Redescription of type species of the genus *Cytaea* Keyserling, 1882 (Araneae: Salticidae) – an integrative approach

Ł. TRĘBICKI ⋅*, B. M. PATOLETA ⋅, M. DABERT ⋅, & M. ŻABKA ⋅

1Faculty of Biology and Environmental Protection, Department of Invertebrate Zoology and Hydrobiology, University of Lodz, Poland, 2Faculty of Exact and Natural Sciences, Siedlce University of Natural Sciences and Humanities, Poland, and 3Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University, Poland

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Abstract
The jumping spider genus *Cytaea* is an iconic member of the Australo-Pacific region. The genus as recognized today is reportedly not monophyletic, most of the forty-two species described here in the 19th century lacking modern diagnoses and adequate illustrations. The genus clearly needs a thorough revision. To avoid future synonyms, species redescriptions based on integrative taxonomy are necessary. Several species of the genus *Cytaea* are studied here in terms of species taxonomy. *Cytaea alburna* Keyserling 1882, the type species, is re-described. We propose *Cytaea severa* (Thorell, 1881) and *Cytaea barbatissima* (Keyserling, 1881) are as junior synonyms of the new combination for *Ascytus asper* (Karsch, 1878), *Cytaea asper* (Karsch, 1878) comb nov. as we find to belong to the genus as well. The integrative approach was used based on diagnostic morphological characters (presented as images and drawings), DNA barcodes and a barcode gap analysis which tested the species distinctness. Genitalic structures are redefined and discussed in terms of function. The conclusion for the 4 species were based on 22 specimens, 14 DNA barcodes, publication supplemented with 46 digital micrographs and 7 drawings.

http://LSIDurn:lsid:zoobank.org:pub:001FB3A0-074E-4290-B2D8-AD246D629E93

Keywords: Integrative taxonomy, DNA barcoding, new synonyms, jumping spiders, genitalic structures

Introduction
Spiders are one of the most successful groups of arthropods and an interesting model in biological studies (Mammola et al. 2017). So far, nearly 50 thousand species in 120 families have been described (WSC 2021), but recent estimates range from 76 to 170 thousand species (Agnarsson et al. 2013; Platnick & Raven 2013). A large part of species described in XIXth and early XXth centuries lack proper diagnoses, documentation, were placed in wrong genera, need verification and revision (Paquin & Duppéré 2009; Dimitrov & Hormiga 2010; Sudhin et al. 2016). Although studies of the type specimens are pivotal in revisional works, several hindering circumstances like the poor condition of the specimens, unknown depository, being destroyed or lost, could be managed (Arnedo 2003; Nentwig et al. 2020). Molecular markers also can help support species delimitation (Hebert et al. 2003). The DNA barcoding has variety of applications in spider research, while is apply for estimate country scale species diversity (Aastrup et al. 2016; Coddington et al. 2016), identification species in tropical regions (Gaikwad et al. 2017; Tahir et al. 2019; Tyagi et al. 2019) in few cases problems arose (Ortiz et al. 2020). With over 6200 species and 646 genera (WSC 2021), salticids are the most diverse family of spiders. Still however, the actual numbers may be 2–3 times higher, especially in tropical regions (Żabka 1991). Almost half of the species have been described from single sex and 367 species still lack illustrations (Metzner 2021). The genus *Cytaea* Keyserling (1882) well illustrates these issues. It includes 42 nominal species (WSC 2021)

*Correspondence: Ł. Trębicki, Faculty of Biology and Environmental Protection, Department of Invertebrate Zoology and Hydrobiology, University of Lodz Banacha 12/16, 90-237, Łódź, Poland. Email: lukasz.trebicki@biol.uni.lodz.pl
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from the Australian and Oriental Regions (Żabka 1991; Patoleta & Trębicki 2015). Many species described over a century ago lack clear diagnoses and documentation (Trębicki et al. 2016). The phylogenetic studies on Salticidae family indicate that genus in its present composition is not monophyletic and has to be revised (Zhang & Maddison 2013, 2015; Maddison 2015). Some *Cytaea* species have already been studied by Prószyński (1976, 1984), Davies and Żabka (1989), Żabka (1991), Berry et al. (1998), Patoleta and Gardzińska (2010), Prószyński and Deeleman-Reinhold (2010, 2013), Cao et al. (2016), Wang and Li (2020); however, the status of type species of the genus and its closest relatives remains unclear. *Cytaea albura* Keyserling 1882 is the generic type. In 1991 Żabka synonymized it with *C. severa* (Thorell 1881), while Prószyński (2017) reinstated the species. Here we revisit the problem based on type and new material for four nominal species: *Cytaea alburna* Keyserling 1882, *Cytaea severa* (Thorell 1881), *Cytaea barbatissima* (Keyserling 1881) and *Ascyllus asper* (Karsch 1878) - all being previously involved in the problem. The taxonomic decisions were supported by DNA barcoding analysis.

The goals of this study are as follows: (I) to redescribe the type species of the genus *Cytaea* and its relatives, using integrative methods, (II) to provide a new reliable documentation enabling species identification, (III) to propose new characters for the genus.

**Material and methods**

**Morphological methods**

The study was based on type and new specimens; the latter being collected during various biodiversity surveys in Australia and preserved in 75% or in 96% ethanol. The specimens used in taxonomic and phylogenetic studies belong to the following institutions: Zoologisches Institut und Zoologisches Museum, Universität Hamburg (ZMH), Museo Civico di Storia Naturale Giacomo Doria, Genoa (MCSN), Zoologisches Museum der Humboldt-Universität, Berlin (ZMB), Muséum National d’Histoire Naturelle, Paris (MNHN), Queensland Museum, Brisbane (QMB). Specimens were examined with Nikon SMZ800, SMZ1000 and Ci microscopes and photographed with Nikon D5100 digital camera. Pictures were digitally processed with HeliconFocus and Adobe Photoshop software. The drawings were made from digital photographs. Epigynes were cleared in 10% potassium hydroxide (KOH) and in methyl salicylate (C₉H₆O₃). All measurements are given in millimetres. Photographs of live spiders and fresh specimens were provided by Robert Whyte, through Queensland Museum Brisbane, Australia. Terminology follows Zhang and Maddison (2015). For further explanations see Figures 3–8. Abbreviation used: CL: cephalothorax length, CW: cephalothorax width, CH: cephalothorax height, AL: abdomen length, AW: abdomen width, AH: abdomen height, EFL: eye field length, AEW: anterior eye row width, PEW: posterior eye row width, DAM: diameter of anterior median eye. The embolic base and course have been described using clock position.

**Molecular methods**

Specimens used in phylogenetic analysis are listed in Table I. New sequence data were generated from 14 *Cytaea* specimens. Total genomic DNA was extracted from single leg of each specimen, using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) according to manufacturer’s instructions. The COI gene fragment was amplified with bcdF01 and bcdR04 primers (Dabert et al. 2010). PCRs were conducted in 10 μL reaction volumes containing 5 μL of the Type-it Microsatellite PCR Kit (Qiagen), 0.5 μM of each primer and 4 μL of DNA template. Thermocycling profile was as follows: one cycle of 5 min at 95°C followed by 35 steps of 30 s each at 95°C, 60 s at 50°C, 1 min at 72°C, and with a final step of 5 min at 72°C.

After amplification, PCR reactions were diluted with 10 μL of MQ water and 5 μL were analysed by electrophoresis on 1% agarose gel. Samples containing visible bands were purified with exoneuclease I and Fast alkaline phosphatase (Thermo Fisher Scientific, USA) and sequenced using the BigDye Terminator v3.1 kit and the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems), following the manufacturer’s instructions. Sequence chromatograms were checked for accuracy in FinchTV 1.3.1 (Geospiza Inc.). Sequences were aligned using MUSCLE (Edgar 2004) with default parameters, performed in Geneious 10.2.3 (Biomatters Ltd.). Genetic distances were estimated using the Kimura 2-parameter model (K2P) (Kimura 1980), Bootstrap values calculated with 500 replicates (Felsenstein 1985) as implemented in MEGA X (Stecher et al. 2020). DNA barcoding-gap was calculated using ABGD method with K2P model and default settings (http://wwwabi.snv.jussieu.fr/public/abgd/) (Puillandre et al. 2012). All new sequences used in this work have been deposited in BOLD Systems (https://www.boldsystems.org/) and GenBank (https://www.ncbi.nlm.nih.gov/) (Table...
Table I. List of specimens studied (F: female, M: male specimen).

| Species     | ID    | Locality                              | Sex | GenBank accession number | BOLD accession number |
|-------------|-------|---------------------------------------|-----|--------------------------|-----------------------|
| 1. Cytaea alburna | TL084  | Alligator creek, QLD, Australia        | M   | MZ479022                 | OZCYT034-21           |
| 2. Cytaea asper   | TL085  | Wollomombi Falls, NSW, Australia       | M   | MZ479053                 | OZCYT035-21           |
| 3. Cytaea asper   | TL086  | Trec Creek, QLD, Australia             | M   | MZ479028                 | OZCYT036-21           |
| 4. Cytaea asper   | TL087  | Townsvile, QLD, Australia              | M   | MZ479041                 | OZCYT037-21           |
| 5. Cytaea alburna | TL088  | Mareeba, QLD, Australia                | M   | MZ479051                 | OZCYT038-21           |
| 6. Cytaea asper   | TL089  | Mareeba, QLD, Australia                | M   | MZ479044                 | OZCYT039-21           |
| 7. Cytaea alburna | TL090  | Mareeba, QLD, Australia                | M   | MZ479029                 | OZCYT040-21           |
| 8. Cytaea alburna | TL091  | Katharine, NT, Australia               | M   | MZ479025                 | OZCYT041-21           |
| 9. Cytaea asper   | TL092  | Cooktown, QLD, Australia               | M   | MZ479007                 | OZCYT042-21           |
| 10. Cytaea alburna| TL093   | Kyogle, NSW, Australia                 | M   | MZ479047                 | OZCYT043-21           |
| 11. Cytaea alburna| TL094   | Grafton, NSW, Australia                | F   | MZ479017                 | OZCYT044-21           |
| 12. Cytaea alburna| TL095   | Myall Park Botanic Garden Glenmorgan, QLD, Australia | F   | MZ479023                 | OZCYT045-21           |
| 13. Cytaea alburna| TL130   | Wongalara Wildlife Sanctuary, Mt. Throsby, NT, Australia | F   | –                     | –                     |
| 14. Cytaea asper   | TL142  | Jandakot, Semple Court, NT, Australia  | M   | –                       | –                     |
| 15. Salticidaesp. | TL124   | Bellthorpe, QLD, Australia             | M   | –                       | –                     |
In addition, comparative pictures of specimens preserved in ethanol are attached to sequences deposited in BOLD.

Results

The final alignment for species delimitation comprised 644 nucleotide positions (nps) for COI gene sequences representing 14 specimens (Table I). The nucleotide sequences could be translated into amino acid sequences without any stop codons. In the dataset, 77 nps out of 644 were variable, and transition-to-transversion ratio (R) amounted to 1.47. Neighbour-Joining (NJ) analysis clustered Cytaea COI sequences into two maximally supported clades (Figure 1) grouping members of C. alburna and C. asper. Genetic distance between C. alburna and C. asper COI sequences amounted to 12.13%. Mean intraspecific distances were 0.91% (SD = 0.27%) and 0.84% (SD = 0.33) for C. alburna and C. asper, respectively. Using ABGD method, we found a 3–10% barcoding gap between the species of C. alburna and C. asper (Figure 2), supporting species distinctiveness.

Closer examination of the type specimens of C. severa, C. barbatissima and Ascyltus asper has revealed their copulatory organs do not differ to put them into separate species; all are here transferred to Cytaea as Cytaea asper comb nov. The analysis (Figures 1, 2) of C. alburna and Cytaea asper revealed that both are separate species, differing by general morphology (Figures 3, 4, 6, 7) and details of gentilic structures (Figures 5, 8).

Taxonomy

Family Salticidae Blackwall 1841
Tribe Euophryini Simon 1901
Genus Cytaea Keyserling 1882

Cytaea Keyserling 1882: 1380–1381; Simon 1903: 810–817; Żabka 1991: 24; Berry et al. 1998: 150–151; Murphy & Murphy 2000: 349; Prószyński & Deeleman-Reinhold 2010: 162; Prószyński & Deeleman-Reinhold 2013: 117–118; Patoleta & Trębicki 2015: 556; Zhang i Maddison 2015: 31; Trębicki et al. 2016: 379.

Type species
Cytaea alburna Keyserling, 1882.

List of transferred species
Ascyltus asper (Karsch 1878) = Cytaea asper (Karsch 1878), comb nov.
Cytaea barbatissima (Keyserling 1881) = C. asper (Karsch 1878), comb nov.
Figure 2. Results of Automatic Barcode Gap Discovery (ABGD) analysis for COI sequences. (a) Distribution of pairwise differences; (b) Ranked pairwise differences.

Figure 3. *Cytaea alburna*. (a–c) female habitus S103096; (d–e) male habitus S103083; (f) male habitus S103032; (g–i) male habitus S103087. Photos: Robert Whyte (QMB).
Figure 4. *Cytaea alburna* habitus. (a) Syntype ZMH dorsal view; (b) S103096 female dorsal view; (c) same, ventral view; (d) same, lateral view; (e) same, frontal view; (f) same, chelicerae dentation; (g) S103079 male dorsal view; (h) same, ventral view; (i) same, lateral view; (j) same, frontal view; (k) same close-up on chelicerae. Scale bars 1 mm.
Figure 5. *Cytaea alburna* copulatory organs. (a) C. female S103096 epigyne ventral view; (b) same, dorsal view; (c) same, schematic drawings (lam. lateral atrial margin; mg. median guide; co. copulatory opening; cd. copulatory duct; s. spermatecae; ag I – ag II. accessory glands; fd. fertilization duct; (d) male S103079 pedipalp ventral view; (e) same, schematic drawing; (f) pedipalp lateral view; (g) same, schematic drawing (c. cymbium; e. embolus; ed. embolic disc; edi. embolic disc indentation; t. tegulum; sd. seminal duct; rta. retrolateral tibial apophysis). Scale bars 0.2 mm.
Cytaea severa (Thorell 1881) = C. asper (Karsch 1878), comb nov.

Cytaea alburna (Keyserling 1882)
Figures 3–5.

Cytaea alburna Keyserling 1882: 1383, Pl. 117, Figs 3–4; Prószyński 1984: Pl. 30; Davies & Żabka 1989: 220, Pl. 30; Żabka 1991: 25; synonymized with Cytaea severa (Thorell 1881), reinstated by Prószyński 2017: 74, Fig. 39C, Fig. 39D; Whyte & Anderson 2017: 238–240.

Material

Cytaea alburna Keyserling 1882, 3♀, syntypes, Peak Downs, Rockhampton, ZMH.

New material examined: 1♂, Queensland, Townsville, Alligator Creak, 11.11.2012, coll. I. R. Macaulay, QMB S103032; 1♂, Queensland, Mareeba, 28.04.2014, coll. R. Whyte, QMB S103079; 1♂, Queensland, Mareeba, 27.11.2013, coll. I. R. Macaulay QMB S103081; 1♀, Queensland, Glenmorgan, Myall Park Botanic Garden, 22.12.2012, coll. R. Whyte, QMB S103222. 1♂, Northern Territory, Katherine, 14.01.2013, coll.
Figure 7. *Cytaea asper* comb nov. (a) female Syntype ZMH of *Hasarius barbatusima* dorsal view; (b) S103029 dorsal view; (c) same, ventral view; (d) same, lateral view; (e) same, frontal view; (f) same, epigyne; (g) male Syntype MCSN of *Plexippus severus* dorsal view; (h) male Syntype of *Euryattus senex* MNHN dorsal view; (i) male S103076 dorsal view; (j) same, ventral view; (k) same, lateral view; (l) same, frontal view; (m) same chelicerae. Scale bars 1 mm.
Figure 8. *Cytaea asper* comb nov. (a) female S103029 epigyne ventral view; (b) same, dorsal view; (c) same, schematic drawings (lam, lateral atrial margin; mg, median guide; co, copulatory opening; cd, copulatory duct; s, spermathecae; ag I – ag II, accessory glands; fd, fertilization duct); (d) male S103076 pedipalp ventral view; (e) same, schematic drawing; (f) pedipalp lateral view; (g) same, schematic drawing (c, cymbium; e, embolus; ed, embolic disc; edi, embolic disc indentation; t, tegulum; sd, seminal duct; rta, retrolateral tibial apophysis). Scale bars 0.2 mm.
I. R. Macaulay, QMB S103083; I♂, New South Wales, Kyogle, 28.10.2014, coll. R. Whyte, QMB S103087; 1♀, New South Wales, Grafton, 25.10.2014, coll. R. Whyte, QMB S103096.

**Diagnosis**

Embolsus shorter than in *C. fibula* (Berland 1938): 2 circles vs. 3.5 circles, longer than in *C. asper* (Karsch 1878): 1.5 circle (Figure 5(d,e)). Embolsus based on round disc, vs. longitudinal disc in *C. nimba* (Thorell 1881). Unlike in *C. laticeps* (Thorell 1878), embolsus is located on the ventral side of the bulb (in *C. laticeps* is perpendicular to the longitudinal axis of the bulb). Spermathecae in *C. alburna* (Figure 5(a–c)) differ from *C. nimba* (Thorell 1881) by presence of sclerotized lateral atrial margins on the epigynal windows and location of copulatory openings: posteriorly in *C. alburna* vs. anteriorly in *C. nimba* (Thorell 1881).

**Female S103096**

Cephalothorax dark orange, eye surroundings black. Whole surface covered with brown numerous setae and white scales, the latter form a belt along the lower margin (Figures 3(a–c), 4(a–f)). Eye field wider than long, its length 54% of CL. PME halfway between PLE and ALE. Fovea located between PLE (Figure 4(a,b)). Abdomen elongate, whitish, covered with sparse brown hairs and orange scales, the latter numerous on sides (Figure 4(a–d)). Spinnerets orange, not distinctive. Clypeus narrower (33%) than AME diameter, pale orange, covered with long, white scales (Figure 4(e)). Chelicerae orange, massive, elongate, inclined downwards, promarg with four teeth, retromargin with a single bicuspidate tooth (Figure 4(e,f)). Endites and labium orange, with pale chewing margins. Sternum yellowish (Figure 4(c)). Legs I orange, others lighter, all legs covered with numerous spines and setae (Figure 4(a–d)). Leg formula: I-IV-III-II.

Epigyne with two circular windows separated by narrow median septum (Figure 5(a–c)) and with sclerotized lateral atrial margins. Copulatory openings located posteriorly and oriented laterally (Figure 5(a–c)). Copulatory ducts short. Spermathecae channel-like, long and twisted, with 2 accessory glands: one located near the copulatory duct entry and another close to the fertilization duct (Figure 5(a–c)).

Dimensions: CL 3.5, CW 2.65, CH 1.55, AL 4.75, AW 2.75, AH 2.5, EFL 1.9, AEW 2.05, PEW 2, DAM 0.6, clypeus 0.2, epigyne length 0.45, epigyne width 0.56, leg I: 8.4 (1 + 0.4 + 2 + 1.1 + 1.8 + 1.2 + 0.9); leg II: 7.65 (0.9 + 0.35 + 1.9 + 1.15 + 1.4 + 1.15 + 0.8); leg III: 8 (0.9 + 0.4 + 2.05 + 1.2 + 1.2 + 1.4 + 0.85); leg IV: 8.2 (0.9 + 0.35 + 1.95 + 1.1 + 1.5 + 1.5 + 0.9).

**Male S103079**

Cephalothorax orange, eye surroundings dark. Whole surface covered with numerous setae and black scales, the latter form two longitudinal thoracic stripes (Figures 3(d–i), 4(g,i)). White scales present on eye field and on sides (Figures 3(d–i), 4(g,i)). Eye field wider than long, narrowing posteriorly (Figure 4(g)). Its length 54% of CL. Fovea short, located between PLE. Abdomen elongate, pale, with dark orange and brown scales on sides, covered with dark brown setae (Figures 3(d–i), 4(g,i)). Spinnerets orange. Clypeus light orange, narrower (44%) than AME diameter (Figures 3(d,g), 4(j–k)), covered with long whitish scales. Chelicerae vertical, elongate, orange with black spots and transverse stripes of white scales at the base (Figure 4(k)). Promargin with three teeth, retromargin with a single bicuspidate tooth. Endites and labium slender, not distinctive, orange, with lighter chewing margins. Sternum longer than wide, pale (Figure 4(h)). Venter dark brown, pale on sides. All legs covered by numerous spines and setae (Figure 4(g,j)). Proximal parts of tibiae pale, the rest of legs pale covered with red shimmering scales. Leg formula: I-III-II-IV.

Palpal organ covered with red, metallic scales and setae (Figures 3(g), 4(k)). RTA of medium length, wider at the base, with an apical hook, with distal notch (Figure 5(f,g)). Embolsus originated at 11th hour, based on a round disc with almost circa 2 loops (Figure 5(d,e)).

Dimensions: CL 2.3, CW 1.7; CH 1.05; AL 2.5; AW 1.3; AH 0.9; EFL 1.1; AEW 1.4; PEW 1.35; DAM 0.45; Clypeus 0.2, leg I: 5.4 (0.5 + 0.2 + 1.35 + 0.65 + 1.15 + 0.9 + 0.65), leg II: 5.15 (0.55 + 0.2 + 1.4 + 0.55 + 1.05 + 0.75 + 0.65), leg III: 5.35 (0.55 + 0.2 + 1.4 + 0.6 + 1 + 0.95 + 0.65), leg IV: 5 (0.6 + 0.2 + 1.45 + 0.45 + 0.9 + 0.8 + 0.6).

**Cytarea asper** Karsch 1878 comb nov. Figures 6–8.

*Attus asper* Karsch 1878: 24; *Euryattus senex* Simon 1885: 90; *Zenodorus asper* Zabka 1988: 476, Figs 151–157; *Ascutis asper* Prószyński 2017: 73–74, Figs 1B: D8+E8 +G8+H8, syn. nov.; *Hasarius barbatissimus* Keyserling 1881: 1272, Pl. 109, Figs 1–2; *Cytarea barbatissima* Keyserling 1883: 1477; *Servaea barbatissima*: Simon 1903: 819, Figs 965; *Cytarea barbatissima*: Zabka 1991: 24, syn. nov.; *Plecoptus severus* Thorell 1881: 596; *Cytarea
severa Žabka 1991: 25; Prószyński 2017: Figs 39C, Figs 39D, syn. nov.

Material

Plexippus severus Thorell 1881 2♂♂, Somerset (Cape York), 1875, L. M. D’Albertis (MCSN). Hasarius barbatissima (Keyserling), 2♀♀, Sydney, New South Wales, Daemul”, ZMB 1684.

New material examined: 1♀ Australian Capital Territory, Canberra, 16.12.2012, coll. I. R. Macaulay, (QMB S103029); 1♂ Queensland, Townsville, R. Whyte, (QMB S103076); 1♂, Queensland, Cooktown, 9.03.2010, I. R. Macaulay, (QMB S103084); 1♂, Queensland, Mareeba, 6.03.2012, R. Whyte, (QMB S103080); 1♂, Queensland, Tree Creek, 29.10.12, I. R. Macaulay, (QMB S103049); 1♂, New South Wales, Wollomombi Falls, 1.11.2012, I. R. Macaulay, (QMB S103045).

Diagnosis

C. asper is grater in size than C. albuna, covered with metallic shimmering scales and is dull in colour (Figures 6–7) vs. red, in alburna (Figures 3–4). Also, the first pair of legs longer in C. severa. Palpal tibia twice longer than in C. albuna, embolus shorter by 0.5 loop length, spermatheca differ by course of ducts (Figure 8(a–g)).

Female S103029

Cephalothorax dark brown, eye surroundings black. Whole surface covered with numerous setae and white scales, the later more numerous along the central part and on sides (Figures 6(a), 7(a–d)). Eye field wider than long, its length 54% of CL. PME halfway between PLE and ALE. Fovea located between PLE (Figure 7b). Abdomen elongate, dark brown, covered with brown setae and white scales (Figure 7a–b). Spinnerets brown. Clypeus brown, covered with long, white scales, narrower (36%) than AME diameter. Chelicerae brown, elongate, inclined downwards (Figure 6(c,e,f), 7(i)). Promargin with three teeth, retromargin with bicuspitate tooth (Figure 7(m)). Endites and labium dark brown, with pale chewing margins, sternum brown (Figure 7(j)). Ventr abdominal brownish. Legs brown covered by numerous spines and setae (Figure 7(g–i)). Leg formula: II-III- IV. Pedipalps brownish, covered with numerous white setae (Figures 6(b,c,e,f), 7(l)). Palpal femur pale, RTA of medium length, wider at the base, hooked apically (Figure 8(d–g)). Embolus set on a rounded embolic disc, starts at the 7th hour and makes 1,5 loop (Figure 8(d,e)).

Dimensions: CL 3.1; CW 2.6; CH 1.7; AL 3.55; AW 1.95; AH 1.7; EFL 1.85; AEW 2.3; PEW 2.25; DAM 0.65; Clypeus 0.2. Leg I: 7.7 (0.95 + 0.55 + 1.75 + 1.05 + 1.4 + 1.25 + 0.75); leg II: 10.05 (0.85 + 0.5 + 2.25 + 1.45 + 2.45 + 2 + 0.55); leg III: 7.4 (0.75 + 0.6 + 1.65 + 0.9 + 1.4 + 1.35 + 0.75); leg: IV: 7.35 (0.8 + 0.55 + 1.9 + 0.9 + 1.2 + 1.25 + 0.75).

Discussion

The nominal type species of the genus Cytacea has been redescribed and 8 COI sequences have been obtained for molecular characterization of the species. Integrated morphological and molecular data allowed us to distinguish several species of the genus Cytacea and to propose 3 synonyms. Cytacea albuna,
type species of the genus is well separated by morphology and DNA barcodes. Ascytus asper, Cytaea severa and C. barbatissima were transferred and synonymized as Cytaea asper comb nov. Diagnostic illustration and DNA barcoding data proved species distinctiveness and clarified that Cytaea alburna is not the junior synonym of Cytaea severa (Zabka 1991; Prószyński 2017) – here proposed as Cytaea asper comb nov. Additionally, genitalic structures of studied species were redefined. To delimit the boundaries between animal species, Hebert et al. (2003) proposed a cytochrome c oxidase subunit 1 (COI) segment of approximately 650 nucleotides and the COI gene fragment has proven to be a good and most widely used marker for animal species delimitation (Hebert et al. 2003; Ratnasingham and Hebert 2007; DeSalle and Goldstein 2019), including spiders (Barrett & Hebert 2005). To establish the boundaries between species, for most taxa, the genetic discrepancy of 3% proved sufficient (Sbordoni 2010). However, due to different rates of evolution, the value may differ between groups of animals and even closely related species. To support species redescription in Salticidae, DNA barcoding method was used by Yamasaki et al. (2018). The 28S gene was additionally sequenced to support the COI results (Vink et al. 2011; Macías-Hernández et al. 2020). The gene 16S was used to designate the species (Rezáč et al. 2008; Marusik et al. 2018), while COI was also used in generic revisions (Richardson & Gunter 2012; Pekar et al. 2017). In our study, we applied DNA barcoding for studied species delimitation. Result of Neighbor-joining and ABGD analysis support distinctiveness of C. alburna and C. asper. COI DNA barcoding revealed that the mean nearest-neighbour distance based on species was 10 times higher than the mean intraspecific divergence in Canadian spiders (7.85% vs. 0.78%), and 7 times higher in Canadian Salticid spiders (7.57% vs. 1.18%) (Blagoev et al. 2016). Other studies on various arthropod taxa including spiders (Hebert et al. 2003; Barrett & Hebert 2005; Robinson et al. 2009) suggested that the interspecific divergence values of COI are usually greater than 2–3%, or the intraspecific divergence values of COI are usually less than 2–3%. Applying these thresholds showed that the distinctiveness of Cytaea alburna and C. asper comb nov. is strongly supported, it has to remembered though that some authors considered the DNA barcoding gap analyze as not conclusive (Puillandré et al. 2012) and suggest using morphological, distributional, environmental, microhabitat and behavioral data. In this research we analyze all available data, including DNA and morphology.

We redefined morphology of genitalic structures in studied species. Long channel-like spermathecae in Cytaea have been so far considered copulatory ducts (Zhang & Maddison 2015; Trębicki et al. 2016). This may be important defining the genus as a monophyletic group. Both Cytaea alburna and C. asper comb nov. have two spermathecal accessory glands, previously only the one nearby copulatory opening was known in those species. We discovered second accessory gland nearby fertilization duct. Accessory glands located in spider epigyne play a nutritive and protective function (desiccation and pathogens) for sperm cells stored in (Michalik & Uhl 2005). Female secretory products also have been suggested to control sperm activation. The accessory glands in Cytaea located nearby fertilization ducts may play similar role. Indentation at the embolus base correspond with anterior lateral atrium in epigyne window are probably part of lock and key mechanism – well known in many arthropods and plays a role of effective reproductive barrier (Masly 2012). All those aspects suggest need for future study on biology of C. alburna, C. asper comb nov. as well as on other species in the genus.

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Geolocation information
Australia.

ORCID
L. Trębicki http://orcid.org/0000-0002-6384-226X
Redescription of type species of the genus Cytaea

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