

ORIGINAL ARTICLE

Discovery of the male of *Colletes yanruae* from Yunnan, China (Hymenoptera: Apoidea: Colletidae)

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Abstract Several wild pollinator bees were found visiting local plants and collected in early spring in dry and eroding lands in Yunnan, China. With both morphology and DNA information, we discovered the hitherto unknown male of *Colletes yanruae* Niu, Zhu & Kuhlmann, 2013. Herein, the male was described and illustrated for the first time.

Key words Apiformes, DNA barcoding, taxonomy.

1 Introduction

Sex association is important and difficult in the taxonomy of the bees, for the bees always exhibit sex dimorphism. In practice, some sex associations have been made based on similar geographic ranges and other associations have been made more rarely by collecting males and females together. As a result, many of these species are likely synonyms of species described from the opposite gender (Pilgrim *et al.*, 2008). It is the best way to get evidences by the mating experiment. However, it has been always difficult to get enough living bees to observe the mating behavior in the field or in the lab. Now, molecular techniques, especially DNA barcoding, have been accepted to be another option to associate genders of the same species by comparing species-specific DNA sequences, because the DNA sequences are identical or nearly identical in the same species (Pilgrim & Pitts, 2006).

The Chinese *Colletes* Latreille, 1802 has been reviewed recently and 66 species were recorded so far (Niu *et al.*, 2013a, b, 2014a, b). However, 8 species are only known from a single gender, including *C. yanruae* Niu, Zhu & Kuhlmann, 2013 that was described from the female (Niu *et al.*, 2013b). Recently, Dr. Zongxin Ren and his colleagues collected bee specimens while studying reproductive biology on native plants in Yunnan, China. Among these wild pollinator bees in early spring, the male of *C. yanruae* Niu, Zhu & Kuhlmann, 2013 was discovered for the first time. Its status was further confirmed by DNA barcoding besides morphological similarity to the female. Here the male of *C. yanruae* Niu, Zhu & Kuhlmann, 2013 is described and illustrated for the first time.

2 Material and Methods

Wild pollinator insects were observed visiting flowers of the host plant, *Cotoneaster subadpressus* and *Berberis* sp. between 14:00 and 15:30, May 7th, 2015. All insects were collected by sweeping net and brought back for studies in lab.

All specimens examined are deposited in the Insect Collection of the Institute of Zoology, Chinese Academy of Sciences (IZCAS), Beijing, China. The specimens were examined with a NIKON SMZ 1500 stereomicroscope. Attributes were recorded with a NIKON D7000 digital camera. The terminology used in the descriptions follows Michener (2007) for general morphology. Absolute measurements, in millimeters (mm), are used for body length. For all other structures, relative measurements are used. Abbreviations used in the descriptions follow Niu *et al.* (2013b).

Multiple specimens (Table 1) were sequenced for DNA barcoding with the primers: 5'-TATCAACCAATCATAAAAA-TATTG-3' and 5'-TAAACTTCTGGATGACCAAAAAATCA-3' (Hastings *et al.*, 2008). We compared the DNA sequences from both sexes of *Colletes yanruae* and used MEGA 5 (Tamura *et al.*, 2011) to reconstruct a NJ tree to confirm the positions and identities of specimens. For comparative purposes, a parsimony tree analysis with the Heuristics search was conducted in NONA ver. 2.0 (Goloboff, 1999), as implemented in WinClada (ver. 1.00.08; Nixon, 2002), using the following parameters: 10000 trees to hold, 100 replications, and the other options follow the default settings. This analysis obtained 4 most parsimonious trees of 297 steps (Ci (consistency) = 80, Ri (retention) = 93). Then unambiguous character optimizations were calculated for all trees. The strict consensus of these 4 trees collapsed 14 nodes and had a length of 299 steps. We select the COI-1935601, COI-1935602, COI-1935603 as the outgroup and reroot the consensus tree.

3 Results

In the dry and eroding lands at high elevation (2900–3000 m) on Yaoshan Mountain, Northeastern Yunnan, China, *Cotoneaster subadpressus* (Rosaceae) have been found quite popular in native flora. It has been very important ecologically to control the loss of water and soil erosion (Fig. 1). However, in early spring, there are just a few bees or flies visiting this plant. Based on the specimens recently collected, we found associated the male specimens with female *C. yanruae* Niu, Zhu & Kuhlmann, 2013 visiting this plant and *Berberis* sp. (Berberidaceae).

The male specimens has narrow apical tergal hair bands and much long hairs on the scutum like the female, and both sexes specimens were collected from the same locality. Furthermore, we sequenced both sexes of different species of *Megachile*, *Hylaeus* and *Colletes*, including 8 specimens of *C. yanruae* (7 females and 1 male). The sequences from different both sexes were grouped together respectively, and both sexes of *C. yanruae* also were grouped in a single highly



Figure 1. Habitat and host plants (*Cotoneaster subadpressus*) of *Colletes yanruae* Niu, Zhu & Kuhlmann, 2013 found in Yunnan, China.

Table 1. Information of specimens for DNA barcoding.

IOZ(E) number	Sex	Collecting site	Coordinates	Coll. Date	Floral association	Leg.	Species	Genbank accession number
1935501	F	Yunnan, Yaoshan	27°12'N, 103°06'E	7.V.2015	<i>Cotoneaster subadpressus</i>	Ren & Tao	<i>Colletes yanruae</i>	KU315129
1935502	F	Yunnan, Yaoshan	27°12'N, 103°06'E	7.V.2015	<i>Cotoneaster subadpressus</i>	Ren & Tao	<i>C. yanruae</i>	KU315130
1935503	F	Yunnan, Yaoshan	27°12'N, 103°06'E	7.V.2015	<i>Cotoneaster subadpressus</i>	Ren & Tao	<i>C. yanruae</i>	KU315131
1935504	F	Yunnan, Yaoshan	27°12'N, 103°06'E	7.V.2015	<i>Cotoneaster subadpressus</i>	Ren & Tao	<i>C. yanruae</i>	KU315132
1935505	F	Yunnan, Yaoshan	27°12'N, 103°06'E	7.V.2015	<i>Berberis</i> sp.	Ren & Tao	<i>C. yanruae</i>	KU315133
1935506	F	Yunnan, Yaoshan	27°12'N, 103°06'E	7.V.2015	<i>Cotoneaster subadpressus</i>	Ren & Tao	<i>C. yanruae</i>	KU315134
1935507	F	Yunnan, Yaoshan	27°12'N, 103°06'E	7.V.2015	<i>Cotoneaster subadpressus</i>	Ren & Tao	<i>C. yanruae</i>	KU315135
1935508	M	Yunnan, Yaoshan	27°12'N, 103°06'E	7.V.2015	<i>Cotoneaster subadpressus</i>	Ren & Tao	<i>C. yanruae</i>	KU315136
1496181	M	Xizang, Linzhi, Bank of Niyang River	29°40'N, 94°21'E	15.VIII.2012	<i>Tamarix chinensis</i>	Niu	<i>C. harreri</i>	KC469665
1496182	M	Xizang, Linzhi, Bank of Niyang River	29°40'N, 94°21'E	15.VIII.2012	<i>Tamarix chinensis</i>	Niu	<i>C. harreri</i>	KC469666
1496185	F	Xizang, Linzhi, Bank of Niyang River	29°40'N, 94°21'E	16.VIII.2012	<i>Trifolium repens</i>	Niu	<i>C. harreri</i>	KC469667
1496234	M	Xizang, Linzhi, Bank of Niyang River	29°38'N, 94°22'E	26.VIII.2012	<i>Tamarix chinensis</i>	Niu	<i>C. collaris</i>	KC469644
1496259	F	Xizang, Linzhi, Ecological Research Institute of Xizang Plateau	29°40'N, 94°21'E	13.X.2009	No record	Pan	<i>C. collaris</i>	KC469649
1495768	F	Beijing, Baiyanggou	40°14'N, 115°57'E	6.IX.2011	No record	Yuan	<i>C. collaris</i>	KC469650
1495782	M	Beijing, Baiyanggou	40°14'N, 115°57'E	6.IX.2011	No record	Yuan	<i>C. collaris</i>	KC469651
1496121	F	Xizang, Linzhi, Bank of Niyang River	29°40'N, 94°21'E	11.VIII.2012	<i>Tamarix chinensis</i>	Niu	<i>C. linzhiensis</i>	KC469653

Table 1 (continued)

IOZ(E) number	Sex	Collecting site	Coordinates	Coll. Date	Floral association	Leg.	Species	Genbank accession number
1496176	F	Xizang, Linzhi, Bank of Niyang River	29°40'N, 94°21'E	15.VIII.2012	<i>Tamarix chinensis</i>	Niu	<i>C. linzhiensis</i>	KC469660
1496204	F	Xizang, Linzhi, Bank of Niyang River	29°39'N, 94°21'E	20.VIII.2012	<i>Tamarix chinensis</i>	Niu	<i>C. linzhiensis</i>	KC469671
1496167	M	Xizang, Linzhi, Bank of Niyang River	29°40'N, 94°21'E	13.VIII.2012	<i>Tamarix chinensis</i>	Niu	<i>C. linzhiensis</i>	KC469659
1496180	M	Xizang, Linzhi, Bank of Niyang River	29°40'N, 94°21'E	15.VIII.2012	<i>Tamarix chinensis</i>	Niu	<i>C. linzhiensis</i>	KC469664
1496218	M	Xizang, Linzhi, Bank of Niyang River	29°39'N, 94°21'E	20.VIII.2012	<i>Tamarix chinensis</i>	Niu	<i>C. linzhiensis</i>	KC469677
1935601	M	Yunnan, Kunming City	25°00'N, 102°14'E	2.VIII.2015	<i>Pueraria lobata</i>	Ren & P. Bernhardt	<i>Megachile subtranquilla</i>	KU315140
1935602	M	Yunnan, Kunming City	25°00'N, 102°14'E	2.VIII.2015	<i>Pueraria lobata</i>	Ren & P. Bernhardt	<i>M. subtranquilla</i>	KU315141
1935603	F	Yunnan, Kunming City	25°00'N, 102°14'E	2.VIII.2015	<i>Pueraria lobata</i>	Ren & P. Bernhardt	<i>M. subtranquilla</i>	KU315142
1935513	M	Yunnan, Yaoshan	27°05'N, 103°02'E	7.V.2015	<i>Cotoneaster subadpressus</i>	Ren & Tao	<i>Hylaeus sp3</i>	KU315137
1935514	M	Yunnan, Yaoshan	27°05'N, 103°02'E	7.V.2015	<i>Taraxacum mongolicum</i>	Ren & Tao	<i>Hylaeus sp3</i>	KU315138
1935515	F	Yunnan, Yaoshan	27°05'N, 103°02'E	7.V.2015	<i>Taraxacum mongolicum</i>	Ren & Tao	<i>Hylaeus sp4</i>	KU315139

supported branch (Fig. 13) with only one female (1935505) has a slight base pair variance at gene level. Furthermore, the strict consensus tree showed the same result (Fig. 12), i.e. the sequences from both sexes of *C. yanruae* were grouped together. All these results confirm the identity of the male specimens as *C. yanruae*. *Colletes yanruae* Niu, Zhu & Kuhlmann, 2013 was originally placed in the *C. clypearis*-group, but the male genitalia and S7 clearly show that it belongs to the *C. foveolaris*-group. Besides, the DNA sequence analyses show that *C. yanruae* grouping with *C. linzhiensis*, one member of the *C. foveolaris*-group.

***Colletes yanruae* Niu, Zhu & Kuhlmann, 2013** (Figs 2–11)

Colletes yanruae Niu, Zhu & Kuhlmann, In: Niu *et al.*, 2013b: 114 (female).

Diagnosis. Like the female, the male *C. yanruae* has narrow apical tergal hair bands (Figs 6–7) and much long hair on the scutum (Fig. 5). The male has a very large and broad S7 (Figs 10–11) that is similar in shape to that of *C. reinigi* Noskiewicz, *C. luzhouensis* Kuhlmann and *C. linzhiensis* Niu, Zhu & Kuhlmann. But *C. yanruae* differs from *C. reinigi* by the finer and denser punctation of terga, the rounded outer apical corners of S7 (shallowly incised in *C. reinigi*) and the rounded gonostylus (pointed in *C. reinigi*), and from *C. linzhiensis* by the distinctly narrower apical tergal hair bands and shorter malar area (Fig. 4). In *C. yanruae*, the gonostylus is broader than long, while it is narrower at its base and, thus, longer than broad in *C. luzhouensis* (Fig. 9). The males of both species are otherwise very similar, with only subtle differences in



Figures 2–7. *Colletes yanruae* Niu, Zhu & Kuhlmann, 2013, male. 2. Body, lateral view. 3. Head, frontal view. 4. Head, lateral view. 5. Mesonotum, dorsal view. 6. Metasoma, dorsal view. 7. T1–2, dorsal view. Scale bars = 1 mm.



Figures 8–11. *Colletes yanruae* Niu, Zhu & Kuhlmann, 2013, Male. 8. Genitalia, dorsal view. 9. Genitalia, lateral view. 10. S7, dorsal view. 11. S7, ventral view. Scale bars=0.5 mm.

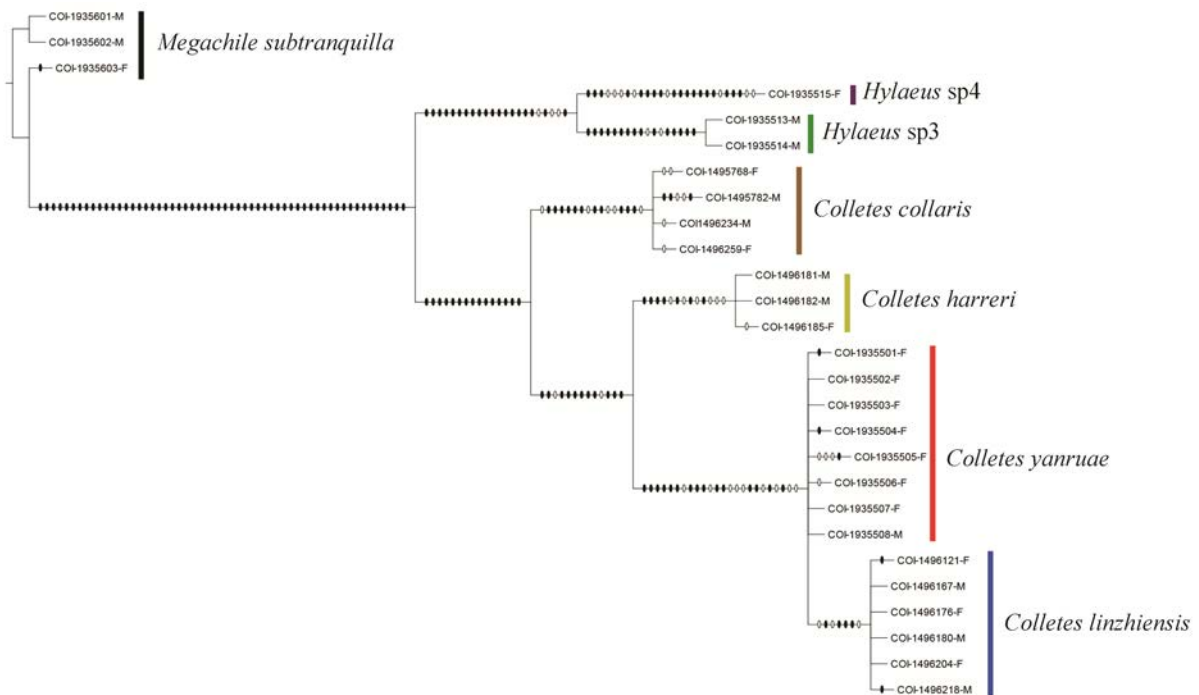


Figure 12. The strict consensus of four most-parsimonious trees based on the DNA sequences of mitochondrial COI from various specimens collected in China. Black circles represent nonhomoplastic characters, white circles represent homoplastic characters. The detailed information of the sequenced specimens and symbols of F and M is identical to that in NJ tree.

tergal punctation (punctures of T2 only slightly smaller than on T1 in *C. luzhouensis* while in *C. yanruae* punctures of T2 are about half the diameter of those on T1). The male of *C. brumalis* Noskiewicz is unknown but likely has distinctly broader apical tergal hair bands than *C. yanruae*, for the female of *C. brumalis* Noskiewicz with broader apical tergal hair bands than *C. yanruae*.

Description. Male, BL=9.5 mm (Fig. 2); head broader than long (Fig. 3), HW : HL=53 : 44; gena slightly narrower than eye in lateral view, GW : EW=9 : 12 (Fig. 4); width of metasoma as broad as that between tegulae, MtW : TW=52 : 52. Clypeus slightly convex, median part with fine oblique spine-shaped punctation (Fig. 3); antenna short, extending to the middle of scutellum, first flagellomere slightly longer than broad, 0.7 time as long as second flagellomere, flagellomeres 2–11 longer than broad, nearly 1.5 times as long as broad and nearly equal to each other in length; malar area medially shorter than width of mandible base, about 1/2 long as width of mandible base; facial fovea shallow and narrow, only half as wide as antennal flagellum; vertex behind eye rounded; propodeum laterally covered with sparse long erect hairs, integumental sculpture completely visible; disc of scutum shiny, with dense punctation, $i=0.5-1.5d$ (Fig. 5); metasomal terga with apical hair bands, the band on T1 slightly narrower medially, that on T2–T5 nearly 1/6 width as that of the related exposed terga (Fig. 6); posterior margin of T1 translucent and orange, punctation on disc of T1 round and dense, $i=0.2-0.5d$ (Fig. 7); sloping anterior and lateral anterior parts of T1 covered with erect long plumose hairs, and disc of T1 also with erect long sparse plumose hairs (Fig. 7); apical lobe of S7 large and broad, outer apical corner rounded, but with inside concave below the apical corner (Fig. 10); genitalia as showing in Fig. 8 and Fig. 9, gonostylus short and broad at its base. Antennal flagellum ventrally black; all legs black. Face covered with dense long paler white plumose hairs, intermixed with black plumose hairs; gena, scutellum and mesepisternum covered with long paler white plumose hairs; scutum covered with dense

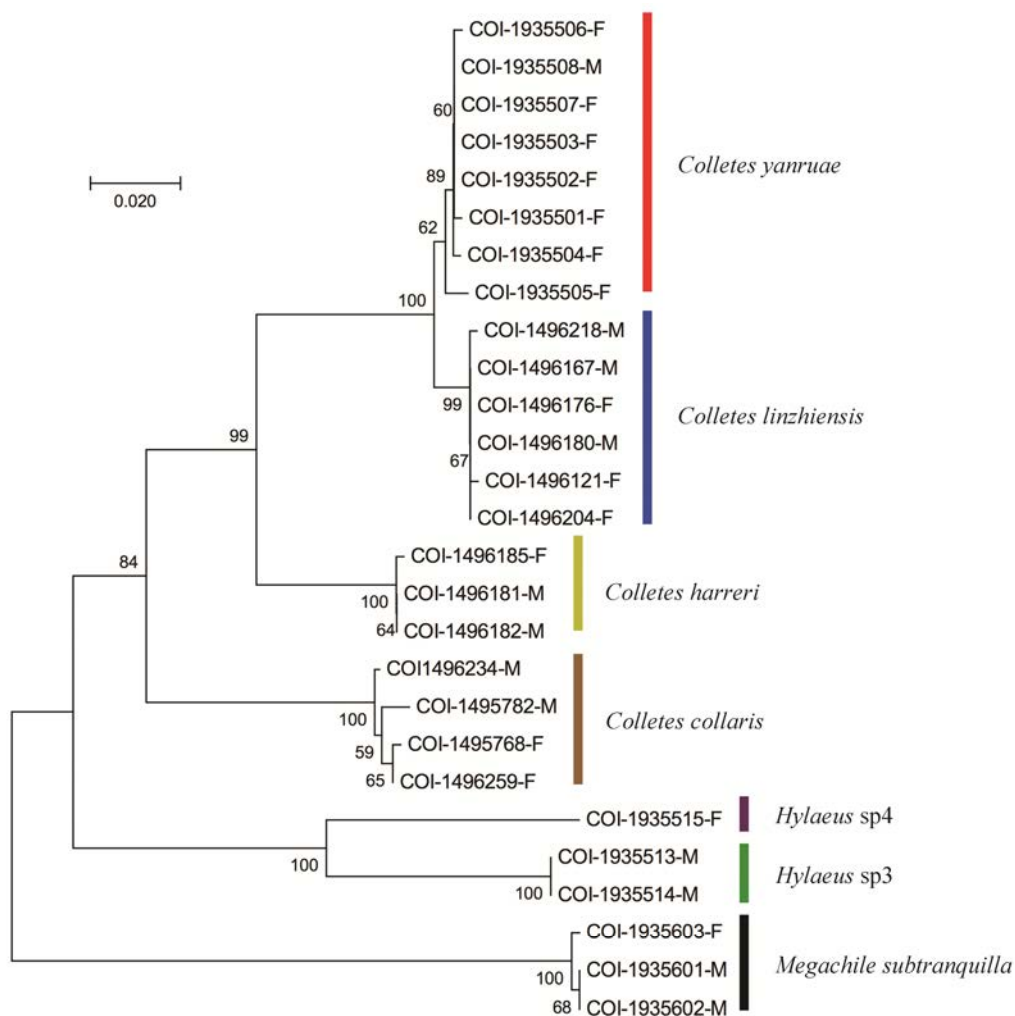


Figure 13. NJ tree based on the DNA sequences of mitochondrial COI from various specimens collected in China. For detailed information of the sequenced specimens see Table 1. The symbols of F and M represent the sex of specimens, female and male respectively.

long paler white plumose hairs, intermixed with black plumose hairs (Figs 4–5).

Material examined. China, Yunnan, Zhaotong City, Qiaojia County, Yaoshan Town (27°12'N, 103°06'E), 7♀1♂ (from *Cotoneaster subadpressus*), 1♀ (from *Berberis* sp.), 7.V.2015, leg. Zongxin Ren, Zhibin Tao.

Type Materials. Holotype. ♀, China, Yunnan, Lijiang, Yulong Shan (100°18'E, 27°06'N; elev. 2850 m), 19.VII. 1984, leg. Changfang Li; Paratypes. 3♀, China, Yunnan, Lijiang, Yulong Shan (100°18'E, 27°06'N; elev. 2850 m), 17.VII. 1984, leg. Changfang Li; 2♀, China, Yunnan, Lijiang, Baishui (103°54'E, 24°30'N; elev. 3200 m), 17.VII.1984, leg. Jianguo Fan.

Distribution. China (Yunnan).

Floral associations. *Berberis* sp. (Berberidaceae), *Cotoneaster subadpressus* (Rosaceae).

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