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Targeted sampling in Ryukyus facilitates species delimitation of the primitively segmented spider genus *Ryuthela* (Araneae: Mesothelae: Liphistiidae)

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Molecular-based species delimitation approaches greatly facilitate discovering species in taxa with considerable intraspecific morphological variation, in those lacking diagnostic morphological characters or in those rare in collections. A combination of molecular species delimitation and morphological diagnosis has recently helped resolve parts of species diversity in primitively segmented spiders (family Liphistiidae), in which female genitalia are highly variable and thus difficult to diagnose, and males are often unknown. Here, we provide a taxonomic revision of the liphistiid genus *Ryuthela* Haupt, 1983, endemic to Ryukyu archipelago, Japan, through a combination of molecular and morphological data in a phylogenetic framework. We barcoded 173 originally collected specimens of 15 putative species using the universal cytochrome *c* oxidase subunit I (*COI*) mitochondrial marker and used these data for species delimitation estimates. The analytical approaches involved two genetic variation-based methods (DNA barcoding gap and automatic barcode gap discovery) and two tree-based methods [species delimitation plugin (P ID(Liberal)) and generalized mixed Yule-coalescent model (GMYC)]. All species delimitation methods except for GMYC suggest that *Ryuthela* consists of the five currently deemed valid species plus one named and as many as nine newly discovered species. Our findings shed light on species diversity of endemic spiders in one of the Asian biodiversity hotspots, and a formal taxonomy of 15 species completes the genus revision.

ADDITIONAL KEYWORDS: DNA barcoding – Okinawa – phylogeny – Ryukyu archipelago – species delimitation – taxonomy.

INTRODUCTION

The application of molecular-based approaches to species delimitation has boosted global species diversity by facilitating species discovery in closely related taxa that lack clear morphological diagnosability (Hamilton, Formanowicz & Bond, 2011; Weigand *et al.*, 2011; Satler, Carstens & Hedin, 2013; Hamilton *et al.*, 2014; Xu *et al.*, 2015a), in taxa that show considerable intraspecific morphological variation or in taxa that are rare in collections (missing a series of both sexes or adult forms) (Xu *et al.*, 2015a). For such taxa, species

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boundaries are often inherently difficult if not impossible to delimit based solely on morphology, calling for more integrative species delimitation approaches (Bickford *et al.*, 2007; Hamilton *et al.*, 2011, 2014; Rittmeyer & Austin, 2012; Carstens *et al.*, 2013; Satler *et al.*, 2013; Fouquet *et al.*, 2014; Shirley *et al.*, 2014; Tanikawa & Miyashita, 2014; Xu *et al.*, 2015a), that is combining evidence from analyses of molecular, morphological, geographical and other kinds of data within a phylogenetic framework.

DNA barcodes, short gene sequences from a standardized portion of the genome, have become a routine tool not only in identifying species, but also in delimiting known species, and discovering new, previously unrecognized and cryptic ones (Hebert *et al.*, 2003a, 2004; Barrett & Hebert, 2005; Hamilton *et al.*, 2014; Xu *et al.*, 2015a). Cytochrome *c* oxidase subunit I (*COI*), the DNA barcode in animals (Hebert *et al.*, 2003a; Hebert, Ratnasingham & deWaard, 2003b; Barrett & Hebert, 2005), is readily obtained and sufficiently variable among species of most animal lineages (Folmer *et al.*, 1994), justifying its overall taxonomic utility at the species level (Hebert *et al.*, 2003a; Hamilton *et al.*, 2011, 2014; Čandek & Kuntner, 2015; Xu *et al.*, 2015a), as well as at higher taxonomic levels (Coddington *et al.*, 2016). Recent years have seen a rapid increase in DNA barcoding applied to taxonomic problems in spiders (Kuntner & Agnarsson, 2011a, b; Hamilton *et al.*, 2014; Agnarsson *et al.*, 2015, 2016; Hedin, 2015; Hendrixson, Guice & Bond, 2015; Li *et al.*, 2015; Toussaint *et al.*, 2015; Xu *et al.*, 2015a).

The use of DNA barcoding in taxonomy and systematics has, however, also been deemed controversial (Hebert *et al.*, 2003a; Moritz & Cicero, 2004; Barrett & Hebert, 2005; Rubinoff & Holland, 2005; Song *et al.*, 2008; Hedin, 2015). One of the controversies is how to objectively determine the barcoding gap (i.e. a species 'threshold'). In an ideal scenario, a clear, non-overlapping DNA barcode gap between intraspecific and interspecific genetic distances can be used as a threshold to delimit species (Hebert *et al.*, 2004; Barrett & Hebert, 2005; Meyer & Paulay, 2005; Weigand *et al.*, 2011). In practice, however, studies have found instead substantial overlap between intraspecific vs. interspecific divergences in different animal groups, which likely reflects different taxonomic practices (Meyer & Paulay, 2005; Hickerson, Meyer & Moritz, 2006; Meier *et al.*, 2006; Čandek & Kuntner, 2015; Kvist, 2014; Dang *et al.*, 2016). Researchers have thus proposed several different ways to identify the barcoding gap, such as a standard interspecific threshold of 3% for all the species (e.g. Hebert *et al.*, 2003a, 2004), an interspecific distance of ten times the mean intraspecific distance (Hebert *et al.*, 2004), the smallest interspecific instead of the mean interspecific distance (Meier, Zhang & Ali, 2008) and, because data are rarely normal, the use of median instead of mean intraspecific and interspecific distances (Čandek & Kuntner, 2015). Because of this challenge, alternative species delimitation methods are useful and these either use genetic distances, for example automatic barcode gap discovery (ABGD) algorithm (Puillandre *et al.*, 2012), or are tree-based, for example species delimitation plugin [P ID(Liberal)] (Masters, Fan & Ross, 2011) and general mixed Yule-coalescent (GMYC) methodology (Pons *et al.*, 2006).

Among lineages where alternative species delimitation methods are useful due to morphological similarity among species and their rarity, are primitively segmented spiders of the family Liphistiidae (Xu *et al.*, 2015a). As the only living family of the spider suborder Mesothelae, a species-poor and ancient lineage dating back to the very origin of spiders in the Carboniferous, Liphistiidae, whose origin is as recent as Palaeogene (Xu *et al.*, 2015c), is important for the study of the spider tree of life. The family is confined to Southeast and East Asia and contains species with restricted, disjunct geographical distributions (Xu *et al.*, 2015b, c, 2016; World Spider Catalog, 2017). The family has recently been revised to contain eight genera, each being endemic to a particular geographical region (Xu *et al.*, 2015a, b, c), where they exhibit high habitat specificity (Haupt, 2003; Xu *et al.*, 2015c). Their biogeographical patterns with many endemic taxa make them an ideal study model for speciation research (Xu *et al.*, 2015a, c, 2016). However, species of liphistiids have historically been difficult to delimit due to the lineage's uniform, conservative morphology with simple yet highly variable female genitalia, and rarity of available adult forms in collections, notably males (Haupt, 2003; Schwendinger & Ono, 2011; Tanikawa, 2013a; Tanikawa & Miyashita, 2014; Xu *et al.*, 2015a). Our recent taxonomic study of the liphistiid genus *Ganthela* has demonstrated that DNA barcodes, if integrated with morphological and geographical data, can provide reliable species delimitation (Xu *et al.*, 2015a).

In this study, we focus on species delimitation of another liphistiid genus, *Ryuthela* Haupt, 1983, endemic to the Japanese Ryukyu archipelago (Xu *et al.*, 2015b, c, 2016; Fig. 1), an Asian biodiversity hotspot with highly endemic floras and faunas (Myers *et al.*, 2000; Crowe *et al.*, 2006). This was facilitated by our original sampling throughout the genus range (Xu *et al.*, 2016; Fig. 2). *Ryuthela* currently has five valid species occurring in central and southern Ryukyus (World Spider Catalog, 2017; see also Taxonomic History). As is the case in other liphistiids, *Ryuthela* species are morphologically alike, exhibit considerable intraspecific variation in female genitalia and are rare in collections either from one or both sexes (Haupt, 2003; Schwendinger & Ono, 2011; Tanikawa, 2013a, b; Tanikawa & Miyashita, 2014; Xu *et al.*, 2015a, c). As such, *Ryuthela* species taxonomy cannot be adequately solved based solely on

Figure 1. Natural history of *Ryuthela*. A, Female *Ryuthela nishihirai s.s.* B, macrohabitat of *R. nishihirai s.l.* (Sheyoshi Park, Shuri, Okinawajima). C, close-up from (B), showing densely positioned trapdoors (arrows).

morphological characters, and instead other species delimitation approaches are necessary. We use four different species delimitation methods based on original DNA barcodes. We specifically test the accuracy of existing species hypotheses (see Taxonomic History) through different barcoding gap methods thereby obtaining estimates of species limits in *Ryuthela*, then use these results to complete the revision of the genus.

MATERIAL AND METHODS

Taxon sampling

We sampled 220 *Ryuthela* individuals from 29 localities from central to southern Ryukyus (Fig. 2) guided by the known type locality information and additional searches in suitable habitats. We collected adults and immature spiders by excavating them from their subterranean burrows. We reared juveniles to adulthood and fixed the adults in absolute ethanol, storing their right-side legs at –80 °C for DNA extraction and preserving the remainder of vouchers in 80% ethanol for identification and morphological examination. Our ingroup consisted of 173 *Ryuthela* specimens, and we used four specimens of *Ganthela jianensis* Xu, Kuntner & Chen, 2015, *Heptathela higoensis* Haupt, 1983, *Songthela hangzhouensis* (Chen, Zhang & Zhu, 1981) and *Vinathela cucphuongensis* (Ono, 1999) as outgroups (Table 1). Voucher specimens are deposited at the Centre for Behavioural Ecology and Evolution (CBEE), College of Life Sciences, Hubei University, Wuhan, China, and all type specimens will be deposited in National Museum of Nature and Science, Tokyo, Japan (NSMT) and National Zoological Museum, Chinese Academy of Sciences, Beijing, China.

Molecular protocols

We extracted total genomic DNA from spider legs using the Animal Genomic DNA Isolation Kit (Dingguo, Beijing, China) following the manufacturer's protocols. We amplified cytochrome *c* oxidase subunit I (*COI*) using the primer pairs LCO1490/HCO2198 (Folmer *et al.*, 1994) and standard protocols (Xu *et al.*, 2015c). The sequencing was done at Sunny Biotechnology Co, Ltd (Shanghai, China) using the ABI 3730XL DNA analyser.

We manually edited the sequences using Geneious 5.6.6 (Biomatters Ltd., 2012), translated nucleotide reads to amino acids to check for stop codons and to ensure proper configuration of codon positions and aligned the sequences in Geneious with gap opening/extension penalties set to 24/3 (Xu *et al.*, 2015a, c, 2016).

Phylogenetic analyses

To investigate the phylogenetic relationships within *Ryuthela* in a Bayesian framework, we used *COI* data from the 173 ingroup specimens representing all sampled localities and 4 outgroups (Table 1). We used PARTITIONFINDER v1.1.1 (Lanfear *et al.*, 2012) to

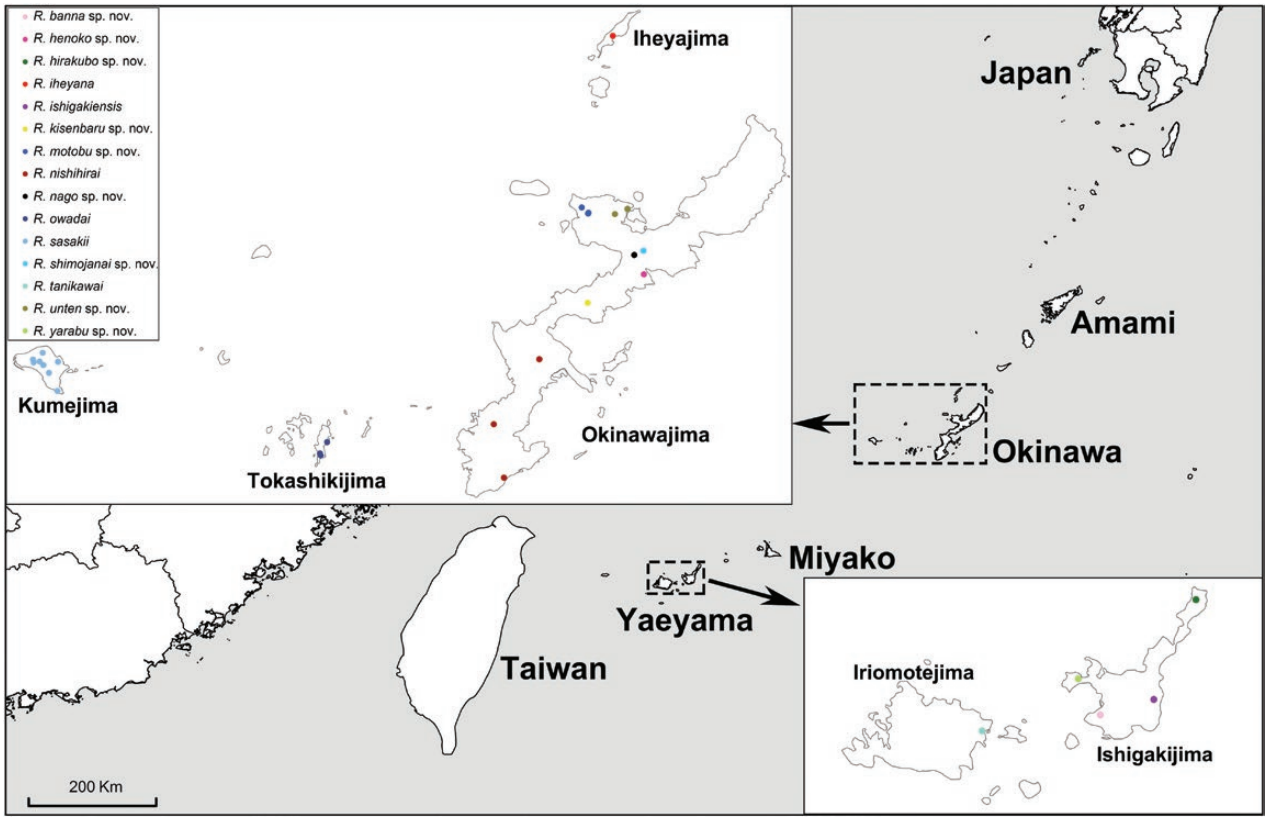

Figure 2. Maps showing the sampling localities for species of *Ryuthela* on Ryukyu archipelago. The map on the upper left corner shows the collection localities and species of *Ryuthela* on Okinawa island group (Iheyajima, Kumejima, Okinawajima, Tokashikijima). The map on the lower right shows the collection localities and species on Ishigakijima and Iriomotejima.

select the best data partitioning schemes using the greedy algorithm based on the Akaike information criterion. GTR + I + G substitution model was assigned to first and third codon positions, and F81 + I was assigned to second codon position in *COI*. We conducted Bayesian inference (BI) analyses in MRBAYES v3.2.1 (Ronquist *et al.*, 2012) by running four Markov chain Monte Carlo (MCMC) chains for 20 million generations, and sampling trees every 2000 generations. We monitored stationarity in Tracer v1.6 (Rambaut *et al.*, 2014) and discarded as 'burnin' the first quarter of cold chain samples with the remaining trees used to build a consensus, as in a prior study (Xu *et al.*, 2015a). We used FigTree v1.4.0 (Rambaut, 2012) to visualize and manipulate trees and manually summarized the results from different approaches using vector graphics in Adobe Illustrator.

Species delimitation

In order to delimit *Ryuthela* species based on the *COI* data matrix, we employed both genetic variation-based methods – DNA barcoding gap (Hebert *et al.*, 2003a) and the ABGD procedure (Puillandre *et al.*, 2012) – and tree-based species delimitation methods – P ID(Liberal) and GMYC methodology (Pons *et al.*, 2006). As DNA barcoding gap (Hebert *et al.*, 2003a) and P ID(Liberal) require a priori species designation, we assigned 173 *Ryuthela* individuals (representing five nominal species) to 15 putative species based on a combination of phylogenetic topology, morphological characters and geographic information. In the DNA barcoding gap analysis, we examined the pairwise genetic distances for both intraspecific and interspecific K2P and uncorrected *p*-distance for each candidate species calculated in Mega v. 5.2.2 (Tamura *et al.*, 2011). We used the species delimitation plugin (Masters *et al.*, 2011) in Geneious v5.6.6 (Biomatters Ltd., 2012) to obtain P ID(Liberal) statistics that calculate the mean probability of genetic distance ratios between and within putative species. The BI tree was used as a guide tree to test the species hypothesis.

The other two methods that we used do not require terminals to be assigned to putative species. ABGD calculates all pairwise distances in the data set, evaluates intraspecific divergences and then sorts the terminals into candidate species with calculated *P*-values. We performed ABGD analyses following our earlier study (Xu *et al.*, 2015a).

Table 1. Samples used in phylogenetic analysis and species delimitation: specimen label, taxon name, sample collection locality with coordinates and GenBank accession numbers

Specimen code	Genus	Species	Locality	County	Coordinates	Elevation (m a.s.l.)	*COI* GenBank accession
3211	***Ryuthela***	***banna* sp. nov.**	Mt. Banna dake, Ishigakijima, Okinawa Prefecture	Japan	24.36928°N, 124.15949°E	100	KT767561
3213	***Ryuthela***	***banna* sp. nov.**	Mt. Banna dake, Ishigakijima, Okinawa Prefecture	Japan	24.36928°N, 124.15949°E	100	KT767562
3216	***Ryuthela***	***banna* sp. nov.**	Mt. Nose dake, Ishigakijima, Okinawa Prefecture	Japan	24.37473°N, 124.14134°E	125	KT767566
3217	***Ryuthela***	***banna* sp. nov.**	Mt. Nose dake, Ishigakijima, Okinawa Prefecture	Japan	24.37473°N, 124.14134°E	125	MF078500
3218	***Ryuthela***	***banna* sp. nov.**	Mt. Nose dake, Ishigakijima, Okinawa Prefecture	Japan	24.37473°N, 124.14134°E	125	MF078501
3219	***Ryuthela***	***banna* sp. nov.**	Mt. Nose dake, Ishigakijima, Okinawa Prefecture	Japan	24.37473°N, 124.14134°E	125	KT767567
3220	***Ryuthela***	***banna* sp. nov.**	Mt. Nose dake, Ishigakijima, Okinawa Prefecture	Japan	24.37473°N, 124.14134°E	125	MF078502
3221	***Ryuthela***	***banna* sp. nov.**	Mt. Nose dake, Ishigakijima, Okinawa Prefecture	Japan	24.37473°N, 124.14134°E	125	MF078503
2463	***Ryuthela***	***henoko* sp. nov.**	Near Henoko Dam, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.54132°N, 128.03017°E	47	MF078504
2465	***Ryuthela***	***henoko* sp. nov.**	Near Henoko Dam, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.54132°N, 128.03017°E	47	MF078505
2466	***Ryuthela***	***henoko* sp. nov.**	Near Henoko Dam, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.54132°N, 128.03017°E	47	MF078506
2468	***Ryuthela***	***henoko* sp. nov.**	Near Henoko Dam, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.54132°N, 128.03017°E	47	KP229907
2471	***Ryuthela***	***henoko* sp. nov.**	Near Henoko Dam, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.54132°N, 128.03017°E	47	KT767555
3222	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.59121°N, 124.31851°E	19	KP229823
3223	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.59121°N, 124.31851°E	19	MF078507
3227	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.58864°N, 124.31858°E	23	MF078508
3232	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.58864°N, 124.31858°E	22	MF078509
3233	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.58864°N, 124.31858°E	22	MF078510
3234	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.58864°N, 124.31858°E	22	MF078511
3236	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.58864°N, 124.31858°E	22	MF078512
3237	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.58864°N, 124.31858°E	22	MF078513
3238	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.58864°N, 124.31858°E	22	MF078514
3239	***Ryuthela***	***hirakubo* sp. nov.**	Hirakubo River, Ishigakijima, Okinawa Prefecture	Japan	24.58864°N, 124.31858°E	22	KT767556

Table 1. *Continued*

Specimen code	Genus	Species	Locality	County	Coordinates	Elevation (m a.s.l.)	*COI* GenBank accession
4067	*Ryuthela*	*iheyana*	Forest Trail, Mt. Koshi dake, Iheyajima, Okinawa Prefecture	Japan	27.04083°N, 127.965°E	NA	MF078515
4068	*Ryuthela*	*iheyana*	Forest Trail, Mt. Koshi dake, Iheyajima, Okinawa Prefecture	Japan	27.04083°N, 127.965°E	NA	MF078516
3240	*Ryuthela*	*ishigakiensis*	Mt. Kara daka, Ishigakijima, Okinawa Prefecture	Japan	24.40331°N, 124.24037°E	75	KT767563
3241	*Ryuthela*	*ishigakiensis*	Mt. Kara daka, Ishigakijima, Okinawa Prefecture	Japan	24.40331°N, 124.24037°E	75	MF078517
3242	*Ryuthela*	*ishigakiensis*	Mt. Kara daka, Ishigakijima, Okinawa Prefecture	Japan	24.40331°N, 124.24037°E	75	MF078518
3245	*Ryuthela*	*ishigakiensis*	Mt. Kara daka, Ishigakijima, Okinawa Prefecture	Japan	24.40331°N, 124.24037°E	75	MF078519
3246	*Ryuthela*	*ishigakiensis*	Mt. Kara daka, Ishigakijima, Okinawa Prefecture	Japan	24.40334°N, 124.24043°E	76	MF078520
3247	*Ryuthela*	*ishigakiensis*	Mt. Kara daka, Ishigakijima, Okinawa Prefecture	Japan	24.40334°N, 124.24043°E	76	MF078521
3249	*Ryuthela*	*ishigakiensis*	Mt. Kara daka, Ishigakijima, Okinawa Prefecture	Japan	24.40334°N, 124.24043°E	76	KT767564
2474	***Ryuthela***	***kisenbaru* sp. nov.**	Kisenbaru, Onna-son, Kin-cho, Okinawajima, Okinawa Prefecture	Japan	26.48157°N, 127.91217°E	31	KT767558
2475	***Ryuthela***	***kisenbaru* sp. nov.**	Kisenbaru, Onna-son, Kin-cho, Okinawajima, Okinawa Prefecture	Japan	26.48157°N, 127.91217°E	31	MF078522
2476	***Ryuthela***	***kisenbaru* sp. nov.**	Kisenbaru, Onna-son, Kin-cho, Okinawajima, Okinawa Prefecture	Japan	26.48157°N, 127.91217°E	31	MF078523
2477	***Ryuthela***	***kisenbaru* sp. nov.**	Kisenbaru, Onna-son, Kin-cho, Okinawajima, Okinawa Prefecture	Japan	26.48157°N, 127.91217°E	31	KP229857
2477A	***Ryuthela***	***kisenbaru* sp. nov.**	Kisenbaru, Onna-son, Kin-cho, Okinawajima, Okinawa Prefecture	Japan	26.48157°N, 127.91217°E	31	MF078524
2538	***Ryuthela***	***motobu* sp. nov.**	Yamazato, Motobu-cho, Okinawajiam, Okinawa Prefecture	Japan	26.67073°N, 127.91328°E	141	KP229916
2539	***Ryuthela***	***motobu* sp. nov**.	Yamazato, Motobu-cho, Okinawajiam, Okinawa Prefecture	Japan	26.67073°N, 127.91328°E	141	MF078531
2540	***Ryuthela***	***motobu* sp. nov.**	Yamazato, Motobu-cho, Okinawajiam, Okinawa Prefecture	Japan	26.67073°N, 127.91328°E	141	MF078532
4085	***Ryuthela***	***motobu* sp. nov.**	Jahana, Motobu-cho, Okinawajima, Okinawa Prefecture	Japan	26.68134°N, 127.89967°E	88	MF078525
4086	***Ryuthela***	***motobu* sp. nov.**	Jahana, Motobu-cho, Okinawajima, Okinawa Prefecture	Japan	26.68134°N, 127.89967°E	88	MF078526
4087	***Ryuthela***	***motobu* sp. nov.**	Jahana, Motobu-cho, Okinawajima, Okinawa Prefecture	Japan	26.68134°N, 127.89967°E	88	MF078527

Table 1. *Continued*

Specimen code	Genus	Species	Locality	County	Coordinates	Elevation (m a.s.l.)	*COI* GenBank accession
4089	***Ryuthela***	***motobu* sp. nov.**	Jahana, Motobu-cho, Okinawajima, Okinawa Prefecture	Japan	26.68134°N, 127.89967°E	88	MF078528
4090	***Ryuthela***	***motobu* sp. nov.**	Jahana, Motobu-cho, Okinawajima, Okinawa Prefecture	Japan	26.68139°N, 127.89973°E	77	MF078529
4091	***Ryuthela***	***motobu* sp. nov.**	Jahana, Motobu-cho, Okinawajima, Okinawa Prefecture	Japan	26.68139°N, 127.89973°E	77	MF078530
4092	***Ryuthela***	***motobu* sp. nov.**	Yamazato, Motobu-cho, Okinawajima, Okinawa Prefecture	Japan	26.67034°N, 127.91368°E	143	MF078533
4098	***Ryuthela***	***motobu* sp. nov.**	Yamazato, Motobu-cho, Okinawajima, Okinawa Prefecture	Japan	26.66869°N, 127.9132°E	160	MF078534
2447	***Ryuthela***	***nago* sp. nov.**	Mt. Nago dake, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.58172°N, 128.00986°E	217	KP229879
2448	***Ryuthela***	***nago* sp. nov.**	Mt. Nago dake, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.58172°N, 128.00986°E	217	MF078535
2449	***Ryuthela***	***nago* sp. nov.**	Mt. Nago dake, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.58172°N, 128.00986°E	217	MF078536
2450	***Ryuthela***	***nago* sp. nov.**	Mt. Nago dake, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.58172°N, 128.00986°E	217	MF078537
2451	***Ryuthela***	***nago* sp. nov.**	Mt. Nago dake, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.58172°N, 128.00986°E	217	MF078538
2452	***Ryuthela***	***nago* sp. nov.**	Mt. Nago dake, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.58172°N, 128.00986°E	217	MF078539
2453	***Ryuthela***	***nago* sp. nov.**	Mt. Nago dake, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.58172°N, 128.00986°E	217	MF078540
2454	***Ryuthela***	***nago* sp. nov.**	Mt. Nago dake, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.58172°N, 128.00986°E	217	KT767565
2455	***Ryuthela***	***nago* sp. nov.**	Mt. Nago dake, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.58172°N, 128.00986°E	217	MF078541
2301	*Ryuthela*	*nishihirai*	Sheyoshi Park, Shuri, Naha, Okinawajima, Okinawa Prefecture	Japan	26.22731°N, 127.71532°E	35	MF078548
2302	*Ryuthela*	*nishihirai*	Sheyoshi Park, Shuri, Naha, Okinawajima, Okinawa Prefecture	Japan	26.22731°N, 127.71532°E	35	KP229854
2303	*Ryuthela*	*nishihirai*	Sheyoshi Park, Shuri, Naha, Okinawajima, Okinawa Prefecture	Japan	26.22731°N, 127.71532°E	35	KT767570

Table 1. *Continued*

Specimen code	Genus	Species	Locality	County	Coordinates	Elevation (m a.s.l.)	*COI* GenBank accession
2304	*Ryuthela*	*nishihirai*	Sheyoshi Park, Shuri, Naha, Okinawajima, Okinawa Prefecture	Japan	26.22731°N, 127.71532°E	35	MF078549
2306	*Ryuthela*	*nishihirai*	Sheyoshi Park, Shuri, Naha, Okinawajima, Okinawa Prefecture	Japan	26.22742°N, 127.71516°E	44	MF078550
2307	*Ryuthela*	*nishihirai*	Sheyoshi Park, Shuri, Naha, Okinawajima, Okinawa Prefecture	Japan	26.22742°N, 127.71516°E	44	MF078551
2309A	*Ryuthela*	*nishihirai*	Sheyoshi Park, Shuri, Naha, Okinawajima, Okinawa Prefecture	Japan	26.22742°N, 127.71516°E	44	MF078552
2309B	*Ryuthela*	*nishihirai*	Sheyoshi Park, Shuri, Naha, Okinawajima, Okinawa Prefecture	Japan	26.22742°N, 127.71516°E	44	MF078553
2541	*Ryuthela*	*nishihirai*	Asato, Yaese-cho, Okinawajima, Okinawa Prefecture	Japan	26.11555°N, 127.73668°E	51	KT767553
2541A	*Ryuthela*	*nishihirai*	Asato, Yaese-cho, Okinawajima, Okinawa Prefecture	Japan	26.11555°N, 127.73668°E	51	MF078542
2542	*Ryuthela*	*nishihirai*	Asato, Yaese-cho, Okinawajima, Okinawa Prefecture	Japan	26.11555°N, 127.73668°E	51	KP229891
2543	*Ryuthela*	*nishihirai*	Asato, Yaese-cho, Okinawajima, Okinawa Prefecture	Japan	26.11555°N, 127.73668°E	51	MF078543
2544	*Ryuthela*	*nishihirai*	Asato, Yaese-cho, Okinawajima, Okinawa Prefecture	Japan	26.11555°N, 127.73668°E	51	MF078544
2545	*Ryuthela*	*nishihirai*	Asato, Yaese-cho, Okinawajima, Okinawa Prefecture	Japan	26.11555°N, 127.73668°E	51	MF078545
4043	*Ryuthela*	*nishihirai*	Chibana, Gusuku, Okinawa-shi, Okinawajima, Okinawa Prefecture	Japan	26.36346°N, 127.81081°E	66	MF078546
4044	*Ryuthela*	*nishihirai*	Chibana, Gusuku, Okinawa-shi, Okinawajima, Okinawa Prefecture	Japan	26.36345°N, 127.81081°E	67	MF078547
2404	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16629°N, 127.35091°E	53	MF078486
2405	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16629°N, 127.35091°E	53	MF078487
2406	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16629°N, 127.35091°E	53	MF078488
2407	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16629°N, 127.35091°E	53	MF078489
2408	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16639°N, 127.35075°E	54	MF078490
2409	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16639°N, 127.35075°E	54	KP229813
2410	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16639°N, 127.35075°E	54	KT767552
2411	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16639°N, 127.35075°E	54	MF078491
2412	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16639°N, 127.35075°E	54	MF078492

Table 1. *Continued*

Specimen code	Genus	Species	Locality	County	Coordinates	Elevation (m a.s.l.)	*COI* GenBank accession
2413	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16639°N, 127.35075°E	54	MF078493
2415	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16195°N, 127.35258°E	100	MF078494
2416	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16195°N, 127.35258°E	100	MF078495
2418	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16195°N, 127.35258°E	100	KT767571
2419	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16195°N, 127.35258°E	100	MF078496
2420	*Ryuthela*	*owadai*	Aharen, Tokashikijima, Okinawa Prefecture	Japan	26.16195°N, 127.35258°E	100	MF078497
2426	*Ryuthela*	*owadai*	Tokashiki Port, Tokashikijima, Okinawa Prefecture	Japan	26.18985°N, 127.36607°E	168	MF078498
2430	*Ryuthela*	*owadai*	Tokashiki Port, Tokashikijima, Okinawa Prefecture	Japan	26.18984°N, 127.3662°E	144	MF078499
2334	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36271°N, 126.7496°E	46	KT767577
2335	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36280°N, 126.74963°E	54	MF078601
2335A	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36280°N, 126.74963°E	54	MF078602
2337	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36280°N, 126.74963°E	54	MF078603
2338	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36280°N, 126.74963°E	54	KT767578
2339	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36280°N, 126.74963°E	54	MF078604
2340	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36285°N, 126.74976°E	48	MF078605
2341	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36285°N, 126.74976°E	48	MF078606
2342	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36285°N, 126.74976°E	48	MF078607
2343	*Ryuthela*	*sasakii*	Yamazato, Kumejima, Okinawa Prefecture	Japan	26.36285°N, 126.74976°E	48	MF078608
2344	*Ryuthela*	*sasakii*	Yamagusuku, Kumejima, Okinawa Prefecture	Japan	26.33484°N, 126.78293°E	30	KT767576
2345	*Ryuthela*	*sasakii*	Yamagusuku, Kumejima, Okinawa Prefecture	Japan	26.33484°N, 126.78293°E	30	MF078593
2346	*Ryuthela*	*sasakii*	Yamagusuku, Kumejima, Okinawa Prefecture	Japan	26.33471°N, 126.78259°E	32	MF078594
2347	*Ryuthela*	*sasakii*	Yamagusuku, Kumejima, Okinawa Prefecture	Japan	26.33471°N, 126.78259°E	32	MF078595
2348	*Ryuthela*	*sasakii*	Yamagusuku, Kumejima, Okinawa Prefecture	Japan	26.33471°N, 126.78259°E	32	MF078596
2350	*Ryuthela*	*sasakii*	Yamagusuku, Kumejima, Okinawa Prefecture	Japan	26.33471°N, 126.78259°E	32	MF078597
2351	*Ryuthela*	*sasakii*	Yamagusuku, Kumejima, Okinawa Prefecture	Japan	26.33471°N, 126.78259°E	32	MF078598
2352	*Ryuthela*	*sasakii*	Yamagusuku, Kumejima, Okinawa Prefecture	Japan	26.33471°N, 126.78259°E	32	MF078599

Table 1. *Continued*

Specimen code	Genus	Species	Locality	County	Coordinates	Elevation (m a.s.l.)	*COI* GenBank accession
2353	*Ryuthela*	*sasakii*	Yamagusuku, Kumejima, Okinawa Prefecture	Japan	26.33471°N, 126.78259°E	32	MF078600
2354	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29711°N, 126.80067°E	71	KT767572
2355	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29711°N, 126.80067°E	71	MF078575
2356	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29711°N, 126.80067°E	71	MF078576
2357	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29711°N, 126.80067°E	71	MF078577
2358	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29711°N, 126.80067°E	71	MF078578
2359	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29711°N, 126.80067°E	71	MF078579
2360	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29716°N, 126.80082°E	77	MF078580
2361	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29718°N, 126.8009°E	78	MF078581
2362	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29704°N, 126.80106°E	77	MF078582
2363	*Ryuthela*	*sasakii*	Tokujimu Nature Park, Kumijima, Okinawa Prefecture	Japan	26.29704°N, 126.80106°E	77	MF078583
2364	*Ryuthela*	*sasakii*	Maja, Nakazato-son, Kumejima, Okinawa Prefecture	Japan	26.35823°N, 126.80168°E	41	KT767559
2365	*Ryuthela*	*sasakii*	Maja, Nakazato-son, Kumejima, Okinawa Prefecture	Japan	26.35823°N, 126.80168°E	41	MF078560
2366	*Ryuthela*	*sasakii*	Maja, Nakazato-son, Kumejima, Okinawa Prefecture	Japan	26.35823°N, 126.80168°E	41	KT767560
2368	*Ryuthela*	*sasakii*	Maja, Nakazato-son, Kumejima, Okinawa Prefecture	Japan	26.35823°N, 126.80168°E	41	MF078561
2369	*Ryuthela*	*sasakii*	Maja, Nakazato-son, Kumejima, Okinawa Prefecture	Japan	26.35823°N, 126.80168°E	41	MF078562
2370	*Ryuthela*	*sasakii*	Maja, Nakazato-son, Kumejima, Okinawa Prefecture	Japan	26.35823°N, 126.80168°E	41	MF078563

Table 1. *Continued*

Specimen code	Genus	Species	Locality	County	Coordinates	Elevation (m a.s.l.)	*COI* GenBank accession
2371	*Ryuthela*	*sasakii*	Maja, Nakazato-son, Kumejima, Okinawa Prefecture	Japan	26.35823°N, 126.80168°E	41	MF078564
2372	*Ryuthela*	*sasakii*	Maja, Nakazato-son, Kumejima, Okinawa Prefecture	Japan	26.35837°N, 126.80177°E	48	MF078565
2373	*Ryuthela*	*sasakii*	Maja, Nakazato-son, Kumejima, Okinawa Prefecture	Japan	26.35837°N, 126.80177°E	48	MF078566
2374	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37685°N, 126.76971°E	302	MF078584
2375	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37685°N, 126.76971°E	302	MF078585
2376	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37685°N, 126.76971°E	302	MF078586
2377	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37685°N, 126.76971°E	302	KT767573
2378	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37685°N, 126.76971°E	302	MF078587
2379	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37698°N, 126.76994°E	302	MF078588
2380	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37698°N, 126.76994°E	302	MF078589
2381	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37698°N, 126.76994°E	302	MF078590
2382	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37698°N, 126.76994°E	302	MF078591
2383	*Ryuthela*	*sasakii*	Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima, Okinawa Prefecture	Japan	26.37548°N, 126.76987°E	247	MF078592
2384	*Ryuthela*	*sasakii*	Daruma Yama Park, Mt. Daruma, Kumejima, Okinawa Prefecture	Japan	26.35865°N, 126.76321°E	144	MF078554

Table 1. *Continued*

Specimen code	Genus	Species	Locality	County	Coordinates	Elevation (m a.s.l.)	*COI* GenBank accession
2386	*Ryuthela*	*sasakii*	Daruma Yama Park, Mt. Daruma, Kumejima, Okinawa Prefecture	Japan	26.35865°N, 126.76321°E	144	MF078555
2387	*Ryuthela*	*sasakii*	Daruma Yama Park, Mt. Daruma, Kumejima, Okinawa Prefecture	Japan	26.35865°N, 126.76321°E	144	KP229870
2388	*Ryuthela*	*sasakii*	Daruma Yama Park, Mt. Daruma, Kumejima, Okinawa Prefecture	Japan	26.35865°N, 126.76321°E	144	MF078556
2389	*Ryuthela*	*sasakii*	Daruma Yama Park, Mt. Daruma, Kumejima, Okinawa Prefecture	Japan	26.35865°N, 126.76321°E	144	MF078557
2391	*Ryuthela*	*sasakii*	Daruma Yama Park, Mt. Daruma, Kumejima, Okinawa Prefecture	Japan	26.35871°N, 126.76321°E	127	MF078558
2392	*Ryuthela*	*sasakii*	Daruma Yama Park, Mt. Daruma, Kumejima, Okinawa Prefecture	Japan	26.35871°N, 126.76321°E	127	KT767554
2393	*Ryuthela*	*sasakii*	Daruma Yama Park, Mt. Daruma, Kumejima, Okinawa Prefecture	Japan	26.35871°N, 126.76321°E	127	MF078559
2394	*Ryuthela*	*sasakii*	Shirasegawa River, Gushikawa-son, Kumejima, Okinawa Prefecture	Japan	26.35126°N, 126.77116°E	44	MF078567
2395	*Ryuthela*	*sasakii*	Shirasegawa River, Gushikawa-son, Kumejima, Okinawa Prefecture	Japan	26.35049°N, 126.76907°E	47	MF078568
2397	*Ryuthela*	*sasakii*	Shirasegawa River, Gushikawa-son, Kumejima, Okinawa Prefecture	Japan	26.35049°N, 126.76907°E	47	MF078569
2398	*Ryuthela*	*sasakii*	Shirasegawa River, Gushikawa-son, Kumejima, Okinawa Prefecture	Japan	26.35049°N, 126.76907°E	47	MF078570
2399	*Ryuthela*	*sasakii*	Shirasegawa River, Gushikawa-son, Kumejima, Okinawa Prefecture	Japan	26.35049°N, 126.76907°E	47	MF078571
2400	*Ryuthela*	*sasakii*	Shirasegawa River, Gushikawa-son, Kumejima, Okinawa Prefecture	Japan	26.35048°N, 126.76911°E	48	KT767569
2401	*Ryuthela*	*sasakii*	Shirasegawa River, Gushikawa-son, Kumejima, Okinawa Prefecture	Japan	26.35048°N, 126.76911°E	48	MF078572
2402	*Ryuthela*	*sasakii*	Shirasegawa River, Gushikawa-son, Kumejima, Okinawa Prefecture	Japan	26.35048°N, 126.76911°E	48	MF078573
2403	*Ryuthela*	*sasakii*	Shirasegawa River, Gushikawa-son, Kumejima, Okinawa Prefecture	Japan	26.35048°N, 126.76911°E	48	MF078574
2329	***Ryuthela***	***shimojanai* sp. nov.**	Taira, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.59078°N, 128.0291°E	98	MF078609
2333	***Ryuthela***	***shimojanai* sp. nov.**	Taira, Nago-shi, Okinawajima, Okinawa Prefecture	Japan	26.59082°N, 128.02928°E	97	KP229830

Table 1. *Continued*

Specimen code	Genus	Species	Locality	County	Coordinates	Elevation (m a.s.l.)	*COI* GenBank accession
3200	*Ryuthela*	*tanikawai*	Mihara, Iriomotejima, Okinawa Prefecture	Japan	24.34522°N, 123.92197°E	26	KP229848
3203	*Ryuthela*	*tanikawai*	Mihara, Iriomotejima, Okinawa Prefecture	Japan	24.34537°N, 123.92207°E	27	MF078613
3204	*Ryuthela*	*tanikawai*	Mihara, Iriomotejima, Okinawa Prefecture	Japan	24.34537°N, 123.92207°E	27	MF078610
3206	*Ryuthela*	*tanikawai*	Mihara, Iriomotejima, Okinawa Prefecture	Japan	24.34537°N, 123.92207°E	27	KT767557
3208	*Ryuthela*	*tanikawai*	Mihara, Iriomotejima, Okinawa Prefecture	Japan	24.34537°N, 123.92207°E	27	MF078611
3209	*Ryuthela*	*tanikawai*	Mihara, Iriomotejima, Okinawa Prefecture	Japan	24.34537°N, 123.92207°E	27	MF078612
2524	***Ryuthela***	***unten* sp. nov.**	Unten Port, Nakijin-son, Okinawajima, Okinawa Prefecture	Japan	26.67828°N, 127.99577°E	24	KT767574
2525	***Ryuthela***	***unten* sp. nov.**	Unten Port, Nakijin-son, Okinawajima, Okinawa Prefecture	Japan	26.67828°N, 127.99577°E	24	KT767575
2528	***Ryuthela***	***unten* sp. nov.**	Unten Port, Nakijin-son, Okinawajima, Okinawa Prefecture	Japan	26.67828°N, 127.99577°E	24	MF078617
2529	***Ryuthela***	***unten* sp. nov.**	Unten Port, Nakijin-son, Okinawajima, Okinawa Prefecture	Japan	26.67828°N, 127.99577°E	24	MF078618
2531	***Ryuthela***	***unten* sp. nov.**	Unten Port, Nakijin-son, Okinawajima, Okinawa Prefecture	Japan	26.67828°N, 127.99577°E	24	MF078619
2532	***Ryuthela***	***unten* sp. nov.**	Mt. Otowa dake, Nakijin-son, Okinawajima, Okinawa Prefecture	Japan	26.66759°N, 127.9694°E	81	MF078614
2533	***Ryuthela***	***unten* sp. nov.**	Mt. Otowa dake, Nakijin-son, Okinawajima, Okinawa Prefecture	Japan	26.66759°N, 127.9694°E	81	MF078615
2536	***Ryuthela***	***unten* sp. nov.**	Mt. Otowa dake, Nakijin-son, Okinawajima, Okinawa Prefecture	Japan	26.66757°N, 127.96935°E	80	KP229898
4100	***Ryuthela***	***unten* sp. nov.**	Mt. Otowa dake, Nakijin-son, Okinawajima, Okinawa Prefecture	Japan	26.66854°N, 127.96889°E	98	MF078616
3251	***Ryuthela***	***yarabu* sp. nov.**	Mt. Yarabu dake, Ishigakijima, Okinawa Prefecture	Japan	24.44173°N, 124.10018°E	111	KT767568
3171	*Songthela*	*hangzhouensis*	Hangzhou City, Zhejiang Province	China	30.22069°N, 120.11679°E	114	KT767579
3008	*Vinathela*	*cucphuongensis*	Cuc Phuong National Park, Nho Quan, Ninh Binh Province	Vietnam	20.26831°N, 105.69324°E	182	KT767580
3530	*Ganthela*	*jianensis*	Qingyuanshan, Ji'an City, Jiangxi Province	China	27.05865°N, 115.04759°E	104	KP875486
3361	*Haptathela*	*higoensis*	Hitoyoshi Ruins Park, Fumotomachi, Hitoyoshi-shi, Kumamoto-ken, Kyushu	Japan	32.21032°N, 130.76677°E	140	KT767524

GMYC uses likelihood to test for species boundaries by detecting the transition point of interspecific vs. intraspecific rates of lineage coalescence. We performed GMYC analyses in the 'splits' package for R (Ezard, Fujisawa & Barraclough, 2009). We used the single (GMYCs) and multiple (GMYCm) threshold models (Pons *et al.*, 2006; Monaghan *et al.*, 2009). We used BEAST v. 1.8.0 (Drummond *et al.*, 2012) to obtain an ultrametric gene tree that GMYC requires as a guide tree, in combination with a strict molecular clock (Zuckerkandl & Pauling, 1962) and Yule process model (Yule, 1924; Gernhard, 2008). We used standard arthropod substitution rates (Brower, 1994) by setting the *COI* substitution rate parameter a normal prior with a mean 0.0115, and ran 50 million generations, sampling every 5000 generations. We used TRACER v 1.6 (Rambaut *et al.*, 2014) to assess the chain convergence and correct mixing of each MCMC chain and discarded as 'burnin' 10% of the trees in each chain, as in a prior study (Xu *et al.*, 2015a), to settle on an ultrametric tree using TreeAnnotator (Drummond *et al.*, 2012).

Taxonomy

All specimens were examined with an Olympus SZX16 stereomicroscope; anatomic details were studied with a Leica M205C stereomicroscope. Male palps and female genitalia were examined and photographed with Leica M205C stereomicroscope and Olympus BX51 compound microscope after being dissected from the spider bodies. Genitalia were cleaned in boiling 10% KOH for a few minutes to dissolve soft tissues. Unless otherwise noted, left palps were depicted. All measurements are in millimetres. Leg and palp measurements are given in the following order: total length (femur + patella + tibia + metatarsus + tarsus).

Abbreviations used are as follows: ALE = anterior lateral eyes, AME = anterior median eyes, BL = body length, CL = carapace length, CT = contrategulum, CW = carapace width, E = embolus, OL = opisthosoma length, OW = opisthosoma width, PC = paracymbium, PLE = posterior lateral eyes, PME = posterior median eyes, RC = receptacular cluster, T = tegulum.

Species were diagnosed based on morphology, and in the cases where this was impossible, additional diagnoses derived from species-specific nucleotide substitutions in the standard DNA barcode alignment (Xu *et al.*, 2015a; Agnarsson *et al.*, 2015, 2016).

RESULTS AND DISCUSSION

Our original *COI* matrix of 644 bp for 173 *Ryuthela* individuals had 225 variable and 222 parsimony informative sites. The Bayesian topology strongly supported *Ryuthela* monophyly (posterior probability, hereafter PP = 1), with a well-supported species-clade *Ryuthela iheyana* sister to all remaining putative *Ryuthela* species (Fig. 3). The latter clade suggested monophyly of taxa on individual islands: Okinawajima + (Kumejima/Tokashikijima + Ishigakijima/Iriomotejima) (Fig. 3). Although this clade was poorly supported, the nodes of each hypothesized species were highly supported (PP = 1) except *Ryuthela ishigakiensis* (PP = 0.94) (Fig. 3). The phylogenetic topology of *Ryuthela* based on *COI* is congruent with a prior, multi-gene analysis that lacked *R. iheyana* (Xu *et al.*, 2016).

When assigning *Ryuthela* specimens to 15 hypothetical species, a distinct gap between intraspecific and interspecific genetic distances was found ranging from 5.62 to 6.68% for K2P and from 5.34 to 6.34% for *p*-distance (Fig. 4). The lowest mean interspecific distance was 7/6.7% (K2P/uncorrected *p*-distance) found between *Ryuthela nishihirai s.s.* and *Ryuthela kisenbaru* sp. nov., and the highest mean intraspecific distance (3.15/3.03% K2P/uncorrected *p*-distance) was estimated for *Ryuthela sasakii s.s.* (Table 2). Compared with the barcode gap of 4–12% (K2P)/4–11% (uncorrected *p*-distance) for the liphistiid genus *Ganthela* (Xu *et al.*, 2015a), the gap in *Ryuthela* is narrower, but similar to that in mygalomorphs, estimated at 5–6% (Hamilton *et al.*, 2011, 2014). The presence of a 'barcode gap' or different barcode gaps in different liphistiid lineages could reflect incomplete sampling across their distribution ranges (Moritz & Cicero, 2004). A wider barcode gap in *Ganthela* (Xu *et al.*, 2015a) might reflect incomplete sampling in that genus since only six species with large geographical distances were included in that analysis. However, our sampling scheme indicates that this is likely not the case in *Ryuthela*, with the distinct clustering of populations and species truly representing a lack of past gene flow, with evolutionary events separating these independently evolved lineages (Xu *et al.*, 2016).

ABGD results using different parameter combinations and the initial 15 species partition agreed on those 15 species (Fig. 3; Table 3), while those under a recursive partition regime yielded more species (Table 3). The settings P_{min}/P_{max} = 0.0001/0.2 yielded the most significant *P*-values. Although ABGD usually generates diverse outcomes (Jörger *et al.*, 2012; Puillandre *et al.*, 2012; Kekkonen & Hebert, 2014), our current ABGD analyses under different assumptions consistently revealed the 15 hypothetical species, thereby lending further support to the utility of the barcoding gap in delimiting species (Weigand *et al.*, 2013; Hamilton *et al.*, 2014; Čandek & Kuntner, 2015; Xu *et al.*, 2015a).

The results based on the Bayesian tree revealed high P ID(Liberal) values ≥ 0.95 (0.90–1.0) (Table 2), thus also recognizing the 15 species (Fig. 3). P ID(Liberal) is used to test species hypotheses on phylogenetic trees (Masters *et al.*, 2011; Hamilton *et al.*, 2014; Xu *et al.*, 2015a). In this study, our results support our previous

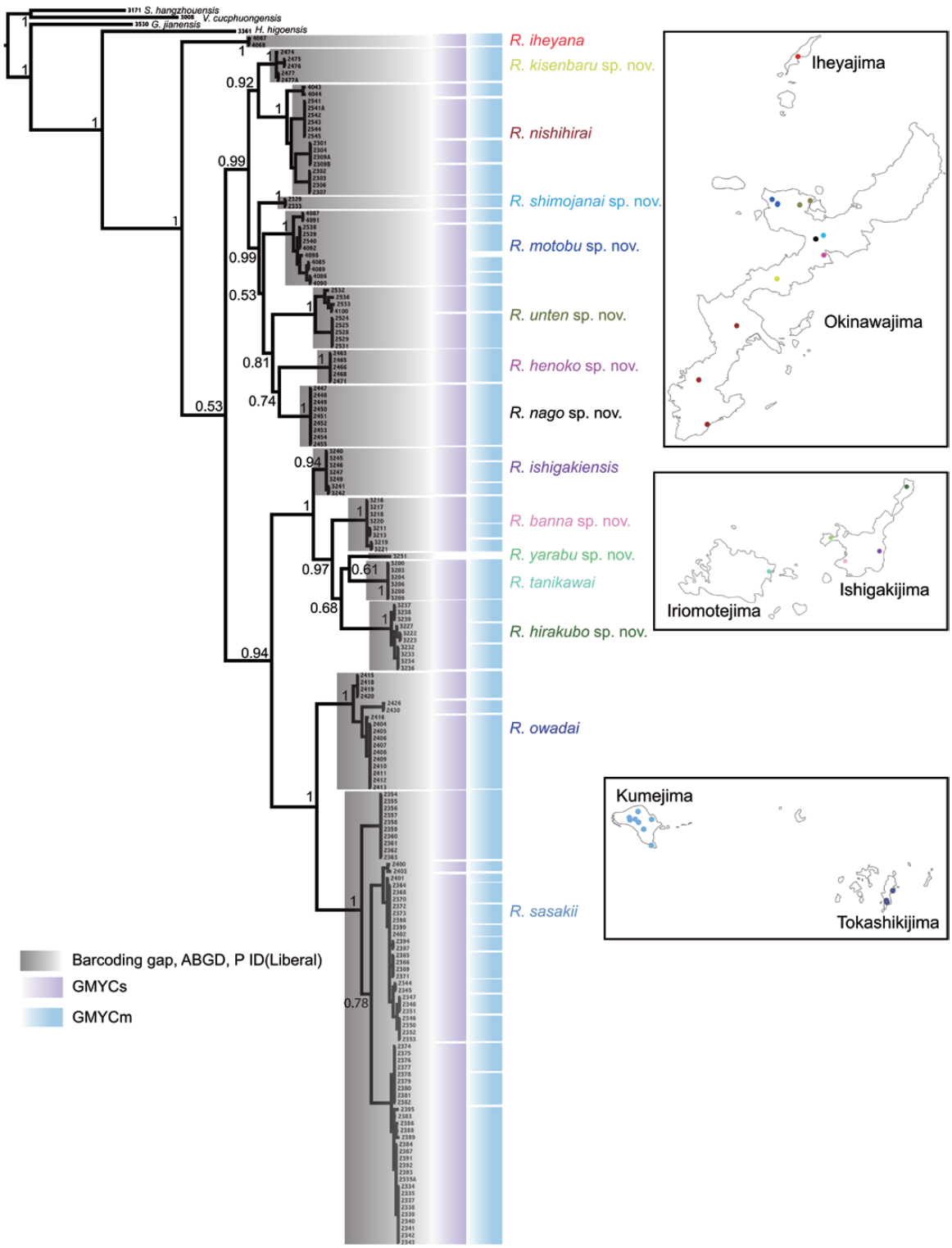

Figure 3. Bayesian *COI* gene tree for 173 terminals of *Ryuthela*, with the results of five species delimitation approaches in addition to morphology. Numbers at nodes represent posterior probabilities. Species names reflect the consensus results, and species colours correspond to those in maps.

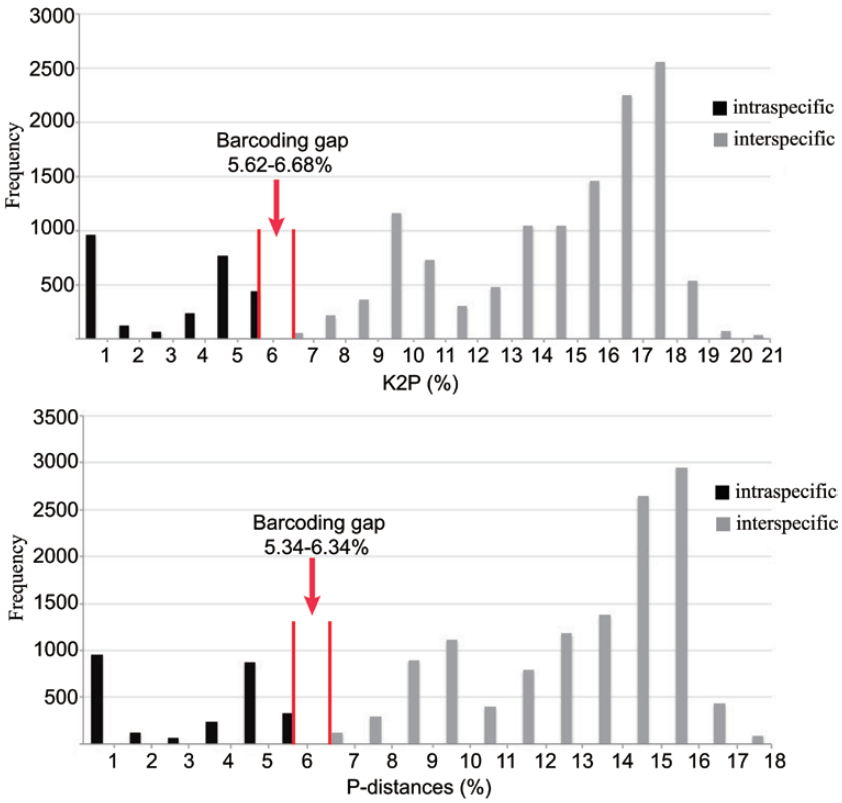

Figure 4. DNA barcoding gap in *Ryuthela*. Histograms show the distribution of intraspecific (black) and interspecific (grey) *COI* sequence variation based on K2P (A) and uncorrected *p*-distance (B).

inference: the cutoff of 95% can be used to delimit liphistiid species (Xu *et al.*, 2015a). GMYC based on both single and multiple threshold models resulted in 24/42 clusters and 25/47 entities for single/multiple threshold models (Fig. 3: GMYCs/GMYCm; Table 4). Both GMYCs and GMYCm overestimate *Ryuthela* species as has also been reported in *Ganthela* and in other taxa (Esselstyn *et al.*, 2012; Paz & Crawford, 2012; Miralles & Vences, 2013; Talavera, Dinca & Vila, 2013; Hamilton *et al.*, 2014; Xu *et al.*, 2015a).

Thus, the results from DNA barcoding gap, ABGD and P ID(Liberal) analyses all consistently support the validity of the 15 putative species (Fig. 3). These analyses agree with the morphological understanding of *Ryuthela* species diversity, and thus elegantly complement traditional taxonomy, particularly for those taxa that are only known from one sex. Our findings indicate that *Ryuthela* species diversity has been grossly underestimated, as it consists of not only six previously described species but also nine new species, thus increasing species richness by 150% in this genus. We formally describe them in the Taxonomy section.

On Okinawajima island, our analyses revealed that what was previously considered one species (*R. nishihirai s.l.*) is actually seven species, a 600% increase in the number of species (Fig. 3). Our analyses also revealed that one previously recognized species (*R. ishigakiensis s.l.*) on Ishigakijima island actually represents four species, a 300% increase in species richness (Fig. 3). Moreover, this may be an underestimate of this lineage as Tanikawa (2013b) found additional clades from other islands and other parts of Okinawajima island where we were unable to sample. Furthermore, this clade is relatively well-studied taxonomically compared with other liphistiid lineages; thus, species diversity may be even higher in the other Ryukyu archipelago liphistiid genus, *Heptathela* Kishida, 1923, and in other liphistiid genera in other regions. Intensive and integrative systematic analyses of additional clades representing all liphistiid genera are thus necessary to estimate total liphistiid species diversity, which may be unexpectedly much higher than previously thought.

We do, however, acknowledge a potential shortcoming of our study that only used mitochondrial and no nuclear loci. The peril of our approach is that in non-vagile organisms, where males and not females move around and thus contribute to gene flow, the maternally inherited mtDNA may not accurately delimit species boundaries and relationships. When only considering mtDNA, the restricted gene flow might lead to artificial deep population genetic structure even in the absence of clear geographical barriers (Hedin, 2015). Thus, using mtDNA-based species,

Table 2. Summary of the mean intraspecific and closest interspecific genetic distance, the mean probability with 95% confidence interval and the intra/inter ratio for the 15 putative species

Putative species	Intraspecific K2P/*p*-distances	Closest interspecific K2P/*p*-distances	Closest P ID(Liberal) species	P ID(Liberal)	Intra/inter
Ryuthela iheyana	0.0017/0.0017	0.1298/0.1177	***Ryuthela kisenbaru* sp. nov.**	0.97 (0.82, 1.0)	0.02
***Ryuthela shimojanai* sp. nov.**	0/0	0.0776/0.0731	***Ryuthela kisenbaru* sp. nov.**	0.97 (0.82, 1.0)	0.02
***Ryuthela motobu* sp. nov.**	0.0115/0.0114	0.0774/0.0718	***Ryuthela shimojanai* sp. nov.**	0.96 (0.90, 1.0)	0.2
***Ryuthela unten* sp. nov.**	0.0228/0.0222	0.1023/0.0940	***Ryuthela nago* sp. nov.**	0.95 (0.90, 1.0)	0.22
***Ryuthela nago* sp. nov.**	0/0	0.0840/0.0785	***Ryuthela shimojanai* sp. nov.**	1.00 (0.95, 1.0)	0.02
***Ryuthela henoko* sp. nov.**	0/0	0.0917/0.0851	***Ryuthela nago* sp. nov.**	0.98 (0.87, 1.0)	0.02
***Ryuthela kisenbaru* sp. nov.**	0.0078/0.0077	0.0776/0.0731	***Ryuthela shimojanai* sp. nov.**	0.97 (0.87, 1.0)	0.12
Ryuthela nishihirai	0.0264/0.0257	0.0701/0.0665	***Ryuthela kisenbaru* sp. nov.**	0.96 (0.94, 0.99)	0.38
Ryuthela ishigakiensis	0.0008/0.0008	0.0726/0.0685	***Ryuthela banna* sp. nov.**	0.99 (0.93, 1.0)	0.04
***Ryuthela banna* sp. nov.**	0.0029/0.0029	0.0726/0.0685	*Ryuthela ishigakiensis*	0.98 (0.92, 1.0)	0.07
***Ryuthela yarabu* sp. nov.**	NA/NA	0.0799/0.0751	*Ryuthela tanikawai*	0.96 (0.83, 1.0)	0.00E+00
Ryuthela tanikawai	0/0	0.0799/0.0751	***Ryuthela yarabu* sp. nov.**	0.98 (0.87, 1.0)	0.02
***Ryuthela hirakubo* sp. nov.**	0.0053/0.0053	0.0914/0.0845	*Ryuthela ishigakiensis*	0.98 (0.93, 1.0)	0.09
Ryuthela sasakii	0.0315/0.0303	0.1003/0.0926	*Ryuthela owadai*	0.97 (0.94, 1.00)	0.27
Ryuthela owadai	0.0145/0.0142	0.1003/0.0926	*Ryuthela sasakii*	0.98 (0.96, 1.0)	0.12

NA, not applicable.

Table 3. Results of the ABGD analyses

Substitution model	P_{min}/P_{max}	X	Partition	Prior intraspecific divergence (P)							
				0.001	0.0017	0.0028	0.0046	0.0077	0.0129	0.0215	0.0359
JC	0.001/0.1	1.5	Initial	15	15	15	15	15	15	15	15
			Recursive	50	45	45	36	25	23	21	17
K2P	0.001/0.1	1.5	Initial	15	15	15	15	15	15	15	15
			Recursive	51	48	48	34	25	23	21	17
Simple	0.001/0.1	1.5	Initial	15	15	15	15	15	15	15	15
			Recursive	26	26	26	26	23	21	18	15
				0.0001	0.0002	0.0005	0.0013	0.0029	0.0068	0.0159	0.0369
JC	0.0001/0.2	1.5	Initial	15	15	15	15	15	15	15	15
			Recursive	50	50	50	50	45	25	23	17
K2P	0.0001/0.2	1.5	Initial	15	15	15	15	15	15	15	15
			Recursive	51	51	51	51	48	25	23	17
Simple	0.0001/0.2	1.5	Initial	15	15	15	15	15	15	15	15
			Recursive	26	26	26	26	26	23	20	15

Table 4. Results of the GMYC analyses

Analysis	Clusters (CI)	Entities (CI)	Likelihood (null)	Likelihood (GMYC)	Likelihood ratio	Threshold
Single	24 (19–27)	25 (20–30)	1383.753	1399.464	31.42142***	–0.01541945
Multiple	42 (32–42)	47 (36–56)	1383.753	1404.423	41.33915***	–0.01445926

CI, confidence interval.
***$P < 0.001$.

delimitation in isolation may lead to overestimation of the number of species (Rubinoff & Holland, 2005; Song *et al.*, 2008; Hedin, 2015). While species delimitation based on multi-locus data may reduce the risk of overestimating species diversity based on DNA barcodes alone (Edwards & Knowles, 2014; Opatova & Arnedo, 2014; Hedin, Carlson & Coyle, 2015), our additional examination of morphological variation, in our view, minimizes this risk in our study.

TAXONOMY

TAXONOMIC HISTORY

Haupt (1983) described the genus *Ryuthela* based on the type species *R. nishihirai* (Haupt, 1979) and recognized two subspecies, *R. nishihirai nishihirai* (Haupt, 1979) and *R. nishihirai ishigakiensis* Haupt, 1983. Ono (1997) elevated *R. ishigakiensis* Haupt, 1983 to species, described four additional *Ryuthela* species (*Ryuthela owadai* Ono, 1997 from Tokashikijima island; *R. sasakii* Ono, 1997 and *Ryuthela secundaria* Ono, 1997 from Okinawajima island; and *Ryuthela tanikawai* Ono, 1997 from Iriomotejima island) and subsequently added *R. iheyana* Ono, 2002 from Iheyajima island (Ono, 2002). However, based on molecular phylogeny and morphological data, Tanikawa (2013a) considered both *R. secundaria* and *R. owadai* as synonyms of *R. sasakii* and *R. tanikawai* as a synonym of *R. ishigakiensis*. Basing the taxonomy entirely on male genitalic characters, Tanikawa lumped all cryptic lineages from Okinawajima island under *R. nishihirai s.l.*, from both Kumejima and Tokashikijima islands under *R. sasakii s.l.*, from both Ishigakijima and Iriomotejima islands under *R. ishigakiensis s.l.*, in addition to *R. iheyana*, recognizing only four species of *Ryuthela* (Tanikawa, 2013a). Recently, Dunlop, Steffensen & Ono (2014) revalidated *R. tanikawai*.

TAXONOMIC REVISION

GENUS *RYUTHELA* HAUPT, 1983

Diagnosis: Ryuthela males can be distinguished from all other Heptathelinae genera by lacking the conductor and by the contrategulum with an elongated spine (i.e. Fig. 8A, C, E). The females differ from *Heptathela*, *Qiongthela*, *Sinothela*, *Songthela* and *Vinathela* by one pair of receptacular clusters close to each other (i.e. Fig. 5A–V), located at the anterior margin of the bursa copulatrix, and from *Ganthela* by receptacular clusters without stems (Xu *et al.*, 2015b). *Ryuthela* body length varies from 6.5 to 14.5 mm, and *Ryuthela* species can have seven or eight spinnerets.

Distribution: Ryuthela species are restricted to Ryukyu archipelago and are found from central Ryukyus (Okinawa group) to southern Ryukyus. *Ryuthela* species on central Ryukyus share habitats with *Heptathela* species (Xu *et al.*, 2016).

Composition: After this revision, *Ryuthela* contains 15 species.

Remarks: Seven of the 15 delimited species with male and female specimens can be diagnosed from each other morphologically. For the other eight species without males, especially for those that are distributed on the same islands, the species-specific nucleotide changes in the standard DNA barcode alignment were used to augment insufficient morphological diagnoses.

RYUTHELA NISHIHIRAI (HAUPT, 1979)

Heptathela nishihirai Haupt, 1979, p. 356, figs 6a–d, 7a–b, 8a–b, 9a–c, 10, 12a–c (description of male and female); Yaginuma, 1979, p. 1, fig. 2; Yaginuma, 1980, p. 44, fig. 2; Yaginuma, 1986, p. 2, figs 1 and 2; Chikuni, 1989, p. 18, fig. 2; Yoo & Kim, 2002, p. 28, fig. 47. *Ryuthela nishihirai* Haupt, 1983, p. 286, fig. 9g–h, 10a–b, 11b, 12b, 12e, 13g (transferred from *Heptathela*); Haupt, 1984, p. 164, figs 3 and 5; Kraus, 1984, p. 375, fig. 2; Haupt, 2003, p. 71, figs 43H, 48A–B, 52A–B, 53.1-24, 55.7–9, 62A; Ono, 2009, p. 80, figs 26–32; Tanikawa, 2013a, p. 33, figs 2A–C, 3A–C, 4A–C.

Type material: Syntypes deposited at NSMT, one female, one male (NSMT-Ar 422–423) from Shuri, Okinawajima island, Okinawa Prefecture, 15 March 1976 (male became adult in October 1977), by M. Nishihira and J. Haupt; examined.

Other material examined: Eight females [XUX-2012-(301–304, 306/307/309A/309B)], Sheyoshi Park, Shuri, Naha, Okinawajima island, Japan, 26.23°N, 127.72°E, 35 m a.s.l., 17 December 2012; 5 females [XUX-2012-(541/541A, 542–544)], Asato, Yaese-cho, Okinawajima island, Japan, 26.12°N, 127.74°E, 28 m a.s.l., 17 December 2012, by D. Li, F. X. Liu and X. Xu; 9 females and 1 juvenile [XUX-2014-(013-022)], Sheyoshi Park, Shuri, Naha, Okinawajima island, Japan, 26.23°N, 127.72°E, 52 m a.s.l., 4 May 2014; 3 females (XUX-2014–041/043/044) from Chibana, Gusuku, Okinawajima island, Japan, 26.36°N, 127.81°E, 67 m a.s.l., 7 May 2014, by D. Li and B. Wu.

Diagnosis: Male *R. nishihirai s.s.* can be distinguished from all other *Ryuthela* species by the contrategulum with sparse denticles on proximal margin (Fig. 5W). Females of *R. nishihirai s.s.* differ from most other island *Ryuthela* species by the short receptacular clusters that are close to each other, and further differ from the other species on Okinawajima island by detailed arrangement of the receptacular clusters. These are either fused into one group with obvious granula or form flat receptacular clusters separated from each other (Fig. 5L, P). However, *R. nishihirai s.s.* can also be diagnosed from all other Okinawajima island *Ryuthela* species by the following unique nucleotide substitutions in the standard DNA barcode alignment: A (16), C (173), T (278), G (304), C (475), C (494), G (496), T (571).

Description: Male (lectotype). Body colour somewhat faded. Carapace light yellowish brown with darker

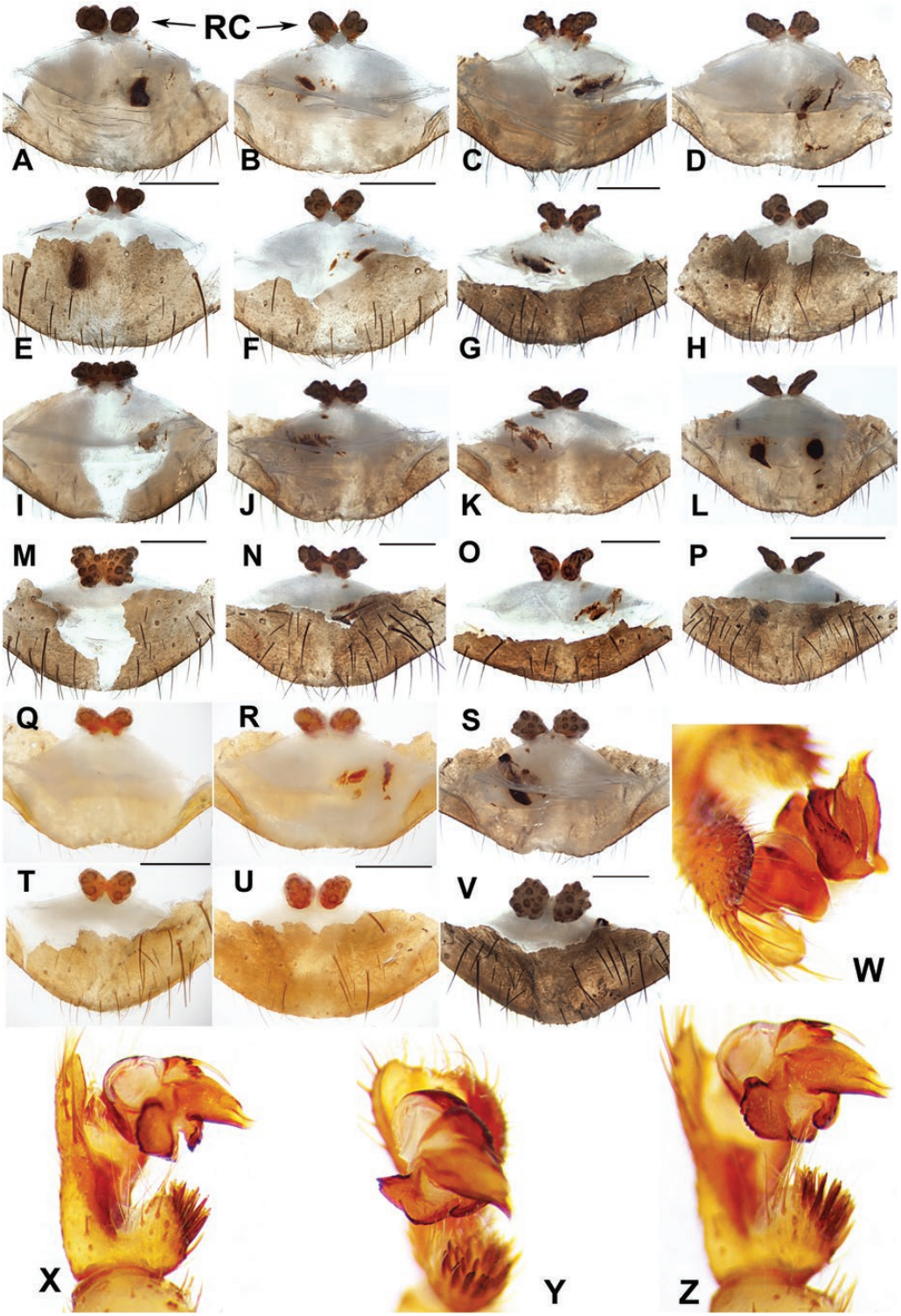

Figure 5. Genital anatomy of *Ryuthela nishihirai* (Haupt, 1979). A, E, 2302 (short for XUX-2012-302). B, F, 2303. C, G, 4014. D, H, 4013. I, M, 2541. J, N, 2542. K, O, 2544. L, P, 2306. Q, T, 4041. R, U, 4043. S, V,4044. W–Z, NSMT-Ar 423. A–D, I–L, Q–S, vulvae dorsal view. E–H, M–P, T–V, vulvae ventral view. W–Z, palp distal view. 2302, 2303, 2306, 4013, 4014, NSMT-Ar 423, Sheyoshi Park, Shuri. 2541, 2542, 2544, Asato, Yaese-cho. 4041, 4043, 4044, Chibana, Gusuku. Scale bars, 0.5 mm.

frontal margin and black ocular area; opisthosoma yellow, with beige tergites; sternum narrow, much longer than wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove with ten denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites, the first tergite forming the superior lorum of the pedicel, the 12th tergite forming the dorsal wall of the anal tubercle, the third widest; seven spinnerets. Measurements: BL 9.05, CL 5.25, CW 4.20, OL 4.20, OW 3.15; ALE > PLE > PME > AME (left PME absent); leg I 17.10 (4.75 + 2.25 + 3.40 + 4.50 + 2.20), leg II 17.15 (4.50 + 2.25 + 3.20 + 4.95 + 2.25), leg III 18.80 (4.25 + 2.10 + 3.45 + 6.00 + 3.00), leg IV 23.90 (5.25 + 2.40 + 4.65 + 7.70 + 3.90).

Palp. Prolateral side of paracymbium unpigmented and unsclerotized; numerous setae and spines at the tip of paracymbium (Fig. 5X). Prolateral contrategulum with a short and curved spine and sparse denticles on proximal margin (Fig. 5W); the distal part of contrategulum blunt (Fig. 5Y, Z); tegulum with a large smooth marginal apophysis (Fig. 5Y, Z); embolus with a narrow basal part (Fig. 5X, Z) (cf. Ono, 2009, p. 79, figs 26–29).

Female. Carapace and opisthosoma brown; tergites slightly dark brown; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove with 13–15 strong denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites; 7 spinnerets. Measurements: BL 9.75–13.92, CL 4.85–6.30, CW 3.80–5.80, OL 5.82–7.28, OW 4.10–5.92; ALE > PLE > PME > AME; palp 10.11 (3.28 + 1.90 + 2.25 + 2.68), leg I 12.15 (3.72 + 2.12 + 2.25 + 2.58 + 1.48), leg II 11.76 (3.55 + 2.13 + 2.03 + 2.55 + 1.50), leg III 12.59 (3.53 + 2.18 + 2.05 + 3.05 + 1.78), leg IV 18.82 (5.21 + 2.43 + 3.25 + 5.18 + 2.75).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix; in the dorsal view, their basal parts close to each other (Fig. 5A–D, K–L, Q–S) or fused to form one large receptacular cluster with obvious granula (Fig. 5I–J); with a high intraspecific variation in the shape and number of the receptacular clusters.

Distribution: Okinawajima island (Asato, Chibana and Shuri), Okinawa Prefecture, Japan.

Remarks: Tanikawa (2013a) considered all species on Okinawajima island as *R. nishihirai s.l.*, but based on our exhaustive collection on Okinawajima island, results from an integrative analysis of the geographic distribution, phylogenetic and population genetic data, and distinct barcoding gaps in the genus *Ryuthela* (5.6–6.7% for K2P and 5.3–6.3% for *p*-distance), we propose seven *Ryuthela* species on Okinawajima island, including *R. nishihirai s.s.* Since the holotype locality of *R. nishihirai s.l.* is Shuri, Okinawajima island (Haupt, 1979), we treated the specimens collected from the southern part of Okinawajima island (from Chibana to the south Okinawajima island) as *R. nishihirai s.s.* We examined the syntype series of *R. nishihirai* deposited at NSMT. According to the remarks in Dunlop, Steffensen & Ono (2014, p. 39), we designated the male specimen deposited at NSMT from syntype series as the lectotype because it is important for describing and classifying all other new species on Okinawajima island. Female genitalia of *R. nishihirai s.s.* are highly variable. The fused receptacular clusters may be caused by a physical moment at forming their female genital instead of variation; however, we considered them as variation in this study.

RYUTHELA HENOKO SP. NOV.

urn:lsid:zoobank.org:act:87781F27-394C-4452-8F3D-BF5C1B93528D

Holotype: Female (XUX-2012-468), collected near Henoko Dam, Nago-shi, Okinawajima island, Japan, 26.54°N, 128.03°E, 50 m a.s.l., 25 December 2012, by D. Li, F. X. Liu and X. Xu.

Paratypes: Female (XUX-2012-471), collected at the same locality, 25 December 2012, by D. Li, F. X. Liu and X. Xu.

Etymology: The species epithet, a noun in apposition, refers to the type locality.

Diagnosis: Females of *Ryuthela henoko* sp. nov. differ from all other *Ryuthela* species except the species on Okinawajima island by the receptacular clusters close to each other at the basal part and separated from each other at the terminal part (Fig. 6A–D); the shape of receptacular clusters of this species is similar to *Ryuthela motobu* sp. nov., but can be distinguished from that species by the receptacular clusters with bases close to each other (Fig. 6A–D) and from *R. nishihirai s.s.* by the shape of receptacular clusters (Fig. 6A–D). However, *R. henoko* sp. nov. can also be diagnosed from all other *Ryuthela* species on Okinawajima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: G (64), G (70), G (157), T (211), C (226), G (247), A (257), T (322), T (328), T (445), T (484), T (547), C (607), C (613).

Description: Female (holotype). Carapace brown; opisthosoma light brown, with dark brown tergites; sternum narrow, much longer than wide; a few long pointed hairs running over ocular mound in a

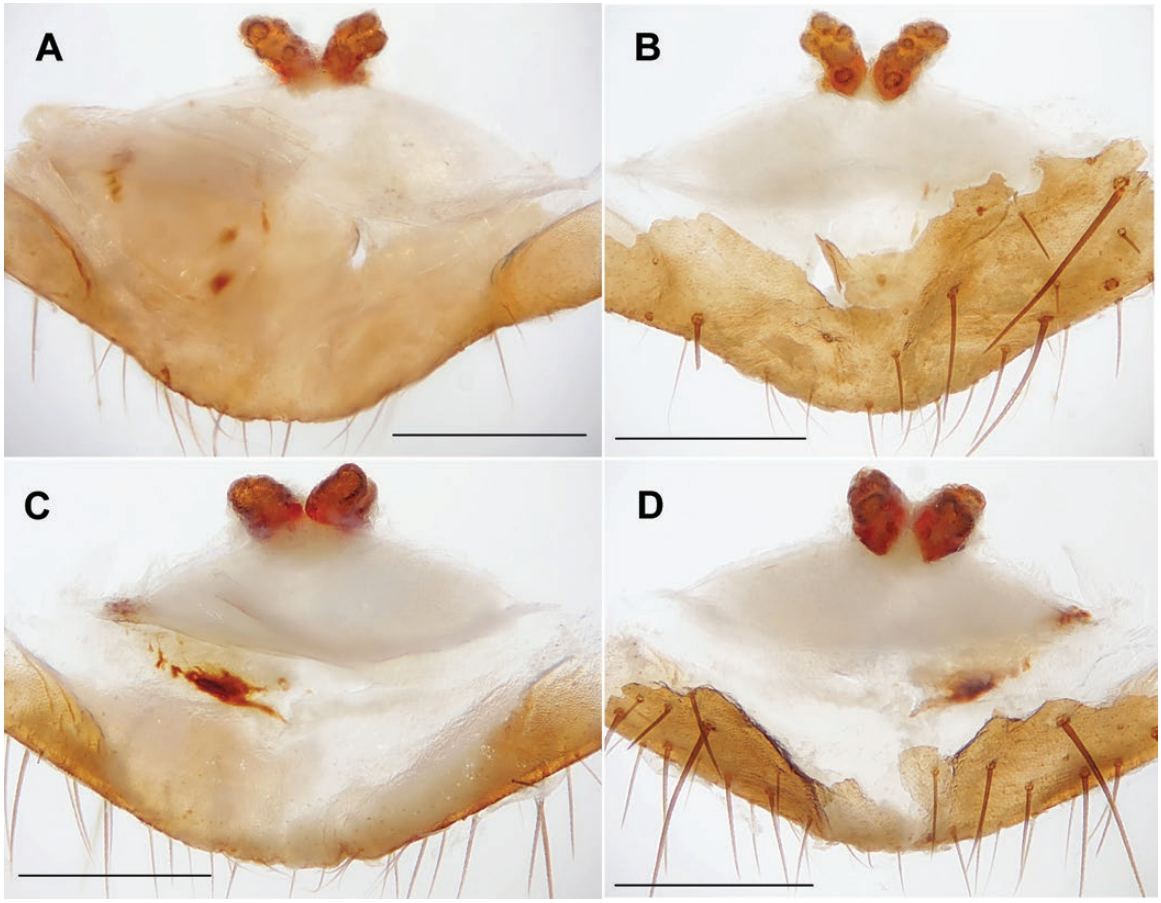

Figure 6. Genital anatomy of ***Ryuthela henoko* sp. nov**. A, B, holotype (XUX-2012-468). C, D, paratype (XUX-2012-471). A, C, vulvae dorsal view. B, D, vulvae ventral view. Scale bars, 0.5 mm.

longitudinal row; chelicerae robust with promargin of cheliceral groove with 13 denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites, the fifth largest; 7 spinnerets. Measurements: BL 11.85, CL 5.38, CW 4.50, OL 6.68, OW 4.82; ALE > PLE > PME > AME; palp 9.40 (3.06 + 1.68 + 2.10 + 2.56), leg I 10.74 (3.25 + 1.82 + 2.15 + 2.30 + 1.22), leg II 10.83 (3.15 + 1.90 + 2.03 + 2.45 + 1.30), leg III 10.77 (2.82 + 1.80 + 1.85 + 2.70 + 1.60), leg IV 15.39 (4.22 + 1.60 + 3.00 + 4.36 + 2.21).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix with their basal parts close to each other; the two receptacular clusters similar in length and their length about two times their width (Fig. 6A, B) or less than two times (Fig. 6C, D).

Male. Unknown.

Distribution: Okinawajima island (Henoko Dam, Nago-shi), Okinawa Prefecture, Japan.

RYUTHELA KISENBARU SP. NOV.

urn:lsid:zoobank.org:act:9367AD6C-D34A-41BE-A9DA-94985D36BF73

Holotype: Female (XUX-2012-474) collected southeast of County Road 104 and 58 junction, Kisenbaru, area between Onna-son and Kin-cho, Okinawajima island, Japan, 26.48°N, 127.91°E, 30 m a.s.l., 25 December 2012, by D. Li, F. X. Liu and X. Xu.

Paratypes: Two females (XUX-2012-476/477), collected at the same locality, 25 December 2012, by D. Li, F. X. Liu and X. Xu.

Etymology: The species epithet, a noun in apposition, refers to the type locality.

Diagnosis: Females of *R. kisenbaru* sp. nov. differ from all other *Ryuthela* species except *R. nishihirai s.s.* by the short and slightly cylindrical receptacular clusters, and can be distinguished from *R. nishihirai s.s.* by the

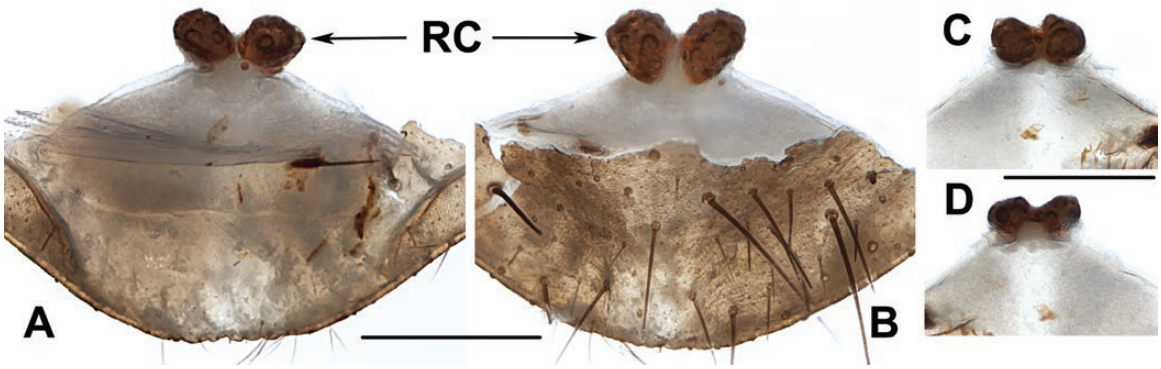

Figure 7. Genital anatomy of ***Ryuthela kisenbaru* sp. nov**. A, B, paratype (XUX-2012-476); C, D, holotype (XUX-2012-474). A, C, vulvae dorsal view; B, D, vulvae ventral view; scale bars: 0.5 mm.

receptacular clusters fused together with fewer granula (Fig. 7C, D). However, *R. kisenbaru* sp. nov. can also be diagnosed from *R. nishihirai s.s.* by the following unique nucleotide substitutions in the standard DNA barcode alignment: T (16), A (19), T (115), A (157), A (160), T (173), C (239), C (262), A (278), A (304), A (310), T (352), G (358), T (382), G (406), G (454), A (475), T (494), A (496), C (554), A (559), C (571), T (574), C (592), T (601).

Description: Female (holotype). Carapace and opisthosoma light brown; tergites slightly dark brown; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove with 12–14 strong denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites; 7 spinnerets. Measurements: BL 8.08–9.90, CL 3.65–4.85, CW 3.38–3.92, OL 3.88–5.50, OW 3.00–3.90; ALE > PLE > PME > AME; palp 8.53 (2.90 + 1.48 + 1.85 + 2.30), leg I 10.08 (3.20 + 1.60 + 2.00 + 2.10 + 1.18), leg II 9.52 (2.85 + 1.62 + 1.60 + 2.15 + 1.30), leg III 10.16 (2.80 + 1.71 + 1.75 + 2.42 + 1.48), leg IV 14.53 (4.05 + 2.00 + 2.15 + 3.95 + 2.38).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix; the basal parts close to each other or fused together; without genital stalks (Fig. 7A–D).

Male. Unknown.

Distribution: Okinawajima island (Kisenbaru), Okinawa Prefecture, Japan.

Ryuthela motobu sp. nov.

urn:lsid:zoobank.org:act:AF642340-2C19-4DE9-B38E-793C3221A261

Holotype: Male (XUX-2014-090, matured 6 September 2014 at CBEE, College of Life Sciences, Hubei University), collected at Jahana, Motobu-cho, Okinawajima island, Japan, 26.68°N, 127.90°E, 77 m a.s.l., 11 May 2014, by D. Li and B. Wu.

Paratypes: Five females and one juvenile [XUX-2014-(085-089/091)], collected at the same locality as the holotype, two females (XUX-2014-092/098), collected at Yamazato, Motobu-cho, Okinawajima island, Japan, 26.67°N, 127.91°E, 143 m a.s.l., 11 May 2014, by D. Li and B. Wu.

Etymology: The species epithet, a noun in apposition, refers to the type locality.

Diagnosis: Male *R. motobu* sp. nov. can be distinguished from *R. nishihirai s.s.* by the short and curved contrategular spine (Fig. 8A, C, E); from *R. iheyana* by the larger marginal apophysis of tegulum (Fig. 8C–E); from other *Ryuthela* species by the short and blunt distal contrategulum, and the narrow basal part of embolus (Fig. 8B, D, E). Females of *R. motobu* sp. nov. can be distinguished from *R. henoko* sp. nov. by the receptacular clusters with bases separated from each other and from the other Okinawajima island species by the receptacular clusters that are longer than their width (Fig. 8F–N). However, *R. motobu* sp. nov. can also be diagnosed from *R. nishihirai s.s.* by the following unique nucleotide substitutions in the standard DNA barcode alignment: G (1), T (16), C (26), A (58), C (76), T (109), C (115), G (119), A (157), A (160), T (173), G (181), C (239), G (241), T (247), C (256), A (278), A (304), C (313), C (332), T (337), T (343), T (352), G (358), A (445), T (475), C (484), T (494), A (496), T (533), G (535), C (571), T (577), C (592), T (601), C (604), G (607), G (619) and differs from all other *Ryuthela* species on Okinawajima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: A (58), C (76), G (181), T (337), T (533), G (535), G (619).

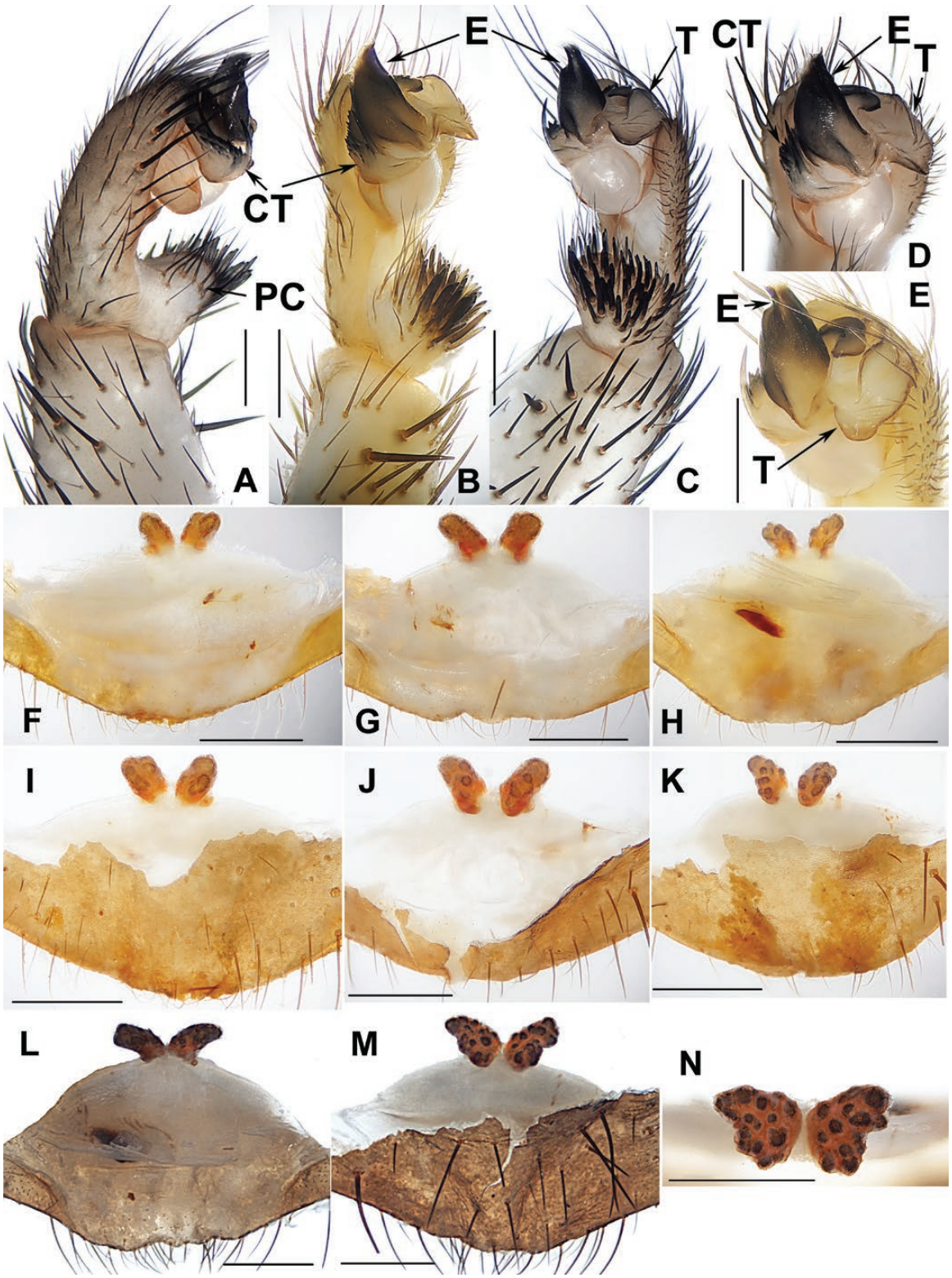

Figure 8. Male and female genital anatomy of ***Ryuthela motobu* sp. nov**. A–E, 4090 (short for XUX-2014-090); F, I, 4085; G, J, 4086; H, K, 4087; L–N, 4098. A, palp prolateral view; B, palp ventral view; C, palp retrolateral view; D, E, palp distal view; F–H, L, vulvae dorsal view; I–K, M, vulvae ventral view; N, vulvae distal view; 4085, 4086, 4087, 4090, Jahana, Motobu-cho; 4098, Yamazato, Motobu-cho; scale bars: 0.5 mm.

Description: Male (holotype). Carapace light brown; opisthosoma brown, with dark brown tergites; sternum narrow, much longer than wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove with 11 denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites, the fourth largest; 7 spinnerets. Measurements: BL 11.50, CL 6.15, CW 5.20, OL 6.60, OW 5.00; ALE > PLE > PME > AME; leg I 16.45 (4.10 + 1.25 + 3.60 + 4.90 + 2.60), leg II 19.80 (5.30 + 1.90 + 4.10 + 5.50 + 3.00), leg III 20.00 (5.20 + 1.60 + 3.90 + 5.60 + 3.70), leg IV 28.00 (6.70 + 2.20 + 5.50 + 8.80 + 4.80).

Palp. Prolateral side of paracymbium unpigmented and unsclerotized; numerous setae and spines at the tip of paracymbium (Fig. 8A). Prolateral contrategulum with a short and curved spine and numerous denticles on proximal margin (Fig. 8A, B); the distal part of contrategulum blunt (Fig. 8E); tegulum with a large smooth marginal apophysis (Fig. 8C, E); embolus with a narrow basal part (Fig. 8C–E).

Female. Carapace and opisthosoma brown; tergites slightly dark brown; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove with 11–15 strong denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites, the fourth largest; 7 spinnerets. Measurements: BL 10.90–14.80, CL 5.00–6.90, CW 4.00–5.80, OL 6.10–9.40, OW 5.00–7.30; ALE > PLE > PME > AME; palp 9.20 (2.90 + 1.40 + 2.20 + 2.70), leg I 11.20 (3.50 + 1.90 + 2.25 + 2.35 + 1.20), leg II 10.95 (3.40 + 1.80 + 2.00 + 2.30 + 1.45), leg III 10.55 (3.15 + 1.85 + 2.15 + 2.10 + 1.30), leg IV 17.55 (4.80 + 2.40 + 3.15 + 4.70 + 2.50).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix; their length longer than their width in dorsal view (Fig. 8F–H, L); without genital stalks (Fig. 8F–N).

Distribution: Okinawajima island (Jahana and Yamazato, Motobu-cho), Okinawa Prefecture, Japan.

Ryuthela nago sp. nov.

urn:lsid:zoobank.org:act:647F993A-A067-4FD5-BE2B-DC087324AD2D

Holotype: Female (XUX-2012-448), collected at Mt. Nago dake, Nago-shi, Okinawajima island, Japan, 26.58°N, 128.01°E, 210 m a.s.l., 24 December 2012, by D. Li, F. X. Liu and X. Xu.

Paratypes: Seven females and one juvenile [XUX-2012-(447/449–455)], collected at the same locality as holotype, 24 December 2012, by D. Li, F. X. Liu and X. Xu.

Etymology: The species epithet, a noun in apposition, refers to the type locality.

Diagnosis: Females of *Ryuthela nago* sp. nov. can be distinguished from other Okinawajima island species by the terminal part of the receptacular clusters, which slightly extends outward (Fig. 9A, C, G, I). *Ryuthela nago* sp. nov. can also be diagnosed from all other *Ryuthela* species on Okinawajima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: A (7), C (43), C (100), C (106), G (124), G (205), C (270), G (322), G (337), T (383), C (433), G (523).

Description: Female (holotype). Carapace and opisthosoma light brown; tergites slightly dark brown; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove with 12–14 strong denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites, the fifth largest; 7 spinnerets. Measurements: BL 8.80–12.00, CL 4.25–5.52, CW 3.70–4.55, OL 4.62–6.50, OW 3.50–4.70; ALE > PLE > PME > AME; palp 8.00 (2.70 + 1.38 + 1.80 + 2.12), leg I 9.52 (3.00 + 1.60 + 1.78 + 1.95 + 1.19), leg II 9.06 (2.75 + 1.50 + 1.65 + 1.98 + 1.18), leg III 9.44 (2.52 + 1.50 + 1.65 + 2.35 + 1.42), leg IV 14.22 (4.02 + 1.82 + 2.50 + 3.88 + 2.00).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix with their basal parts close to each other or separated; the terminal parts slightly extending outward; without genital stalks (Fig. 9A–I).

Male. Unknown.

Distribution: Okinawajima island (Mt. Nago dake, Nago-shi), Okinawa Prefecture, Japan.

Ryuthela shimojanai sp. nov.

urn:lsid:zoobank.org:act:BCFACE25-4C4E-471D-B26F-2EF11B805621

Holotype: Female (XUX-2012-323), collected at Taira near Haneji-Dam, Nago-shi, Okinawajima island, Japan, 26.59°N, 127.03°E, 100 m a.s.l., 18 December 2012, by D. Li, F. X. Liu and X. Xu.

Paratypes: One female (XUX-2012-333), collected at the same locality, 18 December 2012, by D. Li, F. X. Liu and X. Xu.

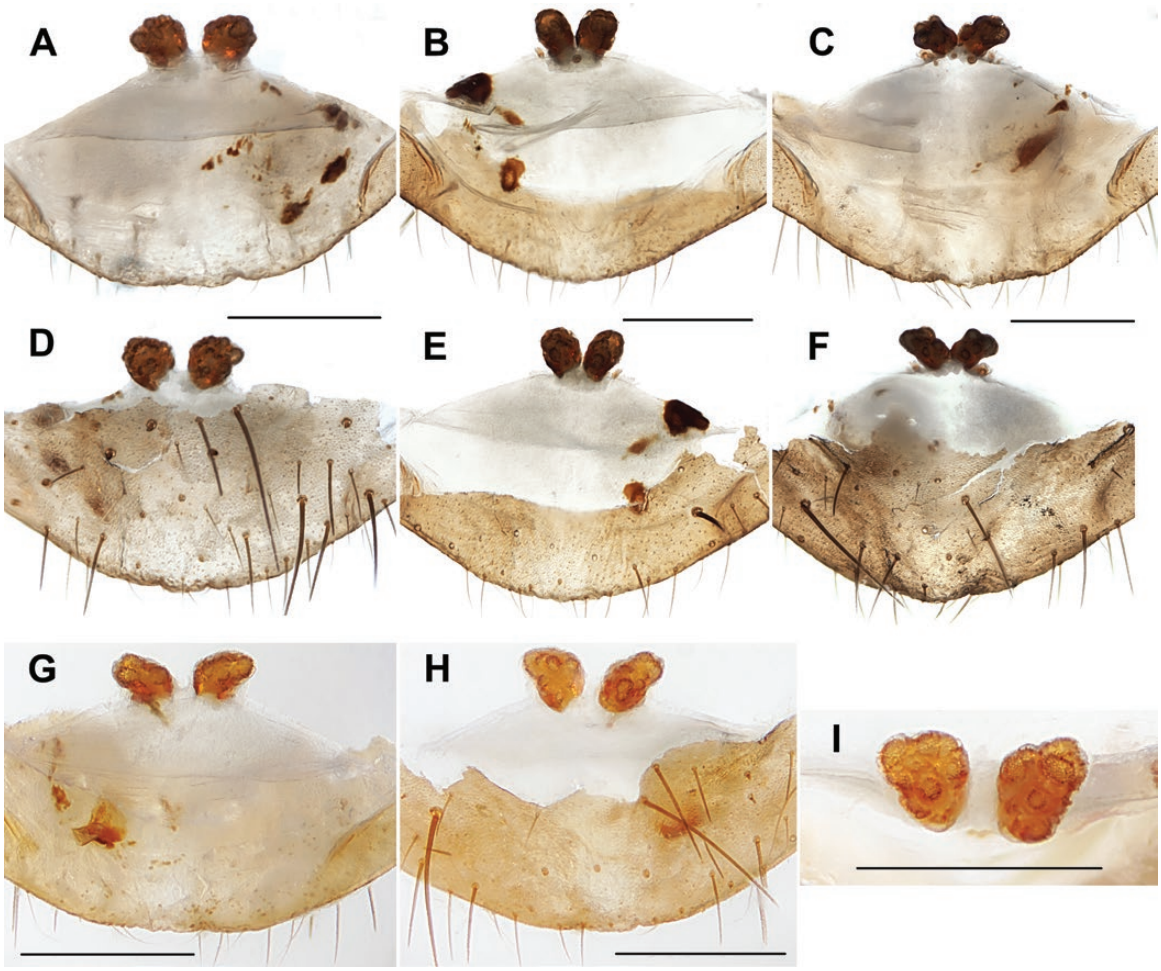

Figure 9. Genital anatomy of ***Ryuthela nago* sp. nov**. A, D, holotype (XUX-2012-448); G–I, paratype (XUX-2012-447); B, E, paratype (XUX-2014-450); C, F, paratype (XUX-2014-453). A–C, G, vulvae dorsal view; D–F, H, vulvae ventral view; I, vulvae distal view; scale bars: 0.5 mm.

Etymology: The species epithet honours Matsuei Shimojana who pioneered the study of liphistiids in Okinawa.

Diagnosis: Females of *Ryuthela shimojanai* sp. nov. can be distinguished from all other *Ryuthela* species on Okinawajima island by the relative length of the receptacular clusters (slightly longer than their width) and the lack of obvious granula in dorsal view (Fig. 10A, C). *Ryuthela shimojanai* sp. nov. can also be diagnosed from all other *Ryuthela* species on Okinawajima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: C (25), T (86), C (244), G (292), C (298), G (319), A (379), T (409), C (424), G (457), T (469).

Description: Female (holotype). Carapace brown; opisthosoma light brown, with dark brown tergites; sternum narrow, much longer than wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove with 13 denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites, the fifth largest; 7 spinnerets. Measurements: BL 12.00, CL 5.33, CW 4.56, OL 6.40, OW 4.61; ALE > PLE > PME > AME; palp 9.62 (3.25 + 1.75 + 2.10 + 2.52), leg I 10.80 (3.55 + 1.73 + 2.12 + 2.15 + 1.25), leg II 10.73 (3.32 + 1.88 + 1.95 + 2.28 + 1.39), leg III 10.29 (3.18 + 1.92 + 1.88 + 1.76 + 1.55), leg IV 16.62 (4.76 + 2.22 + 3.10 + 4.24 + 2.30).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix with their basal parts close to each other or separated; the length of the receptacular clusters slightly longer than their width, with unclear granula in dorsal view; without genital stalks (Fig. 10A–D).

Male. Unknown.

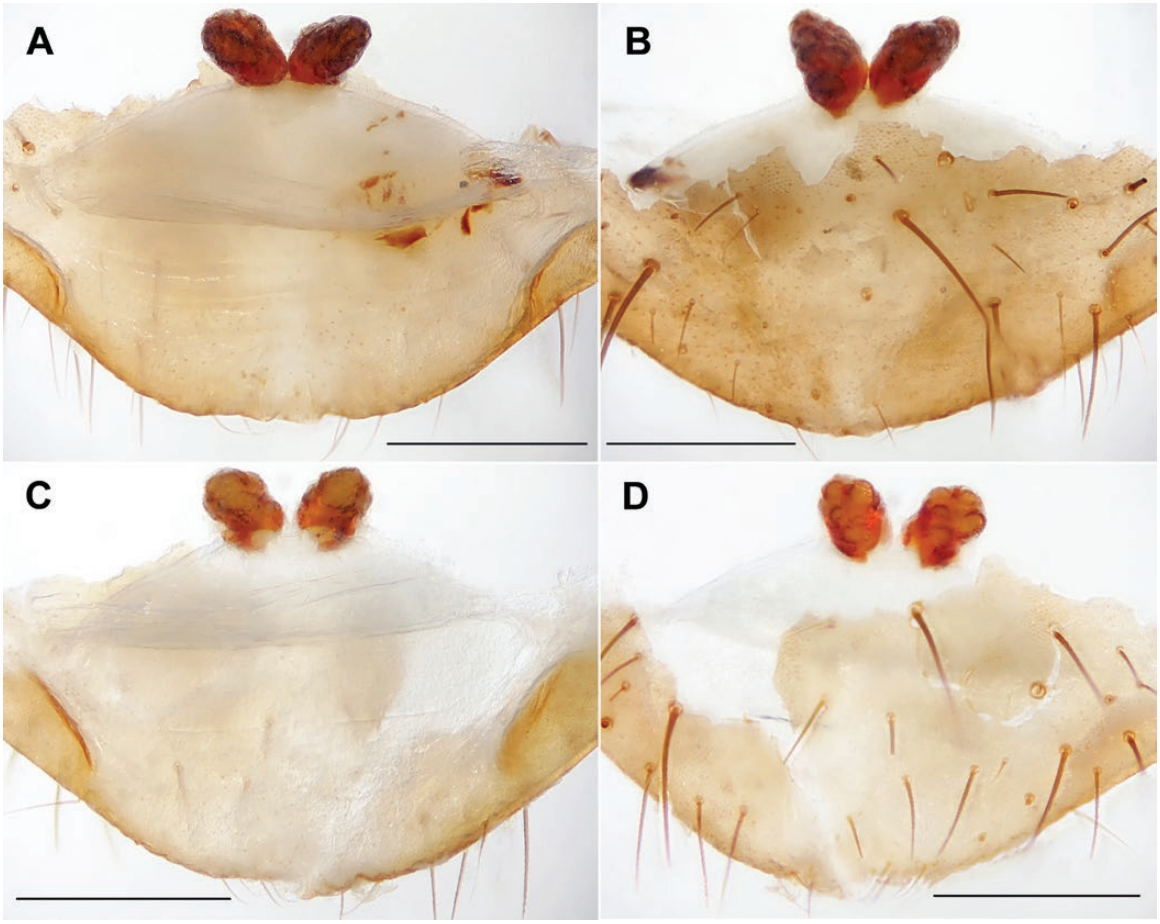

Figure 10. Genital anatomy of ***Ryuthela shimojanai* sp. nov**. A, B, holotype (XUX-2012-323); C, D, paratype (XUX-2012-333). A, C, vulvae dorsal view; B, D, vulvae ventral view; scale bars: 0.5 mm.

Distribution: Okinawajima island (Taira, Nago-shi), Okinawa Prefecture, Japan.

RYUTHELA UNTEN SP. NOV.

urn:lsid:zoobank.org:act:9E8B4944-AD55-4B78-9135-8ACD47F22308

Holotype: Female (XUX-2012-524), collected at Unten Port, Nakijin-son, Okinawajima island, Japan, 26.68°N, 128.00°E, 24 m a.s.l., 27 December 2012, by D. Li, F. X. Liu and X. Xu.

Paratypes: Four females [XUX-2012-(525/528/529/531)], collected at the same locality as holotype, 27 December 2012; two females [XUX-2012-(532/536)] from near Beach Rock Village, Mt. Otowa dake, Nakijin-son, Okinawajima island, Japan, 26.67°N, 127.97°E, 80 m a.s.l., 27 December 2012, by D. Li, F. X. Liu and X. Xu; two females (XUX-2014-084/100), collected from Mt. Otowa dake, Nakijin-son, Okinawajima island, Japan, 26.67°N, 127.97°E, 100 m a.s.l., 11 May 2014, by D. Li and B. Wu.

Etymology: The species epithet, a noun in apposition, refers to the type locality.

Diagnosis: Females of *Ryuthela unten* sp. nov. can be distinguished from other Okinawajima island *Ryuthela* species by the more or less triangular receptacular clusters from both ventral and distal views (Fig. 11A–H). *Ryuthela unten* sp. nov. can also be diagnosed from all other *Ryuthela* species on Okinawajima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: G (97), T (127), C (148), C (160), G (172), G (196), C (202), G (269), C (352), T (364), C (376), C (379), G (430), G (487), C (502), A (514), A (550).

Description: Female (holotype). Carapace and opisthosoma light brown; tergites slightly dark brown; a few

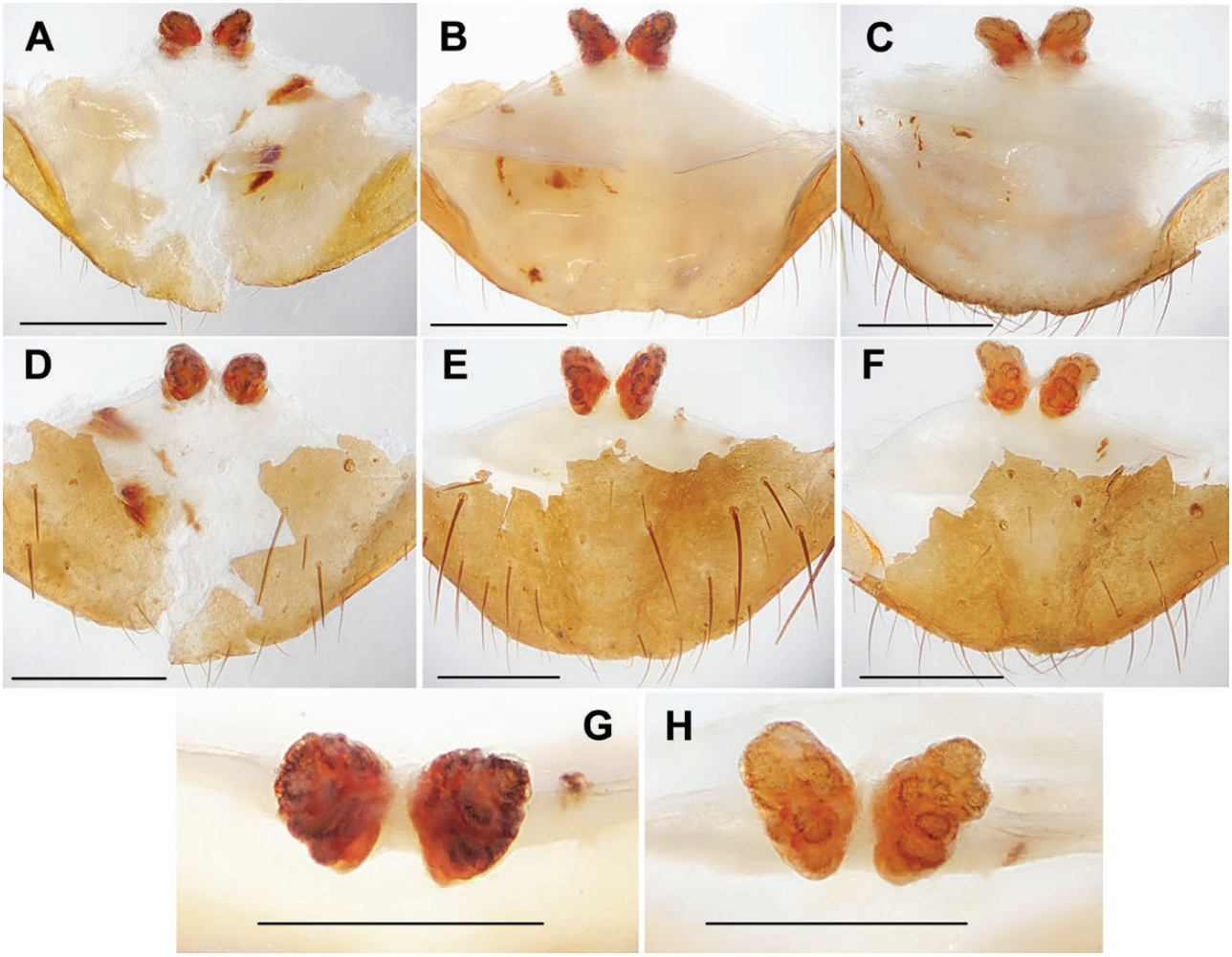

Figure 11. Genital anatomy of ***Ryuthela unten* sp. nov**. A, D, holotype (2524, short for XUX-2012-524); B, E, G, paratype (2525); C, F, H, paratype (4100). A–C, vulvae dorsal view; D–F, vulvae ventral view; G–H, vulvae distal view; 2524, 2525, Unten Port, Nakijin-son; 4100, Mt. Otowa dake, Nakijin-son; scale bars: 0.5 mm.

long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove with 12–13 strong denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites, the fifth largest; 7 spinnerets. Measurements: BL 9.22–11.40, CL 4.40–5.18, CW 3.90–4.62, OL 5.30–7.25, OW 3.88–5.40; ALE > PLE > PME > AME; palp 8.16 (2.81 + 1.42 + 1.75 + 2.18), leg I 9.06 (2.70 + 1.61 + 1.75 + 2.00 + 1.00), leg II 9.23 (2.75 + 1.60 + 1.70 + 1.98 + 1.20), leg III 9.56 (2.60 + 1.61 + 1.65 + 2.30 + 1.40), leg IV 15.01 (3.80 + 2.83 + 2.43 + 3.88 + 2.07).

Female genitalia. A pair of more or less triangular receptacular clusters along the anterior margin of bursa copulatrix, without genital stalks (Fig. 11A–H).

Male. Unknown.

Distribution: Okinawajima island (Unten Port and Mt. Otowa dake, Nakijin-son), Okinawa Prefecture, Japan.

Ryuthela iheyana Ono, 2002

Ryuthela iheyana Ono, 2002, p. 52, figs 1–3 (description of female); Ono, 2009, p. 80, figs 1, 7–8; Tanikawa, 2013a, p. 34, figs 2D–F, 3D–F, 4D–F, 5, 6A–E (description of male).

Holotype: Female deposited at NSMT (NSMT-Ar 5185), from Mt. Gayozan, NE slope, 50 m a.s.l., Iheyajima island, Okinawa Prefecture, Japan, 20 January 1998, by H. Ono; examined.

Other material examined: Male (XUX-2014-067) and female (XUX-2014-068), collected at forest trail, Mt. Koshi dake, Iheyajima island, Okinawa Prefecture,

Japan, 27.04°N, 127.97°E, 10 May 2014, by D. Li and B. Wu.

Diagnosis: Male *R. iheyana* can be distinguished from *R. motobu* sp. nov. by the straight contrategulum spine and the narrower marginal apophysis of tegulum and from all other *Ryuthela* species by the short and straight contrategulum spine (Fig. 12D–F, J) and the rather sharp distal part of contrategulum (Fig. 12E, G), as well as the wider and blunt terminal apophysis of tegulum (Fig. 12F, J). Females of *R. iheyana* can be distinguished from *R. sasakii* and *R. nishihirai s.s.* by the bar-shaped receptacular cluster (Fig. 12A–C). *Ryuthela iheyana* can also be diagnosed from all other *Ryuthela* species by the following unique nucleotide substitutions in the standard DNA barcode alignment: G (2), G (5), G (32), C (68), A (95), T (107), C (112), T (199), T (217), G (307), C (373), T (388), C (397), T (580), C (586).

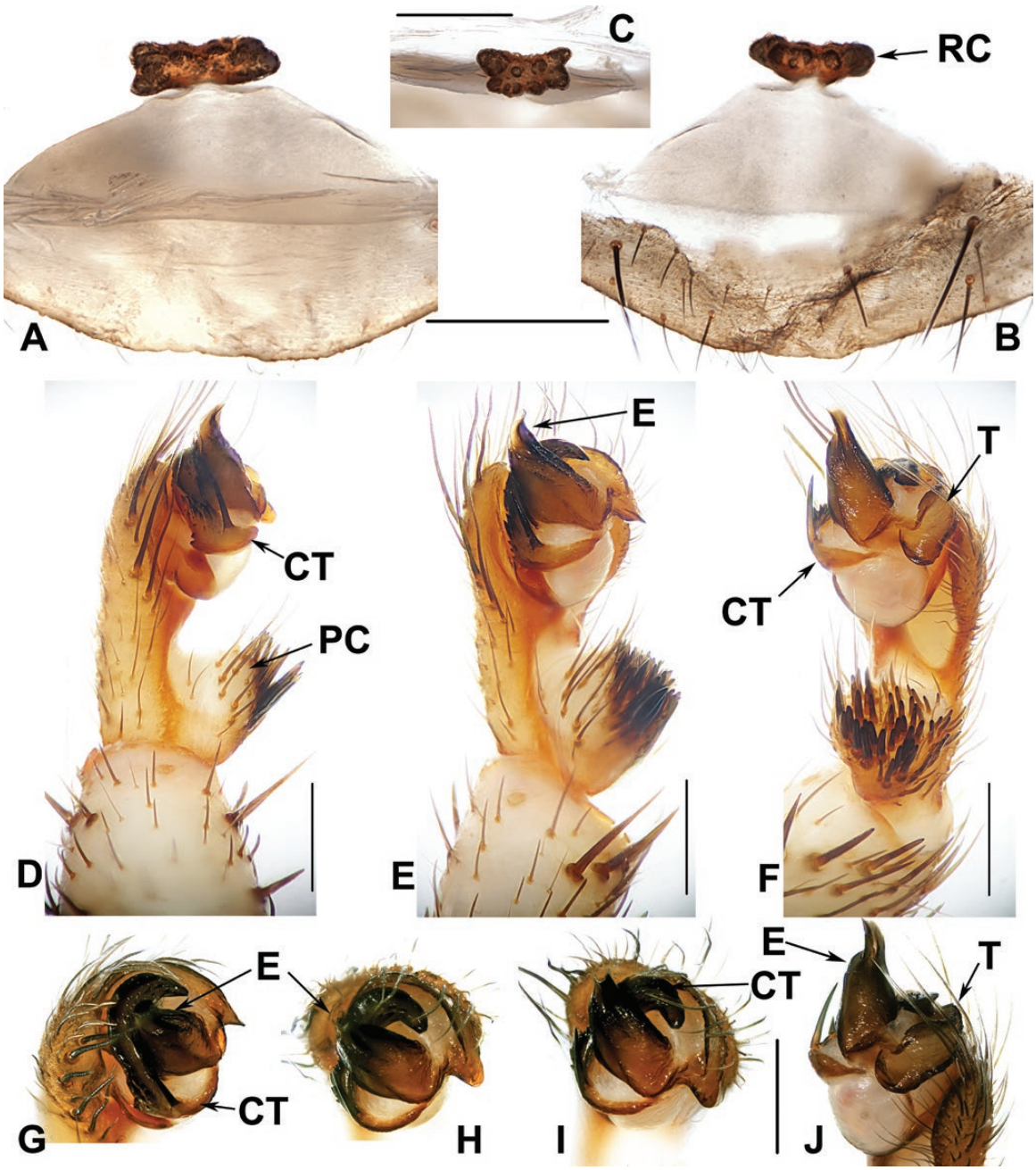

Figure 12. Male (XUX-2014-067) and female (XUX-2014-068) genital anatomy of *Ryuthela iheyana* Ono, 2002. A, vulvae dorsal view; B, vulvae ventral view; C, vulvae distal view; D, palp prolateral view; E, palp ventral view; F, palp retrolateral view; G–J, palp distal view; scale bars: 0.5 mm.

Description: Male. Carapace and opisthosoma light brown; tergites dark brown; sternum narrow, nearly twice as long as wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove with 11 vestigial denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites, close to each other, the first 2–5 larger and the fifth largest; 7 spinnerets. Measurements: BL 9.40, CL 4.80, CW 4.00, OL 5.20, OW 3.70; ALE > PLE > PME > AME; leg I 14.05 (3.90 + 1.70 + 2.75 + 3.70 + 2.00), leg II 14.45 (3.78 + 1.75 + 2.85 + 4.07 + 2.00), leg III 15.53 (3.70 + 1.70 + 2.88 + 4.75 + 2.50), leg IV miss.

Palp. Prolateral side of paracymbium unpigmented and unsclerotized, numerous setae and spines at the tip of paracymbium (Fig. 12D); contrategulum with a short and straight spine and numerous denticles on proximal margin (Fig. 12D–F, J); in the ventral view, the distal part of contrategulum sharp (Fig. 12E, G); the terminal apophysis of tegulum is wide and blunt in retrolateral view (Fig. 12F, J); embolus with a narrow basal part (Fig. 12F).

Female. Coloration similar to that of male; chelicerae robust with promargin of cheliceral groove with 14 strong denticles of variable size; legs and opisthosoma as in the male; 7 spinnerets. Measurements: BL 12.70, CL 5.70, CW 5.00, OL 6.40, OW 4.70; ALE > PLE > PME > AME; palp 9.83 (3.10 + 1.70 + 2.28 + 2.75), leg I 11.70 (3.46 + 1.95 + 2.36 + 2.50 + 1.43), leg II 11.45 (3.45 + 2.00 + 2.00 + 2.50 + 1.50), leg III 12.68 (3.10 + 2.90 + 2.00 + 3.00 + 1.68), leg IV 17.50 (5.00 + 2.25 + 3.10 + 4.65 + 2.50).

Female genitalia. Fused receptacular cluster forming a bar with a very short genital stalk (Fig. 12A–C).

Distribution: Iheyajima island, Okinawa Prefecture, Japan.

Remarks: Based on Tanikawa's (2013a) description, the female genitalia of *R. iheyana* show high intraspecific variation comparable to other species.

Ryuthela owadai Ono, 1997

Ryuthela owadai Ono, 1997, p. 155, figs 15–18 (description of male); Ono, 2001, p. 151, figs 1–3 (description of female); Ono, 2009, p. 80, figs 19–25; Schwendinger & Ono, 2011, p. 616, figs 48–50.

Holotype: Male deposited at NSMT (NSMT-Ar 3459), from Aharen, *c.* 100 m a.s.l., Tokashikijima island, Okinawa Prefecture, Japan, 11 October 1990, by M. Owada; examined.

Other material examined: Fourteen females and five juveniles [XUX-2012-(404–416, 418–420/422/426/429A/430)], collected at Aharen, Tokashikijima island, Okinawa Prefecture, Japan, 26.17°N, 127.35°E, 50 m a.s.l., 22 December 2012, by D. Li, F. X. Liu and X. Xu.

Diagnosis: Females of *R. owadai* differ from all other *Ryuthela* species by the relative length of receptacular clusters (these are slightly longer than wide; Fig. 13A–L); the female genitalia are morphologically similar to *R. sasakii*, but the length of the receptacular clusters is shorter than that of *R. sasakii*. Moreover, *R. owadai* can also be diagnosed from *R. sasakii* by the following unique nucleotide substitutions in the standard DNA barcode alignment: T (10), A (22), A (43), C (163), C (211), C (239), T (241), T (271), T (322), C (343), T (352), T (361), T (364), T (367), C (370), T (415), T (454), A (470), T (502), T (541), G (544), C (601), C (610), C (622).

Description: Female. Carapace and opisthosoma yellow brown; tergites darker brown; sternum narrow, nearly twice as long as wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove containing 12–14 vestigial denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites; 7 spinnerets. Measurements: BL 6.80–10.50, CL 3.50–5.18, CW 2.98–4.20, OL 3.48–5.85, OW 2.60–4.20; ALE > PLE > PME > AME; palp 5.98 (2.05 + 1.08 + 1.30 + 1.55), leg I 8.63 (2.50 + 1.43 + 1.55 + 1.50 + 1.65), leg II 8.29 (2.43 + 1.50 + 1.45 + 1.73 + 1.18), leg III 8.59 (2.40 + 1.37 + 1.35 + 2.15 + 1.32), leg IV 11.89 (2.97 + 1.78 + 2.10 + 3.21 + 1.83).

Female genitalia. A pair of receptacular clusters with the basal parts separated or fused together and with the distal part heart shaped (Fig. 13D, F); the opening part of receptacular clusters is located at the ventral part of bursa copulatrix, but visible in the dorsal view (Fig. 13A–L).

Distribution: Tokashikijima island, Okinawa Prefecture, Japan.

Remarks: Tanikawa (2013a) considered *R. owadai* as synonym of *R. sasakii* based on the lack of distinct morphology to distinguish them. Although we only collected females whose genitalia are highly variable as in other *Ryuthela*, the results from other data sources support the validity of *R. owadai*.

Ryuthela sasakii Ono, 1997

Ryuthela sasakii Ono, 1997, p. 151, figs 1–8 (description of male and female); Ono, 2009, p. 80, figs 9–14;

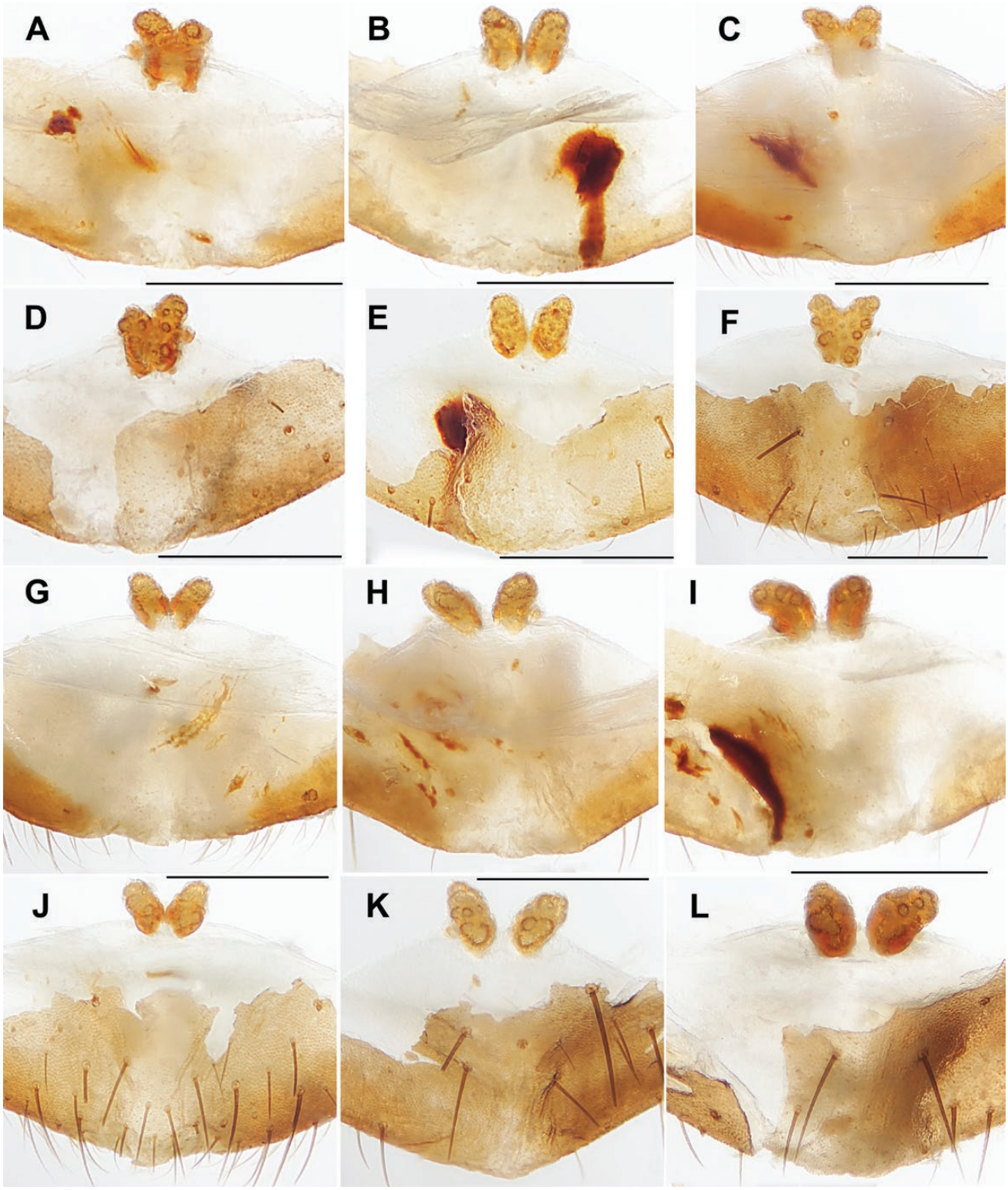

Figure 13. Genital anatomy of *Ryuthela owadai* Ono, 1997. A, D, 2409 (short for XUX-2012-409); B, E, 2410; C, F, 2411; G, J, 2413; H, K, 2426; I, L, 2430. A–C, G–I, vulvae dorsal view; D–F, J–L, vulvae ventral view; scale bars: 0.5 mm.

Tanikawa, 2013a, p. 38, figs 2G–I, 3G–I, 4G–I, 6F–Y. *Ryuthela secundaria* Ono, 1997, p. 153, figs 9–10; Ono, 2009, p. 80, figs 15–18 (description of female).

Type material: Female holotype and male allotype deposited at NSMT (NSMT-Ar 3464–3465), collected from the middle area of Shirasegawa

River, Gushikawa-son, Kumejima island, Okinawa Prefecture, Japan, 2–3 December 1994 (male became adult on 26 October 1995 at laboratory), by H. Ono; female (NSMT-Ar 3471), Mt. Uegusuku dake, 28 m a.s.l., Kumejima island, Okinawa Prefecture, Japan, 10 March 1997, by H. Ono; examined.

Other material examined: Seven females and three juveniles [XUX-2012-(334-335A, 337–343)] and one male (XUX-2012-340A matured 5 November 2013 at CBEE, College of Life Sciences, Hubei University), collected at Yamazato, Kumejima island, Okinawa Prefecture, Japan, 26.36°N, 126.75°E, 50 m a.s.l.; nine females [XUX-2012-(344–348, 350–353)], collected at Yamagusuku, Kumejima island, Okinawa Prefecture, Japan, 26.33°N, 126.78°E, 30 m a.s.l.; ten females [XUX-2012-(354–363)], collected at Tokujimu Nature Park, Kumejima island, Okinawa Prefecture, Japan, 26.30°N, 126.80°E, 75 m a.s.l., 19 December 2012; eight females and one juvenile [XUX-2012-(364–366, 368–373)], collected at Maja, Nakazato-son, Kumejima island, Okinawa Prefecture, Japan, 26.36°N, 126.80°E, 40 m a.s.l.; ten females [XUX-2012-(374–383)], collected at Uegusuku Castle Site, Mt. Uegusuku-son, Kumejima island, Okinawa Prefecture, Japan, 26.38°N, 126.77°E, 300 m a.s.l.; five females and three juveniles [XUX-2012-(384–393)], collected at Near Daruma Yama Park, Mt. Daruma, Kumejima island, Okinawa Prefecture, Japan, 26.36°N, 126.76°E, 140 m a.s.l., 20 December 2012; eight females and two juveniles [XUX-2012-(394–403)], collected at Shirasegawa River, Gushikawa-son, Kumejima island, Okinawa Prefecture, Japan, 26.35°N, 126.77°E, 50 m a.s.l., 21 December 2012, by D. Li, F. X. Liu and X. Xu; five females [XUX-2014-(108/109A/110A/110B/110C)] and one male (XUX-2014-110, matured 28 September 2014 at CBEE, College of Life Sciences, Hubei University), collected at Maja, Nakazato-son, Kumejima island, Okinawa Prefecture, Japan, 26.36°N, 126.80°E, 60 m a.s.l., 12 May 2014; one female (XUX-2014-111B) and six males (XUX-2014-111C/D/E/G/K/L, matured September–October 2014 at CBEE, College of Life Sciences, Hubei University) collected at Uezu, Kumejima island, Okinawa Prefecture, Japan, 26.36°N, 126.75°E, 80 m a.s.l., 13 May 2014, by D. Li and B. Wu.

Diagnosis: Males of *R. sasakii* differ from other *Ryuthela* species by the bifurcated tip of the embolus (Fig. 14C, D, F) and longer contrategulum (Fig. 14E); they can be distinguished from *R. motobu* sp. nov. by the longer contrategular spine (Fig. 14C, F); from *R. iheyana* by the curved contrategular spine (Fig. 14C, F); and from species on Ishigakijima island by the shape of the contrategulum (Fig. 14C, E, F). Females of *R. sasakii* can be distinguished from *R. owadai* by the longer and slender receptacular clusters (Fig. 14G–R). *Ryuthela sasakii* can also be diagnosed from *R. owadai* by the following unique nucleotide substitutions in the standard DNA barcode alignment: A (4), G (10), A (19), T (43), T (163), T (211), A (238), T (239), A (241), A (250), C (271), A (292), T (319), A (322), C (352), A (355), C (361), C (364), C (367), T (370), T (383), C (415), T (433), G (470), T (475), C (502), T (524), A (541), A (601), T (610), T (622).

Description: Male. Carapace and opisthosoma light brown; tergites darker brown; sternum narrow, nearly twice as long as wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove containing 10–14 vestigial denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites; 7 spinnerets. Measurements: BL 9.80–13.60, CL 4.35–6.30, CW 4.10–5.80, OL 4.70–6.90, OW 3.00–4.70; ALE > PLE > PME > AME; leg I 15.68 (4.15 + 1.90 + 3.21 + 4.22 + 2.20), leg II 16.05 (4.10 + 1.90 + 3.05 + 4.50 + 2.50), leg III 15.80 (3.90 + 1.95 + 3.00 + 4.00 + 2.95), leg IV 21.66 (5.30 + 2.00 + 3.95 + 6.65 + 3.76).

Palp. Prolateral side of paracymbium unpigmented and unsclerotized, numerous setae and spines at the tip of paracymbium (Fig. 14A); long contrategulum with a long, curved spine and several denticles on proximal margin (Fig. 14C, E, F); embolus with a wide basal part and bifurcated tip (Fig. 14B, E); in retrolateral view, the marginal apophysis and terminal apophysis of tegulum more or less parallel (Fig. 14C, F).

Female. Coloration similar to that of male; chelicerae robust with promargin of cheliceral groove containing 12–15 strong denticles of variable size; legs and opisthosoma as in the male; 7–8 spinnerets. Measurements: BL 7.68–14.95, CL 3.63–6.63, CW 3.10–5.90, OL 3.70–8.05, OW 2.60–6.28; ALE > PLE > PME > AME; palp 7.69 (2.50 + 1.45 + 1.73 + 2.28), leg I 9.60 (3.10 + 1.60 + 1.78 + 2.00 + 1.12), leg II 9.40 (2.91 + 1.60 + 1.57 + 2.07 + 1.25), leg III 9.85 (2.70 + 1.70 + 1.55 + 2.35 + 1.55), leg IV 14.83 (4.00 + 1.97 + 2.55 + 4.01 + 2.30).

Female genitalia. A pair of receptacular clusters with the basal parts separated or fused and with the heart-shaped distal part (Fig. 14K, O); the opening part of receptacular clusters is located at the ventral part of bursa copulatrix, but visible in the dorsal view; the dorsal view of each receptacular cluster is slender (Fig. 14I, L–N).

Distribution: Kumejima island (Yamazato, Yamagusuku, Tokujimu Nature Park, Maja, Uegusuku Castle Site, Mt. Daruma, Shirasegawa River, Uezu), Okinawa Prefecture, Japan.

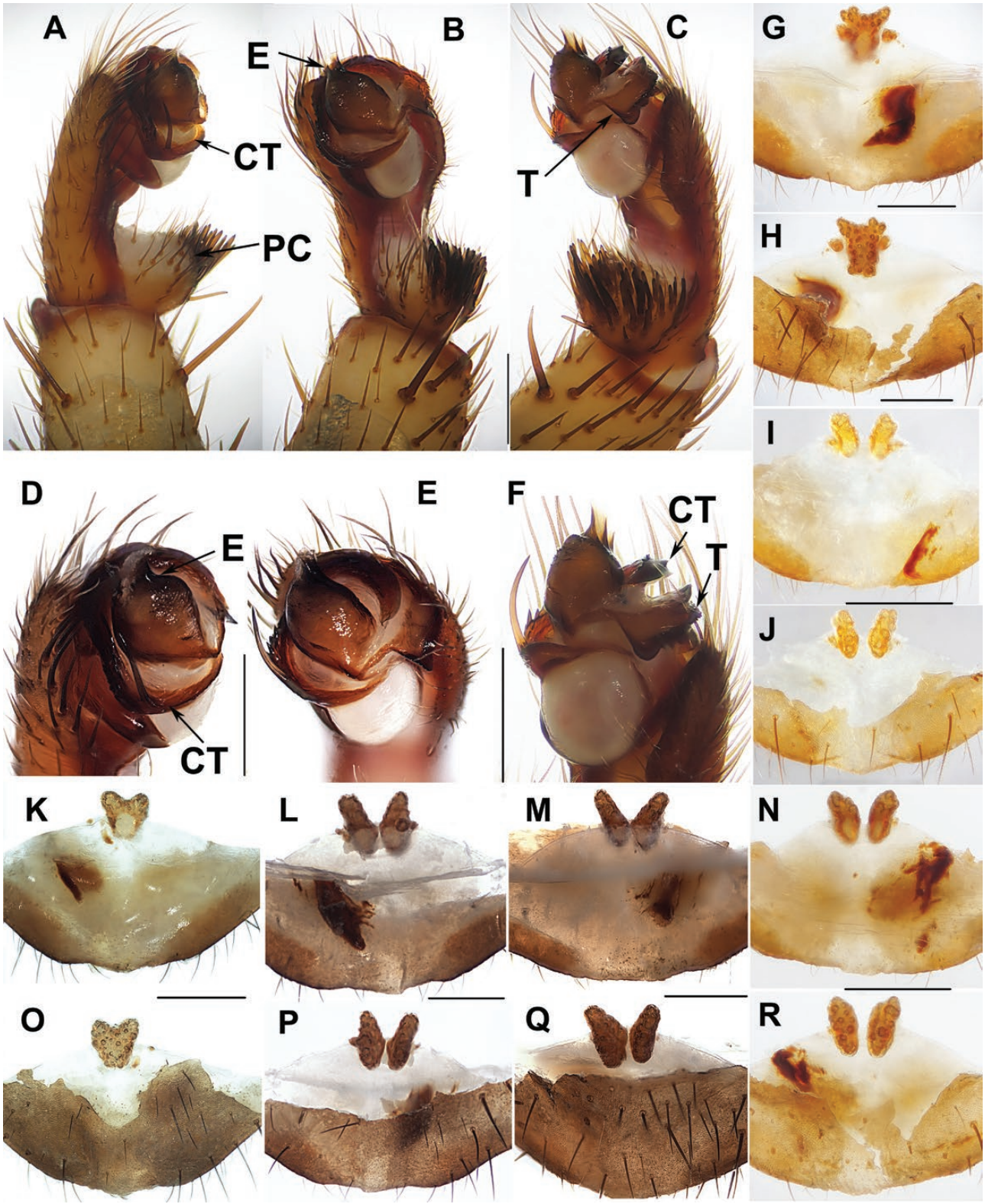

Figure 14. Male and female genital anatomy of *Ryuthela sasakii* Ono, 1997. A–F, 2340A (short for XUX-2012-340A); G, H, 2383; I, J, 2387; K, O, 2364; L, P, 2380; M, Q, 2381; N, R, 4110A. A, palp prolateral view; B, palp ventral view; C, palp retrolateral view; D–F, palp distal view; G, I, K–N, vulvae dorsal view; H, J, O–R, vulvae ventral view; scale bars: 0.5 mm.

Ryuthela ishigakiensis Haupt, 1983

Ryuthela nishihirai ishigakiensis Haupt, 1983, p. 287, figs 9f, 10c–d, 12f, 13h (description of male and female); Haupt, 2003, p. 71, figs 48C–D, 52C-E, 53.25-33, 62B. *Ryuthela ishigakiensis* Ono, 1997, p. 150 (elevated from subspecies); Ono, 2009, p. 80, figs 33–37.

Material examined: Nine females [XUX-2013-(240–242, 244–249)] and one male (XUX-2013-243 matured 5 November 2013 at CBEE, College of Life Sciences, Hubei University), collected at Mt. Kara dake, Ishigakijima island, Okinawa Prefecture, Japan, 24.40°N, 124.24°E, 75 m a.s.l., 10 July 2013, by D. Li and B. Wu.

Diagnosis: Male of *R. ishigakiensis s.s.* is similar to *Ryuthela banna* sp. nov. and *Ryuthela hirakubo* sp. nov. (see below for the description), but it can be distinguished from *R. banna* sp. nov. by the long and curved contrategular spine and the fusion of the inner serrate margin of the upper edge and the lower edge at the distal part of contrategulum (Fig. 15A, C, E); from *R. hirakybensis* sp. nov. by the curved contrategular spine and the sharp lower edge of contrategulum (Fig. 15C, E); and from other *Ryuthela* species by the contrategulum with two edges (Fig. 15C, E) and the wide separation of tegulum from contrategulum (Fig. 15C, E). Female genitalia of *R. ishigakiensis s.s.* lack diagnostic characters from other species on Ishigakijima island and Iriomotejima island (Fig. 15F–K). However, *R. ishigakiensis s.s.* can be diagnosed from all other *Ryuthela* species on Ishigakijima island and Iriomotejima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: C (7), G (16), C (139), C (154), T (188), C (220), T (331), C (343), C (379), G (385), T (454).

Description: Male. Carapace and opisthosoma light brown; tergites darker brown; sternum narrow, nearly twice as long as wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove containing 10 vestigial denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites; 8 spinnerets. Measurements: BL 11.15, CL 5.70, CW 5.30, OL 5.50, OW 4.40; ALE > PLE > PME > AME; leg I 15.10 (3.80 + 2.00 + 2.80 + 4.00 + 2.50), leg II 16.85 (4.70 + 1.35 + 3.50 + 4.40 + 2.90), leg III 15.90 (3.50 + 2.10 + 2.90 + 4.30 + 3.10), leg IV 23.15 (5.80 + 2.00 + 3.90 + 7.35 + 4.10).

Palp. Prolateral paracymbium unpigmented and unsclerotized, numerous setae and spines at the tip of paracymbium (Fig. 15A); contrategulum with a long and curved spine, and with two edges, the upper edge with two margins (inner one with serrate margin and the outer one with smooth margin) and the lower edge (Fig. 15C, E); the distal part of the inner serrate margin fused with the lower edge (Fig. 15C, E); the terminal apophysis of tegulum blunt (Fig. 15C, E); embolus with a wide basal part (Fig. 15E). In retrolateral view, the terminal apophysis of tegulum located approximately at the middle basal part of embolus (Fig. 15C, E).

Female. Coloration darker than that of male; chelicerae robust with promargin of cheliceral groove containing 12–16 strong denticles of variable size; legs and opisthosoma as in the male; 8 spinnerets. Measurements: BL 11.40–14.48, CL 5.88–7.80, CW 5.00–6.25, OL 6.50–7.65, OW 4.60–5.60; ALE > PLE > PME > AME; palp 10.56 (3.64 + 1.82 + 2.30 + 2.80), leg I 12.40 (3.95 + 2.10 + 2.45 + 2.60 + 1.30), leg II 12.07 (3.60 + 2.10 + 2.25 + 2.67 + 1.45), leg III 12.52 (3.65 + 1.90 + 2.40 + 3.32 + 1.25), leg IV 18.20 (5.15 + 1.90 + 3.25 + 5.10 + 2.80).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix, basally separated from each other, cylinder shaped and granulated (Fig. 15G–H, J–K), or basally fused (Fig. 15F, I).

Distribution: Ishigakijima island (Mt. Kara dake), Okinawa Prefecture, Japan.

Remarks: The holotype locality of *R. ishigakiensis* is at Mt. Omoto dake, which is located at the central Ishigakijima island (Haupt, 1983). Our specimens were collected at Mt. Kara dake at the eastern Ishigakijima island. We did not examine the holotype of this species, but based on male and female illustrations, we consider the specimens collected from Mt. Kara dake as *R. ishigakiensis s.s.*

***Ryuthela banna* sp. nov.**

urn:lsid:zoobank.org:act:60FDE0FA-EFC8-436E-8A95-598E4EBD7443

Holotype: Male (XUX-2013-214), collected at Mt. Banna dake, Ishigakijima island, Okinawa Prefecture, Japan, 24.37°N, 124.16°E, 216 m a.s.l., 9 July 2013, by D. Li and B. Wu.

Paratypes: Two females (XUX-2013-211/213), collected at the same locality as holotype; three females and three juveniles [XUX-2013-(216–221)], collected at Mt. Nose dake, Ishigakijima island, Okinawa Prefecture, Japan, 24.37°N, 124.14°E, 125 m a.s.l., 9 July 2013, by D. Li and B. Wu.

Etymology: The species epithet, a noun in apposition, refers to the type locality.

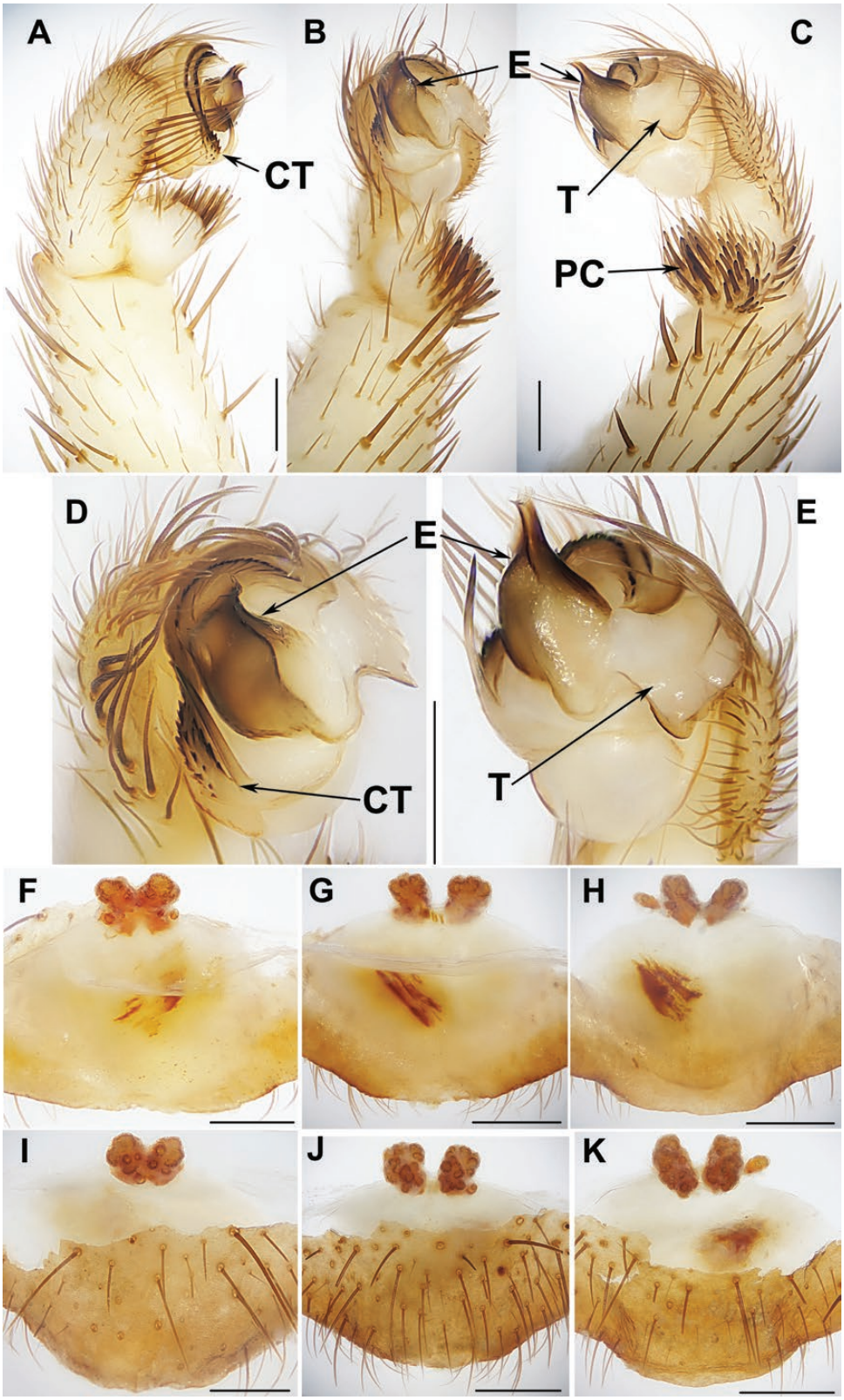

Figure 15. Male and female genital anatomy of *Ryuthela ishigakiensis* Haupt, 1983. A–E, 3243 (short for XUX-2013-243); F, I, 3240; G, J, 3245; H, K, 3246. A, palp prolateral view; B, palp ventral view; C, palp retrolateral view; D, E, palp distal view; F–H, vulvae dorsal view; I–K, vulvae ventral view; scale bars: 0.5 mm.

Diagnosis: Male of *R. banna* sp. nov. is similar to *R. ishigakiensis s.s.* and *R. hirakubo* sp. nov., but it can be distinguished from them by the short and curved contrategular spine, and the fewer denticles at the basal part of the contrategulum (Fig. 16A, D, E); from *R. ishigakiensis s.s.* by the relative location between the basal part of the embolus and the terminal apophysis of the tegulum (Fig. 16F); and from *R. hirakubo* sp. nov. by the fusion of the outer smooth margin of the upper edge and the lower at the distal part of contrategulum (Fig. 16F); it differs from other *Ryuthela* species by the contrategulum with two edges, the wider basal embolus (Fig. 16C, E, F) and the wide separation of the tegulum from the contrategulum (Fig. 16C, F). Female genitalia of *R. banna* sp. nov. lack diagnostic characters from other species on Ishigakijima and Iriomotejima islands (Fig. 16G–L). However, *R. banna* sp. nov. can be diagnosed from all other *Ryuthela* species on Ishigakijima island and Iriomotejima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: G (4), T (61), G (64), G (85), T (157), C (226), T (310), T (328), G (349), C (367), A (376), C (439), G (454), T (533), A (559).

Description: Male (holotype). Carapace and opisthosoma yellow brown; tergites darker brown; sternum narrow, nearly twice as long as wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove containing 14 vestigial denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites; 8 spinnerets. Measurements: BL 13.00, CL 6.50, CW 5.70, OL 5.60, OW 4.35; ALE > PLE > PME > AME; leg I 18.85 (5.00 + 2.30 + 3.60 + 5.30 + 2.65), leg II 20.05 (5.15 + 2.55 + 3.70 + 5.70 + 2.95), leg III 21.45 (5.30 + 2.30 + 3.70 + 6.65 + 3.50), leg IV 27.45 (6.50 + 2.80 + 5.00 + 8.55 + 4.60).

Palp. Prolateral paracymbium unpigmented and unsclerotized, numerous setae and spines at the tip of paracymbium (Fig. 16A); contrategulum with a short and curved spine and fewer denticles at the basal part of contrategulum (Fig. 16A, D, E); the outer smooth margin of the upper edge and the lower fused at the distal part of contrategulum, and lower edge of contrategulum blunt (Fig. 16E, F); the distal part of terminal apophysis of tegulum thumb-shaped in retrolateral view (Fig. 16C, F); embolus with a wide basal part (Fig. 16E, F); in retrolateral view, the terminal apophysis of tegulum is located at two thirds of the basal embolus (Fig. 16F).

Female. Coloration similar to that of the male; chelicerae robust with promargin of cheliceral groove containing 13–14 strong denticles of variable size; legs and opisthosoma as in the male; 7–8 spinnerets. Measurements: BL 10.90–15.50, CL 5.28–7.83, CW 4.11–6.90, OL 5.60–8.28, OW 4.35–6.25; ALE > PLE > PME > AME; palp 13.81 (4.70 + 2.58 + 2.88 + 3.65), leg I 15.35 (4.70 + 2.4 0 + 3.25 + 3.30 + 1.70), leg II 15.78 (4.70 + 2.68 + 2.80 + 3.60 + 2.00), leg III 16.51 (4.25 + 2.88 + 2.70 + 4.20 + 2.48), leg IV 22.80 (6.10 + 2.50 + 4.30 + 6.40 + 3.50).

Female genitalia. A pair of small, ball-shaped receptacular clusters along the anterior margin of bursa copulatrix, basally separated from each other, with many or with unclear granula in ventral view (Fig. 16G–L).

Distribution: Ishigakijima island (Mt. Banna dake, Mt. Nose dake), Okinawa Prefecture, Japan.

RYUTHELA HIRAKUBO SP. NOV.

urn:lsid:zoobank.org:act:88535314-B431-4EF0-AEA2-9871DFB2EFDB

Holotype: Male (XUX-2013-231, matured 12 November 2013, at CBEE, College of Life Sciences, Hubei University) collected at Hirakubo River, Ishigakijima island, Okinawa Prefecture, Japan, 24.59°N, 124.32°E, 125 m a.s.l.; 10 July 2013, by D. Li and B. Wu.

Paratypes: Ten females and four juveniles [XUX-2013-(222–228, 230/232–239)] and one male (XUX-2013-228 matured 5 November 2013, matured 10 October 2013, at CBEE, College of Life Sciences, Hubei University) collected at the same locality as holotype, 10 July 2013, by D. Li and B. Wu.

Etymology: The species epithet, a noun in apposition, refers to the type locality.

Diagnosis: Male of *R. hirakubo* sp. nov. is similar to *R. ishigakiensis s.s.* and *R. banna* sp. nov., but it can be distinguished from them by the straight contrategular spine and the terminal shape of the contrategulum in retrolateral review (Fig. 17A, C, E); it differs from other *Ryuthela* species by the contrategulum with two edges, and the longer contrategulum, and the wide separation of tegulum from contrategulum (Fig. 17C, E). Females *R. hirakubo* sp. nov. can be distinguished from other species of *Ryuthela* on the Ishigakijima island and Iriomotejima island by the paired receptacular clusters basally close to each other and bifurcated (Fig. 17F, G). *Ryuthela hirakubo* sp. nov. can also be diagnosed from all other *Ryuthela* species on Ishigakijima island and Iriomotejima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: C (19), T (31), G (49), G (142), C (169), A (184), T (196), C (202), C (223), A (244), T (370), C (403), G (487), C (499), C (505), T (547), C (595), T (604), T (616).

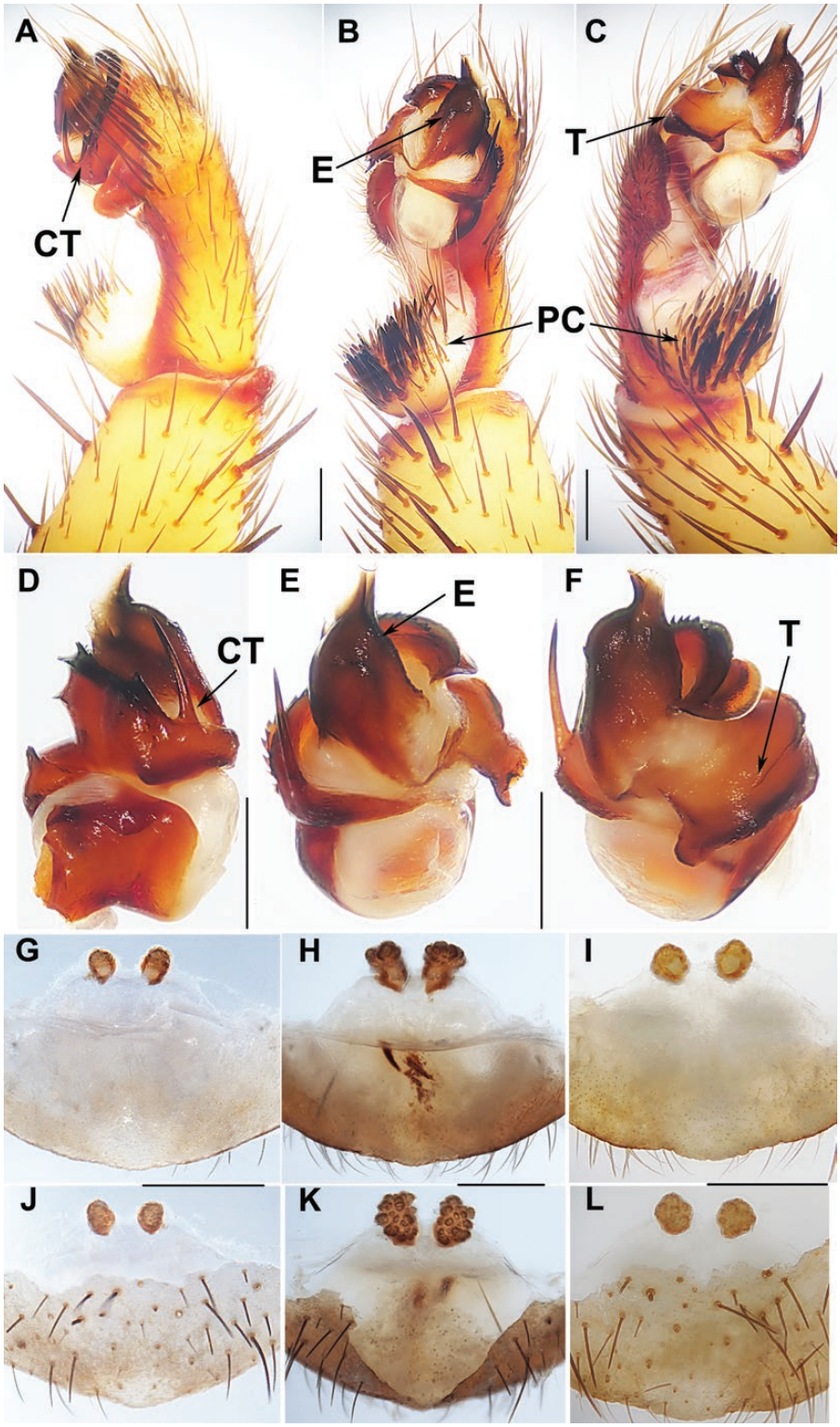

Figure 16. Male and female genital anatomy of ***Ryuthela banna* sp. nov**. A–F, 3214 (short for XUX-2013-214; holotype); G, J, 3220; H, K, 3213; I, L, 3219. A, Right palp prolateral view; B, right palp ventral view; C, right palp retrolateral view; D–F, left palp distal view; G–I, vulvae dorsal view; J–L, vulvae ventral view; 3213, 3214, Mt. Banna dake; 3219, 3220, Mt. Nose dake; scale bars: 0.5 mm.

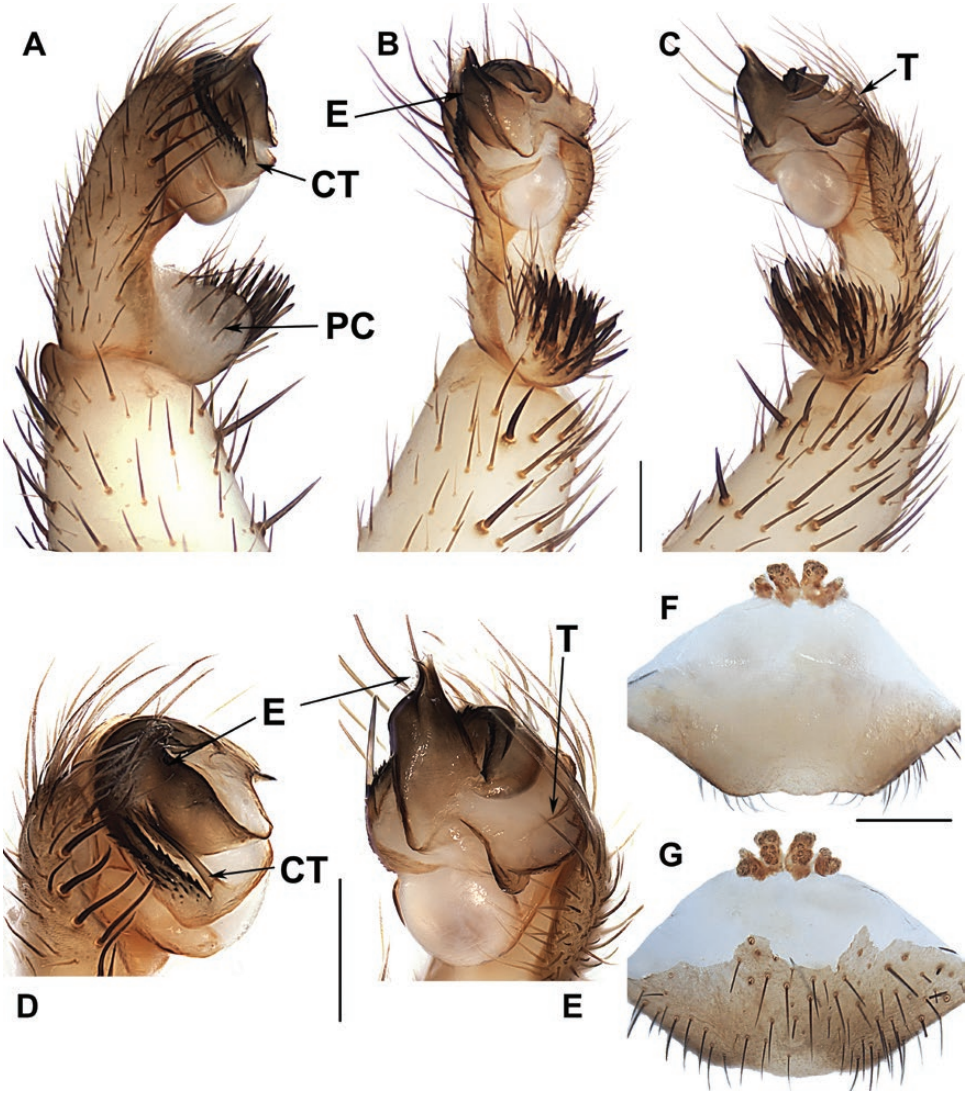

Figure 17. Male (XUX-2013-231, holotype) and female (XUX-2013-232, paratype) genital anatomy of ***Ryuthela hirakubo* sp. nov**. A, palp prolateral view; B, palp ventral view; C, palp retrolateral view; D–F, palp distal view; F, vulvae dorsal view; G), vulvae ventral view; scale bars: 0.5 mm.

Description: Male (holotype). Carapace and opisthosoma light brown; tergites darker brown; sternum narrow, nearly twice as long as wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove containing 10–11 vestigial denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites; 7 spinnerets. Measurements: BL 8.20–10.20, CL 4.80–5.45, CW 4.00–4.50, OL 4.40–5.00, OW 3.10–3.30; ALE > PLE > PME > AME; leg I 14.65 (3.65 + 2.00 + 2.90 + 3.90 + 2.20), leg II 14.80 (3.70 + 1.90 + 2.80 + 4.00 + 2.40), leg III 15.40 (3.90 + 1.60 + 2.60 + 4.50 + 2.80), leg IV 20.00 (4.95 + 2.00 + 3.55 + 5.50 + 4.00).

Palp. Prolateral paracymbium unpigmented and unsclerotized, numerous setae and spines at the tip of paracymbium (Fig. 17A); contrategulum with a long and straight spine and with two edges not fused at the distal parts in retrolateral view (Fig. 17A, C, D, E); the distal part of terminal apophysis of tegulum thumb-shaped in retrolateral view (Fig. 17C, E); embolus with a wide basal part (Fig. 17E). In retrolateral view, the basal tegulum locates approximately at the middle basal part of embolus (Fig. 17C, E).

Female. Coloration darker than that of males; chelicerae robust with promargin of cheliceral groove containing 12–16 strong denticles of variable size; legs and opisthosoma as in the male; 7–8 spinnerets. Measurements: BL 10.00–12.80, CL 5.20–6.50, CW 4.21–5.32, OL 5.00–6.40, OW 3.28–5.25; ALE > PLE >

PME > AME; palp 10.76 (3.81 + 1.95 + 2.20 + 2.80), leg I 12.61 (4.11 + 2.18 + 2.30 + 2.50 + 1.52), leg II 13.03 (4.00 + 2.30 + 2.30 + 2.91 + 1.52), leg III 13.64 (3.72 + 2.30 + 2.28 + 3.45 + 1.89), leg IV 20.23 (5.60 + 2.68 + 3.62 + 5.55 + 2.78).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix, basally close to each other and bifurcated (Fig. 17F, G).

Distribution: Ishigakijima island (Hirakubo), Okinawa Prefecture, Japan.

RYUTHELA YARABU SP. NOV.

urn:lsid:zoobank.org:act:80F64EF2-F931-4C70-AA31-81BFFFBEDD92

Holotype: Female (XUX-2013-251), collected at Mt. Yarabu dake, Ishigakijima island, Okinawa Prefecture, Japan, 24.44°N, 124.10°E, 111 m a.s.l., 11 July 2013, by D. Li and B. Wu.

Etymology: The species epithet, a noun in apposition, refers to the type locality.

Diagnosis: Female *Ryuthela yarabu* sp. nov. differs from all the other *Ryuthela* species except *R. banna* sp. nov. by the slightly globose receptacular clusters separated at the basal part, but cannot be morphologically diagnosed from *R. banna* sp. nov. (Fig. 18A, B). The highly variable female genitalia of *Ryuthela* fail to diagnose species on Ishigakijima island and Iriomotejima island. However, *R. yarabu* sp. nov. can be diagnosed from all other *Ryuthela* species on Ishigakijima island and Iriomotejima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: C (67), C (79), C (91), G (157), G (172), A (250), T (259), T (263), T (322), A (379), T (383), G (406), G (506), G (538), C (577), C (592), A (595), G (604), T (617).

Description: Female (holotype). Carapace and opisthosoma yellow brown; tergites darker brown; sternum narrow, nearly twice as long as wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove containing 15 vestigial denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites; 8 spinnerets. Measurements: BL 11.50, CL 5.60, CW 4.70, OL 5.60, OW 4.60; ALE > PLE > PME > AME; palp 9.08 (3.10 + 1.65 + 1.88 + 2.45), leg I 11.21 (3.60 + 1.98 + 2.05 + 2.30 + 1.28), leg II 10.74 (3.31 + 1.78 + 1.85 + 2.40 + 1.40), leg III 11.61 (3.23 + 2.00 + 1.75 + 2.98 + 1.65), leg IV 17.43 (4.68 + 2.30 + 3.10 + 4.80 + 2.55).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix, basally separated from each other, short cylinder shaped without any granulum in dorsal view (Fig. 18A, B).

Male. Unknown.

Distribution: Ishigakijima island (Mt. Yarabu dake), Okinawa Prefecture, Japan.

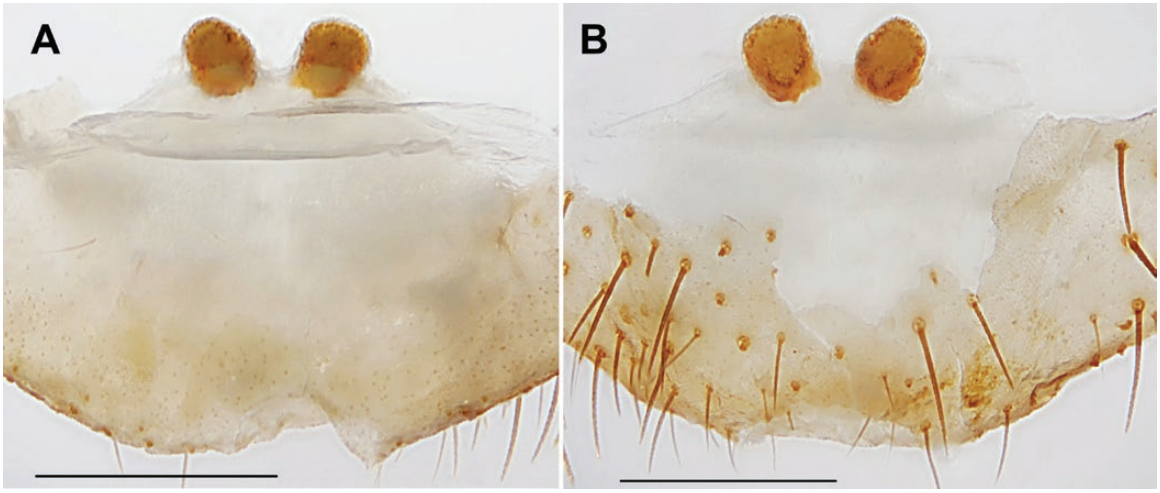

Figure 18. Female (XUX-2013-251, holotype) genital anatomy of ***Ryuthela yarabu* sp. nov**. A, vulvae dorsal view; B, vulvae ventral view; scale bars: 0.5 mm.

RYUTHELA TANIKAWAI ONO, 1997

Ryuthela tanikawai Ono, 1997, p. 157, figs 19–20 (description of female); Ono, 2009, p. 80, figs 38–40; Dunlop, Steffensen & Ono, 2014, p. 39 (removed from synonymy of *R. ishigakiensis*, contra Tanikawa, 2013a, p. 38).

Holotype: Female deposited at NSMT (NSMT-Ar 3484), from Urauchi, Iriomotejima island, Okinawa Prefecture, Japan, 30 March 1983, by A. Tanikawa; examined.

Other material examined: Two females (XUX-2013-200/206), collected at Mihara, Iriomotejima island, Okinawa Prefecture, Japan, 24.35°N, 123.92°E, 30 m a.s.l., 8 July 2013, collected by D. Li and B. Wu.

Diagnosis: Females *R. tanikawai* differ from all other *Ryuthela* species except *R. ishigakiensis s.s.* by short cylinder-shaped and granulated receptacular clusters, but cannot be morphologically diagnosed from *R. ishigakiensis s.s.* (Fig. 19A–D). However, *R. tanikawai* can be distinguished from all other *Ryuthela* species on Ishigakijima island by the following unique nucleotide substitutions in the standard DNA barcode alignment: T (43), G (70), C (121), G (169), T (250), T (257), T (266), C (268), C (292), A (334), A (356), T (358), A (383), T (415), G (470), G (484), T (556).

Description: Female. Carapace and opisthosoma dark brown; tergites darker brown; sternum narrow, nearly twice as long as wide; a few long pointed hairs running over ocular mound in a longitudinal row; chelicerae robust with promargin of cheliceral groove containing 13–15 vestigial denticles of variable size; legs with strong hairs and spines; opisthosoma with 12 tergites; 8 spinnerets. Measurements: BL 11.20–11.50, CL 5.60–6.44, CW 4.38–4.70, OL 6.03–6.52, OW 4.31–4.40; ALE > PLE > PME > AME; palp 10.47 (3.52 + 1.88 + 2.19 + 2.88), leg I 12.57 (3.90 + 2.20 +

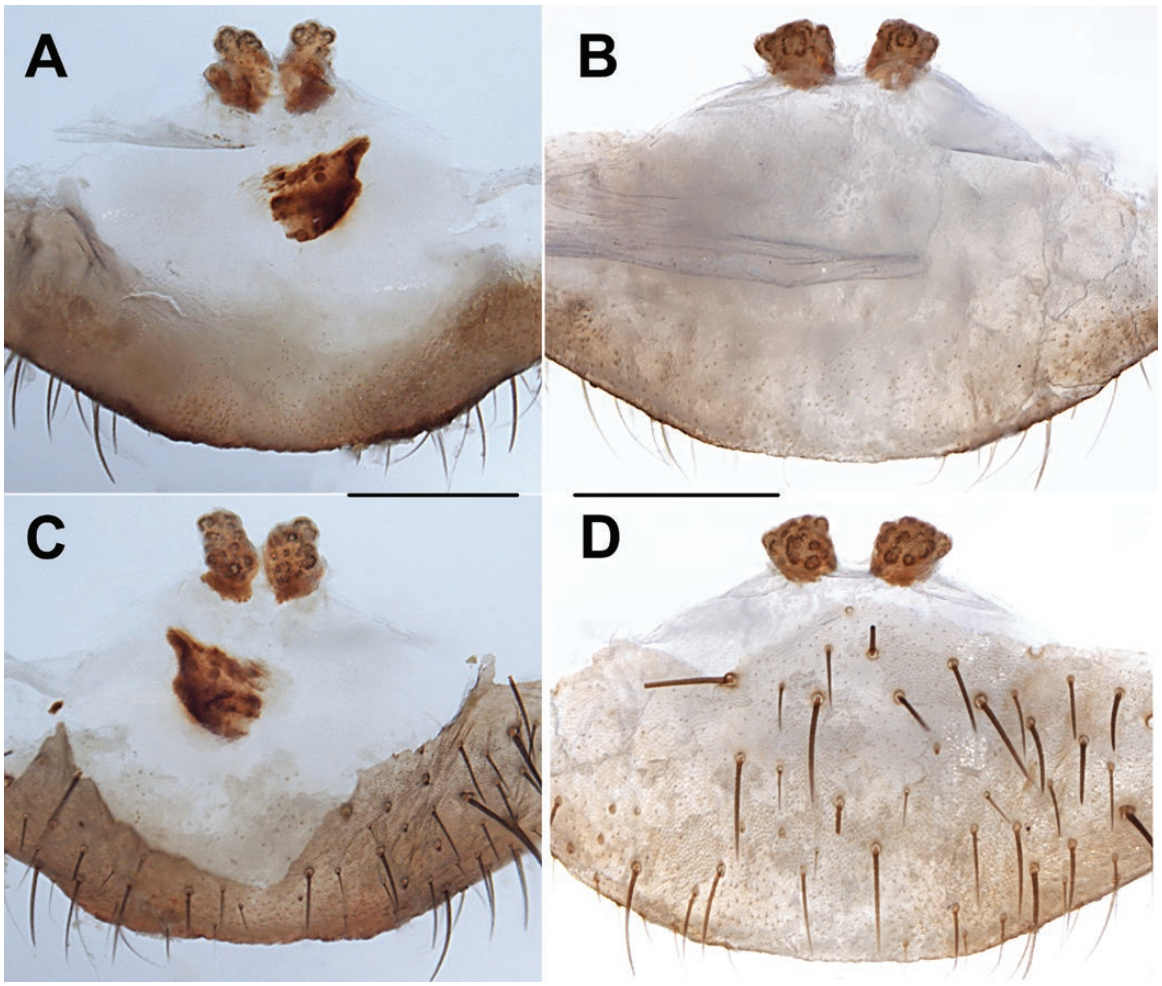

Figure 19. Genital anatomy of *Ryuthela tanikawai* Ono, 1997. A, C, XUX-2013-200; B, D, XUX-2013-206. A, B, vulvae dorsal view; C, D, vulvae ventral view; scale bars: 0.5 mm.

2.30 + 2.68 + 1.49), leg II 12.47 (3.72 + 2.16 + 2.18 + 2.85 + 1.56), leg III 13.26 (3.78 + 2.18 + 2.22 + 3.18 + 1.90), leg IV 18.03 (5.43 + 1.80 + 3.30 + 5.00 + 2.50).

Female genitalia. A pair of receptacular clusters along the anterior margin of bursa copulatrix, basally separated from each other, cylinder-shaped and granulated (Fig. 19B, D), or granulated receptacular clusters close to each other at the basal part (Fig. 19A, C).

Male. Unknown.

Distribution: Iriomotejima island, Okinawa Prefecture, Japan.

Remarks: Ono (2009) indicated some differences in male palpal organ between *R. ishigakiensis s.l.* and *R. tanikawai* based on specimens and photographs made by Haupt (2003). Contrary to this, Tanikawa (2013a) considered *R. tanikawai* as the synonym of *R. ishigakiensis* based on the lack of distinct male and female morphology to distinguish between them. In this study, we collected only two females and no males. Considering highly variable female genitalia in *Ryuthela*, we base our revalidation of *R. tanikawai* on other data sources.

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