*, and *Myriochila melancholica melancholica*. This assemblage represents the largest concentration of tiger beetles in a single 1º latitude / longitude square in Europe. General patterns of spatial and temporal segregation among species are discussed based on observations of 1462 specimens registered during an observation period of one year, from April to August. The different species of Cicindelini appear to be distributed over space and time, with little overlapping among them. Three sets of species replace each other phenologically as the season goes on. Most of the species occupy drying or dried salt lakes and salt marshes, with sparse vegetation cover. Spatial segregation is marked in terms of substrate and vegetation use. *Calomera littoralis* and *M. melancholica* have been observed mainly on wet soils; *C. circumdata* on dry open saline flats; *C. dulcinea* and *C. paludosa* in granulated substrates with typical halophytic vegetation; *C. maura* is often present in man-modified areas. *Cephalota circumdata* and *C. dulcinea* are included as species of special interest in the list of protected species in Castilla–La Mancha. Conservation problems for the Cicindelini assemblage arise from agricultural activities and inadequate use of sport vehicles. Attempts at restoring the original habitat, supressing old semi-industrial structures, may affect the spatial heterogeneity of the lakes, and have an effect on Cicindelinae diversity.

Keywords

Introduction

The geomorphology of the Iberian Peninsula has been modelled through a long and complex paleogeographic history, including the ancient emergence of the western territories, complex Miocene reorganization of the tectonic plates with active orogenic periods and incorporation of a large portion of the Betic–Rifean massif, and the effect of the Pleistocene glacial periods (López Martínez 1989, Barbadillo et al. 1997, Hewitt 2000, Cavazza et al. 2004, Gutiérrez et al. 2008). As a result of this complex history, the diversification of the Iberian biota has attained such a level as to be considered part of one of the 25 world hotspots of biodiversity (Myers et al. 2000). The complexity of the Iberian faunal assemblages is enhanced by the persistence of representatives of ancient lineages (Machordom and Doadrio 2001, Martínez-Solano et al. 2006, Albert et al. 2007, Gonçalves et al. 2007), together with the settlement of Holocene recent immigrants from the African Continent (Carranza et al. 2004, Recuero et al. 2007).

The Central Iberian Plateau, is an almost flat elevated plain which occupies most of the central area of the Peninsula. The Iberian Plateau, is delimited by mountain chains of different height all around its perimeter, and crossed transversally by the Central System Mountain Chain, which divides it into two sub-regions: a northern one, mostly conformed by the Duero River Basin, located in the Spanish Autonomous Region of Castilla – León, and a southern one crossed by the Tagus and Guadiana river systems, in the Spanish Autonomous Region of Castilla – La Mancha (Cirujano 1980, de la Peña and Marfil 1986, Comín and Alonso 1988, Alonso 1998, Gutiérrez et al. 2008, de Luis et al. 2010). In general terms, the biological diversity of the Iberian Plateau is relatively limited with regard to the surrounding mountainous areas. However, the paleogeographic history of the Plateau has many singularities with regard to all other Iberian structural regions. The Iberian Plateau was covered by large endorheic lakes along most of the Tertiary (Macau and Riba 1965, Plans 1969, Ordóñez et al. 1987, 1991, López Martínez 1989). Those lakes were drained during the Pliocene, along with the formation of the current main river systems that crossed the plateau in an east-west direction, leaving thick layers of gypsum soils (Gutiérrez et al. 2008). Because of the relatively high elevation of the Plateau, 650–800 m, the effect of the glaciations was relatively strong, allowing for the development of a typical taiga vegetation, interrupted by large swamps which covered most of the southern Plateau during the coldest periods (Zagwijn 1996, Ravazzi 2002). Some of those swamps and small lakes are still present in the central areas of the southern Plateau, particularly in La Mancha region. Many of the small lakes, endorheic and temporary, are shallow and highly saline, and when dried present a flat surface covered by salt crusts (de la Peña and Marfil 1986, Comín and Alonso 1988, Florín et al. 1993).

Flat, sun exposed areas surrounded by halophytic vegetation, create a typical habitat for tiger beetles (Cicindelinae) (Vives and Vives 1978b, Rueda and Montes 1987, Hoback et al. 2000, Pearson and Vogler 2001, Jaskuła 2007, 2011, 2015). La Mancha lakes, isolated in the centre of the Plateau, are diverse, not only in salt concentration and depth, but also in vegetation cover and land use around them, offering a

wide range of microhabitats suitable for tiger beetle activity. Tiger beetles are active predators, spread over most of the world. In temperate regions most species are terrestrial and hunt actively, running after their prey on sun exposed relatively flat surfaces, such as beaches, lake and river shores, dirt roads, forest clearings, and high elevation denuded areas. Larvae are also predators that hide in self-excavated vertical tunnels, in the same habitats where adult activity takes place (Pearson 1988, Cassola and Pearson 2000, Pearson and Vogler 2001).

The tiger beetle fauna of the Iberian Peninsula is considered well-known in relation to the knowledge on other taxonomic groups. Serrano (2013) indicated the presence of 22 species of Cicindelinae in the Iberian Peninsula, some of them represented by more than one subspecies. However, a large portion of the current knowledge has been generated recently, including the confirmation of the specific differentiation of *Cicindela lagunensis* Gautier des Cottes, 1872, *C. iberica* Mandl, 1935, *C. lusitanica* Mandl, 1935, and *C. hybrida* Linnaeus, 1758 (Matalin 1998, Cardoso and Vogler 2005, Cardoso et al. 2009), the long-awaited description of *Cephalota dulcinea* López, Rosa & Baena, 2006, the analyses of the genetic differentiation of some Iberian species of *Cephalota* (López-López and Galián 2010) and their conservation (Diogo et al. 1999), and the first confirmed reports for Spain of *Cephalota luctuosa* (Dejean, 1831) (Werner 1992) and *Calomera lunulata* (Fabricius, 1781) (López-Pérez and García 2007, López-Pérez 2010). All these new discoveries suggest that we are still far from having a complete knowledge of the Iberian Fauna of tiger beetles. Taxonomic characterization of complex species groups, detailed knowledge on geographic distribution at the local level and basic information on reproductive biology and habitat use are still lacking for most of the species of tiger beetles along most of the Iberian geography.

Previous reports on the Cicindelinae of La Mancha lacustrine areas (Vives and Vives 1978a, Rueda and Montes 1987, Ortíz et al. 1988; Serrano et al. 1990, Lencina et al. 1991, 2001, Andújar et al. 2002, 2009) indicate that the region hosts a particularly large number of tiger beetles when compared to other European regions (Jaskuła 2011). The relevance of salt marshes for tiger beetle diversity and their conservation has been reported often, but the description of *Cephalota dulcinea*, an endemic tiger beetle of La Mancha, restricted to a few salt lakes (López et al. 2006), enhanced the interest on the region as a singular spot for Cicindelinae diversity.

In this study, we monitored, from April to August, the presence and activity of tiger beetles in more than 30 small lakes, salt marshes, and gypsum flats in La Mancha region. Most of these locations (27) were monitored as part of a Life Project: *La Mancha Wetlands* (LIFE+10 NAT/ES/000563), aimed at restoring the *espartal* or *albardinal* (*Lygeum spartum* (L.) Kunth) grasslands and salt flats, by reclaiming Mediterranean salt steppes (*Limonietalia*), designated as a priority habitat (1510) by Habitats Directive 92/43/EEC, and other halophytic formations in the La Mancha wetlands SCI and SPA zones. Most of the locations studied are protected under the laws of the Autonomous Region of Castilla – La Mancha (Spain), and included in the Nature 2000 European Union conservation program (http://ec.europa.eu/environment/nature/natura2000/db_gis/).

Our main objective was to determine the relevance of the lacustrine environments of La Mancha region for tiger beetle diversity and conservation. During the study we found that the number of species inhabiting the region had been underestimated. In comparison to previously published European locality records, La Mancha region harbours the largest number of species of tiger beetles present in a single square (one degree latitude x longitude). This finding prompted us to undertake the following tasks: (1) inventory the tiger beetle species inhabiting each lake or marsh, (2) identify the phenology for each species during the period of study, (3) gather information on general landscape use for each species, and (4) evaluate threats and possible conservation needs, based on observed abundances and observations on human activities around the lakes.

Methods

The study area

The study was carried on the 27 marshes and small lakes included in the Life Project *La Mancha Wetlands*, all located in the traditional demarcation of La Mancha, which include portions of Ciudad Real, Cuenca, and Toledo provinces (Fig. 1; Table 1). Some additional salt marshes, ancient channels, and gypsum flats, located in possible corridor areas between lakes were also studied. Amongst those, the gypsum flats and channels of Cerro San Cristóbal in Quero (Toledo), and salt marshes of the Gigüela River in Quero and Villafranca de los Caballeros (Toledo) were also monitored. Some larger lakes not included in the Life Project, as Laguna del Hito (Cuenca), or La Laguna in Miguel Esteban (Toledo), were also included in the study (Fig. 1; Table 1). Despite their current protection, and their healthy appearance, most of the small lakes and swamps have suffered important modifications and historical aggressions of all kinds, from being ploughed in dry years, to being partially filled with urban waste, and flooded with residual waters when located near villages. Some of the lakes most visited by bird-watchers (La Veguilla in Alcázar de San Juan), were artificially created for human uses, less than four decades ago.

The temporary lakes of La Mancha are a group of wetlands spread across the territory of the Upper Guadiana Basin. According to Jerez García (2010), these wetlands are characterised by the presence of sulphates (magnesium sulphate) and, to a lesser extent, calcium carbonates and chloride (sodium chloride). These water bodies are endorheic and, during summer, water can evaporate completely, thus rendering them saline or hyper-saline.

These singularities – fluctuating saline lakes or hyper-saline lakes, with salt concentrations that can exceed those of seawater, and vegetation adapted to fairly restrictive conditions – make these wetlands and their surroundings particularly interesting for biodiversity conservation. These areas harbour a large number of endemic or endangered halophytic plants [i.e. *Limonium carpetanicum* Erben, *Limonium dichotomum*
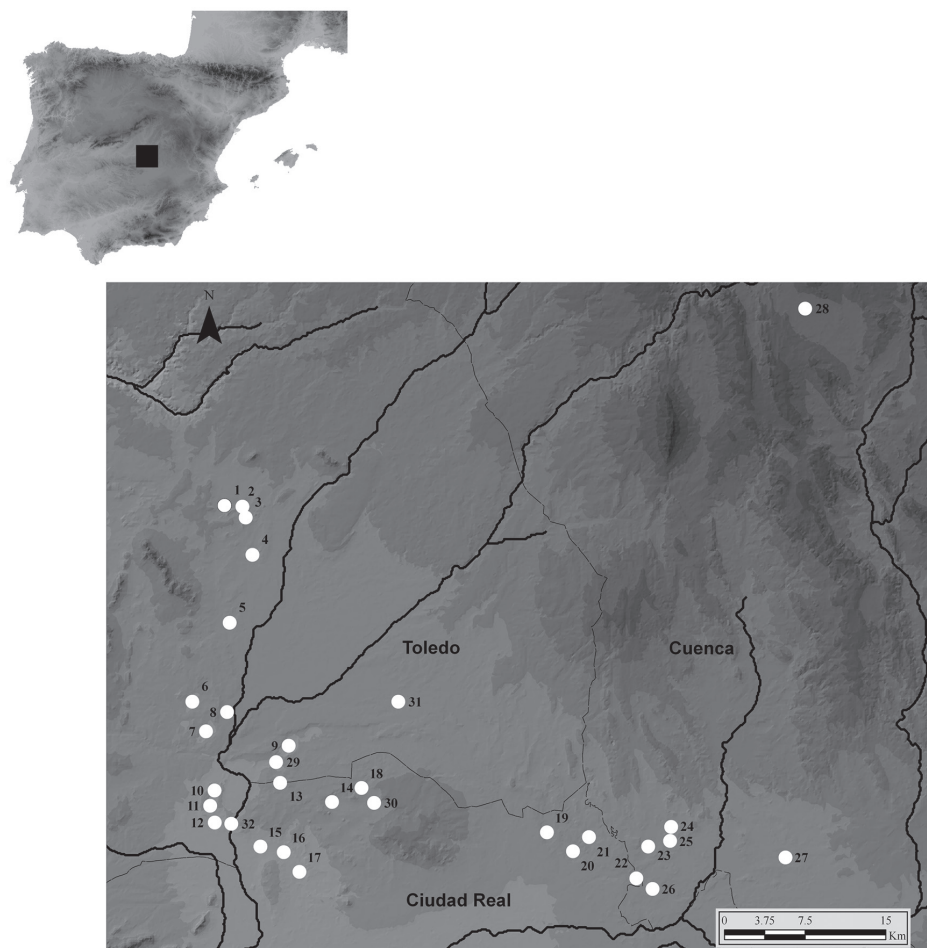
Figure 1. Map of the Iberian Peninsula showing the location of the study area. The inset shows a detail of the 27 marshes and small lakes included in the Life Project "La Mancha Wetlands" (**1–27**), and some other monitored marshes and lakes (**28–32**). Numbers represent the sampled areas: TOLEDO: Lillo: **1** Laguna de El Longar **2** Laguna del Altillo Chica **3** Laguna del Altillo Grande **4** Laguna de la Albardiosa; Miguel Esteban: **31** La Laguna de Miguel Esteban; Villacañas: **5** Laguna Larga **6** Laguna de Tirez **7** Laguna de Peña Hueca; Quero: **8** Laguna del Taray **9** Laguna Grande **29** Cerro de San Cristóbal; Villafranca de Los Caballeros: **10** Laguna Chica **11** Laguna Grande **12** Laguna de la Sal **32** Gigüela marshes. CIUDAD REAL: Alcázar de San Juan: **13** Laguna de los Carros **14** Laguna de Pajares/los Pájaros **15** Laguna de las Yeguas **16** Laguna del Camino de Villafranca **17** Laguna de la Veguilla; Campo de Criptana: **18** Laguna de Salicor **30** Arroyo de San Marcos; Pedro Muñoz: **19** Laguna del Retamar **20** Laguna del Pueblo/de la Vega **21** Laguna de Navalafuente **22** Laguna de Alcahozo. CUENCA: Mota del Cuervo: **23** Laguna de Manjavacas **24** Laguna de Sánchez Gómez **25** Laguna de la Dehesilla; Las Mesas/Las Pedroñeras: **26** Laguna de Alcahozo Chico **27** Laguna del Taray Chico. El Hito: **28** Laguna del Hito.

Table 1. Localities monitored, geographic coordinates and sampling dates for each locality.

Wetland	Town and province	Coordinates	Date
Laguna de El Longar	Lillo (Toledo)	39°42'8.00"N, 3°19'17.86"W	15-VI-2012, 17-VI-2012, 13-IV-2014, 23-V-2014, 13-VI-2014, 11-VII-2014, 2-VIII-2014, 9-VIII-2014, 23-VIII-2014
Laguna del Altillo Chico	Lillo (Toledo)	39°42'4.57"N, 3°18'11.38"W	23-V-2014, 7-VI-2014, 13-VI-2014, 2-VIII-2014
Laguna del Altillo Grande	Lillo (Toledo)	39°41'33.10"N, 3°18'1.03"W	17-V-2014, 23-V-2014, 7-VI-2014, 13-VI-2014
Laguna de la Albardiosa	Lillo (Toledo)	39°39'38.75"N, 3°17'32.35"W	23-V-2014
Laguna Larga	Villacañas (Toledo)	39°36'17.45"N, 3°19'1.58"W	26-IV-2014, 17-V-2014, 23-V-2014, 13-VI-2014, 20-VI-2014, 27-VI-2014, 9-VIII-2014, 23-VIII-2014
Laguna de Tirez	Villacañas (Toledo)	39°32'6.85"N, 3°21'26.73"W	17-VI-2012, 13-IV-2014, 17-V-2014, 20-VI-2014, 27-VI-2014, 2-VIII-2014, 23-VIII-2014, 24-X-2014
Laguna de Peña Hueca	Villacañas (Toledo)	39°30'50.58"N, 3°20'24.98"W	13-IV-2014, 26-IV-2014, 17-V-2014, 23-V-2014, 13-VI-2014, 27-VI-2014, 11-VII-2014, 2-VIII-2014, 9-VIII-2014, 23-VIII-2014, 24-X-2014
Laguna del Taray	Quero (Toledo)	39°31'48.62"N, 3°18'59.79"W	26-IV-2014, 7-VI-2014, 21-VI-2014
Laguna Grande	Quero (Toledo)	39°29'59.25"N, 3°15'12.42"W	26-IV-2014, 24-V-2014, 21-VI-2014, 28-VI-2014, 12-VII-2014
Laguna Chica	Villafranca de los Caballeros (Toledo)	39°27'51.61"N, 3°19'56.61"W	26-IV-2014, 20-VI-2014, 27-VI-2014
Laguna Grande	Villafranca de los Caballeros (Toledo)	39°27'3.42"N, 3°20'14.67"W	26-IV-2014, 20-VI-2014, 27-VI-2014, 11-VII-2014, 2-VIII-2014, 9-VIII-2014
Laguna de la Sal	Villafranca de los Caballeros (Toledo)	39°26'14.71"N, 3°19'54.51"W	26-IV-2014, 24-V-2014, 11-VII-2014
Laguna de los Carros	Alcázar de San Juan / Quero (Toledo)	39°28'12.39"N, 3°15'45.30"W	8-VI-2012, 26-IV-2014, 24-V-2014, 21-VI-2014
Laguna de Pajares	Alcázar de San Juan (Ciudad Real)	39°27'15.47"N, 3°12'20.83"W	25-IV-2014, 17-V-2014, 7-VI-2014
Laguna de las Yeguas	Alcázar de San Juan (Ciudad Real)	39°25'0.58"N, 3°16'58.85"W	26-IV-2014, 17-V-2014, 24-V-2014, 20-VI-2014, 27-VI-2014, 11-VII-2014, 27-VII-2014, 2-VIII-2014, 9-VIII-2014
Laguna del Camino de Villafranca	Alcázar de San Juan (Ciudad Real)	39°24'52.88"N, 3°15'30.63"W	26-IV-2014, 11-VII-2014, 27-VII-2014, 2-VIII-2014, 9-VIII-2014

Wetland	Town and province	Coordinates	Date
Laguna de la Veguilla	Alcázar de San Juan (Ciudad Real)	39°23'45.37"N, 3°14'24.14"W	26-IV-2014, 11-VII-2014, 8-VIII-2014, 9-VIII-2014
Laguna de Salicor	Campo de Criptana (Ciudad Real)	39°28'0.24"N, 3°10'24.78"W	8-VI-2012, 13-IV-2014, 17-V-2014, 6-VI-2014, 7-VI-2014, 28-VI-2014, 8-VIII-2014
Laguna de Retamar	Pedro Muñoz (Ciudad Real)	39°25'42.06"N, 2°58'20.98"W	25-IV-2014, 21-VI-2014
Laguna del Pueblo	Pedro Muñoz (Ciudad Real)	39°24'39.52"N, 2°56'47.20"W	25-IV-2014, 6-VI-2014, 28-VI-2014, 12-VII-2014, 8-VIII-2014
Laguna de Navalafuente	Pedro Muñoz (Ciudad Real)	39°25'31.02"N, 2°55'44.17"W	25-IV-2014
Laguna de Alcahozo	Pedro Muñoz (Ciudad Real)	39°23'27.46"N, 2°52'31.21"W	25-IV-2014, 16-V-2014, 6-VI-2014, 21-VI-2014 , 8-VIII-2014
Laguna de Manjavacas	Mota del Cuervo (Cuenca)	39°25'0.21"N, 2°51'55.06"W	25-IV-2014, 16-V-2014, 21-VI-2014, 28-VI-2014, 12-VII-2014, 8-VIII-2014
Laguna de Sánchez Gómez	Mota del Cuervo (Cuenca)	39°25'55.79"N, 2°50'22.26"W	25-IV-2014, 16-V-2014, 6-VI-2014, 21-VI-2014, 28-VI-2014, 8-VIII-2014,
Laguna de la Dehesilla	Mota del Cuervo (Cuenca)	39°25'20.28"N, 2°50'25.51"W	16-V-2014, 6-VI-2014, 20-VI-2014, 8-VIII-2014
Laguna de Alcahozo chico	Mota del Cuervo (Cuenca)	39°23'26.99"N, 2°52'32.64"W	25-IV-2014, 16-V-2014, 6-VI-2014, 12-VII-2014
Laguna del Taray Chico	Las Mesas / Las Pedroñeras (Cuenca)	39°24'30.19"N, 2°42'55.98"W	25-IV-2014, 16-V-2014,
Laguna de El Hito	Montalbo / El Hito (Cuenca)	39°52'4.22"N, 2°41'34.02"W	15-VI-2014
Cerro San Cristóbal	Quero (Toledo)	39°29'16.10"N 3°15'58.62"W	8-VI-2012 , 24-V-2014, 21-VI-2014
Arroyo de San Marcos	Campo de Criptana (Ciudad Real)	39°27'13.66"N 3°09'37.29"W	8-VI-2012, 17-V-2014,
La Laguna	Miguel Esteban (Toledo)	39°32'19.15"N 3°08'03.70"W	12-VII-2014
Gigüela marshes	Villafranca de los Caballeros (Toledo)	39°26'09.36"N 3°18'52.00"W	24-V-2014

(Cav.) Kuntze Revis., *Limonium squarrosum* Erben, *Gypsophila tomentosa* L., *Lygeum spartum*, *Microcnemum coralloides* (Loscos & J.Pardo) Font Quer] and aquatic plants [*Lamprothamnium papulosum* (K.Wallroth) J.Groves, *Tolypella salina* R. Corillion, *Althenia orientalis* (Tzvelev) P.García-Murillo and Talavera, and *Ruppia drepanensis* Tineo ex Guss.] (Cirujano and Medina 2002, Cirujano et al. 2014).

The lakes of La Mancha and the protective steppes that surround them, provide extensive ecosystem services (Florín and Montes 1999) and function as habitats for many species of endangered invertebrates and vertebrates (de la Cruz 2009). However, the saline wetlands of La Mancha are as important as they are unknown throughout Spain and the rest of Europe. In the last decade, the increase in cultivated land in the area, together with the construction of ditches and canals to prevent waterlogging, has contributed to the destruction of much of the natural vegetation surrounding the cuvettes (Aguirre et al. 2013).

Sampling and monitoring strategy

Tiger beetle sampling and monitoring was designed to cover the period in which most saline lakes are totally or partially dry, from April to September. Monitoring in 2014 consisted in ½ to 1 hour walks for visual census in favourable habitats within marshes and lakes, including halophytic vegetation, shores of lakes, denuded salt flats, dirt roads with or without saline surfaces, draining channel banks, and margins of culti-vated fields. Walks were carried out by one or two people (occasionally more) (MG-P, PCR-F, and collaborators mentioned in the acknowledgments). In particular situations (e.g. behavioural observations) walks took longer than 1 hour. At a first stage, we estab-lished transects to evaluate abundance and population density, but populations moved or changed locations to nearby areas along the monitoring season, rendering transects unsuitable. As a consequence, sampling effort and dates changed across patches, so sta-tistical analyses of tiger beetle densities were no longer adequate. This particular situa-tion is quite different from that observed in other studies (Mazzei et al. 2014), in which specimens were located along defined transects during the entire period of survey.

Most species of cicindelids in the region are easy to identify *de visu* without the need of seizing the specimens. Only *Cicindela campestris* and *Cicindela maroccana* are difficult to differentiate at a distance. In those cases, and in any other particularly dif-ficult situation, specimens were captured with an entomological hand-net. Captured specimens were released on site, except for a few per location that were kept as vouchers or reference, or for future confirmation of identification based on molecular analyses (*C. campestris* and *C. maroccana* difficult cases). Specimens captured are preserved dry or in ethanol at the Museo Nacional de Ciencias Naturales (MNCN–CSIC). Walks started no earlier than 11 AM and ended around sunset (from 18:30 to 21:30 PM depending on the season).

Each sampled area was visited between 1 and 11 times during the period of study (Table 1), the number varying in accordance with its potential to harbour undetected

species in previous visits, or to follow individual site changes in species phenology. Day samplings were complemented with occasional night observations to detect resting places or possible nocturnal activity. Larval sampling was not undertaken. Additional surveys were performed by car in peripheral areas. These surveys consisted of low speed searches along dirt roads (about 180 km), with the objective of detecting populations inhabiting areas far from the larger marshes and lakes. Additional observations from a 2012 preliminary survey (Villacañas, Lillo, Alcázar de San Juan, and Campo de Criptana) (MG-P), and from local collaborators in Alcázar de San Juan (Pablo Pichaco), were also taken into account.

Sampling was not adequately designed to monitor species with late winter and early spring activity, such as *C. campestris*, *C. maroccana*, and possibly *L. flexuosa* that could be already active around completely water- filled lakes. Therefore, presence and abundances recorded for these three species are not representative of their actual temporal and spatial yearly distribution.

Unpredictable climate changes have a strong influence on the phenology and patterns of activity of tiger beetles. Consequently, in these current years in which rain and temperature patterns are often erratic, the results obtained can only be considered representative of the season studied. Climate changes may have a particularly strong effect on the species that develop most of their activity in dry lake basins, which may suffer strong delays or acceleration during the drying period.

Each specimen was geo-referenced and the general types of soil and vegetation were recorded. Categories used for soil structure were: salt crust (coarse or sheet salt layers on lake basins), salt patches in trails (small salt flats outcropping on patches on trails), granulated salty soils (areas with a thick layer of non-crystalline salty soils, usually gritty or dusty); compact soils in flat trails, compact soils in the banks of channels, saprobe shores (soils formed by decomposed organic matter in lake basins with heavy avian populations), and muds (wet soils with lower salt concentration) (Table 2).

Categories used for vegetation cover were: denuded (areas without vegetation) or vegetated. We split vegetated areas into halophytic and non-halophytic prairies. Within the halophytic category, two main groups were selected: *albardinal*, open or dense, dominated by *Lygeum spartum* (considered apart because of its ecological singularities and because it is the main target of the conservation and restoration programs); and other halophytic prairies, open or dense (which include all types of prairies located on salt soils, from the temporarily flooded edges of the saline basins, to the never flooded margin of the lakes. Their vegetation include vegetal communities dominated mainly by *Salicornia ramossisima* J.Woods, *Limonium* Mill., *Suaeda vera* Forssk. ex J.F.Gmel., *Salsola vermiculata* L., *Microcnemum coralloides* (Loscos & J. Pardo) Font Quer, *Plantago maritima* L., *Suaeda splendens* (Pourr.) Gren. & Godr., *Frankenia pulverulenta* L., *Hordeum marinum* Huds., *Sphenopus divaricatus* (Gouan) Reichenb., *Lolium rigidum* (Gaudin) Weiss ex Nyman, and *Bolboschoenus maritimus* (L.) Palla at the shore (Cirujano 1981) (Table 2).

The non-halophytic category, open or dense, includes vegetation not necessarily associated with salty soils, either on relatively dry soils [dominated in the area by

Table 2. Number of specimens of tiger beetles found on each of the different substrate and vegetation types considered. See text for details.

Soil type / Species	C. littoralis	C. maura	C. dulcinea	C. circumdata	C. campestris	C. paludosa	M. melancholica
Salt crust	227	15	5	82		1	
Granulated salty soils	116	12	279	19	45	41	
Salt patches in trails		20	153			24	
Trails		11	87		7		
Banks		27			13	1	
Sapropel shores	52						
Mud	166	11			13		35
Vegetation type / Species							
Denuded areas	274	63	113	83	12	4	10
Halophytic prairies							
open	132	15	289	18	21	28	
dense	2	11			33	8	
Non halophytic prairies							
open	47				5		
Albardinal							
open			122			26	
Shore hidrophytic vegetation							
open	106	1			3	1	25
dense		6			4		

Scirpus holoschoenus L., *Cirsium monspessulanus* (L.) Hill., *Plantago major* L., *Trifolium fragiferum* L., *Cynodon dactylon* (L.) Pers.] (Cirujano 1981), or on wet soils [formed by hydrophytic communities dominated by *Scirpus littoralis* Shrad. *Phragmites australis* (Cav.) Trin. ex Steud., *Typha angusifolia* L., etc.] (Cirujano 1980) (Table 2).

Results

Presence and number of tiger beetles observed at each locality visited is recorded in Table 3 and Suppl. material 1. We observed a total of 1462 specimens corresponding to 9 species of Cicindelinae (Figs 2–3): *Calomera littoralis littoralis* (Fabricius, 1787), *Cephalota* (*Cassolaia*) *maura maura* (Linnaeus, 1758), *Cephalota* (*Taenidia*) *circumdata imperialis* Klug, 1834, *Cephalota* (*Taenidia*) *dulcinea* López, Rosa & Baena, 2006, *Cicindela* (*Cicindela*) *campestris campestris* Linnaeus, 1758, *Cicindela* (*Cicindela*) *maroccana* Fabricius, 1801, *Cylindera* (*Cylindera*) *paludosa* (Dufour, 1820), *Lophyra* (*Lophyra*) *flexuosa flexuosa* (Fabricius, 1787), and *Myriochila* (*Myriochila*) *melancholica melancholica* (Fabricius, 1798). This assemblage of tiger beetles in La Mancha represents 41% of the overall diversity of tiger beetles present in the Iberian Peninsula (based on Serrano 2013). Taxonomic assignation at subspecific level for La Mancha populations of *C. maroccana* is problematic based on the scarce material studied. Nomenclature used follows Putchkov and Matalin (2003) and Serrano (2003, 2013).

Observations on local geographic distribution, general landscape selection (soil structure and vegetation types) and adult activity are described in the following paragraphs, sorted by species.

Calomera littoralis littoralis

Calomera l. littoralis (Fig. 2a) was found in 19 localities (Fig. 4a). A total of 561 specimens were observed. Most specimens were found in relatively humid situations: in the humid shore of small lakes (45.6%), around drying pools (25.7%), in drying channels communicating saline lakes (3.6%), and a few in totally dried out lakes (25.1%). Most specimens were found in saline areas (66.8%) (Table 2) (Figs 5–6), but they were also present in ponds with much less salt concentration (33.2%), located near a salt lake (El Longar), or water bodies resulting from regulated flooding (south end of Laguna del Camino de Villafranca, and Laguna del Pueblo de Pedro Muñoz) (Fig. 6d, e).

Most populations of *C. littoralis* changed location within the same locality during the period of study. We followed these intra-populational movements at some lakes. At Laguna de Manjavacas, specimens were active in late April in the salty marshes surrounding the main, totally water-filled, salt lake. As the lake started to dry out, specimens appeared along the shoreline, and disappeared completely from the dried out salty marshes. Subsequently, and until the complete drying out of the main lake,
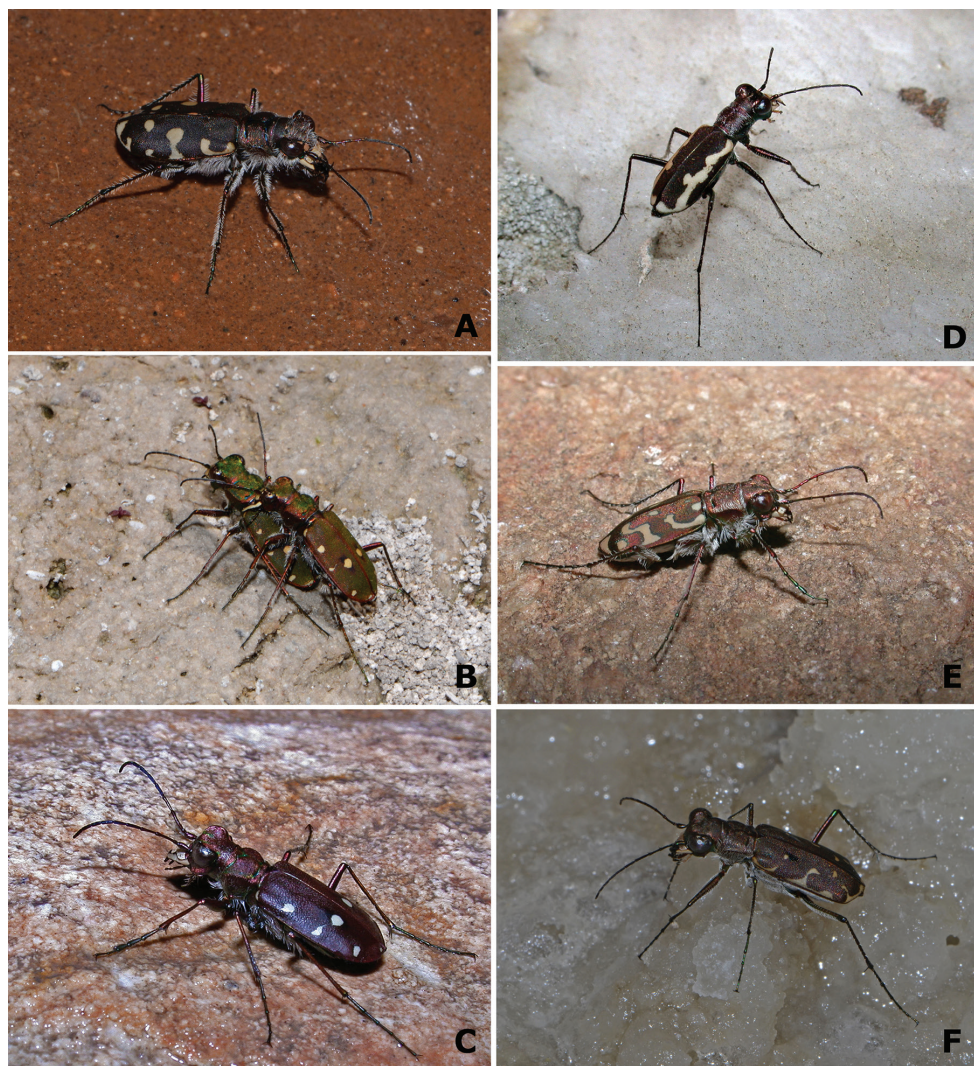
Figure 2. Representative specimens of tiger beetles from La Mancha wetlands **A** *Calomera littoralis littoralis* (La Dehesilla, Cuenca) **B** *Cicindela campestris campestris* (La Dehesilla, Cuenca) **C** *Cicindela maroccana* (La Sal, Toledo) **D** *Cylindera paludosa* (El Longar, Toledo) **E** *Lophyra flexuosa flexuosa* (El Pardo, Madrid) **F** *Myriochila melancholica melancholica* (Tirez, Toledo). Photographs by MG-P.

specimens of *C. littoralis*, moved from nearby peripheral vegetated areas towards central flat drying areas (Fig. 7). In general terms, the spatial positioning of specimens of *C. littoralis* accompanied the retreat of water, following the wet soil. After total drying, some specimens remained active in local humid patches at different positions within the lakes, until their total disappearance.

Most specimens were located on salt crust (40.5%), in open denuded areas (48.8%) (Table 2). No specimens were found on roads, or in shallow marshes or channels lo-

Table 3. Number of specimens of tiger beetles found at each sampled locality.

No.	Lake name / Species	*C. littoralis*	*C. maura*	*C. circumdata*	*C. dulcinea*	*C. campestris*	*C. maroccana*	*C. paludosa*	*L. flexuosa*	*M. melancholica*
1	Laguna de El Longar	133	8	3	39	9		8		6
2	Laguna del Altillo Chico	2		43	12			16		
3	Laguna del Altillo Grande			11	27			16		
4	Laguna de la Albardiosa									
5	Laguna Larga	49						1		
6	Laguna de Tirez	2		21	35	9	4			4
7	Laguna de Peña Hueca	15	1	4	30		2			
8	Laguna del Taray				11			1		
9	Laguna Grande	22								
10	Laguna Chica									
11	Laguna Grande	68	8		1					
12	Laguna de la Sal	2			6		2			
13	Laguna de los Carros				68					
14	Laguna de Pajares		10		40			3		
15	Laguna de las Yeguas	22	1	6	66	1		8		
16	Laguna Camino de Villafranca	48	13		2					11
17	Laguna de la Veguilla									
18	Laguna de Salicor	11		1		10				
19	Laguna de Retamar									
20	Laguna del Pueblo	51								14
21	Laguna de Navalafuente					1				
22	Laguna de Alcahozo	4		12	10					
23	Laguna de Manjavacas	25	20							
24	Laguna de Sánchez Gómez	4			95	1				
25	Laguna de la Dehesilla	52			30	12		2		

No.	Lake name / Species	C. littoralis	C. maura	C. circumdata	C. dulcinea	C. campestris	C. maroccana	C. paludosa	L. flexuosa	M. melancholica
26	Laguna de Alcahozo Chico	42				28		4	3	
27	Laguna del Taray Chico					3				
28	Laguna de El Hito				29					
29	Cerro San Cristóbal				23					
30	Arroyo de San Marcos		3			4		1		
31	La Laguna de Miguel Esteban	9	30							
32	Gigüela marshes							7		
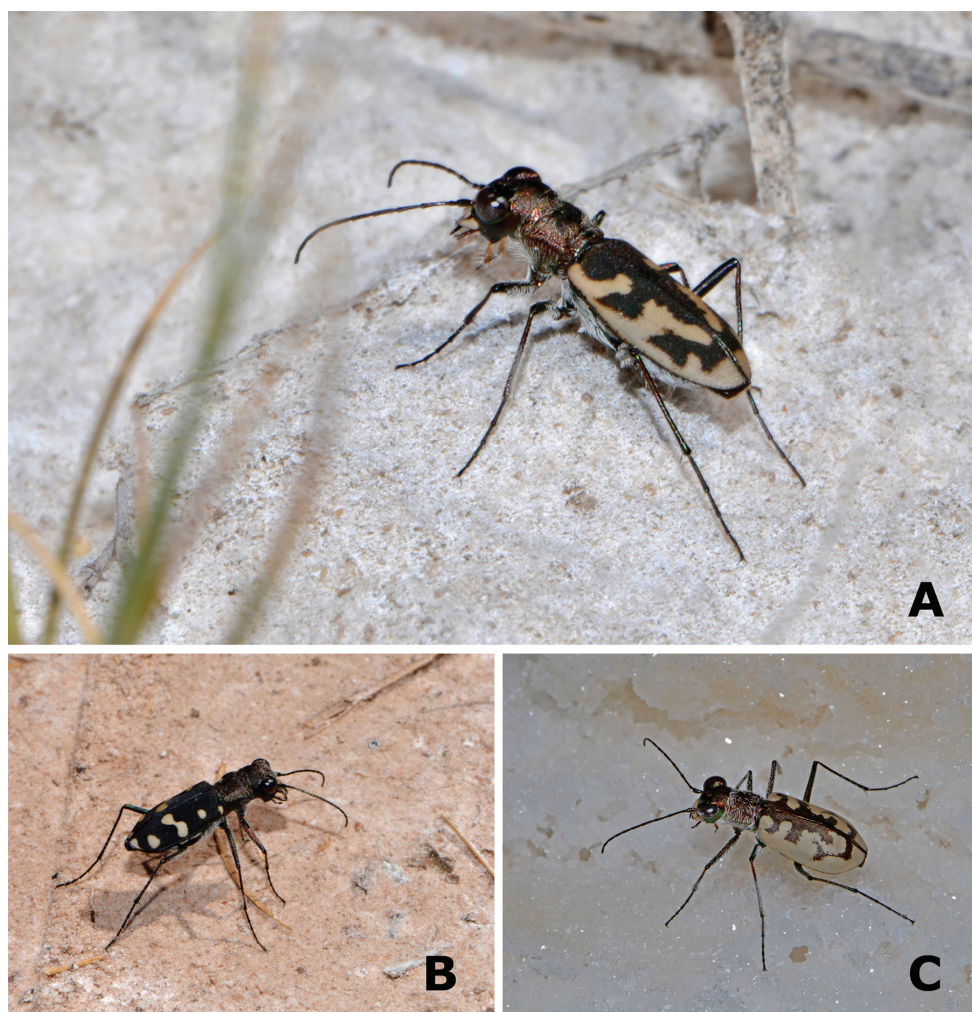
Figure 3. Representative specimens of *Cephalota* from La Mancha wetlands. **A** *Cephalota* (*Taenidia*) *dulcinea* (Sánchez Gómez, Cuenca) **B** *Cephalota* (*Cassolaia*) *maura* (Arroyo San Marcos, Ciudad Real) **C** *Cephalota* (*Taenidia*) *circumdata imperialis* (Tirez, Toledo). Photographs by MG-P.

cated far from large lakes, but they were present around all large water bodies, including nearby freshwater reservoir shores (Carrascosa del Campo, Cuenca).

Specimens of *C. littoralis* were present in the area all over the study period (Fig. 8), although active adults were almost never continuously present in the same location through the entire sampling, with marked local absences following the complete drying out of each lake. Specimens were observed active in February 2015 (Las Yeguas, Fig. 1: 15, P. Pichaco pers. com.). Our phenological observations are comparable with previous data for the region (Lencina et al. 1991), partially coincident with those from Tunisian populations (Jaskuła and Rewicz 2015), but quite different from published
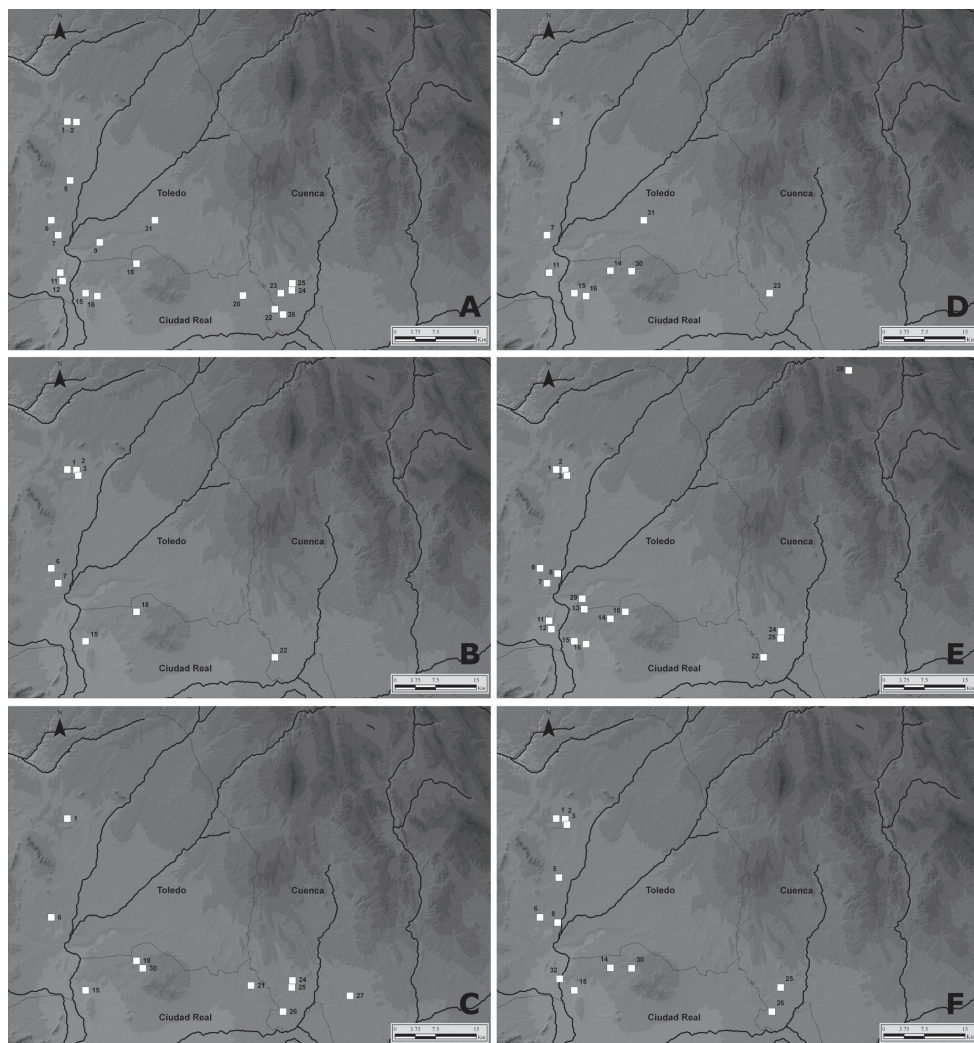
Figure 4. Maps showing the location of species of Cicindelinae found in the wetlands of La Mancha. Numbers correspond to Fig. 1. **A** *Calomera littoralis littoralis* **B** *Cephalota* (*Cassolaia*) *maura* **C** *Cephalota* (*Taenidia*) *circumdata imperialis* **D** *Cephalota* (*Taenidia*) *dulcinea* **E** *Cicindela campestris campestris* **F** *Cylindera paludosa*.

data from other geographic regions (Vives and Vives 1978b, Zanella 2010, both for *C. l. nemoralis* Olivier, 1790).

Abundance of *C. littoralis* is highly variable across the study area (Table 3; Suppl. material 1). Since specimens are generally present near water, some pools on the drying lakes might have extraordinary concentrations of specimens (more than 6 per square metre), while they are generally less concentrated when water is largely available, especially at lake shores. Aggregation of *C. littoralis* adult specimens has been correlated with habitat disturbance and prey availability (Mazzei et al. 2014); our data suggest
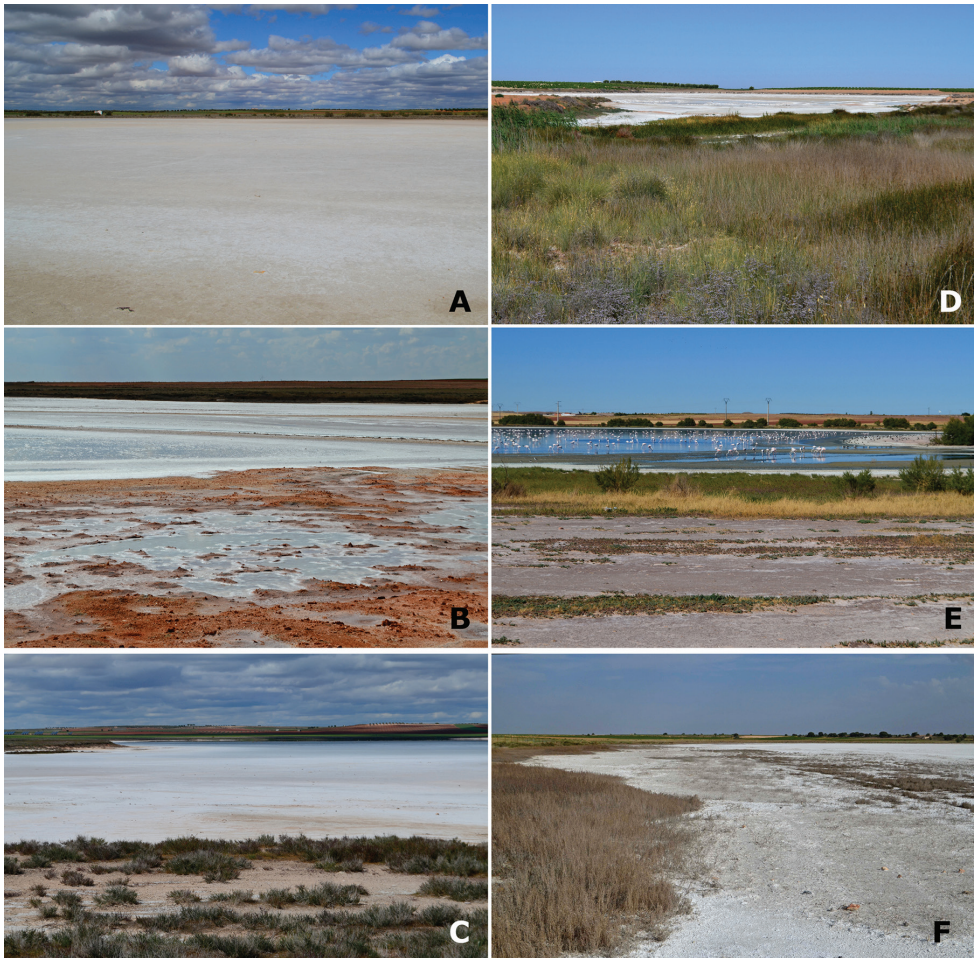
Figure 5. Representative habitats of Cicindelinae in La Mancha. **A** Laguna de la Sal (Toledo) in May. Salt flats occupied by *Calomera l. littoralis* **B** Laguna de Peña Hueca (Toledo) in April. Wet salt flats, with gypsum outcrops, occupied by *C. l. littoralis*; when totally dry, in summer, occupied by *Cephalota circumdata imperialis* **C** Laguna de Las Yeguas (Ciudad Real) in June. Salt flats, occupied by *C. l. littoralis* and *C. c. imperialis*; and halophytic prairies occupied by *Cephalota dulcinea* and *Cylindera paludosa* **D** La Laguna de Miguel Esteban (Toledo) in July. Salt flats, occupied by *C. l. littoralis*; and halophytic prairies with "albardinal" occupied by *Cephalota m. maura* **E** Laguna del Camino de Villafranca (Ciudad Real), with flamingos, in July. Sapropel shores occupied by *C. l. littoralis* **F** Laguna de Alcahozo (Ciudad Real) in June. Salt flats, occupied by *C. l. littoralis* and *C. c. imperialis*; and open areas near dry halophytic prairies occupied by *C. dulcinea* and also by *C. c. imperialis*. Photographs by N. Percino and MG-P.

that concentration seems to be a direct effect of availability of wet soils, rather than a response to food or disturbances.

Calomera littoralis was occasionally found in sympatry with *C. campestris* in late spring (La Dehesilla, Camino de Villafranca Channel, Alcahozo Chica), with *C. ma-*

Figure 6. Representative habitats of Cicindelinae in La Mancha. **A** Channel at Laguna de las Yeguas (Ciudad Real) in May. Wet areas occupied by *Calomera l. littoralis* and *Cicindela campestris*; when drier, occupied by *Cephalota m. maura* **B** Road side pools and halophytic vegetation in Laguna de Manjavacas (Cuenca) in April. *C. l. littoralis* was the only species observed **C** Wells and deep pools in Laguna de Manjavacas (Cuenca) in June. *Cephalota m. maura* is frequent in these structures **D** Laguna del Pueblo de Pedro Muñoz (Ciudad Real) in July. Mud areas amongst hydrophytic matts, occupied by *C. l. littoralis* and *Myriochila m. melancholica* **E** Temporary pools at Laguna del Longar (Toledo), in July. *Calomera l. littoralis*, *Myriochila m. melancholica* and *Cephalota maura* co-occcur at the sides **F** Laguna del Taray Chico (Cuenca) in May. Trails and non-halophytic prairies near the fresh water lake, occupied by *C. c. campestris*. Photographs by N. Percino and MG-P.

roccana (La Sal), and with *C. circumdata* (Altillo Chica, Tirez, Peña Hueca), but strict syntopy, sharing a unique patch, was only observed with *M. melancholica* at the end of summer (El Longar, Tirez, Camino Villafranca, and Pedro Muñoz), and occasionally with *C. maura* (El Longar, Camino de Villafranca Channel) and with *L. flexuosa* (Alcahozo Chica) in summer. When present, *C. littoralis* is generally the dominant species, ex-

Figure 7. Observations of *Calomera l. littoralis* at Laguna de Manjavacas (left) and La Dehesilla (upper right). Colours indicate the month in which observations were made (see legend). Note seasonal changes in specimen's location as the water front retreats or following changes in humidity of the soil. Blue squares correspond to the habitat shown in Fig. 6b.

cept in extreme drying-out situations at the lake basins or channels, when *C. circumdata* or *C. maura* might be temporally dominant.

    Calomera littoralis is a conspicuous and very active species, displaying its activity in plain sunlight. When approached, they run fast, and when disturbed they are able to start a quite long and sustained, relatively elevated, flight, sometimes changing direction in mid- flight. Flight is never directed to vegetated areas. At some localities (Manjavacas, Peńahueca, Alcahozo Chico) many specimens remain quiet among the sparse vegetation of the basin shore, running towards the central open areas when disturbed. One specimen was observed at night, hidden, but alert, in the crevices of a thick salt crust layer (Peña Hueca). Jaskuła et al. (2005) mentioned that some specimens climb small bushes for the night. Nocturnal activity may be extended but not common for this species, since attraction to artificial lights was reported for *C. l. nemoralis* in Northern Italy (Zanella 2010). Copulatory behaviour was observed during most of the study period. Predatory behaviour was observed towards ants and small flies. Some specimens captured their prey while running away from our chase.

Cephalota (Cassolaia) maura maura

Cephalota m. maura (Fig. 3b) was found in 12 localities (Fig. 4b). The 96 specimens observed were found in relatively humid situations, including salt marshes (37.5%) (Laguna de Miguel Esteban; Peña Hueca), inclined saline sides of channels (7.3%) (Salicor; San Cristóbal; El Longar), drying channels communicating saline lakes (15.6%) (Camino de Villafranca), around drying pools and pits (24%) (Manjavacas, El Longar), but also in quite dry areas, such as dirt roads covered by a flat salt crust (15.6%) (Pajares trail) (Fig. 5d and 6a,c,e). All the specimens were found in saline areas (96.9%), but they were also present in less saline drying pools (El Longar) (Fig. 6e). They avoided the basins of large salt lakes, with most of the populations located in small saline areas separated from the main lake. Many populations, particularly the denser ones, were found in man-modified habitats, such as ancient ditches with salty sides, or the inclined banks of old pits excavated not far from the lakes. Márquez Rodríguez (2014) also reported the presence of the species in degraded habitats in southern Spain. Most places where they were present in La Mancha kept water through all the sampling period. Additional specimens were found under similar conditions in man-modified salt marshes not included in the regional study (Belinchón in Cuenca and Yepes in Toledo).

Most specimens were located on salt patches in trails (20.8%), and on banks and other compact soils (39.6%). According to Márquez Rodríguez (2014), *C. maura* uses banks of streams for reproduction. Most specimens were found on bare ground (65.6%) and secondarily in halophytic prairies (27.1%) (Table 2). No specimens were found in the basins of large lakes.

Adult specimens of *C. maura* were observed through most of the study period (Fig. 8). However, specimens of *C. maura* were not continuously present in the same location during the entire period. Absences did not follow a clear pattern as in *C. littoralis*, since populations disappeared even if humidity was still present. Population densities of *C. maura* are variable, but generally low in comparison to *C. littoralis*. It is not a rare species, but its presence in non-conventional habitats requires further exploration. Besides, some populations occupy habitats of minimal extension (saline sides of isolated pits in halophytic prairies), making their localization difficult.

Cephalota m. maura was occasionally found in strict syntopy with *C. campestris* in late spring (El Longar, Salicor and San Cristóbal channels), with *C. dulcinea* and *C. paludosa* (road to Pajares) in early summer, with *C. littoralis* in mid and late summer (Laguna Grande de Villafranca, El Longar, Camino Villafranca channel), and with *M. melancholica* at the end of summer (El Longar).

Specimens of *C. m. maura* are very active, displaying most of its activity in plain sunlight, although some specimens were observed resting on the ground between the twigs of *Scirpus* at mid-day, near the water (La Laguna de Miguel Esteban, Laguna Grande de Villafranca). When approached, they run fast, displaying a short low flight when threatened, often directed towards vegetated areas, or even into the water surface, from where they reach the shore.

Figure 8. Frequency of observation of Cicindelinae during the sampling period (April to August), all sites pooled. From top to bottom: *Calomera l. littoralis*, *Cephalota m. maura*, *Cicindela c. campestris*, and *Myriochila m. melancholica*.

Cephalota (*Taenidia*) *circumdata imperialis*

Cephalota circumdata imperialis (Fig. 3c) was found in 8 localities (Fig. 4c). The 101 specimens observed were found inside the saline basin of small lakes, distributed both near the shore, or in central areas (Fig. 5b, c, f). No specimens were found outside the salt flats. Specimens were found mainly on salt crusts (81.2%) and in sun exposed granulated salty soils (82.2%) (Table 2).

Specimens of *C. circumdata* were present in the area during summer, from late June to August (Fig. 9), as previously reported (Vives and Vives 1978b, Lencina et
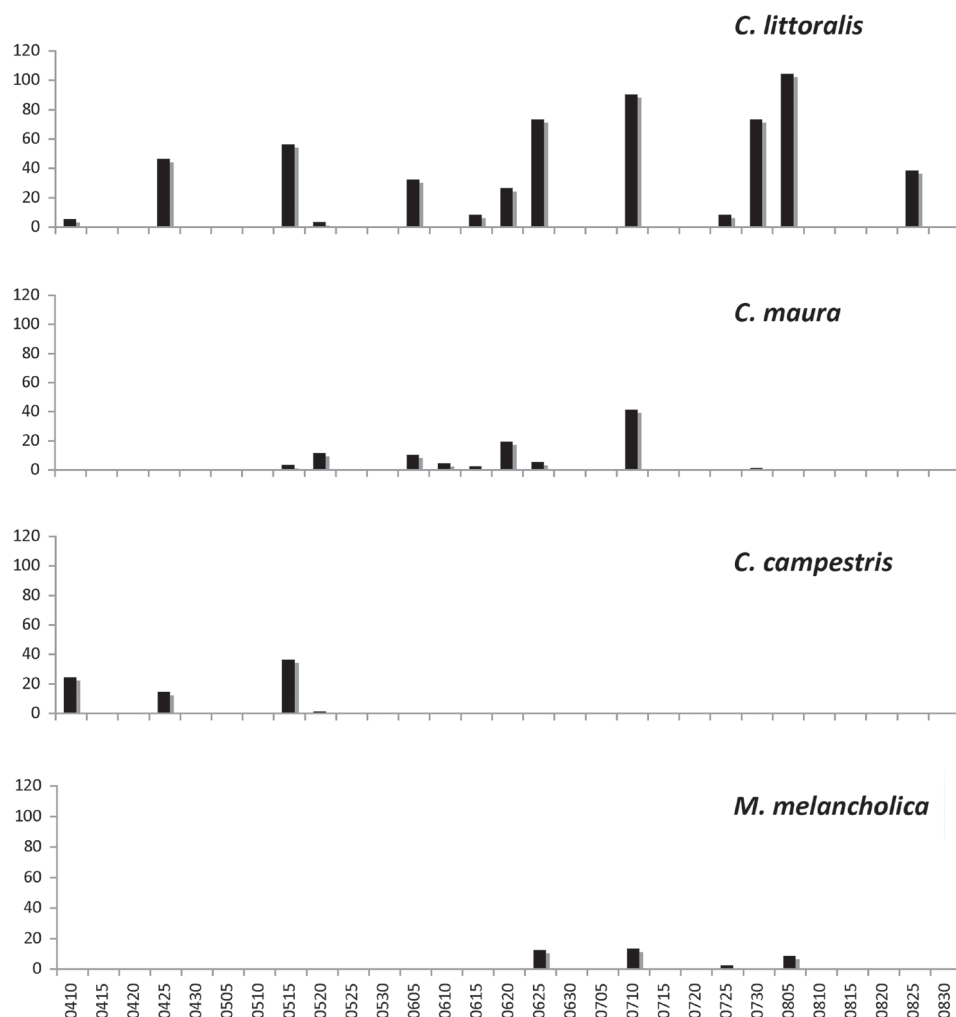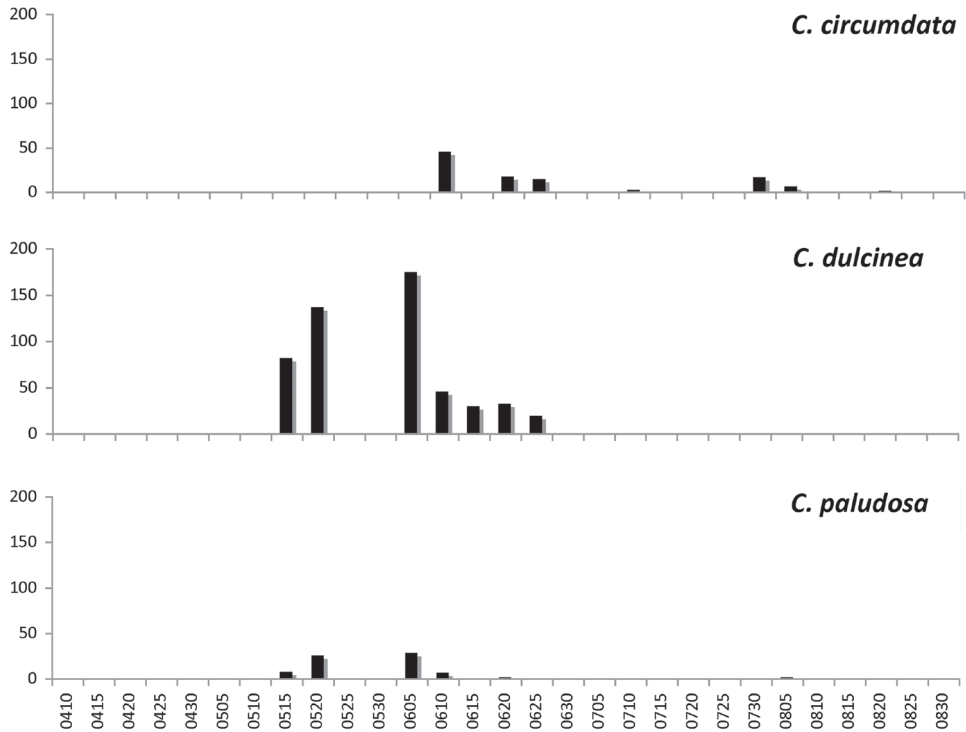
Figure 9. Frequency of observation of Cicindelinae during the sampling period (April to August), all sites pooled. From top to bottom: *Cephalota c. imperialis*, *Cephalota dulcinea*, and *Cylindera paludosa*.

al. 1991). Active adults were almost never continuously present in the same location throughout the entire period, with marked local absences following the complete drying of the mud beneath the salt layer.

Population densities of *C. circumdata* are variable, but normally low. Single specimens were often found alone, far from any other specimen, and when close, they were usually relatively isolated, and well distributed along favourable habitat. *C. circumdata* was occasionally found in sympatry with *C. littoralis* (Altillo Chica, Tirez, Peña Hueca, Salicor), and with *C. dulcinea* at the start of summer (El Longar, Las Yeguas, Alcahozo).

Most specimens were observed displaying their activity in plain sunlight, in agreement with previous observations of circadian activity of *C. circumdata leonschaeferi* in Italy (Eusebi et al. 1989). They run fast when approached, or fly relatively far away towards the centre of the lake. Flight was not directed to vegetated areas. However, most specimens started running from relatively hidden positions, either from below dry twigs, or from under isolated plant mats. The earliest observations of the species in late June, were made on Altillo Grande and Chico lakes. All the specimens observed at that time were located under a few random objects (stones, bricks, wood, plastic bags) present in the salt flats. The specimens were, either aggregated under the shade
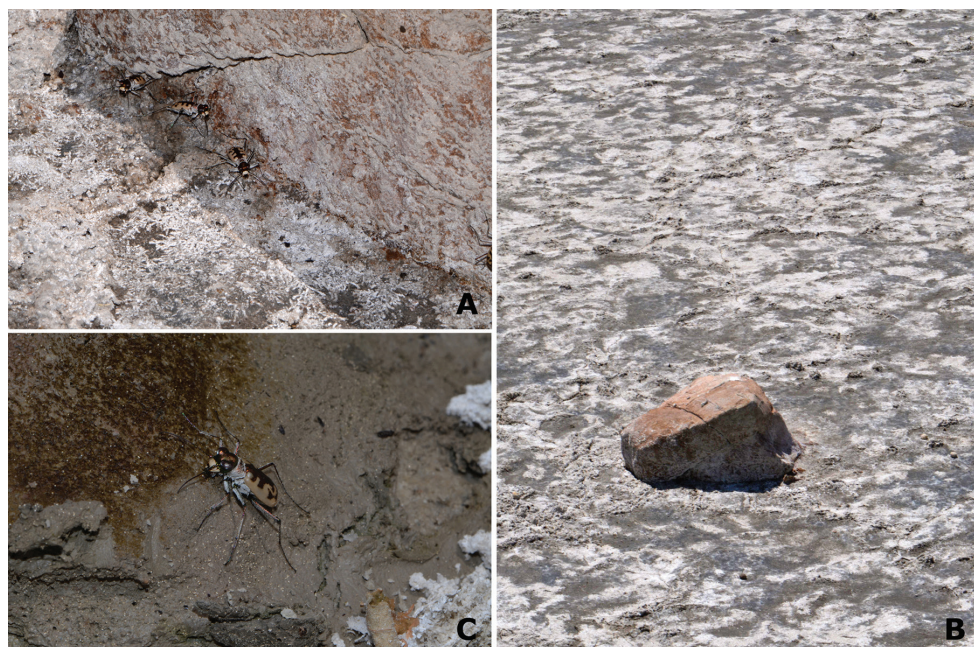
Figure 10. Behavioural observations on *Cephalota circumdata imperialis*. **A, B** A few specimens of *C. circumdata* located at the shade of a rock in the middle of the exposed salt flat **C** Individual photographed on the wet mud under the remains of a plastic bag, also in the salt flat. This behaviour is atypical for the species, which is generally active at full sunlight in mid summer in the area. The observations were made the 13th of June, at mid-day, the first date in which activity of the species was recorded. It is possibly that the specimens had just eclosed and wait in the shade while hardening their integument. Photographs by MG-P.

produced by the objects (Fig. 10a), or aggregated under the objects, in direct contact with the wet mud (Fig. 10b). Further searches during the species activity period failed to disclose any other specimens hidden or aggregated under objects. Lencina et al. (1991) indicated that specimens of *C. circumdata* aggregate under large stones at dusk.

Cephalota (*Taenidia*) *dulcinea*

Cephalota dulcinea (Fig. 3a) was found in 18 localities (Fig. 4d). The 524 specimens observed were found in relatively dry situations. Most of the specimens were found on small open ground areas, generally covered by a loose, granulated, sometimes dusty soil layer (53.2%), which gets a little more compact after typical summer storms. Other specimens were located on dirt roads and trails (45.8%), some of them covered by salt patches (29.2%). All of the specimens were found in saline areas, either lake shores and surrounding trails, or salt marshes (Figs 5c, f and 11a–f). Vegetation cover was never dense, but almost consistently present, except along trails; 55.2% of the specimens
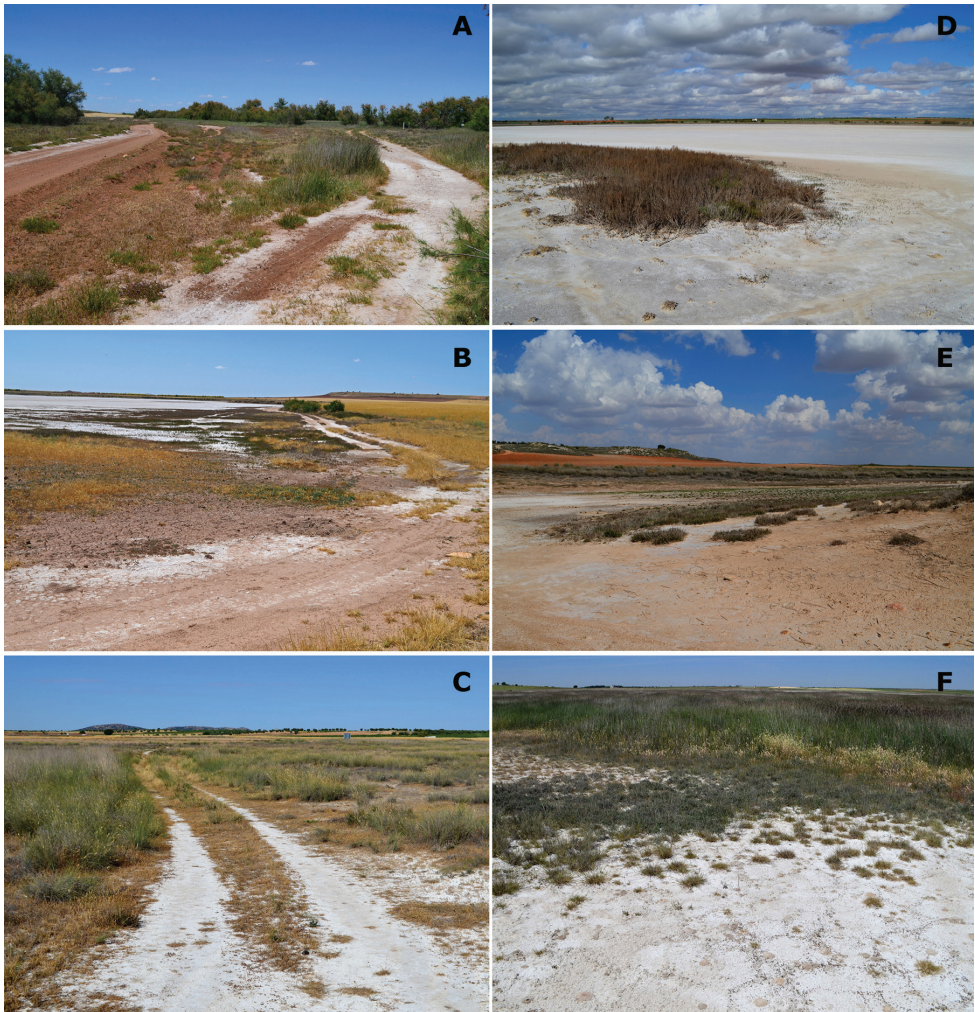
Figure 11. Characteristic habitat of the La Mancha endemic tiger-beetle *Cephalota* (*Taenidia*) *dulcinea*. **A** Trails at laguna del Taray (Toledo) **B** Trails and halophytic vegetation in Laguna de Pajares (Ciudad Real), where it was found in company of *C. m. maura* **C** Trails and "albardinal" in Laguna del Altillo Chica (Toledo), where it co–occurs with *C. paludosa* **D** Open areas amongst halophytic vegetation at laguna de La Sal (Toledo), *Calomera l. littoralis* and *Cicindela maroccana* occur in this habitat at the end of the spring **E** Halophytic vegetation at Laguna de Peña Hueca (Toledo), in this spot, *C. l. littoralis* and *C. maroccana* are present at the end of the spring **F** Halophytic vegetation and open areas with salty granulated soils at Laguna de Sánchez Gómez (Cuenca). Photographs MG-P.

were located around typical halophytic vegetation, 23.3% on open *albardinal*, and 21.6% in open denuded areas.

Active adults were observed from late spring to midsummer (Fig. 9), although they were almost never continuously present in the same location during the entire period, disappearing completely or changing locations within the same locality. The latest ob-

servations correspond to slow moving specimens, missing tarsal segments, likely corresponding to the actual end of the activity period.

Changes in population extension and local positioning were marked in some lakes, particularly at Los Carros, Sánchez Gómez and La Dehesilla, while in other areas the specimens occupy constantly at least a portion of the locality (Tirez, Peña Hueca, Las Yeguas). At Los Carros, La Dehesilla and Las Yeguas, large concentrations of specimens were present along dirt roads and trails in late spring (Fig. 11e, f). These trails were totally abandoned by midsummer, and the remaining active specimens were located in open small salt flats, in vegetated dry areas. Frequency of observation of *C. dulcinea* is variable, but in general densities are relatively high, especially in late spring and early summer, and the species is generally easy to detect (Table 3). We failed to find the species at Salicor, where one specimen was found in 2012.

Cephalota dulcinea was occasionally found in sympatry with *C. circumdata*, but strict syntopy, sharing a unique patch, was only observed with *C. paludosa* at the end of spring in open flats within vegetated areas, and occasionally with *C. maura* along trails. When present, *C. dulcinea* is generally the dominant species, and it was the only species found in some small lakes (Los Carros).

Cephalota dulcinea is an active species, displaying its activity in plain sunlight. When approached, they run fast and fly relatively short distances, sometimes changing direction in mid- flight. Flight can be directed to vegetated areas, but usually they fly farther along trails or saline flats. Occasionally, we found some specimens resting in the shade of objects, at midday (El Longar). Preying behaviour was observed towards ants, other small hymenopterans, and small dipterans. Some specimens seem to keep a hunting spot for a period, as suggested by the insect remains scattered around them (Fig. 12a). Copulatory behaviour was observed during most of the study period (Fig. 12b).

Cicindela (*Cicindela*) *campestris campestris*

Cicindela c. campestris (Fig. 2b) was found at 11 localities (Fig. 4e). All 78 specimens found were present in relatively humid situations, most of them in the granulated salty soil areas around small lakes (57.7%), some in drying channels communicating saline lakes (16.7%), or in small open areas (15.4%), and roads near prairies or cultivated fields (9.0%). Specimens were found around saline or freshwater areas, in densely vegetated areas (42.3%), or relatively open (26.9%) (Table 2) (Fig. 6a, f).

Specimens of *C. campestris* were present in the area in late spring (Fig. 8). According to previous studies, the species is also present during fall and in early spring in Castilla–La Mancha (Lencina et al. 1991, 2001), consequently in this study we missed a large part of its possible activity period. Three additional specimens were observed in October at Tirez, outside the monitoring period.

Population abundances of *C. campestris* are highly variable (Table 3). They can be locally abundant (La Dehesilla, Salicor, Alcahozo Chica), or represented by isolated specimens, sometimes found in the middle of other species populations (*C. maura*).
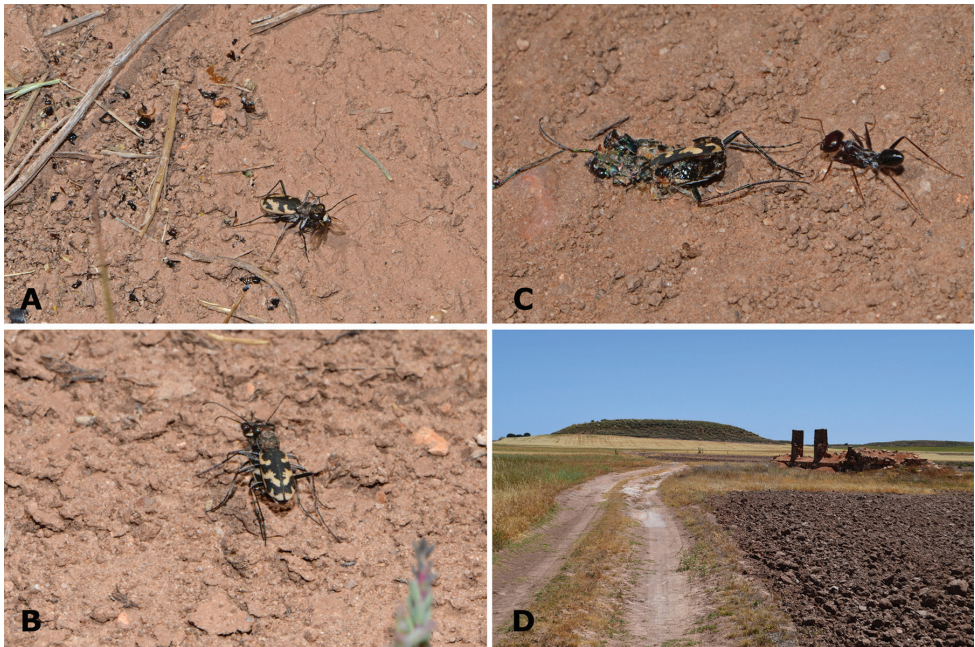
Figure 12. Behavioural observations on *Cephalota dulcinea* **A** Specimen of *C. dulcinea* located in a trail devouring a winged hymenopteran. Note the number of insect remains located behind the specimen (ants, other Hymenoptera and Coleoptera), suggesting that the spot is used as a hunting post **B** A couple of *C. dulcinea* involved in courship **C** A recently killed specimen of *C. dulcinea* in a trail, about to be seized by ants **D** Trail located near laguna de Pajares with a large colony of *C. dulcinea*, *C. m. maura* and scattered *C. paludosa*. We hypothesize that these trails, despite of being surrounded by unfavourable habitat, might be used as dispersal corridors between lakes, facilitating the maintenance of metapopulation systems. Photographs by MG-P.

Cicindela campestris was occasionally found in strict sympatry with *C. littoralis* in late spring (La Dehesilla, Camino de Villafranca Channel, Alcahozo Chica), with *C. maura* in drying channels (El Longar, Camino de Villafranca Channel), and with *L. flexuosa* (Alcahozo Chica) in early summer. It is the only species found in some largely vegetated less saline or non-saline areas (Taray Chico, Navalafuente).

Cicindela campestris is an active but not conspicuous species in the area, displaying its activity in small patches and trails amongst vegetation. When approached, they run along the trails or into vegetation, and when flying, they show a tendency to land on vegetation. Copulatory behaviour was observed during most of the study period.

Cicindela (*Cicindela*) *maroccana*

Cicindela maroccana (Fig. 2c) was only found in three localities (Fig. 1: 6, 7, 12). Only 8 specimens were found, all of them located in open or poorly vegetated areas of the saline shore of small lakes and salty marshes, on granulated soils (Fig. 11d, e).

Specimens of *C. maroccana* were present in the area during late spring. According to previous studies, the species is also present in early spring (Lencina et al. 1991, Ortuño and Toribio 1996). These reports are now confirmed for the area since the species was observed active in February 2015 (Las Yeguas, Fig. 1: 15, P. Pichaco pers. com.). Frequency of observation of *C. maroccana* is low in the study area, but the species requires an earlier sampling (Table 3). *Cicindela maroccana* was found in sympatry with *C. campestris* (Tirez), and with *C. littoralis* (La Sal, Peña Hueca). The specimens found displayed their activity in plain sunlight, flying readily when disturbed.

Cylindera (*Cylindera*) *paludosa*

Cylindera paludosa (Fig. 2d) was found in 11 localities (Fig. 4f). 67 specimens were found in areas with a relatively dense vegetation cover, most of them in saline flats in the *albardinal* (*Lygeum spartum* prairies) (38.8%), in open halophytic prairies (41.8%), or in dense halophytic prairies (11.9%) at the shore of small lakes (Table 2) (Figs 5c and 12c).

Specimens of *C. paludosa* were present from late spring to midsummer (Fig. 9), although one isolated specimen was found at the end of August. Population densities of *C. paludosa* are variable. The species may be locally abundant in salt flats around or inside the *albardinal* (Table 3).

Cylindera paludosa was occasionally found in sympatry with *C. dulcinea*, around salt flats and vegetated saline marshes (Altillo Chica, El Longar, Las Yeguas, La Dehesilla), with *C. campestris* in late spring (Salicor), and with *C. maura* (El Longar channel, Pajares trail). *Cylindera paludosa* is generally the dominant species in salt marshes, and often it is the only species present in marshes of limited extension. *Cylindera paludosa* is an inconspicuous but very active species, displaying its activity in plain sunlight. When approached, they fly readily towards the nearby vegetated areas, rendering its observation difficult.

Lophyra (*Lophyra*) *flexuosa flexuosa*

Lophyra f. flexuosa (Fig. 2e) was only found at one locality (Alcahozo Chico, Fig. 1: 26) (n=3) during the study period, but it was observed in another locality (Salicor, Fig. 1: 18) (n=5) during the preliminary samplings in 2012. The specimens were found in the saline shore of a small lake (2012), or in a drying- out salt marsh (2014). We failed to find the species at Salicor in 2014, despite successive searches.

We found the specimens in late spring (mid May 2014, and early June 2012). Despite successive samplings, the observations were made in a single occasion. These observations are puzzling, because, according to the literature and personal observations, the species has a relatively long period of activity, from April to July, in other areas of Castilla – La Mancha and nearby Madrid (Lencina et al. 1991, Ortuño and

Toribio 1996, unpub. observ.). The population of *L. flexuosa* at Alcahozo Chico was found in strict sintopy with *C. littoralis*, and very close to *C. campestris* and *C. paludosa*. At Salicor it was the only species present on the shore. *Lophyra flexuosa* is a very active species, displaying its activity in plain sunlight, and flying readily when disturbed.

Myriochila (*Myriochila*) *melancholica melancholica*

35 specimens of *Myriochila m. melancholica* (Fig. 2f) were found in 4 localities (Fig. 1: 1, 6, 16, 20). All specimens were found in relatively humid situations, all of them on the shore of small lakes or around drying pools. Some of the specimens were found in saline areas (Laguna de Tirez), but most of them were present in drying pools, with much less salt concentration, located near a salt lake (El Longar), or lakes resulting from regulated flooding (south end of Laguna del Camino de Villafranca, and Laguna del Pueblo de Pedro Muńoz) (Fig. 6d, e). Most of the specimens were observed on mud amongst open hydrophytic vegetation (71.4%), or in denuded shores (28.6%) (Table 2).

The period of activity of *M. melancholica* was restricted to the end of the study period, from late June to early August (Fig. 8). The population of Laguna del Pueblo de Pedro Muńoz moved along the shoreline, following the retreating water front, as the small lake was drying out once the forced flooding was stopped. Populations disappeared following the complete drying- out of the pools (Tirez, El Longar).

Frequency of observation of *M. melancholica* in the area is low, with only a few specimens found per visit. *Myriochila melancholica* was always and only found in syntopy with *Calomera littoralis*. In these cases *C. littoralis* was the dominant species. *Myriochila melancholica* displayed its activity in plain sunlight, but it is also attracted to artificial lights (Ortuńo and Toribio 2002). When approached, they usually run fast towards wetter areas, but generally stop at a relatively short distance from the starting point. They only fly when distressed.

Discussion

Tiger beetle assemblage at La Mancha wetlands

The tiger beetle taxocenosis in La Mancha wetlands is composed of 9 species (Table 3; Figs 2–3). This is a high number of cicindelids for any small geographic area in Europe. In fact, Jaskuła (2011) recorded a maximum of 8 species per square of 1º latitude and longitude in the Balkan Peninsula. This region, together with the Iberian Peninsula, displays the largest diversity of tiger beetles in Europe. The studied region in La Mancha fits entirely inside a single 1º latitude/longitude square (longitude west 2º40'at Laguna del Hito, to 3º22' at Laguna de Tirez; and latitude north 39º21' at Laguna de Alcahozo Chico, to 39º53' at Laguna del Hito) (Fig. 1). All the entire tiger beetle com-

munity is actually represented in a surface of less than 2 km². According to these data, La Mancha is likely the European geographic area with the most complex assemblage of tiger beetles (see Cassola 1970, 1972, Aliquò 1992, Franzen 2005, Jaskuła et al. 2005, Jaskuła 2007, 2011, Kirichenko et al. 2001).

The cicindelid assemblage at La Mancha wetlands could be even more complex, since two additional species of tiger beetles, not found in the study area, inhabit the region of Castilla – La Mancha. *Cicindela lagunensis* has been located in sandy soil areas, relatively close to La Mancha wetlands (Ortuño and Toribio 1996, Matalin 1998, Cardoso and Vogler 2005). We have not located the species in the study area, but we cannot exclude the possibility that it could be present along the banks of the rivers (Riansares and Gigüela) that cross the region. *Grammognatha euphratica euphratica* (Dejean, 1822), has been recorded at the extreme south-eastern area of Castilla – La Mancha, about 150 km south east of the study area (Ortiz et al. 1988, Andújar et al. 2002). Although *G. euphratica* inhabits salt marshes, its ecological characteristics seem to differ from those available at La Mancha wetlands. However, in current scenarios of climate change, the possibility of colonization of La Mancha salt marshes cannot be excluded.

Such a concentration of cicindelid beetles, 9 species in an area of less than 1º latitude/longitude square, is likely the result of a combination of both historical (paleogeographic) and ecological factors. The presence of one endemic species, *C. dulcinea*, suggests that, on the one hand, the region has maintained a relative level of historical isolation with respect to other Iberian areas. On the other hand, some areas acting as sanctuaries of diversity (*sensu* Recuero and García-París 2011), might have persisted in the high plateau of La Mancha, despite the strong changes suffered during the Pleistocene glacial periods (Zagwijn 1996). It seems possible that these sanctuaries were precisely the saline lakes, which currently harbour a relative high level of endemic and relict species (e.g. carabid beetles as *Broscus uhagoni* Bolívar, 1912; bradyporine orthopterans as *Pycnogaster graellsii* Bolívar, 1873) (Gómez et al. 1991, Toribio and Pichaco 2014).

The diversity of environments available for tiger beetles at La Mancha wetlands, also contributes to the maintenance of such a high number of cicindelids, since most of the species seem to occupy particular ecological niches within the area (Fig. 14, Table 2). Spatial segregation of tiger beetles based on habitat structure has been reported in previous studies (Willis 1967, Cassola 1970, Ganeshaiah and Belavadi 1986, Ortíz et al. 1988, Schultz 1989, Lencina et al. 1991, Hoback et al. 2000, Satoh and Hori 2005, Jaskuła 2011, 2015), suggesting that it is a common situation for the group.

Habitat partitioning among species in La Mancha is evident at temporal and spatial scales. On the temporal scale, peaks of activity differ markedly among species (Figs 8–9). Only one species, *C. littoralis*, was present throughout the entire period of study. A set of two species, *C. campestris* and *C. maroccana*, were exclusively present during spring, and fall in the case of *C. campestris* (although, as mentioned above, their period of activity likely started before our earliest sampling date), sharing a minimum overlap, with the second set. The second set of taxa, composed by *C. maura*, *C. dulcinea*, *C. paludosa*, and *L. flexuosa*, started their activity in late spring, overlapping occasionally with isolated

late specimens of *C. campestris*. *Cephalota dulcinea*, *C. paludosa*, and possibly *L. flexuosa*, ended their activity quite abruptly, during mid-late June, with isolated individuals of *C. paludosa* observed at later dates. *Cephalota maura* activity overlapped with a third set of species, composed of *C. circumdata* and *M. melancholica*. This third group of taxa started their adult activity at mid and late June respectively, and were observed until late and early August (Figs 8–9). These observations are similar to those reported by Lencina et al. (1991) in a saline area in southeastern Castilla – La Mancha (Salinas de Pinilla).

Cephalota dulcinea, *C. circumdata*, *M. melancholica*, and possibly *C. maroccana*, concentrate adult activity in a short period of time, in a single adult emergence period (Figs 8–9). *Cephalota maura*, *C. campestris*, and possibly *C. paludosa*, seem to present an extended period of emergence of adults, in two distinct periods (*C. campestris* and possibly *C. paludosa*), or in a temporally, but not geographic, continued single period (*C. maura*). Emergence in *C. littoralis* seems to follow the same pattern as in *C. maura*: temporally defined emergence at single localities, but temporally continuous in the whole geographic area (Figs 8–9).

Overlap in habitat use is maximal for *M. melancholica* and *C. littoralis* when *M. melancholica* is present, as previously reported by Lencina et al. (1991) in salt lakes from Albacete (Laguna de Pinilla) and Jaskuła et al. (2012) in Albania. *Myriochila melancholica* coexists in time and space with *C. littoralis*, and share the wet shores of drying pools and small lakes. We did not observe any kind of interaction between these two species, even though specimens of each species were found less than 30 cm apart from each other. Marked differences in size between *M. melancholica* and *C. littoralis* may facilitate resource partitioning between them (Schoener 1974).

All other species show little overlap in habitat use (see Fig. 14 as a particular example where 7 species co-occur), as is typical for cicindelids all over the world (Freitag 1979, Knisley and Pearson 1984, Ganeshaiah and Belavadi 1986, Acciavatti and Pearson 1989). *Cephalota dulcinea* and *C. paludosa*, are often present in the salt flats and trails within the *albardinal* (*L. spartum* prairies), but *C. dulcinea* is usually found in the less vegetated patches, where open spaces dominate, while *C. paludosa* is usually located in narrower and smaller clearings, in denser patches (Table 2). *Cicindela campestris* and *C. littoralis* also occasionally share the humid margins of lakes, but in these situations *C. littoralis* occupies the open areas while *C. campestris* is active around the vegetated patches. Frequency of syntopy is very low for all other species, usually represented by single observations, or by very low number of specimens.

Populations of some species remained on the same spot throughout their whole period of activity, from the earliest observations to the latest. Amongst those, *C. circumdata*, *C. paludosa* and *C. maura* are the most representative, but also some populations of *C. dulcinea* remained at the same location during their whole active period. Populations of *C. littoralis* and some of *C. dulcinea*, moved to different patches within the same location throughout their period of activity. Populations of *C. littoralis* shifted their location following the retreat of water during the continuous drying-out of lakes and pools (Fig. 7). Location- shifting is a continuous process, in which the already active adult specimens and the newly emerged ones from successive cohorts are likely

involved. Since we did not mark each individual, we cannot ascertain the contribution of each population group to the actual shifting. When the water surface of the lake dries out, there is a new shift in location; the beetles move to wetter patches in the salt basin, or disappear completely. We have not been able to determine if disappearances are a consequence of specimens' deaths or migration to nearby lakes.

Apparent location shifts of *C. dulcinea* were observed in dry lakes surrounded by trails and dirt roads, particularly at Las Yeguas and Los Carros, but also in other lakes on a smaller scale. Soon after the start of their activity, we observed large concentrations of specimens of *C. dulcinea* along trails and roads around salt lakes, and less numerous at the salt basin shore in the typical halophytic prairies used by the species throughout the area. The number of specimens located on roads and trails declined as the season advanced, and completely disappeared well before the end of the general activity period of the species (Fig. 9). At the time of their disappearance, a reduced, but significant presence of specimens was located at the halophytic prairies, where they remained until the end of the activity period. Trails and roads are contiguous to prairies, so movements between them are likely to occur, but concentration of specimens at the prairies did not suffer dramatic changes, so it is possible that specimens occupying the roads die or move away, rather than move on to the prairies. A concentration of *C. dulcinea* was found on a dirt road located between Laguna de Pajares, and Laguna de Salicor, about 600 m (air distance) from Pajares (Fig. 12d). This population might represent a migratory movement from Pajares, since we were only able to detect a couple of specimens of *C. dulcinea* at the shore of the lake (Fig. 11b), and the road was totally flanked by unfavourable cultivated or ploughed fields (Fig. 12d).

These observations, coupled with the absence of *C. dulcinea* at Laguna de Salicor, where it was found in 2012, suggest that, as a working hypothesis, the whole *C. dulcinea* deme might function as a metapopulational system, with high probabilities of local extinction matched by frequent recolonization events (Diogo et al. 1999, Omland 2002, Cornelisse et al. 2013).

Local threats for tiger beetle conservation at La Mancha wetlands

Populations of tiger beetles in La Mancha seems to present a relatively healthy status. Population densities are locally high for *C. l. littoralis*, *C. maura*, *C. dulcinea*, *C. paludosa*, and *C. c. campestris*, a set of species with a relatively extensive area of occupation at La Mancha wetlands. *Cephalota circumdata imperialis* and *M. m. melancholica*, present less dense populations, and also a more restricted local distribution, but they are nonetheless well distributed across the area. *Lophyra f. flexuosa* and *C. maroccana*, two widespread species in Central Spain (Ortuño and Toribio 1996), are scarce at La Mancha wetlands and their populations are represented by a low number of specimens. Two species, *C. circumdata* and *C. dulcinea*, are protected under Castilla – La Mancha legislation (620–CMA 20 Decreto 33/1998 Catálogo Regional de Especies Amenazadas de Castilla-La Mancha).

Most of the studied lakes and marshes are legally protected (http://ec.europa.eu/environment/nature/natura2000/db_gis/). Conservation efforts over the last decade have been directed at restoring the original ecological system, and also at eliminating the remains of human activity, particularly the removal and restoration of drainage channels, removal of urban waste, closure and restoration of village dumpsters, control of artificial flooding, and, more recently, restoration of native vegetation (the *albardinal* and the *Limonium* prairies), restoration of adjacent agricultural lands around the lakes, removal of salt- related semi-industrial activities, and road tracks removal and restoration (Fig. 13).

We have detected a series of threats that may affect the whole cicindelid (and other salt- associated animals) community. These threats are mostly derived from current agricultural practices around marshes, non-adequate use of trails and dirt roads, and insufficient regulation of water quality. Other threats might be derived from the restoration process itself, particularly the removal of abandoned semi-industrial infrastructures and trails.

Ploughing and cultivation of cereal, legumes, garlic or grapes, in fields adjacent to the lakes (Fig. 13f), and sheep grazing on the vegetated areas of lakes or around them (Fig. 13e) are still usual practices over most of the region. Consequences of these activities include the incorporation into the lake basin of large amounts of agricultural soil, drained from ploughed fields during the rainy periods, concentration of fertilizers or pesticides in the lake, washed from adjacent treated fields, direct changes in soil structure as sheep step on the salt shores (Fig. 13e), and nitrification and vegetation changes by sheep activity (Kiehl et al. 2001, Knisley 2011). Trails and dirt roads are used traditionally by agricultural vehicles (Fig. 13b). These slow moving vehicles pose little threat to tiger beetles, other than compacting a portion of the already compacted road. However, in recent years, some trails and roads have been heavily used by fast quads, and 4x4 vehicles, which no doubt have a direct impact on tiger beetle populations, either by direct killing (Fig. 12c) (Spomer and Higley 1993, Knisley 2011, Cornelisse 2013), or by habitat modification, including a clear increment in dust and erosion. Some lakes located near villages are receiving treated fresh water during most of winter and spring (García-Ferrer et al. 2003, Pérez and García 2004). This practice involves minor fluctuations in water levels, which seem to be favourable for species such as *C. l. littoralis* and *M. m. melancholica*, which maintain healthy populations in these areas (Pedro Muñoz, pools at Camino de Villafranca southeast end) (Fig. 6d). On the other hand, the addition of freshwater reduces salinity, and might have a negative effect on other species, which in fact are not present in such systems.

Restoration practices might have a significant impact on the larger saline lakes that were subjected in the past to semi-industrial activities, predominantly salt extraction. Salt extraction industries modified the salt flats by creating evaporation pools, scattered deep pits, and some channels (Figs 6a, c and 13a, c, d). The removal of these manmade structures may represent a direct impact on some species that use the sides of the pools and the inclines of the pit sides and channels. These are places highly favoured by *C. maura*, and sometimes used by *C. campestris*, *C. littoralis* that *M. melancholica*,
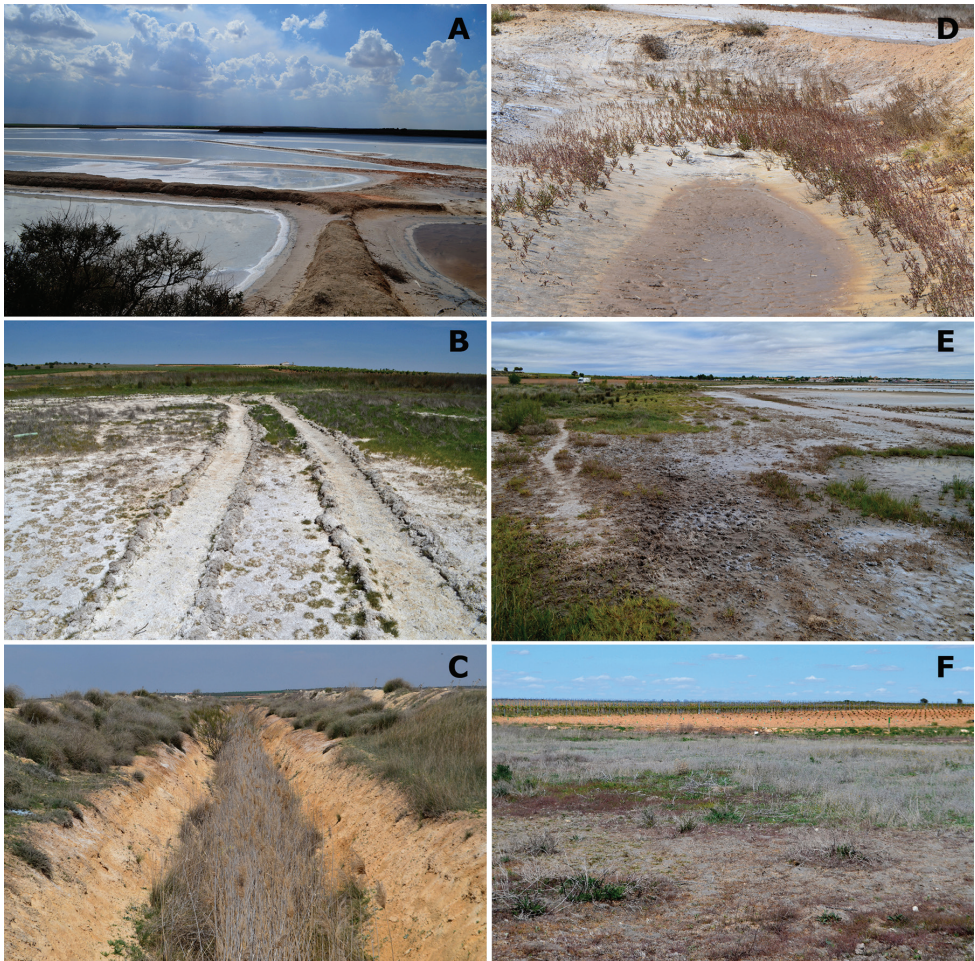
Figure 13. Conservation problems for tiger beetles in La Mancha, and remains of old semi-industrial activities which provide additional structural heterogenity **A** Saline pools at Laguna de Peña Hueca (Toledo) occupied at different seasons by *C. l. littoralis*, *C. dulcinea*, *C. circumdata imperialis* and *C. m. maura* **B** Trails on salty soils at Laguna de la Dehesilla (Cuenca), where *C. l. littoralis*, *C. c. campestris* and *C. dulcinea* are present along different periods of the year **C** Old channel in Laguna del Longar (Toledo), where *C. m. maura*, *C. campestris* and *C. paludosa* co-occur **D** Saline pool at Laguna de Tirez (Toledo), where *C. l. littoralis* and *M. m. melancholica* co-occur, while populations of *C. dulcinea* and *C. circumdata imperialis* are established not far from this point **E** Effect of sheep along the shores of the saline Laguna Grande de Quero (Toledo), in this spot *C. l. littoralis* was present **F** Vineyards at the edge of Laguna de Alcahozo Chico (Cuenca), note the diverse halophytic vegetation and nearby open areas where *C. l. littoralis*, *C. c. campestris*, *C. paludosa*, and *L. f. flexuosa* live in close proximity. Photographs by N. Percino and MG-P.

and seem to add a component of structural diversity to the system. Mazzei et al. (2014) indicate that population densities of *C. littoralis* in disturbed habitats are lower than in non-disturbed ones, although for these authors disturbance is caused directly by hu-

Figure 14. Location of seven species of Cicindelinae at Laguna del Longar (Lillo, Toledo). Colour dots indicate the spatial position of each specimen (often, more than one specimen are presented under a single dot) (see legend). Note the spatial segregation of most species. Dots in lower right area, where *M. m. melancholica* was found, correspond to the habitat shown in Fig. 6e. Dots in the upper left area, where *C. c. campestris* was present, correspond to the habitat shown in Fig. 13c.

man presence (beaches in summertime), rather than a consequence of ancient habitat modifications.

Closure and restoration of trails and roads around the lakes might pose a threat for species such as *C. dulcinea*, that consistently use them for hunting and mating. Further studies are required to evaluate the role of these man-made structures for the species, and particularly whether they have any impact on larval development.

As general recommendations, we propose that the current practices of governmental buying of agricultural land around the lakes are absolutely necessary, both to avoid potentially dangerous agricultural practices and to create a buffer zone from other activities such as sheep grazing. Trails and minor roads must be closed for high risk sport practices (quads and 4x4 vehicles), while they can be used for other less aggressive activities. We recommend the maintenance of trails around lakes until their effect on populations of *C. dulcinea* is properly evaluated. Old semi-industrial structures, evaporation pools, pits and channels, may be retained to increase structural diversity of the lakes. Finally, and especially considering the possible metapopulation system of *C. dulcinea*, we consider necessary the implementation of legal protection for all salt

marshes and saline lakes, of the occupation area of *C. dulcinea*, including all minor entities that could act as migrational steps during recolonization events.

Our data support the status of protected species for *C. circumdata*, a definite indicator of well-preserved salt lake flats, with relatively low population densities, and *C. dulcinea*, an endemism to the salt marshes and lakes of La Mancha. A species appearing as a good indicator of protected halophytic prairies (*Limonium*, *Lygeum*) is *C. paludosa*, but we do not consider necessary additional specific protection for this species.

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Supplementary material 1

Appendix

Authors: Paula C. Rodríguez-Flores, Jorge Gutiérrez-Rodríguez, Ernesto F. Aguirre-Ruiz, Mario García-París

Data type: Occurrence

Explanation note: Field observations.

RESEARCH ARTICLE

ZooKeys
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Pempheris gasparinii, a new species of sweeper fish from Trindade Island, southwestern Atlantic (Teleostei, Pempheridae)

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Abstract

Pempheris gasparinii **sp. n.** is described from five specimens, 59.1–68.0 mm in standard length. It is only known to occur in the shallow reefs of Trindade Island, 1200 km east of the Brazilian coast, in the southwestern Atlantic. *Pempheris gasparinii* is the third recognized species of *Pempheris* in the Atlantic Ocean. This new species is morphologically similar to its close relative, *P. poeyi*, differing by the number of lateral-line scales (51–54 in *P. gasparinii* vs. 47–49 in *P. poeyi*), scales below lateral line (10–11 vs. 9), circumpeduncular scales (11–12 vs. 13), head and caudal peduncle lengths (2.7–3.3 vs 3.5–4.0 in head length). Moreover, *Pempheris gasparinii* shows a 4% genetic divergence from *P. poeyi* at the cytochrome oxidase I locus (COI), consistent with a lineage split at the beginning of the Pleistocene. This new species represents the 12th endemic fish species from Trindade Island.

Keywords

Introduction

The genus *Pempheris* Cuvier contains 69 valid species (Randall and Victor 2015), with two species known from the Atlantic Ocean: *Pempheris poeyi* Bean 1885 and *P. schomburgkii* Müller & Troschel, 1848. *Pempheris* species generally display similar body shapes, however, these two species strongly differ by coloration (*P. poeyi* is silvery while *P. schomburgkii* is yellowish) and number of anal-fin rays (22–24 in *P. poeyi* vs. 31–34 in *P. schomburgkii*). *Pempheris schomburgkii* is widely distributed in the western Atlantic, from Bermuda (32°N) to Santa Catarina, Brazil (29°S). In contrast, a disjunct distribution has been previously assigned to *Pempheris poeyi*, with populations occurring in the Greater Caribbean and in Trindade Island, Brazil (Pinheiro et al. 2015), at least 5,000 km apart.

Pempheris poeyi was described from Cuba in 1885 (Bean 1885), and since then few specimens have been collected from localities such as the Bahamas, Grenada, Trinidad and Tobago, and Venezuela. Specimens identified as *P. poeyi* were found at Trindade Island in the early 20th century (Miranda Ribeiro 1919), and rediscovered 90 years later by Pinheiro et al. (2009). There is overlap in counts of dorsal- and anal-fin rays between the Greater Caribbean and Trindade populations, a common observation among *Pempheris* species (Mooi and Randall 2014), which likely explains why the new Trindade species described herein was misidentified in two recent checklists (Simon et al. 2013, Pinheiro et al. 2015, Randall and Victor 2015). Recent publications revealed that there is an abundance of undescribed cryptic species in *Pempheris* (Koeda et al. 2013, Mooi and Randall 2014, Randall and Bineesh 2014, Randall and Victor 2014, Randall et al. 2014). In this study, the use of genetic tools allowed us to revisit the Trindade specimens and update their taxonomic status. Thus, herein we describe a third Atlantic Ocean *Pempheris* species, so far only known from Trindade Island, at the end of the Vitória-Trindade Chain, 1200 km off the Brazilian coast.

Methods

All specimens were collected using hand nets. Counts were performed using a microscope, and morphological characters were measured to the nearest 0.1 mm following Mooi and Randall (2014). Morphometric and meristic data for the type series are presented in Table 1. In the description, meristic values for the holotype are provided first, followed by the range of counts of paratypes in parentheses. Type specimens were deposited in the fish collections of the Universidade Federal do Espírito Santo (CIUFES), California Academy of Sciences (CAS), and Universidade Estadual de Campinas (ZUEC).

Mitochondrial Cytochrome c oxidase subunit I (COI) DNA was analyzed for the new species. DNA extraction and PCR amplification of the COI were performed following Weigt et al. (2012). DNA sequences were then compared to those of the other two Atlantic *Pempheris* (*P. poeyi* and *P. schomburgkii*) downloaded from GenBank. Phy-

Table 1. Counts and measurements of selected type specimens of *Pempheris gasparinii* sp. n. and comparative specimens of *P. poeyi* and *P. schomburgkii* (data range) as percentages of standard length. "Circumped" = circumcaudal peduncular.

	Holotype	Paratypes				Pempheris poeyi	Pempheris schomburgkii
	CIUFES 3127	CAS 238409	CAS 238410	CIUFES 2432	ZUEC PIS 11233		
LL Scales	54LR	51L,52R	53L,54R	53L,52R	52LR	47–49	56–59
Scales above LL	2	2	2	2	2	1	3
Scales below	11	11	10	11	10	8–9	15–17
Circumped scales	12	11	11	12	12	13	14–17
Gill rakers	25	24	24	23	25	24	24–25
Dorsal fin	IV 8	IV 8	IV 9	IV 8	IV 9	IV 7–8	IV 8
Anal fin	III 24	III 24	III 24	III 24	III 25	III 24–26	III 31–34
Pectoral fin	15	15	15	15	15	14–15	I 15
Pelvic rays	I 5	I 5	I 5	I 5	I 5	I 5	I 5
SL (mm)	64.5	68.0	60.5	64.6	59.1	28.6–52.7	91.6–101.9
Body depth	36.5	37.1	37.2	37.3	36.9	36.2–41.0	41.4–48.4
Body width	14.1	13.5	15.0	14.2	15.2	14.6–17.1	15.0–16.6
Head length	31.5	27.2	30.6	31.4	31.3	32.1–36.1	26.3–33.3
Snout length	5.4	3.4	3.7	5.0	4.1	4.3–6.1	4.0–5.2
Orbit diameter	11.8	14.3	13.9	13.3	13.7	12.9–14.8	13.4–13.9
Interorbital width	12.6	12.7	9.8	9.4	8.5	8.4–11.2	8.5–9.3
Caudal-penducle depth	9.6	10.0	10.5	10.5	10.9	8.4–10.2	8.7–9.0
Caudal penducle length	7.1	10.1	8.1	7.7	9.1	6.6–9.7	7.9–8.5
Predorsal length	41.2	40.6	43.1	42.9	41.6	38.9–45.1	36.4–42.3
Pre-anal length	58.8	54.3	58.7	55.4	59.9	53.5–60.5	44.7–48.6
Prepelvic length	39.1	34.9	37.4	35.1	38.1	36.4–38.6	27.3–28.6
Base of dorsal fin	15.6	15.0	16.9	16.3	17.4	16.4–18.2	15.0–16.6
First dorsal spine	4.9	4.1	4.4	4.5	3.9	3.5–3.8	3.0–7.3
Fourth dorsal spine	17.1	16.5	21.7	18.2	17.7	16.6–18.9	18.5–20.9
Longest dorsal ray	22.0	19.5	22.3	23.1	21.8	17.6–21.9	20.3–23.3
Base of anal fin	35.0	34.9	35.0	34.3	37.6	33.5–42.6	47.2–47.8
First anal spine	intern	intern	intern	intern	intern	intern	1.9–2.7
Third anal spine	4.3	3.2	4.5	4.3	3.6	4.5–7.6	8.9–11.5
Longest anal ray	9.1	8.8	9.8	7.6	9.0	8.1–11.8	10.6–12.2
Caudal fin length	28.5	22.9	25.3	broken	broken	broken	25.4–29.6
Caudal concavity	17.1	17.1	17	broken	broken	broken	8.6–9.5
Pectoral-fin length	24.5	23.3	24.4	25.9	24.2	25.8–31.8	25.5–27.5
Pelvic-spine length	9.5	9.4	8.1	8.7	9.1	10.2–11.7	9.7–10.5
Pelvic fin length	14.9	13.2	13.7	13.0	14.0	13.1–18.5	10.5–13.6
Upper jaw length	16.1	18.4	16.8	17.5	18.8	15.9–18.4	15.3–17.4

logenetic relationships were assessed by Maximum Likelihood (ML, GARLI software, Zwickl 2006), and Neighbor-Joining (MP, PAUP* software, Swofford 2003) methods. For Maximum Likelihood topologies, we conducted 10 independent runs in GARLI, using default settings and the automated stopping criterion, terminating the search when

the ln score remained constant for 20,000 consecutive generations. Statistical confidence in nodes was evaluated using 1,000 non-parametric bootstrap replicates (Felsenstein 1985) for the NJ method, and 100 replicates for ML in GARLI, using the automated stopping criterion set at 10,000 generations. *Parapriacanthus* is the only other genus of Pempheridae and was used as an outgroup as in Azzurro et al. (2015) (sequence from GenBank, accession number JF494087). *Pempheris* sequences are from Azzurro et al. (2015), except for *P. poeyi*, which is from this study. GenBank access numbers are KJ609406 (*Pempheris gasparinii* sp. n.), KT634057 (*Pempheris poeyi*), and KJ609388.1 and KJ609389.1 (*Pempheris schomburgkii*).

Results

Pempheris gasparinii sp. n.
http://zoobank.org/E4965E3A-F4BB-44DF-AE9B-51135B822B41
Figs 1–2, Trindade sweeper

Pempheris poeyi: Miranda-Ribeiro 1919: 173
Pempheris poeyi: Pinheiro et al. 2009: 49
Pempheris poeyi: Simon et al. 2013: 2121
Pempheris poeyi: Pinheiro et al. 2015: 20

Type locality. Trindade Island, Espírito Santo State, Brazil.

Holotype. CIUFES 2432, 64.5 mm SL, GenBank KJ609406, Parcel pool, Trindade Island, Espírito Santo, Brazil. 20°30'S, 29°20'W, depth 1 m, collected by HT Pinheiro and JL Gasparini, 25 Jun 2009 (Figure 2).

Paratypes. CAS 238409, 68.0 mm SL, CAS 238410, 60.5 mm SL, CIUFES 2432, 64.6 mm SL, ZUEC PIS 11233, 59.1 SL. Same data as holotype.

Comparative material. *Pempheris poeyi*: CAS 238411 (one specimen, 52.7 mm SL, Curaçao), USNM 318952 (two specimens, 34.6 and 39.1 mm SL, Tobago), USNM 354575 (two specimens, 28.6 and 29.0 mm SL, Tobago); *Pempheris schomburgkii*: CAS 1595 (one specimen, 92.7 mm SL, Bahia, Brazil), CAS 32060 (two specimens, 48.7 and 39.7 mm SL, Barbados), CAS 236600 (one specimen, 99.2 mm SL, Curaçao), CAS 236604 (one specimen, 103.4 mm SL, Curaçao).

Diagnosis. *Pempheris gasparinii* differs from its congeners by the following combination of character states: Head 3.2–3.7 in SL; body depth 2.7 in SL; head length 3.2–3.7 in SL; orbit diameter 1.9–2.7 in HL; caudal-peduncle depth 2.7–3.3 in HL; dorsal rays IV, 8–9; anal-fin rays III, 24–25; pectoral rays 15; lateral-line scales 51–54; scales below the lateral line 10–11; circumpeduncular scales 11–12; and gill rakers 23–25. Color in life mostly silvery, darker from mid-body to the lateral line and greenish above; fins are translucent with a darker tail. Color in alcohol light brown to silvery with darker dorsum and translucent fins; caudal fin darker. Additionally, mitochondrial DNA COI sequences show a divergence of at least 4% from all Atlantic congeners.

Figure 1. Underwater picture of *Pempheris gasparinii* sp. n. at the Parcel Pool, Trindade Island (top; photo J.L. Gasparini), and a specimen shortly after death (bottom; photo T. Simon).

Description. Dorsal-fin rays IV, 8 (IV, 8–9), all segmented rays branched; anal rays III, 24 (III, 24–25), first internal, all segmented rays branched; pectoral rays 15, the first rudimentary, second unbranched, remaining rays branched; pelvic rays I V (I V); principal caudal rays 9+9 (9–10+9), the median 16 branched (16–17); upper+lower procurrent caudal rays 6+6 (6–8+5–6), the most posterior of each side segmented dis-

tally; lateral-line scales 54 (51–53) to the base of the caudal fin, pored scales continuing to the end of the fin; gill rakers 6+19 = 25 (4–6+19–21).

Body moderately deep and compressed. Depth 2.7 (2.7) in SL and width 2.6 (2.4–2.7) in body depth; head length 3.2 (3.2–3.7) in SL and dorsal profile of head moderately convex; snout very short, 5.8 (6.3–8.2) in HL; orbit diameter 2.7 (1.9–2.4) in HL; interorbital width 2.5 (2.1–3.7) in HL; caudal-peduncle depth 3.3 (2.7–3.1) in HL; caudal-peduncle length 4.5 (2.7–4.1) in HL.

Mouth oblique, forming ~60°angle to horizontal axis of body; lower jaw slightly protruding when mouth fully closed; maxilla expanding posteriorly to a width two-thirds pupil diameter; upper jaw teeth very small, sharp, incurved, in two irregular rows anteriorly, narrowing to a single row posteriorly; lower jaw teeth small, in a patch with three to four irregular rows, outer one or two rows sharply nodular, inner two rows strongly recurved and narrowly sharp; vomer with expanded V-shaped patch (three to four irregular rows) of very small nodular teeth; palatines with a long and narrow patch (two irregular rows anteriorly to one posteriorly) of very small and medially curved teeth; tongue diamond shaped, upper surface with small papillae.

Gill rakers long, longest gill filaments two-thirds the length of longest gill raker. Gill opening extending dorsally, near level of center of orbit. Margin of preopercle smooth. Anterior and posterior nostril apertures vertically oval, positioned in front of dorsal edge of pupil, and separated by narrow septum; no membranous flap on nostril edges. Most scales cycloid, except finely ctenoid posteriorly in interorbital area, on nape, on dorsal area posteriorly to dorsal fin, and above lateral line and chest. Small scales covering slightly more than basal half of anal fin and slightly less than half of caudal fin.

Origin of dorsal fin posterior to vertical alignment of rear base of pectoral fin; predorsal length 2.4 (2.3–2.5) in SL; dorsal-fin base 6.4 (5.7–6.7) in SL; first dorsal spine short, 6.4 (6.6–8.0) in HL; fourth dorsal spine longest, 1.8 (1.4–1.8) in HL; first or second dorsal segmented rays longest, 1.4 (1.4) in HL; origin of anal fin aligned with end of dorsal fin base, preanal length 1.7 (1.7–1.8) in SL; anal-fin base short, 2.9 (2.7–2.9) in SL; first anal spine internal and very short; third anal spine short, 7.3 (6.7–8.8) in HL; longest anal segmented ray 3.4 (3.1–3.5) in HL; caudal-fin length 3.5 (4.0–4.4) in SL; caudal concavity 1.8 (1.6–1.8) in HL; second or third branched pectoral ray usually longest, 4.1 (3.9–4.3) in SL; origin of pelvic fins slightly posterior to rear base of pectoral fins, prepelvic length 2.6 (2.6–2.9) in SL; pelvic spine 3.3 (2.9–3.8) in HL; pelvic-fin length 2.1 (2.1–2.4) in HL.

Color in life. Body mostly silvery, darker from mid-body to lateral line and greenish above; fins translucent with a darker tail (Figure 1).

Color in alcohol. Body light brown, darker dorsally; fins pale except caudal fin with basal melanophores (Figure 2).

Etymology. The specific name honors our ichthyologist colleague and friend João Luiz Rosetti Gasparini, one of the pioneers on the study of taxonomy and biodiversity of reef fishes in Brazil and Trindade Island. "Gaspa" has contributed to nearly half of the descriptions of reef-fish species from Brazilian waters in the last two decades. To be treated as a noun in apposition.

Figure 2. *Pempheris gasparinii* sp. n., preserved holotype, 64.5 mm SL, CIUFES 3127 (photo A. Barber).

Figure 3. Parcel Pool, Trindade Island, Brazil, type locality of *Pempheris gasparinii* sp. n. (photo H.T. Pinheiro).
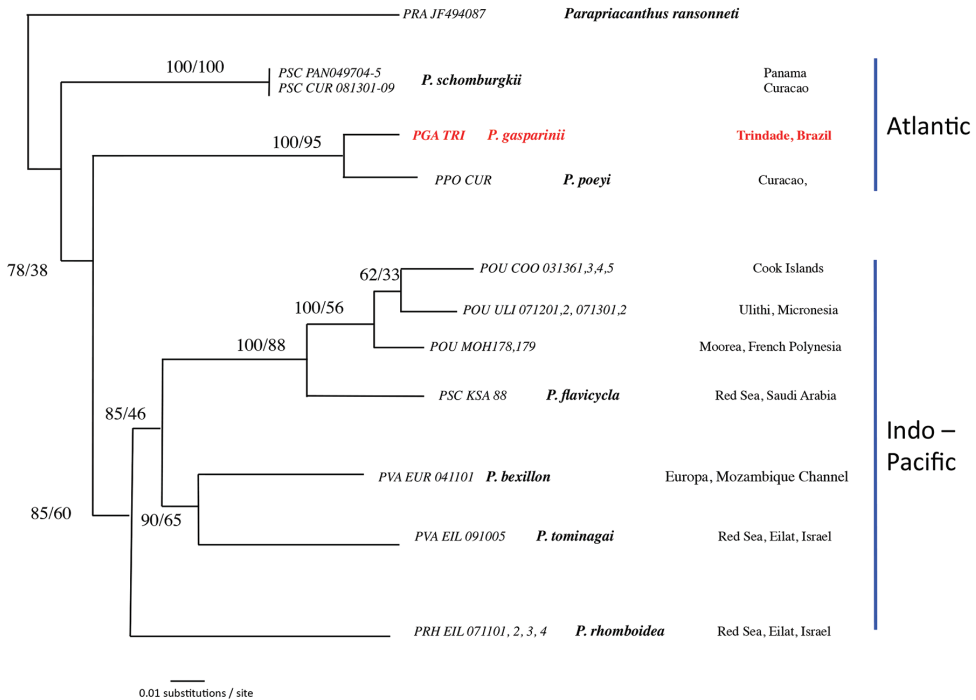
Figure 4. Maximum Likelihood tree of Atlantic *Pempheris* based on the mitochondrial cytochrome oxi-dase 1 (CO1) marker. Labels correspond to sample names (following Azzurro et al. 2015), species names (when identified), collection locality, and biogeographic region. Bootstrap support (1000 replicates) for Neighbor-Joining and Maximum Likelihood are given at the node, in this order. The new species, *P. gasparinii*, is labeled in red.

Distribution and habitat. *Pempheris gasparinii* sp. n. is known only from the type locality, Trindade Island, Espírito Santo, Brazil. It has only been found schooling in the very shallow waters of the rocky Parcel pools (Figure 3).

Genetic data. A maximum likelihood phylogenetic reconstruction of Atlantic *Pempheris*, based on the mitochondrial cytochrome oxidase 1 (CO1) marker, is presented in Figure 4. The Kimura-2 genetic distance between *Pempheris gasparinii* sp. n. and its closest relative (*P. poeyi*) is 4%.

Comparative remarks. *Pempheris gasparinii* differs from its Atlantic Ocean congeners by the smaller number of soft anal-fin rays (24–25) compared to *P. schomburgkii* (31–34), and greater number of lateral-line scales (51–54) and scales below the lateral line (10–11) compared to *P. poeyi* (47–49, and 9 scales respectively). *Pempheris gasparinii* also has fewer circumpeduncular scales (11–12 vs 13), a smaller head (3.2–3.7 vs 2.8–3.1 in SL) and higher caudal peduncle depth (2.7–3.3 vs 3.5–4.0 in head length) compared to *P. poeyi*.

Discussion

As stated by Mooi and Randall (2014), assignments of original or new names to *Pempheris* species is remarkably difficult because: 1) the poor condition and descriptions of type material; and 2) traditional meristics (e.g. fin ray counts) are not very informative at the species level due either to too little or too much character variability. For instance, because of its similarity to *P. poeyi*, *P. gasparinii* is misidentified in the most recent biodiversity checklists of Trindade Island (Pinheiro et al. 2009, 2015, Simon et al. 2013).

A phylogenetic tree of *Pempheris* of the Atlantic based on COI placed *P. poeyi* and *P. gasparinii* as sister species (Figure 4). The two species show a 4% sequence divergence at this locus, which is consistent with a speciation process initiated in the beginning of the Pleistocene. With only one sequence from each of the two species and thus no information on intraspecific genetic divergences, we cannot definitively use the genetic data to separate the two species. However, assuming that intraspecific divergence is low, as it is in *P. schomburgkii* from Panama and Curacao (Fig. 4), the combined genetic and morphological data easily distinguish the two species. The disjunct distribution associated with the genetic divergence of the species suggests that *P. gasparinii* might be a relict species. Similarly to other recently described species from Trindade Island (Pinheiro et al. 2010, Rocha et al. 2010), *P. gasparinii* seems to have been preserved in the Vitória-Trindade Chain while the Pleistocene sea-level changes extirpated the lineages along the adjacent Brazilian coast. This species represents the 12th endemic fish from Trindade Island. Thus, following the diversity reported by Pinheiro et al. (2015) and criteria of reef fishes established by Floeter et al. (2008), the proportion of endemics found in the Vitória-Trindade Chain (191 species) and Trindade Island (137 species) today is 6% and 9% respectively.

Acknowledgements

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RESEARCH ARTICLE

Cryptomyrus: a new genus of Mormyridae (Teleostei, Osteoglossomorpha) with two new species from Gabon, West-Central Africa

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Abstract

We use mitochondrial and nuclear sequence data to show that three weakly electric mormyrid fish specimens collected at three widely separated localities in Gabon, Africa over a 13-year period represent an unrecognized lineage within the subfamily Mormyrinae and determine its phylogenetic position with respect to other taxa. We describe these three specimens as a new genus containing two new species. *Cryptomyrus,* new genus, is readily distinguished from all other mormyrid genera by a combination of features of squamation, morphometrics, and dental attributes. *Cryptomyrus ogoouensis,* new species, is differentiated from its single congener, *Cryptomyrus ona*, new species, by the possession of an anal-fin origin located well in advance of the dorsal fin, a narrow caudal peduncle and caudal-fin lobes nearly as long as the peduncle. In *C. ona*, the anal-fin origin is located only slightly in advance of the dorsal fin, the caudal peduncle is deep and the caudal-fin lobes considerably shorter than the peduncle. Continued discovery of new taxa within the "Lower Guinea Clade" of Mormyridae highlights the incompleteness of our knowledge of fish diversity in West-Central Africa. We present a revised key to the mormyrid genera of Lower Guinea.

Résumé

Nous avons démontré avec des marqueurs mitochondriaux et nucléaires que trois spécimens de poissons mormyridés faiblement électriques, collectés à trois localités au Gabon sur une période de 13 ans, forment une lignée inconnue au sein de la sous famille des Mormyrinae dont nous avons déterminé la position phylogénétique. Nous décrivons un nouveau genre et deux nouvelles espèces pour ces trois spécimens. *Cryptomyrus*, nouveau genre, se distingue des autres genres de mormyridés par une combinaison de caractères de l'écaillure, morphométriques, et dentaires. *Cryptomyrus ogoouensis*, nouvelle espèce, se distingue de sa seule espèce congénérique par sa nageoire anale qui commence nettement en avant de sa nageoire dorsale, son pédoncule caudal gracile et les lobes de sa nageoire caudale pratiquement aussi longs que son pédoncule caudal. Chez *Cryptomyrus ona*, nouvelle espèce, la nageoire anale commence légèrement en avant de l'origine de la nageoire dorsale, le pédoncule caudal est large, et les lobes de la nageoire caudale sont plus courts que le pédoncule caudal. La découverte ininterrompue de nouveaux taxa dans le «clade de basse Guinée» des Mormyridae illustre notre connaissance incomplète de la diversité des poissons de l'ouest de l'Afrique centrale. Nous présentons une clé révisée des genres de Mormyridae de basse Guinée.

Introduction

Mormyrids are nocturnally active fishes endemic to the continental freshwaters of Africa that produce weak electric impulses from a muscle-derived organ located in the caudal peduncle, anterior to the caudal fin. Using specialized electroreceptors distributed over the skin, mormyrids sense nearby objects and prey organisms as distortions to their self-produced electric field (von der Emde and Schwarz 2002). The electric organ discharge, or EOD, is also used for communication. In many mormyrids the waveform of each short (0.2–12 millisecond) pulse encodes the species identity and sex of the signaler while patterns in the timing of pulses convey motivational states (Baker et al. 2013; Hopkins 1986, 1999; Hopkins and Bass 1981). Due to their frequent species-specificity, recorded EOD waveforms can provide valuable characters for the taxonomy of these fishes (Sullivan et al. 2002; Arnegard and Hopkins 2003; Hopkins et al. 2007). There are currently 223 valid species of Mormyridae placed in 20 genera (Sullivan and Lavoué 2015). The division of Mormyridae into two subfamilies, Mormyrinae (19 genera) and Petrocephalinae (one genus), is supported by both morphological and molecular evidence (Taverne 1972; Sullivan et al. 2000; Lavoué et al. 2003).

Here we describe two new species and a new genus of Mormyrinae, based on only three specimens collected over a period of 13 years at three widely separated localities in Gabon, West Central Africa. Despite significant fish collection effort at a number of sites in Gabon since the late 1990s often specifically targeting mormyrid fishes, we know of no other specimens belonging to this unrecognized mormyrid lineage in museum collections. The fishing effort required to produce these three

individuals suggests that these species may be extremely rare in nature or that their precise habitat has yet to be discovered. In either case, we have little confidence that additional material will become available soon and believe description of these taxa should not be further postponed.

In July 2001 the first specimen (MNHN 2003-0425) was collected in a gill net placed in the Moukalaba River close to its confluence with the Nyanga River in southern Gabon (Figs 1, 2). No EOD was recorded, but a tissue sample was taken. The morphological distinctiveness of this fish was noted, but a second trip to the locality was unsuccessful in collecting more specimens. The head, body shape, and nearly equal median fins of the Moukalaba River specimen reminded us of *Hippopotamyrus castor* Pappenheim, 1906 from the Lokoundjé and Sanaga Rivers of Cameroon and to the Nilo-Sudanic species *Hippopotamyrus pictus* (Marcusen, 1864). However, an unpublished phylogenetic analysis of DNA sequences from the mitochondrial 12S, 16S, and cytochrome *b* genes added to a matrix of sequences from other mormyrid taxa including *H. castor* and *H. pictus* did not support a close relationship with *Hippopotamyrus*. Description of this fish was deferred in the hope that more specimens would become available for study.

In 2012 a second specimen resembling the Moukalaba River fish was collected in a gill net sample from the Mabounié River, a small, right-bank tributary of the lower Ngounié River (Ogooué basin, Ngounié Province; Figs 1, 2). The specimen was photographed and preserved in ethanol enabling DNA extraction.

Finally, in September 2014 during the course of an ichthyofaunal survey sponsored by The Nature Conservancy in the "Rapids of Mboungou Badouma and Doumé" Ramsar site located along the Ogooué River upstream of Lastoursville (Ogooué-Lolo Province), a single live specimen somewhat resembling the two other fish was collected in an earthworm-baited fish trap at Doumé falls, just beside the village of Doumé (Figs 1, 2). EOD recordings, photographs, and a tissue sample were obtained.

Materials and methods

As detailed above, the three specimens described here were collected on three separate expeditions to Gabon in 2001, 2012 and 2014. Specimens of other species used in this study are listed below in the comparative material examined section. Institutional abbreviations follow Sabaj Pérez (2014).

Specimen handling and EOD recording

Specimens were collected, handled and euthanized in accordance with guidelines published by the Use of Fishes in Research Committee (1987, 2004, 2014). EODs of the Doumé specimen (tag no. JPS-1194) were recorded in a small aquarium filled with
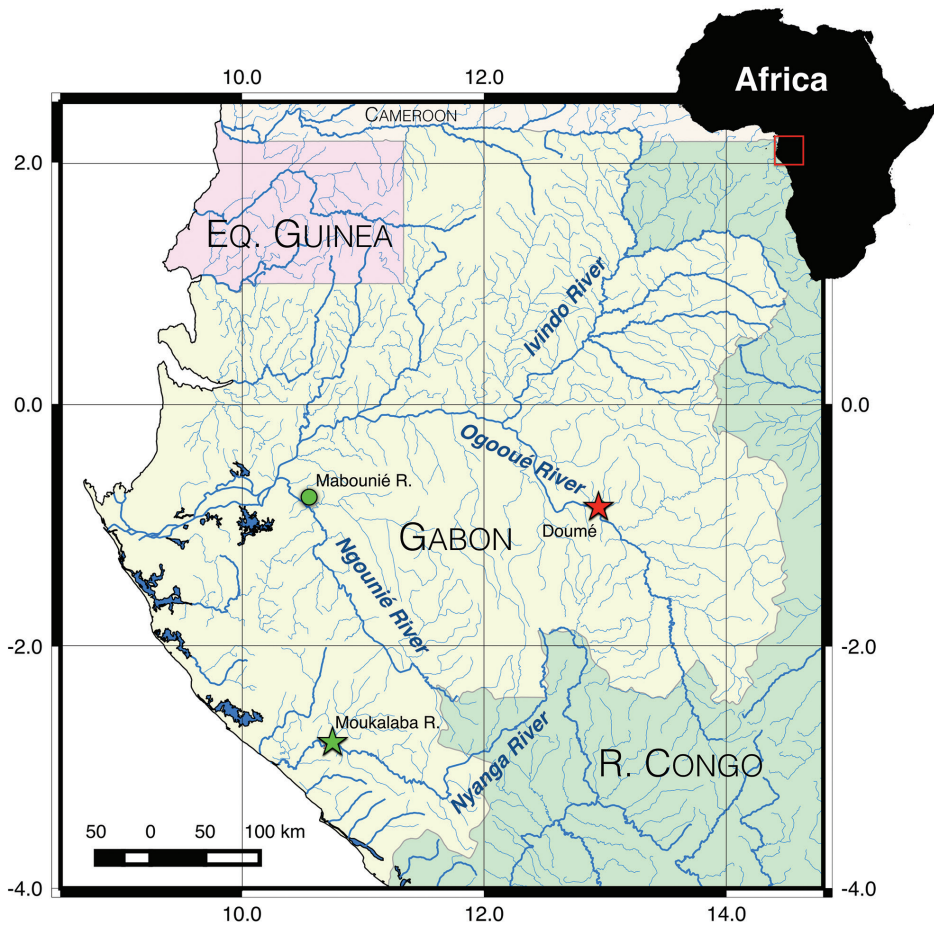
Figure 1. Geographic location of collection sites for the three mormyrid specimens treated in this study. Red star = type locality of *Cryptomyrus ogoouensis* sp. n. at Doumé, Ogooué River; green star = type locality of *Cryptomyrus ona* sp. n. at the Moukalaba River; green circle = locality of second specimen of *C. ona* at the Mabounié River.

water from the collection site, using chloridized-silver wire electrodes connected to an Echo 2 USB analog to digital converter (Echo Audio, Inc.) sampling at 192 kHz/16 bits. We visualized and saved signals using SignalScope virtual oscilloscope software (Faber Acoustical, LLC). We recorded head positivity of the fish in the upward direction and noted water temperature at time of recording. After recording, the fish was euthanized with an overdose of the anesthetic MS222 (tricaine methanesulfonate), tagged, and fixed in 10% formalin. The specimen was subsequently transferred to 70% ethanol and is deposited in the Cornell University Museum of Vertebrates as CUMV 98155.
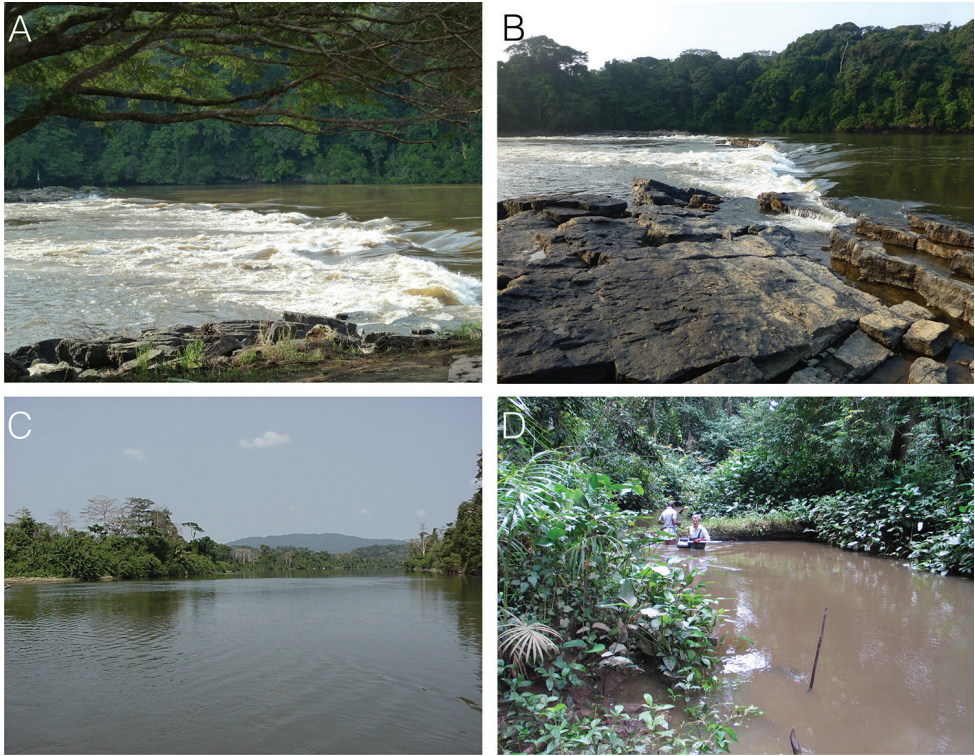
Figure 2. Photographs of the collection localities of the three mormyrid specimens treated in this study. **A** Doumé falls on the Ogooué River, Ogooué-Lolo, Gabon, type locality of *Cryptomyrus ogoouensis* sp. n. during high water in May 2011 **B** same locality in low water, September 2014 **C** Nyanga River at confluence with Moukalaba River near collection site of holotype of *Cryptomyrus ona* sp. n., July 2001 **D** Collection site of *Cryptomyrus ona* specimen CUMV 98647 in Mabounié River, Ngounié Province, February 2012.

DNA sequencing and phylogenetic analysis

In order to investigate the phylogenetic relationships of the three novel mormyrid specimens, we sequenced from each the complete cytochrome *b* (cyt-*b*) gene and portions of the 12S and 16S genes from the mitochondrial genome, as well as partial *rag*2 and the complete S7 introns 1 & 2 and the short intervening exon from the nuclear genome. We added these sequences to an existing 4209 bp alignment of these markers from 38 species belonging to 17 mormyrin genera published in Lavoué et al. (2003), the most complete molecular phylogenetic study of the Mormyrinae to date. In addition to these markers used for phylogenetic analysis, we sequenced a 636 bp fragment of the cytochrome oxidase I (COI) gene, the "barcode" marker from the three specimens for comparison to sequences found in the Barcode of Life Database (Ratnasingham and Hebert 2007).

Because the possible relationship of these new taxa to the genus *Hippopotamyrus* Pappenheim, 1906 was of special interest, we also sequenced all of these markers from a specimen of *Hippopotamyrus castor* from Cameroon, the type species of this genus, a species not included in Lavoué et al. (2003). In addition, we added to the expanded matrix cyt-*b* sequences of a specimen of *H. pictus* from the White Nile of Ethiopia (complete data for a *H. pictus* from the Niger basin of West Africa was already included in the dataset) as well as cyt-*b* sequences retrieved from GenBank for *Hippopotamyrus ansorgii* (Boulenger, 1905) and *H. szaboi* Kramer, van der Bank & Wink, 2004, coding missing data as "?" in the matrix.

DNA was extracted from fin clips or epaxial muscle tissue preserved in 95% ethanol using a QIAGEN (QIAGEN, Inc., Valencia, CA) DNeasy kit. Primer sequences for cyt-*b*, 12S, 16S, *rag*2, S7 and COI are listed in Table 1. For each marker we carried out PCR in 10 to 30µl reactions with components at the following concentrations: 1x Sigma PCR buffer (Sigma–Aldrich, St. Louis, MO), 0.02 U/µl Sigma JumpStart *Taq*, 2 mM MgCl$_2$ (3 mM MgCl$_2$ for *rag*2), 0.4 µM of forward and reverse primer, 200 µM of each dNTP and approximately 200 pg/µl template DNA. We used an initial denaturation step of 1 min at 94 °C followed by 35 cycles of 94 °C for 30 s, annealing at 54 °C (59 °C for *rag*2) for 30 s, and extension at 72 °C for 1.5 min, followed by a final 72 °C extension step for 10 min. We evaluated amplification success on ethidium bromide-stained agarose gels, purified PCR products using Exonuclease I and Shrimp Alkaline Phosphatase and dye-deoxy termination cycle sequencing using ABI Big Dye chemistry followed by Sephadex column purification and data collection on an Applied Biosystems Automated 3730xl analyzer (PE Applied Biosystems, Foster City, CA).

Sequences were edited and combined into contigs for each fragment with Sequencher 4.2 (GeneCodes Corporation, Ann Arbor, MI). Requiring no gaps, alignment of the new coding gene sequences (cyt-*b* and *rag*2) to the Lavoué et al. (2003) dataset was trivial; for 12S, 16S and S7 we were able to align these sequences by eye to the previously optimized alignment without need for insertion of additional gaps.

We performed a maximum likelihood phylogenetic analysis of the matrix from Lavoué et al. (2003) with the added taxa in RAxML v.8, implemented on XSEDE (Stamatakis 2014) via the CIPRES Science Gateway web server (Miller et al. 2010) using separate GTRGAMMA evolutionary models for each gene partition and performed a non-parametric bootstrap analysis to estimate support for nodes. Bootstrapping was auto-terminated using the autoMRE criterion. All other settings were left at their default values. The tree was rooted using *Myomyrus macrops* Boulenger, 1914 as outgroup (Lavoué et al. 2003).

Morphometrics and meristics

Because the three specimens under study do not obviously belong to any one of the 19 described mormyrin genera, choosing mormyrin species for comparison was not

Table 1. Forward and reverse primers used to amplify six genetic markers used in this study shown in 5'
to 3' orientation.

cytbF-L14724	GAC TTG AAA AAC CAC CGT TG
cytbR-H15915	CTC CGA TCT CCG GAT TAC AAG AC
COIF-ZPeng	TCT CAA CCA ACC ATA AAG ACA TTG G
COIR-ZPeng	TAT ACT TCT GGG TGC CCA AAG AAT CA
12S-L1067	AAA CTG GGA TTA GAT ACC CCA CTA T
12S-H1478	GAG GGT GAC GGG CGG GCG GTG TGT
16S-L2510	CGC CTG TTT ATC AAA AAC AT
16S-H3080	CCG GTC TGA ACT CAG ATC ACG T
rag2F2	ArA CGC TCm TGT CCm ACT GG
rag2R6	TGr TCC ArG CAG AAG TAC TTG
S7RPEX1F	TGG CCT CTT CCT TGG CCG TC
S7RPEX3Ralt	ACC TTT GCT GCA GTG ATG TT

straightforward. We collected counts and measurements from seven sympatric taxa
with which the new taxa could conceivably be confused in the field and two extralimi-
tal taxa with (in our estimation) the most overall resemblance to the three specimens,
both of which are type species of genera not known to occur in Gabon. The sympat-
ric taxa used for comparison are *Ivindomyrus marchei* (Sauvage, 1879), *I. opdenboschi*
Taverne & Géry, 1975, *Marcusenius moorii* (Günther, 1867), *Stomatorhinus walkeri*
(Günther, 1867), *Paramormyrops kingsleyae* (Günther, 1896) and two undescribed spe-
cies of *Paramormyrops* Taverne, Thys van den Audenaerde & Heymer, 1977. The extra-
limital taxa used for comparison are *Hippopotamyrus castor* (from coastal drainages of
Cameroon) and *Cyphomyrus psittacus* (Boulenger, 1897) from the Congo basin. Most
of these species are illustrated in Figure 3.

　　We took 29 point-to-point measurements with a digital caliper on each of the
three specimens under study for comparison to those taken from 115 individuals of
nine species listed above. Twenty-seven of these measurements are defined in Boden et
al. (1997): total length (TL), standard length (SL), body depth (BD), caudal pedun-
cle depth (CPD), middle caudal peduncle depth (MCPD), caudal peduncle length
(CPL), head length (HL), head depth (HD), head width (HW), snout length (SNL),
interorbital width (IOW), eye diameter (ED), postorbital length (POL), internarine
distance (DNN), posterior nares-eye distance (DNE), predorsal distance (PDD), prea-
nal distance (PAD), prepelvic distance (PPLD), prepectoral distance (PPCD), dor-
sal-fin length (DFL), dorsal-fin height (DFH), anal-fin length (AFL), anal-fin height
(AFH), pelvic-fin length (PLFL), pectoral-fin length (PCFL), pelvic-to-anal-fin dis-
tance (DPLAF), and pectoral-to-anal-fin distance (DPCAF). To these we added two
additional measures: head length to the end of the opercle bone (HLBO), and mouth
width measured at the inner corner of the front of the mouth (MW).
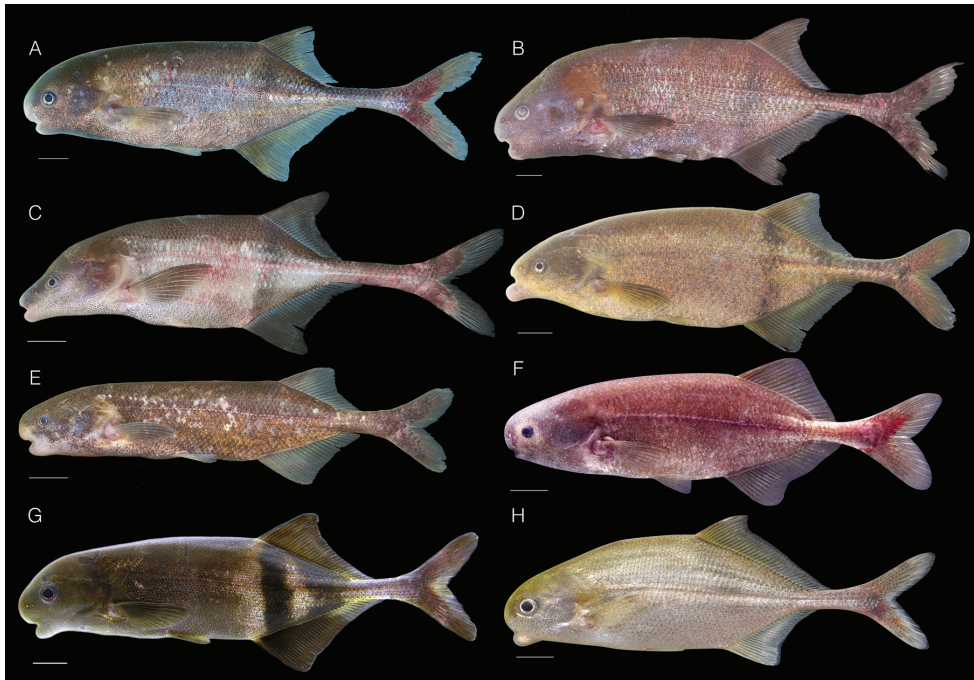
Figure 3. Some mormyrid species used for morphological comparison in this study. **A** *Ivindomyrus marchei* female, 135 mm SL, CUMV 98172, tag no. JPS-1233, Sébé River, Ogooué-Lolo, Gabon **B** *Ivindomyrus opdenboschi* female, 160 mm SL, CUMV 96829, tag no. JPS-1057, Ivindo River at Loa Loa, Ogooué-Ivindo, Gabon **C** *Boulengeromyrus knoepffleri* juvenile 107 mm SL, CUMV 96838, tag no. JPS-1055, Ivindo River at Loa Loa, Ogooué-Ivindo, Gabon **D** *Marcusenius moorii* female, 147 mm SL, CUMV 96836, tag no. JPS-1110, Ogooué River at Franceville, Haut-Ogooué, Gabon **E** *Paramormyrops* sp. (undescribed species) female, 100 mm SL, CUMV 98119, tag no. JPS-1148, Moumba Creek, Ogooué-Lolo, Gabon **F** *Stomatorhinus walkeri* female 95 mm SL, CUMV 80227, tag no. 2814, Diengui Creek, Moyen-Ogooué, Gabon **G** *Hippopotamyrus castor* female, 122 mm SL, CUMV 89955, tag no. 6018, Sanaga River at Nachtigal Falls, Centre, Cameroon **H** *Cyphomyrus psittacus* female, 109 mm SL, CUMV 96767, tag no. JPS-0438, Congo River at Yangambi, Orientale, D.R. Congo.

To facilitate dorsal- and anal-fin ray comparisons among individuals, we report the number of unbranched rays (3) + number of branched rays. (The first two rays are small, the first often only visible in radiographs.)

Specimens were radiographed using a Faxitron Model LX-60 cabinet x-ray system and Kodak Industrex MX125 film. Film images were scanned on a flatbed scanner.

We follow Taverne (1968) in reporting features of the axial skeleton although we exclude the demi-centrum fused to hypurals 3+4 in our vertebral counts. Our nomenclature for hypural bones follows Teugels and Hopkins (1998) and that for intermuscular bones follows Patterson and Johnson (1995).

Sex of the specimens was determined by presence/absence of an "anal-fin notch": a dorsally directed indentation along the anterior base of the anal fin present in all mature male mormyrids (Pezzanite and Moller 1998).

Results

EOD recording of the Doumé specimen

The EOD waveform of the specimen from Doumé is triphasic and very brief (Fig. 4A). Among mormyrids of Lower Guinea, this EOD is most similar to that recorded from *Hippopotamyrus castor* from Cameroon (Hopkins et al. 2007), but dissimilar to that of every other species recorded so far in Gabon. Details of the EOD are provided in the species description below, and the recording is available in the archive of the Macaulay Library at the Cornell Lab of Ornithology under accession number ML197475.

DNA sequences

GenBank numbers for the new sequences generated for this study and their appropriate GenSeq codes (Chakrabarty et al. 2013) are given in Table 2. The sequence data (Table 3) confirm that the three specimens are very close relatives while genetically distant from other mormyrins. The cyt-*b* sequences of the Doumé and Moukalaba specimens differ at 15 sites across the 1140 base pairs, or a p-distance of 1.32%. Between the Doumé specimen and the Mabounié specimen this difference is 17 sites or 1.49%. Between the Moukalaba and the Mabounié specimens the difference is only six sites, or 0.53%. COI genetic distance is smaller between the three specimens: a p-distance of only 0.6% between the Doumé and Moukalaba specimens, 0.8% between the Doumé and the Mabounié specimen, and 0.2% between the Moukalaba and Mabounié specimens. Genetic distances for the 12S and 16S markers are similar to COI, and nuclear *rag*2 and S7 sequences were identical among all three (Table 3) with the exception of base 564 in the S7 sequence which is heterozygous for C and T in the Doumé specimen (coded as "Y" in the sequence), while homozygous for T in the other two specimens. For all markers, sequences from the two species of *Ivindomyrus* Taverne & Géry, 1975 and from *Boulengeromyrus knoepffleri* Taverne & Géry, 1968 are closest to those of the new taxa, with cyt-*b* p-distances between 6.1 and 6.5% (Table 3).

We note that genetic differentiation observed between the Doumé specimen and the Moukalaba and Mabounié specimens, while small, is within the range seen between some closely related, but distinct mormyrid species. For example cyt-*b* sequences from the morphologically divergent *Paramormyrops gabonensis* Taverne, Thys van den Audenaerde & Heymer, 1977 and *P. hopkinsi* Taverne & Thys van den Audenaerde, 1985 – two species for which there is no evidence of mitochondrial introgression – similarly differ by 1.3%. However, in other cases, populations from different river basins regarded as conspecific can differ by this much. For example, *Ivindomyrus marchei* from the Ogooué and Ivindo Rivers differ from those in the Nyanga River by just slightly less than 1.3% (Lavoué et al. 2008) as do Congo and Ogooué basin populations of *Marcusenius moorii* (unpublished data). The degree of genetic differentiation between the Moukalaba River and Mabounié River specimen (0.53%), on the other hand, is
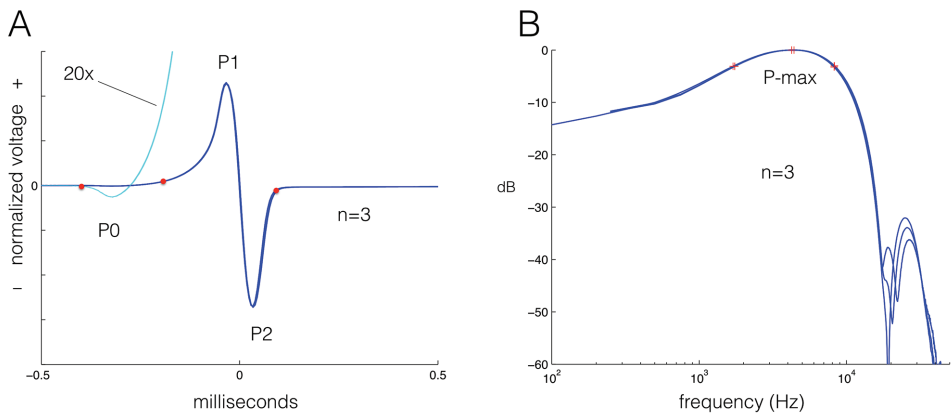
Figure 4. A Three superimposed electric organ discharge (EOD) waveform recordings of *Cryptomyrus ogoouensis* holotype CUMV 98155 (Macaulay Library #197475) recorded at 23.2 °C, head positivity upwards, X-axis = 1 millisecond, Y-axis tick marks indicate 20% of EOD peak-to-peak height; P0, P1, P2 mark the positive and negative excursions in the waveform, P0 highlighted by 20× amplification; red dots indicate onset of P0, onset of P1 and offset of P2, respectively **B** Power spectrum of EOD waveforms in A; red "+" symbols mark peak frequency and frequencies -3 dB below peak at 4300 Hz.

squarely within the normal range of intraspecific variation for cyt-*b*, with the exception of cases where introgressive hybridization is suspected.

Among the more than 300 mormyrid COI sequences in the Barcode of Life Database (BOLD; Ratnasingham and Hebert 2007), the closest match to those from the new taxa is from an *Ivindomyrus marchei*, with 92% sequence identity. To further explore the relationship of mitochondrial sequence differentiation within and between mormyrid species, we downloaded all of the mormyrid COI sequences archived in BOLD and calculated intraspecific variation in COI for species represented by more than one sequence. Intraspecific distances in the COI dataset are well above the 0.6–0.8% observed between the Doumé specimen and the other two individuals for several nominal species with wide distributions spanning multiple major watersheds, e.g. *Brevimyrus niger* (Günther, 1866), *Gnathonemus petersii* (Günther, 1862), and *Mormyrops anguilloides* (Linnaeus, 1758). However, there are also examples of species pairs with COI sequence differentiation below 1% p-distance within genera *Marcusenius* Gill, 1862, *Campylomormyrus* Bleeker, 1874, *Cyphomyrus* Myers, 1960, and *Petrocephalus* Marcusen, 1854. While some of these findings could be attributed to identification errors and/or mitochondrial introgression between sympatric species, morphologically distinct species with very low mitochondrial genetic divergence are documented within the riverine species flocks of *Campylomormyrus* (Feulner et al. 2007) and *Paramormyrops* (Sullivan et al. 2002, 2004).

Turning to the nuclear markers, the near identity of the nuclear S7 intron sequences among the Mabounié, Doumé and Moukalaba specimens might be thought

Table 2. GenBank numbers and specimen information for DNA sequences generated in this study. All but COI were added to alignment of Lavoué et al. (2003) to investigate phylogenetic relationships of new taxa. GenSeq nomenclature follows Chakrabarty et al. (2013).

Specimen	Catalog no.	COI	cyt-*b*	12S	16S	rag2	S7	GenSeq Status
Cryptomyrus ogoouensis holotype	CUMV 98155	KT875221	KT875226	KT875213	KT875217	KT875230	KT875233	genseq-1 COI, cyt-*b*, 12S, 16S, *rag2*, S7
Cryptomyrus ona holotype	MNHN 2003-0425	KT875222	KT875227	KT875214	KT875218	KT875231	KT875235	genseq-1 COI, cyt-*b*, 12S, 16S, *rag2*, S7
Cryptomyrus ona Mabounié specimen	CUMV 98647	KT875223	KT875228	KT875215	KT875219	KT875232	KT875234	genseq-3 COI, cyt-*b*, 12S, 16S, *rag2*, S7
Hippopotamyrus castor	CUMV 89959-6072	KT875220	KT875224	KT875212	KT875216	KT875229	KT875236	genseq-3 COI, cyt-*b*, 12S, 16S, *rag2*, S7
Hippopotamyrus pictus (White Nile)	CUMV 94598-1159		KT875225					genseq-4 cyt-*b*

Table 3. Genetic distances (uncorrected p-distances) between three *Cryptomyrus* specimens and nearest relatives *Boulengeromyrus knoepffleri* and *Ivindomyrus marchei* for mitochondrial and nuclear markers sequenced, shown in order as COI/cyt-*b*/combined 12S-16S/rag2/S7. *Boulengeromyrus knoepffleri* CUMV 92903 and *Ivindomyrus marchei* CUMV 92346 used for COI comparison.

		1	2	3	4	5
1	*Boulengeromyrus knoepffleri*					
2	*Ivindomyrus marchei*	0.033/0.051/0.014/0.004/0.011	•			
3	*Cryptomyrus ogoouensis* holotype	0.085/0.065/0.028/0.022/0.021	0.083/0.064/0.025/0.017/0.015	•		
4	*Cryptomyrus ona* holotype	0.085/0.063/0.028/0.021/0.021	0.080/0.064/0.028/0.017/0.015	0.006/0.013/0.006/0.0/0.0	•	
5	*Cryptomyrus ona* Mabounié specimen	0.083/0.061/0.027/0.021/0.021	0.079/0.064/0.026/0.017/0.015	0.008/0.015/0.005/0.0/0.0	0.002/0.005/0.001/0.0/0.0	•

surprising if these three specimens represent more than one species (less surprising for the identity of the *rag*2 sequences, as this marker evolves more slowly). However, distinct mormyrid species having identical or near-identical nuclear S7 intron sequences is not unprecedented, as similarly identical S7 intron sequences were found among three species of *Campylomormyrus* by Feulner et al. (2007).

Phylogenetics

The aligned dataset and tree described here have been archived on TreeBASE and can be accessed at http://purl.org/phylo/treebase/phylows/study/TB2:S18468?format=html.

In the maximum likelihood tree produced in RAxML (Fig. 5) the three specimens form a monophyletic group, with the Moukalaba River and Mabounié River specimens paired together, sister to the Doumé specimen, as one would predict from the genetic distances. Together, the three specimens appear as the sister lineage to the clade formed by *Boulengeromyrus knoepffleri* plus the two *Ivindomyrus* species. All of these together are in turn sister to the clade formed by species of *Paramormyrops* plus *Marcusenius ntemensis* (Pellegrin, 1927). Bootstrap support at all of these nodes is 100% with the exception of a 91% value at the node joining the Moukalaba River and Mabounié River specimens. This larger clade that includes taxa either endemic to or most diverse in Lower Guinea is sister to a clade formed by species of genus *Stomatorhinus* Boulenger, 1898 plus species of genus *Pollimyrus* Taverne, 1971, with lower bootstrap support. *Hippopotamyrus castor* from Cameroon, the type species of *Hippopotamyrus*, clusters far separately from the new taxa from Gabon, sister to two *Hippopotamyrus pictus* individuals, one from the White Nile River and one from the Niger River. These are sister to a large clade containing species of *Marcusenius*, *Campylomormyrus*, *Gnathonemus* Gill, 1863, *Cyphomyrus*, *Genyomyrus* Boulenger, 1898 and a separate pair of species from southern Africa classified as *Hippopotamyrus*: *H. ansorgii* and *H. szaboi*. While incidental to the description of the new taxa treated here, this result implies the "*Hippopotamyrus ansorgii* complex" of southern Africa treated by Kramer et al. (2004) and Kramer and Swartz (2010) does not belong in *Hippopotamyrus* Pappenheim and requires classification in a different genus.

To explore the relative contribution of mitochondrial and nuclear data to the phylogenetic result, we ran two additional analyses, one with only mitochondrial data included for the three specimens and the other with only nuclear data included for them. In each case, excluded bases were recoded as missing data, while the full dataset was used for all other specimens. In the nuclear data-only analysis, we used a single OTU for the three specimens since *rag*2 and S7 sequences were identical among them. In both experimental analyses, the lineage of the three new taxa was resolved as sister to *Boulengeromyrus* plus *Ivindomyrus* with a 100% bootstrap proportion, indicating strong signal contributing to this result from both the nuclear and mitochondrial data partitions.

Figure 5. Maximum likelihood phylogenetic tree calculated in RAxML for 46 mormyrin specimens based on DNA sequences from mitochondrial cytochrome *b*, 12S, 16S and nuclear *rag*2 and S7 intron, rooted with *Myomyrus macrops*. Bootstrap support values at nodes. Some clades collapsed for simplicity. Position of new taxa (magenta) within the "Lower Guinea Clade" (green) and distance from *Hippopotamyrus castor*, type species of genus *Hippopotamyrus* (red), highlighted.

Morphometrics and meristics

Consistent with the genetic distances reported above, measures and counts of the Moukalaba and Mabounié specimens are more similar to each other than they are to those of the Doumé specimen. In particular, dorsal- and anal-fin bases are short in the former two, with low fin-ray counts, 20/25 and 21/24 total rays respectively, compared to 24/30 in the Doumé specimen, in which the anal-fin origin is situated much further in advance of the dorsal-fin origin. Predorsal distance is nearly equal to preanal distance in the Moukalaba and Mabounié specimens, while it is markedly shorter in the Doumé specimen due to its longer anal fin. Additionally, the caudal peduncle is much deeper and the caudal-fin lobes are shorter in the Moukalaba and Mabounié specimens than in the Doumé specimen. We have never observed a range of four dorsal-fin rays, six anal-fin rays, and differences of 25 percent in anal-fin length relative to standard length among individuals of a single mormyrid species.

While our initial hypothesis that the new taxa are close relatives of *Hippopotamyrus castor* from Cameroon was ruled out by the molecular result, substantial morphological differences are also obvious between the new taxa and *H. castor*. The new taxa have fewer scales (44–45 lateral line scales/12 around the caudal peduncle vs. 72–81/16 in *H. castor*), fewer total vertebrae (40–43 vs. 47), fewer dorsal-fin rays (20–24 vs. 31–33), and a longer anal-fin base (dorsal 78–88% of anal-fin base vs. nearly equal), among other significant differences. Also, while *H. castor* has elongate paired incisor teeth in the lower jaw (from which its specific epithet derives), their morphology is quite different from the lower teeth of *Cryptomyrus*. In the three specimens under study, the central incisors are flattened and spatulate. The neighboring pair of teeth are smaller, but similarly spatulate and close or appressed to the inner pair which assume a sort of trowel shape, pointing outward (Fig. 6). In *H. castor*, the central incisors are cylindrical and club-like and the lateral teeth are greatly reduced (Fig. 6).

With its moderately swollen chin and small mouth, the head of *Cyphomyrus psittacus* appears somewhat similar to those of the new taxa and like them *C. psittacus* has a diffuse band of dark pigment between the origin of the dorsal and anal fins, but the new taxa differ from *C. psittacus* in being less deep bodied (BD 21–24% SL vs. 30–34%), having a shallower head (HD close to 79% HL vs. 63–75%), a smaller eye (ED 19–20% HL vs. 24–26%), a dorsal fin somewhat shorter than the anal fin (vs. far longer than the anal fin) and fewer midlateral scales (44–45 vs. 54–56). Unlike the spatulate dentary teeth of the new taxa, dentary teeth in species of *Cyphomyrus* examined are very small, feebly notched, and embedded in the gums.

The three specimens also differ morphologically from individuals of the two species of *Ivindomyrus* to which the phylogenetic study shows they are closely related. Compared to *Ivindomyrus*, all three specimens have shorter, deeper caudal peduncles (CPD 31–35% CPL vs. 21–28%), greater interorbital width compared to head length (IOW 33–35% HL vs. 21–30%), a higher dorsal-fin to anal-fin length ratio (DFL 78–83% AFL vs. 65–76%), and considerably fewer midlateral scales (44–45 vs. 53–58). Like the dentary teeth in the new taxa, those in *Ivindomyrus* are spatulate, however the
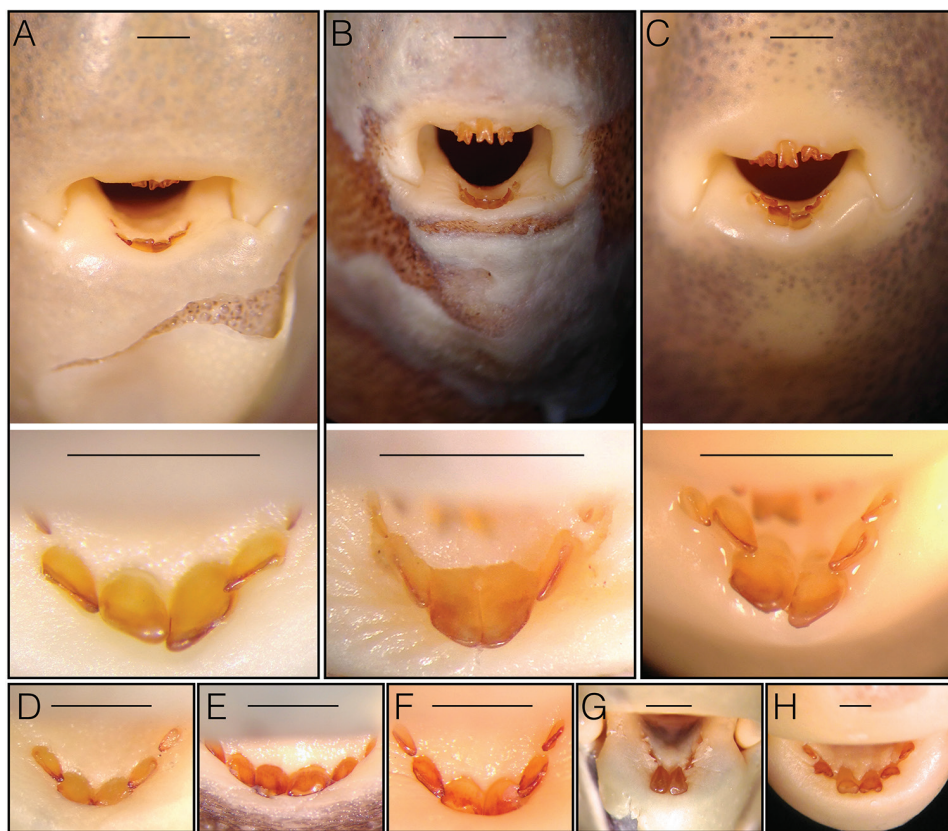
Figure 6. Mouth and dentary teeth in **A** *Cryptomyrus ogoouensis* holotype CUMV 98155 **B** *Cryptomyrus ona* holotype MNHN 2003-0425 **C** *Cryptomyrus ona* non-type CUMV 9864; dentary teeth in **D** *Boulengeromyrus knoepffleri* CUMV 81643 tag no. 2254 **E** *Ivindomyrus marchei* CUMV 96827 tag no. JPS-1043 **F** *Ivindomyrus opdenboschi* CUMV 89324 tag no. 5654 **G** *Hippopotamyrus castor* CUMV 89955 tag no. 6033 **H** *Paramormyrops* sp. "SN4" CUMV 81322. Scale bars = 1 millimeter.

central pair of incisors are not noticeably elongate along their inner margins as they are in the new taxa (Fig. 6).

Compared to species of *Paramormyrops* the new taxa are generally deeper bodied (BD 21–25% SL vs. 15–21%) with deeper heads (HD close to 79% HL vs. 63–75%), larger eyes (ED 19% HL vs. 9–17%) and shorter postorbital head length (POL 59–60% HL vs. 61–70%) in addition to having far fewer midlateral scales (44–45 vs. 56–71). Dentary teeth in all *Paramormyrops* are bicuspid, not spatulate (Fig. 6).

Compared to *Marcusenius moorii*, a species with a similarly low midlateral line scale count, the new taxa most notably lack a chin swelling that protrudes forward beyond the tip of the snout, have a much more subdued and diffuse area of pigment between the dorsal-fin and anal-fin origins, a larger eye (ED 19–20% HL vs. 15–18%) and unicuspid (vs. bicuspid or notched) dentary teeth.

Two of the three new taxa are of larger size than the largest *Stomatorhinus walkeri* examined and all three have a smaller head relative to standard length (HL 22–23% SL vs. 28–31%) a larger eye (ED 19% HL vs. 9–13%), a modest chin swelling (vs. none), and the posterior nares are remote from the rictus of the mouth (vs. in close proximity).

Generic and specific classification

Based on the molecular phylogenetic analysis, we conclude that these three morphologically distinctive specimens represent an undescribed lineage of Mormyrinae sister to the clade formed by *Boulengeromyrus* plus *Ivindomyrus*. This phylogenetic finding in combination with the morphological differences that exist between these new taxa and *Boulengeromyrus* and *Ivindomyrus* precludes placing them in either genus. Creation of an enlarged *Boulengeromyrus* Taverne & Géry via synonymy in which to subsume all of these taxa is ruled out by the significant morphological differences between them and lack of known morphological synapomorphies. Hence a new genus is required.

We recognize two species: one for the Doumé specimen and the other comprising the Moukalaba and Mabounié specimens, reflecting the greater morphological and genetic similarity of these latter two and their notable morphological differences from the Doumé specimen, discussed above. We choose the Moukalaba River specimen as type for the second species and treat the Mabounié River specimen as a non-type. We describe these new taxa below.

Taxonomy

***Cryptomyrus* gen. n.**
http://zoobank.org/E7F2B850-5FEB-4859-AD41-0DE6A079398C

Type species. *Cryptomyrus ogoouensis* sp. n.
Included species. *Cryptomyrus ogoouensis* sp. n., *Cryptomyrus ona* sp. n.
Diagnosis. *Cryptomyrus* gen. n. is distinguished from all other mormyrid genera by combination of the following features. Scales large: 44 or 45 along the midlateral line, with about 42 pierced lateral line scales; mouth subinferior; broad but nonprotrusive chin swelling that does not extend beyond snout; snout expansive and rounded in lateral profile with slight inflection point visible above anterior margin of eye in lateral view; snout somewhat v-shaped in dorsal view; eye large, 19–20% HL; middle four teeth on dentary squarish, broad and spatulate, oriented nearly horizontally, central two lower teeth longest along inner edges and in contact with each other, jutting in advance of neighboring pair and forming a trowel-like shape (Fig. 6); ventral profile of head with marked concavity between gular region and chin, body depth increasing rapidly from there to pelvic-fin origin, body depth at pelvic-fin origin 21–24% SL;

interorbital width 32–35% HL; dorsal-fin length 78–88% of anal-fin length; caudal-peduncle depth at end of anal fin greater than 30% of caudal-peduncle length; faint, wide and diffuse band of pigment between anterior portion of dorsal fin and anal-fin bases, darkest from midlateral region dorsally.

Comparisons. Very few Mormyrinae have so few midlateral scales. Only some of the large-scaled *Marcusenius* such as *Marcusenius moorii* (Boden et al. 1997) and smaller species of *Stomatorhinus* and *Pollimyrus* are in the same range. *Cryptomyrus* has a more pronounced chin swelling than *Ivindomyrus* and *Paramormyrops*, although it does not protrude forward beyond the snout as in *Marcusenius*. *Cryptomyrus* has a noticeably more fusiform body shape and larger eye (19–20% HL) than *Paramormyrops* (eye 11–16% HL), the most speciose mormyrin genus in Gabon and Lower Guinea, which also lacks the band of pigment between the dorsal and anal fins.

Etymology. Gender masculine; from the Greek *kryptos* meaning secret or hidden referring to the rarity of these fishes in collections and the Greek *myros*, a kind of fish, a suffix used in the names of many other mormyrid genera.

Cryptomyrus ogoouensis sp. n.
http://zoobank.org/61AA9C32-4368-47D8-98DD-DF7FA8A279CC
Fig. 7; Table 4

Holotype. CUMV 98155, tag no. JPS-1194, 132 mm TL, 111.6 mm SL, female; Ogooué–Lolo Province, Gabon: Ogooué River at Doumé falls, off rocks on left bank near village of Doumé, approx. 1.5 meters depth, 0°50.4822'S, 12°57.9288'E, earthworm-baited fish trap, J.P. Sullivan, B. Sidlauskas, J.H. Mve Beh, J. Cutler, & A. Dolé, 17 September 2014.

Diagnosis. *Cryptomyrus ogoouensis* sp. n. is readily differentiated from its sole congener, *C. ona* sp. n., in the possession of an anal-fin origin located well in advance of the dorsal fin (first dorsal ray above anal-fin ray 7 vs. first dorsal ray above anal-fin ray 3), a narrow caudal peduncle (depth 5.1% SL vs. 6.0–6.8% SL), and lobes of caudal fin nearly as long as caudal peduncle (vs. markedly shorter).

Description. Morphometric and meristic data for holotype (female, 111.6 mm SL) presented in Table 4. Maximum size of this species unknown. Body fusiform with dorsal and ventral profiles gently convex, greatest body depth between terminus of pelvic fin and urogenital pore. Body compressed, widest at head. Dorsal head profile very nearly straight from back of head to above eye, a slight indentation above anterior margin of eye and abruptly angled at tip of snout. In lateral view, upper lip to tip of snout nearly a straight line, forming a 90° angle with top of head. Ventral profile of head with marked concavity between gular region and chin, body depth increasing rapidly from there to below mid level of pectoral fin. Snout blunt, deep and broad, rounded from above. Tip of snout on level with center of eye, projecting beyond upper lip. Nostrils well separated from each other and from eye; posterior naris at level of bottom of orbit, anterior naris just below line through center of eye; straight line drawn through nostrils

Figure 7. Holotype of *Cryptomyrus ogoouensis* CUMV 98155, female, 112 mm SL, Ogooué River at Doumé falls, Ogooué-Lolo Province, Gabon. Left and right views of preserved whole specimen and radiograph shown below photo of specimen immediately post-mortem. Scale bar = 1 centimeter.

Table 4. Descriptive morphometrics and meristics. Data shown for holotype of *Cryptomyrus ogoouensis* sp. n. (CUMV 98155) and the holotype (MNHN 2003-0425) and second specimen (CUMV 98647) of *Cryptomyrus ona* sp. n. expressed in millimeters (mm) and as a percent of standard measures.

Character	*Cryptomyrus ogoouensis* sp. n. Holotype CUMV 98155 Ogooué River at Doumé	*Cryptomyrus ona* sp. n. Holotype MNHN 2003-0425 Moukalaba River	*Cryptomyrus ona* sp. n. Non-type CUMV 98647 Mabounié River
Standard length (SL), mm	111.6	107.8	98.1
Head length (HL), mm	24.3	24.3	22.6
Percent of SL			
Body depth at pelvic fin (BD)	23.4	24.2	20.8
Head length (membrane) (HL)	21.8	22.6	23.0
Head length (opercle bone) (HLBO)	18.1	20.3	19.6
Predorsal distance (PDD)	62.7	62.4	63.1
Preanal distance (PAD)	55.0	62.3	58.7
Prepelvic distance (PPLD)	35.0	40.2	35.7
Prepectoral distance (PPCD)	21.5	24.5	21.8
Caudal peduncle depth (CPD)	5.1	6.8	6.6
Caudal peduncle length (CPL)	16.4	18.0	18.6
Head width (HW)	10.1	10.7	11.0
Pectoral-fin length (PCFL)	18.8	18.5	17.8
Pelvic-fin length (PLFL)	11.6	10.9	12.7
Pelvic-anal-fin distance (DPLAF)	19.8	22.9	22.3
Pectoral-anal-fin distance (DPCAF)	33.9	37.8	37.3
Anal-fin base length (AFL)	29.5	22.1	24.3
Dorsal-fin base length (DFL)	23.2	19.4	20.0
Percent of HL			
Snout length (SNL)	26.6	26.3	23.1
Post-orbital length (POL)	59.0	59.6	59.5
Head width (HW)	46.1	47.5	47.7
Interorbital width (IOW)	33.2	31.9	34.5
Head depth (HD)	79.4	78.9	78.7
Eye diameter (ED)	19.5	19.9	19.0
Inter-nostril distance (DNN)	4.7	4.2	5.4
Nares-eye distance (DNE)	7.2	5.9	3.1
Mouth width (MW)	14.8	15.8	11.8
Ratios and angles			
Inter-orbital width as % head width	72.0	67.2	72.3
Pre-anal distance/pre-dorsal distance	87.8	100.0	93.0
Inter-nostril width as % interorbital width	14.2	13.1	15.6
Caudal peduncle depth as % CP length	31.2	37.7	35.3
Length of dorsal/length of anal	78.5	87.7	82.5
Counts			
Dorsal rays (simple+branched)	3+21=24	3+18=21	3+17=20
Anal rays (simple +branched)	3+28=31	3+21=24	3+22=25
Anal rays before dorsal	7	2	4
Anal rays beyond last dorsal ray	3	2	2
Pectoral rays	10	10	10
Pelvic rays	6	6	6

Character	*Cryptomyrus ogoouensis* sp. n. Holotype CUMV 98155 Ogooué River at Doumé	*Cryptomyrus ona* sp. n. Holotype MNHN 2003-0425 Moukalaba River	*Cryptomyrus ona* sp. n. Non-type CUMV 98647 Mabounié River
Total midlateral scales (pierced+unpierced)	43+2=45	42+2=44	42+2=44
Rows scales above lateral line to dorsal	9	9	7
Rows scales below lateral line to pelvic	9	9	10
Caudal peduncular scales	12	12	12
Teeth upper jaw/lower jaw	5/6	5/6	5/6
Total vertebrae	43	41	40
Hypurals 1 & 2	unfused	unfused	unfused

passes through tip of snout and just below pectoral-fin origin. Mouth subinferior, small, rictus beneath posterior naris in advance of eye. Chin swelling present: expansive, but not protruding beyond snout. Premaxillary teeth 5, notched, dentary teeth 6, broad, squarish, spatulate, center four oriented horizontally. Center two projecting beyond neighboring pair, longest along inner edges (Fig. 6). Eye large, laterally positioned on head. Predorsal distance greater than preanal distance; anal-fin origin well in advance of dorsal fin: first dorsal ray above anal-fin ray 7 (branched ray 4); dorsal-fin base shorter than anal-fin base; dorsal-fin rays 3+21, anal-fin rays 3+28; 3 anal-fin rays beyond last dorsal-fin ray. Dorsal fin with strongly falcate posterior margin. Longest dorsal-fin ray is second branched ray; next 10 rays successively shorter; final 8 rays equal in length. Anal fin with gently falcate posterior margin. Longest anal-fin ray is third branched ray; next 13 rays successively shorter, final 12 rays equal in length. Pectoral fin long and pointed distally, with 10 rays, extending to vertical through middle of pelvic fin. Pelvic fin with 6 rays, closer to pectoral-fin origin than to anal-fin origin. Caudal peduncle narrow, depth at middle equal to depth at origin. Caudal fin deeply forked with 20 rays in each lobe, lobes long with rounded ends, scaled at bases; distance from caudal flexion to caudal tips roughly equal to CPL. Body covered by thin, cycloid scales, head naked. Body scales large along sides, smaller dorsally: 43 pierced lateral line scales + 2 unpierced along midlateral line, 19 scales in transverse series between origins of dorsal and anal fins, 9 from pelvic fin to midlateral line, 12 around caudal peduncle. Total vertebrae 43; epineurals associated with vertebrae 1–9, vertebral centra 2–13 with pleural ribs directly attached, vertebrae 14–17 with pleural ribs displaced beneath haemal arches; caudal vertebrae 18–43. Hypurals 1 & 2 unfused.

Color. In life, a light cinnamon brown along sides, darker along dorsum, reflective coppery pigment on lower half of head and along belly to anal fin, mouth and chin whitish. Eye with golden iris, dark center. Faint, diffuse broad band of pigment between dorsal and anal fin occupying 4–5 scales, darker above. Numerous depigmented spots over electroreceptors conspicuous on snout, top of head, belly and upper back. Fins with very lightly pigmented rays, interradial membranes hyaline. In preservation, light brown.

Electric organ discharge. EOD waveform recorded from the holotype specimen is very short, about 0.55 millisecond total duration (recorded at 23.2 °C) if measured

from the onset of the first very weak head-negative phase (P0) and only 0.28 milliseconds if measured from the rising phase of the head-positive phase (P1) to the end of the large head negative phase (P2) using a 2% departure-from-baseline threshold (Fig. 4A). P1 is twice the duration and 85% of the amplitude of P2. Both P1 and P2 rise and fall smoothly with no inflection points. In advance of P1 there is a weak head-negative (P0) phase, only 0.2% of the peak-to-peak height of P1 and P2, difficult to see without additional amplification. The presence of a P0 indicates that the electrocytes of this species' electric organ have penetrating stalks innervated on the posterior side of the electrocyte (type Pa) unlike the non-penetrating stalk electrocytes (type NPp) of species of sister genera *Boulengeromyrus* and *Ivindomyrus* (Sullivan et al. 2000). The power spectrum of the EOD (Fig. 4B) exhibits a broad plateau between 1700 and 8200 Hz with peak power at 4300 Hz.

Etymology. The specific epithet is a Latinized noun in the genitive case and refers to the Ogooué River of Gabon.

Distribution and ecology. Currently known only from the Ogooué River at Doumé falls. At the collection site, we recorded a water temperature of 26.7 °C, a pH of 6.89, water conductivity of 13.8 µS/cm and dissolved oxygen of 84.7%.

Cryptomyrus ona sp. n.
http://zoobank.org/D75443F1-97B6-4A89-B1C4-EC06167D47A5
Figs 8, 9; Table 4

Holotype. MNHN 2003-0425, 120.6 mm TL, 107.8 SL, male; Nyanga Province, Gabon: Moukalaba River very near its confluence with the Nyanga River, just above ferry landing on Tchibanga-Digoudou road, 2°47.3400'S, 10°43.7160'E, gill net at night, S. Lavoué & V. Mamonekene, 23 July 2001.

Other (non-type) specimen. CUMV 98647, 115.1 mm TL, 98.1 mm SL, male; Ngounié Province, Gabon: Mabounié River (Lower Ngounié-Ogooué River basin), Station HBG-010, 0°45.1692'S, 010°32.9202'E, gill net at night, Y. Fermon, J.H. Mve Beh, & J.D. Mbega, 21 February 2012.

Diagnosis. *Cryptomyrus ona* sp. n. is readily differentiated from its sole congener, *C. ogoouensis* sp. n., in having an anal-fin origin located only just in advance of the dorsal fin (first dorsal ray above anal-fin ray 3 vs. first dorsal-fin ray above anal-fin ray 7), a deep caudal peduncle (6.0–6.8% SL vs. 5.1% SL), and lobes of caudal fin markedly shorter than caudal peduncle (vs. nearly as long as peduncle).

Description. Morphometric and meristic data for holotype (male, 107.8 mm SL) presented in Table 4. Maximum size of this species unknown. Body fusiform with dorsal and ventral profiles gently convex, greatest body depth between terminus of pelvic fin and urogenital pore. Body compressed, widest at head. Dorsal head profile gently convex with shallow slope downwards to snout, slight inflection between snout and head above eye. Ventral profile of head with marked concavity between gular region and chin, with body depth increasing rapidly from here to below pelvic fin origin. Snout bulbous, rounded, tip

Figure 8. Holotype of *Cryptomyrus ona* MNHN 2003-0425, male, 110 mm SL, Moukalaba River near confluence with Nyanga River, Nyanga Province, Gabon. Preserved specimen shown above radiograph and photo of specimen shortly after collection. Scale bar = 1 centimeter.

below horizontal through center of eye, projecting above upper lip. Nostrils well separated from each other and from eye; posterior naris at level of bottom of orbit, anterior naris below line through center of eye; straight line drawn through nostrils passes through tip of snout and just below pectoral-fin origin. Mouth subinferior, small; rictus below posterior naris, in advance of eye. Chin swelling modest, but broad, nonprotrusive. Premaxillary teeth 5, strongly notched, dentary teeth 6, spatulate, center four oriented horizontally, center two with broad contact between inner edges which are longer than outer edges, projecting well beyond neighboring pair to which they are closely appressed, trowel-like (Fig. 6). Eye large, laterally positioned on head. Predorsal distance equal to preanal distance; anal fin only slightly in advance of dorsal fin: first dorsal-fin ray above first or second branched anal-fin ray; dorsal and anal fins with short bases. Dorsal-fin rays 3+18, anal-fin

rays 3+21; 2 anal-fin rays beyond last dorsal-fin ray. Dorsal fin with falcate posterior margin. Longest dorsal-fin ray is first branched ray; next 10 rays successively shorter; next 5 rays approximately equal in length, final 2 rays longer. Anal-fin ray with gently falcate posterior margin. Longest anal-fin ray is second branched ray; next 10 rays successively shorter, next 6 rays more or less equal in length, final 3 successively longer. Pectoral fin with 10 rays, tip pointed, extending beyond the origin of the pelvic fin, but short of halfway. Pelvic fin with 6 rays, closer to pectoral than to anal. Caudal peduncle deep, depth at middle of peduncle slightly deeper than at origin. Caudal fin deeply forked with 20 rays in each lobe, lobes short, scaled at their bases with bluntly pointed ends; distance from caudal flexion to caudal tips shorter than CPL. Body scales thin, cycloid, head naked. Body scales large along sides, smaller dorsally: 42 pierced lateral line scales + 2 unpierced along midlateral line, 18 scales in transverse series between origins of dorsal and anal fins, 9 from pelvic fin to midlateral line, 12 around caudal peduncle. Total vertebrae 41, epineurals associated with vertebrae 1–8, pleural ribs directly attached to vertebral centra 2–13, ribs displaced beneath haemal arches on vertebrae 14–17, caudal vertebrae 18–41. Hypurals 1 & 2 unfused.

Color. Photo of holotype specimen recovered from gill net, several hours postmortem, shows a purplish-gray body, darker along dorsum, with whitish marbling/speckling on lower half of head and along belly to anal fin and diffuse band of pigment below dorsal fin occupying 4 scales, darker above, lighter or absent above anal fin. Numerous depigmented spots over electroreceptors conspicuous on snout, top of head, belly and upper back. Fins with darkly pigmented rays, interradial membranes hyaline. In preservation, body yellowish tan.

Electric organ discharge. Unknown.

Etymology. The specific epithet is a noun in apposition that honors Marc Ona Essangui, Gabonese environmental and civic activist, founder and executive director of the NGO Brainforest and recipient of the 2009 Goldman Environmental Prize, in appreciation for his efforts to protect Gabon's equatorial forests and wetlands.

Distribution and ecology. Known from the type locality in the Moukalaba River at its confluence with the Nyanga River. A second specimen referred to this species comes from the Mabounié River, a small right-bank affluent of the lower Ngounié River, below Samba Falls. Both specimens were caught by gill net at night. At the type locality we recorded water temperature of 23.6 °C, pH of 8.0, water conductivity of 116.0 µS/cm and dissolved oxygen of 4.98 mg/l. Water conductivity at the Mabounié River at time of collection of that specimen was 48 µS/cm.

Revised key to the mormyrid genera of Lower Guinea, West-Central Africa.

Lower Guinea is defined as the Atlantic drainages of Africa from the Cross River of Cameroon in the north to the Chiloango River of Cabinda/D.R. Congo in the south (Roberts 1975). This artificial key is modified after Hopkins et al. (2007) in which some character states are illustrated. Distribution of each genus within Lower Guinea is indicated in parentheses.

1   Nostrils close to one another and to the eye; mouth inferior, below the level of the eye; body short and rather deep ....**genus *Petrocephalus*** (subfamily Petrocephalinae; widespread)
–   Nostrils separated from each other and from the eye; mouth terminal or inferior, in advance of the eye, body deep or elongate ... (subfamily Mormyrinae)
2   Teeth extending along the entire edge of both jaws in a single series, 10–36 in each jaw; mouth terminal, well in advance the eye; body elongate, depth more than 5.2 times into SL ....***Mormyrops*** (widespread)
–   Teeth restricted to middle of each jaw, 3–10 in each jaw **3**
3   Dorsal fin more than twice the length of anal, originating in advance of pelvic fin insertion .... ***Mormyrus*** (Cross, Sanaga, Nyong)
–   Dorsal fin 0.35–1.25 times the length of the anal, originating behind pelvic fin insertion **4**
4   Pelvic fin insertion closer to the anal than to the pectoral fins; body very elongate, at least 8–11 times as long as deep .... ***Isichthys*** (widespread)
–   Pelvic fin insertion mid-way between anal and pectoral fins or closer to pectorals; body less elongate to short **5**
5   Posterior nostril located close to the border of the mouth .... ***Stomatorhinus*** (Ogooué, Ivindo, Kouilou-Niari)
–   Neither nostril close to the border of the mouth **6**
6   Snout elongated and tubular, its length greater than the post-orbital length of the head; snout turned downward....................***Campylomormyrus*** (Sanaga)
–   Snout non-tubular, its length less than the post-orbital length of the head....**7**
7   Prominent tapered cylindrical barbel-like appendage under the chin, extending forward from below lower jaw................ ***Gnathonemus*** (Cross, Sanaga)
–   Submental appendage reduced to fleshy swelling or absent altogether.........**8**
8   Submental appendage prominent, extending slightly beyond the end of the upper jaw, mouth terminal ....***Marcusenius*** (widespread)
–   Submental appendage not extending beyond end of upper jaw or absent altogether, mouth terminal or inferior **9**
9   Scales along midlateral line 44 or 45, broad submental swelling, mouth subinferior... ***Cryptomyrus*** (Ogooué, Nyanga)
–   Scales along midlateral line 47 or more, chin swelling only slightly developed or absent; mouth terminal or inferior...**10**
10   Dorsal and anal fins approximately equal in length and originating at the same vertical level, dorsal fin with 31–34 rays, anal fin with 31–35 rays, mouth inferior ....***Hippopotamyrus*** (Cross, Sanaga, Wouri, Lokoundjé)
–   Dorsal fin shorter than anal fin and with fewer than 30 rays **11**
11   Body moderately elongate, depth 18–22% SL **12**
–   Body moderately deep, more than 23% SL...**13**
12   Anal and dorsal fins terminate at about the same level. Distal tips of last anal and dorsal rays not offset ....***Paramormyrops*** (widespread)

–   Anal fin terminates beyond the end of dorsal. Distal tips of last anal and dorsal fin rays offset...**Brienomyrus** (widespread)

13   Mouth terminal **14**

–   Mouth subterminal...**15**

14   Snout straight, short and blunt, no darkly pigmented transverse band between dorsal and anal fins ....**Brevimyrus** (Cross)

–   Snout turned downward, long, conical; darkly pigmented transverse band between dorsal and anal fins.....................**Boulengeromyrus** (Ntem, Ivindo)

15   Posterior nostril closer to anterior nostril than to eye; darkly pigmented transverse band between dorsal and anal fins ....**Ivindomyrus** (Ntem, Ivindo, Ogoué, Nyanga)

–   Posterior nostril closer to eye than to anterior nostril; no darkly pigmented transverse band between dorsal and anal fins...**Pollimyrus** (Cross, Wouri, Kouilou-Niari)

Discussion

Cryptomyrus is the first new genus of Mormyridae to be described since *Paramormyrops*, from the same region of Africa, in the late 1970s (Taverne et al. 1977). Given the perception that Gabon is better sampled for fishes than other parts of Central Africa (Stiassny and Hopkins 2007; Stiassny et al. 2011), having no more than three individuals of this mormyrid lineage in collections may seem surprising. In reality, only a small percentage of Gabon's aquatic environments have been visited by ichthyologists. Tropical freshwaters harbor a high proportion of narrowly distributed fish species and in poorly inventoried regions like Gabon such species may long go undetected (Pelayo-Villamil et al. 2014). We have too few data to infer whether these two *Cryptomyrus* species are rare throughout their range, or simply rare where ichthyologists have chosen to collect (Hercos et al. 2012). Because of the "commonness of rarity" in the tropics, description of singletons remains a common taxonomic practice (Lim et al. 2012). Any rule requiring a minimum number of specimens to erect a new taxon would leave a significant proportion of tropical biodiversity undocumented, including taxa most at risk of extinction.

The Moukalaba-Nyanga system of Gabon, the type locality of *Cryptomyrus ona*, remains understudied and is likely to produce additional taxonomic novelties for Mormyridae and other groups. Doumé, the Ogooué River collection site for *C. ogoouensis*, is already an important type locality for fishes. From collections made here in 1876–77 by Alfred Marche, naturalist on the first of Pierre Savorgnan de Brazza's expeditions that explored the sources of the Ogooué (see Sullivan 2007), Henri Émile Sauvage described the mormyrids *Mormyrops sphekodes* (now *Paramormyrops sphekodes*), *Petrocephalus marchei* (now *Ivindomyrus marchei*) and *Petrocephalus simus* along with five non-mormyrid species that remain valid today (Sauvage 1879, 1880). Until our two brief collecting trips in 2011 and 2014, Doumé had apparently not been revisited by ichthyologists. Our

intention was to clarify the identity of *Paramormyrops sphekodes*, a taxon whose name is often erroneously applied. In the course of making new collections of *P. sphekodes* from Doumé in 2014, we collected the single *C. ogoouensis* specimen as well as specimens of a new *Paramormyrops* being described separately.

Despite its provenance from a part of the greater Ogooué basin, not the Nyanga basin, we treat the Mabounié River specimen as a non-type specimen of *Cryptomyrus ona* on the basis of its morphological and genetic similarity to the *C. ona* type specimen. It is worth noting that headwaters of the Ngounié abut those of the Nyanga and that at least two other fish species, *Synodontis ngouniensis* De Weirdt, Vreven & Fermonm, 2008 and *Aphyosemion primigenium* Radda & Huber, 1977 appear to have exclusive distribution in these two river basins, having never been collected elsewhere in the wider Ogooué system. *Synodontis punu* Vreven & Milondo, 2009 is found in these two river basins plus the Kouilou-Niari basin to the south within the Republic of Congo.

The distinctive morphology of these specimens drew our attention to them, but provided few clues about their affinities to other mormyrid species. While we have an EOD waveform recording only from the Doumé specimen, its uniqueness among those known from Gabon's mormyrids also helped to highlight its special status. Sequence data were necessary for confirming that these three individuals are indeed closest relatives and for placing them phylogenetically within the Mormyrinae. The combination of these datasets– morphology, electric signals and DNA– provides a practical, integrative, and evolutionary framework in which to evaluate the status of candidate mormyrid species. The utility of this approach has been demonstrated in a number of other recent publications on Mormyridae (e.g. Kramer and Swartz 2010; Lavoué et al. 2010; Lavoué and Sullivan 2014).

Morphological synapomorphies remain to be discovered for the "Lower Guinea Clade" of Mormyridae to which *Cryptomyrus* belongs (Fig. 5), a group that has been recognized from a series of molecular phylogenetic studies (Alves-Gomes and Hopkins 1997; Lavoué et al. 2000, 2003; Sullivan et al. 2000, 2002, 2004). All are species endemic to the river systems of Lower Guinea with the exception of two species of *Paramormyrops* described from the Congo basin and a third species (*Paramormyrops kingsleyae*) common to the Ogooué and the Congo (Sullivan et al. 2002; Hopkins et al. 2007). A fourth species of *Paramormyrops*, *P. jacksoni* (Poll, 1967) from the upper Zambesi basin of Angola, has never been sequenced and is likely misclassified. In the Lower Guinea watersheds, mormyrid species of Lower Guinea Clade genera *Paramormyrops*, *Ivindomyrus*, *Boulengeromyrus* and *Cryptomyrus* appear in some ways to be eco-morphological equivalents to species of *Mormyrus*, *Marcusenius*, *Cyphomyrus*, and *Pollimyrus* that occur the Congo and Nilo-Sudanic drainages. *Cryptomyrus ogoouensis* and *C. ona* represent only a small portion of the unrecognized diversity within the Lower Guinea Clade of Mormyridae as more than a dozen species of *Paramormyrops* from Gabon still await description.

Biodiversity unknown to science is invisible to conservation efforts. The rivers of Gabon, like others across Africa, are increasingly impacted by logging, road-building, mining, and hydropower dam construction (Brummett et al. 2011). Grand Poubara

Figure 9. Non-type specimen of *Cryptomyrus ona* CUMV 98647, male, 98 mm SL, Mabounié River, tributary of Ngounié River, Ogooué River basin, Ngounié Province, Gabon. Left and right views of preserved specimen above radiograph. Scale bar = 1 centimeter.

Dam on the upper Ogooué River is already completed and construction may begin soon on dams on the Okano and Ngounié Rivers (Ministry of Energy and Water Resources of Gabon 2015). The discovery of *Cryptomyrus ogoouensis* and *C. ona* from just three specimens collected over 13 years reveals the incompleteness of our knowledge of this fish fauna and should motivate us to finish the task of documenting it before human activity further alters these ecosystems.

Comparative material examined

Cyphomyrus psittacus: D.R. Congo, Orientale, Wagenia Falls, CUMV 97543, 4, 88–133 mm SL, tag nos. JPS-0537, JPS-0538, 2 untagged.

Hippopotamyrus castor: Cameroon, Centre, Sanaga R. at Nachtigal Falls, CUMV 89955, 6, 104–136 mm SL, tag nos. 6018, 6019, 6020, 6037, 6038, 6039.

Ivindomyrus marchei: Gabon, Moyen-Ogooué, Ogooué R. at Lambaréné, CUMV 80252, 2, 113 & 115 mm SL, tag nos. 2940, 2941; CUMV 80244, 1, 115 mm SL, tag no. 2908; Ngounié, Loétsi R., CUMV 95128, 3, 105–114 mm SL, tag nos. 6665, 6673, 6674; Ogooué-Ivindo, Ivindo R. at Loa Loa, CUMV 96827, 4, 90–133 mm SL, tag nos. JPS-1101, JPS-1101, JPS-1038, JPS-1043, JPS-1086; Ogooué-Lolo, Sébé R., CUMV uncat., 1, 105 mm SL, field no. TNC14-061; CUMV 89325, 1, 95 mm SL, tag no. 5668.

Ivindomyrus opdenboschi: Gabon, Ogooué-Ivindo, Ivindo R. at Loa Loa, CUMV 83107, 2, 89 & 97 mm SL, tag nos. 4800, 4847; CUMV 89324, 4, 101–114 mm SL, tag no. 5599, 5653, 5654, 5682.

Marcusenius moorii: Gabon, Haut-Ogooué, Ogooué R., CUMV 80460, 1, 123 mm SL, tag no. 3453; Okoloville, CUMV 81623, 1, 124 mm SL, no tag; Moyen-Ogooué, Ogooué R. off point of Lambaréné Island, CUMV 83100, 4, 111–125 mm SL, tag nos. 4950, 4951, 4954, 4972; Ngounié, Louétsi R., CUMV 80346, 1, 126 mm SL, tag no. 3107; CUMV 84517, 1, 122 mm SL, tag no. 2693; Ogooué-Ivindo, Ivindo R., CUMV 89372, 2, 100 & 115 mm SL, tag nos. 5556, 5576; CUMV 96830, 1, 107 mm SL, tag no. JPS-1020; CUMV 81658, 1, 112 mm SL, tag no. 2082; Ogooué R. at Lopé, CUMV 83084, 2, 85 & 85 mm SL, tag nos. 4760, 4761.

Paramormyrops kingsleyae: Gabon, Moyen-Ogooué, Lambaréné, Mikouma Creek, CUMV 80232, 8, 86–108 mm SL, tag nos. 2846–2851, 2854, A3; Ngounié, Biroundou Creek, CUMV 80342, 2, 94 & 121 mm SL, tag nos. 3214, 3215; Bambomo Creek, CUMV 80343, 2, 102 & 109 mm SL, tag nos. 3121, 3128; Woleu-Ntem, Bikagala Creek, CUMV 80348, 2, 102 & 118 mm SL, tag nos. 3209, 3210; CUMV 80251, 7, 85–118 mm SL, tag nos. 3123, 3125, 3129, 3192, 3196, 3197, 3199; Bakanda Creek CUMV 80527, 3, 93–120 mm SL, tag nos. 3221, 3223, 3224.

Paramormyrops sp. (undescribed species): Gabon, Haut-Ogooué, Okoloville, CUMV 80474, 3, 84–101 mm SL; CUMV 80812, 8, 73–99 mm SL; Creek at Bibassa, MRAC 9450 P 0074, 1, 95 mm SL; Ogooué-Ivindo, Balé Creek, CUMV 83110, 1, 90 mm SL; Ivindo R. at Loa Loa rapids, CU89380, 3, 87–115 mm SL.

Paramormyrops sp. "SN4": Gabon, Haut-Ogooué, Ogooué R. at Franceville, CUMV 80458, 1, 127 mm SL, tag no. 3396; CUMV 80463, 2, 126 & 126 mm SL, tag nos. 3466, 3477; CUMV 80901, 2, 118 & 125 mm SL, tag nos. AG, AH; Mpassa R., CUMV 80501, 3, 112–121 mm SL, tag nos. 3747, 3750, 3755; CUMV 80507, 2, 127, 128 mm SL, tag nos. 3707, 3710; Ogooué-Lolo, Doumé, CUMV 96811, 2, 108 & 117 mm SL, tag nos. JPS-1117, JPS-1119; Woleu-Ntem, Okano R. at Na, CUMV 83059, 3, 86–106 mm SL, tag nos. 4680, 4681, 4684; CUMV 83078, 2, 85 & 86 mm SL, tag nos. 4728, 4729; CUMV 83085, 2, 85 & 87 mm SL, tag nos. 4723, 4725; CUMV 83155, 1, 103 mm SL, tag no. 5.

Stomatorhinus walkeri: Gabon, Ogooué-Lolo, Ogooué R., Haut-Ogooué, Ogooué R., CUMV 80477, 2, 86 & 89 mm SL, tag nos. 3605. 3608; CUMV 81631, 1, 79

mm SL, no tag. Ogooué-Lolo, Doumé, MNHN A-0894 (holotype of *Petrocephalus affinis* Sauvage), 1, 82 mm SL. Moyen-Ogooué, Ogooué R., BMNH 1867.5.3.15, .16 (syntypes of *Mormyrus walkeri* Günther), 2, 85, 86 mm SL; CUMV 80237, 2, tag nos. 2877, 2880; CUMV 80248, 1, 82 m mSL, tag no. 2912; CUMV 80255, 1, 75 mm SL, tag no. 2922; Ogooué-Ivindo, Ogooué R. at Lopé, CUMV 83071, 1, 80 mm SL, tag no. 4770.

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