ACTA ENTOMOLOGICA
MUSEI NATIONALIS PRAGAE
2024
64(2): 365–381
doi: 10.37520/aemnp.2024.027
ISSN 1804-6487 (online) – 0374-1036 (print)
www.aemnp.eu

RESEARCH PAPER

Contribution to the study of the genus *Dicyphus*: the case of *D. bolivari* and *D. tamaninii* (Hemiptera: Heteroptera: Miridae)

Armand MATOCQ[1)], Berend AUKEMA[2)], Guénaëlle GENSON[3)], Peter DE KNIJFF[4)], Éric PIERRE[3)] & Jean-Claude STREITO[3)]

[1)] Muséum national d'Histoire naturelle, Département Adaptation du Vivant, MECADEV, UMR7179, MNHN/CNRS, C. P. 50, Entomologie, 57 rue Cuvier, F-75231 Paris cedex 05, France; e-mail: matocq.armand@wanadoo.fr
[2)] Naturalis Biodiversity Center, PO Box 9517, 2300 RA Leiden, The Netherlands; e-mail: berendaukema@outlook.com
[3)] CBGP, INRAE, CIRAD, IRD, Institut Agro, Univ Montpellier, Montpellier, France; e-mails: guenaelle.genson@inrae.fr; eric.pierre@inrae.fr; jean-claude.streito@inrae.fr
[4)] Department of Human Genetics, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands; e-mail: p.de_knijff@lumc.nl

Accepted:
4th November 2024

Published online:
26th December 2024

Abstract. The genus *Dicyphus* Fieber, 1858 (Miridae: Bryocorinae: Dicyphini) comprises small plant bugs well known for their zoophytophagous diet and predatory activity, making them useful in biological control. Despite their importance in plant protection, many species remain difficult or impossible to identify. This is the case of both species *Dicyphus* (*Dicyphus*) *bolivari* Lindberg, 1934 and *Dicyphus* (*Dicyphus*) *tamaninii* Wagner, 1951, commonly found in vegetable crops in Western Europe. We have conducted a taxonomic study integrating morphological (including male and female genitalia), molecular, biogeographical, and biological data. The following new synonymy is proposed: *Dicyphus bolivari* Lindberg, 1934 = *Dicyphus tamaninii* Wagner, 1951, syn. nov. The synonymies established by Sanchez & Cassis (2018), *Dicyphus bolivari* Lindberg, 1934 = *Dicyphus bolivari atlanticus* Wagner, 1951 = *D. marroccanus* Wagner, 1951, are confirmed. *Dicyphus bolivari* is compared to closely related species. The following country records are added to *D. bolivari* range: Channel Islands: Guernsey, Italy: Sardinia, Monaco, Asian part of Turkey and Cyprus.

Key words. Hemiptera, Heteroptera, Cimicomorpha, Miridae, Bryocorinae, Dicyphini, COI barcoding, Cytb, integrative taxonomy, female genitalia, male genitalia, mtDNA, new synonymy, Europe, Palaearctic Region

Zoobank: http://zoobank.org/urn:lsid:zoobank.org:pub:B65913AF-4CF9-4229-823A-0476C1210018

Introduction

The genus *Dicyphus* Fieber, 1858 comprises small plant bugs (Hemiptera: Heteroptera: Miridae) well known for their zoophytophagous diet and their predatory activity, which are used in biological control (e.g., Ingegno et al. 2017, Abraços-Duarte et al. 2021). It includes 48 described Palaearctic species divided into four subgenera: *Brachyceroea* Fieber, 1858 (18 species), *Dicyphus* (23 species), *Idolocoris* Douglas & Scott, 1865 (3 species), and *Mesodicyphus* Wagner, 1951 (4 species) (Kerzhner & Josifov 1999, Aukema 2018), plus five species from China not attributed to a subgenus (Liu et al. 2022). Although their usefulness and effectiveness in agrobiocenoses are well established, many species remain difficult or even impossible to identify. This is the case of *Dicyphus* (*Dicyphus*) *bolivari* Lindberg, 1934 and *Dicyphus* (*Dicyphus*) *tamaninii* Wagner, 1951 which are commonly found in vegetable crops in Western Europe.

Dicyphus bolivari was described by Lindberg (1934) based on the following discriminating characters: 'it differs from *D. hyalinipennis* in the dimorphic development of the hemelytra, orange-red colouration on the head, pronotum and hemelytra, as well as in smaller size. Like *D. escalerae*, *D. bolivari* is dimorphic, but it is distinguished from the latter by the different colour of the first antennal segment, the pronotum, and the femora'.

In fact, none of these characters can be used to separate *D. bolivari* from related species, especially *D. hyalinipennis* (Burmeister, 1835). Wagner (1951, 1974) and Wagner & Weber (1964) illustrated the left paramere of the male, providing a good character to differenciate *D. bolivari* from

other species of the subgenus *Dicyphus* s. str. (except for *D. tamaninii* and *D. lindbergi* Wagner, 1951). Wagner (1951) described *Dicyphus bolivari atlanticus* from the Canary Islands and *Dicyphus maroccanus* from Morocco.

Sanchez & Cassis (2018) established the synonymies between *D. bolivari*, *D. bolivari atlanticus* and *D. maroccanus*. Based on their new understanding of the species, they gave a set of characters to discriminate *D. bolivari* from *D. tamaninii*: body size and external characters strongly overlap; however, *D. bolivari* has endosoma with generally 8–12 small lobal sclerites, rarely 6–7 (versus 2–5 for *D. tamaninii*) and the left paramere moderately elongated with an apophysis 440–450 µm long (versus 530–600 µm for *D. tamaninii*). They did not study females but provided 9 sequences of the standard COI barcodes for specimens of *D. bolivari* from Spain and the Canary Islands.

Jung & Kim (2023) recently published a World phylogeny of the genus *Dicyphus* in which they proposed several taxonomical changes but without establishing them formally. Their phylogeny is based on a matrix of 52 morphological characters, focusing on the size and colouration of vestiture, body, head, and appendages. Based on very few specimens they proposed to restore *D. maroccanus* and *D. bolivari atlanticus*.

Dicyphus tamaninii was described by Wagner (1951: 16) who differentiated it using the following characters: 'very close to *D. stachydis* Reut., but differs from it externally in the strong, dark pubescence and punctation of the hemelytra. Moreover, the vertex is narrower, the 3rd+4th antennal segments are shorter and the antennae are overall more robust, with the hind tibiae being somewhat shorter. Most notably, the genitalia of the male are quite different'. Surprisingly, Wagner did not compare this species to *D. bolivari* which has very similar male genitalia. He did so indirectly by comparing *D. tamaninii* with *D. maroccanus* (synonym of *D. bolivari*): '*D. maroccanus* nov. spec. also belongs to the *hyalinipennis* group of the subgenus *Dicyphus* s.str. It is most similar to *D. tamaninii* nov. spec. in the morphology of the genitalia, but clearly differs from this species in the shape of the left paramere and the number and size of the chitinous sclerites of the vesica'.

Sanchez et al. (2006) provided a phylogeny including some species of the '*hyalinipennis* group' in which five Cytb sequences of *D. tamaninii* specimens from Spain and the Canary Islands are included. Sanchez & Cassis (2018) argued that 'the body size and external characters of *D. bolivari* and *D. tamaninii* strongly overlap, and species separation is based on differences in the male genitalia, especially the size of the left paramere and the number of endosomal lobal sclerites' (see the previous paragraph dealing with *D. bolivari*). In that study they did not sequence specimens of *D. tamaninii*. It is therefore not possible to compare *D. bolivari* and *D. tamaninii* molecularly, as so far they have been sequenced on different genes.

In their character matrix, Jung & Kim (2023) coded 10 characters differently for *D. bolivari* and *D. tamaninii*: nine concern external morphology and only one male genitalia (character 49: *D. bolivari* was coded to have the endosoma 'without sclerification' and *D. tamaninii* 'multiple sclerites') in contradiction with Sanchez & Cassis (2018) who consider that both species are externaly similar, observed sclerites in the endosoma of both species and made their number a distinctive character.

The taxonomy of *D. bolivari* and *D. tamaninii* is therefore confused. None of the morphological characters used by the authors, either externally or on the male genitalia, allow reliable differentiation of these two species. The female genitalia have never been studied. In fact, none of the available identification keys, including those of Wagner (1951, 1974), Wagner & Weber (1964), Sanchez & Cassis (2018), and Jung & Kim (2023), allow an accurate identification. The DNA sequences currently available in the Bold and GenBank databases cannot be used to identify these two taxa, since they have been sequenced on different genes. They have similar, largely overlapping distributions and both live on a variety of host plants belonging to different families, some of which they share.

In this context, we have conducted a taxonomic study integrating morphological, molecular, biogeographical, and biological data to test whether *D. bolivari* and *D. tamaninii* are two distinct species or not. We studied both sexes and increased the number of specimens and the geographic range of the sampling, as recommended by Doorenweerd et al. (2023).

Material and methods

Collection abbreviations. The material examined is deposited in the following collections:

AMPF	Armand Matocq's private collection, Paris, France;
BABN	Berend Aukema's private collection, Bennekom, the Netherlands;
CBGP-INRAE	Continental Arthropod Collection, Centre de Biologie pour la Gestion des Populations, Montpellier, France (https://doi.org/10.15454/D6XAKL);
HSDG	Helga Simon's private collection, Dienheim, Germany;
JSMF	Jean-Claude Streito's private collection, Montpellier, France;
MLGU	Mark Lawlor's private collection, Guernsey, United Kingdom;
MNHN	Muséum national d'histoire naturelle, Paris, France;
MTDU	Mark Telfer's private collection, Dunstable, United Kingdom;
MZHF	Zoological Museum, University of Helsinki, Finland;
RMNH	Naturalis Biodiversity Center, Leiden, the Netherlands;
ZMUH	Zoologisches Museum, Universität Hamburg, Germany.

Morphological studies. Identifications to species level were based on Wagner (1951), Wagner & Weber (1964), Wagner (1974), and Sanchez & Cassis (2018). Both male and female genitalia were dissected: abdomens were cleared in hot KOH (10%) for about 10 minutes, washed and cleaned in distilled water, stained with chlorazol black in the case of females, then dried in ethanol and transferred to glycerine for further dissection and observation. After being photographed in glycerine, the genital segments were preserved in glycerine in microvials pinned with the specimens. Observations were made with a Leica MZ16 stereomicroscope. Photographs of both habitus and genitalia were taken with a Keyence VHX5000 microscope. Terminology follows Pluot-Sigwalt & Matocq (2017) for the females and Sanchez & Cassis (2018) for the males.

Morphometrical analyses. The total lengths of the specimens were measured from the tip of the clypeus to the

end of hemelytron in macropters and from the tip of the clypeus to the end of the abdomen in brachypters using a calibrated ocular micrometer. As described by Sanchez & Cassis (2018: fig. 9) we measured the total length of the left paramere apophysis [apo] and the length of the apophysis shaft 'from outer margin of the base to tip of shaft' [apo(s)] using the measurement tool integrated into the Keyence VHX5000 microscope correctly calibrated (Fig. 2A).

Molecular analysis. We sequenced the mitochondrial COI (cytochrome c oxidase subunit I) fragment for 33 specimens representative of the range of these two taxa. This gene has been adopted as a universal standard barcode by the Consortium for the Barcode of Life (Hebert et al. 2003) for animals (5' end of COI); it was shown to be relevant for the discrimination of *Dicyphus* species (Sanchez & Cassis 2018), and will allow us to compare our results with those of these authors.

Sanchez et al. (2006) generated a Cytb portion of 381 bp from five specimens of *Dicyphus* identified as *D. tamaninii*. These specimens were different from those sequenced for COI in Sanchez & Cassis (2018), but were also collected in Spain and Tenerife (Canary Islands). These authors did not sequence these specimens for COI, so it is not possible to compare both taxa from their publications. To make such a comparison we also generated Cytb sequences with the same primers as Sanchez et al. (2006) for twenty specimens whose previously sequenced COI corresponds to *D. bolivari* sensu Sanchez & Cassis (2018).

We have also sequenced COI for *D. escalerae* and *D. rubicundus* Blöte, 1929 as the closest species to *D. bolivari/D. tamaninii* complex, based on the analyses published by Sanchez & Cassis (2018: 28–29).

Two specimens of *Macrolophus* (*M.*) *pygmaeus* (Rambur, 1839) and *Macrolophus* sp. and two of *Nesidiocoris tenuis* (Reuter, 1895) were used as outgroups.

Some specimens were sequenced on the molecular platform of CBGP-INRAE, others at the Department of Human Genetics, Leiden.

Using the CBGP molecular platform, we sequenced the COI gene for 13 specimens of *D. bolivari/tamaninii*, 7 *D. escalerae* and 2 outgroups (JSTR in Table 1). Total genomic DNA was extracted non-destructively from the whole specimens using the Qiagen DNeasy 96 Blood & Tissue extraction kits according to the supplier's protocol. The sequenced reference specimens and their DNA are deposited in the CBGP-INRAE collection. The methods used for sequencing, alignment and processing of the sequences are the same as those described by Streito et al. (2018).

At the Department of Human Genetics, Leiden we sequenced two mitochondrial genes, COI and Cytb, from twenty *D. bolivari*/*tamaninii* specimens (FLBO-DBT in Table 1). DNA was extracted from one hind leg. Sanger sequences were generated following the protocol and primers published by Sanchez & Cassis (2018) for COI and Sanchez et al. (2006) for Cytb. Specimens will be returned to their respective collections (see Table 1).

All resulting barcodes are deposited in the CBGP Arthemis database (https://doi.org/10.15454/TBGRIB), the international databases Bold Systems (http://www.boldsystems.org) and GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Accession numbers are given in Table 1.

Phylogenetic analysis. For this study, we used sequences from the CBGP, Montpellier and the Department of Human Genetics, Leiden (i.e. 33 COI sequences and 20 Cytb sequences) and sequences published by Sanchez et al. (2006) (i.e., 11 Cytb sequences) and Sanchez & Cassis (2018) (i.e., 9 COI sequences).

The same protocol was used for both genes. Sequences were aligned using the default parameters of ClustalW (1.81) (Thompson et al. 1997). Phylogenetic trees were constructed using the maximum likelihood (ML) method. The most appropriate evolutionary model (GTR+I+Γ) for our dataset was identified using MrAIC.pl 1.4.3 software, based on Akaike's criteria (Nylander 2004). Maximum likelihood analyses were performed using the parallelized version (MPI-parallelized) of the RAxML 7.2.8 software (Stamatakis 2006a). The GTRCAT approximation was used to compute the statistical support values (bootstrap values) for each of the bootstrap value nodes (BP) (Stamatakis 2006b) (1000 replicates).

The distance matrices were calculated using a Neighbour-Joining distance method with the K2P (Kimura two parameter) evolution model, which distinguishes transitions from transversions in the substitution matrix.

Haplotype networks were constructed for COI gene. Mean-link networks (Bandelt et al. 1999), chosen in this study, integrate the information contained in several trees of minimum size; connections are made not only on the haplotypes present in the sampling but also on missing haplotypes, thus increasing the diversity of the network. PopArt software version 1.7 (Population Analysis with Reticulate Trees) was used with the Median-Joining method (default setting: epsilon = 0).

Sequencing of the full mtDNA genome. Sanchez et al. (2006) published a 381 bp fragment of the mtDNA gene Cytb for *D. tamaninii* but not for *D. bolivari*. In a subsequent study (Sanchez & Cassis 2018), partial mtDNA gene COI sequences were provided for *D. bolivari* but not for *D. tamaninii*. This effectively prevents a direct comparison of the relevance of both studies, at least with respect to the underlying genetic data. In order to solve this, we sequenced the full mtDNA genome of a single *D. tamaninii* individual, FLBO-DBT05 (see Table 1) (Genbank acession number PP746700) using the following protocol: DNA extract of a single hind leg was sheared using the Covaris S2 to an average target length of approximately 600 bp. Adapters were ligated using the Kapa Hyper Prep kit according to the manufacturer's protocol (07962371001; Roche Diagnostics). A library amplification was used to boost the library yield. Sequencing was performed using the MiSeq® Sequencer according to the manufacturer's protocol (Illumina, 600 cycles v3 chemistry). Paired-end reads were adapter-trimmed by the Miseq reporter Software. We used MITObim version 1.8 with the COI fragment of 816 bp as seed-sequence to filter a read pool that resembles mitochondrial reads in multiple iterations rendering a draft mtDNA genome consisting of overlapping reads. Subsequently we created a Bam file with SAMtools

Table 1. List of species and specimens used for phylogenetic analysis (COI, Cytb): Field data, deposit location and accession numbers of specimens sequenced in this study and already published (Sanchez et al. 2006, Sanchez & Cassis 2018). The exact sampling localities that were missing in the publications of the previous authors, which complement and harmonise the present table, were estimated in Google Maps from the GPS coordinates that these authors provided in their respective papers. (*) corresponds to the complete mtDNA genome. (Og) represents the outgroup species used to root and consolidate the phylogenetic tree.

Species	Specimen	Country	Region	Sampling locality	Latitude (Decimal degree)	Longitude (Decimal degree)	Sampling date	Sex	Collection	GenBank COI Access. num.	GenBank Cytb access. num.	BOLD ID COI
D. bolivari	JSTR00085_0201	France	Pyrénées-Orientales	Saint-Estève	42.725150	2.829914	29/06/2011	♂	CBGP-INRAE	PQ100039		BTBUG0021-24
D. bolivari	JSTR00466_0101	France	Hérault	Fraisse-sur-Agout	43.606055	2.797722	19/08/2012	♂	CBGP-INRAE	PQ100046		BTBUG0022-24
D. bolivari	JSTR03352_0101	France	Cher	Marmagne	47.107931	2.270888	23/08/2016	♂	CBGP-INRAE	PQ100016		BTBUG0023-24
D. bolivari	JSTR03352_0102	France	Cher	Marmagne	47.107931	2.270888	23/08/2016	♂	CBGP-INRAE	PQ100050		BTBUG0024-24
D. bolivari	JSTR08327_0101	France	Finistère	Kerlouan	48.658678	-4.377627	27/08/2019	♂	CBGP-INRAE	PQ100020		BTBUG0025-24
D. bolivari	JSTR08415_0101	France	Loire-Atlantique	Nantes	47.189980	-1.531910	10/08/2017	♂	CBGP-INRAE	PQ100048		BTBUG0026-24
D. bolivari	JSTR08416_0101	France	Sarthe	Dissé-sous-le-Lude	47.595500	0.135000	07/07/2019	♂	CBGP-INRAE	PQ100010		BTBUG0027-24
D. bolivari	JSTR08423_0101	France	Marne	Saron-sur-Aube	48.569192	3.734134	13/07/2019	♂	CBGP-INRAE	PQ100018		BTBUG0028-24
D. bolivari	JSTR08423_0102	France	Marne	Saron-sur-Aube	48.569192	3.734134	13/07/2019	♂	CBGP-INRAE	PQ100023		BTBUG0029-24
D. bolivari/ tamaninii	JSTR08457_0101	France	Côtes-d'Armor	Hillion	48.524682	-2.653969	30/08/2019	♂	CBGP-INRAE	PQ100026		BTBUG0030-24
D. bolivari	JSTR09750_0101	France	Côtes-d'Armor	Pléneuf-Val-André	48.574720	-2.516944	19/08/2016	♂	CBGP-INRAE	PQ100019		BTBUG0031-24
D. bolivari	JSTR09751_0101	France	Côtes-d'Armor	Pléneuf-Val-André	48.574720	-2.516944	19/08/2016	♂	CBGP-INRAE	PQ100035		BTBUG0032-24
D. bolivari	JSTR10841_0102	Netherlands	Limburg	Weert	51.257800	5.696800	10/09/2019		CBGP-INRAE	PQ100027		BTBUG0033-24
D. bolivari	FLDO-DBT01	Spain	Alicante	Altea			17/31-10-1987		RMNH	PQ100030	PP996100	BTBUG0034-24
D. bolivari	FLDO-DBT02	France	Corsica	Calvi			25/27-08-1971		RMNH	PQ100009	PP996101	BTBUG0035-24
D. bolivari	FLDO-DBT03	Spain	Alicante	Altea			17/31-10-1987		RMNH	PQ100029	PP996102	BTBUG0036-24
D. bolivari	FLDO-DBT04	Spain	Alicante	Altea			17/31-10-1987		RMNH	PQ100041	PP996103	BTBUG0037-24
D. tamaninii	FLDO-DBT05	Spain	Almería	Níjar, Faro del Cabo del Gata			30-04-2016	♀	RMNH	PQ100014	PP996104	BTBUG0038-24
										PP746700 (*)		
D. tamaninii	FLDO-DBT06	Spain	Araba-Álava	Iruraitz-Gauna, Laku Gazeo			15-07-2007	♂	RMNH	PQ100013	PP996105	BTBUG0039-24
D. tamaninii	FLDO-DBT07	Spain	Araba-Álava	Santa Cruz de Campezo / Santikurutze Kanpezu			07-07-2003	♀	RMNH	PQ100038	PP996106	BTBUG0040-24
D. tamaninii	FLDO-DBT08	Spain	Araba-Álava	Santa Cruz de Campezo / Santikurutze Kanpezu			07-07-2003	♀	RMNH	PQ100022	PP996107	BTBUG0041-24
D. bolivari/ tamaninii	FLDO-DBT09	Netherlands	Limburg	Weert	51.257800	5.696800	10-09-2019		BABN	PQ100024	PP996108	BTBUG0042-24
D. bolivari/ tamaninii	FLDO-DBT10	Netherlands	Limburg	Weert	51.257800	5.696800	10-09-2019		BABN	PQ100033	PP996109	BTBUG0043-24
D. bolivari/ tamaninii	FLDO-DBT11	Netherlands	Limburg	Weert	51.257800	5.696800	10-09-2019		BABN	PQ100011	PP996110	BTBUG0044-24

continues on next page

Table 1. Continuation.

D. bolivari/ tamaninii	FLDO-DBT10	Netherlands	Limburg	Weert	51.257800	5.696800	10-09-2019		BABN	PQ100033	PP996109	BTBUG0043-24
D. bolivari/ tamaninii	FLDO-DBT11	Netherlands	Limburg	Weert	51.257800	5.696800	10-09-2019		BABN	PQ100011	PP996110	BTBUG0044-24
D. bolivari/ tamaninii	FLDO-DBT12	Netherlands	Limburg	Weert	51.257800	5.696800	10-09-2019		BABN	PQ100017	PP996111	BTBUG0045-24
D. bolivari/ tamaninii	FLDO-DBT13	Netherlands	Limburg	Weert	51.257800	5.696800	10-09-2019		BABN	PQ100028	PP996112	BTBUG0046-24
D. bolivari/ tamaninii	FLDO-DBT14	Channel Islands	Guernsey	Moulin Huet			03-08-2020		MLGU	PQ100034	PP996113	BTBUG0047-24
D. tamaninii	FLDO-DBT15	England	London	Crystal Palace			23-08-2013	♂	MTDU	PQ100021	PP996114	BTBUG0048-24
D. tamaninii	FLDO-DBT16	England	London	Silvertown Quays			03-10-2013	♂	MTDU	PQ100031	PP996115	BTBUG0049-24
D. tamaninii	FLDO-DBT17	Spain	Almería	Bédar			27-04-2015	♂	HSGD	PQ100025	PP996116	BTBUG0050-24
D. tamaninii	FLDO-DBT18	Spain	Almería	Bédar			27-04-2015	♀	HSGD	PQ100049	PP996117	BTBUG0051-24
D. bolivari	FLDO-DBT19	Germany	Rheinland-Pfalz	Hornbach	49.184060	7.366050	24-09-2019	♂	HSGD	PQ100042	PP996118	BTBUG0052-24
D. bolivari	FLDO-DBT20	Germany	Rheinland-Pfalz	Hornbach	49.184060	7.366050	24-09-2019	♀	HSGD	PQ100044	PP996119	BTBUG0053-24
D. bolivari	D133-1	Spain	Canary Islands	La Palma,Puntallana	28.742778	-17.743611	2009		Sanchez & Cassis (2018)	KY274620.1		
D. bolivari	D134-1	Spain	Canary Islands	La Gomera, Las Rosas	28.185278	-17.221111	2009		Sanchez & Cassis (2018)	KY274621.1		
D. bolivari	D137-1	Spain	Canary Islands	Gran Canaria, Teror	28.056944	-15.538611	2009		Sanchez & Cassis (2018)	KY274622.1		
D. bolivari	D26-1	Spain	Málaga	Nerja	36.758056	-3.8480556	2006		Sanchez & Cassis (2018)	KY274623.1		
D. bolivari	D40-1	Spain	Zaragoza	Daroca	41.108611	-1.414167	2006		Sanchez & Cassis (2018)	KY274624.1		
D. bolivari	D46-1	Spain	León	Vidanes	42.744167	-5.139167	2006		Sanchez & Cassis (2018)	KY274625.1		
D. bolivari	D49-1	Spain	Teruel	Linares de Mora	40.322222	-0.574722	2006		Sanchez & Cassis (2018)	KY274626.1		
D. bolivari	D97-1	Spain	Canary Islands	Tenerife, Punta del Hidalgo	28.571389	-16.316111	2008		Sanchez & Cassis (2018)	KY274627.1		
D. bolivari	D97-2	Spain	Canary Islands	Tenerife, Punta del Hidalgo	28.571389	-16.316111	2008		Sanchez & Cassis (2018)	KY274628.1		
D. tamaninii	D1	Spain	Murcia	Cehegín	38.1086111	-1.7836111		♂	Sanchez et al. (2006)		DQ232678.1	
D. tamaninii	D14	Spain	Murcia	Benizar	38.275000	-1.985000		♂	Sanchez et al. (2006)		DQ232679.1	
D. tamaninii	D14-1	Spain	Murcia	Benizar	38.275000	-1.985000		♂	Sanchez et al. (2006)		DQ232675.1	

continues on next page

Table 1. Continuation.

Species	Specimen	Country	Region	Sampling locality	Latitude (Decimal degree)	Longitude (Decimal degree)	Sampling date	Sex	Collection	GenBank COI Access. num.	GenBank Cytb access. num.	BOLD ID COI
D. tamaninii	D14-1	Spain	Murcia	Benizar	38.275000	-1.985000		♂	Sanchez et al. (2006)		DQ232675.1	
D. tamaninii	D17	Spain	Canary Islands	Tenerife, Punta del Hidalgo	28.573333	-16.331111		♂	Sanchez et al. (2006)		DQ232677.1	
D. tamaninii	D17-1	Spain	Canary Islands	Tenerife, Punta del Hidalgo	28.573333	-16.331111		♂	Sanchez et al. (2006)		DQ232676.1	
D. escalerae	JSTR00047_0101	Spain	Huesca	Broto	42.603935	-0.121963	15/08/2011	♀	CBGP-INRAE	PQ100015		BTBUG054-24
D. escalerae	JSTR00047_0102	Spain	Huesca	Broto	42.603935	-0.121963	15/08/2011	♀	CBGP-INRAE	PQ100037		BTBUG055-24
D. escalerae	JSTR00047_0103	Spain	Huesca	Broto	42.603935	-0.121963	15/08/2011	♀	CBGP-INRAE	PQ100047		BTBUG056-24
D. escalerae	JSTR00047_0104	Spain	Huesca	Broto	42.603935	-0.121963	15/08/2011	♀	CBGP-INRAE	PQ100045		BTBUG057-24
D. escalerae	JSTR00273_0401	France	Alpes-de-Haute-Provence	Uvernet-Fours	44.318171	6.631361	01/09/2012	♂	CBGP-INRAE	PQ100036		BTBUG058-24
D. escalerae	JSTR00273_0402	France	Alpes-de-Haute-Provence	Uvernet-Fours	44.318171	6.631361	01/09/2012	♀	CBGP-INRAE	PQ100043		BTBUG059-24
D. escalerae	JSTR10720_0101	France	Alpes-Maritimes	Roure	44.146440	7.097420	11/07/2020	♀	CBGP-INRAE	PQ100032		BTBUG060-24
D. escalerae	D32-2	Spain	Granada	Bubión	36.955278	-3.355834	2006		Sanchez & Cassis (2018)	KY274605.1		
D. escalerae	D47-1	Spain	Cádiz	Grazalema	36.759722	-5.365556	2006		Sanchez & Cassis (2018)	KY274606.1		
D. escalerae	D53-1	Spain	Huesca	Torla, Valle de Bujaruelo	42.707222	-0.120000	2005		Sanchez & Cassis (2018)	KY274607.1		
D. escalerae	D22	Spain	Cáceres	Trujillo	39.463889	-5.881945		♂	Sanchez et al. (2006)		DQ232680.1	
D. rubicundus	D100-1	Spain	Canary Islands	Tenerife, Tegueste	28.533889	-16.326389	2008		Sanchez & Cassis (2018)	KY274633.1		
D. rubicundus	D132-1	Spain	Canary Islands	La Gomera, Barranco de las Lajas	28.115833	-17.199445	2009		Sanchez & Cassis (2018)	KY274634.1		
D. rubicundus	D132-2	Spain	Canary Islands	La Gomera, Barranco de las Lajas	28.115833	-17.199445	2009		Sanchez & Cassis (2018)	KY274635.1		
D. rubicundus	D138-1	Spain	Canary Islands	La Palma, Breña Alta	28.665833	-17.808889	2009		Sanchez & Cassis (2018)	KY274636.1		
D. rubicundus	D138-2	Spain	Canary Islands	La Palma, Breña Alta	28.665833	-17.808889	2009		Sanchez & Cassis (2018)	KY274637.1		
D. rubicundus	D139-2	Spain	Canary Islands	La Palma, Tenagua	28.721944	-17.750834	2009		Sanchez & Cassis (2018)	KY274638.1		

continues on next page

Table 1. Continuation.

D. rubicundus	D139-2	Spain	Canary Islands	La Palma, Tenagua	28.721944	-17.750834	2009		Sanchez & Cassis (2018)	KY274638.1		
D. rubicundus	D98-1	Spain	Canary Islands	Tenerife, Los Realejos	28.380278	-16.598611	2008		Sanchez & Cassis (2018)	KY274639.1		
D. rubicundus	D18	Spain	Canary Islands	Tenerife, Los Realejos	28.392778	-16.610278		♂	Sanchez et al. (2006)		DQ232673.1	
D. rubicundus	D18-1	Spain	Canary Islands	Tenerife, Los Realejos	28.392778	-16.610278		♂	Sanchez et al. (2006)		DQ232674.1	
Macrolophus pygmaeus (Og)	JSTR00210_0801	France	Val d'Oise	Saint-Prix	49.022720	2.272140	02/08/2012		CBGP-INRAE	PQ100012		BTBUG061-24
Macrolophus sp.	JAS-2005	Spain	Murcia						Sanchez et al. (2006)		DQ232683.1	
Nesidiocoris tenuis (Og)	JSTR00218_0101	France	Bouches-du-Rhône	Tarascon	43.814800	4.656338	26/09/2012	♂	CBGP-INRAE	PQ100040		BTBUG062-24
Nesidiocoris tenuis (Og)		Spain	Murcia						Sanchez et al. (2006)		DQ232682.1	

Table 2. Mean intraspecific distance values calculated with the K2P evolution model which distinguishes transitions and transversions in substitutions between specimens studied for the clades *D. bolivari* Lindberg, 1934 / *D. tamaninii* Wagner, 1951, *D. escalerae* Lindberg, 1934 and *D. rubicundus* Blöte, 1929.

	Number of samples	**Mean intraspecific distance**	**Maximum distance**
D. bolivari/ tamaninii	42	0.004	[0–0.0076]
D. escalerae	10	0.009	[0–0.0157]
D. rubicundus	7	0.008	[0–0.0122]

Table 3. Mean interspecific distance values calculated with the K2P evolution model which distinguishes transitions and transversions in substitutions between specimens studied for the clades *D. bolivari* Lindberg, 1934 / *D. tamaninii* Wagner, 1951, *D. escalerae* Lindberg, 1934 and *D. rubicundus* Blöte, 1929.

	Number of samples	***D. bolivari/ tamaninii***	***D. escalerae***	***D. rubicundus***	**Outgroup 1**	**Outgroup 2**
D. bolivari/ tamaninii	42					
D. escalerae	10	0.12 [0.108–0.125]				
D. rubicundus	7	0.09 [0.083–0.098]	0.11 [0.109–0.136]			
Outgroup 1 *Nesidiocoris*	1	0.27 [0.267–0.276]	0.29 [0.293–0.296]	0.26 [0.257–0.261]		
Outgroup 2 *Macrolophus*	1	0.25 [0.248–0.264]	0.23 [0.230–0.235]	0.23 [0.233–0.237]	0.25 [0.246]	

version 1.10 of the relevant reads. Raw reads were aligned against this first *Dicyphus* mtDNA genome by means of BWA v. 0.7.17 and the consensus genome was subsequently re-aligned using BioEdit 7.2.5, and manually inspected for possible inconsistencies.

Protein coding genes (PCG), tRNAs and rRNAs were annotated by the MITOS WebServer (http://mitos2.bioinf.uni-leipzig.de/index.py) and blasted against the NCBI database of mitochondrial sequences. Further finetuning of the PCG sequences was done by means of the Open Reading Frame Finder portal of NCBI (https://www.ncbi.nlm.nih.gov/orffinder/). tRNA prediction by MITOS was verified by tRNAscan-SE v1.3.172.

Results

Dicyphus (*Dicyphus*) *bolivari* Lindberg, 1934

Dicyphus bolivari Lindberg, 1934: 12
= *Dicyphus* (*Dicyphus*) *bolivari atlanticus* Wagner, 1951: 29 (syn. Sanchez & Cassis 2018: 39). **Confirmed synonymy.**
= *Dicyphus* (*Dicyphus*) *maroccanus* Wagner, 1951: 19 (syn. Sanchez & Cassis 2018: 39). **Confirmed synonymy.**
= *Dicyphus* (*Dicyphus*) *tamaninii* Wagner, 1951: 16. **New synonymy.**

Type material examined. ***Dicyphus bolivari*** Lindberg, 1934. Holotype: **SPAIN:** ♂ (brachypterous): '[S:a Morena. St:a Helena 4 — 8 4 26 Lindberg] / [Coll. Lindberg] / [Spec. typ. *Dicyphus bolivari* Lindberg] / [MUS ZOOL HELSINKI loan nr HE 06-44.' (MZHF)]. Specimen not dissected, the left paramere was visible in lateral view (Figs 1A–B) and we have opted not to take the risk of damaging it. – Paratypes: **CANARY ISLANDS:** ♀ (macropterous) [GZ50300], Spain: Canary Islands, Tenerife, Santa Ursula 18.V.1947 Lindberg, Hakan leg. (MZHF); ♂ (macropterous) [GZ50301], Spain: Canary Islands, Tenerife, Santa Ursula 18.V.1947 Lindberg, Hakan leg. (MZHF); ♀ (macropterous) [GZ50309], Spain: Canary Islands, Tenerife, Santa Cruz 4.IV.1949 Lindberg, Hakan leg. / Photographied 2022 Pekka Maninen (MZHF); ♀ (macropterous) [GZ50313], Spain: Canary Islands, Tenerife, Santa Cruz 4.IV.1949 Lindberg, Hakan leg. (MZHF); ♀ (macropterous) [GZ50314], Spain: Canary Islands, Tenerife, Santa Cruz 4.IV.1949 Lindberg, Hakan leg. (MZHF); ♂ (macropterous, dissected): Spain: Canary Islands, Tenerife, Santa Cruz 4.IV.1949 Lindberg, Hakan leg. (Figs 1E–G) (MZHF); ♀ (macropterous, dissected) [GZ50316], Spain: Canary Islands, Tenerife, Santa Cruz 14.I.1949 Lindberg, Hakan leg. (Figs 1H, J) (MZHF).

Dicyphus bolivari atlanticus Wagner, 1951. Paratype: **CANARY ISLANDS:** ♀ (macropterous) [GZ50324], Spain: Canary Islands, Tenerife, Santa Cruz 4.IV.1949 Lindberg, Hakan leg. / Photographied 2022 Pekka Maninen. Paratype *D. bolivari atlanticus* E.Wagn / coll. Lindberg. (MZHF).

Dicyphus tamaninii Wagner, 1951. Paratypes: **CROATIA:** ♀ (macropterous, dissected): [Dalmatien, Split 13.5.43 Novak leg. *Hyoscyamus niger* L] / [Paratypus *Dicyphus tamaninii* n.sp. E.Wagner det. Paratypoid *Dicyphus tamaninii* n.sp.] / [ZMH 838481] (Figs 1I, K) (ZMUH); ♀ (macropterous, dissected), [Palagruža D. Novak 19.5.49 *Hyoscyamus niger* L] / [Paratypoid *Dicyphus tamaninii* n.sp. E. Wagner det.] / [ZMH 838485] (ZMUH); ♂ (brachypterous), [O Sušac D., 20.6.49, Novak leg. *Hyoscyamus niger* L] / [Paratypoid *Dicyphus tamaninii* n.sp. E. Wagner det.] / [ZMH 838488] (ZMUH). Not dissected, the left paramere was visible in lateral view (Figs 1C–D) and we have opted not to take the risk of damaging it. ♀ (macropterous), [Dalmatien Split 19.5.43 Novak leg. *Hyoscyamus niger* L] / [Paratypus *Dicyphus tamaninii* n.sp. E.Wagner det. Paratypoid *Dicyphus tamaninii* n.sp.] / [ZMH] / [ZMH 838489] (ZMUH).

Additional material identified as *D. bolivari* or *D. tamaninii*. More than 700 specimens of both sexes from the following collections were studied: AMPF, BABN, CBGP-INRAE, JSMF, MLGU, MNHN, MTDU and RMNH, from the following locations: Northern Cyprus (new record: Famagouste, 25 V 2007, AMPF), England, France including Corsica (departments: Aisne, Alpes-Maritimes, Aude, Bouches-du-Rhône, Charente, Cher, Côtes d'Armor, Drôme, Finistère, Haute-Garonne, Gironde, Hérault, Haute-Loire, Loire-Atlantique, Lozère, Manche, Marne, Orne, Pyrénées-Orientales, Paris, Sarthe, Tarn, Var, Vaucluse), Germany, Greece including Crete, Channel Islands: Guernsey (new record: Moulin Huet, 3.viii.2020, BABN), Lebanon, Monaco (new record: Glacis du Palais, 10/18.vii.2010, AMPF), Morocco, the Netherlands (from 52 different localities), Sardinia (new record: Sassari, road 292 near Villanova Monteleone, 1.vi.2001, AMPF), Spain, and Asian Turkey (new record: Burdur, road from Köseler to Kargi, bridge across the Aksu Cayi, 7.vii.1999, AMPF). Fresh material was collected from 2005 to 2022 and preserved in 96% ethanol for DNA analyses. All specimens sequenced are listed in Table 1.

Diagnosis. Adults. Males and females not very different. Macropterous and brachypterous forms known in both sexes. Habitus (Fig. 2B) similar to most *Dicyphus* species of the subgenus *Dicyphus* s. str., with no real distinguishing characters. Identification requires dissection of the male or female genitalia.

Size. Males: brachypterous 2.8–3.2 mm, macropterous 3.8–4.3 mm; females: brachypterous 2.9–3.5 mm; macropterous 4.0–4.4 mm.

Habitus usually pale, macropterous sometimes with reddish tinge. Head marked by two broken dark lines, eyes brownish. Antennal segment I bicoloured, base and apex with reddish-brown annulations, II basally lightly stained with faded brown, apex more or less dark brown, III and IV greyish to dark. Pronotum: underside of mesothorax sometimes black. Legs pale with short dark pilosity, hind tibiae with long dark spines, widely spaced, last tarsal segment black. Hemelytron pale or iridescent with semi-erect pale brown pilosity, two dark spots anterior to cuneal fracture, apex of cuneus black. Male genitalia: left paramere apophysis elongate (245 to 367 µm from outer margin of base to tip of shaft); shaft clearly demarcated and weakly sinuate (Figs 2A, C); endosoma with 2 to 13 small sclerites (Fig. 2D). Female genitalia: genital chamber characterised by cordiform shape, sometimes simply rounded, with edge of the sac folded into very characteristic accordion shape. Oviducts are swollen at base, well separated and generally directed downward (Figs 1J–K).

Morphological variability. ***External characters*.** We have studied more than 700 specimens identified as *D. bolivari* or *D. tamaninii* and sometimes both depending on the specialist responsible for the identification, of which 103 males and 55 females were dissected. We concur with Sanchez & Cassis (2018) that the body size and external characters of *D. bolivari* and *D. tamaninii* overlap strongly and we did not find any morphological characters such as size, proportions of different appendages, segments, head, pronotum etc., nor colouration, wing polymorphism that would justify a separation into two or more taxa.

Male genitalia. Sanchez & Cassis (2018) continue their diagnosis of *D. tamininii* versus *D. bolivari* by "species separation is based on differences in the male genitalia, especially the size of the left paramere and the number of endosomal lobal sclerites". In fact, the shape of the left paramere does not vary very much between the two species. The differences reported by Wagner (1951, 1974) and Wagner & Weber (1964) can be interpreted as different orientations of the paramere or, in the best

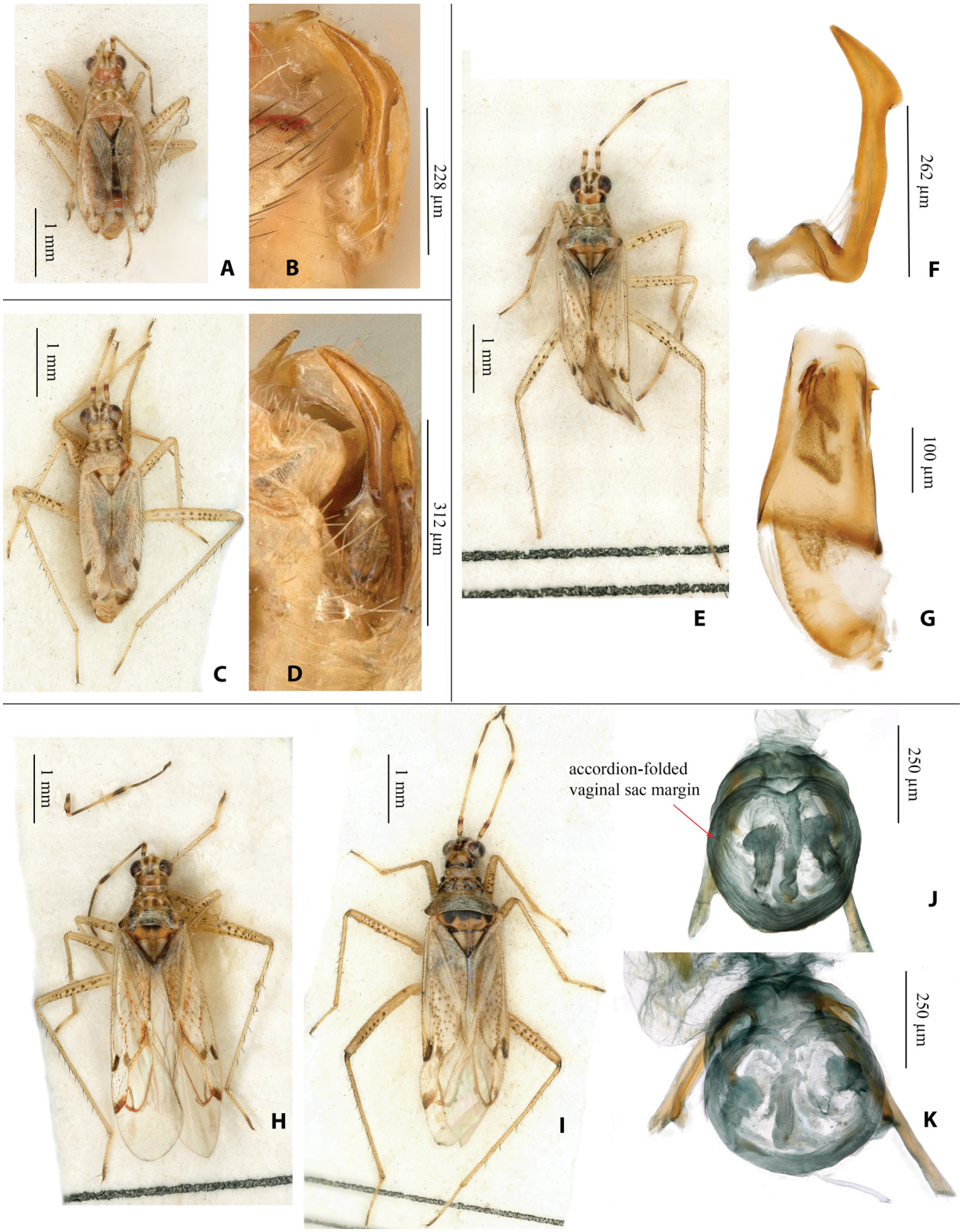

Fig. 1. Type material examined for *Dicyphus bolivari* Lindberg, 1934 and *D. tamaninii* Wagner, 1951. A–B – *D. bolivari*, holotype, brachypterous male (A – habitus, B – left paramere). C–D – *D. tamaninii*, paratype ZMH838488, brachypterous male (C – habitus, D – left paramere). E–G – *Dicyphus bolivari*, paratype GZ50315, macropterous male (E – habitus, F – paramere, G – endosoma). H – *D. bolivari*, paratype GZ50316, macropterous female. I – *D. tamaninii*, paratype ZMH838481, macropterous female. J – *Dicyphus bolivari*, paratype GZ50316, female genitalia. K – *D. tamaninii*, paratype ZMH838481, female genitalia.

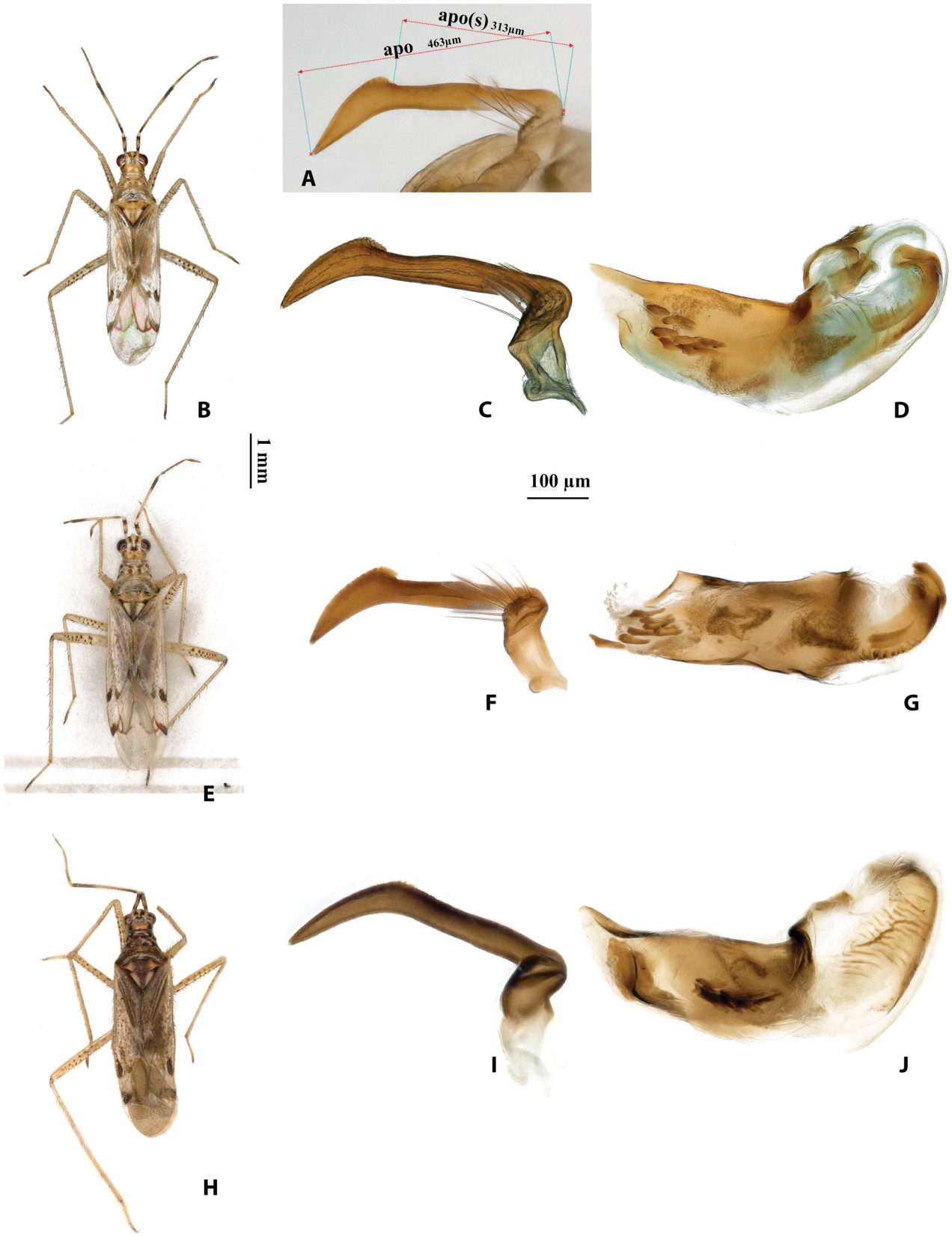

Fig. 2. Habitus and male genitalia of *Dicyphus* (*Dicyphus*) species with a long left paramere apophysis. A – *Dicyphus bolivari* Lindberg, 1934, JSTR08457_0101 from France, left paramere and measures. B – *D. bolivari*, male from Morocco. C–D – *D. bolivari*, from Greece (C – left paramere, D – endosoma). E–G – *D. lindbergi* Wagner, 1951, male from Cyprus (E – habitus, F – left paramere, G – endosoma). H–J – *D. rubicundus* Blöte, 1929, male from the Canary Islands (H – habitus, I – left paramere, J – endosoma). Abbreviations: apo – apophysis length; apo(s) – shaft length. Scale bar = 1 mm for habitus; 100 µm for parameres and endosoma.

Fig. 3. Molecular phylogeny of three *Dicyphus* species inferred by maximum likelihood (RAxML) using sequence data of COI mitocondrial gene. *Nesidiocoris tenuis* (Reuter, 1895) and *Macrolophus pygmaeus* (Rambur, 1839) were used as outgroups to root the tree. Numbers above the branches are bootstrap (BS) values for 1000 replicates (only BS > 75 have been retained).

case, as intra-specific variability (e.g. Figs 2A and 2C). Sanchez & Cassis (2018) measured the apophysis from "outer margin of the base to tip of shaft" and gave the following measurement ranges to distinguish the two species: 440–450 µm for *D. bolivari* and 530–600 µm for *D. tamaninii*. We estimated these measurements on the holotype of *D. bolivari* (a brachypterous male not dissected) and the paratype ZMH838488 (also a brachypterous male) and found 228 µm for the holotype of *bolivari* and 312 µm for the paratype of *tamaninii* which is a larger specimen (Figs 1A–D). We also measured the shaft of apophysis of 20 males identified as *D. bolivari*/*tamaninii*. It varies between 245 and 367 µm. Obviously, Sanchez & Cassis (2018) gave measurements that do not correspond to what they have defined in their material and methods (Sanchez & Cassis 2018: 343: fig. 9f) and used in their key. Another character given by these authors is the number of sclerites of the endosoma: 2–5 for *D. tamaninii*; 8–12 (rarely 6–7) for *D. bolivari.* These sclerites are easy to observe (Fig. 2D) but much more difficult to count, especially when the penis is not inflated. We managed to evaluate the number of endosomal sclerites in 35 males and found that the number of sclerites varied from 4 to 13. We noticed that we could not verify that a low number of sclerites (2–5) was associated with a long apophysis (for *D. tamaninii*) and a high number of sclerites (8-12) was associated with a short apophysis (for *D. bolivari*). The apophysis of the male with 4 sclerites measured 275 µm, of that with 13 sclerites 347 µm. Under these conditions it is not possible to distinguish the two species, nor to use Sanchez & Cassis's (2018) key. There is an intraspecific variation in the male genitalia but none of the criteria used by the authors allow the delimitation of two or more taxa.

Female genitalia. The female genitalia of the subgenus *Dicyphus* have never been studied. They will be described and illustrated in another paper to be published. We have dissected one paratype of *D. bolivari* (GZ50316) and two of *D. tamaninii* (ZMH838481 and ZMH838485) as well as 55 other females. The genital chamber is illustrated for *D. bolivari* paratype GZ50316 (Fig. 1J) and for *D. tamaninii* paratype ZMH838481 (Fig. 1K). The female genitalia of these two putative taxa are very homogeneous and of a single type. This is particularly true for the two paratypes (compare Figs 1J to 1K). This genital chamber is characterised by a cordiform shape, sometimes simply rounded, but above all by the margin of the sac, which is folded into a very characteristic accordion shape. The two oviducts are swollen at the base, well separated and generally directed downwards. This type of vaginal sac is very similar to those of *D. tumidifrons* Ribes, 1997 and *D. escalerae*, the closest relatives, but allows to separate these taxa from most other species in the subgenus *Dicyphus* (study in press). Again, this character cannot be used to separate *D. bolivari* and *D. tamaninii*.

Differential diagnosis. *Dicyphus bolivari* has the left paramere with a long apophysis and an endosoma with several small sclerites. It shares these characters with two other species, *D. rubicundus* and *D. lindbergi* (Fig. 2). *Dicyphus rubicundus* can be easily separated from other species by the shape of its left paramere where the apex of the apophysis is continuous with the shaft without a clear demarcation unlike *D. bolivari* (Fig. 2I compared to Figs 2C and 2F). It can also be differenciated by its barcode sequence. The vaginal sac of the *D. bolivari* female is very similar to that of *D. rubicundus*. The case of *D. lindbergi* is less obvious. Sanchez & Cassis (2018) commentated on *D. lindbergi*: '*Dicyphus lindbergi* and *D. bolivari* are very similar in shape and colour (fig. 33), and the former species can only be confidently separated by the apophysis of the left paramere being shorter (figs 9C–F, 10E) and the fewer small endosomal lobal sclerites (N = 5 cf. N = 6–12) (figs 12C, 14C, D)'. We saw previously that these characters (size of the apophysis of the left paramere and the number of endosoma sclerites) were not relevant to differentiate *D. bolivari* from *D. tamaninii* because of their rather high variability. We were only able to examine two males of *D. lindbergi* but unfortunately no females. Furthermore, neither COI nor Cytb have been sequenced in this species to date. The male we have dissected from Cyprus (Figs 2E–G) has a 248 µm shaft of the apophysis and 7 endosomal sclerites which is in the range of *D. bolivari*. Therefore, the key provided by Sanchez & Cassis (2018) does not allow us to differentiate this species from *D. bolivari*.

Molecular results. We produced 33 sequences of the COI standard barcode marker from specimens identified morphologicaly as *D. bolivari* or *D. tamaninii* from the following countries: England, France (Bretagne, Corse, Pays-de-la-Loire, Centre-Val-de-Loire, Grand-Est, Occitanie), Germany, Guernsey, the Netherlands, Spain and for the analysis we added the sequences published by Sanchez & Cassis (2018) under the name *D. bolivari* from Spain and the Canary Islands. All specimens and their sequences are listed in Table 1.

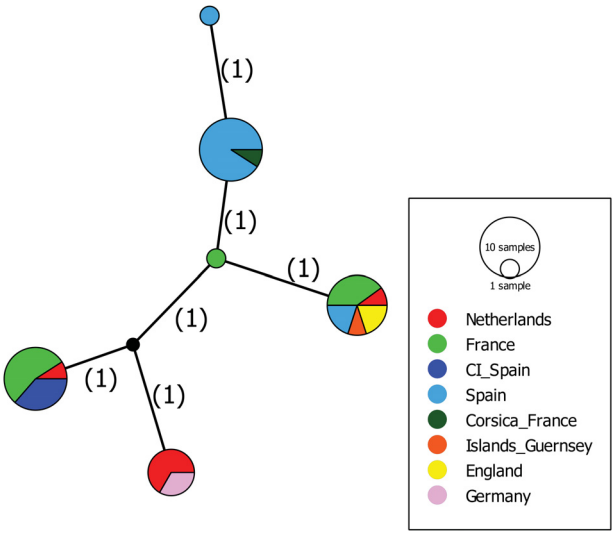

Fig. 4. Haplotype network of 40 sequenced *Dicyphus bolivari* Lindberg, 1934 / *D. tamaninii* Wagner, 1951 specimens. Each circle corresponds to a haplotype, the size of the circle is proportional to the frequency of the haplotype in the dataset. The length of the segments between each haplotype is proportional to the number of mutations between them (mutational steps). The dark square symbolizes a haplotype not detected during the study but necessary to build the network (missing haplotypes or lost through evolutionary drift).

Fig. 3 shows the phylogenetic tree obtained. All specimens from Spain to the Netherlands are grouped in a single well-supported cluster (bootstrap 100%) and clearly differentiated from *D. escalerae* and *D. rubicundus*. The intraspecific distances are 0.4% with a maximum of 0.76% for the *D. bolivari*/*tamaninii* clade, comprising 42 specimens from the Canary Islands to the Netherlands, in comparison with the intraspecific variation of *D. escalerae* (0.9%) and *D. tumidifrons* (0.8%), for which sampling is much more limited (Table 2). Interspecific distances between the clades defined by the phylogenetic tree (Fig. 3) are 9% (minimum 8.3%) between *D. bolivari*/*tamaninii* and *D. rubicundus* and 12% (minimum 10.8%) between *D. bolivari*/*tamaninii* and *D. escalerae* (Table 3). There are no significant differences between our sequences from northern Europe and those from Spain and the Canary Islands published by Sanchez & Cassis (2018). We constructed a network of haplotypes from a dataset of 40 sequences of *D. bolivari* and/or *D. tamaninii* (Fig. 4). We eliminated two specimens with sequences too short to construct the haplotype network. This network comprises 6 haplotypes differing by a maximum of 4 mutations and shows no obvious geographical structuring.

Sanchez et al. (2006) produced a Cytb fraction of 381 bp from 5 specimens of *Dicyphus* identified as *D. tamaninii*. We constructed a phylogenetic tree (Fig. 5) using their sequences and 20 sequences from our dataset (Table 1). The Cytb sequences obtained from our specimens are exactly the same as those identified as *D. tamaninii* by Sanchez et al. (2006). The COI sequences of our 20 specimens are the same as those identified as *D. bolivari* by Sanchez & Cassis (2018).

We also generated the complete mitochondrial genome for a specimen of *D. bolivari*/*tamaninii* (NCBI accession number: PP746700). The sequences identified as *D. bolivari* for COI by Sanchez & Cassis (2018) and *D. tamaninii* for Cytb by Sanchez et al. (2006) are associated in the mitochondria of a single specimen.

Host plants. *Dicyphus bolivari* and *D. tamaninii* as defined by previous authors have a wide range of host plants, especially Solanaceae and Asteraceae, and at least two common host plant genera, i.e. *Hyoscyamus* and *Solanum*. For a list of the known host plant species see Table 4.

Distribution. Europe: Belgium (Aukema 2020), Bosnia and Hercegovina (Protić 1998), Channel Islands: Guernsey (new record), Croatia (Sanchez & Cassis 2018),

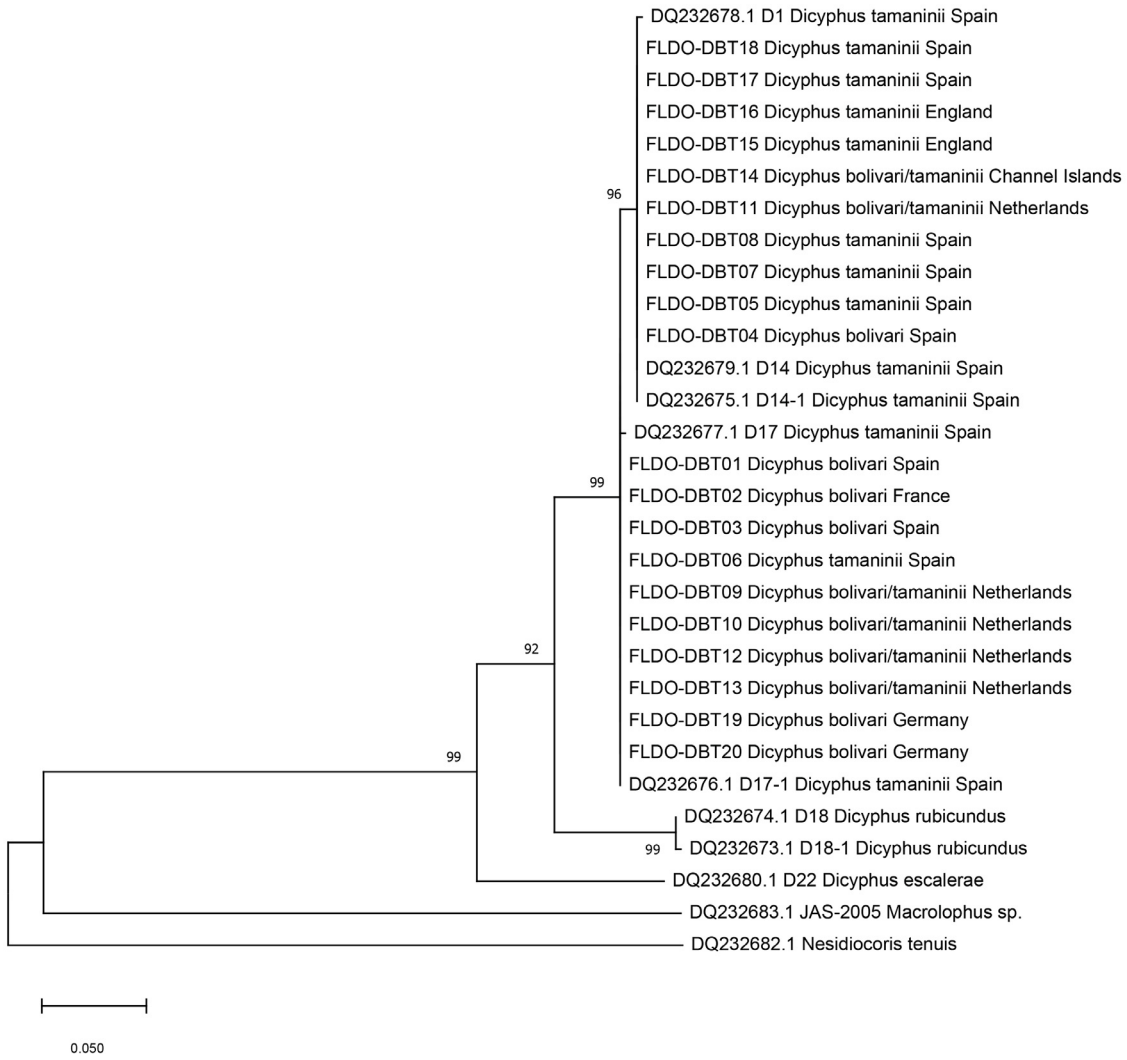

Fig. 5. Molecular phylogeny of three *Dicyphus* species inferred by maximum likelihood (RAxML) using sequence data of Cytb mitochondrial gene. *Nesidiocoris tenuis* (Reuter, 1895) and *Macrolophus* sp. were used as outgroups to root the tree. Numbers above the branches are bootstrap (BS) values for 1000 replicates (only BS > 75 have been retained).

France including Corsica (Wagner & Weber 1964), Germany (Simon 2020), Great Britain (Telfer 2015), Greece (Sanchez & Cassis 2018), including Crete (Heiss et al. 1993), Italy (Wagner 1951) including Sardinia (new record), Luxembourg (Aukema 2020), Malta (Carapezza & Mifsud 2015), Monaco (new record), the Netherlands (Aukema 2020), Spain (Lindberg 1934). Asia: Cyprus (new record), Iran (Abd-Rabou & Ghahari 2006), Israel (Linnavuori 1961), Lebanon (Matocq & Azard 2023), Asian part of Turkey (new record). North Africa: Canary Islands (Wagner 1951), Morocco (Wagner 1951, as *D. maroccanus*), Tunisia (Wagner 1951).

Discussion

We studied more that 700 specimens identified as *D. bolivari* or *D. tamaninii* representative of the known geographic area of these two taxa. We did not find any external characters to differentiate them as two taxonomic entities. The study of male genitalia and, for the first time, female genitalia also failed to separate the two taxa. The differences highlighted by previous studies (Wagner, 1951, 1974; Wagner & Weber, 1964; Sanchez & Cassis 2018) should be considered intraspecific variability.

To support our morphological results, we conducted a molecular study on two mitochondrial genes (COI and Cytb). In the whole geographical area, we found only one mitochondrial type for the two genes. These sequences vary little, with no geographical structure, which can be interpreted as intraspecific variability at the same level as that observed in the other *Dicyphus* species studied. Our sequences are identical to those published by Sanchez et al. (2006) and Sanchez & Cassis (2018), but unlike these authors, we have sequenced these two genes from the same specimens, which shows that both sequences are present in the same individuals. This result is confirmed by the sequencing of the mitogenome where the two sequences named differently in the previous publications are found in the same mitochondrion.

The COI gene was studied here as the marker chosen by the International Barcode of Life Consortium to describe animal biodiversity. As a result, it is the gene with the most extensive data in international genetic databases (Pentinsaari et al. 2016) and it has also proven its effectiveness in species delimitation and identification of cryptic species, including Heteroptera (Raupach et al. 2014; Namyatova et al. 2024). The fact that it had previously been successfully

Table 4. Host plants of *D. bolivari* Lindberg, 1934 / *D. tamaninii* Wagner, 1951. Plant taxonomy follows the World Flora Online list (https://www.worldfloraonline.org/).

Familly	Host plants	Reference	Taxon
Amaranthaceae	*Amaranthus* sp.	Alomar et al. (1994)	*D. tamaninii*
Amaranthaceae	*Atriplex* sp.	Alomar et al. (1994)	*D. tamaninii*
Asteraceae	*Calendula arvensis* Batt.	Alomar et al. (1994)	*D. tamaninii*
Asteraceae	*Centaurea* sp.	Alomar et al. (1994)	*D. tamaninii*
Asteraceae	*Dittrichia viscosa* (L.) Greuter	Alomar et al. (1994)	*D. tamaninii*
Asteraceae	*Erigeron annuus* Sessé & Moc.	Alomar et al. (1994)	*D. tamaninii*
Asteraceae	*Erigeron canadensis* Ten.	Alomar et al. (1994)	*D. tamaninii*
Asteraceae	*Galactites tomentosa* Moench	Alomar et al. (1994)	*D. tamaninii*
Asteraceae	*Sonchus* sp.	Alomar et al. (1994)	*D. tamaninii*
Borraginaceae	*Borago officinalis* L.	Alomar et al. (1994)	*D. tamaninii*
Borraginaceae	*Cynoglossum* sp.	Alomar et al. (1994)	*D. tamaninii*
Cistaceae	*Cistus monspeliensis* L.	Alomar et al. (1994)	*D. tamaninii*
Cistaceae	*Cistus salviifolius* L.	Alomar et al. (1994)	*D. tamaninii*
Cucurbitaceae	*Ecballium elaterium* (L.) A.Rich.	Jung & Kim (2023)	*D. bolivari*
Cucurbitaceae	*Lagenaria siceraria* (Molina) Standl.	Sanchez & Cassis (2018)	*D. bolivari*
Euphorbiaceae	*Euphorbia* sp.	Alomar et al. (1994)	*D. tamaninii*
Euphorbiaceae	*Mercurialis* sp.	Alomar et al. (1994)	*D. tamaninii*
Fabaceae	*Ononis natrix* L.	Sanchez & Cassis (2018)	*D. bolivari*
Geraniaceae	*Geranium* sp.	Alomar et al. (1994)	*D. tamaninii*
Malvaceae	*Lavatera* sp.	Alomar et al. (1994)	*D. tamaninii*
Onagraceae	*Epilobium hirsutum* L.	Wagner & Weber (1964)	*D. bolivari*
Plantaginaceae	*Digitalis atlantica* Pomel	Wagner (1974)	*D. maroccanus*
Rubiaceae	*Galium* sp.	Alomar et al. (1994)	*D. tamaninii*
Solanaceae	*Datura stramonium* L. test	Sanchez & Cassis (2018)	*D. bolivari*
Solanaceae	*Hyoscyamus albus* L.	Sanchez & Cassis (2018)	*D. bolivari*
Solanaceae	*Hyoscyamus niger* L.	Wagner (1974)	*D. tamaninii*
Solanaceae	*Solanum lycopersicum* L.	Sanchez & Cassis (2018)	*D. bolivari*
Solanaceae	*Solanum nigrum* Tausch ex Dunal	Alomar et al. (1994)	*D. tamaninii*
Urticaceae	*Parietaria officinalis* L.	Alomar et al. (1994)	*D. tamaninii*
Urticaceae	*Urtica* sp.	Alomar et al. (1994)	*D. tamaninii*

used by Sanchez & Cassis (2018) to delimit *Dicyphus* species also provided an opportunity to compare our results with theirs. However, being a mitochondrial gene, COI has major limitations when it comes to tracing evolutionary processes, especially due to introgression and *Wolbachia* (Toews & Brelsford 2012). Sequencing of nuclear genes can be useful to overcome such biases. However, our molecular results were consistent with morphological, biogeographical and biological results; they confirmed results from previous molecular studies, and the aim was to establish a synonymy rather than a phylogeny. Under these conditions, we felt it would be pointless to sequence nuclear genes.

Jung & Kim (2023) in their World phylogeny of the genus *Dicyphus* suggested several taxonomic changes but without formally establishing them. Their phylogeny is based on a matrix of 52 morphological characters, focusing on the size and colouration of the vestiture, body, head, and appendages. Many of these characters, such as colouration, exhibit high variability within species, while others, such as the size of segment IV of the antennae, are often distorted or broken by desiccation. These authors used only four characters from male genitalia and two from female genitalia (based on the original descriptions, as they did not examine female genitalia). The number of specimens studied is low (e.g. only one male of *D. bolivari* and three males of *D. tamaninii*), and only macropterous specimens were coded; if such specimen was not available original descriptions were used. All species were illustrated in Plate 2, with some illustrated only by brachypterous specimens [e.g. *D. errans*, which is known only from macropterous specimens (Sanchez & Cassis 2018, Konstantinov & Neimorovets 2021)]. We have little confidence in the results obtained due to: i) the inappropriate choice of characters, which exhibit high intraspecific variability and low discriminatory power between species, ii) the insufficient consideration of intraspecific variability with too few coded individuals, iii) concerns about the identification of certain specimens. The clades recovered are surprising and not consistent with previous studies based on either molecular or morphological data. Some taxa previously considered synonymous are placed in distant clades and male and female genitalia which are relevant for species delimitation are not discussed. Under these conditions, we adopted the synonymies proposed by Sanchez & Cassis (2018) (i.e., *D. maroccanus* and *D. bolivari altanticus* synonymised with *D. bolivari*), which are better supported by a much larger sampling, better consideration of intraspecific variability, more relevant morphological characters, molecular and morphometric data.

Jung & Kim (2023) also propose in their paper the description of a new *Dicyphus* species which they do not formally name until more specimens are avalaible but which they refer to as *Dicyphus* n. sp. 1. The diagnosis given uses non-informative characters to distinguish these specimens from other species of *Dicyphus* with the exception of the description of the left paramere illustrated in Plate 4. They give no information on the endosoma and its sclerites but code this character in 0 'multiple sclerites'. The two specimens studied from Spain (Barcelona) are compared with *D. alkannae* from Turkey which has very different male genitalia and with *D. tamaninii* but not with the other taxa in the authors' possession (*D. bolivari*, *D. bolivari atlanticus* and *D. maroccanus*) which have the same male genitalia and are known to occur in Spain. As stated in their paper, the number of specimens observed (only 2 males) is insufficient to draw conclusions, but the description of the paramere corresponds exactly to *D. bolivari*, a species well known in the Barcelona region and currently the only one with this type of paramere in all of Europe.

So far, there are three species of *Dicyphus* that have male genitalia with a very long apophysis of the left paramere and an endosoma with a series of small sclerites: *D. bolivari*, *D. lindbergi* and *D. rubicundus*. *Dicyphus rubicundus* is an endemic species of the Canary Islands and seems to be associated with several plants of the genus *Aeonium* (Crassulaceae). *Dicyphus lindbergi* is restricted to middle Asia (Cyprus, Lebanon, Jordan and Syria) and lives on the genus *Hyoscyamus* (Solanaceae). As interpreted here, *D. bolivari* is widespread in Europe, the Near East and North Africa on a variety of plants from different families.

Conclusion

After examination of the types of *D. bolivari* and *D. tamaninii* and specimens from all over Europe identified by several specialists as *D. bolivari* or/and *D. tamaninii*, we did not find any morphological character (externally, on male and female genitalia) that could justify separation of the two taxa. The results of our molecular analyses (COI, Cytb and mtDNA) and those of previous publications are the same: throughout Europe there is only one clade for these two taxa, composed of haplotypes that are little different from each other. Furthermore, no biogeographic or biological evidence has been observed to help separate the two taxa. Under these conditions we propose the following synonymy: *Dicyphus* (*Dicyphus*) *bolivari* Lindberg, 1934 = *Dicyphus* (*Dicyphus*) *tamaninii* Wagner, 1951, syn. nov.

Dicyphus bolivari is a widespread species and one of the most frequently observed in agrosystems (e.g. Castañé et al. 1996, Alomar et al. 1994). It has often been cited as *D. tamaninii* in an agronomic context when studied both in the laboratory and for biological control through conservation (Barnadas et al. 1998, Castañé et al. 2004, Messelink et al. 2015). In Google Scholar more than 1300 citations refer to these two species, either directly (as the main subject of the study) or indirectly (as a term of comparison, taxa included in species lists, etc.), but almost 9 out of 10 citations are actually dedicated to *D. tamaninii.* Despite this the identification of both taxa remained doubtful. Crop beneficials often belong to genera whose taxonomy is difficult (e.g. *Macrolophus* Fieber, 1858 (Miridae), *Orius* Wolff, 1811 (Anthocoridae) for true bugs, *Chrysoperla* Steinmann, 1964 for neuropterans), but it is essential to distinguish the different species precisely as their biology is not equivalent even between closely related ones. For example, *Macrolophus pygmaeus* (Rambur, 1839) and *M. melanotoma* (A. Costa, 1853) which were confused for many years, do not share the same host plants. The former

establishes itself on tomato crops while the latter remains restricted to *Dittrichia viscosa*; leading agronomists who confused the two species advised planting *D. viscosa* around tomato greenhouses in the hope that *Macrolophus* would transfer to the crop. In fact, none of these beneficial insects ever reached the tomato (Bout et al. 2019). On the other hand, our integrative taxonomic studies of *D. bolivari* and *D. tamaninii* have shown that they form a single species, which will clarify the situation and allow the use of the abundant literature on them in a sustainable plant health context.

Acknowledgements

We are indebted to Eileen Nguyen (ZMUH) and Heidi Viljanen (MZHF) for providing access to type specimens from MZHF and ZMUH. We are also obliged to colleagues who shared their specimens with us, increasing the geographical area studied, particularly Thomas Cherpitel and Philippe Loncle from the French association of Heteropterists 'Zicrona', and to Max Caspers (RMNH), Mark Lawlor (Guernsey), Helga Simon (Germany) and Mark G. Telfer (United Kingdom) for providing material for sequencing.

References

ABD-RABOU S. & GHAHARI H. 2006: Predators of whiteflies (Homoptera: Aleyrodidae) in Iran. *Trends in Entomology* **5**: 41–46.

ABRAÇOS-DUARTE G., RAMOS S., VALENTE F., BORGES da SILVA E. & FIGUEIREDO E. 2021: Functional response and predation rate of *Dicyphus cerastii* Wagner (Hemiptera: Miridae). *Insects* **12 (6) (530)**: 1–15.

ALOMAR O., GOULA M. & ALBAJES R. 1994: Mirid bugs for biological control: identification, survey in non-cultivated winter plants, and colonization of tomato fields. *IOBC/WPRS Bulletin* **17**: 217–223.

AUKEMA B. (ed) 2024: *Catalogue of the Palearctic Heteroptera*. Available at https://catpalhet.linnaeus.naturalis.nl (Last access February 10, 2024.)

AUKEMA B. 2020: Nieuwe en interessante Nederlandse wantsen X (Hemiptera: Heteroptera). (New and interesting Dutch bugs X (Hemiptera: Heteroptera)). *Nederlandse Faunistische Mededelingen* **55**: 49–72 (in Dutch, English summary).

BARNADAS I., GABARRA R. & ALBAJES R. 1998: Predatory capacity of two mirid bugs preying on *Bemisia tabaci*. *Entomologia Experimentalis et Applicata* **86**: 215–219.

BOUT A., LAMBION J., QUAGLIETTI B., ION-SCOTTA M., RUIZ I., LE GOFF I., RIS N. & STREITO J.-C. 2019: *M. pygmaeus*, une punaise auxiliaire pleine de ressources. *Phytoma* **724**: 41–44.

CARAPEZZA A. & MIFSUD D. 2015: New records of true bugs (Hemiptera, Heteroptera) from the Maltese Islands. *Bulletin of the Entomological Society of Malta* **7**: 27–50.

CASTAÑÉ C., ALOMAR O., GOULA M. & GABARRA R. 2004: Colonization of tomato greenhouses by the predatory mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii*. *Biological Control* **30**: 591–597.

CASTAÑÉ C., ALOMAR O. & RIUDAVETS J. 1996: Management of Western Flower Thrips on Cucumber with *Dicyphus tamaninii* (Heteroptera: Miridae). *Biological Control* **7 (1)**: 114–120.

DOORENWEERD C., SAN JOSE M., LEBLANC L. & RUBINOFF D. 2023: Inadequate molecular identification protocols for invasive pests threaten biosecurity. *Systematic Entomology* **48 (3)**: 355–360.

HEBERT P. D. N., CYWINSKA A., BALL S. L. & De WAARD J. R. 2003: Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* **270**: 313–321.

HEISS E., GÜNTHER H., RIEGER Ch. & MALICKY H. 1993: Heteroptera collected by light traps in Crete (Heteroptera from the Island of Crete VIII). *Biologia Gallo-Hellenica* **20**: 107–114.

INGEGNO B. L., CANDIAN V., PSOMADELIS I., BODINO N. & TAVELLA L. 2017: The potential of host plants for biological control of *Tuta absoluta* by the predator *Dicyphus errans*. *Bulletin of Entomological Research* **107 (3)**: 340–348.

JUNG S.-H. & KIM J.-G. 2023: *Monograph of the plant bug genus Dicyphus*. National Institute of Biological Resources, Incheon, 111 pp.

KERZHNER I. M. & JOSIFOV M. 1999: Cimicomorpha II: Miridae. Pp. 1–577. In: AUKEMA B. & RIEGER C. (eds): *Catalogue of the Heteroptera of the Palaearctic Region. Vol. 3*. The Netherlands Entomological Society, Amsterdam, xiv + 577 pp.

KONSTANTINOV F. V. & NEIMOROVETS V. V. 2021: Bryocorinae Baerensprung, 1860 (Hemiptera: Heteroptera: Miridae) of European Russia and the Caucasus: synopsis and key to species. *Zootaxa* **4920 (3)**: 301–338.

LINDBERG H. 1934: Inventa entomologica itineris Hispanici et Maroccanni quod a. 1926 fecerunt Harald et Hakan Lindberg. XX. In Spanien gesammelte Miriden. *Societatis Scientiarium Fennica, Commentationes Biologicae* **4 (12)**: 1–23.

LINNAVUORI R. 1961: Hemiptera of Israel II. *Annales Zoologici Societatis Zoologicae Botanicae Fennicae 'Vanamo'* **22 (7)**: 1–51.

LIU G.-Q, MU Y.-R., XU J.-Y. & LIN L. 2022: *Hemiptera. Miridae III. Bryocorinae, Cylapinae, Deraeocorinae, Isometopinae and Psallopinae. Fauna Sinica, Insecta, Vol. 73*. Science Press, Beijing, xiii + 606 + 17 pls.

MATOCQ A. & AZAR D. 2023: Preliminary inventory of terrestrial Heteroptera from Lebanon (Hemiptera: Leptopodomorpha, Cimicomorpha and Pentatomomorpha). *Zootaxa* **5230 (1)**: 1–26.

MESSELINK G. J., BLOEMHARD C. M. J., HOOGERBRUGGE H., van SCHELT J., INGEGNO B. L. & TAVELLA L. 2015: Evaluation of mirid predatory bugs and release strategy for aphid control in sweet pepper. *Journal of Applied Entomology* **139**: 333–341.

NAMYATOVA A. A., DZHELALI P. A. & KONSTANTINOV F. V. 2024: Delimitation of the widely distributed Palearctic *Stenodema* species (Hemiptera, Heteroptera, Miridae): insights from molecular and morphological data. *ZooKeys* **1209**: 245–294.

NYLANDER J. A. A. 2004: *MrAIC.pl. Program distributed by the author*. Evolutionary Biology Centre, Uppsala University.

PENTINSAARI M., SALMELA H., MUTANEN M. & ROSLIN T. 2016: Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. *Scientific Reports* **6 (35275)**: 1–12.

PLUOT-SIGWALT D. & MATOCQ A. 2017: An investigation of the roof of the genital chamber in female plant-bugs with special emphasis on the "dorsal sac" (Hemiptera: Heteroptera: Miridae). *Annales de la Société Entomologique de France (Nouvelle S*érie*)* **53 (1)**: 1–16.

PROTIĆ L. 1998: Catalogue of the Heteroptera fauna of Yugoslav countries. Part One. *Prirodnjački Muzej u Beogradu, Posebna Izdanja* **38**: 1–215.

RAUPACH M. J., HENDRICH L., KÜCHLER S. M., DEISTER F., MORINIÈRE J. & GOSSNER M. M. 2014: Building-up of a DNA barcode library for true bugs (Insecta: Hemiptera: Heteroptera) of Germany reveals taxonomic uncertainties and surprises. PLOS ONE **9 (9) (e106940)**: 1–13 + on-line supplements.

SANCHEZ J. A. & CASSIS G. 2018: Towards solving the taxonomic impasse of the biocontrol plant bug subgenus *Dicyphus* (*Dicyphus*) (Insecta: Heteroptera: Miridae) using molecular, morphometric and morphological partitions. *Zoological Journal of the Linnean Society* **184**: 330–406.

SANCHEZ J. A., MARTINEZ-CASCALES J. I. & CASSIS G. 2006: Description of a new species of *Dicyphus* Fieber (Insecta: Heteroptera: Miridae) from Portugal based on molecular data. *Insect Systematics and Evolution* **37**: 281–300.

SIMON H. 2020: 4. Nachtrag zum Verzeichnis der Wanzen in Rheinland-Pfalz (Insecta: Heteroptera). *Fauna Flora Rheinland-Pfalz* **14 (2)**: 659–686.

STAMATAKIS A. 2006a: *Phylogenetic models of rate heterogeneity: A High Performance Computing Perspective*. International Parallel and Distributed Processing Symposium (IPDPS 2006), Rhodes Island, Greece, 8 pp.

STAMATAKIS A. 2006b: RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22 (21)**: 2688–2690.

STREITO J.-C., FONTAINE O., MORGUEN A., GENSON G., PIERRE E., SADEYEN J., FRAGO E. & NIBOUCHE S. 2018: Présence sur l'île de La Réunion de deux espèces de punaises prédatrices potentiellement utilisables pour la lutte biologique: *Orius naivashae* et *Cyrtopeltis callosus* (Hemiptera, Anthocoridae et Miridae). *Bulletin de la Société Entomologique de France* **123 (1)**: 29–42.

TELFER M. G. 2015: *Dicyphus tamaninii* (Hemiptera: Miridae) new to Britain. *British Journal of Entomology and Natural History* **28 (2)**: 71–74.

THOMPSON J. D., GIBSON T. J., PLEWNIAK F., JEANMOUGIN F. & HIGGINS D. G. 1997: The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25 (24)**: 4876–4882.

TOEWS D. P. L. & BRELSFORD A. 2012: The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology* **21**: 3907–3930.

WAGNER E. 1951: Zur Systematik der Gattung *Dicyphus* (Hem. Het., Miridae). *Commentationes Biologicae* **12 (6)**: 1–36.

WAGNER E. 1974: Die Miridae Hahn, 1831, des Mittelmeerraumes und des Makaronesischen Inseln (Hemiptera, Heteroptera). Teil 1. *Entomologische Abhandlungen Staatlichen Museum für Tierkunde Dresden, Supplementum* **37**: [1970–1971]: i–ii + 1–484.

WAGNER E. & WEBER H. H. 1964: *Hétéroptères Miridae. Faune de France. Vol. 67*. Fédération Française des Sociétés de Sciences Naturelles, Paris, 591 pp.