# Three new species of Sesioctonus Viereck (Hymenoptera, Braconidae, Agathidinae) from Peru 

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Academic editor: C. van Achterberg | Received 15 March 2012 | Accepted 15 May 2012 | Published 21 May 2012
urn:lsid:zoobank.org:pub:0345EA97-A583-4CAB-9AE8-D8463A6D063B
Citation: Sulca L, Sharkey MJ (2012) Three new species of Sesioctonus Viereck (Hymenoptera, Braconidae, Agathidinae) from Peru. ZooKeys 196: 1-10. doi: 10.3897/zookeys.196.3086


#### Abstract

Three new species of Sesioctonus (Braconidae: Agathidinae) are described and illustrated, i.e., Sesioctonus huggerti, S.wayquecha, and S. bina. Two new Peruvian species records for Sesioctonus are reported: S. longinoi and $S$. diazi. A revised key to all known species of Sesioctonus is presented.


## Keywords

Insecta, taxonomy, biodiversity, parasitoid

## Introduction

Sesioctonus Viereck, 1912 is a Neotropical genus of Agathidinae which for which the biology is largely unknown, only $S$. parathyridis is recorded as a larval parasitoid of Parathyris perspicilla Stall (Lepidoptera: Arctiidae) (Viereck 1912). Briceño (2003) revised the species of Sesioctonus and included twenty six species and Sharkey and Briceño (2005) described five new species. Here we describe three new species from Peru and two additional species are reported for the first time from Peru.

## Methods

Morphological terminology follows that of Sharkey and Wharton (1997). Figures in this paper that are followed by the letter 'B' refer to those in Briceño (2003). The species descriptions are of the holotypes with variation given in parenthesis.

Unless otherwise stated specimens are deposited in the Natural History Museum, University of San Marcos, Lima, Peru (MUSM), with duplicates deposited in the Hymenoptera Institute Collection at the University of Kentucky, USA (HIC).

## Results and discussion

## Diagnosis

Members of Sesioctonus are restricted to the Neotropical realm of the New World and may be distinguished from all other agathidine braconids with the following combination of characters: Mesoscutum smooth, lacking notauli; tarsal claws simple, lacking a basal claw; hind coxal cavities open, sharing a common opening with the metasomal foramen.

## Key to Sesioctonus species of the world, modified from Sharkey and Briceño (2003)

1 Occipital tubercles present (Figs. 16B-18B) ..... 2

- Occipital tubercles absent. (Figs. 19B) ..... 17
2(1) Epicnemial carina straight medially or absent, not indented at midline, be- tween fore coxae) (Figs. 4B, 23B) ..... 3
- Epicnemial carina bilobed medially (indented at midline, between fore coxae) (Figs. 3B, 22B) ..... 6
3(2) Epicnemial carina complete laterally (Figs. 3B, 22B) ..... 4
- Epicnemial carina incomplete or absent laterally (Fig. 23B) ..... 5
4 (3) Interantennal space with longitudinal rounded keel; face without medianlongitudinal carinaeS. garciai Briceño
- Interantennal space lacking longitudinal keel; face with median longitudinalcarinaeS. huggertii sp. n.
5(3) Face with median longitudinal carina (Fig. 13B)........S. acrolophus Briceńo- Face without median longitudinal carina (similar to Figs. 12B, 14B)
6(3) Mid coxa color variable, but not completely melanic ..... 7
- Mid coxa completely melanic ..... 10
7(6) Fore wing banded from base: yellow, black, yellow, black...S. chaconi Briceño- Fore wing infuscate (melanic)8
8(7) Fore tibia with spines; mid femur yellowish orange ..... 9

- Fore and hind coxa melanic (Fig. 1aB)...........S. philipi Sharkey \& Briceño
22(19) Median areola of metanotum with lateral carinae meeting posteriorly (Figs. 25B, 26B) ..... 23
- Median areola of metanotum with lateral carinae absent or, if present, not meeting posteriorly (Figs. 27B, 28B) ..... 32
23(22) Epicnemial carina present (Figs. 3B, 4B) ..... 24
- Epicnemial carina absent ..... 28
24(23) Epicnemial carina complete laterally (Fig. 3B) ..... 25
- Epicnemial carina incomplete laterally (Fig. 4B) ..... 30
25(24) Hind tibia entirely melanic S. amazonensis Briceńo
- Hind tibia mostly yellowish orange ..... 26
26(25) Propodeum with central areola absent. ..... 27
- Propodeum with central areola present S. areolatus Briceño
27(26) Antenna with more than 29 flagellomeres; interantennal space with roundedlongitudinal keel (similar to Fig. 12B); hind tibia yellowish orange in basalhalf, melanic apicallyS. miyayensis Briceño
- Antenna with less than 28 flagellomeres; interantennal space without longi-tudinal keel; hind tibia mostly yellowish orange, melanic apically
S. clavijoi Briceño
28(23) Scutellar depression with longitudinal carinae; body color yellow, white, and black (Fig. 1cB) S. torresi Sharkey \& Briceño
- Scutellar depression without longitudinal carinae; body color yellowish or- ange and black ..... 29
29(28) (RS+M)a vein of fore wing complete, median tergite of first metasomal seg- ment with pair of lateral longitudinal carinae S. ammosakron Briceńo
$-\quad(R S+M) a$ vein fore wing incomplete, median tergite of first metasomal seg- ment without pair of lateral longitudinal carinae S. wayquecha sp. n.
30 (24) Epicnemial carina straight medially (between fore coxae) (Fig. 4B); body length less than 3 mm . S. dominicus Briceńo
- Epicnemial carina bilobed medially (indented at midline, between fore coxae) (Fig. 3B); body length more than 3 mm ..... 31
31(30) Fore wing (RS +M )a vein complete (Fig. 10aB) S.armandoi Briceño
- $\quad$ Fore wing (RS +M )a vein incomplete (Fig. 9aB) S. biospleres Briceño
32(22) Epicnemial carina present, complete or incomplete laterally (Figs. 3B, 4B)... ..... 33
- Epicnemial carina completely absent ..... S. chrestos Briceńo
33(32) Fore wing banded, yellow, black, yellow, black; labial palpus 3-segmented....S.galeos Briceño
- Fore wing infuscate; labial palpus 4-segmented ..... S. theskelos Briceño34(18) Third and fourth labial palpomeres not fused; first metasomal median tergitewith depression posteriad spiracle (Figs. 36B, 37B).........S. grandis Briceño- Third and fourth labial palpomeres fused, first metasomal median tergite withor without depression posteriad spiracle.35

35(36) First metasomal median tergite with depression posteriad spiracle (similar to Figs. 3, 36) S. qui Briceño

- First metasomal median tergite without depression posteriad spiracle $\qquad$
S. parathyridis Viereck


## New Species Descriptions

Sesioctonus huggerti Sulca \& Sharkey, sp. n.
urn:lsid:zoobank.org:act:A198E0BC-7DFE-42CB-B5AD-9DF7E63597E6
http://species-id.net/wiki/Sesioctonus_huggerti
Figure 1

Diagnosis. Distinguished from all other known species of Sesioctonus by the following suite of characters: Interantennal space lacking longitudinal keel, epicnemial carinae straight medially.

Description. $q$ Length. Length of body, excluding ovipositor, 5 mm .
Head. Flagellum with 30 flagellomeres. Interantennal space lacking longitudinal keel. Antennal sockets moderately excavated. Face with median longitudinal carina. Gena not expanded posteroventrally. Occipital tubercles present. Occiput not excavated. Mandible concave. Outer tooth of mandible not longer than inner tooth. Maxillary palpus with 4 palpomeres. Third and fourth labial palpomeres not fused. Mesosoma. Subpronope elongate-oval. Longitudinal carinae of scutellar depression absent. Scutellum convex. Median areola of metanotum smooth, with median longitudinal carina, and with lateral carinae present and not meeting posteriorly. Propodeum convex. Median longitudinal carina of propodeum absent. Epicnemial carina complete, sharp, straight medially (between fore coxae). Hind femur 6 times as long as wide. ( $R S+M$ )a vein of fore wing incomplete. 3RSa vein of fore wing absent. 2-1A vein of hind wing not tubular. Cub vein of hind wing not tubular. Metasoma. Median tergite of first metasomal segment without pair of lateral longitudinal carinae. Hind wing with 4 hamuli. First metasomal median tergite without depression posteriad spiracle. Length/width ratio of first metasomal median tergite 0.63 . Ovipositor 4 mm .

Color. Head melanic. Maxillary palpomeres melanic. Labial palpomeres melanic. Pronotum melanic. Mesoscutum yellowish orange. Scutellum yellowish orange. Metanotum yellowish orange. Propodeum melanic. Propleuron melanic. Mesopleuron yellowish orange. Metapleuron melanic. Fore coxa melanic. Fore trochanter melanic. Fore trochantellus melanic. Fore femur melanic. Fore tibia melanic. Fore tarsus melanic. Mid coxa melanic. Mid trochanter melanic. Mid trochantellus melanic. Midfemur melanic. Mid tibia melanic. Mid tarsus melanic. Hind coxa melanic. Hind trochanter melanic. Hind trochantellus melanic. Hind femur melanic. Hind tibia melanic. Hind tarsus melanic. Fore wing entirely infuscate. Stigma melanic. Hind wing entirely infuscate. First metasomal tergum melanic. Second metasomal tergum me-


Figure I. Sesioctonus huggerti. Ia dorsal habitus Ib lateral habitus Ic wings Id propodeum and first metasomal segment.
lanic. Third metasomal tergum melanic. Fourth metasomal tergum melanic. Fifth to eighth metasomal terga melanic. Ovipositor yellowish orange.
${ }^{\top}$ Unknown.
Etymology. Named in honor of the late Lars Huggert who collected the type specimen.
Holotype. PERU, Madre de Dios, Puerto Maldonado, 6-11.i.1984, L. Huggert Leg. (Canadian National Collection).

Distribution. Known only from the type locality in Peru.

## Sesioctonus wayquecha Sulca \& Sharkey, sp. n.

 urn:lsid:zoobank.org:act:19BD24A0-162D-405A-8BF0-5CDA62C5FE86 http://species-id.net/wiki/Sesioctonus_wayquechaFigure 2 a,b,c,d

Diagnosis. Distinguished from all other known species of Sesioctonus by the following suite of characters: occipital tubercles absent, epicnemial carina completely absent, antennal socket not excavated, gena moderately expanded posteroventrally .

Description. $P$ Length. Length of body, excluding ovipositor, $4.3-5.5 \mathrm{~mm}$.
Head. Flagellum with 31 flagellomeres. Interantennal space lacking longitudinal keel. Antennal sockets not excavated. Face without median longitudinal carina. Gena moderately expanded posteroventrally. Occipital tubercles absent. Occiput not excavated. Mandible concave. Outer tooth of mandible not longer than inner tooth. Maxillary palpus with 5 palpomeres. Third and fourth labial palpomeres not fused. Mesosoma. Subpronope elongate-oval. Longitudinal carinae of scutellar depression absent. Scutellum convex. Median areola of metanotum smooth, without median longitudinal carina, and with lateral carinae present and meeting posteriorly. Propodeum convex. Median longitudinal carina of propodeum absent. Epicnemial carina completely absent. Fore tibial spines present. Mid tibia with 7 spines. Hind tibia with 15 spines. Hind femur 3.3-4 times as long as wide. (RS+M)a vein of fore wing complete. 3RSa


Figure 2. Sesioctonus wayquecha. 2a lateral habitus $\mathbf{2 b}$ wings $\mathbf{2 c}$ dorsal scutellum and propodeum $\mathbf{2 d}$ anterior head $\mathbf{2 e}$ dorsal habitus.
vein of fore wing absent. $2-1 \mathrm{~A}$ vein of hind wing not tubular. Cub vein of hind wing not tubular. Metasoma. Median tergite of first metasomal segment without pair of lateral longitudinal carinae. Hind wing with 4 hamuli. First metasomal median tergite without depression posteriad spiracle. Length/width ratio of first metasomal median tergite 0.63 . Ovipositor $0.5-0.6 \mathrm{~mm}$.

Color. Head melanic. Antenna melanic. Maxillary palpomeres yellowish orange. Labial palpomeres yellowish orange. Pronotum melanic. Mesoscutum melanic. Scutellum melanic. Metanotum melanic. Propodeum mostly yellowish orange with melanic spots. Propleuron mostly melanic with yellowish orange areas. Mesopleuron melanic. Metapleuron yellowish orange. Fore coxa yellowish orange. Fore trochanter yellowish orange. Fore trochantellus yellowish orange. Fore femur yellowish orange. Fore tibia melanic with yellowish orange ends. Fore tarsus melanic. Mid coxa yellowish orange. Mid trochanter yellowish orange. Mid trochantellus yellowish orange. Mid femur yellowish orange. Mid tibia melanic. Mid tarsus melanic. Hind coxa yellowish orange. Hind trochanter yellowish orange. Hind trochantellus yellowish orange. Hind femur yellowish orange. Hind tibia melanic with a yellow orange apical spot. Hind tarsus melanic. Fore wing entirely infuscate. Stigma melanic. Hind wing entirely infuscate. First metasomal tergum yellowish orange. Second metasomal tergum yellowish orange. Third metasomal tergum yellowish orange. Fourth metasomal tergum yellowish orange but median tergum melanic. Fifth to eighth metasomal terga melanic. Ovipositor yellowish orange.
$\delta$. As in the female (above).

Etymology．Named after the type locality，Wayquecha which means＇brother＇in Quechua．

Holotype．PERU．Q，Cusco，Wayquecha， $13^{\circ} 11^{\prime} 21^{\prime \prime} \mathrm{S}, 71^{\circ} 35^{\prime} 04^{\prime \prime} \mathrm{W} 2837 \mathrm{~m}$ ，6－ 20．x．2007，C．Castillo．Leg．

Paratypes：PERU：Cusco： 2 早果，Wayquecha， $13^{\circ} 11^{\prime} 21^{\prime \prime} \mathrm{S}, 71^{\circ} 35^{\prime} 4^{\prime \prime} \mathrm{W}, 2837 \mathrm{~m}, \mathrm{Ma}-$ laise，20．x．2007，C．Castillo Leg．； 3 q q，$^{\text {T，Wayquecha，}} 13^{\circ} 10^{\prime} 31^{\prime \prime} \mathrm{S}, 71^{\circ} 34^{\prime} 53^{\prime \prime} \mathrm{W}$ ， 2692 m, Malaise，10．ix．2007，C．Castillo Leg．；$q$ ，Wayquecha $13^{\circ} 11^{\prime} \mathrm{S}, 71^{\circ} 35^{\prime} \mathrm{W}$ ， 2800 m ，sweep，12．ix．2007，C．Castillo Leg；đo Wayquecha， $13^{\circ} 10^{\prime} 311^{\prime \prime} \mathrm{S}, 71^{\circ} 34^{\prime} 53^{\prime \prime}$ ， 2692m，Malaise，22．x．2007，C．Castillo Leg．

Distribution．Known only from one locality in Peru．

## Sesioctonus bina Sulca \＆Sharkey，sp．n． urn：lsid：zoobank．org：act：5AE5EEED－ACF8－47D4－8189－531FFDBB1209 <br> http：／／species－id．net／wiki／Sesioctonus＿bina

Figure 3

Diagnosis．Distinguished from all other known species of Sesioctonus by the follow－ ing suite of characters：occiput not excavated，subpronope oval，median tergite of first metasomal segment with pair of lateral longitudinal carinae．

Description．$Q$ Length．Length of body，excluding ovipositor， 3.35 mm ．Flagel－ lum broken after flagellomere 28．Interantennal space with longitudinal rounded keel． Antennal sockets moderately excavated．Face without median longitudinal carina． Gena not expanded posteroventrally．Occipital tubercles present．Occiput not exca－ vated．Mandible concave．Outer tooth of mandible not longer than inner tooth．Max－ illary palpus with 4 palpomeres．Mesosoma．Subpronope oval．Longitudinal carinae of scutellar depression absent．Scutellum convex．Median areola of metanotum smooth， without median longitudinal carina，and with lateral carinae present and meeting pos－ teriorly．Propodeum convex．Median longitudinal carina of propodeum present．Epic－ nemial carina complete，blunt，bilobed medially（between fore coxae）．Fore tibial spines present．Mid tibia with 3 spines．Hind tibia with 12 spines．Hind femur 4 times as long as wide．$(R S+M)$ a vein of fore wing incomplete． $3 R S$ a vein of fore wing present．2－1A vein of hind wing tubular．Cub vein of hind wing not tubular．Metasoma．Median tergite of first metasomal segment with pair of lateral longitudinal carinae．Hind wing with 3 hamuli．First metasomal median tergite without depression posteriad spiracle． Length width ratio of first metasomal median tergite 0.5 ．Ovipositor 1.68 mm ．

Color．Head black and yellowish orange．Antenna melanic．Maxillary palpomeres yellowish orange．Labial palpomeres yellowish orange．Pronotum melanic．Mesoscutum melanic．Scutellum melanic．Metanotum melanic．Propodeum melanic．Propleuron me－ lanic．Mesopleuron melanic．Metapleuron melanic．Fore coxa yellowish orange．Fore trochanter yellowish orange．Fore trochantellus yellowish orange．Fore femur yellow－ ish orange．Fore tibia yellowish orange．Fore tarsus mostly yellowish orange，but apical tarsomere melanic．Mid coxa yellowish orange．Mid trochanter yellowish orange．Mid


Figure 3. Sesioctonus bina. 3a lateral head and mesosoma $\mathbf{3} \mathbf{b}$ lateral habitus $\mathbf{3} \mathbf{c}$ wings $\mathbf{3 d}$ propodeum and first metasomal segment.
trochantellus yellowish orange. Mid femur yellowish orange. Mid tibia yellowish orange in basal half, melanic apically, or yellowish orange basally, otherwise melanic. Mid tarsus melanic. Hind coxa melanic. Hind trochanter melanic. Hind trochantellus melanic. Hind femur melanic. Hind tibia melanic in basal and apical third, yellowish orange medially. Hind tarsus melanic. Fore wing entirely infuscate. Stigma melanic. Hind wing entirely infuscate. First metasomal tergum melanic. Second metasomal tergum yellowish orange but median tergite melanic. Third metasomal tergum melanic. Fourth metasomal tergum melanic. Fifth to eighth metasomal terga melanic. Ovipositor yellowish orange.

ふunknown
Etymology. Bina means 'wasp' in Shipibo, an indigenous language of the Peruvian Amazon.

Holotype. Q, PERU, Cusco, Rocotal, 16.ix.2007, Sweep, C. Castillo Leg.

## New Peruvian Distribution Records

## Sesioctonus longinoi

+, Cusco, Cosñipata valley, San Pedro, $13^{\circ} 03^{\prime} 23^{\prime \prime} \mathrm{S}, 71^{\circ} 32^{\prime} 55^{\prime \prime} \mathrm{W}, 1520 \mathrm{~m}$, Malaise,
 laise, C. Castillo. leg.

## Sesioctonus diazi

Q , Cusco, Reserva Comunal Amarakaeri, Rio Azul, $12^{\circ} 49,8^{\prime} 24^{\prime \prime} \mathrm{S}, 71^{\circ} 05^{\prime} 55^{\prime \prime} \mathrm{W}, 507 \mathrm{~m}$, 11.x.2010. C.Castillo. leg. P, Loreto, Alto Nanay, Albarenga north, 18M 0533605 $9645694,142 \mathrm{~m}, \mathrm{C}$. Castillo leg.

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# Ethanol fuel improves arthropod capture in pitfall traps and preserves DNA 

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Academic editor: Terry Erwin | Received 26 March 2012 | Accepted 15 May 2012 | Published 21 May 2012
Citation: Szinwelski N, Fialho VS, Yotoko KSC, Seleme LR, Sperber C (2012) Ethanol fuel improves arthropod capture in pitfall traps and preserves DNA. ZooKeys 196: 11-22. doi: 10.3897/zookeys.196.3130


#### Abstract

We tested the value of ethanol fuel as a killing solution in terms of sampling efficiency (species richness and accumulated abundance) and DNA preservation of Ensifera ground-dwelling specimens. Sampling efficiency was evaluated comparing abundance and species richness of pitfall sampling using $100 \%$ ethanol fuel, with two alternative killing solutions. We evaluated the DNA preservation efficiency of the killing solutions and of alternative storage solutions. Ethanol fuel was the most efficient killing solution, and allowed successful DNA preservation. This solution is cheaper than other preserving liquids, and is easily acquired near field study sites since it is available at every fuel station in Brazil and at an increasing number of fuel stations in the U.S. We recommend the use of ethanol fuel as a killing and storage solution, because it is a cheap and efficient alternative for large-scale arthropod sampling, both logistically and for DNA preservation. For open habitat sampling with high day temperatures, we recommend doubling the solution volume to cope with high evaporation, increasing its efficacy over two days.


## Keywords

Killing solutions, molecular tools, taxonomy, large-scale fieldwork, Brazil

## Introduction

Several sampling techniques are used to assess biodiversity of different animal species (King and Porter 2005). All present advantages and disadvantages, so the choice is at the discretion of the researcher. Small organisms (e.g. arthropods) are frequently hand-
sampled, which provides information on the organism's habits and behavior, but this method is of little use for ecological comparisons, because of collector interference (Krebs 1999, Southwood and Henderson 2000).

Pitfall traps are a good alternative for collecting ground-dwelling arthropods (Dahl 1896). This kind of trap is inexpensive and easy to handle, allowing both rich and abundant samples. It can be used for taxonomic (although some coloration characters may be lost), ecological, morphological and molecular studies (Gurdebeke and Maelfait 2002, Schoereder et al. 2004, Sperber et al. 2007, Mews et al. 2008, Pereira et al. 2010). One of the main challenges is deciding which killing solution to use in the pitfall traps, which depends on the objectives of each study. As far as sampling involves financial, environmental and researcher's effort costs, the ideal solution should minimize those costs and maximize the utility of the sampled material. The utility of the samples may extrapolate strictly ecological purposes, and should involve other scientific areas, such as morphology and molecular biology. Therefore an ideal should also preserve the specimens' tissues and DNA (Stevens et al. 2011).

Regarding methodological necessities in pitfall sampling, a good killing solution should minimize evaporation, as far as many pitfall trap regimes check traps every 2 weeks or more. A good solution should not be toxic to the researcher nor environmentally harmful. Regarding sampling efficiency, a good solution should kill quickly so as to reduce the escape of specimens. In addition, the trap solution cannot be prohibitively expensive, and must be readily available.

Finding a solution that meets all of these specifications is not easy. Many types of solutions have been used and tested, for example water and detergent, which is inexpensive but accelerates the decomposition of tissues and genetic material (Schmidt et al. 2006). Mixtures of formaldehyde and ethylene glycol (Barber 1931, Sperber et al. 2003b, Schmidt et al. 2006), are efficient in killing and preserving tissue, but are toxic and do not preserve DNA (Aristophanous 2010). Other solutions contain salt brines (Sasakawa 2007) and acetic acid (Gurdebeke and Maelfait 2002), which do not preserve tissues and can alter gonads, genitalia and eggs (Sasakawa 2007). An additional class of solutions contains different concentrations of commercial alcohol (Sperber et al. 2003a, Paquin 2008, Chen et al. 2011), which evaporates faster than the other solutions, but preserves the internal and external organs through tissue dehydration.

It has been shown that at concentrations higher than $95 \%$, commercial alcohol preserves DNA (Nagy 2010), but the use of highly concentrated commercial alcohol as a killing solution may be prohibitively expensive when needed in large quantities, such as in large-scale biodiversity sampling. In Brazil, for example, it is illegal to carry large amounts of commercial alcohol on long journeys, which could hinder its use in extensive field expeditions. Here we propose the use of ethanol fuel as a cheaper and logistically feasible alternative.

In Brazil, ethanol fuel and commercial alcohol have some differences. While the alcoholic concentration ( 92.6 to $93.8 \%$ ) and the amount of water ( 6.2 to $7.4 \%$ ) varies in ethanol fuel, in commercial alcohol the alcoholic concentration (92.8\%) and the amount
of water $(7.2 \%)$ is fixed. The largest difference is, however, the quantity of gasoline present in ethanol fuel (up to 30 milliliters per liter), that is absent in commercial alcohol (BR0029 2011). In the United States, the highest concentration of ethanol fuel includes $85 \%$ ethanol and $15 \%$ gasoline (Tatum 2010). Ethanol fuel is available throughout Brazil, at all fuel stations, and at an increasing number of fuel stations in the U.S. (Méjean and Hope 2010, Sorda et al. 2010) and is at least $50 \%$ cheaper than commercial alcohol.

In this study, we tested the value of ethanol fuel as a pitfall trap killing solution in terms of sampling efficiency (richness and abundance) and DNA preservation of Ensifera ground-dwelling specimens, comparing $100 \%$ ethanol fuel with two alternative killing solutions.

## Material and methods

## Sampling efficiency

Field sampling site
To evaluate sampling efficiency, we conducted field sampling in a primary Atlantic Forest reservoir, the Iguaçu National Park, in Foz do Iguaçu municipality ( $25^{\circ} 32^{\prime}$ S, $54^{\circ} 35^{\prime} \mathrm{W}, 195 \mathrm{~m}$ above sea level), Paraná State, in January 2010. The vegetation is mostly tropical semideciduous forest and Araucaria forest, within the Atlantic Forest biome (Rizzini 1997, Dias et al. 1998). The climate is mesothermal subtropical superhumid, with average annual temperatures between 18 and $20^{\circ} \mathrm{C}$ and an average rainfall of 1600 mm (Peel et al. 2007).

## Sampling design

We compared the efficiency of $100 \%$ ethanol fuel pitfall killing solution (Solution 1) for ground-dwelling Orthoptera, against the conventional killing solution, comprised of $80 \%$ commercial alcohol $\left(80^{\circ} \mathrm{GL}\right)+10 \%$ glycerin (P.A) $+10 \%$ formaldehyde (P.A) (Sperber et al. 2003b) (Solution 2), and a solution of $90 \%$ commercial alcohol $\left(80^{\circ} \mathrm{GL}\right)+10 \%$ glycerin (P.A) (Solution 3). GL is the amount, in milliliters, of absolute alcohol contained in 100 milliliters of hydro-alcoholic solution. P.A., or 'Pro Analysis' means that the sample is of a very high purity, sufficient to be used in chemical analyses. Formaldehyde is recommended for better preservation; glycerin is used to prevent stiffening of the sampled specimens.

For this comparison, we designed the following field experiment. We established a transect of 5 km , starting at a distance of 100 m from the forest's edge. At the beginning of the transect a set of five pitfall traps, containing one of the three killing solutions chosen randomly, were placed perpendicularly to the transect, 2 m apart from one another. After the next 30 m on the transect, we placed the second set with a different, randomly
chosen, killing solution. After another 30 m along the transect, we placed the third set, with the third killing solution. After an additional forty meters we began the procedure again, and repeated it a total of 50 sampling stations. In summary each sampling station contained five pitfall traps with each of the three killing solutions, for a total sampling effort of 750 pitfall traps. Traps consisted of polyethylene vials, 20 cm in diameter and 22 cm deep, filled with 500 ml of killing solution. After 48 hours, specimens were removed from the the traps, identified and stored in ethanol fuel, after gathering the data.

## Data analysis

To evaluate sampling efficiency of ethanol fuel as a pitfall killing solution, we compared cricket species richness and accumulated abundance (= total number of individuals per pitfall set) among the three solutions. Each pitfall set was considered one sampling unit, rendering 150 replicates. We performed one-way analysis of variance (ANOVA), adjusting generalized linear models (GLMs) with Poisson error distribution, correcting for over- or under-dispersion using quasi-Poisson when necessary. We considered cricket species richness and accumulated abundance in each set of five pitfall traps as response variables ( $\mathrm{n}=150$ ), and the type of killing solution as the explanatory factor. We used contrast analyses to evaluate effect differences among the kinds of solution, simplifying the complete models by amalgamating non-significantly different factor levels (Crawley 2007). We used Chi-square ( $\chi^{2}$ ) test for Poisson error distributions, and the $F$ test in cases where there was a correction for over- or under-dispersion, as recommended by Zuur et al. (2009). We checked residuals for homoscedasticity. All analyses were undertaken within the R 2.15 environment (R Development Core Team 2012).

## DNA preservation

## Killing and storage

To test the DNA preservation properties of each pitfall killing solution, we placed each of 18 living cricket specimens of Gryllus sp. (not identified) into one of the three pitfall killing solutions, totaling six specimens per solution. As a control, we separately placed another six crickets into undiluted commercial alcohol ( $92.8^{\circ} \mathrm{GL}$ ), which is considered a good preservative of DNA (Nagy 2010). Twenty-four hours later, we took one leg of each individual and extracted its DNA. Twenty-four hours later (i.e. 48 hours after immersion into the killing solution), we removed a second leg off the crickets to evaluate DNA preservation, analogous to in the field procedure collecting time of 48 hours, as recommended by Sperber et al. (2003a) for ground-dwelling Orthoptera sampling.

To evaluate the efficiency of ethanol fuel as a storage solution, we stored each cricket specimen, after 48 hours in the killing solution, in one of two storage solutions: undiluted commercial alcohol $\left(92.8^{\circ} \mathrm{GL}\right)$ or undiluted ethanol fuel. To test
the effect of time and type of storage solution on the DNA preservation efficiency, we removed a third leg off each cricket after 15 days, and a fourth leg after 30 days in the storage solution.

We evaluated efficiency of DNA preservation for the 24 crickets used in the above procedure. Each set of six individuals was submitted to one of four different killing solutions, and each individual provided two samples (= legs) for DNA extraction before storage ( 24 and 48 hours in the killing solution). Individuals from each killing solution were transferred to either commercial alcohol or ethanol fuel for storage, providing three replicates (individuals) per storage solution, and two further samples (= legs) per individual, 15 and 30 days in the storage solution. All specimens were maintained at room temperature for 30 days.

## DNA extraction

Total DNA was isolated from each individual using the protocol described in Waldschmidt et al. (1997) but without the deproteinization step with phenol:chloroform (1:1). Preliminary analysis of fresh specimens killed by freezing showed that tissue extractions from the thorax or legs were equally effective. Therefore, we chose to use only the legs, allowing maximum preservation of anatomical parts for further studies, and repeated sampling of the same individuals with minimum tissue damage.

DNA extractions were verified via agarose gel ( $0.8 \%$ ) electrophorese, prepared and run in 1X TBE Buffer, stained with ethidium bromide and viewed under UV light. The quality of the extractions was checked by comparison with the extract made from fresh material (specimens that were killed by freezing, with immediate DNA extraction). Extractions from fresh material presented two bands, the first clearly marked and bright, corresponding to genomic DNA and the second smaller, more opaque, corresponding to RNA. We considered DNA as properly preserved when we detected a well-defined single band of DNA without apparent trawlers.

## Results

## Sampling efficiency

We collected 3,528 individuals of 14 species from four different families of Orthoptera, following the classification of Desutter-Grandcolas (1987, 1988): Phalangopsidae (2,090 individuals of eight species), Trigonidiidae ( 835 individuals of two species), Gryllidae (394 individuals of two species) and Eneopteridae (209 individuals of two species). Species richness ( $\mathrm{F}_{2,147}=177.09 ; \mathrm{p}<0.001$ ) and abundance $\left(\mathrm{F}_{2,147}=104.64\right.$; $\mathrm{p}<0.001$ ) were significantly higher in pitfalls with ethanol fuel killing solution (Figure $1 \mathrm{~A}, \mathrm{~B})$ than in those containing the other two solutions. Sampling efficiency was not different between killing solution 2 and 3 (richness: $\mathrm{F}_{2,147}=0.34 ; \mathrm{p}=0.55$; abundance: $\mathrm{F}_{2,147}=2.87 ; \mathrm{p}=0.09$ ).


Figure I. Boxplot showing sampling efficiency of different kinds of pitfall traps' killing solution. Traps with Solution 1 ( $100 \%$ ethanol fuel) captured more species and individuals than Solution 2 ( $80 \%$ commercial alcohol ( $80^{\circ} \mathrm{GL}$ ) + 10\% glycerin (P.A) + 10\% formaldehyde (P.A)) and Solution 3 ( $90 \%$ commercial alcohol $\left(80^{\circ} \mathrm{GL}\right)+10 \%$ glycerin (P.A)). A Total number of species per pitfalls' set. B Total number of individuals per pitfalls' set. Different lower case letters correspond to significant differences between killing solution levels, evaluated through contrast analyses.

## DNA Preservation

Table 1 indicates that both solution 1 and solution 3 were efficient in preserving DNA and are appropriate for use as killing solutions in pitfall traps that must remain in the field for up to 48 hours, with no visible damage to DNA. In addition, these samples can be stored at room temperature for up to 30 days in either commercial alcohol or ethanol fuel. On the other hand, our results suggest that just 24 hours in solution 2 (commercial alcohol + glycerin + formaldehyde) are enough to destroy the DNA of the samples (Figure 2).

Table I. Success (yes) or failure (no) of DNA extractions after different periods (Time in the solution) in Killing solution (Pitfall: 24 h and 48 h ) and in storage solution (C.A. and E.F.: 15 and 30 days). C.A. $=$ undiluted commercial alcohol ( $92.8^{\circ} \mathrm{GL}$ ); E.F. $=$ undiluted ethanol fuel; Solution $1=$ E.F.; Solution $2=$ $80 \%$ commercial alcohol $\left(80^{\circ} \mathrm{GL}\right)+10 \%$ glycerin (P.A.) $+10 \%$ formaldehyde (P.A.); Solution $3=90 \%$ commercial alcohol $\left(80^{\circ} \mathrm{GL}\right)+10 \%$ glycerin (P.A.). All material was maintained at room temperature. Asterisks mark the treatments shown in Figure 2.

| Killing solutions | Time in the solution |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Pitfall |  | C.A. |  | E.F. |  |
|  | 24h | 48h | 15days | 30days | 15days | 30days |
| C.A. | yes | yes | yes | yes* | yes | yes* |
| Solution 1 | yes | yes | yes | yes* | yes | yes* |
| Solution 2 | no* | - | - | - | - | - |
| Solution 3 | yes | yes | yes | yes* | yes | yes* |



Figure 2. Electrophoresis of all 24 analyzed individuals. M represents the lambda DNA marker ( 100 ng / ul ) and F represents the control extraction made using fresh tissue. A) Lanes $01-06$, individuals killed in C.A. (undiluted commercial alcohol), maintained in the killing solution for 48 hours and then transferred to closed vials containing C.A. $(01-03)$ and E.F. $(03-06)$ and maintained in these storage solutions for 30 days. Lanes $07-12$, individuals killed in Solution 1 (= E.F.), maintained in the killing solution for 48 hours and transferred to C.A. $(07-09)$ and E.F. $(10-12)$ and maintained in these storage solutions for 30 days. B) Lanes $13-18$, individuals killed in the Solution 2 and maintained in this solution for 24 hours. Lanes $19-24$, individuals killed in Solution 3, maintained in this solution for 48 hours, than transferred to C.A. $(19-21)$ and E.F. $(22-24)$ and maintained in these solutions for 30 days. All DNA extractions where successful, but those of crickets killed in solution 2 (lanes $13-18$ ).

## Discussion

In this study, we investigated the efficiency of ethanol fuel as a pitfall killing solution in terms sampling efficiency, as measured by species richness and accumulated abundance, and in terms of DNA preservation. Our results indicate increased sampling and preservation efficiency of ethanol fuel, compared to the commonly used alternatives. Below we discuss the advantages and disadvantages of using ethanol fuel as a pitfall killing and storage solution, with particular emphasis on large-scale field expeditions.

## Financial costs

Of the solutions tested in our study, ethanol fuel is the least expensive option: 1 liter of ethanol fuel (US\$ 1.25 on average) costs less than half the price of 1 liter of commercial alcohol (US\$ 3.15), which does not include the other components, such as glycerin and formaldehyde, which cost around US\$ 15.00 a liter (prices for Brazil).

## Field logistics

The transportation of flammable or toxic liquids is dangerous and illegal under Brazilian and international law. This danger increases with the distance, and consequently time spent in transportation. Ethanol fuel presents a partial solution to this limitation: as it can be bought near the field study sites, at any fuel station in Brazil, the distance of transportation is diminished, decreasing the danger. Large field expeditions can use these facilities to reduce the distances of ethanol transportation, thus reducing the risks of accidents, and simplifying expedition logistics. Even so, for transportations and storage of collected material, we recommend using firm, pressure-resistant bottles, with sealed caps, fully filled with ethanol, so as to minimize oxygen within the bottle, reducing explosion risks. We used PET tubes, which have low costs and may be bought in large quantities.

Commercial alcohol has to be purchased in large shops when bought in large quantities, and is hardly available in the small towns that border most of the large conservation areas. Therefore it would require long-distance transportation and represent huge environmental and personal risks. The additional components of the tested killing solutions (glycerin and formaldehyde), are only available in specialized establishments, restricted to a few large cities in Brazil (Brazilian Federal Law n ${ }^{\circ} 10.357 / 2001$ ).

## Sampling efficiency

We showed that ethanol fuel presented higher sampling efficiency, both for species richness and accumulated abundance of ground-dwelling Orthoptera species, therefore maximizing the gains of the sampling effort. We hypothesize that this higher sampling efficiency is related to the lower density and surface tension of the solution 1 (density $=0.81 \mathrm{~g} / \mathrm{cm}^{3}$; surface tension $=21.55 \mathrm{mN} / \mathrm{m}^{-1}$ ) than solution 2 (density $=0.92 \mathrm{~g} / \mathrm{cm}^{3}$; surface tension $=48.56 \mathrm{mN} / \mathrm{m}^{-1}$ ) and solution 3 (density $=0.97 \mathrm{~g} / \mathrm{cm}^{3}$; surface tension $=55.34 \mathrm{mN} / \mathrm{m}^{-1}$ ) (Adamson and Gast 1997), which could cause the crickets to sink and die faster in ethanol fuel, reducing their chances of escape from the trap.

One piece of evidence in favor of our hypothesis is that all winged cricket species captured in this study died exclusively within pitfalls that used ethanol fuel as the killing solution (94 individuals of Eneoptera sp. and 183 individuals of Gryllus sp.). These genera contain species of large body size, which are powerful jumpers as nymphs and powerful fliers as adults, and are rarely captured in conventional pitfall traps killing solution (N. Szinwelski, personal observation). Indeed, C.F. Sperber, in other field collections, has observed adults of Eneoptera sp. flying out of pitfalls with water + detergent killing solution. The alternative pitfall design used to prevent escape from traps, using an inverted funnel at the trap's top (Melbourne et al. 1997), may reduce sampling efficiency, especially for good jumpers and fliers.

## DNA preservation efficiency

To obtain DNA samples, it is recommended that the sampled organisms be removed from the pitfall killing solution as soon as possible and placed in vials containing highly concentrated alcohol, preferably at low temperatures (Nagy 2010). Based on the results presented here, we suggest that sampled organisms may be safely stored in undiluted ethanol fuel at room temperature, without major damage to DNA quality, for up to 30 days.

Indeed, we were able to obtain sequences of mitochondrial DNA (COI) and nuclear (18S rRNA) of Orthoptera specimens kept for two weeks in ethanol fuel killing solution, before being sorted and stored in undiluted commercial ethanol $\left(92.8^{\circ} \mathrm{GL}\right)$, where they remained at $38^{\circ} \mathrm{C}-45^{\circ} \mathrm{C}$ room temperature for another 45 days (in Manaus - AM) and 70 days at similar temperature (in Cuiabá - MT).

## Counterarguments

One of the main arguments against the use of ethanol fuel as a pitfall trap killing solution is that it evaporates faster than other solutions, making its use limited to high temperature areas. We were, however, able to use ethanol fuel pitfall traps successfully in Amazon forest sampling $\left(38^{\circ} \mathrm{C}-45^{\circ} \mathrm{C}\right)$, where the traps were kept for 48 h in the field without significant volume reduction of the killing solution.

Solution evaporation is a limiting factor in open habitat with high temperatures as Brazilian "Campo Cerrado", for example. In such field conditions, we recommend increasing the killing solution volume by $100 \%$, from 500 ml to 1000 ml , to maintain sufficient killing solution volume in the traps after 48 h in the field.

Another problem with ethanol fuel is the fact that it can be denatured. In Brazil, that means that every liter of ethanol fuel can contain up to 30 ml of gasoline. In the United States every liter of ethanol E85 contain 150 ml of gasoline. This may represent an environmental problem if the pitfall is damaged and the solution is spread in the environment. Moreover, gasoline might hinder DNA preservation. For Brazilian ethanol fuel we showed that this did not occur. Even specimens collected in ethanol fuel, were successfully preserved and we were able to extract DNA and run PCR reactions obtaining sequences of mitochondrial COI and nuclear rRNA18S .

## Acknowledgments

We thank I. Brol, L. Szinwelski, S. Oliveira for assistance in the field and N. S. Cardias and I. L. Brol for help in cricket screening. Field facilities were provided by CCZ - Foz do Iguaçu and Iguaçu National Park. This paper is part of Ph.D. theses by N. Szin-
welski and M.Sc. theses by V.S. Fialho to be presented to the Postgraduate Program in Entomology at UFV. N. Szinwelski and V.S. Fialho were sponsored by CNPq. This study was supported by research grants by CNPq, CAPES, FAPEMIG and SISBIOTA (CNPq/FAPEMIG - 5653360/2010-0).

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# A new golden frog species of the genus Diasporus (Amphibia, Eleutherodactylidae) from the Cordillera Central, western Panama 

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Academic editor: F. Andreone | Received 27 January 2012| Accepted 7 May $2012 \mid$ Published 21 May 2012
urn:lsid:zoobank.org:pub:41280300-1E31-4062-A7FA-62F23C34D004


#### Abstract

Citation: Hertz A, Hauenschild F, Lotzkat S, Köhler G (2012) A new golden frog species of the genus Diasporus (Amphibia, Eleutherodactylidae) from the Cordillera Central, western Panama. ZooKeys 196: 23-46. doi: 10.3897/ zookeys.196.2774


#### Abstract

We describe the frog species Diasporus citrinobapheus sp. n. from the Cordillera Central of western Panama . The new species differs from all other species in its genus in coloration, disk cover and disk pad shape, skin texture, advertisement call, and size. It is most similar to $D$. tigrillo, from which it differs in dorsal skin texture, relative tibia length, number of vomerine teeth, ventral coloration, dorsal markings, and relative tympanum size, and to $D$. gularis, from which it can be distinguished by the lack of membranes between the toes, adult size, posterior thigh coloration, and position of the choanae. We provide data on morphology, vocalization, and distribution of the new species, as well as brief information on its natural history.


## Resumen

Describimos la especie de rana Diasporus citrinobapheus sp. n. de la Cordillera Central, occidente de Panamá. La nueva especie se distingue de otras especies del género por su coloración, su forma de la cubierta y la almohadilla de los discos, textura de la piel, canto de anúncio, y tamaño corporal. Se asemeja mas a D. tigrillo, del cual se distingue por la textura de la piel dorsal, longitud relativa de la tibia, número de dientes vomerianos, coloración ventral, patrón dorsal, y tamaño relativo del tímpano, y a D. gularis, del
cual se diferencia por la ausencia de membranas entre los dedos de pie, tamaño corporal, coloración de la parte trasera del muslo, y posición de las coanas. Presentamos datos de la morfología, vocalización, y distribución de la nueva especie, así como notas concisas de su historia natural.

## Keywords

Central America, Anura, diversity of species, taxonomy, vocalization

## Palabras clave

América Central, Anura, diversidad de especies, taxonomía, vocalización

## Introduction

Panama's herpetofauna is known to be the most diverse in consideration of its size in Central America, with only Mexico being more diverse in absolute species count (Myers and Duellman 1982; Jaramillo et al. 2010). Although herpetological research has been conducted in Panama for more than a hundred years (Ibánez et al. 2001), the knowledge of amphibian species diversity is still far from being completed. This is demonstrated impressively by the multitude of amphibian species described from this country within the last years (e.g. Wake et al. 2005, 2007; Köhler et al. 2007; Mendelson et al. 2008; Bolaños and Wake 2009; Crawford et al. 2010b; Mendelson and Mulcahy 2010; Ryan et al. 2010a, 2010b).

The genus Diasporus (Hedges et al. 2008) is the closest relative of the predominantly Caribbean genus Eleutherodactylus. The species of Diasporus are distributed from eastern Honduras to western Ecuador (Frost 2011; Köhler 2011). The genus contains nine described species, five of which (Diasporus diastema Cope, D. hylaeformis Cope, D. tigrillo Savage, D. ventrimaculatus Chaves, García-Rodríguez, Mora and Leal, and D. vocator Taylor) are currently known to occur in western Panama and/or southern Costa Rica. The remaining four species ( $D$. anthrax Lynch, D. gularis Boulenger, D. quidditus Lynch, and D. tinker Lynch) are distributed in Panama east of the Canal, and further along the Pacific coast of northern South America southwards to northwestern Ecuador (Frost 2011; IUCN 2011). However, differences in body size, male advertisement call, and coloration in the genus Diasporus suggest that there are several undescribed species (Lynch and Duellman 1997; Ibáńez et al. 1999; Savage 2002). Recent fieldwork in the Serranía de Tabasará of western Panama resulted in the discovery of a remarkable new species of Diasporus. The purpose of this paper is to describe this new species.

## Materials and methods

Field work was carried out between May and August 2010 at several sites along both slopes of the Serranía de Tabasará between the Fortuna depression and Santa Fé, Veraguas, Panama. All specimens were encountered during opportunistic searches at night.

Preparation and preservation of voucher specimens follows Köhler (2001). Tissue samples, usually the left forearm, were stored in $98 \%$ undenatured ethanol and deposited in the tissue collection of the Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Germany (SMF). Geographic coordinates and altitude above sea level were recorded with a handheld Garmin GPS MAP 60 CSx GPS receiver. All georeferences are in the geographical coordinate system and WGS 1984 datum, given in decimal degrees rounded to the fourth decimal place. Elevations are rounded to the next tenth. The map was created using ArcGIS 10 (ESRI).

We took additional morphological data from all Central American species currently assigned to the genus Diasporus in the SMF collection. We list all specimens examined for comparison in the Appendix I. Abbreviations for museum collections follow those of Sabaj Pérez (2010) except MHCH (Museo Herpetológico de Chiriquí, the herpetological collection of the Universidad Autónoma de Chiriquí, David, Panama). Specimens in the Appendix labeled with AH field numbers will be deposited at MHCH.

The sex of the male holotype and the paratypes was determined by the presence of vocal slits and vocal sac. Measurements were made with a dial caliper with the aid of a dissecting microscope and rounded to the nearest 0.1 mm . Measurements of the holotype (LACM 146212) and paratype (LACM 146241) of Diasporus tigrillo were taken from Savage (1997), those of D. ventrimaculatus from Chaves et al. (2009). Additionally, we examined photographs of the type specimens of $D$. tigrillo. If possible, missing measurements were calculated on the basis of data presented in the respective descriptions. In the case of $D$. ventrimaculatus, no individual measurements are available, for which reason calculations were made using the average values given for the paratypes by Chaves et al. (2009). We follow Savage (1997, 2002) in the terminology of disk cover and disk pad shape, dorsal outline of head, and snout profile shape. Abbreviations used for measurements are: snout-vent length (direct line distance from the tip of the snout to the posterior margin of the vent): SVL; length of Finger III (from distal end of the Finger III including disk to the base of the second subarticular tubercle): LF III; length of Toe IV (from distal end of the toe IV including disk to the base of third subarticular tubercle): LT IV; disk width at Finger III (at greatest width): DWF III; disk width at Toe IV (at greatest width): DWT IV; head length (from quadratojugal region to tip of the snout): HL; head width (between angles of jaw): HW; tibia length (straight length of the tibia): TL; horizontal eyelid diameter (greatest length of the upper eyelid): EL; interorbital distance (the width of frontoparietal bone between the orbits): IOD; horizontal tympanum diameter (at greatest length): TD; and horizontal eye diameter (at greatest length): ED. The capitalized colors and color codes (the latter in parentheses) in color descriptions in life are those of Smithe (1975-1981). Drawings of head, hands, and feet were made using a camera lucida attachment for a Leica MZ 12 dissecting microscope. Values provided for morphometric and acoustic parameters are minimum, maximum, and mean value $\pm$ standard deviation.

Advertisement calls were recorded using a Sennheiser ME 66 shotgun microphone capsule with a Sennheiser K6 powering module in combination with the Marantz PMD 620 solid-state recorder. A minimum distance from microphone to frog of one
meter was kept while recording to prevent disturbance. As needed, the microphone was attached to branches with the aid of a Joby Gorillapod in order to minimize disturbance of the calling frog. Calls were recorded in PCM format at a sampling rate of 48 kHz with 24 bit resolution and stored as wav files on a SD Card. Call editing and analysis were performed using SOUND RULER 0.9.6.0 (Gridi-Papp 2003-2007) for frequency analysis and to generate figures of oscillograms and audiospectrograms. We measured temporal parameters by hand using ADOBE AUDITION 3.0, because SOUND RULER has difficulties in accurately and precisely measuring temporal parameters (Bee 2004). Frequency information was obtained through Fast Fourier Transformation (FFT length 512 points, overlap between FFTs 0.8 ) at Hanning window function. Air temperature and humidity were measured immediately after each sound recording using the digital device Voltcraft HT-200 to the nearest $1^{\circ} \mathrm{C}$ and $3.5 \%$ relative humidity ( RH ). An alcohol extraction of skin secretions of the new species has been examined for alkaloids using Liquid Chromatography-Time-Of-FlightMass Spectrometry (LC-TOF-MS) at the Center of Forensic Medicine of the GoetheUniversity Frankfurt am Main.

For the complementary molecular analysis, we extracted DNA following the protocol of Ivanova et al. (2006). To eliminate potential PCR-inhibiting contaminants, the tissue samples were incubated for one hour in TE-buffer (10 parts Tris- $\mathrm{HCl}(\mathrm{pH}=$ 8.0) and one part EDTA) before digestion for at least 10 hours in $50 \mu$ l Vertebrate Lysis Buffer and $5.2 \mu$ Proteinase K at $56^{\circ} \mathrm{C}$. After extraction, DNA was eluted in $50 \mu \mathrm{~L}$ TE buffer. A fragment of the mitochondrial 16 S rRNA gene was amplified in an MJ Research Dyad Deciple ${ }^{\text {ru }}$ Peltier Thermal Cycler using the following program: initial denaturation for 180 s at $94^{\circ} \mathrm{C}$; followed by 39 cycles with denaturation for 15 s at 94 ${ }^{\circ} \mathrm{C}$, hybridization for $60 s$ at $51^{\circ} \mathrm{C}$, and elongation for $60 s$ at $72^{\circ} \mathrm{C}$; final elongation for 120 s at $72^{\circ} \mathrm{C}$. Reaction mix for each sample contained $1 \mu \mathrm{~L}$ DNA template, $2.5 \mu \mathrm{~L}$ Reaction Buffer (PeqGold), $2.5 \mu \mathrm{~L} 2.5 \mathrm{mM}$ dNTPs, $0.5 \mu \mathrm{~L}$ Taq Polymerase (PeqLab), $16.5 \mu \mathrm{~L} \mathrm{H}_{2} \mathrm{O}$, and $1 \mu \mathrm{~L}$ of each primer (forward: 16SA-L, 5'-CGCCTGTTTAT-CAAAAACAT-3’; reverse:16SB-H, 5’-CCGGTCTGAACTCAGATCACGT-3’). The achieved 16 S sequences were deposited in GenBank. We compared 21 sequences of the genus Diasporus in our analysis, three of the type series of the new species and one referred specimen, four specimens referred to as $D$. aff. hylaeformis from Cerro Pando, as well as all 16 S sequences of the genus Diasporus available on GenBank. We used an additional 16 S sequence of Pristimantis ridens as an outgroup (see Appendix II for examined specimens and GenBank accession numbers). Sequences were aligned with ClustalW (Larkin et al. 2007) using the default settings in Geneious (Drummond et al. 2010). The manually refined final alignment contained 535 positions. Using MEGA5 (Tamura et al. 2011), we computed uncorrected pairwise genetic distances, determined the Tamura 3-parameter model (Tamura 1992) as the best-fitting substitution model, and conducted Maximum Likelihood as well as Maximum Parsimony analyses (each with 10000 bootstrap replicates). Using TCSv1.21 (Clement et al. 2000), we conducted a statistical parsimony network analysis, with gaps considered as a fifth character state and a connection limit of $95 \%$.

## Results

## Diasporus citrinobapheus sp. n.

urn:lsid:zoobank.org:act:4A526693-CA45-44FC-9D9D-4F3064A47341
http://species-id.net/wiki/Diasporus_citrinobapheus
Figures 1 A, B; 2; 3; 5

Holotype. Adult male SMF 89814: collected on June 26, 2010 at 19:13 by Andreas Hertz and Sebastian Lotzkat at Quebrada Rasca $\left(8.4851^{\circ} \mathrm{N}, 81.1727^{\circ} \mathrm{W}, 790 \mathrm{~m}\right.$ elevation), near Paredón, Comarca Ngöbe-Buglé, western Panama, approximately 50 airline km NNW of the city of Santiago and 20 airline km N of Cañazas, Veraguas.

Paratypes. All collected by Andreas Hertz and Sebastian Lotzkat at the type locality on June 26, 2010: MHCH 2370-71; SMF 89816; all adult males.

Referred specimens. Adult males SMF 89817 and MHCH 2372: collected on July 01, 2010 by Andreas Hertz and Sebastian Lotzkat at the private reserve Willie Mazú, Comarca Ngöbe-Buglé ( $8.7903^{\circ} \mathrm{N}, 82.1989^{\circ} \mathrm{W}, 681 \mathrm{~m}$ elevation); female SMF 89820: collected on March 31, 2009 by Andreas Hertz, Sebastian Lotzkat and Arcadio Carrizo at Cerro Negro, Parque Nacional Santa Fé, Veraguas ( $8.5691^{\circ} \mathrm{N}, 81.0988^{\circ} \mathrm{W}$, 730 m elevation).

Diagnosis. A member of the genus Diasporus based on the following combination of characters: vocal slits and a single subgular vocal sac present, adult males without nuptial thumb pads; Finger I shorter than Finger II; Toe III much shorter than Toe V; subarticular tubercles on hands and feet flattened; no supernumerary tubercles on hands and feet; no tarsal fold or tubercle. Diasporus citrinobapheus differs from all described members of its genus by the following combination of characters (for accounts, see Table 1): coloration bright yellow to orange in life (Fig. 1 A); head almost as broad as long, but comparatively broad in relation to SVL; skin of dorsum smooth; venter coarsely areolate; tympanum covered by skin but annulus clearly visible; TD about $41 \%$ of ED; EL on average narrower than IOD; snout subacuminate in profile and rounded to subovoid in dorsal outline; disks of fingers and toes slightly expanded, disk covers of most fingers and toes spadate, but lacking papillae; disk pads of most fingers and toes triangular; subarticular tubercles of hands and feet rounded, very flat, almost not visible; vomerine odonthophores longish oval and widely separated; vomerine teeth weakly developed; upper eyelid usually smooth, very low pustules in some individuals; heel smooth. Its bright yellow to orange coloration distinguishes $D$. citrinobapheus from almost all described Central American Diasporus, which, in spite of considerable variation, are all tan to gray or brownish to almost black. In $D$. hylaeformis and $D$. ventrimaculatus, the dorsal ground color can be suffused with pink or red. Only D. tigrillo from Costa Rica, a species known only from two specimens, shows a yellowish coloration in life according to the original description (Savage 1997). Diasporus citrinobapheus differs from the two known specimens of $D$. tigrillo in the following characters (character for $D$. tigrillo in parentheses): SVL in adult males $17.3-19.7 \mathrm{~mm}$ (16.0-17.5 mm); dorsal skin absolutely smooth (dorsal skin with scattered low pus-


Figure I. A-B Holotype of Diasporus citrinobapheus (SMF 89814, adult male): A in life B in preservative. C Diasporus tigrillo in preservative (LACM 146212, holotype, adult male), note dark brown spots. Pictures are not at the same scale.
tules, best developed on dorsum); TD 32-45\% of ED (54-57\%); TL $40 \%$ of SVL (about 48\%); distal subarticular tubercle of Finger and Toe I flat and rounded (weakly bifid); many weakly developed vomerine teeth in three to four close rows (a few vomerine teeth in two obliquely aligned and widely separated rows); dorsal surface uniformly bright yellow to orange, sometimes with irregularly distributed dark blotches (yellow
Table I. Morphological measurements of Diasporus citrinobapheus in comparison with other described species of the genus from western Panama and southern Costa Rica (mean $\pm$ SD, min-max). See Materials and Methods for abbreviations.

| Character | D. citrinobapheus |  | D. diastema |  | D. hylaeformis |  | D. ventrimaculatus |  | D. vocator |  | D. tigrillo |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\begin{array}{\|l} \hline \text { male } \\ (\mathrm{n}=6) \end{array}$ | female $(\mathrm{n}=1)$ | male ( $\mathrm{n}=20$ ) | $\begin{aligned} & \text { female } \\ & (\mathrm{n}=22) \\ & \hline \end{aligned}$ | male $(\mathrm{n}=9)$ | $\begin{array}{\|l\|} \hline \text { female } \\ (\mathrm{n}=5) \end{array}$ | male ( $\mathrm{n}=6$ ) | female ( $\mathrm{n}=2$ ) | male $(\mathrm{n}=4)$ | female $(\mathrm{n}=6)$ | male $(\mathrm{n}=2)$ |
| SVL (mm) | $\begin{aligned} & 18.7 \pm 0.63 \\ & (17.3-19.7) \\ & \hline \end{aligned}$ | 21.8 | $\begin{aligned} & 18.7 \pm 1.62 \\ & (15.9-22.1) \end{aligned}$ | $\begin{aligned} & 18.7 \pm 2.58 \\ & (15.0-23.5) \\ & \hline \end{aligned}$ | $\begin{array}{\|l\|} \hline 19.1 \pm 1.30 \\ (16.9-20.9) \end{array}$ | $\begin{aligned} & 21.2 \pm 0.97 \\ & (19.2-21.7) \end{aligned}$ | $\begin{aligned} & 21.8 \pm 1.2 \\ & (20.2-23.5) \\ & \hline \end{aligned}$ | $\begin{aligned} & 23.9 \pm 0.8 \\ & (23.2-24.7) \end{aligned}$ | $\begin{aligned} & 15.3 \pm 2.18 \\ & (12.2-17.2) \end{aligned}$ | $\begin{aligned} & 14.7 \pm 2.18 \\ & (13.6-15.7) \end{aligned}$ | $\begin{aligned} & 16.8 \\ & (16.0-17.5) \end{aligned}$ |
| DW/LF III | $\begin{aligned} & 0.23 \pm 0.03 \\ & (0.18-0.26) \end{aligned}$ | 0.23 | $\begin{aligned} & 0.36 \pm 0.06 \\ & (0.21-0.44) \end{aligned}$ | $\begin{aligned} & 0.32 \pm 0.07 \\ & (0.21-0.44) \\ & \hline \end{aligned}$ | $\begin{array}{\|l\|} \hline 0.31 \pm 0.03 \\ (0.27-0.36) \\ \hline \end{array}$ | $\begin{array}{\|l\|} \hline 0.32 \pm 0.02 \\ (0.29-0.34) \\ \hline \end{array}$ |  | - | $\begin{array}{\|l\|} \hline 0.26 \pm 0.06 \\ (0.19-0.32) \\ \hline \end{array}$ | $\begin{aligned} & 0.32 \pm 0.06 \\ & (0.24-0.42) \end{aligned}$ |  |
| DW/LT IV | $\begin{aligned} & 0.14 \pm 0.03 \\ & (0.11-0.18) \\ & \hline \end{aligned}$ | 0.17 | $\begin{aligned} & 0.23 \pm 0.05 \\ & (0.15-0.32) \end{aligned}$ | $\begin{aligned} & 0.22 \pm 0.05 \\ & (0.11-0.29) \\ & \hline \end{aligned}$ | $\begin{array}{\|l\|} \hline 0.22 \pm 0.03 \\ (0.18-0.26) \\ \hline \end{array}$ | $\begin{array}{\|l\|} \hline 0.22 \pm 0.02 \\ (0.20-0.24) \\ \hline \end{array}$ |  | - | $\begin{aligned} & 0.17 \pm 0.03 \\ & (0.13-0.19) \end{aligned}$ | $\begin{aligned} & 0.17 \pm 0.03 \\ & (0.14-0.19) \end{aligned}$ |  |
| HL/SVL | $\begin{aligned} & \hline 0.41 \pm 0.01 \\ & (0.39-0.44) \\ & \hline \end{aligned}$ | 0.40 | $\begin{aligned} & 0.39 \pm 0.02 \\ & (0.35-0.44) \end{aligned}$ | $\begin{aligned} & 0.41 \pm 0.02 \\ & (0.36-0.44) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.39 \pm 0.02 \\ & (0.35-0.43) \end{aligned}$ | $\begin{aligned} & 0.39 \pm 0.02 \\ & (0.37-0.42) \\ & \hline \end{aligned}$ | 0.33 | 0.35 | $\begin{aligned} & 0.38 \pm 0.02 \\ & (0.35-0.41) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.38 \pm 0.02 \\ & (0.35-0.42) \end{aligned}$ | $\begin{array}{\|l\|} \hline 0.39 \\ (0.38-0.40) \\ \hline \end{array}$ |
| HW/SVL | $\begin{aligned} & 0.37 \pm 0.01 \\ & (0.35-0.38) \\ & \hline \end{aligned}$ | 0.36 | $\begin{aligned} & 0.36 \pm 0.02 \\ & (0.33-0.39) \end{aligned}$ | $\begin{aligned} & 0.36 \pm 0.02 \\ & (0.32-0.39) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.37 \pm 0.01 \\ & (0.35-0.39) \end{aligned}$ | $\begin{aligned} & 0.36 \pm 0.02 \\ & (0.35-0.40) \\ & \hline \end{aligned}$ | 0.39 | 0.40 | $\begin{aligned} & 0.34 \pm 0.02 \\ & (0.31-0.36) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.34 \pm 0.02 \\ & (0.31-0.36) \end{aligned}$ | $\begin{aligned} & 0.36 \\ & (0.34-0.37) \end{aligned}$ |
| HW/HL | $\begin{aligned} & 0.91 \pm 0.03 \\ & (0.88-0.97) \\ & \hline \end{aligned}$ | 0.90 | $\begin{aligned} & 0.91 \pm 0.06 \\ & (0.79-1.01) \end{aligned}$ | $\begin{array}{\|l\|} \hline 0.90 \pm 0.06 \\ (0.78-1.04) \\ \hline \end{array}$ | $\begin{aligned} & 0.94 \pm 0.05 \\ & (0.85-1.00) \end{aligned}$ | $\begin{aligned} & 0.92 \pm 0.04 \\ & (0.85-0.96) \\ & \hline \end{aligned}$ | 1.15 | 1.14 | $\begin{aligned} & 0.89 \pm 0.08 \\ & (0.79-0.96) \end{aligned}$ | $\begin{aligned} & 0.89 \pm 0.08 \\ & (0.86-0.95) \end{aligned}$ | $\begin{aligned} & 0.92 \\ & (0.85-0.99) \end{aligned}$ |
| TL/SVL | $\begin{array}{\|l\|} \hline 0.41 \pm 0.01 \\ (0.40-0.42) \\ \hline \end{array}$ | 0.42 | $\begin{aligned} & 0.40 \pm 0.04 \\ & (0.35-0.51) \end{aligned}$ | $\begin{aligned} & 0.42 \pm 0.05 \\ & (0.36-0.56) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.39 \pm 0.01 \\ & (0.37-0.42) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.39 \pm 0.05 \\ & (0.35-0.47) \\ & \hline \end{aligned}$ | 0.50 | 0.51 | $\begin{aligned} & 0.40 \pm 0.02 \\ & (0.38-0.43) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.38 \pm 0.02 \\ & (0.36-0.42) \end{aligned}$ | $\begin{aligned} & 0.48 \\ & (0.46-0.50) \end{aligned}$ |
| EL/IOD | $\begin{array}{\|l\|} \hline 0.98 \pm 0.12 \\ (0.83-1.12) \\ \hline \end{array}$ | 0.94 | $\begin{aligned} & 1.04 \pm 0.10 \\ & (0.89-1.24) \end{aligned}$ | $\begin{array}{\|l\|} \hline 1.12 \pm 0.18 \\ (0.89-1.62) \\ \hline \end{array}$ | $\begin{array}{\|l\|} \hline 1.01 \pm 0.12 \\ (0.88-1.24) \\ \hline \end{array}$ | $\begin{array}{\|l\|} \hline 1.07 \pm 0.10 \\ (0.88-1.19) \\ \hline \end{array}$ | 0.86 | 1.00 | $\begin{aligned} & 1.07 \pm 0.12 \\ & (0.95-1.24) \\ & \hline \end{aligned}$ | $\begin{aligned} & 1.43 \pm 0.12 \\ & (1.25-1.59) \end{aligned}$ |  |
| ED/HL | $\begin{aligned} & 0.32 \pm 0.03 \\ & (0.28-0.36) \\ & \hline \end{aligned}$ | 0.32 | $\begin{aligned} & 0.29 \pm 0.04 \\ & (0.22-0.35) \end{aligned}$ | $\begin{aligned} & 0.29 \pm 0.04 \\ & (0.21-0.37) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.30 \pm 0.03 \\ & (0.27-0.35) \end{aligned}$ | $\begin{aligned} & 0.28 \pm 0.03 \\ & (0.22-0.30) \\ & \hline \end{aligned}$ | 0.37 | 0.39 | $\begin{aligned} & 0.33 \pm 0.01 \\ & (0.32-035) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.34 \pm 0.01 \\ & (0.33-0.37) \end{aligned}$ | $\begin{aligned} & 0.32 \\ & (0.28-0.35) \\ & \hline \end{aligned}$ |
| TD/ED | $\begin{aligned} & 0.39 \pm 0.07 \\ & (0.32-0.45) \\ & \hline \end{aligned}$ | 0.32 | $\begin{aligned} & 0.38 \pm 0.09 \\ & (0.27-0.65) \end{aligned}$ | $\begin{aligned} & 0.37 \pm 0.08 \\ & (0.19-0.52) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.42 \pm 0.07 \\ & (0.30-0.52) \end{aligned}$ | $\begin{aligned} & 0.45 \pm 0.03 \\ & (0.42-0.50) \\ & \hline \end{aligned}$ | 0.48 | 0.47 | $\begin{aligned} & 0.36 \pm 0.07 \\ & (0.30-0.44) \end{aligned}$ | $\begin{aligned} & 0.43 \pm 0.07 \\ & (0.33-0.50) \end{aligned}$ | $\begin{aligned} & 0.55 \\ & (0.54-0.57) \end{aligned}$ |
| ED/SVL | $\begin{aligned} & 0.13 \pm 0.01 \\ & (0.11-0.15) \\ & \hline \end{aligned}$ | 0.13 | $\begin{array}{\|l} 0.11 \pm 0.20 \\ (0.08-0.13) \\ \hline \end{array}$ | $\begin{aligned} & 0.12 \pm 0.13 \\ & (0.09-0.15) \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.12 \pm 0.01 \\ & (0.10-0.14) \end{aligned}$ | $\begin{aligned} & 0.11 \pm 0.01 \\ & (0.09-0.12) \\ & \hline \end{aligned}$ | 0.12 | 0.13 | $\begin{aligned} & 0.13 \pm 0.01 \\ & (0.12-0.13) \\ & \hline \end{aligned}$ | $\begin{array}{\|l} 0.13 \pm 0.01 \\ (0.12-0.14) \\ \hline \end{array}$ | $\begin{aligned} & 0.12 \\ & (0.11-0.13) \end{aligned}$ |

to orange dorsal coloration with dark brown spots confined to the pustules); ventral surfaces almost colorless and transparent, in some individuals with a fine dirty white speckling, except male vocal sac that is suffused with yellow (undersurfaces, including venter, yellow); coloration in preservative grayish-white with only a suggestion of yellow (brownish-ocher with dark brown dots; see comments in Discussion section for the usage of different preservation methods). Furthermore, D. citrinobapheus superficially resembles the South American D. gularis from western Ecuador and western Colombia in coloration (see photo in Lynch 2001, page 295 Fig. 7). Diasporus gularis has been described comprehensively by Lynch and Duellman (1997). According to them, adult $D$. gularis are larger (SVL in males $20.2-21.6 \mathrm{~mm}$, in females $23.3-24.8 \mathrm{~mm}$ ) than $D$. citrinobapheus (males $17.3-19.7 \mathrm{~mm}$, single known female 21.8 mm ). Moreover, $D$. gularis shows basal webbings between toes and some specimens have papillae at the apex of the disk pad on some toes, whereas there are no such papillae, and no webbing between toes of $D$. citrinobapheus. The posterior surfaces of thighs are brown in $D$. gularis, but yellow to orange in D. citrinobapheus. Moreover, the choanae are long, oval, and not concealed by the palatal shelf of the maxillary arch in D. gularis, whereas they are round, orientated extremely laterally on palate, and partially concealed by the palatal shelf of the maxillary arch in $D$. citrinobapheus.

Description of the holotype. An adult male; measurements (in mm): SVL 18.4, LF III 2.4, LT IV 4.2, DWF III 0.6, DWT IV 0.5, HL 7.2, HW 7.0, TL 7.8, EL 2.6, IOD 2.9, TD 0.8 , ED 2.4; dorsal skin smooth; venter coarsely areolate; no discoidal fold; upper eyelid smooth; snout subovoid in dorsal outline and subacuminate in profile; nostrils weakly protuberant, directed dorsolaterally; head slightly longer than wide, width $97 \%$ of length; HW 38\% of SVL; canthus rostralis indistinct; ED 36\% of HL and $13 \%$ of SVL; EL $90 \%$ of IOD; TD $33 \%$ of ED (Fig. 2 A); choanae round, orientated extremely laterally on palate, partially concealed by palatal shelf of maxillary arch; elliptical vomerine odonthophores, posteromedian to choanae, which are widely separated from each other, with four rows of weakly developed, short teeth; legs short in relation to body; TL $42 \%$ of SVL; relative finger length: $\mathrm{I}<\mathrm{II}=\mathrm{IV}<\mathrm{III}$; all fingers with disks, slightly wider than digits, on Fingers II-IV wider than on Finger I; relative toe length: $\mathrm{I}<\mathrm{II}<\mathrm{III}<\mathrm{V}<\mathrm{IV}$, Toe V much longer than toe III; tip of Toe V extending to distal subarticular tubercle on Toe IV; tip of Toe III extending to penultimate subarticular tubercle on Toe IV; disks on Toes III-V larger than on I-II; disk covers spadate, lacking papillae; no supernumerary tubercles (Figs 2 B,C).

Etymology. The specific name citrinobapheus is a noun in apposition and is derived from the Greek words citrinos (citrin-yellow) and bapheus (dyer) referring to the yellow body color that dyes one's fingers yellowish when the frog is handled. Although we could observe this phenomenon in a few other species of Diasporus too, it is notably evident in the new species.

Coloration in life. All examined specimens show shades of bright yellow and orange dorsally; some have dark grayish and/or whitish-grayish spots (Fig. 3). Ventral surfaces are almost achlorophyllaceous and transparent apart from the yellow male vocal sac.


Figure 2. Holotype of Diasporus citrinobapheus (SMF 89814, adult male): A Lateral view of head B Ventral view of right hand. C Ventral view of right foot. Scale bars $=1 \mathrm{~mm}$.


Figure 3. Variation in coloration pattern in life of Diasporus citrinobapheus from different localities: A Female SMF 89820 from Cerro Negro, Parque Nacional de Santa Fé (Veraguas, Panama) with dirty orange coloration B Male SMF 89816 from type locality Paredón (Comarca Ngöbe-Buglé, Panama) with immaculate yellow coloration C Male MHCH 2372 from Willie Mazú (Comarca Ngöbe-Buglé, Panama) with intense mottling D Male SMF 89817 from Willie Mazú (Comarca Ngöbe-Buglé, Panama) with intermediate mottling.

MHCH 2372 (Fig. 3 C): Dorsal ground color Orange Yellow (18); posterior and anterior surfaces of thighs Chrome Orange (16); Raw Umber (23) interorbital and postocular stripes formed by very fine mottling; dorsum with five Dark Grayish Brown (20) blotches, forming a pattern like the five dots on a dice; scattered Dark Grayish Brown (20) blotches on dorsal surfaces of limbs; disk covers Blackish Neutral Gray (82), with white rings at the base; ventral surface of hind limbs Chrome Orange (16); ventral surface of body transparent with dirty white mottling; vocal sac white with a suggestion of Spectrum Yellow (55).

SMF 89820 (Fig. 3 A): In the only female, coloration in life has been recorded as follows: Dorsal surface Yellow Ocher (123 C); a Chamois (123 D) interorbital bar; anterior and posterior surfaces of thighs Chrome Orange (16); venter almost transparent; upper surfaces of disks Sepia (119) with dirty white spots and a dirty white ring around base; gular region Smoke Gray (44).

Coloration in preservative ( $\mathbf{7 0 \%}$ alcohol). In preservation the bright yellow and orange colors fade rapidly to a pale grayish yellow (Fig. 1 B) with scattered dark gray-


Figure 4. Results of 16 SmtDNA analysis. A Consensus tree from Maximum Likelihood analysis. Scale bar refers to substitutions per site. Bootstrap support values before the slash correspond to Maximum Likelihood analysis, those after the slash to the Maximum Parsimony consensus tree of exactly the same topology. Numbers behind branches refer to respective museum numbers B Parsimony network derived from the same alignment, with each node representing a unique haplotype separated by one substitutional step from its nearest neighbor. Rectangles are haplotypes of analyzed sequences, circles are haplotypes missing in our sample $\mathbf{C}$ Tentative taxonomic implication. Bar breaks indicate assumed species boundaries. Names refer to morphological determination or GenBank taxonomic identity.
ish blotches in some individuals. Legs pale orange; vocal sac pale yellow in males; gular area in females pale gray; tips of digits dark grayish black. Dark grayish black eyeballs shining through skin when head is viewed dorsally.

Variation. Compared to other species of this genus, the individuals of Diasporus citrinobapheus available to us exhibit only little variation in their coloration (Fig. 3). All show a yellow to orange dorsal ground color in life. This can either appear bright and clear or somewhat dirty, depending on the pigment translocation within the melanophores in the frog's skin. In some individuals, higher concentrations of melanophores in certain areas of the dorsum form dark blotches or stripes. This is especially the case in the two specimens from Willie Mazú (Figs 3 C, D). The most frequent pattern of


Figure 5. A Visualizations of an advertisement call (Hanning window function, FFT 512, 0.8 overlap) of Diasporus citrinobapheus (holotype, SMF 89814) recorded in Paredón, Comarca Ngöbe-Buglé, Panama, at $24.5^{\circ} \mathrm{C}$ air temperature and $95.3 \%$ relative humidity. Clockwise from top left: Oscillogram of a call group; Oscillogram of the penultimate call in the shown call group; Power spectrum showing the dominant frequency of the penultimate call in the shown call group; Spectrogram of the penultimate call in the shown call group B-D Different call positions of male D. citrinobapheus: B Male holotype (SMF 89814) from Paredón calling on dead leaves in dense vegetation about 2 meters above ground level; C Male paratype (MHCH 2371) from Paredón on green leaf about 3 m above ground level D Male specimen (SMF 89817) from Willie Mazú referred to as $D$. citrinobapheus calling from an elevated position on the underside of a leaf.
this type is an interorbital bar, which in most cases is darker than ground color along the anterior edge of the bar and lighter than ground color along the posterior edge. In addition, some individuals show dark brown blotches on the limbs and less frequently also on the dorsum. Most individuals show additional small whitish spots, in particular under and around the eyes, as well as scattered across the forelimbs. In the male SMF 89816 from the type locality (Fig. 3 B) the dark and white markings on and around the disk covers are not as pronouncedly contrasting as in the other individuals examined.


Figure 6. Distribution map of Diasporus citrinobapheus and type localities of other species in the genus in Panama and Costa Rica. Solid triangle: Paredón, Comarca Ngöbe-Buglé, type locality of $D$. citrinobapheus. Hollow triangles: Additional collection sites of D. citrinobapheus: Private Reserve Willie Mazú in the west, and Cerro Negro (Parque Nacional Santa Fé, Veraguas) in the east. Inverted triangle: Agua Buena, Puntarenas, Costa Rica, type locality of $D$. vocator. Pentagon: Valle del Silencio, at the provincial boarder between Puntarenas and Limón, Costa Rica, type locality of D. ventrimaculatus. Square: Cerro Utyum, Limón, Costa Rica, type locality of D. hylaeformis. Circle: Río Lari, Limón, Costa Rica, type locality of $D$. tigrillo. Diamond: Margarita, Colón, Panama, type locality of D. diastema. Dashed lines represent provincial borders. Solid lines represent coast line and national border.

Molecular genetics. The distinctiveness of Diasporus citrinobapheus is supported by the analysis of the 16 S mitochondrial rRNA gene (Fig. 4). The four individuals we examined form a distinct cluster that appears separated from the other members for which 16 S sequences are available. The mean genetic distance among the four specimens of $D$. citrinobapheus is $0.3 \%$. In our consensus tree (Fig. 4 A ) it appears to be most closely related to the candidate species $D$. aff. diastema from El Copé, from which it is separated by a mean genetic distance of $1.8 \%$. In the haplotype network analysis (Fig. 4 B) both clades form unconnected subnetworks, indicating a differentiation at species level (Fig. 4 C ). The mean genetic distance to the next closest relative $D$. quidditus is $6.6 \%$ for $D$. citrinobapheus and $7 \%$ for $D$. aff. diastema.

Vocalization of holotype. We recorded a $3 \mathrm{~min}, 43.5$ seconds portion of the advertisement call of the holotype that yielded a total of 63 calls. An exemplary visualization of the call structure is given in Fig. 5 A . Relative humidity during recording was 95.3\%
at an air temperature of $24.5^{\circ} \mathrm{C}$. As in other members of the genus, the call consists of a single note, even though calls sound like a "whistle," rather than the typical "tink" usually emitted by members of the genus Diasporus (Savage 2002; Chaves et al. 2009). The 63 recorded calls are organized in five call groups of $8-17$ calls per group (12.8 $\pm 3.2$ ). A call group lasts $19.8-34.1 \mathrm{~s}(25.0 \pm 5.7)$. Intervals between call groups range from 15.7$33.2 \mathrm{~s}(21.6 \pm 8.0)$ and intervals between calls within a call group range from 0.57-5.77 $s(1.93 \pm 1.2)$. Call group rate is 1.34 call groups per minute; call rate within a call group varies from $23.4-40.8$ calls per minute ( $32.0 \pm 6.3$ ). Call duration varies from $0.13-0.18$ $s(0.16 \pm 0.01)$. There is a rather weak frequency modulation of $190-470 \mathrm{~Hz}(370 \pm 65)$. The spectrum of frequencies within a call range from a mean minimum of $2890 \pm 44$ Hz to a mean maximum of $3260 \pm 44 \mathrm{~Hz}$. Fundamental and dominant frequencies are identical at about 2950 Hz . The dominant frequency, as the frequency with the greatest energy in the signal, is reached about 0.05 s after initiation of the call.

Vocalizations of paratypes and referred specimens. In addition to the holotype, we recorded and analyzed the advertisement calls of two paratypes (SMF 89816, MHCH 2371) and one referred specimen (SMF 89817). Summing up, the advertisement call of Diasporus citrinobapheus sounds like a whistle, is organized in call groups, has a call duration of $0.14-0.16 \mathrm{~s}$ in average and a dominant frequency of 2860-3040 Hz (see all parameters in Table 2). While the paratypes vary only little in call parameters, SMF 89817 shows obvious differences regarding call duration, call interval, and call rate (see Discussion section for details).

Geographical distribution and natural history notes. So far, Diasporus citrinobapheus has been found on the Caribbean slopes of the western Serranía de Tabasará and on both Pacific and Caribbean slopes of the eastern Serranía de Tabasará (Fig. 6) at intermediate elevations from 680 to 790 m.a.s.l. Males call from very dense vegetation and are difficult to spot. They are almost only detectable by following their characteristic vocalization. Vocal activity is highest just after dusk and finally stops when it

Table 2. Variation in advertisement call parameters in four male specimens referred to as Diasporus citrinobapheus (mean $\pm$ SD, min-max).

|  | SMF 89814 | SMF 89816 | MHCH 2371 | SMF 89817 |
| :--- | :--- | :--- | :--- | :--- |
| Temperature / RH during <br> recording | $24.5^{\circ} \mathrm{C} / 95.3 \%$ | $24.3^{\circ} \mathrm{C} / 93.5 \%$ | $24.6^{\circ} \mathrm{C} / 93.6 \%$ | $21.8^{\circ} \mathrm{C} / 100 \%$ |
| Total recording time (min) | 3.73 | 1.35 | 1.66 | 3.03 |
| Number of call groups <br> recorded | 5 | 2 | 1 | 4 |
| Number of calls recorded | 63 | 26 | 11 | 68 |
| Call group rate (call groups / <br> min) | 1.34 | 1.48 | 0.6 | 1.32 |
| Call group duration (s) | $25.0 \pm 5.7$ <br> $19.8-34.1$ | $23.0 \pm 9.5$ <br> $16.3-29.7$ | 19.0 | $20.6 \pm 8.5$ <br> $12.5-28.5$ |
| Calls per group | $12.8 \pm 3.2$ <br> $8-17$ | $11-15$ | 11 | $16.6 \pm 5.7$ <br> $10-22$ |


|  | SMF 89814 | SMF 89816 | MHCH 2371 | SMF 89817 |
| :---: | :---: | :---: | :---: | :---: |
| Call group interval (s) | $\begin{aligned} & \hline 21.6 \pm 8.0 \\ & 15.7-33.2 \end{aligned}$ | 26.84 | >78 | $\begin{aligned} & \hline 25 \pm 16.7 \\ & 10.9-43.5 \end{aligned}$ |
| Call rate over entire recording (calls/min) | 16.9 | 19.3 | 6.63 | 22.4 |
| Call rate within a call group (calls/min) | $\begin{array}{\|l\|} \hline 32 \pm 6.3 \\ 23.4-40.8 \\ \hline \end{array}$ | $\begin{aligned} & 35.4 \pm 7.1 \\ & 30.3-40.4 \end{aligned}$ | 35 | $\begin{aligned} & 50 \pm 6.8 \\ & 44.2-60 \end{aligned}$ |
| Call duration (s) | $\begin{aligned} & 0.157 \pm 0.01 \\ & 0.126-0.178 \end{aligned}$ | $\begin{array}{\|l\|} \hline 0.162 \pm 0.01 \\ 0.143-0.174 \end{array}$ | $\begin{aligned} & \hline 0.156 \pm 0.003 \\ & 0.151-0.162 \\ & \hline \end{aligned}$ | $\begin{aligned} & 0.141 \pm 0.01 \\ & 0.114-0.167 \end{aligned}$ |
| Call interval (s) | $\begin{array}{\|l\|} \hline 1.93 \pm 1.2 \\ 0.57-5.77 \end{array}$ | $\begin{aligned} & 1.74 \pm 1.4 \\ & 0.63-3.77 \end{aligned}$ | $\begin{aligned} & 1.71 \pm 0.75 \\ & 0.85-2.85 \end{aligned}$ | $\begin{aligned} & 1.15 \pm 0.49 \\ & 0.55-2.58 \end{aligned}$ |
| Dominant frequency (Hz) | $2953 \pm 0$ | $\begin{aligned} & \hline 3010 \pm 75 \\ & 2859-3140 \\ & \hline \end{aligned}$ | $2859 \pm 0$ | $\begin{aligned} & 2965 \pm 32 \\ & 2953-3046 \end{aligned}$ |
| Minimum frequency (Hz) | $\begin{array}{\|l\|} \hline 2889 \pm 44 \\ 2859-2953 \end{array}$ | $\begin{array}{\|l\|} \hline 2776 \pm 31 \\ 2765-2859 \end{array}$ | $2671 \pm 0$ | $\begin{aligned} & 2939 \pm 33 \\ & 2895-2953 \end{aligned}$ |
| Maximum frequency (Hz) | $\begin{array}{\|l\|} \hline 3257 \pm 44 \\ 3140-3328 \\ \hline \end{array}$ | $\begin{aligned} & \hline 3184 \pm 61 \\ & 3064-3234 \end{aligned}$ | $\begin{aligned} & 3029 \pm 38 \\ & 2953-3046 \end{aligned}$ | $\begin{aligned} & 3290 \pm 74 \\ & 3140-3421 \end{aligned}$ |
| Frequency modulation (Hz) | $\begin{array}{\|l\|} \hline 367 \\ 188-469 \end{array}$ | $\begin{array}{\|l\|} \hline 407 \\ 281-468 \end{array}$ | $\begin{aligned} & 358 \\ & 281-375 \end{aligned}$ | $\begin{aligned} & 351 \\ & 281-375 \end{aligned}$ |

becomes dark. Calling height ranges from near ground level up to three meters above ground. Calling position can be either on the upper side of a leaf or on its underside (Figs 4 B-D). The only female (SMF 89820) was found at daytime ( $15: 00 \mathrm{~h}$ ) inside an involute, young plantain leaf that apparently served as a daytime hiding place. The species does not seem to be limited to mature forest, but is also found in secondary growth and plantations. However, it appears to avoid open habitats like pasture land.

## Discussion

Diasporus citrinobapheus is easily distinguishable from all other known frogs of the genus in Lower Central America by its bright yellow to orange coloration. The only described species of the genus that somewhat resemble the new species in coloration are D. gularis from Colombia and Ecuador and D. tigrillo from the Caribbean slopes of the Costa Rican part of the Serranía de Talamanca. The latter species is known only from two specimens, both collected in 1964 at a single locality and there are no photographs of the species in life, tissue samples, or call recordings available to clarify the systematic relationships of this species. The different ground coloration in preservative between $D$. tigrillo and $D$. citrinobapheus is certainly due to different preservation techniques, because the fixation process in $10 \%$ formalin darkens the complete specimen. However, this does not influence the general color pattern, so we treat the dark brown spots on the dorsum of $D$. tigrillo as a diagnostic feature to differentiate between $D$. tigrillo and D. citrinobapheus. Additional material is required, preferably from near the type locality of $D$. tigrillo to conduct further studies. In contrast, $D$. gularis is known
from a number of specimens from Colombia and Ecuador. However, the presence and development of papillae at the apex of the pad on the underside of the disk cover, one of the main characters that has been used to distinguish this species from its congeners, is a controversial issue. Lynch (1976, page 12, Fig. 3 B) provided a drawing of the left hand of a specimen (LACM 73239) from Chocó, Colombia, that shows long papillae. In a later work, the same author presented drawings of finger disk pads of two $D$. gularis (ICN 45168, 45171) from Valle del Cauca, Colombia, that show knobbed disk covers (Lynch 2001, Figs 2 A, B), but he also noted that there are specimens lacking this character. He further considered the presence or absence of this knob at the underside of the disk cover might be due to preparation technique. Lynch and Duellman (1997) noted that the holotype of $D$. gularis from Ecuador does not have papillae at the tip of any digit, while they stated that specimens from southern Colombia and Ecuador have a rounded knob at the apex of the pad on the underside of the disk cover of toe II-IV. In Chocoan Colombia, specimens referred to as D. gularis have larger papillae. Depending on their diagnosis, Lynch and Duellman (1997) argued that several species might currently be referred to $D$. gularis, three in western Colombia and only one in Ecuador, where the type locality is. Based on our genetic analysis, D. citrinobapheus is closely related to, and may even be conspecific with, the candidate species $D$. aff. diastema from El Copé (Crawford et al. 2010a). Albeit the comparably small p-distance, the haplotype network analysis yields a separate network for each of the two clades, supporting the assumption of two distinct species. However, genetic evidence revealed only from mitochondrial markers alone is not strong enough to support either one or the other hypothesis (Vences et al. 2005). Further integrative taxonomic studies, including morphology, bioacoustics, and nuclear genes are needed to clarify this matter.

Besides various records of other amphibians and reptiles, we found no additional species of the genus Diasporus at the type locality. At Willie Mazú, a locality approximately 120 km NW of the type locality of $D$. citrinobapheus, we collected a single specimen of Diasporus that we refer to $D$. vocator based on size, coloration, disk shape, and male advertisement call. At Cerro Negro, D. citrinobapheus occurs sympatrically with $D$. diastema. Based on our current concept of its distribution, the possibility remains that also $D$. vocator occurs at this locality, although the species has not been recorded from this site.

The eponymous, readily soluble yellow coloration of Diasporus citrinobapheus lead us to the assumption that this might serve a defensive function against predators. On this account, an alcohol extraction was analyzed for alkaloids, but no active substances were found. Probably, the yellow pigment is just highly soluble and therefore easily washed out. Nevertheless, one could speculate that it has a bitter or otherwise unpalatable taste that might deter certain predators.

Various studies have shown that the advertisement call represents a premating isolating mechanism in anurans (e.g., Duellman and Trueb 1986), which makes it a valuable tool in taxonomy. Having in mind that there are great morphological overlaps between members of the genus Diasporus, analyses of vocalizations might form the most powerful taxonomic approach to decipher its species diversity. Unfortunately,
the calls of most species have never been formally described. Fouquette (1960) was the first to describe the call of $D$. diastema from the Panama Canal area, about 10 km northwest of Panama City, not far from the type locality (Cope 1876; Dunn 1942). Later, Wilczynski and Brenowitz (1988) presented another call description based on calls recorded in the surroundings of Gamboa in Central Panama, about 24 km NNW of Panama City, and even closer to the type locality. Interestingly, the call descriptions of Fouquette (1960) and Wilczynski and Brenowitz (1988) are incongruent in terms of call duration, frequency range, and dominant frequency, rendering it possible that different species were recorded. Unfortunately, none of these papers cited any voucher specimens, so it is impossible to determine which species they actually recorded. The most recent contribution on vocalizations of $D$. diastema is that of Ibánez et al. (1999), also from the environs of the Panama Canal. They provided a rough sonogram, but did not give any numerical values. The dominant frequency in all three papers is roughly described as $3000-4000 \mathrm{~Hz}$, thus considerably higher than in D. citrinobapheus. Furthermore, all three papers (Fouquette 1960; Wilczynski and Brenowitz 1988; Ibánez et al. 1999) present sonograms, which show an obvious frequency modulation expressed by a rapid rise of frequency over time with approximately 1000 Hz difference between beginning and end of the call. In contrast, the call of D. citrinobapheus is characterized by only a moderate frequency rise over time, on average $350-400 \mathrm{~Hz}$. Confusing are the data of call duration provided by Fouquette (1960) and Wilczynski and Brenowitz (1988), respectively. Fouquette (1960) reports on mean call duration of 0.2 s for $D$. diastema. Yet, in the accompanying sonogram (Fig. 2 A in Fouquette 1960), the call seems to be only slightly longer than 0.1 s. Wilczynski and Brenowitz (1988) even mentioned a call duration of more than 0.3 s , but in the accompanying oscillogram (Fig. 1 B in Wilczynski and Brenowitz 1988), the call does not exceed 0.1 s on the time axis. Although difficult to assess precisely, the duration of the call pictured in the sonogram provided by Ibánez et al. (1999) is clearly shorter than 0.2 s. Furthermore, Ibáñez et al. (1999) present a sonogram of $D$. vocator, recorded also in the Canal Zone. According to their analysis, the call of $D$. vocator has a frequency range between 6000 and 7000 Hz , is very short, and shows a strong frequency modulation, thus being very different from the calls of $D$. diastema and $D$. citrinobapheus. However, the type locality of $D$. vocator is Agua Buena in the Peninsula de Osa, Costa Rica. Thus, it is advisable to record comparative call material from the type locality for future analyses. The most recent contribution on Diasporus vocalizations was made by Chaves et al. (2009) in the course of the original description of $D$. ventrimaculatus. This species' voice differs in all standard parameters from that of D. citrinobapheus, as it has much shorter call durations of about 0.08 s , a low dominant frequency of about 2550 Hz , and a lower frequency range between 2140 and 2995 Hz . Furthermore, the dominant frequency is reached at the very beginning at the call. The same authors presented a preliminary analysis of calls emitted by specimens assigned to D. hylaeformis. According to this analysis, call duration in $D$. hylaeformis is on average 0.214 s , while it resembles $D$. ventrimaculatus in spectral parameters. Regarding the vocalizations of $D$. tigrillo, the least known species in the genus, only a field note citation appearing in the original
description describes it as "similar to the dink dink of Eleutherodactylus [Diasporus] diastema" (Savage 1997).

Nevertheless, there is also an intraspecific variation among calls of specimens referred to Diasporus citrinobapheus. The call of the single male recorded at Willie Mazú (SMF 89817) differs from the calls of the members of the type series in temporal parameters, such as shorter call duration and call interval that result in a higher call rate. These differences are minor, but lead us to not include specimens from localities other than the type locality in the type series. However, various studies have shown that call parameter variation is linked to ambient temperature (e.g., Zweifel 1959; Schneider 1977; Gerhardt 1978). According to these studies, call duration and call interval are negatively correlated with temperature, which in turn leads to an increased call rate at higher temperatures. As SMF 89817 was recorded at lower temperatures than for the other three specimens, one would expect the opposite pattern. Nevertheless, these studies used data from many individuals, built scatter diagrams of parameters against temperature and fitted least-squares regression, and there are always outliers that do not follow the general trend. In our case, individual differences may be stronger than tem-perature-related ones, but this assumption needs further research to be reliably assessed.

Apart from morphology which apparently is not the best tool to identify species of Diasporus, neither DNA nor bioacoustics, both of paramount importance for contemporary anuran taxonomy, have been consistently analyzed among geographically and taxonomically wide-ranging samples. While the Panamanian and Costa Rican 16S barcodes compared in this study reveal the existence of more infrageneric lineages than names are available, the doubtless assignation of a given Diasporus "aff. hylaeformis" or "aff. diastema" is likely to be highly challenging if one is to rely on the existing treatments, which mostly provide only partial or even contradicting information. In conclusion, the complex and cryptic diversity within the genus Diasporus requires a thorough revision of as many "quality vouchers" (collected specimens associated with both well-preserved tissue samples and call recordings) from as many localities throughout the generic range as possible.

## Key to the species of Diasporus in Central America

1a Disk covers lanceolate or papillate .............................................................. 2
1b Disk covers palmate or spadate ................................................................... 3
2a Dorsum shagreened; fingers without thick lateral fringes; Toe V not partially fused with Toe IV; SVL of adult males 14.0-16.0 mm, of adult females 16.518.0 mm

Diasporus vocator
2b Dorsum with scattered low warts; fingers with thick lateral fringes; Toe V partially fused with Toe IV; SVL of adult males $10.9-14.8 \mathrm{~mm}$, of adult females $13.2-16.9 \mathrm{~mm}$.

Diasporus quidditus
3a Fingers II and III with palmate disk covers and broadened, non-triangular disk pads; adults with vomerine teeth

Diasporus diastema

3b Fingers II and III with spadate disk covers and triangular disk pads; adults with or without vomerine teeth
4 a Venter in most individuals with distinct black and white blotches; dorsum and dorsal surfaces of arms and legs in some individuals bright red in life.....

## Diasporus ventrimaculatus

$4 b \quad$ Venter patternless or with a few small black dots; dorsum and dorsal surfaces of arms and legs brown, cream, or yellow in life 5
5a Posterior surface of thigh pigmented (brownish, often suffused with red in life); overall dorsal coloration bright cream, grayish or reddish brown in life; adults without vomerine teeth ..................................Diasporus hylaeformis
5b Posterior surface of thigh unpigmented (yellow in life); overall dorsal coloration bright yellow to orange in life; adults with vomerine teeth 6

6a Dorsum with scattered low pustules; ratio tympanum length / eye length 0.54-0.57; distal subarticular tubercle on Fingers and Toes I weakly bifid; dorsum yellow to orange with dark brown spots confined to pustules; SVL of adult males $16.0-17.5 \mathrm{~mm}$

Diasporus tigrillo
6b Dorsum smooth; ratio tympanum length / eye length 0.32-0.45; distal subarticular tubercle on Fingers and Toes I flat and rounded; dorsum uniformly bright yellow to orange, sometimes with irregularly distributed dark blotches; SVL of adult males $17.3-19.7 \mathrm{~mm}$

Diasporus citrinobapheus

## Acknowledgments

Collecting permits SC/A-8-09 and SC/A-21-10, as well as the corresponding exportation permits were issued by the Dirección de Áreas Protegidas y Vida Silvestre of the Autoridad Nacional del Ambiente (ANAM), Panama City, Panama. An additional permit for the Comarca Ngöbe-Buglé was issued by Rogelio Moreno (Cazique General of the Ngöbe), San Félix. We are especially grateful to the inhabitants of Paredón for their exceptional hospitality, to Wilberto Martínez, owner of the private reserve Willie Mazú, for his permission to survey on his ground, as well as to the family Peńa Solís, Alto de Piedra, for logistical support. For assistance in the field we thank Arcadio Carrizo, Joe Bienentreu and Smelin Abrego. Particular thanks go to Dietrich Mebs and Werner Pogoda, Center for Forensic Medicine, University of Frankfurt am Main, who kindly performed an analysis of the new species' skin secretions, to Neftali Camacho, Natural History Museum of Los Angeles County, Los Angeles, who provided photographs of the type material of Diasporus tigrillo, and to Linda Acker, Senckenberg Forschungsinstitut and Naturmuseum, who prepared the illustrations of the holotype of D. citrinobapheus. We thank Franco Andreone, Larry Wilson and two anonymous reviewers for insightful comments on an early draft of the manuscript. This paper is based upon work funded to AH by the FAZITStiftung, and to SL by the Studienstiftung des deutschen Volkes, as well as the Vereinigung von Freunden und Förderern der Goethe-Universität.

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## Appendix I

## Comparative material for morphological examination

Diasporus diastema - Costa Rica: Heredia: Puerto Viejo de Sarapiquí, entrance to La Selva, 30 m: SMF 81812; Rara Avis, 700 m: SMF 81811; Honduras: Gracias a Dios: Region La Mosquitia, Rio Plátano Biosphere Reserve, Raudal Kiplatara, 162 m: SMF 85938; El Ocotilla, 410 m: SMF 85939; Nicaragua: Atlántico Norte: PN Saslaya, 920 m: SMF 82031-82035; Jinotega: Bosawas Biosphere Reserve: SMF 78561; National Park Saslaya, 188 m: SMF 78965; Matagalpa: Selva Negra, 1300 m: SMF 77231, 77235, 78184-78191; Río San Juan: Bartola, 30-70 m: SMF 80977-80979, SMF 79799-79800; Río Sarnoso, 25 m: SMF 79796-79798; Boca de San Carlos, 20 m : SMF 79794-5; ridge near Río Las Cruces, near Caño Negro, 415 m: SMF 83389; Cerro el Bolívar, near Río Machado, 280 m: SMF 83390; Lomas de Tambor, 210 m: SMF 83391; Panama: Bocas del Toro: Bosque Protector Palo Seco, 1148 m: SMF 84997; Archipelago Bocas del Toro, Isla Colón, 30 m: SMF 85068; Chiriquí: El Chorogo, 295 m: SMF 92008; Coclé: Cerro Gaitál, El Valle de Antón, 800 m: SMF 80781; Comarca Kuna Yala: Reserva Natural Nusagandi, 280 m: SMF 81961; Panamá: Canal Zone: SMF 29859, 29874.
Diasporus aff. hylaeformis - Panama: Bocas del Toro: Parque Internacional La Amistad, northern slope of Cerro Pando, 2400 m: SMF 89868, 89869, 89873, 89874, AH 263, AH 266. Chiriquí: Parque Internacional La Amistad, Jurutungo, southern slope of Cerro Pando, 2070-2330 m: SMF 89867, 89872, 89875, 89876, AH 126, 127; Cerro Punta, Las Nubes Ranger Station, 2070 m: SMF 89870, 89871.
Diasporus vocator - Panama: Bocas del Toro: Humedal de San San Pond Sak, 5 m: SMF 89865, AH 364; Comarca Kuna Yala: Reserva Natural Nusagandi, 350 m: SMF 81970-81976.

## Appendix II

Corresponding information of sequenced Diasporus specimens.

| Species | Collection <br> number | Field <br> number | GenBank <br> accession <br> no. | Country | Province | Latitude | Longitude |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| D. citrinobapheus | SMF 89814 | AH 449 | JQ927333 | Panama | Comarca <br> Ngöbe-Buglé | 8.485 | -81.173 |
| D. citrinobapheus | SMF 89820 | AH 211 | JQ927334 | Panama | Veraguas | 8.569 | -81.099 |
| D. citrinobapheus | MHCH 2370 | AH 450 | JQ927335 | Panama | Comarca <br> Ngöbe-Buglé | 8.485 | -81.173 |
| D. citrinobapheus | MHCH 2371 | AH 452 | JQ927336 | Panama | Comarca <br> Ngöbe-Buglé | 8.485 | -81.173 |
| D. aff. hylaeformis | SMF 89868 | AH 267 | JQ927337 | Panama | Bocas del <br> Toro | 8.931 | -82.714 |


| Species | Collection <br> number | Field <br> number | GenBank <br> accession <br> no. | Country | Province | Latitude | Longitude |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| D. aff. hylaeformis | SMF 89869 | AH 268 | JQ927338 | Panama | Bocas del <br> Toro | 8.931 | -82.714 |
| D. aff. hylaeformis | SMF 89872 | AH 124 | JQ927339 | Panama | Chiriquí | 8.911 | -82.713 |
| D. aff. hylaeformis | SMF 89875 | AH 282 | JQ927340 | Panama | Chiriquí | 8.912 | -82.713 |
| D. aff. diastema <br> 'orange' | USNM 572442 | KRL 0902 | FJ784425 | Panama | Coclé | 8.667 | -80.592 |
| D. aff. diastema <br> 'orange' | USNM 572443 | KRL 1181 | FJ784484 | Panama | Coclé | 8.667 | -80.592 |
| D. aff. diastema <br> 'orange' | USNM 572454 | KRL 0900 | FJ784423 | Panama | Coclé | 8.667 | -80.592 |
| D. aff. diastema <br> 'orange' | USNM 572455 | KRL 0901 | FJ784424 | Panama | Coclé | 8.667 | -80.592 |
| D. aff. diastema <br> 'orange' | MVUP 1783 | KRL 0694 | FJ784338 | Panama | Coclé | 8.667 | -80.592 |
| D. aff. diastema <br> 'orange' | MVUP 1830 | KRL 0840 | FJ784395 | Panama | Coclé | 8.667 | -80.592 |
| D. quidditus | USNM 572444 | KRL 0647 | FJ784326 | Panama | Coclé | 8.667 | -80.592 |
| D. quidditus | MVUP 1832 | KRL 0856 | FJ784405 | Panama | Coclé | 8.667 | -80.592 |
| D. vocator | FMNH 257769 | AJC 0127 | JN991419 | Costa Rica | Puntarenas | 8.79 | -82.96 |
| D. aff. diastema | USNM 572546 | KRL 0782 | FJ784369 | Panama | Coclé | 8.667 | -80.592 |
| D. aff. diastema | MVUP 1826 | KRL 0831 | FJ784390 | Panama | Coclé | 8.667 | -80.592 |
| D. diastema | MVZ 203844 | 1999 | EU186682 | Costa Rica | Cartago | 9.75 | -83.804 |
| D. hylaeformis | UCR 16264 | AJC 0468 | JN991418 | Costa Rica | Alajuela | 10.22 | -84.54 |
| Pristimantis ridens | UTA-A 57014 | ENS <br> 10722 | JN991464 | Honduras | Olancho | 14.93 | -86.14 |

## Appendix III

Audio samples of the advertisement calls of specimens of Diasporus citrinobapheus. (doi: 10.3897/zookeys.196.2774.app1) File format: MP3 Audio file (MP3).

Explanation note: MP3 audio samples of specimens of Diasporus citrinobapheus recorded at the type locality (Paredón) and Willie Mazú, Serranía de Tabasará, Panama.

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[^0]
# Eriophyoid mites from Qinghai Province, northwestern China with descriptions of nine new species (Acari, Eriophyoidea) 

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Academic editor: A. Bochkov | Received 23 January 2012 | Accepted 10 May 2012 | Published 21 May 2012
urn:lsid:zoobank.org:pub:3648605E-11DD-4E95-8D86-9E91E90055EA
Citation: Li H-S, Xue X-F, Hong X-Y (2012) Eriophyoid mites from Qinghai Province, northwestern China with descriptions of nine new species (Acari, Eriophyoidea). ZooKeys 196: 47-107. doi: 10.3897/zookeys.196.2726


#### Abstract

Eriophyoid mites from Qinghai Province, northwestern China were studied herein. Up to now, only six species have been reported from Qinghai Province. In field surveys, 17 eriophyoid mite species were collected, among which nine species were found new to science. The new species and their host plants are listed as follows: Acaphyllisa tuberculumae sp. n. on Populus sp. (Salicaceae); Proiectus xiningensis sp. n. on Pinus sp. (Pinaceae); Phyllocoptes beishaniensis sp. n. on Spiraea mongolica Maxim. (Rosaceae); Tetra pruniana sp. n. on Prunus tomentosa Thunb. (Rosaceae) Rupr. (Berberidaceae); Tetra pyriana sp. n. on Pyrus calleryana Decne. (Rosaceae); Tetra simonia sp. n. on Populus simonii Carr. (Salicaceae); Diptacus berberinus sp. n. on Berberis amurensis Rupr. (Berberidaceae); Diptacus mengdaensis sp. n. on Lonicera elisae Franch. (Caprifoliaceae); Rhyncaphytoptus spinus sp. n. on Lonicera rupicola Hook. f. et Thoms. (Caprifoliaceae). Aculops ulmi Hong \& Xue, 2005 was re-described.


## Keywords

Eriophyoid mites, Qinghai, taxonomy, new species

[^1]
## Introduction

Qinghai Province ( $89^{\circ} 35^{\prime} \mathrm{E}-103^{\circ} 04^{\prime} \mathrm{E}, 13^{\circ} 39^{\prime} \mathrm{N}-39^{\circ} 19^{\prime} \mathrm{N}$ ), located in the northwest of the People's Republic of China, is a part of the Qinghai-Tibet Plateau, with an average elevation of over $3000 \mathrm{~m}(1650 \mathrm{~m}-6860 \mathrm{~m})$. The average temperature ranges from $0.4^{\circ} \mathrm{C}$ to $7.4^{\circ} \mathrm{C}$. (Qinghai Province Government Website).

Up to now, six species of eriophyoid mites from Qinghai have been reported. They are Aceria paramacrodonis Kuang, 1988 on Lycium sp. (Solanaceae), Aceria qinghaiensis Kuang, 1997 on Salix babylonica L. (Salicaceae), Aculodes salicis Kuang, 1997 on Salix babylonica L. (Salicaceae), Aculops xiningensis Kuang, 2000 on Malus pumila P. Mill. (Rosaceae), Aculus huangzhongensis Kuang, 2000 on Syringa oblata Lindl. (Oleaceae) and Tetraspinus syringae Lin \& Kuang, 2001 on Syringa oblata Lindl. (Oleaceae). In July 2007, field surveys were conducted in Qinghai Province, northwestern China. Twenty-five eriophyoid mite samples were collected and 17 eriophyoid mite species were identified, among which nine species were found new to science. No species reported earlier from Qinghai were collected in this survey. In total, there are 23 species of the Eriophyoidea from Qinghai Province, belonging to two families and 12 genera. A list of eriophyoid mites from Qinghai Province is given (Table 1).

Table I. List of eriophyoid mites and their hosts in Qinghai Province.

| Family | Subfamily | Tribe | Species | Host |
| :---: | :---: | :---: | :---: | :---: |
| Eriophyidae | Eriophyinae | Aceriini | Aceria paramacrodonis Kuang, 1988 | Lycium sp. (Solanaceae) |
|  |  |  | Aceria qinghaiensis Kuang, 1997 | Salix babylonica L. (Salicaceae) |
|  | Phyllocoptinae | Acaricalini | Acaphyllisa tuberculumae sp. n. | Populus sp. (Salicaceae) |
|  |  | Phyllocoptini | Proiectus xiningensis sp. n . | Pinus sp. (Pinaceae) |
|  |  |  | Phyllocoptes beishaniensis sp. n. | Spiraea mongolica Maxim. (Rosaceae) |
|  |  |  | Phyllocoptes asperatae Song, Xue \& Hong, 2006 | Picea meyeri Rehd. Et Wils. (Pinaceae) |
|  |  |  | Phyllocoptes dangchangi Song, Xue \& Hong, 2006 | Picea sp. (Pinaceae) |
|  |  |  | Phyllocoptes gansunensis Kuang \& Luo, 1998 | Potentilla parvifolia Fisch. ap. Lehm. (Rosaceae) |
|  |  |  | Phyllocoptruta platycladusa Xue, Song, Amrine \& Hong, 2007 | Juniperus chinensis L. (Cupressaceae) |
|  |  | Anthocoptini | Aculus changbais Xue, Song \& Hong, 2008 | Salix chaenomeloides Kimura ( Salicaceae) |
|  |  |  | Aculus huangzhongensis Kuang, 2000 | Syringa oblata Lindl. (Oleaceae) |
|  |  |  | Aculodes salicis Kuang, 1997 | Salix babylonica L. (Rosaceae) |
|  |  |  | Aculops umli Hong \& Xue, 2005 | Ulmus sp. (Ulmaceae) |



## Materials and methods

In the field, eriophyoid mites were collected by the aid of a hand-lens (30X) from the lower surface of host plant leaves. Eriophyoid mites, together with host plants, were immersed in $75 \%$ alcohol and kept in vials. Each vial was marked with the collection data, such as specimen number, collection date, host plant, mite color, location, collector, and mite relationship to host plant. The collection data were also recorded in the collection notebook for further use. The host plants were kept in a plant specimen folder in a dry environment.

The morphological terminology used here follows that of Lindquist (1996) and the generic classification was made according to Amrine et al. (2003). Slides were mounted using Keifer's F-medium and modified Berlese medium (Amrine and Manson 1996). Specimens were measured based on the methods outlined by de Lillo et al. (2010). Specimens were examined with a Zeiss A2 (Germany) research microscope with phase contrast and semi-schematic drawings were made. Photos of slide mounted mites were taken with the same microscope ( $100 \times$ oil immersion objective with $10 \times$ eyepieces), connected to a computer using Axiovision image analysis software. For each species, the holotype female measurement precedes the corresponding range for paratypes (given in parentheses). All measurements are in micrometres ( $\mu \mathrm{m}$ ), and are lengths when not otherwise specified. All the type materials are deposited at Arthropod/Mite collection of, the Department of Entomology, Nanjing Agricultural University, Jiangsu Province, China.

## Taxonomy

Family Eriophyidae Nalepa, 1898
Subfamily Eriophyinae Nalepa, 1898
Tribe Aceriini Amrine \& Stasny, 1994
Genus Aceria Keifer, 1944

## Aceria paramacrodonis Kuang, 1988

http://species-id.net/wiki/Aceria_paramacrodonis
Aceria paramacrodonis Kuang 1988: 49-50, figures 1-6.
Aceria paramacrodonis; Amrine and Stasny 1994: 73.
Aceria paramacrodonis; Kuang 1995: 61, figure 45.
Aceria paramacrodonis; Amrine and Stasny 1996: 295-304.
Aceria paramacrodonis; Hong and Zhang 1996: 25, figure 43.
Aceria paramacrodonis; Song et al. 2008: 4.

Host. Lycium sp. (Solanaceae).
Relation to host. Leaf gall; mites produce pocket galls on the lower side of leaves. Distribution. China (Gansu, Ningxia, Qinghai, Shandong).

## Aceria qinghaiensis Kuang, 1997

http://species-id.net/wiki/Aceria_qinghaiensis
Aceria qinghaiensis Kuang and Pang 1997: 231-232, figures 6-11.
Aceria qinghaiensis; Kuang et al. 2005: 31-32, figure 29.
Aceria qinghaiensis; Song et al. 2008: 14.

Host. Salix babylonica L. (Salicaceae).
Relation to host. The mites produce pockets on the lower surface of the leaves. Distribution. China (Gansu, Qinghai).

Subfamily Phyllocoptinae Nalepa, 1892
Tribe Acaricalini Amrine \& Stasny, 1994
Genus Acaphyllisa Keifer, 1978

## Acaphyllisa tuberculumae sp. n.

urn:lsid:zoobank.org:act:90E42ADF-5C3A-449D-A4C1-BA012B3D09F1
http://species-id.net/wiki/Acaphyllisa_tuberculumae
Figures 1-3

Description. Female. $(\mathrm{n}=8)$ Body fusiform, light yellow, 171 (171-195), 72 (70-75) wide. Gnathosoma 21 (20-21), projecting obliquely down, suboral plate present, pe-


Figure I. Acaphyllisa tuberculumae sp. n.: D dorsal view of female CG coxae and female genitalia CMG coxae and male genitalia.
dipalp coxal seta (ep) 4 (4-5), dorsal pedipalp genual seta (d) 7 (7-8), cheliceral stylets 16 (16-18). Prodorsal shield 49 (45-49), 53 (53-60) wide, subtriangular; frontal lobe 6 (5-8); median, admedian and submedian lines present, median line ending at


Figure 2. Acaphyllisa tuberculumae sp. n.: V ventral view of female em empodium IG female internal genitalia L1 leg I L2 leg II.


Figure 3. Acaphyllisa tuberculumae sp. n.: A dorsal view of female $\mathbf{B}$ ventral view of female $\mathbf{C}$ dorsal view of female posterior part $\mathbf{D}$ ventral view of female posterior part $\mathbf{E}$ prodorsal shield $\mathbf{F}$ coxae and female genitalia.
basal $1 / 2$ of prodorsal shield, admedian lines connected at basal $1 / 2$ and $2 / 3$ of prodorsal shield, forming three cells on each side of the median line. Scapular tubercles ahead of rear shield margin, $2(2-3), 18(18-19)$ apart, scapular setae (sc) $8(6-8)$, projecting centrad. Coxigenital region with 10 smooth annuli. Coxisternal plates with granules, anterolateral setae on coxisternum I (1b) 7 (7-8), 14 (14-15) apart, proximal setae on coxisternum I (1a) 19 (19-21), 11 (10-11) apart, proximal setae on coxisternum II (2a) $51(51-53), 26(26-28)$ apart, tubercles $1 b$ and $1 a 8$ (8-9) apart, tubercles $1 a$ and $2 a 8$ (8-9) apart. Prosternal apodeme combined, 7 (6-7). Leg I 36 (35-36), femur $10(9-10)$, basiventral femoral seta $(b v) 8(8-10)$; genu $5(5-6)$, antaxial genual seta ( $l^{\prime \prime}$ ) $29(29-31)$; tibia $8(7-8)$, paraxial tibial seta ( $l$ ) $7(7-8)$, located at $1 / 4$ from dorsal base; tarsus $8(7-8)$, seta $f t^{\prime} 17(17-19)$, seta $f t^{\prime \prime} 22(22-23)$, seta $u^{\prime} 5(5-6)$; tarsal empodium (em) 6 (6-7), divided, 2-rayed on each side, tarsal solenidion ( $\omega$ ) 7 (6-7), knobbed. Leg II 28 (28-33), femur $10(9-10)$, basiventral femoral seta ( $b v$ ) $10(9-10)$; genu $5(4-5)$, antaxial genual seta ( $\left.l^{\prime \prime}\right) 6(6-8)$; tibia $6(6-7)$; tarsus 7 (6-7), seta ft'5 (5-7), seta ft" 21 (21-22), seta $u^{\prime} 5(5-6)$; tarsal empodium (em) 6 (5-6), divided, 2-rayed on each side, tarsal solenidion ( $\omega$ ) 6 (6-7), knobbed. Opisthosoma dorsally with 55 (55-57) annuli, with round microtubercles, ventrally with 77 (74-77) annuli, with round microtubercles. Setae c2 31 (28-31) on ventral annulus 14 (14-16), 56 (54-56) apart; setae $d 55$ (55-60) on ventral annulus 34 (33-34), 38 (33-38) apart; setae e 17 (16-17) on ventral annulus 52 (52-53), 20 (18-20) apart; setae $f 28(25-28)$ on ventral annulus $71(69-71), 26(25-26)$ apart. Setae h1 $2(2-3)$, h2 70 (70-75). Female genitalia 18 (17-18), 24 (24-25) wide, coverflap smooth, setae $3 a 33$ (33-35), 14 (14-15) apart.

Male. $(\mathrm{n}=1)$ Body fusiform, light yellow, 150, 57 wide. Gnathosoma 17, projecting obliquely down, suboral plate present, pedipalp coxal seta (ep) 5, dorsal pedipalp genual seta (d) 5 , cheliceral stylets 13 . Prodorsal shield has the same design as female, 44, 48 wide, subtriangular; frontal lobe 5 . Scapular tubercles ahead of rear shield margin, 3, 18 apart, scapular setae ( $s c$ ) 6, projecting centrad. Coxigenital region with 10 smooth annuli. Coxisternal plates with granules, anterolateral setae on coxisternum I (1b) 7, 12 apart, proximal setae on coxisternum I (1a) 18, 10 apart, proximal setae on coxisternum II (2a) 45, 23 apart, tubercles $1 b$ and $1 a 7$ apart, tubercles $1 a$ and $2 a$ 7 apart. Prosternal apodeme combined, 5. Legs with usual series of setae. Leg I 29, femur 9, basiventral femoral seta $(b v)$ 8; genu 5, antaxial genual seta ( $l^{\prime \prime}$ ) 31; tibia 7, paraxial tibial seta ( $l$ ) 6 , located at $1 / 4$ from dorsal base; tarsus 6 , seta $f t^{\prime} 17$ ), seta $f t^{\prime \prime}$ 18, seta u'4; tarsal empodium (em) 6, divided, 2-rayed on each side, tarsal solenidion $(\omega)$, knobbed. Leg II 28, femur 10, basiventral femoral seta (bv) 8; genu 4, antaxial genual seta ( $l^{\prime \prime}$ ) 8 ; tibia 6 ; tarsus 6 , seta $f t^{\prime} 5$, seta $f t^{\prime \prime} 18$, seta $u^{\prime} 4$; tarsal empodium (em) 5, divided, 2-rayed on each side, tarsal solenidion $(\omega)$ 6, knobbed. Opisthosoma dorsally with 51 annuli, with round microtubercles, ventrally with 66 annuli, with round microtubercles. Setae c2 21 on ventral annulus 12, 42 apart; setae $d 42$ on ventral annulus 27, 28 apart; setae $e 16$ on ventral annulus 43,16 apart; setae $f 25$ on ventral annulus 61, 21 apart. Setae $h 12$, $h 2$ 60. Male genitalia forming a " Y " like structure in the middle, 19 wide, setae $3 a 22$, 15 apart.

Type material. Holotype, female (slide number NJAUEri789B, marked Holotype), from Populus sp. (Salicaceae), Xining City, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime N}$, $101^{\circ} 45^{\prime} 27^{\prime \prime}$ E, elevation $2241 \mathrm{~m}, 21$ July 2007, coll. Xiao-Feng Xue. Paratypes, 7 females and 1 male (slide number NJAUEri789B), with the same data as holotype.

Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Etymology. The specific designation tuberculumae is from the character of dorsal opisthosomal microtubercles, "tuberculum" in Latin; masculine in gender.

Differential diagnosis. This species is similar to Acaphyllisa populi Xue \& Hong, 2006, but can be differentiated from the latter by prodorsal shield with six cells in the middle (prodorsal shield without cells in $A$. populi), opisthosoma dorsally with round microtubercles (opisthosoma dorsally with elliptical microtubercles only on ridges in A. populi), female genitalia coverflap smooth (female genital coverflap with 10 longitudinal ridges in $A$. populi).

## Tribe Phyllocoptini Nalepa, 1892 <br> Genus Proiectus Huang, 2001

## Proiectus xiningensis sp. n .

urn:lsid:zoobank.org:act:21DA68FE-18B9-4305-B088-20BF3A865A3C
http://species-id.net/wiki/Proiectus_xiningensis
Figures 4-7

Description. Female. $(\mathrm{n}=5)$ Body fusiform, light yellow, 248 (223-308), 100 (100$110)$ wide, 90 (90-91) thick. Gnathosoma 33 (33-34), projecting obliquely down, suboral plate present, pedipalp coxal seta (ep) 3 (3-5), dorsal pedipalp genual seta (d) 15 (11-15), cheliceral stylets 33 (33-34). Prodorsal shield 73 (65-73), 100 (100110 ) wide, subtriangular, with a large projection on each lateral margin 7 (6-7); frontal lobe broad 24 (22-24); median, admedian and submedian lines obscure, median and admedian lines connected at base. Scapular tubercles ahead of rear shield margin, $2(2-3), 30(27-30)$ apart, scapular setae (sc) 8 (6-8), projecting centrad. Coxigenital region with $17(14-17)$ annuli, with round microtubercles, with deep seam under coxisternal plate II. Coxisternal plates with short lines, anterolateral setae on coxisternum I (1b) $10(10-11), 18(18-19)$ apart, proximal setae on coxisternum I (1a) 22 (20-22), 13 (12-13) apart, proximal setae on coxisternum II (2a) 52 (50-52), 32 (32-33) apart, tubercles $1 b$ and $1 a 11(10-11)$ apart, tubercles $1 a$ and $2 a 10(10-11)$ apart. Prosternal apodeme separated, 4 (4-5). Leg I 43 (40-45), femur 9 (8-10), basiventral femoral seta ( $b v$ ) $14(12-14)$; genu 9 ( $8-10$ ), antaxial genual seta ( $l^{\prime}$ ) 28 (28-34); tibia $15(13-15)$, paraxial tibial seta (l) $5(5-6)$, located at $1 / 2$ from dorsal base; tarsus $10(9-10)$, seta ft' $17(17-18)$, seta $f t^{\prime \prime} 30(30-32)$, seta $u^{\prime} 5(5-6)$; tarsal empodium (em) 8 (7-8), simple, 5-rayed, tarsal solenidion ( $\omega$ ) 10 ( $9-10$ ), knobbed. Leg II 40 ( $40-41$ ), femur 13 (13-16), basiventral femoral seta $(b v) 13(10-13)$; genu 6 (6-8), antaxial genual seta ( $l^{\prime \prime}$ ) $8(7-8)$; tibia $10(10-12)$; tarsus 8 (8-9), seta ft'9 (8-9),


Figure 4. Proiectus xiningensis sp. n.: D dorsal view of female CMG coxae and male genitalia CG coxae and female genitalia.


Figure 5. Proiectus xiningensis sp. n.: $\mathbf{L}$ lateral view of female LO lateral microtubercles IG female internal genitalia em empodium L1 leg I L2 leg II.


Figure 6. Proiectus xiningensis sp. n.: A dorsal view of female $\mathbf{B}$ ventral view of female $\mathbf{C}$ lateral microtubercles D empodium $\mathbf{E}$ dorsal view of female posterior part $\mathbf{F}$ ventral view of female posterior part $\mathbf{G} \operatorname{leg} \mathrm{I}$ and $\operatorname{leg}$ II.


Figure 7. Proiectus xiningensis sp. n.: $\mathbf{H}$ lateral view of female $\mathbf{I}$ coxae and female genitalia $\mathbf{J}$ coxae and male genitalia $\mathbf{K}$ lateral view of female posterior part $\mathbf{L}$ prodorsal shield $\mathbf{M}$ female internal genitalia.
seta $f t^{\prime \prime} 27$ (27-28), seta $u^{\prime} 5(5-6)$; tarsal empodium (em) 8 (7-8), simple, 5-rayed, tarsal solenidion $(\omega) 10$ (9-10), knobbed. Opisthosoma dorsally with 41 (39-41) annuli, with weak filamentous microtubercles, ventrally with 92 (92-100) annuli, with round microtubercles. Setae $c 221$ (21-22) on ventral annulus 15 (15-20), 70 (70-73) apart; setae $d 100(80-100)$ on ventral annulus 34 (34-39), 37 (37-46) apart; setae $e 55$ (55-65) on ventral annulus 56 (56-61), 21 (21-22) apart; setae $f 33$ (30-33) on ventral annulus 84 (84-90), 28 (28-29) apart. Setae h1 7 (6-7), h2 65 (55-65). Female genitalia 28 (28-30), 33 (33-34) wide, coverflap with 19 longitudinal ridges, setae $3 a 10$ (10-12), 23 (21-22) apart.

Male. $(\mathrm{n}=1)$ Body fusiform, light yellow, 250, 90 wide. Gnathosoma 34, projecting obliquely down, suboral plate present, pedipalp coxal seta (ep) 3, dorsal pedipalp genual seta (d) 11, cheliceral stylets 50 . Prodorsal shield has the same design as female, 70, 90 wide, subtriangular; with a large projection on each lateral margin, 6 ; frontal lobe broad, 21. Scapular tubercles ahead of rear shield margin, 2, 23 apart, scapular setae $(s c) 6$, projecting centrad. Coxigenital region with 16 annuli, with round microtubercles, with deep seam under coxisternal plate II. Coxisternal plates with short lines, anterolateral setae on coxisternum I (1b) 10, 15 apart, proximal setae on coxisternum I (1a) 15,12 apart, proximal setae on coxisternum II (2a) 52, 29 apart, tubercles $1 b$ and 1a 10 apart, tubercles $1 a$ and $2 a 11$ apart. Prosternal apodeme separated, 5. Leg I 40, femur 10, basiventral femoral seta $(b v) 13$; genu 8 , antaxial genual seta $\left(l^{\prime}\right) 31$; tibia 13 , paraxial tibial seta ( $l$ ) 5 , located at $1 / 2$ from dorsal base; tarsus 8 , seta ft' 17 , seta $f t^{\prime \prime} 30$, seta $u$ ' 5 ; tarsal empodium (em) 7, simple, 5-rayed, tarsal solenidion ( $\omega$ ) 9, knobbed. Leg II 37, femur 15, basiventral femoral seta ( $b v$ ) 12; genu 6, antaxial genual seta ( $l l^{\prime \prime}$ ) 7 ; tibia 10 ; tarsus 8 ), seta $f t^{\prime} 7$, seta $f t^{\prime \prime} 25$, seta $u^{\prime} 5$; tarsal empodium (em) 7 , simple, 5-rayed, tarsal solenidion $(\omega) 9$, knobbed. Opisthosoma dorsally with 41 annuli, with weak filamentous microtubercles, ventrally with 94 annuli, with round microtubercles. Setae $c 221$ on ventral annulus 18, 60 apart; setae $d 80$ on ventral annulus 36, 38 apart; setae $e 60$ on ventral annulus 58, 20 apart; setae $f 27$ on ventral annulus 88,25 apart. Setae $h 16$, h2 65. Male genitalia 25 wide, setae $3 a 12$, 22 apart.

Type material. Holotype, female (slide number NJAUEri790, marked Holotype), from Pinus sp. (Pinaceae), Xining City, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime N}$, $101^{\circ} 45^{\prime} 27^{\prime \prime}$ E, elevation $2241 \mathrm{~m}, 21$ July 2007, coll. Xiao-Feng Xue. Paratypes, 4 females and 1 male (slide number NJAUEri790), with the same data as holotype.

Relation to host. Vagrant on terminal part of the needles. No damage to the host was observed.

Etymology. The specific designation xiningensis is from the place name Xining City, where this new species was collected; feminine in gender.

Differential diagnosis. This species is similar to Proiectus thunbergis Xue, Song, Amrine \& Hong, 2007, but can be differentiated from the latter by median and admedian lines of prodorsal shield simple (median and admedian lines with granules in $P$. thunbergis), opisthosoma dorsally annuli with weak filamentous microtubercles (opisthosoma dorsally annuli with round microtubercles in $P$. thunbergis), tarsal empodium (em) 5-rayed (4-rayed in $P$. thunbergis).

## Genus Phyllocoptes Nalepa, 1887

## Phyllocoptes beishaniensis sp. n.

urn:lsid:zoobank.org:act:0ABE4028-A29D-4B4A-B7FE-133603A01C99
http://species-id.net/wiki/Phyllocoptes_beishaniensis
Figures 8-11

Description. Female. ( $\mathrm{n}=13$ ) Body fusiform, light yellow, 168 (160-178), 63 (6667 ) wide, 61 (61-68) thick. Gnathosoma 20 (20-25), projecting obliquely down, suboral plate present, pedipalp coxal seta (ep) 6 (5-6), dorsal pedipalp genual seta (d) 6 (6-7), cheliceral stylets 16 (16-20). Prodorsal shield 48 (46-48), 65 (60-65) wide, subtriangular; frontal lobe 10 ( $9-10$ ); median, admedian and submedian lines present, median line ending at basal $1 / 2$ of prodorsal shield and connected with admedian lines at basal $1 / 4$. Scapular tubercles ahead of rear shield margin, $2(2-3), 22$ (21-22) apart, scapular setae ( $s c$ ) 10 ( $8-11$ ), projecting centrad. Coxigenital region with 5 (4-5) smooth annuli. Coxisternal plates smooth, anterolateral setae on coxisternum I (1b) 8 (7-8), 13 (13-14) apart, proximal setae on coxisternum I (1a) 21 (17-21), 10 (10-11) apart, proximal setae on coxisternum II (2a) 42 (42-45), 30 (30-31) apart, tubercles $1 b$ and $1 a 9(9-10)$ apart, tubercles $1 a$ and $2 a 10(10-11)$ apart. Prosternal apodeme combined 6 (6-7). Leg I 33 (33-34), femur 11 (10-11), basiventral femoral seta (bv) 12 (12-13); genu 6 (5-6), antaxial genual seta $\left(l^{\prime \prime}\right) 23$ (22-23); tibia 8 ( $8-9$ ), paraxial tibial seta ( $l$ ) 7 ( $6-7$ ), located at $1 / 3$ from dorsal base; tarsus 6 (6-7), seta ft' 18 (18-19), seta ft" 22 (22-23), seta $u^{\prime} 5$ (5-6); tarsal empodium (em) 8 (8-9), simple, 6-rayed, tarsal solenidion ( $\omega$ ) 7 (6-7), knobbed. Leg II 31 (29-31), femur 10 (10-11), basiventral femoral seta (bv) 10 (9-10); genu $6(5-6)$, antaxial genual seta $\left(l^{\prime}\right) 10(8-10)$; tibia $6(6-7)$; tarsus $6(6-7)$, seta ft' 7 (6-7), seta $f t^{\prime \prime} 21$ (21-22), seta u' 5 (5-6); tarsal empodium (em) 8 (8-9), simple, 6 -rayed, tarsal solenidion ( $\omega$ ) 8 ( $8-9$ ), knobbed. Opisthosoma dorsally with 45 (45-53) annuli, smooth; ventrally with $52(51-52)$ annuli, with round microtubercles. Setae c2 30 (29-30) on ventral annulus 10 (9-13), 55 (54-55) apart; setae $d 60(55-60)$ on ventral annulus $20(19-20), 30(30-31)$ apart; setae e 40 (40-45) on ventral annulus $31(30-31), 15(15-16)$ apart; setae $f 25(25-27)$ on ventral annulus 46 (45-46), 24 (24-25) apart. Setae h1 5 (4-5), h2 80 (80-85). Female genitalia 17 (17-18), 22 (22-23) wide, coverflap with 10 longitudinal ridges, setae 3a 56 (55-56), 16 (16-17) apart.

Male. $(\mathrm{n}=9)$ Body fusiform, light yellow, 169-195, 56-67 wide. Gnathosoma 19-22, projecting obliquely down, suboral plate present, pedipalp coxal seta (ep) $4-5$, dorsal pedipalp genual seta (d) 6-7, cheliceral stylets 17-18. Prodorsal shield has the same design as female, 42-50, 49-56 wide, subtriangular; frontal lobe 8-9. Scapular tubercles ahead of rear shield margin, 2-3, 18-19 apart, scapular setae (sc) $8-9$, projecting centrad. Coxigenital region with 5 smooth annuli. Coxisternal plates smooth, anterolateral setae on coxisternum I (1b) 5-6, 12-15 apart, proximal setae on coxisternum I (1a) 13-14, 10-11 apart, proximal setae on coxister-


Figure 8. Phyllocoptes beishaniensis sp. n.: D dorsal view of female CMG coxae and male genitalia em empodium L1 leg I L2 leg II.



Figure 10. Phyllocoptes beishaniensis sp. n.: A dorsal view of female B ventral view of female $\mathbf{C}$ lateral microtubercles $\mathbf{D}$ empodium $\mathbf{E}$ dorsal view of female posterior part $\mathbf{F}$ ventral view of female posterior part $\mathbf{G} \operatorname{leg} \mathrm{I}$ and leg II.


Figure I I. Phyllocoptes beishaniensis sp. n.: H lateral view of female I lateral view of female posterior part J female internal genitalia $\mathbf{K}$ prodorsal shield $\mathbf{L}$ coxae and female genitalia $\mathbf{M}$ coxae and male genitalia.
num II (2a) 22-27, 27-31 apart, tubercles $1 b$ and $1 a 8-9$ apart, tubercles $1 a$ and 2a 9-10 apart. Prosternal apodeme combined, 5-7. Leg I 27-32, femur 10-11, basiventral femoral seta ( $b v$ ) 11-12; genu 5-6, antaxial genual seta ( $l^{\prime \prime}$ ) 21-22; tibia 6-7, paraxial tibial seta (l) 5-6, located at $1 / 3$ from dorsal base; tarsus 6-7, seta $f t^{\prime} 18-19$, seta $f t^{\prime \prime} 21-22$, seta $u^{\prime} 5-6$; tarsal empodium (em) 7-9, simple, 6-rayed, tarsal solenidion $(\omega)$ 6-7, knobbed. Leg II 27-30, femur 9-10, basiventral femoral seta (bv) 9-10; genu 5-6, antaxial genual seta ( $l^{\prime}$ ) 7-9; tibia 5-6; tarsus 5-6, seta $f t^{\prime} 5-6$, seta $f t^{\prime \prime} 17-19$, seta $u^{\prime} 5-6$; tarsal empodium (em) 7-8, simple, 6-rayed, tarsal solenidion $(\omega) 7-8$, knobbed. Opisthosoma dorsally with 52 annuli, smooth, ventrally with 58 annuli, with round microtubercles. Setae $c 221-23$ on ventral annulus 11, 46-53 apart; setae $d 29-30$ on ventral annulus $21,25-28$ apart; setae $e$ $25-28$ on ventral annulus $35,12-13$ apart; setae $f 20(20-23)$ on ventral annulus 54, 20-21 apart. Setae h1 4-5, h2 42-45. Male genitalia 19-21 wide, setae 3a 13-14, 17-19 apart.

Type material. Holotype, female (slide number NJAUEri815, marked Holotype), from Spiraea mongolica Maxim. (Rosaceae), Beishan National Forest Park, Huzhu County, Qinghai Province, P. R. China, $36^{\circ} 53^{\prime} 35^{\prime \prime} \mathrm{N}, 102^{\circ} 25^{\prime} 56^{\prime \prime} \mathrm{E}$, elevation 2610m, 22 July 2007, coll. Xiao-Feng Xue. Paratypes, 7 females and 2 males (slide number NJAUEri815), with the same data as holotype.

Relation to host. Vagrant on leaf lower surface.
Etymology. The specific designation beishaniensis is from the place name Beishan National Forest Park, where the new species were collected; feminine in gender.

Differential diagnosis. This species is similar to Phyllocoptes adalius (Keifer, 1939) from Rosa sp., but can be differentiated from the latter by prodorsal shield front lobe stout (prodorsal shield front lobe pointed in $P$. adalius), dorsal opisthosoma annuli smooth (opisthosoma annuli entirely covered with spinuliferous microtubercles in $P$. adalius), Coxisternal plates smooth (coxisternal plates with short lines in $P$. adalius).

## Phyllocoptes asperatae Song, Xue \& Hong, 2006

http://species-id.net/wiki/Phyllocoptes_asperatae
Phyllocoptes asperatae Song et al. 2006: 36-38, figure 2.
Phyllocoptes asperatae; Xue et al. 2009:134.
Phyllocoptes asperatae; Song et al. 2009c: 37.

Material examined. 4 females and 2 males (slide number NJAUEri810), from a new host, Picea meyeri Rehd. Et Wils. (Pinaceae), Beishan National Forest Park, Huzhu County, Qinghai Province, P. R. China, $36^{\circ} 53^{\prime} 35^{\prime \prime} \mathrm{N}, 102^{\circ} 25^{\prime} 56^{\prime \prime} \mathrm{E}$, elevation 2610 m , 22 July 2007, coll. Xiao-Feng Xue.

Host. Picea asperata Mast. (Pinaceae); Picea meyeri Rehd. Et Wils. (Pinaceae)
Relation to host. Vagrant on leaf surface. No damage to the host was observed. Distribution. China (Shaanxi, Qinghai).

## Phyllocoptes dangchangi Song, Xue \& Hong, 2006

http://species-id.net/wiki/Phyllocoptes_dangchangi
Phyllocoptes dangchangi Song et al. 2006: 38-40, figure 3 .
Phyllocoptes dangchangi; Song et al. 2008: 29.
Phyllocoptes dangchangi; Xue et al. 2009:134.
Phyllocoptes dangchangi; Song et al. 2009c: 36.

Material examined. 11 females and 1 male (slide number NJAUEri811), from Picea sp. (Pinaceae), Beishan National Forest Park, Huzhu County, Qinghai Province, P. R. China, $36^{\circ} 53^{\prime} 35^{\prime \prime} \mathrm{N}, 102^{\circ} 25^{\prime} 56^{\prime \prime} \mathrm{E}$, elevation 2610m, 22 July 2007, coll. Xiao-Feng Xue.

Host. Picea asperata Mast. (Pinaceae); Picea sp. (Pinaceae).
Relation to host. Vagrant on terminal part of the needles. No damage to the host was observed.

Distribution. China (Gansu, Qinghai).

## Phyllocoptes gansuensis Kuang \& Luo, 1998

http://species-id.net/wiki/Phyllocoptes_gansuensis
Phyllocoptes gansuensis Kuang et al. 1998: 201-203, figures 25-29.
Phyllocoptes gansuensis; Huang 2001: 45.
Phyllocoptes gansunensis; Kuang et al. 2005: 66-67, figure 64.
Phyllocoptes gansuensis; Song et al. 2008: 30.
Phyllocoptes gansuensis; Xue et al. 2009:134.
Phyllocoptes gansuensis; Song et al. 2009c: 37.
Material examined. 8 females and 2 males (slide number NJAUEri824), from a new host, Potentilla parvifolia Fisch. ap. Lehm. (Rosaceae), Beishan National Forest Park, Huzhu County, Qinghai Province, P. R. China, $36^{\circ} 53^{\prime} 35^{\prime \prime N}$, $102^{\circ} 25^{\prime} 56^{\prime \prime} \mathrm{E}$, elevation 2610m, 22 July 2007, coll. Xiao-Feng Xue.

Host. Potentilla glabra Lodd. (Rosaceae); Potentilla parvifolica Fisch. et. Lehm. (Rosaceae).

Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Distribution. China (Gansu, Qinghai).

## Genus Phyllocoptruta Keifer, 1938

Phyllocoptruta platyclada Xue, Song, Amrine \& Hong, 2007
http://species-id.net/wiki/Phyllocoptruta_platyclada
Phyllocoptruta platyclada Xue et al. 2007: 340-342, figure 3.
Phyllocoptruta platyclada; Xue et al. 2010: 698.

Material examined. 8 females (slide number NJAUEri814), from a new host, Juniperus chinensis L. (Cupressaceae), Beishan National Forest Park, Huzhu County, Qinghai Province, P. R. China, $36^{\circ} 53^{\prime} 35^{\prime \prime} \mathrm{N}, 102^{\circ} 25^{\prime} 56^{\prime \prime} \mathrm{E}$, elevation $2610 \mathrm{~m}, 22$ July 2007, coll. Xiao-Feng Xue.

Host. Platycladus orientalis (Linn.) Franco (Cupressaceae); Juniperus chinensis L. (Cupressaceae).

Relation to host. Vagrant on terminal part of the needles. No damage to the host was observed.

Distribution. China (Shaanxi, Qinghai).

Tribe Anthocoptini Amrine \& Stasny, 1994
Genus Aculus Keifer, 1959
Aculus changbais Xue, Song \& Hong, 2008
http://species-id.net/wiki/Aculus_changbais
Aculus changbais Xue et al. 2008: 41-42, figure 3.
Aculus changbais; Song et al. 2009b: 3.

Material examined. 8 females and 1 male (slide number NJAUEri778), from a new host, Salix chaenomeloides Kimura (Salicaceae), Mengda Natural Reserve, Xunhua County, Qinghai Province, P. R. China, $35^{\circ} 47^{\prime} 38^{\prime \prime} \mathrm{N}, 102^{\circ} 40^{\prime} 40^{\prime \prime} \mathrm{E}$, elevation 2523 m , 19 July 2007, coll. Xiao-Feng Xue.

Host. Salix gracilistyla Miq. (Salicaceae); Salix chaenomeloides Kimura (Salicaceae).
Relation to host. Vagrant on leaf lower surface. No damage to the host was observed. Distribution. China (Jilin, Qinghai).

Aculus huangzhongensis Kuang, 2000
http://species-id.net/wiki/Aculus_huangzhongensis
Aculus huangzhongensis Kuang 2000: 392-393, figures 13-18.
Aculus huangzhongensis; Kuang et al. 2005: 91-92, figure 91.

Aculus huangzhongensis; Song et al. 2009b: 2.
Host. Syringa oblata Lindl. (Oleaceae).
Relation to host. Vagrant on leaf surface. No damage to the host was observed. Distribution. China (Qinghai).

## Genus Aculodes Keifer, 1966

Aculodes salicis Kuang, 1997
http://species-id.net/wiki/Aculodes_salicis
Aculodes salicis Kuang 1997: 232-233, figures 12-15.
Aculodes salicis; Skoracka 2003: 43.
Aculodes salicis; Kuang et al. 2005: 80-81, figure 79.

Host. Salix babylonica L. (Salicaceae).
Relation to host. Forming galls on the leaf of the host.
Distribution. China (Qinghai), Poland.

## Genus Aculops Keifer, 1966

Aculops ulmi Hong \& Xue, 2005
http://species-id.net/wiki/Aculops_ulmi
Figures 12-15
Aculops ulmi Hong and Xue 2005: 205, 209.

Redescription. Female. $(\mathrm{n}=10)$ Body fusiform, light yellow, 192 (192-230), 70 (62-72) wide, 80 (80-81) thick. Gnathosoma 23 (23-25), projecting obliquely down, suboral plate present, pedipalp coxal seta (ep) $3(2-4)$, dorsal pedipalp genual seta (d) $6(5-6)$, cheliceral stylets 22 (22-25). Prodorsal shield 33 (33-34), 48 (48-51) wide, subtriangular; median, admedian and submedian lines present, median line ending at basal $1 / 4$ of prodorsal shield, median and admedian lines connected at basal $1 / 4$ of prodorsal shield, admedian and submedian lines connected at basal $2 / 3$ of prodorsal shield, forming two cells on both sides of median line. Scapular tubercles on rear shield margin, 4 (4-5), 26 (26-28) apart, scapular setae ( $s c$ ) 55 (55-60), projecting posteriorly, knobbed at the end. Coxigenital region with $7(6-7)$ annuli, with triangular microtubercles. Coxisternal plates with short lines and granules, anterolateral setae on coxisternum $\mathbf{I}(16) 13$ (10-13), 14 (14-15) apart, proximal setae on coxisternum I (1a) $30(27-30), 11(11-13)$ apart, proximal setae on coxisternum II (2a) 54 (54-57), 26 (26-28) apart, tubercles $1 b$ and $1 a 7$ (7-8) apart,


Figure I2. Aculops ulmi Hong \& Xue: D dorsal view of female L1 leg I L2 leg II em empodium IG female internal genitalia.


Figure 13. Aculops ulmi Hong \& Xue: L lateral view of female LO lateral microtubercles CG coxae and female genitalia.


Figure 14. Aculops ulmi Hong \& Xue: A dorsal view of female $\mathbf{B}$ ventral view of female $\mathbf{C}$ lateral view of female $\mathbf{D}$ dorsal view of female posterior part $\mathbf{E}$ ventral view of female posterior part $\mathbf{F}$ lateral microtubercles $\mathbf{G}$ lateral view of female posterior part $\mathbf{H}$ female internal genitalia.


Figure I5. Aculops ulmi Hong \& Xue: I female genitalia Jfemale genitalia from holotype.
tubercles $1 a$ and $2 a 9(9-10)$ apart. Prosternal apodeme combined, 7 (5-11). Leg I 31 (31-35), femur 10 (9-12), basiventral femoral seta (bv) 12 (11-14); genu 5 (5-6), antaxial genual seta ( $l^{\prime \prime}$ ) 22 (22-29); tibia $7(7-9)$, paraxial tibial seta $(l) 6(6-8)$, located at $1 / 3$ from dorsal base; tarsus 8 (8-11), seta ft' 16 (16-18), seta ft" 20 (20-25), seta $u^{\prime} 6(5-6)$; tarsal empodium (em) 6 (6-7), simple, 2-rayed, tarsal solenidion ( $\omega$ ) 9 (8-10), slightly knobbed. Leg II 28 (28-31), femur 9(9-11), basiventral femoral seta (bv) 12 (11-12); genu 5 (5-6), antaxial genual seta $\left(l^{\prime}\right) 9(9-13)$; tibia $5(5-7)$; tarsus $9(9-10)$, seta ft' $7(6-7)$, seta ft" 18 (18-20), seta $u^{\prime} 5$ (4-5); tarsal empodium (em) 6(6-7), simple, 2-rayed, tarsal solenidion ( $\omega$ ) 8 ( $8-11$ ), slightly knobbed. Opisthosoma dorsally with 35 (22-38) annuli, with triangular microtubercles, ventrally with 55 (55-56) annuli, with triangular microtubercles. Setae c2 16 (14-16) on ventral annulus 10 (10-11), 61 (61-69) apart; setae d57 (55-65) on ventral annulus 21 (21-22), $50(48-50)$ apart; setae $e 14(12-19)$ on ventral annulus 33 (32-34), 23 (23-24) apart; setae $f 27$ (26-30) on ventral annulus 51 (49-53), 21 (19-21) apart. Setae h1 3 (3-4), h2 90 (85-90). Female genitalia 11 (11-14), 23 (22-23) wide, coverflap with 8 longitudinal ridges, setae $3 a 17$ (17-22), 17 (16-17) apart.

Male. Unknown.
Type material. Hong and Xue (2005) described types as follows: Holotype female (slide number 17.viii.2003), from Ulmus sp. (Ulmaceae), Xingtai city, Hebei Province, P. R. China, coll. Xiao-Feng Xue. Paratypes (slide number 17.2003). 9 females and 2 males. All types here were re-examined. Female genitalia coverflap with 10-12 longitudinal ridges.

Additional material. 6 females (slide number NJAUEri792) from Ulmus sp. (Ulmaceae), Xining, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime} \mathrm{N}, 101^{\circ} 45^{\prime} 27^{\prime \prime} \mathrm{E}$, elevation 2241 m, 21 July 2007, coll. Xiao-Feng Xue.

Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Distribution. China (Hebei, Qinghai).
Notes. Instead of the original description: female genitalia coverflap smooth, female genitalia coverflap is with 8 longitudinal ridges in this redescription.

## Aculops xiningensis Kuang, 2000

http://species-id.net/wiki/Aculops_xiningensis
Aculops xiningensis Kuang 2000: 392, figures 7-12.
Aculops xiningensis; Kuang et al. 2005: 86-87, figure 86.

Host. Malus pumila P. Mill. (Rosaceae).
Relation to host. Vagrant on leaf lower surface. No damage to the host was observed. Distribution. China (Qinghai).

## Genus Tetraspinus Boczek, 1961

Tetraspinus syringae Lin \& Kuang, 2001
http://species-id.net/wiki/Tetraspinus_syringae
Tetraspinus syringae Lin and Kuang 2001: 351-353, figures 11-16.
Tetraspinus syringae; Kuang et al. 2005: 125-126, figure 128.

Host. Syringa oblata Lindl. (Oleaceae).
Relation to host. Vagrant on leaf lower surface. No damage to the host was observed. Distribution. China (Qinghai).

## Genus Tetra Keifer, 1944

Tetra pinnatifidae Xue, Song \& Hong, 2006
http://species-id.net/wiki/Tetra_pinnatifidae
Tetra pinnatifidae Xue et al. 2006a: 6-8, figure 2.

Material examined. 8 females and 2 males (slide number NJAUEri793) from a new host, Prunus armeniaca Linn. (Rosaceae), Xining, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime} \mathrm{N}, 101^{\circ} 45^{\prime} 27^{\prime \prime} \mathrm{E}$, elevation $2241 \mathrm{~m}, 21$ July 2007, coll. Xiao-Feng Xue.

Host. Crataegus pinnatifida Bunge (Rosaceae); Prunus armeniaca Linn. (Rosaceae).
Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Distribution. China (Shaanxi, Qinghai).

## Tetra pruniana sp. n.

urn:lsid:zoobank.org:act:D0B42704-F828-4F47-9265-47B3EF8E603A
http://species-id.net/wiki/Tetra_pruniana
Figures 16-18

Description. Female. ( $\mathrm{n}=9$ ) Body fusiform, light yellow, 215 (210-225), 77 (7578 ) wide. Gnathosoma 21 (21-23), projecting obliquely down, suboral plate present,
pedipalp coxal seta (ep) $4(4-5)$, dorsal pedipalp genual seta (d) $7(7-8)$, cheliceral stylets 16 (16-18). Prodorsal shield 54 (54-55), 75 (75-78) wide, subtriangular; frontal lobe 12 (11-12); median and submedian lines absent, admedian lines connected by two weak transverse lines. Scapular tubercles near rear shield margin, 3 (3-4), $34(32-34)$ apart, scapular setae (sc) 14 (13-15), projecting posteriorly. Rear shield with wave-like margin. Coxigenital region with 9 annuli. Coxisternal plates with short lines, anterolateral setae on coxisternum I (1b) 11 (10-13), 14 (13-14) apart, proximal setae on coxisternum I (1a) $20(20-25), 10(10-11)$ apart, proximal setae on coxisternum II (2a) $50(45-50), 28(28-29)$ apart, tubercles $1 b$ and $1 a 7(7-8)$ apart, tubercles $1 a$ and $2 a 10(10-12)$ apart. Prosternal apodeme combined, 8 (6-8). Leg I 34 (32-35), femur 12 (12-13), basiventral femoral seta ( $b v$ ) 12 (12-14); genu 7 (67), antaxial genual seta ( $l^{\prime \prime}$ ) 20 (19-20); tibia 10 ( $9-10$ ), paraxial tibial seta ( $l$ ) 6 (5-6), located at $1 / 3$ from dorsal base; tarsus $7(7-8)$, seta $f t^{\prime} 19(19-21)$, seta $f t " 25(24-25)$, seta u'5 (4-5); tarsal empodium (em) 7 (6-7), simple, 4-rayed, tarsal solenidion ( $\omega$ ) 6 (6-7), knobbed. Leg II 27 (27-31), femur 10 (10-11), basiventral femoral seta (bv) 12 (12-13); genu 5 (5-6), antaxial genual seta $\left(l^{\prime}\right) 8(7-8)$; tibia $6(6-7)$; tarsus $6(6-7)$, seta ft'6 (6-7), seta ft" $25(23-25)$, seta $u^{\prime} 5(5-6)$; tarsal empodium (em) 6 (5-6), simple, 4-rayed, tarsal solenidion ( $\omega$ ) 6 (6-7), knobbed. Opisthosoma dorsally with 25 (24-25) annuli, with weak filamentous microtubercles, ventrally with 53 (53-59) annuli, with round microtubercles. Setae c2 25 (24-26) on ventral annulus 9 (9-11), 58 (58-59) apart; setae $d 60$ (57-65) on ventral annulus 22 (22-24), 33 (32-33) apart; setae e 18 (17-20) on ventral annulus 37 (37-40), 18 (17-18) apart; setae $f 29(29-33)$ on ventral annulus $50(50-56), 25(22-25)$ apart. Setae h1 $3(2-3)$, h2 85 (80-95). Female genitalia 15 (15-17), 25 (24-25) wide, coverflap with 10 longitudinal ridges, setae $3 a 18$ (16-19), 17 (17-18) apart.

Male. ( $\mathrm{n}=6$ ) Body fusiform, light yellow, 170-186, 73-74 wide. Gnathosoma 16-17, projecting obliquely downwards, suboral plate present, pedipalp coxal seta (ep) 4-5, dorsal pedipalp genual seta (d) 7-8, cheliceral stylets 14-16. Prodorsal shield has the same design as female, 47-50, 72-73 wide, subtriangular; frontal lobe 10-12. Scapular tubercles on rear shield margin, 3-4, 30-33 apart, scapular setae (sc) 7-9, projecting posteriorly. Coxigenital region with 9 smooth annuli. Coxisternal plates with short lines, anterolateral setae on coxisternum $\mathbf{I}$ (1b) 10-11, 13-14 apart, proximal setae on coxisternum I (1a) 21-22, 10-11 apart, proximal setae on coxisternum II (2a) 45-48, 28-30 apart, tubercles $1 b$ and $1 a 7-8$ apart, tubercles $1 a$ and $2 a$ 9-10 apart. Prosternal apodeme combined, 7-8. Leg I 34-35, femur 10-12, basiventral femoral seta ( $b v$ ) 9-10; genu 6-7, antaxial genual seta ( $l^{\prime}$ ) 19-22; tibia 9-10, paraxial tibial seta (l) 6-7, located at $1 / 3$ from dorsal base; tarsus $7-8$, seta $f t^{\prime} 15-17$, seta $f t^{\prime \prime}$ 21-24, seta $u^{\prime} 4-5$; tarsal empodium (em) 7-8, simple, 4-rayed, tarsal solenidion ( $\omega$ ) 6-7, knobbed. Leg II 28-29, femur 9-10, basiventral femoral seta ( $b v$ ) 10-11; genu 5-6, antaxial genual seta ( $l^{\prime}$ ) 6-7; tibia 7-8; tarsus 6-7, seta ft' 6-7, seta $f t^{\prime \prime} 22-25$, seta $u$ ' 5-6; tarsal empodium (em) 6-7, simple, 4-rayed, tarsal solenidion ( $\omega$ ) 7-8, knobbed. Opisthosoma dorsally with 26-27 annuli, with weak filamentous microtubercles, ventrally with 53-54 annuli, with round microtubercles. Setae c2 18-20 on ventral annulus $10-11,55-65$ apart; setae $d 32-35$ on ventral annulus 20-22, 30-45


Figure 16. Tetra pruniana sp. n.: D dorsal view of female em empodium L1 leg I L2 leg II.
apart; setae $e 18-20$ on ventral annulus 33-35, 18-30 apart; setae $f 25-28$ on ventral annulus 50-51, 20-22 apart. Setae h1 2-3, h2 80-90. Male genitalia 13-14, 23-28 wide, setae $3 a 17-18$, 20-21 apart.


Figure 17. Tetra pruniana sp. n.: V ventral view of female IG female internal genitalia CMG coxae and male genitalia.

Type material. Holotype, female (slide number NJAUEri808, marked Holotype), from Prunus tomentosa Thunb. (Rosaceae), Xining City, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime} \mathrm{N}, 101^{\circ} 45^{\prime} 27^{\prime \prime} \mathrm{E}$, elevation 2241m, 21 July 2007, coll. Xiao-Feng Xue. Paratypes, 2 females and 4 males (slide number NJAUEri808), with the same data as holotype.


Figure 18. Tetra pruniana sp. n.: A dorsal view of female $\mathbf{B}$ ventral view of female $\mathbf{C}$ dorsal view of female posterior part $\mathbf{D}$ ventral view of female posterior part $\mathbf{E}$ prodorsal shield $\mathbf{F}$ coxae and female genitalia G female internal genitalia $\mathbf{H}$ male genitalia $\mathbf{I}$ empodium.

Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Etymology. The specific designation pruniana is from the generic name of host plant, Prunus; feminine in gender.

Differential diagnosis. This species is similar to Tetra pinnatifidae Xue, Song \& Hong, 2006a, but can be differentiated from the latter by median and submedian lines absent (median and submedian lines present in T. pinnatifidae), admedian lines connected by two weak transverse lines (admedian lines separated in T. pinnatifidae).

## Tetra pyriana sp. n.

urn:lsid:zoobank.org:act:99B1B2A9-DB3F-466C-8900-951BB3B35485
http://species-id.net/wiki/Tetra_pyriana
Figures 19-22

Description. Female. ( $\mathrm{n}=11$ ) Body fusiform, light yellow, 180 (169-185), 73 (7276) wide, 65 (64-65) thick. Gnathosoma 20 (20-23), projecting obliquely down, pedipalp coxal seta (ep) 4 (3-4), dorsal pedipalp genual seta (d) 6 (5-7), cheliceral stylets 20 (20-21). Prodorsal shield 47 (47-49), 68 (68-72) wide, subtriangular; frontal lobe $12(11-13)$; median, admedian and submedian lines present, median line obscure and ending at basal $1 / 3$ of prodorsal shield. Scapular tubercles on rear shield margin, 4 (3-4), 36 (33-36) apart, scapular setae (sc) 12 (12-13), projecting posteriorly. Coxigenital region with $13(12-13)$ smooth annuli. Coxisternal plates with short lines, anterolateral setae on coxisternum I (1b) 7 (7-9), 12 (11-12) apart, proximal setae on coxisternum $\mathbf{I}(1 a) 21(20-21), 10(9-10)$ apart, proximal setae on coxisternum II (2a) $40(40-45)$, $27(25-27)$ apart, tubercles $1 b$ and $1 a 6$ (6-7) apart, tubercles $1 a$ and $2 a 10(9-10)$ apart. Prosternal apodeme combined, 7 (6-7). Leg I 32 (30-32), femur $12(11-12)$, basiventral femoral seta ( $b v$ ) 11 (10-11); genu 6 (5-6), antaxial genual seta ( $l^{\prime}$ ) $20(20-21)$; tibia $7(7-8)$, paraxial tibial seta ( $l$ ) $5(5-6)$, located at $1 / 3$ from dorsal base; tarsus 8 (7-8), seta ft' 19 (17-19), seta ft" 25 (21-25), seta $u$ ' $5(4-5)$; tarsal empodium (em) $6(6-7)$, simple, 4-rayed, tarsal solenidion ( $\omega$ ) 6 (6-7), knobbed. Leg II 28 (27-28), femur 11 (10-11), basiventral femoral seta ( $b v$ ) 8 (8-11); genu 5 (4-5), antaxial genual seta ( $l^{\prime \prime}$ ) 8 (7-8); tibia 7 (6-7); tarsus 7 (6-7), seta ft'5 (5-6), seta ft" $20(19-20)$, seta $u^{\prime} 4(4-5)$; tarsal empodium (em) 6 (6-7), simple, 4-rayed, tarsal solenidion ( $\omega$ ) 5 (5-6), knobbed. Opisthosoma dorsally with 32 (30-32) annuli, with filamentous microtubercles, ventrally with 58 (58-60) annuli, with round microtubercles. Setae $c 225(24-30)$ on ventral annulus 11 (11-13), 57 (54-57) apart; setae $d 45$ (45-50) on ventral annulus 22 (22-24), 32 (32-33) apart; setae $e 18(17-18)$ on ventral annulus $37(37-41), 17(16-17)$ apart; setae $f 29$ (28-30) on ventral annulus 53 (53-54), 25 (24-25) apart. Setae h1 3 (2-3), h2 85 (80-90). Female genitalia 15 (14-16), $22(22-23)$ wide, coverflap with $11(10-12)$ longitudinal ridges, setae $3 a 22$ (19-24), 14 (14-15) apart.


Figure 19. Tetra pyriana sp. n.: D dorsal view of female $\mathbf{L O}$ lateral microtubercles em empodium $\mathbf{L 1}$ leg I L2 leg II.


Figure 20. Tetra pyriana sp. n.: L lateral view of female IG female internal genitalia CG coxae and female genitalia CMG coxae and male genitalia.


Figure 21. Tetra pyriana sp. n.: A dorsal view of female B ventral view of female $\mathbf{C}$ lateral microtubercles D empodium $\mathbf{E}$ dorsal view of female posterior part $\mathbf{F}$ ventral view of female posterior part $\mathbf{G}$ leg I and leg II.


Figure 22. Tetra pyriana sp. n.: H lateral view of female $\mathbf{I}$ lateral view of female posterior part $\mathbf{J}$ female internal genitalia $\mathbf{K}$ prodorsal shield $\mathbf{L}$ coxae and female genitalia $\mathbf{M}$ coxae and male genitalia.

Male. $(\mathrm{n}=2)$ Body fusiform, light yellow, 150-176 63-65 wide. Gnathosoma 21-22, projecting obliquely down, pedipalp coxal seta (ep) 2-3, dorsal pedipalp genual seta (d) 6-7, cheliceral stylets 19-20. Prodorsal shield has the same design as female, 42-47, 63-65 wide, subtriangular; frontal lobe 10-11. Scapular tubercles on rear shield margin, 3-4, 30-31 apart, scapular setae (sc) 11-13, projecting posteriorly. Coxigenital region with $12-13$ smooth annuli. Coxisternal plates with short lines, anterolateral setae on coxisternum I (1b) 7-8, 12-13 apart, proximal setae on coxisternum I (1a) 18-20, 9-10 apart, proximal setae on coxisternum II (2a) 45-46, 24-26 apart, tubercles $1 b$ and $1 a$ 6-7 apart, tubercles $1 a$ and 2a 7-9 apart. Prosternal apodeme combined, 5-6. Leg I 29-32, femur 7-9, basiventral femoral seta ( $b v$ ) 9-12; genu 5-6, antaxial genual seta ( $l^{\prime \prime}$ ) 20-23; tibia 6-8, paraxial tibial seta ( $l$ ) 5-6, located at $1 / 3$ from dorsal base; tarsus $7-8$, seta ft' $17-18$, seta $f t$ " 21-23, seta u'3-4; tarsal empodium (em) 6-7, simple, 4-rayed, tarsal solenidion $(\omega) 5-6$, knobbed. Leg II 25-27, femur 7-9, basiventral femoral seta (bv) 9-10; genu $4-5$, antaxial genual seta ( $l^{\prime \prime}$ ) 6-8; tibia $7-8$; tarsus $6-7$, seta $f t^{\prime} 2-3$, seta $f t^{\prime \prime}$ 20-21, seta $u^{\prime} 2-3$; tarsal empodium (em) 6-7, simple, 4-rayed, tarsal solenidion ( $\omega$ ) 6-7, knobbed. Opisthosoma dorsally with 29-31 annuli, with filamentous microtubercles, ventrally with 60-61 annuli, with round microtubercles. Setae $c 2$ 24-25 on ventral annulus $13-14,50-51$ apart; setae $d 36-37$ on ventral annulus 24-25, 29-30 apart; setae e 15-16 on ventral annulus 41-42, 16-17 apart; setae $f 28-29$ on ventral annulus 55-56, 20-21 apart. Setae h1 2-3, h2 85-90. Male genitalia 13-14, 19-20 wide, setae $3 a 17-18$, 16-17 apart.

Type material. Holotype, female (slide number NJAUEri796, marked Holotype), from Pyrus calleryana Decne. (Rosaceae), Xining, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime N}$ N, $101^{\circ} 45^{\prime} 27^{\prime \prime} \mathrm{E}$, elevation $2241 \mathrm{~m}, 21$ July 2007, coll. Xiao-Feng Xue. Paratypes, 10 females and 2 males (slide number NJAUEri796), with the same data as holotype.

Additional material. 13 females (slide number NJAUEri807), from Pyrus betulifolia Bunge. (Rosaceae), Xining, Qinghai Province, P. R. China, 36 ${ }^{\circ} 38^{\prime} 18^{\prime \prime} \mathrm{N}$, $101^{\circ} 45^{\prime} 27^{\prime \prime} \mathrm{E}$, elevation $2241 \mathrm{~m}, 21$ July 2007, coll. Xiao-Feng Xue; 14 females and 1 male (slide number NJAUEri806), from Pyrus calleryana Decne. (Rosaceae), Xining, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime N}$ N, $101^{\circ} 45^{\prime} 27^{\prime \prime} \mathrm{E}$, elevation $2241 \mathrm{~m}, 21$ July 2007, coll. Xiao-Feng Xue.

Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Etymology. The specific designation pyriana is from the generic name of host plant, Pyrus; feminine in gender.

Differential diagnosis. This species is similar to Tetra pinnatifidae Xue, Song \& Hong, 2006a, but can be differentiated from the latter by median line weak and ending at basal $1 / 3$ of prodorsal shield (median normal and does not disappear at basal $1 / 3$ of prodorsal shield in $T$. pinnatifidae), coxigenital region with smooth annuli (coxigenital region annuli with round microtubercles in T. pinnatifidae).

## Tetra simonia sp. n.

urn:lsid:zoobank.org:act:11D2D601-440B-4A7D-AD50-18F7B91B3A33
http://species-id.net/wiki/Tetra_simonia
Figures 23-26

Description. Female. ( $\mathrm{n}=14$ ) Body fusiform, light yellow, 196 (196-224), 72 (72-77) wide, 78 (78) thick. Gnathosoma 22 (20-22), projecting obliquely down, pedipalp coxal seta (ep) 3 (3-4), dorsal pedipalp genual seta (d) 12 (10-12), cheliceral stylets 19 (19-20). Prodorsal shield 46 (42-46), 72 (72-77) wide, subtriangular; frontal lobe 11 (11-12); median, admedian and submedian lines robust and connected, forming an "M" shape in the middle and two "H" shapes anterior to the "M". Scapular tubercles on rear shield margin, $3(3-4), 46(44-46)$ apart, scapular setae (sc) 14 (14-15), projecting posteriorly. Coxigenital region with $14(14-15)$ smooth annuli. Coxisternal plates smooth, anterolateral setae on coxisternum I (1b) 11 (11-12), 14 (14-19) apart, proximal setae on coxisternum $\mathbf{I}(1 a) 17(17-23), 12(12-13)$ apart, proximal setae on coxisternum II (2a) 53 (53-58), 28 (27-31) apart, tubercles $1 b$ and $1 a 8$ (8-9) apart, tubercles $1 a$ and $2 a 9(9-10)$ apart. Prosternal apodeme combined, 6 (6-8). Leg I 34 (34-37), femur $12(11-12)$, basiventral femoral seta $(b v) 12(12-13)$; genu 8 (8-9), antaxial genual seta ( $l^{\prime}$ ) $24(22-24)$; tibia $13(11-13)$, paraxial tibial seta ( $l$ ) $7(6-7)$, located at $1 / 3$ from dorsal base; tarsus $7(7-8)$, seta ft' $22(21-22)$, seta ft" $23(22-23)$, seta $u^{\prime} 6(6-7)$; tarsal empodium (em) 6 (6-7), simple, 4-rayed, tarsal solenidion ( $\omega$ ) 6 (6-7), knobbed. Leg II $30(30-34)$, femur $11(11-12)$, basiventral femoral seta ( $b v$ ) 13 (12-13); genu 6 (6-7), antaxial genual seta ( $l^{\prime \prime}$ ) 11 (11-13); tibia 9 (7-9); tarsus $7(6-7)$, seta $f t^{\prime} 5(5-6)$, seta $f t^{\prime \prime} 22(22-24)$, seta $u^{\prime} 6(6-7)$; tarsal empodium (em) 6 (6-7), simple, 4-rayed, tarsal solenidion ( $\omega$ ) 6 (6-7), knobbed. Opisthosoma dorsally with 31 (30-31) annuli, with dark shading on rear annular margins, ventrally with 58 (58-65) annuli, with round microtubercles. Setae $c 236$ (36-42) on ventral annulus $11(11-13), 52(52-56)$ apart; setae $d 77(71-77)$ on ventral annulus $21(21-23), 33$ (33-38) apart; setae e 23 (20-23) on ventral annulus 37 (37-39), 18 (18-19) apart; setae $f 40(36-40)$ on ventral annulus $51(51-54), 30(30-32)$ apart. Setae h1 $5(4-5)$, h2 150 (135-150). Female genitalia 20 (18-22), 25 (24-25) wide, coverflap with 10 (10-13) longitudinal ridges, setae $3 a 17$ (17-18), 18 (17-18) apart.

Male. $(\mathrm{n}=1)$ Body fusiform, light yellow, 188, 74 wide. Gnathosoma 21, projecting obliquely downwards, pedipalp coxal seta (ep) 3, dorsal pedipalp genual seta (d) 11, cheliceral stylets 19. Prodorsal shield has the same design as female, 42,74 wide, subtriangular; frontal lobe 11. Scapular tubercles on rear shield margin, 44 apart, scapular setae (sc) 13, projecting posteriorly. Coxigenital region with 14 smooth annuli. Coxisternal plates smooth, anterolateral setae on coxisternum I (1b) 13), 14 apart, proximal setae on coxisternum I (1a) 23, 12 apart, proximal setae on coxisternum II (2a) 49, 30 apart, tubercles $1 b$ and $1 a$ apart 8, tubercles $1 a$ and $2 a 10$ apart. Prosternal apodeme combined, 6. Leg I 35, femur 11, basiventral femoral seta ( $b v$ ) 12; genu 8, antaxial genual seta ( $l$ ')


Figure 23. Tetra simonia sp. n.: D dorsal view of female LO lateral microtubercles em empodium L1 leg I L2 leg II.


Figure 24. Tetra simonia sp. n.: L lateral view of female IG female internal genitalia CG coxae and female genitalia CMG coxae and male genitalia.


Figure 25. Tetra simonia sp. n.: A dorsal view of female $\mathbf{B}$ ventral view of female $\mathbf{C}$ lateral microtubercles D empodium $\mathbf{E}$ dorsal view of female posterior part $\mathbf{F}$ ventral view of female posterior part $\mathbf{G} \operatorname{leg} \mathrm{I}$ and $\operatorname{leg}$ II.


Figure 26. Tetra simonia sp. n.: H lateral view of female $\mathbf{I}$ lateral view of female posterior part $\mathbf{J}$ female internal genitalia $\mathbf{K}$ prodorsal shield $\mathbf{L}$ coxae and female genitalia $\mathbf{M}$ coxae and male genitalia.

26; tibia 12, paraxial tibial seta (l) 6 , located at $1 / 3$ from dorsal base; tarsus 7 , seta ft' 21 , seta $f t^{\prime \prime} 24$, seta $u^{\prime} 6$; tarsal empodium (em) 6, simple, 4-rayed, tarsal solenidion ( $\omega$ ) 6, knobbed. Leg II 32, femur 10, basiventral femoral seta ( $b v$ ) 14; genu 6, antaxial genual seta $\left(l^{\prime \prime}\right) 11$; tibia 8 ; tarsus 8 , seta $f t^{\prime} 5$, seta $f t^{\prime \prime} 18$, seta $u^{\prime} 6$; tarsal empodium (em) 6 , simple, 4-rayed, tarsal solenidion $(\omega) 6$, knobbed. Opisthosoma dorsally with 31 annuli, with dark shading on rear annular margins, ventrally with 67 annuli, with round microtubercles. Setae $c 242$ on ventral annulus 13, 58 apart; setae $d 70$ on ventral annulus 27, 40 apart; setae $e 25$ on ventral annulus 47, 20 apart; setae $f 40$ on ventral annulus 63, 30 apart. Setae $h 1$ 5, h2 130. Male genitalia 15, 25 wide, setae $3 a 18$, 19 apart.

Type material. Holotype, female (slide number NJAUEri799, marked Holotype), from Populus simonii Carr. (Salicaceae), Xining, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime} \mathrm{N}, 101^{\circ} 45^{\prime} 27$ "E, elevation 2241m, 21 July 2007, coll. Xiao-Feng Xue. Paratypes, 13 females and 1 male (slide number NJAUEri799), with the same data as holotype.

Additional material. 4 females (slide number NJAUEri789A), from Pориlus simonii Carr. (Salicaceae), Xining, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime N}$, $101^{\circ} 45^{\prime} 27^{\prime \prime} \mathrm{E}$, elevation $2241 \mathrm{~m}, 21$ July 2007, coll. Xiao-Feng Xue. 4 females and 2 males (slide number NJAUEri823), from Populus simonii Carr. (Salicaceae), Beishan National Forest Park, Huzhu County, Qinghai Province, P. R. China, $36^{\circ} 53^{\prime} 35^{\prime \prime} \mathrm{N}$, $102^{\circ} 25^{\prime} 56^{\prime \prime}$ E, elevation 2610m, 22 July 2007, coll. Xiao-Feng Xue.

Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Etymology. The specific designation simonia is from the species name of host plant, simonii; feminine in gender.

Differential diagnosis. This species is similar to Tetra smilaxis Xue, Song \& Hong, 2006a, but can be differentiated from the latter by opisthosoma with dark shading on rear annular margins (dark shading absent in T. smilaxis), prodorsal shield as wide as opisthosoma (opisthosoma wider than prodorsal shield in T. smilaxis), admedian lines connected at basal $1 / 3$ but separate at basal $2 / 3$ of prodorsal shield (admedian lines connected at basal $1 / 3$ and basal $2 / 3$ of prodorsal shield in $T$. smilaxis).

## Family Diptilomiopidae Keifer, 1944 <br> Subfamily Diptilomiopinae Keifer, 1944 Genus Diptacus Keifer, 1951

## Diptacus berberinus sp. n.

urn:lsid:zoobank.org:act:3EF374D2-74CD-46A0-833C-A80FC1EAE1E2
http://species-id.net/wiki/Diptacus_berberinus
Figures 27-30

Description. Female. $(\mathrm{n}=9)$ Body fusiform, light yellow, 283 (280-360), 110 (102$110)$ wide, 115 (114-115) thick. Gnathosoma 26 (25-27), projecting downwards, pedipalp coxal seta (ep) 6 (5-6), dorsal pedipalp genual seta (d) 12 (11-12), cheliceral stylets 65 (65-66). Prodorsal shield 40 (40-46), 80 (77-80) wide, with wide and broad
frontal lobe, 7 (7-8); median, admedian and submedian lines present, admedian lines connected at the basal $1 / 3$ and $2 / 3$ of prodorsal shield, forming 3 cells on each side, submedian lines connected with the median and admedian at the basal $2 / 3$ of prodorsal shield, forming the cell-like pattern at anterior shield margin. Scapular tubercles ahead of rear shield margin, $3(2-3), 30(27-30)$ apart, scapular setae (sc) $5(4-5)$, projecting centrad to forward. Coxigenital region with 13 (13-15) annuli, with triangular microtubercles. Coxisternal plate I with granules, coxisternal plate II smooth, anterolateral setae on coxisternum I (1b) 20 (18-20), 17 (17-18) apart, proximal setae on coxisternum I (1a) 43 (43-45), 19 (17-19) apart, proximal setae on coxisternum II (2a) 70 (70-80), 49 (43-56) apart, tubercles $1 b$ and $1 a 12(12-13)$ apart, tubercles $1 a$ and $2 a$ 17 (14-17) apart. Prosternal apodeme separated, 5 (5-6). Leg I 65 (60-65), femur 20 (20-22), basiventral femoral seta ( $b v$ ) absent; genu 8 ( $8-9$ ), antaxial genual seta ( $l^{\prime \prime}$ ) 48 (48-52); tibia $18(17-19)$, paraxial tibial seta $(l) 11(10-11)$, located at $1 / 2$ from dorsal base; tarsus 11 (11-12), seta ft'30 (29-30), seta ft' 40 (37-40), seta u'7 (6-7); tarsal empodium (em) 11 (10-11), divided, 7-rayed on each side, tarsal solenidion ( $\omega$ ) 10 (10-14), knobbed. Leg II 55 (54-55), femur 20 (19-20), basiventral femoral seta ( $b v$ ) absent; genu $8(7-8)$, antaxial genual seta ( $l^{\prime}$ ) $18(15-18)$; tibia 17 (16-17); tarsus 11 (10-11), seta ft' $11(10-11)$, seta $f t^{\prime \prime} 44(44-50)$, seta $u^{\prime} 8(7-8)$; tarsal empodium (em) 11 (10-11), divided, 7-rayed on each side, tarsal solenidion ( $\omega$ ) 10 (10-11), knobbed. Opisthosoma dorsally with 59 (54-62) annuli, smooth, ventrally with 106 (101-106) annuli, with triangular microtubercles. Setae $c 2115$ (110-115) on ventral annulus 18 (18-20), 74 (74-75) apart; setae $d 100(100-120)$ on ventral annulus 40 (37-40), 56 (49-56) apart; setae $e 60$ (55-60) on ventral annulus 65 (60-65), 31 (29-35) apart; setae $f 65(60-70)$ on ventral annulus 93 (87-93), 35 (35-37) apart. Setae h1 2 (1-2), h2 103 (95-165). Female genitalia 35 (31-40), 35 (34-42) wide, coverflap with short lines on base, and 4 longitudinal ridges in 2 ranks, 1 ridge near the base and 3 ridges at distal margin, setae $3 a 12(11-15), 24(24-25)$ apart.

Male. $(\mathrm{n}=1)$ Body fusiform, light yellow, 269, 86 wide. Gnathosoma 60, projecting downwards, pedipalp coxal seta ( $e p$ ) 5, dorsal pedipalp genual seta (d) 11, cheliceral stylets 65 . Prodorsal shield has the same design as female, 38,71 wide, with wide and broad frontal lobe, 7. Scapular tubercles ahead of rear shield margin, 3, 27 apart, scapular setae ( $s c$ ) 4, projecting centrad to forward. Coxigenital region with 14 annuli, with triangular microtubercles. Coxisternal plate I with granules, coxisternal plate II smooth, anterolateral setae on coxisternum I (1b) 22, 16 apart, proximal setae on coxisternum I (1a) 36, 16 apart, proximal setae on coxisternum II (2a) 60, 41 apart, tubercles $1 b$ and la 11 apart, tubercles $1 a$ and $2 a 12$ apart. Prosternal apodeme separated, 6. Leg I 46, femur 17 , basiventral femoral seta $(b v)$ absent; genu 7 , antaxial genual seta $\left(l^{\prime \prime}\right) 46$; tibia 13, paraxial tibial seta ( $l$ ) 10 , located at $1 / 2$ from dorsal base; tarsus 8 , seta $f t^{\prime} 30$, seta $f t^{\prime \prime} 37$, seta $u^{\prime} 6$; tarsal empodium (em) 10, divided, 7-rayed on each side, tarsal solenidion ( $\omega$ ) 10, knobbed. Leg II 38, femur 17, basiventral femoral seta ( $b v$ ) absent; genu 7, antaxial genual seta ( $l^{\prime \prime}$ ) 15 ; tibia 13; tarsus 6 , seta $f t^{\prime} 10$, seta $f t^{\prime \prime} 38$, seta $u^{\prime} 7$; tarsal empodium (em) 10, divided, 7 -rayed on each side, tarsal solenidion $(\omega) 11$, knobbed. Opisthosoma dorsally with 56 annuli, smooth, ventrally with 83 annuli, with triangular microtuber-


Figure 27. Diptacus berberinus sp. n.: D dorsal view of female IG female internal genitalia LO lateral microtubercles L1 leg I L2 leg II em empodium.


Figure 28. Diptacus berberinus sp. n.: L lateral view of female CMG coxae and male genitalia CG coxae and female genitalia.


Figure 29. Diptacus berberinus sp. n.: A dorsal view of female B ventral view of female $\mathbf{C}$ lateral microtubercles $\mathbf{D}$ empodium $\mathbf{E}$ dorsal view of female posterior part $\mathbf{F}$ ventral view of female posterior part G leg I and leg II.


Figure 30. Diptacus berberinus sp. n.: H lateral view of female I coxae and female genitalia J lateral view of female posterior part $\mathbf{K}$ female internal genitalia $\mathbf{L}$ coxae and male genitalia $\mathbf{M}$ prodorsal shield.
cles. Setae $c 295$ on ventral annulus 15, 63 apart; setae $d 100$ on ventral annulus 30, 49 apart; setae e 55 on ventral annulus 46, 27 apart; setae $f 60$ on ventral annulus 71, 25 apart. Setae h1 2, h2 120. Male genitalia 23, 30 wide, setae $3 a 10,23$ apart.

Type material. Holotype, female (slide number 783, marked Holotype), from Berberis amurensis Rupr. (Berberidaceae), Mengda Natural Reserve, Xunhua County, Qinghai Province, P. R. China, $35^{\circ} 47^{\prime} 38^{\prime \prime}$ N, $102^{\circ} 40^{\prime} 40^{\prime \prime}$ E, elevation 2523m, 19 July 2007, coll. Xiao-Feng Xue. Paratypes, 8 females and 1 male (slide number 783), with the same data as holotype.

Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Etymology. The specific designation berberinus is from the generic name of host plant, Berberis; masculine in gender.

Differential diagnosis. This species is similar to Diptacus maddenis Song, Xue \& Hong, 2007a, but can be differentiated from the latter by opisthosomal dorsal annuli smooth (opisthosomal dorsal annuli with elongated microtubercles in $D$. maddenis), female genital coverflap with short lines at the base (genital coverflap with granules in D. maddenis), tarsal empodium 7-rayed (4-rayed in D. maddenis).

## Diptacus mengdaensis sp. $\mathbf{n}$.

urn:lsid:zoobank.org:act:12904959-7EE9-4780-BCC1-EF886FA54F98
http://species-id.net/wiki/Diptacus_mengdaensis
Figures 31-33

Description. Female. ( $\mathrm{n}=13$ ) Body fusiform, light yellow, 215 (210-232), 104 (104$105)$ wide, 71 ( $71-74$ ) thick. Gnathosoma 25 (24-25), projecting downwards, pedipalp coxal seta (ep) $4(4-5)$, dorsal pedipalp genual seta (d) 14 (14-15), cheliceral stylets 62 (6162). Prodorsal shield $50(50-51)$, $76(71-76)$ wide, with wide and broad frontal lobe, 7 (7-8); median, admedian and submedian lines present, admedian lines connected at the base of prodorsal shield, ending at basal $1 / 3$ of prodorsal shield. Scapular tubercles ahead of rear shield margin, $3(3-4), 34(34-35)$ apart, scapular setae $(s c) 6(6-7)$, projecting centrad. Coxigenital region with $16(15-16)$ annuli, with microtubercles. Coxisternal plates with short lines, anterolateral setae on coxisternum I (1b) 16 (16-18), 18 (18-20) apart, proximal setae on coxisternum I (1a) 25 (25-26), 17 (17-18) apart, proximal setae on coxisternum II (2a) $60(60-70), 44(44-45)$ apart, tubercles $1 b$ and $1 a 12(12-13)$ apart, tubercles $1 a$ and $2 a 16$ (16-17) apart. Prosternal apodeme separated, 10 (9-10). Leg I 60 (58-60), femur 16 (16-17), basiventral femoral seta ( $b v$ ) absent; genu 10 (8-10), antaxial genual seta ( $l^{\prime}$ ) $52(52-53)$; tibia $17(16-17)$, paraxial tibial seta (l) $10(9-10)$, located at $1 / 3$ from dorsal base; tarsus $11(10-11)$, seta $f t^{\prime} 30(25-30)$, seta $f t^{\prime \prime} 40(32-40)$, seta $u^{\prime} 6$ (5-6); tarsal empodium (em) 8 (8-9), divided, 5-rayed at each side, tarsal solenidion ( $\omega$ ) 9 (9-10), knobbed. Leg II 54 (50-54), femur $18(17-18)$, basiventral femoral seta ( $b v$ ) absent; genu 7 (7-8), antaxial genual seta ( $l^{\prime \prime}$ ) 16 (15-16); tibia 11 ( $9-11$ ); tarsus 8 ( $8-10$ ), seta ft' $12(12-13)$, seta $f t^{\prime \prime} 34(34-34)$, seta $u^{\prime} 5(5-6)$; tarsal empodium (em) 9 (8-9),


Figure 31. Diptacus mengdaensis sp. n.: D dorsal view of female $\mathbf{L O}$ lateral microtubercles em empodium L1 leg I L2 leg II.


Figure 32. Diptacus mengdaensis sp. n.: L lateral view of female IG female internal genitalia CG coxae and female genitalia.


Figure 33. Diptacus mengdaensis sp. n.: A dorsal view of female $\mathbf{B}$ ventral view of female $\mathbf{C}$ dorsal view of female posterior part $\mathbf{D}$ ventral view of female posterior part $\mathbf{E}$ prodorsal shield $\mathbf{F}$ lateral microtubercles $\mathbf{G}$ empodium $\mathbf{H}$ coxae and female genitalia.
divided, 5-rayed at each side, tarsal solenidion ( $\omega$ ) 9 (9-10), knobbed. Opisthosoma dorsally with 44 (44-48) annuli, smooth, ventrally with 112 (112-115) annuli, with round microtubercles. Setae $c 255$ (54-55) on ventral annulus 16 (16-18), 76 (70-76) apart; setae $d 90(90-93)$ on ventral annulus 39 (39-42), 51 (51-53) apart; setae e 70 (65-70) on ventral annulus 67 (67-69), 29 (29-31) apart; setae $f 50(50-55)$ on ventral annulus 98 (98-101), 37 (36-37) apart. Setae h1 2 (2-3), h2 152 (150-152). Female genitalia 24 (24-25), 37 (37-39) wide, coverflap with 4 longitudinal ridges in 2 ranks, 1 near the base and 3 at distal margin, setae $3 a 12$ (10-12), 20 (20-21) apart.

Male. Unknown.
Type material. Holotype, female (slide number NJAUEri777, marked Holotype), from Lonicera elisae Franch. (Caprifoliaceae), Mengda Natural Reserve, Xunhua County, Qinghai Province, P. R. China, $35^{\circ} 47^{\prime} 38^{\prime \prime} \mathrm{N}, 102^{\circ} 40^{\prime} 40$ " E, elevation 2523m, 19 July 2007, coll. Xiao-Feng Xue. Paratypes, 12 females (slide number NJAUEri777), with the same data as holotype.

Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Etymology. The specific designation mengdaensis is from the place name Mengda Natural Reserve, where this new species was collected; feminine in gender.

Differential diagnosis. This species is similar to Diptacus lonicerae Kuang, 2001, but can be differentiated from the latter by prodorsal shield with admedian lines and submedian lines separated (admedian lines and submedian lines connected in D. lonicerae), prodorsal shield frontal lobe wide and broad (frontal lobe small in D. lonicerae), female genital coverflap with 4 longitudinal ridges in 2 ranks, 1 near the base and 3 far from the base (female genital coverflap with 6-8 longitudinal ridges in $D$. lonicerae).

## Subfamily Rhyncaphytoptinae Roivainen, 1953

Genus Rhyncaphytoptus Keifer, 1939

## Rhyncaphytoptus ulmi Xin \& Dong, 1981

http://species-id.net/wiki/Rhyncaphytoptus_ulmi
Rhyncaphytoptus ulmi Xin and Dong 1981: 216-217, figures 2-3.
Rhyncaphytoptus ulmi; Amrine and Stasny 1994: 277.
Rhyncaphytoptus ulmi; Amrine and Stasny 1996: 300.
Rhyncaphytoptus ulmi; Hong and Zhang 1996: 79, figures 188-1-188-2.
Rhyncaphytoptus ulmi; Kuang et al. 2005: 155-156, figure 158.
Rhyncaphytoptus ulmi; Xue et al. 2006b: 3.
Rhyncaphytoptus ulmi; Song et al. 2007b: 59.
Rhyncaphytoptus ulmi; Xue et al. 2009: 3.

Material examined. 16 females (slide number NJAUEri792B and NJAUEri795), from Ulmus sp. (Ulmaceae), Xining, Qinghai Province, P. R. China, $36^{\circ} 38^{\prime} 18^{\prime \prime} \mathrm{N}$, $101^{\circ} 45^{\prime} 27^{\prime \prime}$ E, elevation $2241 \mathrm{~m}, 21$ July 2007, coll. Xiao-Feng Xue.

Host. Ulmus sp. (Ulmaceae).
Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Distribution. China (Jiangsu, Gansu, Jilin, Liaoning, Shaanxi, Shandong, Xinjiang, Qinghai).

## Rhyncaphytoptus spinus sp. n.

urn:lsid:zoobank.org:act:F3227702-A360-40D4-9367-E395D5F361DE
http://species-id.net/wiki/Rhyncaphytoptus_spinus
Figures 34-36

Description. Female. ( $\mathrm{n}=6$ ) Body fusiform, light yellow, 270 (232-323), 82 (82-91) wide, 105 (104-105) thick. Gnathosoma 58 (56-59), projecting downwards, suboral plate present, pedipalp coxal seta (ep) $4(4-5)$, dorsal pedipalp genual seta (d) 10 (9-11), palp tarsus ventral seta $(v) 4(3-4)$, cheliceral stylets 71 (71-72). Prodorsal shield 38 (38-40), 60 (57-64) wide, with broad frontal lobe, 7 (6-7); median, admedian and submedian lines present, median, admedian lines connected at basal $1 / 3$ and $2 / 3$ of prodorsal shield, forming three cells each both side. Scapular tubercles ahead of rear shield margin, 6 (5-6), 34 (34-40) apart, scapular setae (sc) 21 (21-25), projecting forward, knobbed at the end. Coxigenital region with 15 (14-15) annuli, with microtubercles. Coxisternal plates smooth, anterolateral setae on coxisternum I ( $1 b$ ) 28 (26-28), 16 (16-18) apart, proximal setae on coxisternum I (1a) 46 (44-46), 10 (10-13) apart, proximal setae on coxisternum II (2a) 80 (71-80), 30 (30-36) apart, tubercles $1 b$ and $1 a 6(5-7)$ apart, tubercles $1 a$ and $2 a 9$ (9-13) apart. Prosternal apodeme combined, 6 (6-7). Leg I 44 (43-49), femur 16 (16-17), basiventral femoral seta $(b v) 18$ (15-20); genu 8 ( $8-9$ ), antaxial genual seta (l') 30 (30-32); tibia 12 (12-13), paraxial tibial seta (l) $14(14-17)$, located at $1 / 3$ from dorsal base; tarsus 9 (9-10), seta ft'26 (24-28), seta ft' $35(34-35)$, seta $u^{\prime} 8(6-8)$; tarsal empodium (em) 12 (12-13), simple, 8-rayed, tarsal solenidion ( $\omega$ ) 11 ( $10-11$ ), tapered. Leg II 41 (41-46), femur 14 (14-16), basiventral femoral seta ( $b v$ ) 18 (18-19); genu 7 (7-8), antaxial genual seta ( $l^{\prime}$ ) 10 ( $10-11$ ); tibia $11(10-11)$; tarsus 8 ( $\left.8-10\right)$, seta $f t ' 12$ (12-13), seta $f t " 34(34-34)$, seta $u^{\prime} 7(6-7)$; tarsal empodium (em) 12 (12-13), simple, 8 -rayed, tarsal solenidion $(\omega) 11(10-11)$, tapered. Opisthosoma dorsally with 38 (38-42) annuli, with long spiny microtubercles, ventrally with 98 (97-98) annuli, with triangle microtubercles. Setae c2 33 (31-33) on ventral annulus 17 (17-19), 76 (71-76) apart; setae $d 97$ (95-97) on ventral annulus 38 (38-40), 53 (53-69) apart; setae $e 41(41-45)$ on ventral annulus $60(59-61), 31$ (31-39) apart; setae $f 36$ (36-38) on ventral annulus 92 (92-93), $28(28-30)$ apart. Setae h15 (5-6), h2 110 (110-115). Female genitalia 21 (18-21), 32 (32-35) wide, coverflap smooth, setae 3a 75 (70-75), 20 (20-23) apart.

Male. Unknown.
Type material. Holotype, female (slide number NJAUEri820, marked Holotype), from Lonicera rupicola Hook. f. et Thoms. (Caprifoliaceae), Beishan National Forest


Figure 34. Rhyncaphytoptus spinus sp. n.: D dorsal view of female L1 leg I L2 leg II IG female internal genitalia em empodium.


Figure 35. Rhyncaphytoptus spinus sp. n.: V ventral view of female CG coxae and female genitalia LO lateral microtubercles.


Figure 36. Rhyncaphytoptus spinus sp. n.: A dorsal view of female $\mathbf{B}$ ventral view of female $\mathbf{C}$ prodorsal shield $\mathbf{D}$ coxae and female genitalia $\mathbf{E}$ dorsal view of female posterior part $\mathbf{F}$ ventral view of female posterior part $\mathbf{G}$ empodium $\mathbf{H} \operatorname{leg}$ I and leg II I female internal genitalia.

Park, Huzhu County, Qinghai Province, P. R. China, $36^{\circ} 53^{\prime} 35^{\prime \prime} N, 102^{\circ} 25^{\prime} 56^{\prime \prime}$ E, elevation 2610m, 22 July 2007, coll. Xiao-Feng Xue. Paratypes, 5 females (slide number NJAUEri820), with the same data as holotype.

Relation to host. Vagrant on leaf lower surface. No damage to the host was observed.
Etymology. The specific designation spinus is from the character of the dorsal opisthosomal microtubercles, spiny, "spina, spinus" in Latin; masculine in gender.

Differential diagnosis. This species is similar to Rhyncaphytoptus guanegounis Song, Xue \& Hong, 2007b, but can be differentiated from the latter by median, admedian and submedian lines present on prodorsal shield (prodorsal shield smooth in R. guanegounis), prodorsal shield with wide and broad frontal lobe (prodorsal shield with long and broad frontal lobe in $R$. guanegounis), opisthosomal dorsal annuli with spiny microtubercles (opisthosomal dorsal annuli smooth in $R$. guanegounis), tarsal empodium (em) 8-rayed (6-rayed in R. guanegounis).

## Discussion

Although Qinghai has climate, vegetation and biological diversity similar to Ti bet, we did not find any eriophyoid mite species already reported in Tibet (Song et al. 2011). On the contrary, 6 of 23 species were reported from Gansu Province or Shaanxi Province, east neighboring provinces. As of 2010, 932 eriophyoid mite species have been described from China (Hong et al. 2010), and the number is still increasing. Qinghai has about 8\% of the land of China, but only about $2 \%$ eriophyoid mites were reported to date. Furthermore, only two investigations have been conducted. More systematic collections of eriophyoid mites from Qinghai Province are needed in the near future.

## Acknowledgements

This research was funded by the National Natural Science Foundation of China (31172132). We thank Xiao Han of NJAU for early review of the manuscript. We also thank Professor Xin-Hua Li of the Dept of Botany at NJAU for identifying host plants. We are very grateful to Professor James W. Amrine Jr. for his valuable suggestions on the manuscript.

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