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A systematic study of the genus *Atlanticus* Scudder, 1894 from Zhejiang, China (Orthoptera: Tettigoniidae: Tettigoniinae)

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Abstract

Six species of genus *Atlanticus* have been recorded from Zhejiang, China prior to this study. We describe 2 new species, *A. fallax* **sp. nov.** and *A. interval*. **sp. nov.** Their morphology, songs, COI genes and distributions are compared. The type specimens are deposited in East China Normal University, Biology of History Museum (ECNU).

Key words: Zhejiang, China, *Atlanticus*, taxonomy, song, COI, phylogeny

Introduction

There are 46 species of genus *Atlanticus* recorded from China (Cheng *et al.* 2016; Du & Shi 2005; Liu 2013). Almost all species have extraordinarily small geographic ranges (usually isolated on mountains). This is reflected in the high diversity in the genus. However, the appearance of *Atlanticus* spp. is very uniform, and only the features of male tegmen, cerci, tenth abdominal tergites can be used to distinguish them. From 2015–2017, *Atlanticus* spp. from Zhejiang, China were investigated. Not only morphology, but also calling songs, COI genes and geography are combined for classification and identification. Our results are quite different from those reported by the authors cited above. Eight species are recorded here.

Materials and methods

Taxonomic sampling. We discovered and located these katydids by listening for their songs at night.

Song analysis. All specimens were kept singly in a plastic box with small holes (diameter: 20 mm, height: 50 mm) in the laboratory. We recorded the song over night by placing a Sony ICD-PX440 recorder near the box, and replayed the songs in computer for analysis by Cool Edit software.

DNA extraction and amplification. The total genomic DNA was extracted from the muscles of one hind leg by AxyPrep Genomic DNA Miniprep Kit (AXYGEN), according to the manufacturer's instructions. The fragments of the mitochondrial cytochrome-c oxidase subunit I gene (COI, 680bp) were sequenced. Primers COBL TYTCAACAAAYCAYAARGATATTGG and COBU TAAACTTCWGGRTGWCCAAARAATCA were used (Huang *et al.* 2013). Parameters for PCR were as follow: pre-denaturation for 5min at 94°C, 10 cycles of 30s at 94°C, 30 s at 45°C, then 30s at 72°C, followed by 25 cycles of 30s at 94°C, 30s at 51°C, 30s at 72°C and a final extension at 72°C for 7min. GenBank accession number are showed in Table 1.

Molecular phylogenetic analyses. *Tarragoilus diuturnus* (GenBank accession number JQ999995.1) and *Anabrus simplex* (GenBank accession number EF373911.1) were used as the outgroup taxa. We used Bayesian inference (BI) as phylogenetic methods to reconstruct phylogenetic tree. The BI tree was conducted using the GTR+G+I model strategy with MrBayes 3.2.6. The analyses run for two millions generations, sampling every 100 generations, the first 25% of samples were discarded as burn-in. The remaining samples were used to estimate the Bayesian posterior probability (Ronquist & Huelsenbeck 2003; Ronquist *et al.* 2012). All specimens deposited in East China Normal University, Shanghai.

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TABLE 1. Collecting information and GenBank accession number

Species	Collection site	Collection time	COI
magnificus (1)	Zhejiang, Qingliangfeng	29-vii-2016	MG787195
magnificus (2)	Zhejiang, Moganshan	15-vii-2017	MG787204
magnificus (3)	Zhejiang, Tianmushan	20-vii-2016	MG787190
pieli (1)	Zhejiang, Qingliangfeng	26-vii-2017	MG787198
pieli (2)	Zhejiang, Qingliangfeng	26-vii-2017	MG787199
pieli (3)	Zhejiang, Qingliangfeng	29-vii-2016	MG787196
pieli (4)	Zhejiang, Tianmushan	7-v-2015	MG787189
fengyangensis (1)	Zhejiang, Tianmushan	15-vii-2017	MG787192
fengyangensis (2)	Zhejiang, Baimashan	9-ix-2017	MG787202
fengyangensis (3)	Zhejiang. Baishanzu	16-vii-2017	MG787206
fengyangensis (4)	Zhejiang, Hangzhou	15-vii-2017	MG787207
fengyangensis (5)	Zhejiang, Tiantongshan	3-vii-2016	MG787208
fengyangensis (6)	Zhejiang, Baimashan	9-ix-2017	MG787203
fengyangensis (7)	Zhejiang, Jiulongshan	10-ix-2016	MG787210
fengyangensis (8)	Zhejiang, Qingliangfeng	24-vii-2017	MG787197
fallax (1)	Zhejiang, Wangdongyang	15-vii-2017	MG787188
fallax (2)	Zhejiang, Wangdongyang	15-vii-2017	MG787185
fallax (3)	Zhejiang, Wangdongyang	15-vii-2017	MG787186
fallax (4)	Zhejiang, Wuyanling	14-vii-2017	MG787193
fallax (5)	Zhejiang, Wuyanling	14-vii-2017	MG787194
interval (1)	Zhejiang, Baimashan	9-ix-2017	MG787200
interval (2)	Zhejiang, Baimashan	9-ix-2017	MG787201
interval (3)	Zhejiang, Shaoxing	10-vii-2017	MG787205
interval (4)	Shanghai, Jinshan	27-vi-2016	MG787209
huangshanensis (1)	Zhejiang, Tianmushan	1-vii-2017	MG787191
huangshanensis (2)	Zhejiang, Tianmushan	1-vii-2017	MG787187

Result

1 Molecular study

When *Anabrus simplex* and *Tarragoilus diuturnus* were set as out group, *Atlanticus* spp. formed a monophyletic group. The same species from different collecting locations are also monophyletic with high value of bayesian posterior probability (>99). *A*. (*S*.) *pieli* was first separated with other species. *A*. (*A*.) *huangshanensis* and *A*. (*S*.) *magnificus* was the second and third. *A*. (*S*.) *fallax*, *A*. (*S*.) *interval and A*. (*S*.) *fengyangensis* were close to each other.

2 Taxonomy

Order Orthoptera

family Tettigoniidae

subfamily Tettigoniinae

genus *Atlanticus* Scudder, 1894

Key to species of genus *Atlanticus* known from Zhejiang, China (based on males)

1 tegmen short (<5mm, Fig. 2F) . . . . . *A.* (*Atlanticus*) *huangshanensis*
- tegmen long (>9mm, Fig. 2A–E) 2
2 cerci straight with a small tooth on inner side vertically (Fig. 3B) . . . . . *A.* (*Sinpacificus*) *pieli*
- cerci more or less curved. 3
3 four lines of spines on fore tibiae . . . . . *A.* (*S.*) *fallax*
- three lines of spines on fore tibiae. 4
4 internal tooth on the middle of cerci (Fig. 3A) . . . . . *A.* (*S.*) *magnificus*
- internal tooth on the apical of cerci (Fig. 3CE). 5
5 tegmen almost same color and long (>11mm) . . . . . *A.* (*S.*) *fengyangensis*
- left tegmen with a white spot on the right and short (<11mm). . . . . *A.* (*S.*) *interval*

Key to species of genus *Atlanticus* known from Zhejiang, China (based on calling songs)

1 interval between two trills. 2
- no interval in trill, constant 3
2 interval more than 25s. . . . . *A.* (*S.*) *interval*
- interval less than 10s . . . . . *A.* (*A.*) *huangshanensis*
3 visible pulses in each chirp 4
- invisible pulses in each chirp 5
4 chirp cycle duration <3s, pulse number>10 . . . . . *A.* (*S.*) *fallax*
- chirp cycle duration >6s, pulse number<10 . . . . . *A.* (*S.*) *pieli*
5 chirp cycle duration >5s . . . . . *A.* (*S.*) *fengyangensis*
- chirp cycle duration <3s . . . . . *A.* (*S.*) *magnificus*

subgenus *Atlanticus*

Type species: *Decticus pachymerus* Burmeister, 1838=*Atlanticus* (*Atlanticus*) *pachymerus* (Burmeister, 1838)

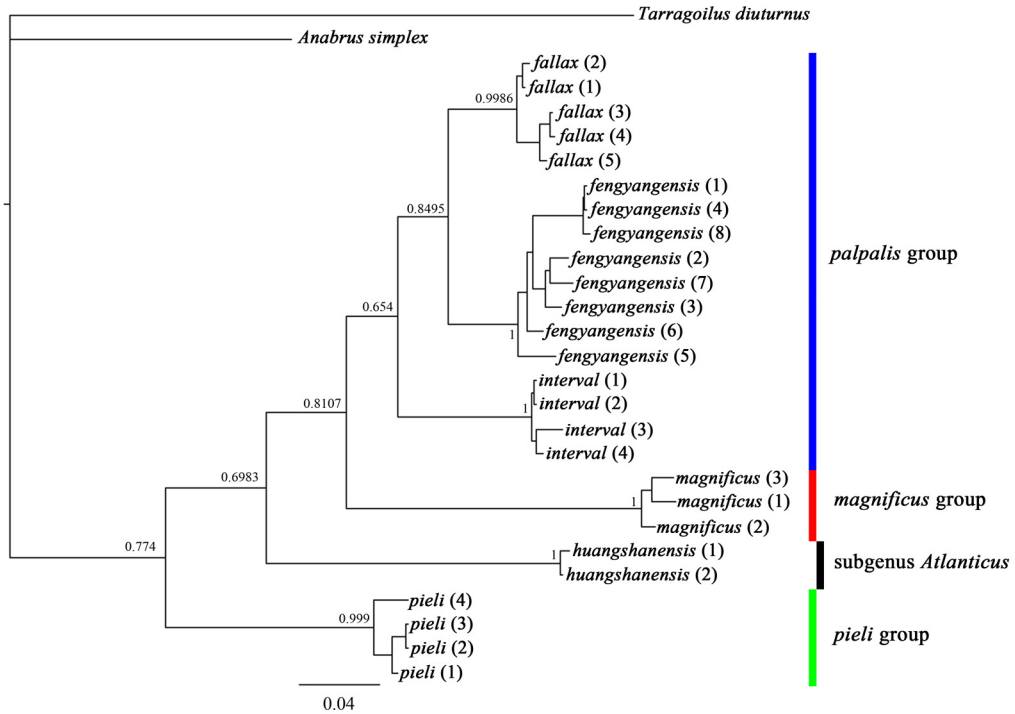


FIGURE 1. Phylogenetic tree of *Atlanticus* spp. reconstructed using bayesian inference analysis (BI) based on COI. Bayesian posterior probability is indicated for main nodes.

2.1 *Atlanticus* (*Atlanticus*) *huangshanensis* Shi & Zheng, 1994

Atlanticus huangshanensis Shi & Zheng, 1994
Atlanticus (*Atlanticus*) *huangshanensis* Liu, 2013

Material examined. 1 male, CHINA, Zhejiang Prov., Lin'an, Tianmushan, 27-vii-2017, coll. He Zhu-Qing; 1 female, CHINA, Zhejiang Prov., Lin'an, Tianmushan, 27-vii-2017, coll. He Zhu-Qing.

Measurements (in mm) male: body length 34.5, pronotum length 10.3, tegmen length 4.6, hind femur length 29.6; female: body length 28.2, pronotum length 9.8, hind femur length 28.8, ovipositor length 21.3.

Distribution. China (Anhui, Zhejiang)

Song. This species sings constantly and quietly. The trill duration is about 4.71s with 7.89s interval. In every trill, it includes about 70 number of chirps/pulses.

Note. Male tegmen is very short (Fig. 2F), which is the feature of this subgenus in China. Female without tegmen. The species in subgenus *Atlanticus* are mainly distributed in North China and this species is the only one in this subgenus found in Zhejiang.

subgenus *Sinpacificus*

Type species: *Atlanticus kiangsu* Ramme, 1939

species group *magnificus* Tinkham, 1941

2.2 *Atlanticus* (*Sinpacificus*) *magnificus* Tinkham, 1941

Atlanticus magnificus Tinkham, 1941
Atlanticus (*Sinpacificus*) *magnificus* Bey-Bienko, 1955; Liu, 2013

Material examined. 1 male, CHINA, Zhejiang Prov., Lin'an, Qingliangfeng, 29-vii-2016, coll. He Zhu-Qing & Lu Hui.

Measurements (in mm) male: body length 33.2, pronotum length 11.7, tegmen length 18.6, hind femur length 31.6.

Distribution. China (Zhejiang)

Song. This species sings fast and constantly. Chirp cycle duration is short as 0.25–0.30s.

Note. Male tegmen is very big and wide compared with other species (Fig. 2A).

species group *pieli* Tinkham, 1941

2.3 *Atlanticus* (*Sinpacificus*) *pieli* Tinkham, 1941

Atlanticus pieli Tinkham, 1941
Atlanticus (*Sinpacificus*) *pieli* Liu, 2013
Atlanticus (*Sinpacificus*) *kiangsu* Bey-Bienko. 1955 paratype syn.

Material examined. 1 male, CHINA, Zhejiang Prov., Lin'an, Qingliangfeng, 29-vii-2016, coll. He Zhu-Qing & Lu Hui; 1 male, CHINA, Zhejiang Prov., Lin'an, Qingliangfeng, 26-vii-2017, coll. He Zhu-Qing; 1 male, CHINA, Zhejiang Prov., Lin'an, Qingliangfeng, 26-vii-2017, coll. He Zhu-Qing; 1 female, CHINA, Zhejiang Prov., Lin'an, Qingliangfeng, 26-vii-2017, coll. He Zhu-Qing.

Measurement (in mm) male: body length 23.3–26.9, pronotum length 9.3–9.4, tegmen length 11.9–12.6, hind femur length 25.7–27.2; female: body length 30.4, pronotum length 9.5, hind femur length 29.2, ovipositor length 16.4.

Distribution. China (Zhejiang)

Song. This species sings constantly. Every chirp cycle duration is 0.67s with about 14 pulses.

Note. Body is small when compared with other species. The apical part of male tegmina are distinctly separated. There is a yellow band on each tegmen. (Fig. 2B).

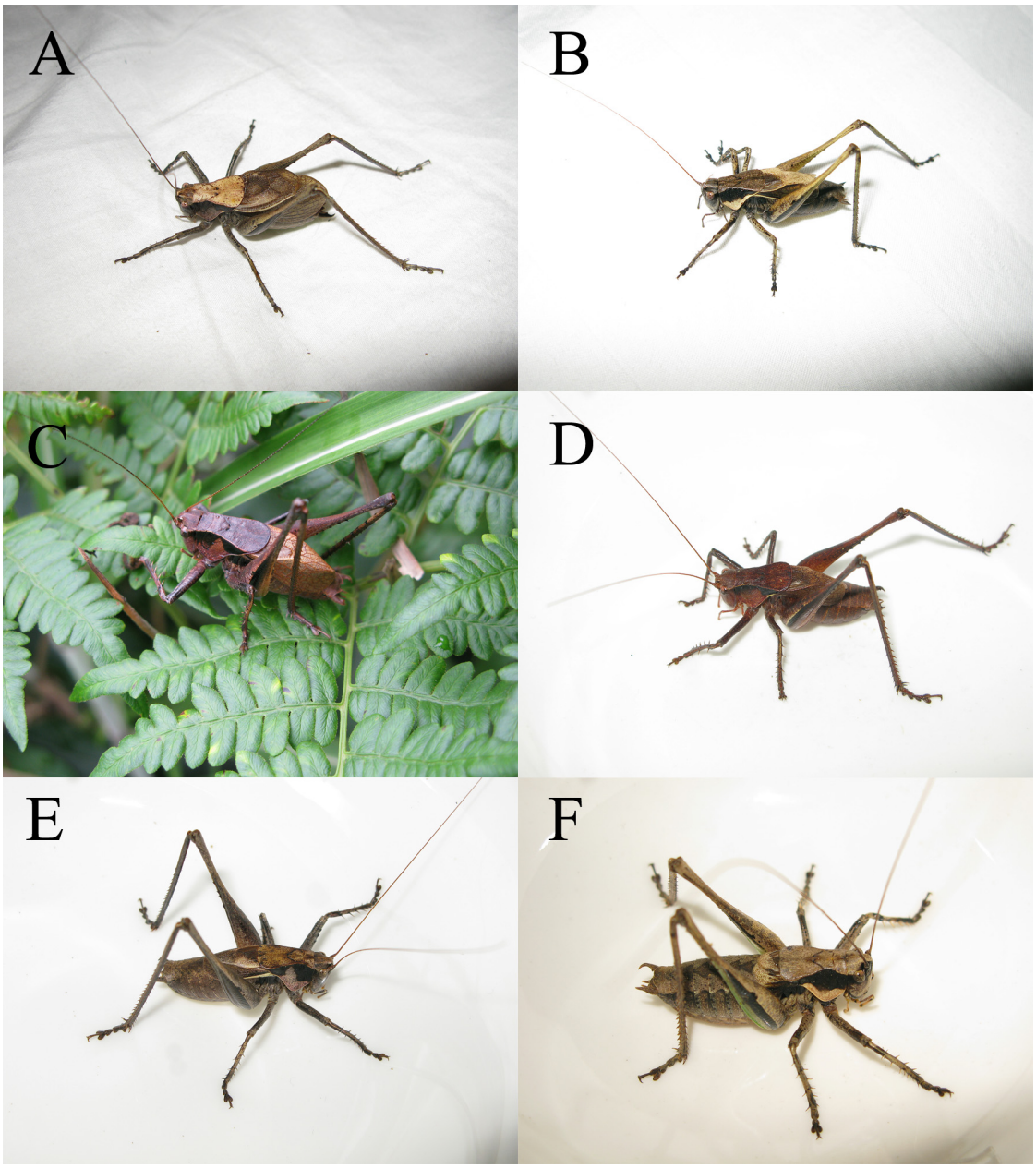


FIGURE 2. *Atlanticus* spp. in living condition: A—*A. magnificus*; B—*A. pieli*; C—*A. fengyangensis*; D—*A. fallax*; E—*A. interval*; F—*A. huangshanensis*.

2.4 *Atlanticus* (*Sinpacificus*) *karnyi* Ebner, 1939

Ebner. 1939. Lingnan Sci. J. 18:294

Material not seen.

Distribution. China (Zhejiang).

2.5 *Atlanticus* (*Sinpacificus*) *brevicaudus* Bey-Bienko, 1955

Bey-Bienko. 1955. Zool. Zhur. 34:1266

Material not seen.

Distribution. China (Zhejiang).

species group *palpalis* Rehn & Hebard, 1920

2.6 *Atlanticus* (*Sinpacificus*) *fengyangensis* Liu, 2013

Atlanticus (*Sinpacificus*) *fengyangensis* Liu, 2013

Material examined. 1 male, CHINA, Zhejiang Prov., Lin'an, Qingliangfeng, 24-vii-2017, coll. He Zhu-Qing; 1 male, CHINA, Zhejiang Prov., Suichang, Baimashan, 9-ix-2017, coll. He Zhu-Qing; 1 female, CHINA, Zhejiang Prov., Suichang, Baimashan, 9-ix-2017, coll. He Zhu-Qing; 2 males, CHINA, Zhejiang Prov., Ningbo, Tiantongshan, 1-ix-2015, coll. He Zhu-Qing & Lu Hui; 1 male, CHINA, Zhejiang Prov., Suichang, Jiulongshan, 30-viii-2016, coll. He Zhu-Qing; 1 male, CHINA, Zhejiang Prov., Qingyuan, Baishanzu, 16-vii-2017, coll. He Zhu-Qing.

Measurements (in mm) male: body length 29.0–32.5, pronotum length 10.8–12.4, tegmen length 11.5–14.9, hind femur length 31.0–34.9; female: body length 34.6–36.6, pronotum length 11.7–12.5, hind femur length 34.5–36.4, ovipositor length 27.0–28.6.

Distribution. China (Zhejiang).

Song. The song of this species is similar to that of *A*. (*S*.) *magnificus* but it sings slowly and constantly. Chirp cycle duration is about 0.50–0.55s.

Note. This species is wide distributed in Zhejiang. It is very similar with *A*. (*S*.) *fallax*. See their differences in *A*. (*S*.) *fallax* part.

2.7 *Atlanticus* (*Sinpacificus*) *fallax* He sp. nov.

Holotype: 1 male, CHINA, Zhejiang Prov., Taishun, Wuyanling, 14-vii-2017, coll. He Zhu-Qing.

Paratypes: 1 male, nymph, CHINA, Zhejiang Prov., Jingning, Wangdongyang, 15-vii-2017, coll. He Zhu-Qing; 1 female, nymph, CHINA, Zhejiang Prov., Jingning, Wangdongyang, 15-vii-2017, coll. He Zhu-Qing.

Description. Pronotum saddle-shaped, disc rectangle in 1/3, and widened gradually with the posterior margin arc, lateral lobes of pronotum deepest in middle, posterior margin oblique with central concavity, tegmen as long as pronotum and extending to the 7th or 8th abdominal tergum. Leg spination, fore femora with 2 interior ventral spines on left and 1 on right, middle femur with 1 external ventral spines, hind femur with 3 interior spines on outer ventral margin and 4 on inner ventral margin, fore tibiae with 3 dorsal spines on outer exterior margin, and 1 spine on the inner exterior margin, 6 spines on both ventral margins, middle tibiae with 3 dorsal spines on outer exterior margin, and 2 spine on the inner exterior margin, 6 ventral spines on both margins, hind tibiae with 21 exterior and 17 interior spines on dorsal margins, 7 and 9 spines on ventral margins. Tenth abdominal tergum with a small U shape notch on central, cerci curved with a small tooth in the middle (Fig. 3D).

Female Nymph, similar to male, no tegmen, ovipositor nearly straight.

Coloration. Brown, upper side of lateral lobes of pronotum black, head and hind femur yellow.

Variation. Neither two nymphs has spine on the inner exterior margin on fore tibiae. It is unclear whether it will appear as it matures.

Etymology. The name *fallax* is used to emphasize its similarity to *A*. (*S*.) *fengyangensis* Liu, 2013.

Measurements (in mm) male: body length 31.2, pronotum length 11.9, tegmen length 13.6, hind femur length 32.9; female (nymph): body length 23.3, pronotum length 10.5, hind femur length 29.8, ovipositor length 22.5.

Distribution. China (Zhejiang)

Song. The song of this species is similar to that of *A*. (*S*.) *magnificus* with 0.25s chirp cycle duration. But in every chirp, 6 or 8 pulses are clearly present.

Note. This new species is very similar to *A*. (*S*.) *fengyangensis* Liu, 2013 (Fig. 2CD), but the new species has one more row of spines on the fore tibia. There are also differences in song and the shape of the cerci (Table 2, Fig.

3CD). The ventral surface of the abdomen is red when alive, while that of *fengyangensisi* is yellow or lime. This species is distributed in the southernmost part of Zhejiang, and there seems to be geographic isolation on two species (Fig. 4).

TABLE 2. Calling songs of *Atlanticus* spp.

species	Area	Temperature (°C)	Trill cycle duration (s)	Trill interval (s)	chirp number	chirp cycle duration (s)	pulse number
huangshanensis	Zhejiang, Tianmushan	27	4.71±0.21	7.89±2.03	70.2±3.03	/	/
magnificus	Zhejiang, Tianmushan	27	/	/	/	0.30±0.01	/
magnificus	Zhejiang, Moganshan	27	/	/	/	0.25±0.02	/
pieli	Zhejiang, Tianmushan	27	/	/	/	0.67±0.06	13.5±0.58
fengyangensis	Zhejiang, Tianmushan	26	/	/	/	0.54±0.02	/
fengyangensis	Zhejiang, Baimashan	26	/	/	/	0.55±0.02	/
fengyangensis	Zhejiang, Tiantongshan	27	/	/	/	0.52±0.02	/
fengyangensis	Zhejiang, Jiulongshan	26	/	/	/	0.50±0.05	/
fallax	Zhejiang, Wangdongyang	27	/	/	/	0.25±0.02	6.57±0.98
interval	Zhejiang, Baimashan	26	17.58±2.27	25.36±3.34	30±5.48	0.67±0.03	5.78±0.44
interval	Zhejiang, Shaoxing	27	6.60±0.59	60.35±21.77	10.86±0.69	0.59±0.02	4.44±0.53

2.8 *Atlanticus* (*Sinpacificus*) *interval* He sp. nov.

Holotype: 1 male, CHINA, Zhejiang Prov., Suichang, Baimashan, 9-ix-2017, coll. He Zhu-Qing.

Paratypes: 1 female, CHINA, Zhejiang Prov., Suichang, Baimashan, 9-ix-2017, coll. He Zhu-Qing; 1 male, CHINA, Zhejiang Prov., Shaoxing, 10-viii-2017, coll. He Zhu-Qing; 1 male, CHINA, Zhejiang Prov., Ningbo, Tiantongshan, 30-v-2015, coll. He Zhu-Qing.

Description. Pronotum saddle-shaped, disc narrower from anterior margin in 1/5, and widened gradually with the posterior margin arc, lateral lobes of pronotum deepest in middle, posterior margin oblique with central concavity, tegmen slightly shorter than length of pronotum and extending to the 5th or 6th abdominal tergum. Leg spination, fore femora with 3 interior ventral spines, middle femur with 3 external ventral spines on left and 2 on right, hind femur with 6 interior spines on outer ventral margin, fore tibiae with 4 dorsal spines on exterior margin, 6 spines on both ventral margins, middle tibiae with 6 interior spines on both margins, 2 exterior and 5 interior spines on dorsal margins, hind tibiae with 24 exterior and 21 interior spines on dorsal margins, 9 and 13 spines on ventral margins. Tenth abdominal tergite with a flat U shaped notch in median portion, cerci curved with a small apical tooth (Fig. 3E).

Female similar to male, tegmina covered by pronotum, but visible from lateral, ovipositor nearly straight with dorsal margin curved in basal 1/2, and ventral margin curved in basal 1/3.

Coloration. Brown, upper side of lateral lobes of pronotum black with middle brown, Sc. vein white, left tegmen with a white spot on right (Fig. 2E), ovipositor yellow.

Variation. External ventral spines on middle femur ranging from 0–4, interior spines on outer ventral margin of hind femur ranging from 2–3, interior spines on dorsal margins of middle tibiae ranging from 4–5.

FIGURE 3. Tenth abdominal tergum and cerci in dorsal view: A—*A. magnificus*; B—*A.pieli*; C—*A. fengyangensis*; D—*A. fallax*; E—*A. interval*.

Etymology. The new species name *interval* refers to the pause in the male calling song.

Measurements (in mm) male: body length 29.0–30.8, pronotum length 10.8–11.0, tegmen length 9.0–10.5, hind femur length 33.3–33.9; female: body length 30.4, pronotum length 11.4, hind femur length 33.0, ovipositor length 21.7.

Distribution. China (Zhejiang)

Song. The trill is about 17.58s with 25.36s interval. In every trill, there are about 30 chirps. Every chirp is about 0.67s with 6 pulses (individual from Baimashan). The song of individual from Shaoxing is quit different in most parameter, but there is also a long interval between two trills as the feature of this species.

Note. The new species is similar to *A.* (*S.*) *fengyangensis* Liu, 2013 in the shape of the cerci, but the tegmen is short. A white spot on the right of tegmen is another unique feature of this species. Song is very special with long interval, which makes it very difficult to catch.

Discussion

(1) According to the molecular result, subgenus *Sinpacificus* is paraphyletic. But this conclusion should be confirmed after more genes are checked. (2) The species *fengyangensis*, *fallax* and *interval* are very similar in morphology and all belong to species group *palpalis*, but the COI genes indicate they are distinct species. Song is another clue to distinguish them (Fig. 5). (3) Contrary to Liu's findings, at least in Zhejiang, all six species we studied were not seen to have restricted distributions. Furthermore, several species are synpatric and can be found in same area (e.g. 4 species in Tianmushan and 3 species in Qingliangfeng and Tiantongshan). As a result, topotypes may represent multiple species.

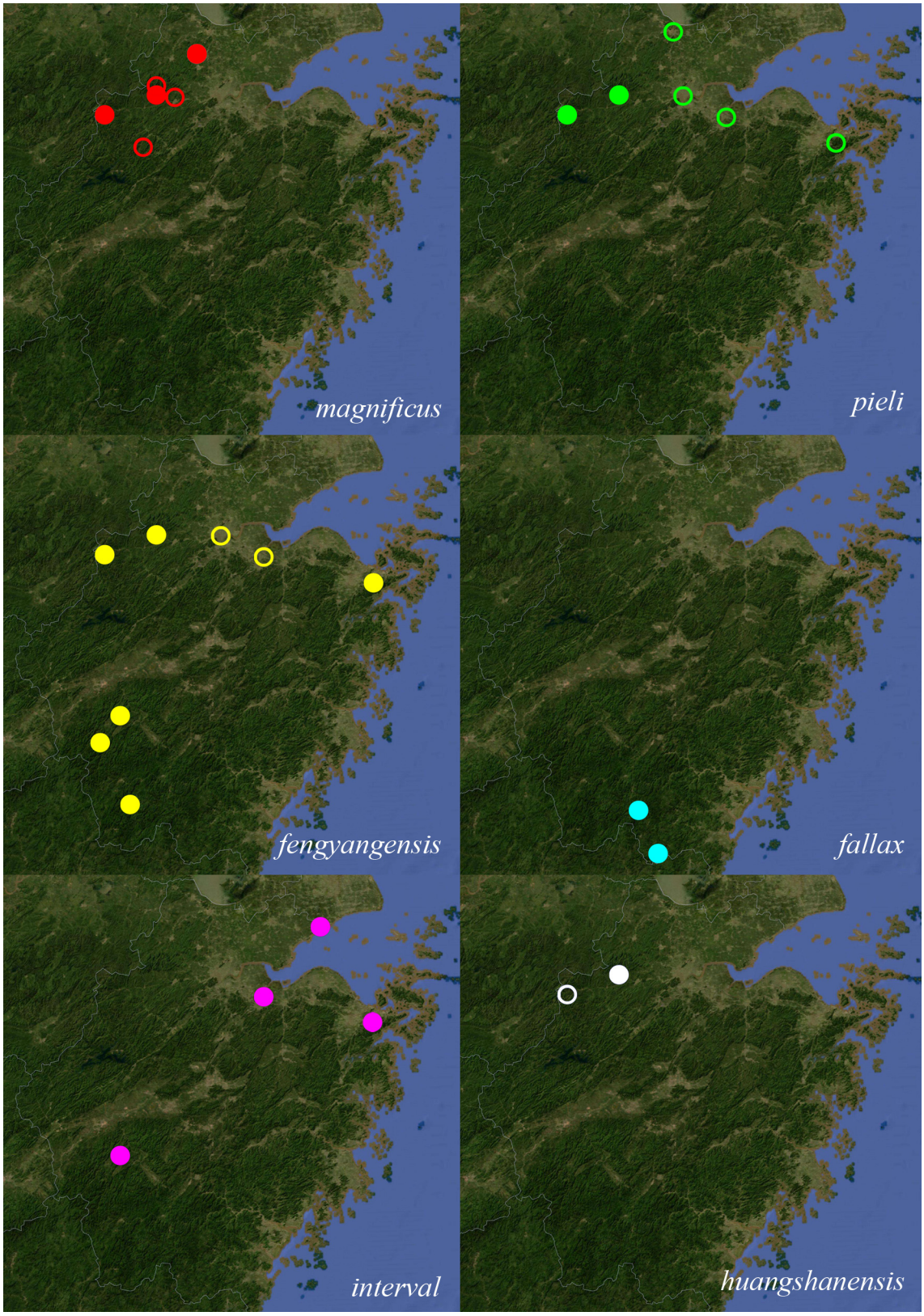
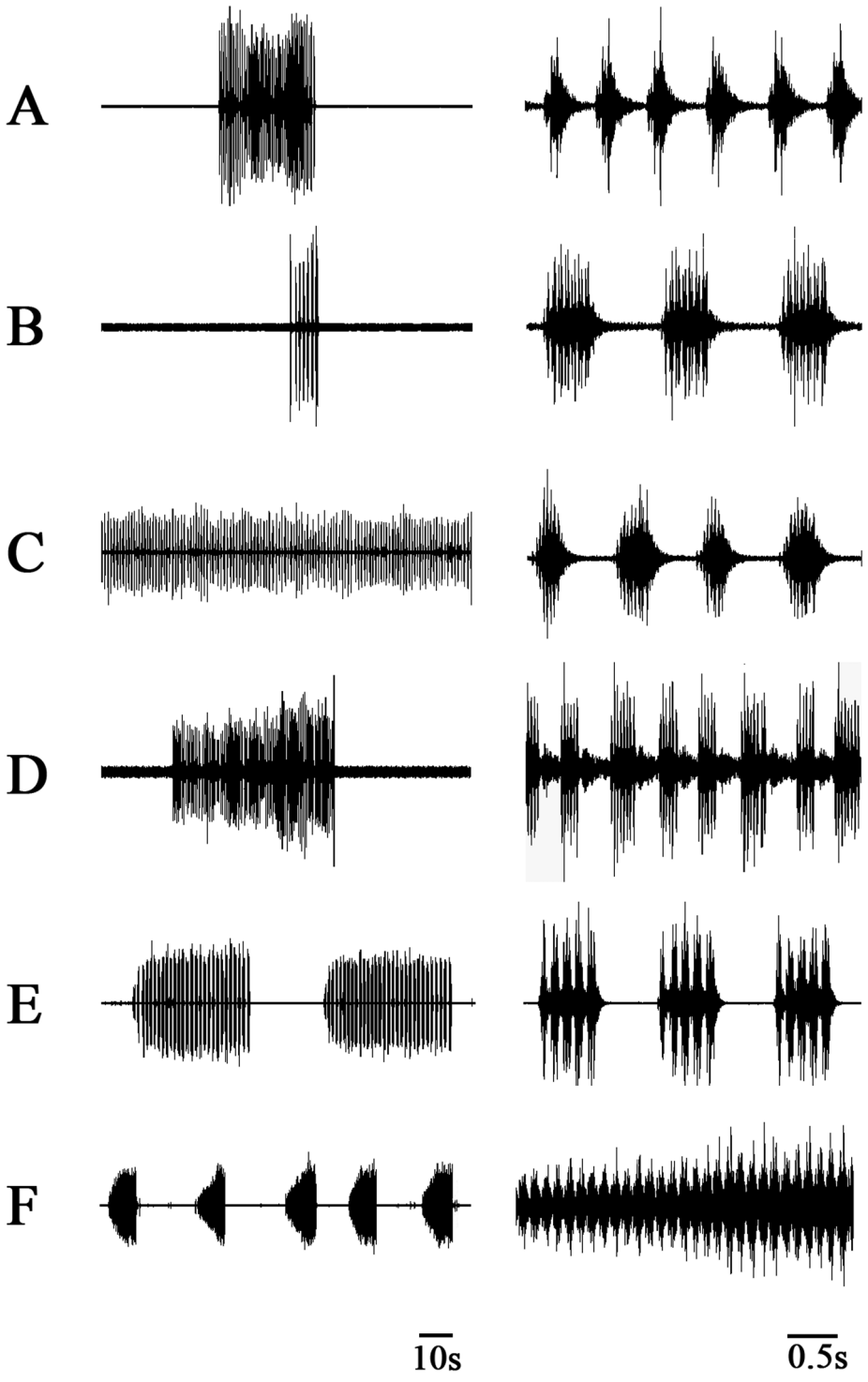


FIGURE 4. Distribution of *Atlanticus* spp. in Zhejiang: dot-species distribution; circle-song records.



FIGRUE 5. Calling songs of *Atlanticus* spp. A—*A. magnificus* (from Qingliangfeng); B—*A. pieli* (from Tianmushan); C—*A. fengyangensis* (from Tianmushan); D—*A. fallax* (from Wangdongyang); E—*A. interval* (from Baimashan); F—*A. huangshanensis* (from Tianmushan), scale bar=60 and 0.2 seconds respectively.

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