



Slow genetal and genetic but rapid non-genetal and ecological differentiation in a pair of spider species (Araneae, Pholcidae)

Bernhard A. Huber^{a,*}, Dimitar Dimitrov^b

^a Alexander Koenig Research Museum of Zoology, Adenauerallee 160, 53113 Bonn, Germany

^b Natural History Museum, University of Oslo, PO Box 1172, Blindern, NO-0318 Oslo, Norway

ARTICLE INFO

Article history:

Received 2 January 2014
Received in revised form 3 April 2014
Accepted 3 April 2014
Available online 13 April 2014
Corresponding Editor: Peter Michalik

Keywords:

Species specificity
Genitalia
Ecological speciation
Barcoding gap
Taiwan
Pholcidae
Pholcus

ABSTRACT

Like most animals with internal fertilization, spiders tend to have species-specific genitalia, allowing closely related species to be identified by their reproductive morphology more easily than by non-genetal characters. This implies that genitalia evolve on average more rapidly than non-genetal morphological traits. Here we describe two putative species of Pholcidae from Taiwan (*Pholcus pingtung*, n. sp.; *Pholcus chengpoi*, n. sp.) that differ conspicuously in their microhabitat (rocks vs. leaves), coloration, color pattern, and body proportions, but have almost indistinguishable genitalia and cytochrome oxidase I (co1) sequences. The two species have identical yet highly unusual male cheliceral modifications, strongly arguing for sister species. Despite the almost identical genitalia and co1 sequences, we treat the two 'morphs' as species for three reasons: (1) they are easily distinguished by several characters; no intermediate specimens were found; (2) subtle yet consistent differences in genetal (uncus) shape support the idea of reproductively isolated entities beyond the more conspicuous non-genetal differences; (3) each locality provided both types of microhabitat but only one of the two species, arguing against environmental plasticity or polymorphism. We conclude that probably a very recent expansion into a novel microhabitat has led to speciation and rapid ecological and non-genetal differentiation, with insufficient time to accumulate significant genetal and genetic differences.

© 2014 Elsevier GmbH. All rights reserved.

1. Introduction

In animals with internal fertilization, genitalia are usually considered to be species-specific. This implies that they are (1) relatively constant within species; and (2) relatively divergent among species, even in species groups where general body shape and color is near-identical or variable and widely overlapping (Eberhard, 1985; Eberhard et al., 1998; Hosken and Stockley, 2004). In spiders, genitalia usually provide a wealth of characters for species discrimination, and spider taxonomy has made undeniably good progress aloof of cumbersome theoretical questions about species-specificity, species concepts, and inherent methodological biases (Huber, 2003, 2004).

However, as soon as an absolute congruence between genetal differences and species limits is questioned, borderline cases emerge where decisions become ambiguous. This ambiguity about species limits persists regardless if we adopt a species concept

emphasizing epistemology (such as the Phylogenetic Species Concept *sensu* Wheeler and Platnick, 2000) or ontology (such as the Biological Species Concept *sensu* Mayr, 2000). Convincing conclusions are often aggravated by low sample sizes and by the lack of biological, experimental, and genetic data. In the spider family Pholcidae, several such cases of problematic species limits have been highlighted recently where future research, building on adequate samples and additional information beyond morphology may prove initial taxonomic conclusions wrong. For example:

- Genetal variation has been interpreted to be intraspecific in 37 of the 106 species (35%) represented by more than one population in a revision of *Pholcus* and close relatives (Huber, 2011); the same tentative conclusion has been made for several species in African *Smeringopina* (most notably *S. fang*; *S. moudouma*; *S. ebolowa*; Huber, 2013), in the Brazilian *Litoporus iguassuensis* (Huber et al., 2013), and the Hispaniolan *Tainonia serripes* (Huber and Astrin, 2009).
- Indistinguishable genitalia have been interpreted to belong to different species in the Colombian species pair *Pomboa quindio* and *P. pallida* based on their conspicuous differences in color and non-genetal morphology (Huber, 2000); a similar case was made

* Corresponding author.

E-mail addresses: b.huber@zfmk.de (B.A. Huber), dimitard.gwu@gmail.com (D. Dimitrov)

Table 1

Material used for sequencing (for detailed collection data see descriptive section), with collection codes, GenBank accession numbers, and ID of vouchered DNA.

Species	Origin (Taiwan)	Code	CO1	ZFMK-DNA-
<i>Pholcus pingtung</i>	Pingtung: Sheding Nature Park	Tai 60-1 (♂)	KJ650069	0100418574
		Tai 60-2 (♀)	KJ650065	0100418547
		Tai 60-3 (j)	KJ650075	0100417916
		Tai 60-4 (j)	KJ650072	0100417941
		Tai 60-5 (j)	KJ650058	0100418017
	Pingtung: near Danlu	Tai 65-1 (♀)	KJ650068	0100417869
		Tai 65-2 (♀)	KJ650059	0100417599
		Tai 65-3 (♀)	KJ650062	0155622354
		Tai 65-4 (♀)	KJ650064	0155622353
		<i>Pholcus chengpoi</i>	Pingtung: near Chouqia	Tai 63-1 (♀)
Tai 63-2 (j)	KJ650073			0155622351
Tai 63-3 (j)	KJ650063			0155622350
Tai 63-4 (j)	KJ650067			0155622349
Tai 63-5 (j)	KJ650066			0155622348
Tai 63-6 (j)	KJ650057			0155622336
<i>Pholcus spilis</i>	Taichung: E Dongshi	Tai 75-1 (♀)	KJ650070	0155622337
		Tai 75-2 (♀)	KJ650061	0155621564
<i>Pholcus fragillimus</i>	Taichung: Tunghai Univ. campus	Tai 78-1 (♂)	KJ650076	0155622339
<i>Uthina luzonica</i>	Pingtung: Sheding Nature Park	Tai 59-1 (♀)	KJ650071	0155622340
		Tai 59-2 (♀)	KJ650060	0155622341

for the Brazilian species pair *Psilochorus itaguyrussu* and *P. ybytyriguara* where identical genitalia shape was interpreted to be shared by two species based on size and color pattern differences (Huber et al., 2005a).

- In the Kenyan species *Spermophora minotaura* conspicuous color pattern variation associated with ecological parameters (ground-dwelling versus leaf-dwelling) was considered to be intraspecific based on the lack of genital differences and on low genetic distances (Huber and Warui, 2012; Dimitrov et al., 2013).
- Molecular data have suggested cryptic species in the Hispaniolan *Modisimus makandal* and *M. vittatus* where morphology failed to show any consistent differences (Huber et al., 2010).

The present paper adds a highly conspicuous case to this list of debatable identities and species limits. Rather than pretending to give a satisfying solution (which is probably impossible with the available material) we aim to present the case and establish the basis for an in-depth analysis involving more specimens, more localities, and more data.

2. Materials and Methods

This study is based on material collected in Taiwan in June 2013 and deposited in Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) and Moscow State University, Moscow (ZMMU). Descriptions follow the style in the recent revision of *Pholcus* (Huber, 2011). The following abbreviations are used in the main text: ALE (anterior lateral eye); ALS (anterior lateral spinneret); AME (anterior median eye); L/d (length by diameter); PME (posterior median eye); PMS (posterior median spinneret).

Two legs each of 20 specimens were detached for molecular analysis (Table 1). Since we had no material available for sequencing of the possibly closest known relatives (*halabala* species group; see below), we used all known Taiwanese *Pholcus* species as out-groups plus the closely related genus *Uthina*. DNA extraction and amplification followed the protocols described in Dimitrov, et al. (2008). Purified PCR product was sequenced at the ABI-lab at the University of Oslo. For both, PCR and sequencing reactions, we used the most widely utilized barcoding primers in invertebrates: LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al., 1994) that target the Cytochrome oxidase I (co1) mitochondrial gene. Our

efforts to amplify a fragment of the 16S gene along with tRNA^{Leu} and a fragment of the NADH dehydrogenase subunit 1 using the primers N1-J-12585 and LR-N-13398 (Simon et al., 1994) were not successful.

Multiple sequences alignment was trivial as there is no length variation and was performed in MAFFT v 7.110 (Katoh and Standley, 2013) using the L-ins method. Uncorrected p-distances and Neighbor Joining (NJ) were calculated in the software package MEGA v.5.2.2 (Tamura et al., 2011). Maximum Likelihood (ML) analysis was carried out in RaxML-HPC ver. 7.2.8 (Stamatakis, 2006) using the GTR+ Γ model for sequence evolution. We ran two analyses, one in which the 1st and 2nd codon position were placed in a separate partition and one without partitioning the data.

3. Results

3.1. Taxonomy

3.1.1. *Pholcus pingtung* new species (Figs. 1–2, 5–21)

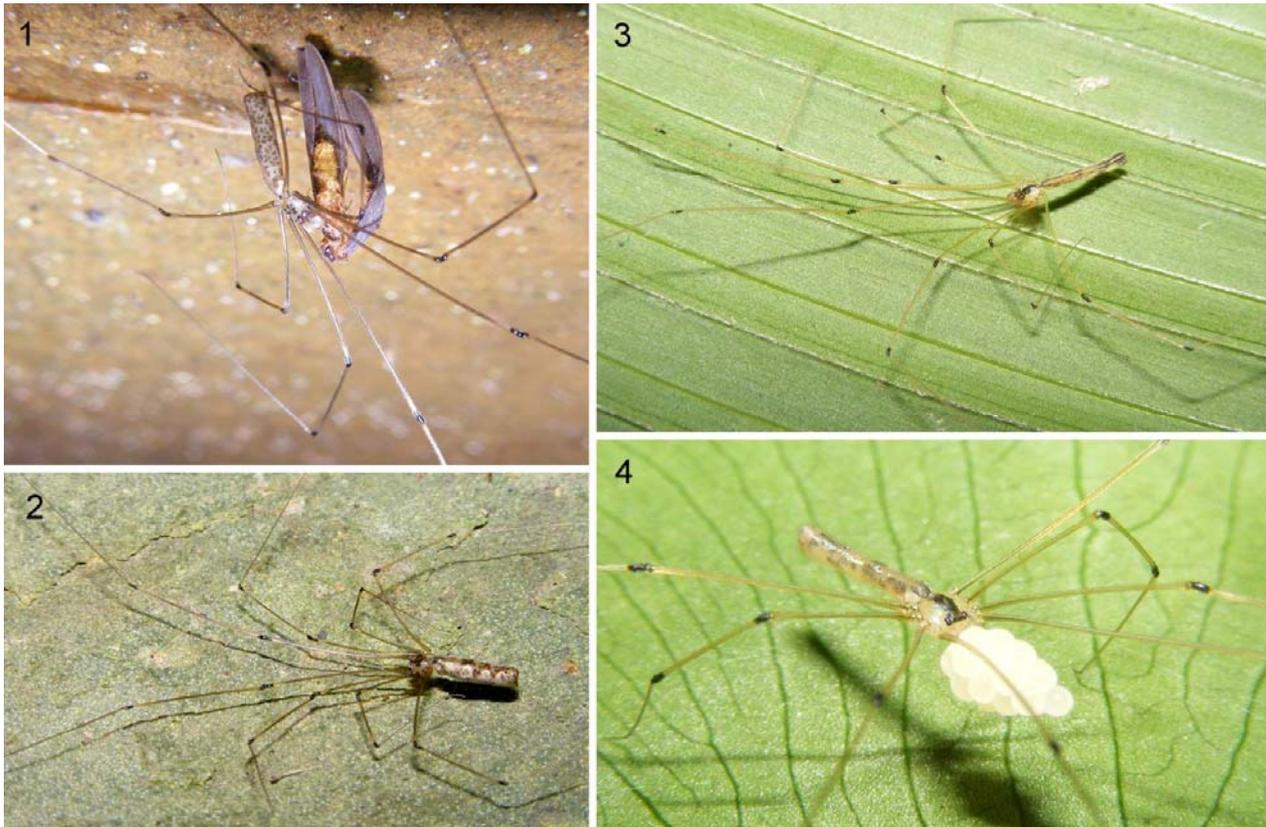
Type. Male holotype from Taiwan, Pingtung County, Sheding Nature Park near Kenting (21.9573°N, 120.8208°E), 200 m a.s.l., at rocks, 26.vi.2013 (B.A. Huber, S. Aharon), in ZFMK (Ar 11959).

Other material examined. Taiwan: Pingtung County: Sheding Nature Park near Kenting, same data as holotype, 11♂11♀ in ZFMK (Ar 11960); same data, 1♂1♀3 juvs. in pure ethanol, in ZFMK (Tai 60); same locality, 26.vi.2013 (S. Huber), 2♂1 juv. in ZFMK (Ar 11961); same locality, 26.vi.2013 (Yu.M. Marusik), 1♂1♀ in ZMMU. Near Danlu (22.2097°N, 120.7595°E), 130 m a.s.l., at brook, at rocks near water, 22.vi.2013 (B.A. Huber), 5♂3♀ in ZFMK (Ar 11962); same data, 4♀ in pure ethanol, in ZFMK (Tai 65).

Etymology. The species name is a noun in apposition, derived from the type locality.

Diagnosis. Six-eyed *Pholcus*, easily distinguished from most known congeners by unique armature of male chelicerae (Figs. 10 and 19); from the very similar *P. chengpoi* (which has slightly smaller but otherwise indistinguishable chelicerae) by patterns on carapace (pair of dark V-marks diverging posteriorly; Figs. 5 and 8) and sternum (distinct dark margins; Figs. 7 and 9).

Description. Male (holotype). Total body length 5.3; carapace width 1.27. Leg 1: 44.4 (10.6 + 0.5 + 10.5 + 20.7 + 2.1), tibia 2: 7.0, tibia 3: 4.2, tibia 4: 6.2, tibia 1 L/d: 99. Habitus as in Figs. 5–7; prosoma light ochre with distinctive pair of dark brown V-marks



Figs. 1-4. *Pholcus pingtung* (1, 2) and *P. chengpoi* (3, 4) in their natural microhabitats, i.e. on rocks (1, male feeding on two Plecoptera; 2, female in resting position), and on leaves (3, male in resting position; 4, female with eggsac).

posteriorly, ocular area brown, clypeus not darkened; legs light ochre, patellae and tibia-metatarsus joints dark brown; abdomen pale gray with dark spots visible through cuticle dorsally and laterally. Distance PME-PME 390 μ m, diameter PME 125 μ m, distance PME-ALE 35 μ m; no trace of AME. Ocular area slightly elevated, each triad on low stalk directed towards lateral; thoracic furrow absent; clypeus and sternum unmodified. Chelicerae with pair of weakly sclerotized lateral apophyses and two pairs of distinctive frontal apophyses, proximal pair flat and pointed, distal pair finger-shaped, both without modified hairs (Figs. 10 and 19). Palps as in Figs. 11-13 and 17 and 18; coxa unmodified, trochanter with retrolatero-ventral apophysis strongly curved towards femur; femur with three proximal projections (ventral, retrolateral, dorsal); procurus relatively simple, with distinct ventral 'knee', without dorsal spines, with distinctive distal elements and ventral spine-like process; bulb with weakly sclerotized embolus, simple appendix distally curved towards retrolateral; uncus very similar to *P. chengpoi* but distal element more distinct (Fig. 35). Legs without spines and curved hairs, with few vertical hairs; retrolateral trichobothrium on tibia 1 at 3%; prolateral trichobothrium absent on tibia 1, present on other tibiae; tarsus 1 with >30 pseudosegments, distally fairly distinct.

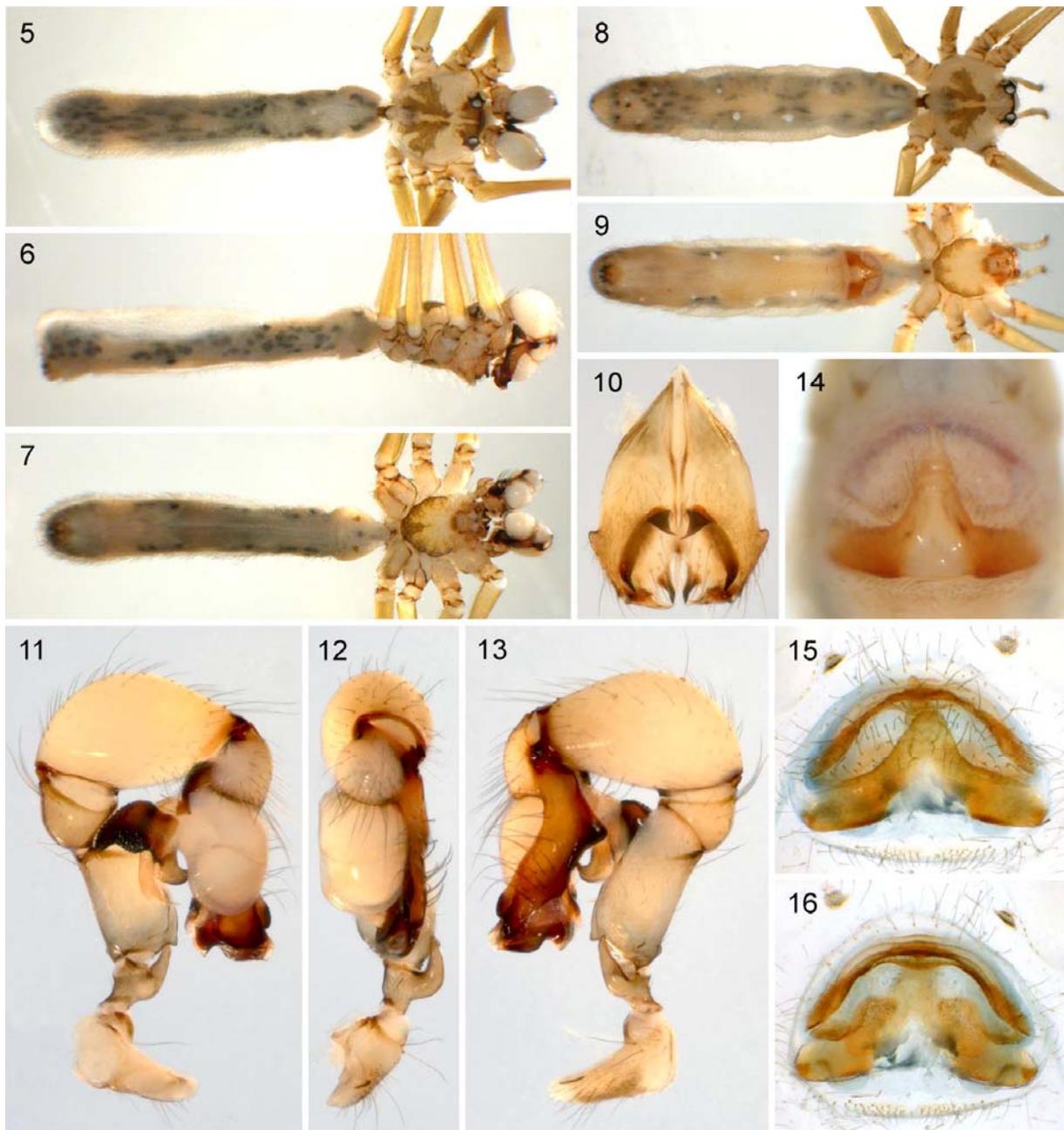
Female. In general similar to male, including carapace and sternum pattern (Figs. 8 and 9); prosoma and legs smaller/shorter (means of tibia 1, tibia 3, and ocular area width 81-85% of male means; see Variation below); abdomen as long as in males but wider (mean width 139% of male mean width). Epigynum transversal plate weakly sclerotized medially, with distinctive frontal scape (Figs. 14 and 20); internal genitalia as in Figs. 15, 16, and 21.

Variation. Males from near Danlu (D; $N=5$) were larger than males from Sheding (S; $N=15$) in all 12 characters measured (cf.

Figs. 38 and 40); tibia 1 length in all specimens: 9.4-12.1 (means, D: 11.6; S: 10.4); tibia 3: 3.8-4.7 (means, D: 4.6; S: 4.1); carapace width: 1.07-1.47 (means, D: 1.34; S: 1.22); carapace length: 0.97-1.27 (means, D: 1.18; S: 1.07); ocular area width: 0.77-0.90 (means, D: 0.89; S: 0.82); chelicerae width: 0.42-0.48 (means, D: 0.48; S: 0.44); sternum width: 0.72-0.87 (means, D: 0.84; S: 0.77); abdomen length: 3.6-5.1 (means, D: 4.9; S: 4.1); abdomen width: 0.63-0.90 (means, D: 0.87; S: 0.76); palpal femur length: 0.50-0.59 (means, D: 0.59; S: 0.52); palpal femur width: 0.27-0.34 (means, D: 0.33; S: 0.29); uncus length: 0.27-0.32 (means, D: 0.31; S: 0.28). Except for size variation, palps and chelicerae were indistinguishable in the specimens examined.

Females from near Danlu (D; $N=7$) were larger than females from Sheding (S; $N=11$) in all six characters measured (cf. Figs. 39 and 41): tibia 1 in all specimens: 7.9-9.8 (means, D: 9.4; S: 8.5); tibia 3: 3.2-4.1 (means, D: 3.9; S: 3.5); ocular area width: 0.66-0.75 (means, D: 0.74; S: 0.68); abdomen length: 3.8-5.2 (means, D: 4.66; S: 4.31); abdomen width: 0.72-1.73 (means, D: 1.15; S: 1.07); epigynum width: 0.65-0.87 (means, D: 0.78; S: 0.71). Epigynum shape was indistinguishable in the specimens examined.

Relationships. The highly unusual yet identical male cheliceral modifications of *P. pingtung* and *P. chengpoi* strongly suggest they are sister species. Molecular data also supports a very close relationship and the barcoding region of the *col* gene does not contain enough information that could be used to distinguish the two taxa. Other than that, relationships to other congeners remain obscure. There are certain similarities to six-eyed members of the Southeast Asian *halabala* species group (e.g. anterior epigynal scape in *P. elongatus*; see Huber, 2011) but no convincing morphological putative synapomorphies seem to exist and we did not have available any material of the *halabala* species group suitable for molecular work.



Figs. 5-16. *Pholcus pingtung*. 5-7. Male, dorsal, lateral, and ventral views. 8-9. Female, dorsal and ventral views. 10. Male chelicerae, frontal view. 11-13. Left male palp, prolateral, dorsal, and retrolateral views. 14. Epigynum, ventral view. 15-16. Cleared female genitalia, ventral and dorsal views.

Natural history. All specimens at both localities were found among large rocks, either in dark spaces close to the ground or in small crevices or caverns in the rocks.

Distribution. Known from two localities in southern Taiwan (Fig. 34). More northern localities visited (in Taichung and Tainan Municipalities and Nantou County) did not reveal this species.

3.1.2. *Pholcus chengpoi* new species (Figs. 3-4, 22-33)

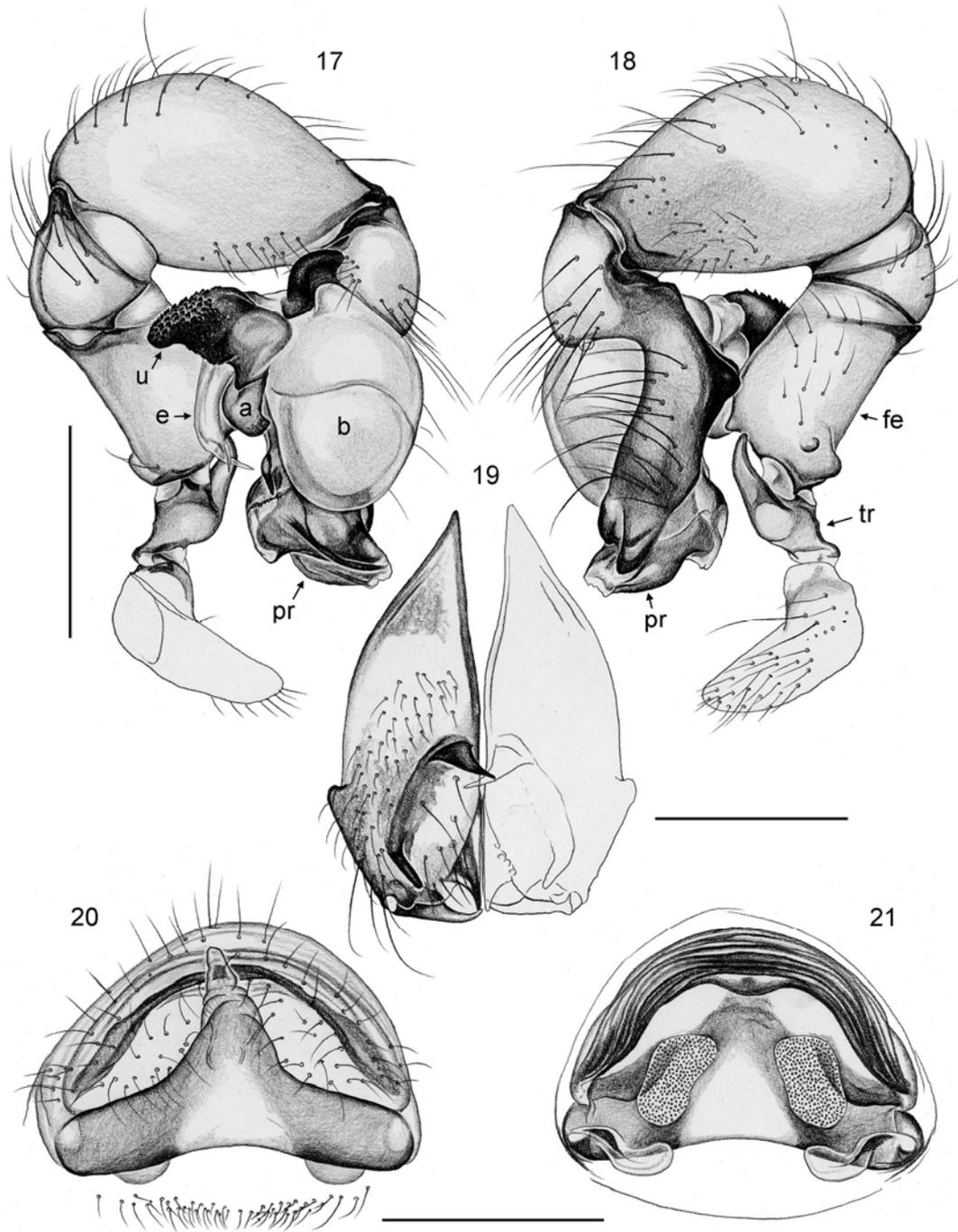
Type. Male holotype from Taiwan, Pingtung County, near Chouqia (22.2409°N, 120.8552°E), 440 m a.s.l., at brook, underside of large leaves, 22.vi.2013 (B.A. Huber), in ZFMK (Ar 11963).

Other material examined. Taiwan: Pingtung County: near Chouqia, same data as holotype, 7♂ 3♀ in ZFMK (Ar 11964); same data, 1♀ 5 juvs. in pure ethanol, in ZFMK (Tai 63).

Etymology. The species is named for Taiwanese painter Chen Cheng-po (1895–1947).

Diagnosis. Six-eyed *Pholcus*, easily distinguished from most known congeners by unique armature of male chelicerae (Fig. 27); from the very similar *P. pingtung* (which has slightly larger but otherwise indistinguishable chelicerae) by pattern on carapace (median dark band; Figs. 22 and 25) and monochromous sternum (Figs. 24 and 26).

Description. Male (holotype). Total body length 5.6; carapace width 1.07. Leg 1: 38.3 (8.8+0.5+8.7+18.3+2.0), tibia 2: 5.9, tibia

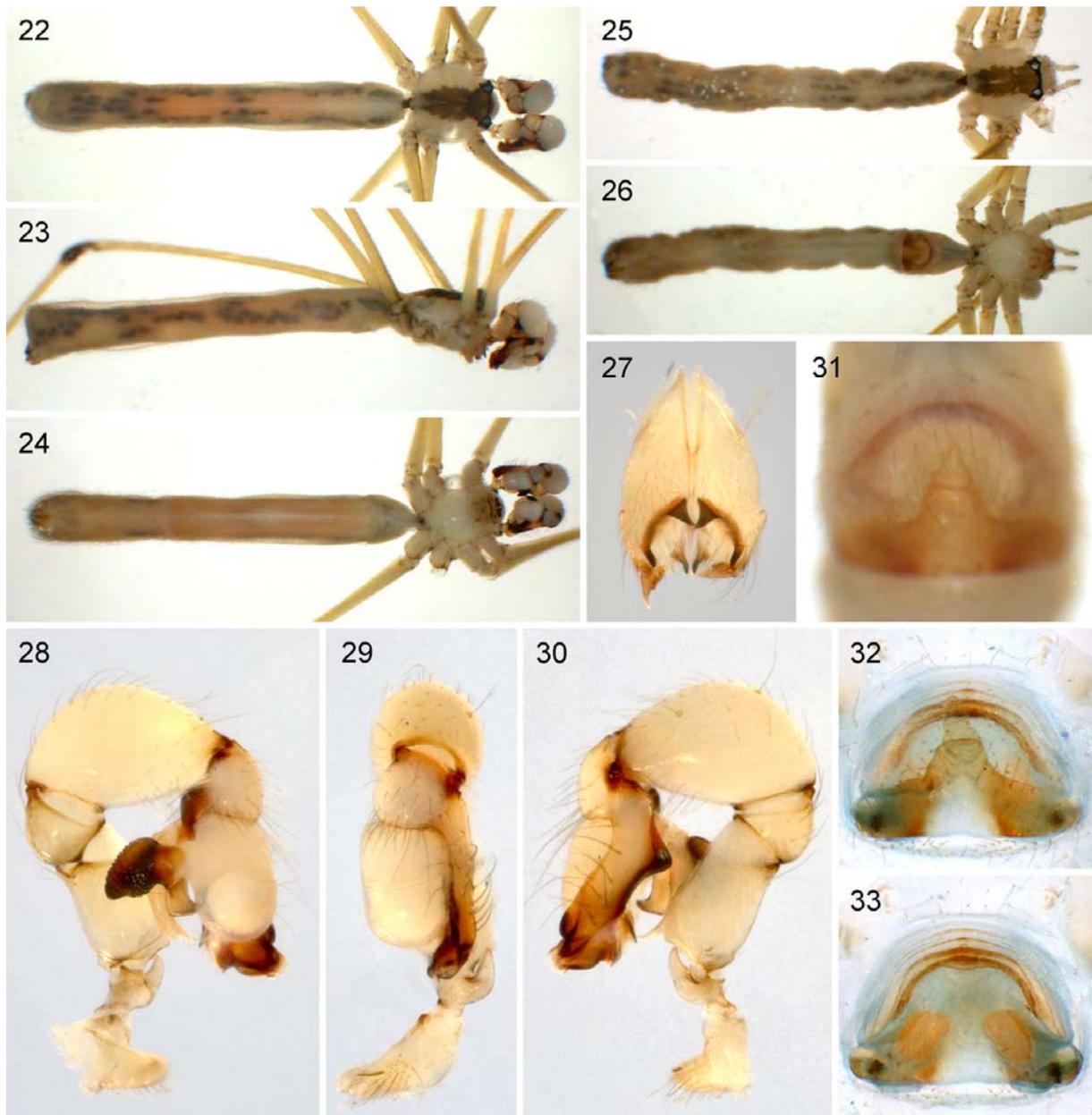


Figs. 17-21. *Pholcus pingtung*. 17-18. Left male palp, prolateral and retrolateral views. 19. Male chelicerae, frontal view. 20-21. Cleared female genitalia, ventral and dorsal views. Abbreviations: a: appendix; b: bulb; e: embolus; fe: femur; pr: procurus; tr: trochanter; u: uncus. Scale lines: 0.5 mm (17-18, 20-21); 0.3 mm (19).

3: 3.5, tibia 4: 5.3, tibia 1 L/d: 89. Habitus as in Figs. 22-24; prosoma light ochre with distinctive dark brown median band, ocular area brown, clypeus not darkened; legs light ochre, patellae and tibia-metatarsus joints dark brown; abdomen pale gray with dark spots visible through cuticle dorsally and laterally. Distance PME-PME 345 μ m, diameter PME 105 μ m, distance PME-ALE 35 μ m; no trace of AME. Ocular area slightly elevated, each triad on low stalk directed towards lateral; thoracic furrow absent; clypeus and sternum unmodified. Chelicerae and palps smaller but otherwise mostly indistinguishable from *P. pingtung*, only shape of uncus

slightly different (distal element less distinct; Fig. 35). Legs without spines and curved hairs, with few vertical hairs; retrolateral trichobothrium on tibia 1 at 3%; prolateral trichobothrium absent on tibia 1, present on other tibiae; tarsus 1 with >30 pseudosegments, distally fairly distinct.

Female. In general similar to male, including carapace pattern and monochromous sternum (Figs. 25 and 26); prosoma and legs smaller/shorter (means of tibia 1, tibia 3, and ocular area width 84-87% of male means; see Variation below); abdomen as long as in males but wider (mean width 114% of male mean width). Epigynum



Figs. 22-33. *Pholcus chengpoi*. 22-24. Male, dorsal, lateral, and ventral views. 25-26. Female, dorsal and ventral views. 27. Male chelicerae, frontal view. 28-30. Left male palp, prolateral, dorsal, and retrolateral views. 31. Epigynum, ventral view. 32-33. Cleared female genitalia, ventral and dorsal views.

smaller but otherwise indistinguishable from *P. pingtung* (Fig. 31); internal genitalia as in Figs. 32 and 33.

Variation. Males ($N=7$): tibia 1: 8.7-10.5 (mean 9.5); tibia 3: 3.5-4.1 (mean 3.8); carapace width: 0.93-1.13 (mean 1.07); carapace length: 0.93-1.03 (mean 0.98); ocular area width: 0.73-0.78 (mean 0.75); chelicerae width: 0.36-0.40 (mean 0.38); sternum width: 0.66-0.72 (mean 0.69); abdomen length: 4.4-5.2 (mean 4.8); abdomen width: 0.45-0.67 (mean 0.56); palpal femur length: 0.42-0.45 (mean 0.43); palpal femur width: 0.25-0.27 (mean 0.26); uncus length: 0.24-0.26 (mean 0.25). Except for size variation, palps and chelicerae were indistinguishable in the specimens examined.

Females ($N=4$): tibia 1: 7.7-8.3 (mean 8.0); tibia 3: 3.3-3.5 (mean 3.3); ocular area width: 0.64-0.65 (mean 0.64); abdomen length: 4.5-5.1 (mean 4.8); abdomen width: 0.47-0.87 (mean

0.64); epigynum width: 0.54-0.58 (mean 0.57). Epigynum shape was indistinguishable in the specimens examined.

Natural history. All specimens were collected from the undersides of large leaves from different monocot plant species.

Distribution. Known from type locality in southern Taiwan only (Fig. 34).

3.2. Molecular data

P-distances between the two sampled populations of *Pholcus pingtung* ranged between 2.9 and 3.1%. This distance was higher than that found between the geographically closer northern population of *P. pingtung* (Danlu population) and *P. chengpoi* (0.0-0.8%) (Fig. 34). Within localities, we found no variation among the five specimens of *P. pingtung* from Sheding; 0.0-0.6% among the four

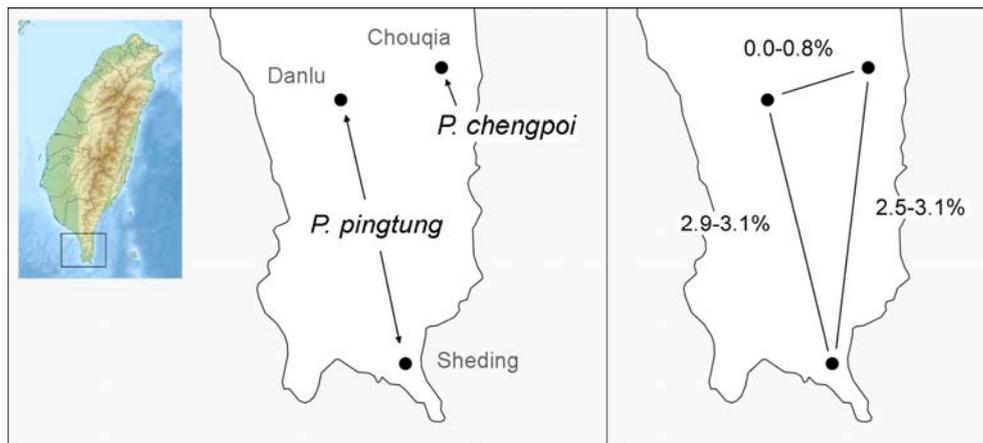


Fig. 34. Known distributions of *Pholcus pingtung* and *P. chengpoi*, and col p-distances among the specimens from different localities; inset: Taiwan.

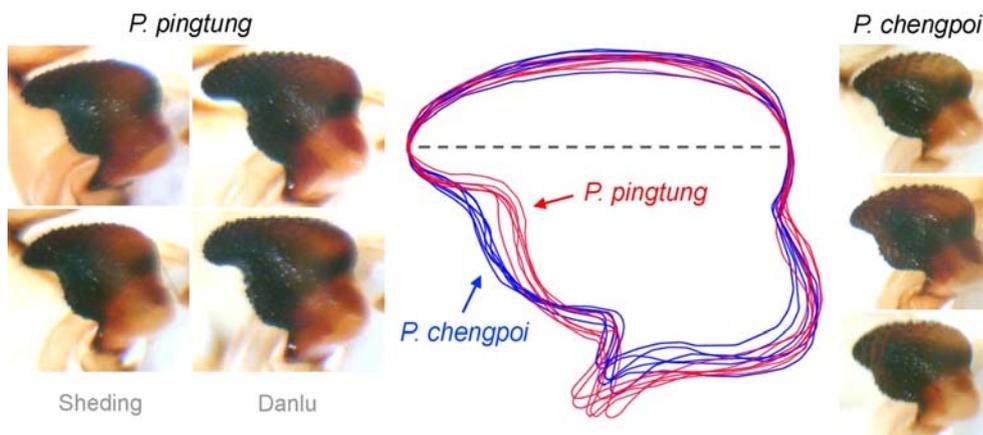


Fig. 35. Differences in uncus size and shape between *Pholcus pingtung* and *P. chengpoi*. All photos at same scale to illustrate size differences. The drawing shows the outlines of the unci of six specimens from each species (in *Pholcus pingtung*: three from Sheding, three from Danlu) rescaled using uncus length (dotted line) as baseline to illustrate shape differences.

specimens of *P. pingtung* from Danlu; and 0.0–1.0% among the six specimens of *P. chengpoi* from Chouqia.

The trees shown in Fig. 42 reflect these low genetic distances, and in particular the lack of divergence between the northern population of *P. pingtung* (Danlu population) and *P. chengpoi*. Partitioned and un-partitioned ML analyses produced very similar results but in the partitioned analyses some of the unsupported nodes within the *P. chengpoi* + *P. pingtung* group were collapsed and an unsupported clade composed of *P. spilis* + *P. fragillimus* appeared (Fig. 42).

4. Discussion

4.1. One species or two?

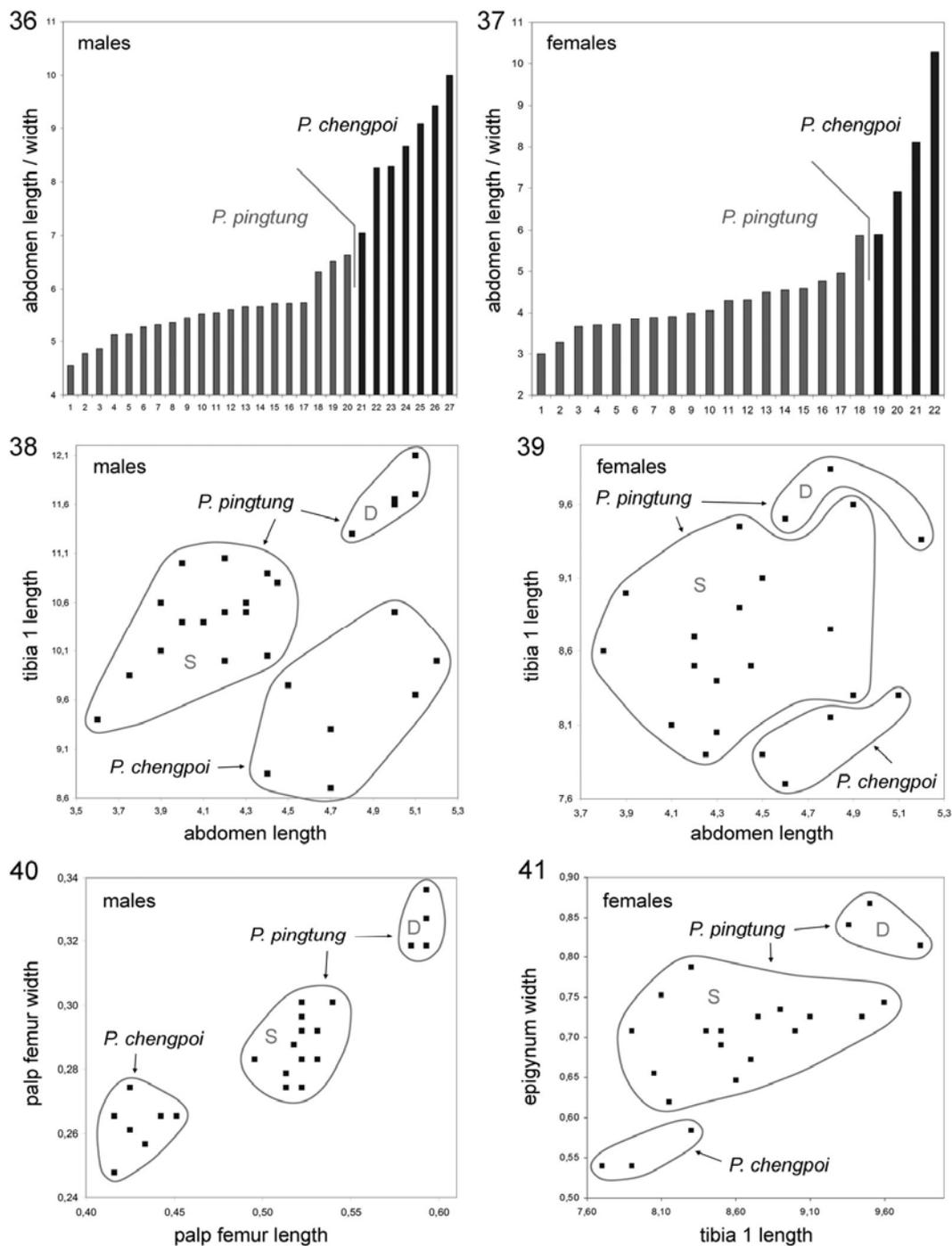
Ecological speciation, where divergence is driven by divergent natural selection between environments, is probably a common means by which new species arise (Rundle and Nosil, 2005; Schluter, 2009). Morphological and ecological divergence can be more rapid than genetic divergence (Vitt et al., 1997) and less than 20,000 generations may be enough for the process to be ‘completed’ (Gavrilets et al., 2007). However, there may be multiple stages in the process of speciation (Lowry, 2012) and a sound documentation of the actual stage in a given case requires data far beyond what is available for the two putative new species described herein.

Our provisional argument for two separate species that have recently originated by ecological speciation rests on the following observations:

1. Even without a comprehensive phylogenetic analysis, there is little doubt that the two species are indeed sister taxa. The unique yet identical male cheliceral armature is very different from any described congener (cf. Huber, 2011) and clearly constitutes a shared derived character. The near-identical shapes of palpal structures and female genitalia also argue for a close relationship, even though symplesiomorphy can not be ruled out in these cases. *Pholcus pingtung* and *P. chengpoi* are the only endemic species of the genus known from Taiwan, suggesting that their similarity is the result of speciation on the island rather than of independent colonization. Our genetic data are congruent with the idea that the two species are indeed sister taxa.
2. The two putative species are easily distinguished by several morphological traits (mainly pattern on carapace; coloration of sternum; ratio of abdomen length by tibia 1 length) as well as by their microhabitat (rocks vs. leaves). No intermediates were found even though all three localities provided both rocks and suitable large leaves. Even though the shape of the uncus is only slightly different between the two species and this constitutes the only apparent genitalic shape difference, the small but consistent gap seems to support the proposed species limit by traditional standards.

4.2. Microhabitat shifts

Shifts among microhabitats have repeatedly occurred in Pholcidae (Dimitrov et al., 2013) but the direction is often difficult



Figs. 36-41. Diagrams illustrating differences between *Pholcus pingtung* and *P. chengpoi*. 36-37. Abdomen length/width in all specimens examined, showing the relatively longer abdomens of *P. chengpoi*. 38-39. Abdomen length scaled against tibia 1 length, showing that *P. chengpoi* specimens do not fall on the same regression line as small and large specimens of *P. pingtung*. 40-41. Genital measures scaled against each other and against tibia 1 length; the absence of a clear trend towards different regression lines suggests that these genital characters are not good indicators for species limits. S: Shedding; D: Danlu.

to establish. Shifts such as in the current case (between near-ground habitats and the underside of leaves higher among the vegetation) seem to have occurred in both directions in pholcids; some of them are well supported also by molecular data. Examples include the leaf-dwelling *Smeringopus cylindrogaster* that has derived from near-ground-dwelling ancestors (Huber, 2012; Dimitrov et al., 2013); the leaf-dwelling morph of *Spermophora*

minotaura that has evolved from near-ground-dwelling ancestors (Huber and Warui, 2012; Dimitrov et al., 2013); and the ground-dwelling *Metagonia* species that have evolved from leaf-dwelling ancestors (Huber et al., 2005b; Dimitrov et al., 2013). All these shifts have been accompanied by conspicuous changes in body shape and coloration: leaf-dwelling species are lighter and more slender. The same differences are apparent in the present case: *Pholcus chengpoi*

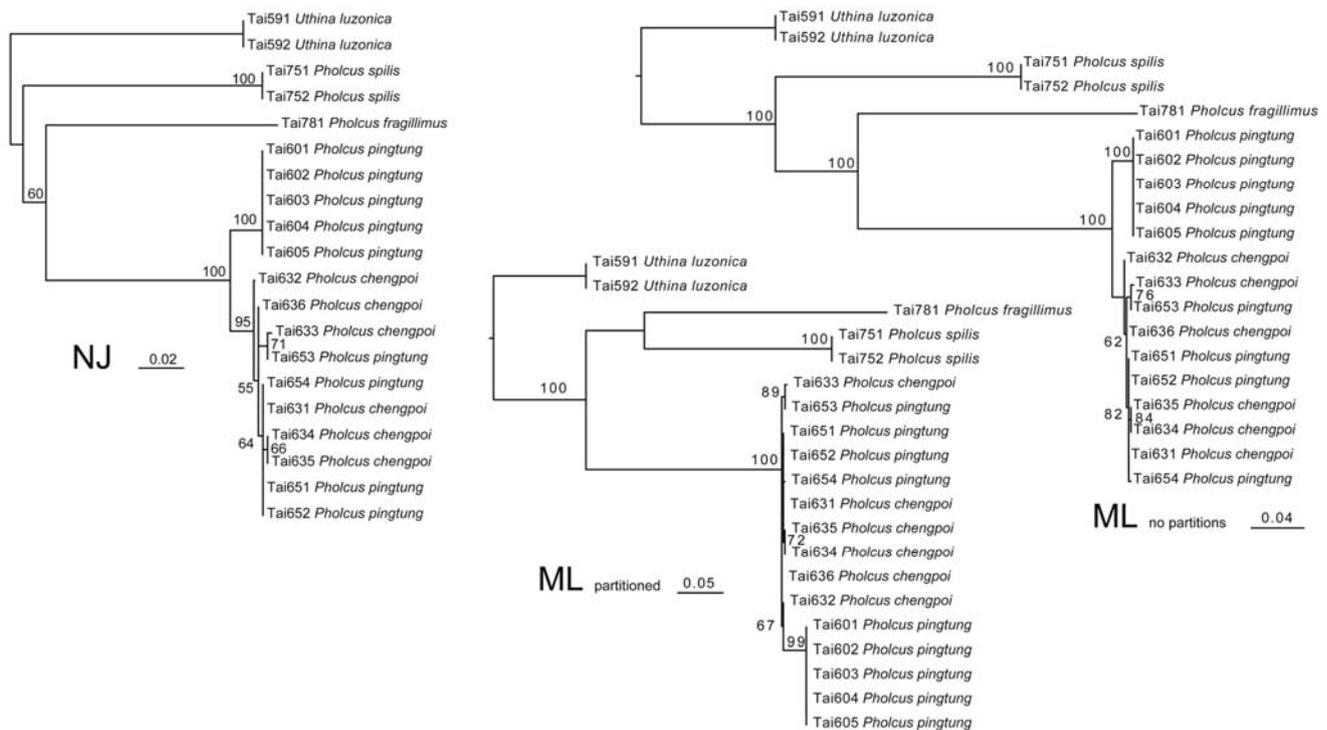


Fig. 42. Results from NJ and ML analyses of the molecular dataset. Branch annotations represent bootstrap values (in all cases 1000 replicates were used to calculate bootstrap support).

is clearly lighter than *P. pingtung* and has a relatively more slender abdomen (Figs. 1-4, 36 and 37). As shown in Figs. 38 and 39, the abdomens of *P. chengpoi* specimens are longer than expected for the sizes of the specimens (as indicated by tibia 1 length which is highly correlated with other indicators of body size like carapace width, ocular area width, or tibia 3 length). Since the closest relatives of the new species are unknown (as is the biology of the *halabala* species group that we speculate might include the closest relatives; see above), the direction of shift in the current case remains unknown.

5. Conclusions

The most reasonable conclusions from the available data are that (1) the two proposed sister species have adapted conspicuously to their different microhabitats; (2) they have attained reproductive isolation, explaining conspicuous differences in color patterns and body proportions and subtle but consistent genitalic (uncus shape) differences; and (3) this split was recent, explaining the otherwise near-identical genitalia and the low level of genetic divergence. If our conclusion about the existence of two species is correct, then standard barcoding (e.g. Ratnasingham and Hebert, 2013) would clearly have failed to discover the species split.

Acknowledgements

BAH thanks I-Min Tso for help with preparing the collecting trip and with permits, Angela Chuang for making the trip start smoothly, and Chih-Wei Lai for being a cheerful and reliable companion in the field. Further material was kindly made available by Yu.M. Marusik and S. Huber. We also thank A. Schröder-Nielsen for assistance with lab work and DNA voucher manipulation. Two anonymous reviewers provided constructive criticism that helped us improve the manuscript.

References

- Dimitrov, D., Arnedo, M.A., Ribera C., 2008. Colonization and diversification of the spider genus *Pholcus* Walckenaer, 1805 (Araneae, Pholcidae) in the Macaronesian archipelagos: Evidence for long-term occupancy yet rapid recent speciation. *Mol. Phylogenet. Evol.* 48, 596-614.
- Dimitrov, D., Astrin, J.J., Huber B.A., 2013. Pholcid spider molecular systematics revisited, with new insights into the biogeography and the evolution of the group. *Cladistics* 29, 132-146.
- Eberhard, W.G., 1985. *Sexual Selection and Animal Genitalia*. Harvard Univ. Press, Cambridge, MA.
- Eberhard, W.G., Huber, B.A., Rodriguez, R.L., Briceño, D., Salas, I., Rodriguez, V., 1998. One size fits all? Relationships between the size of genitalia and other body parts in 20 species of insects and spiders. *Evolution* 52, 415-431.
- Folmer, O., Black, M., Hoch, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294-297.
- Gavrilets, S., Vose, A., Barluenga, M., Salzburger, W., Meyer, A., 2007. Case studies and mathematical models of ecological speciation. 1. Cichlids in a crater lake. *Mol. Ecol.* 16, 2893-2909.
- Hosken, D.J., Stockley, P., 2004. Sexual selection and genital evolution. *Trends Ecol. Evol.* 19, 87-93.
- Huber, B.A., 2000. New World pholcid spiders (Araneae: Pholcidae): a revision at generic level. *Bull. Am. Mus. Nat. Hist.* 254, 1-348.
- Huber, B.A., 2003. Rapid evolution and species-specificity of arthropod genitalia: fact or artifact? *Organ. Diver. Evol.* 3, 63-71.
- Huber, B.A., 2004. The significance of copulatory structures in spider systematics. In: Schult, J. (Ed.), *Studien zur Theorie der Biologie, Band 5, Biosemiotik – Praktische Anwendung und Konsequenzen für die Einzeldisziplinen*. VWB-Verlag für Wissenschaft und Bildung, Berlin.
- Huber, B.A., 2011. Revision and cladistic analysis of *Pholcus* and closely related taxa (Araneae, Pholcidae). *Bonner Zool. Monogr.* 58, 1-509.
- Huber, B.A., 2012. Revision and cladistic analysis of the Afrotropical endemic genus *Smeringopus* Simon, 1890 (Araneae: Pholcidae). *Zootaxa* 3461, 1-138.
- Huber, B.A., 2013. Revision and cladistic analysis of the Guineo-Congolian spider genus *Smeringopina* Kraus (Araneae, Pholcidae). *Zootaxa* 3713, 1-160.
- Huber, B.A., Astrin, J.J., 2009. Increased sampling blurs morphological and molecular species limits: revision of the Hispaniolan endemic spider genus *Tainonia* (Araneae: Pholcidae). *Inver. Systemat.* 23, 281-300.
- Huber, B.A., Fischer, N., Astrin, J.J., 2010. High level of endemism in Haiti's last remaining forests: a revision of *Modisimus* (Araneae: Pholcidae) on Hispaniola, using morphology and molecules. *Zool. J. Linn. Soc.* 158, 244-299.
- Huber, B.A., Pérez-González, A., Astrin, J.J., Blume, C., Baptista, R., 2013. *Litoporus iguassuensensis* Mello-Leitão, 1918 (Araneae, Pholcidae): camouflaged retreat, sexual dimorphism, female color polymorphism, intra-specific genital variation, and description of the male. *Zool. Anz.* 252, 511-521.

- Huber, B.A., Rheims, C.A., Brescovit, A.D., 2005a. Speciation without changes in genital shape: a case study on Brazilian pholcid spiders (Araneae: Pholcidae). *Zool. Anz.* 243, 273-279.
- Huber, B.A., Rheims, C.A., Brescovit, A.D., 2005b. Two new species of litter-dwelling *Metagonia* spiders (Araneae, Pholcidae) document both rapid and slow genital evolution. *Acta Zool. (Stock.)* 86, 33-40.
- Huber, B.A., Warui, C.M., 2012. East African pholcid spiders: an overview, with descriptions of eight new species (Araneae, Pholcidae). *Eur. J. Taxon.* 29, 1-44.
- Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772-780.
- Lowry, D.B., 2012. Ecotypes and the controversy over stages in the formation of new species. *Biol. J. Linn. Soc.* 106, 241-257.
- Mayr, E., 2000. The biological species concept. In: Wheeler, Q.D., Meier, R. (Eds.), *Species Concepts and Phylogenetic Theory. A Debate*. Columbia University Press, New York.
- Ratnasingham, S., Hebert P.D.N., 2013. A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS ONE* 8(8), e66213, <http://dx.doi.org/10.1371/journal.pone.0066213>.
- Rundle, H.D., Nosil, P., 2005. Ecological speciation. *Ecol. Lett.* 8, 336-352.
- Schluter, D., 2009. Evidence for ecological speciation and its alternative. *Science* 323, 737-741.
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* 87, 651-701.
- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731-2739.
- Vitt, L.J., Caldwell, J.P., Zani, P.A., Titus T.A., 1997. The role of habitat shift in the evolution of lizard morphology: evidence from tropical *Tropidurus*. *Proc. Natl. Acad. Sci. U.S.A.* 94, 3828-3832.
- Wheeler, Q.D., Platnick, N.I. 2000. The phylogenetic species concept (sensu Wheeler and Platnick). In: Wheeler, Q.D., Meier, R. (eds.), *Species Concepts and Phylogenetic Theory. A Debate*. Columbia University Press, New York.