International Journal of Odonatology, 2016
Vol. 19, No. 4, 257–274, http://dx.doi.org/10.1080/13887890.2016.1259662

Distribution, ecology and status of a threatened species *Ischnura intermedia* (Insecta: Odonata), new for Europe

Geert De Knijf[a*], David J. Sparrow[b], Andreas C. Dimitriou[c], Roger Kent[b], Heather Kent[b], Klaus Siedle[b], Jenny Lewis[b] and Linda Crossley[b]

[a]*Department of Biodiversity and Natural Environment, Research Institute for Nature and Forest, Brussels, Belgium;* [b]*Cyprus Dragonfly Study Group, Pafos, Cyprus;* [c]*Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*

(*Received 24 June 2016; final version received 3 November 2016*)

The dragonfly genus *Ischnura* has been the subject of numerous studies and is well studied in Europe and the Middle East. Nevertheless, information on the ecology, habitat preferences and phylogenetic relationships of some species is deficient. One species lacking such data is *Ischnura intermedia*, a near endemic species of the Middle East, found for the first time in Europe on Cyprus in 2013, where it occurs in five river valleys. In this study, we monitored *I. intermedia* in Cyprus where the species has a long flight period from the end of March until mid-November. Our results show that it has two and possibly even three generations a year, with the males of the first generation having reduced blue coloration on abdominal segments 8 and 9. *Ischnura intermedia* is confined to small secondary channels adjacent to streams and rivulets where the current slows and water is retained. It appears that populations can only become established at sites that have permanent water. It is therefore anticipated that the species will be under severe pressure within its range. We suggest listing this species as "Endangered" in Europe and globally as "Vulnerable" following the IUCN Red List Categories and Criteria and to prepare a species action plan for the European population of *Ischnura intermedia* on Cyprus. Two partial DNA fragments, mtDNA cytochrome b (Cytb) and cytochrome oxidase subunit I (COI) were used to gain insights into the phylogenetic position within *Ischnura*, especially within the *I. pumilio* clade. We demonstrate that *I. intermedia* is clearly separated from *I. pumilio*, but closely related to *I. forcipata*.

Keywords: dragonfly; Zygoptera; phylogeny; flight period; conservation; habitat; Red List; mitochondrial DNA; *Ischnura pumilio*

Introduction

The genus *Ischnura* Vander Linden, 1820 is probably the most widespread genus of Zygoptera (Odonata), occurring almost everywhere Odonata are found (Westfall & May, 1996). Nearly 70 species *of Ischnura* have been described, including 13 species in the Western Palaearctic (Dijkstra & Kalkman, 2012). The Persian Bluetail *Ischnura intermedia* Dumont, 1974 has been described relatively recently from Turkey (Dumont, 1974). Following the original description from eastern Anatolia, *I. intermedia* has been reported from a few locations, ranging from south-central Turkey, northern Syria, northern Iraq, Iran and the region of the Kopet Dagh in southern Turkmenistan (Bakhshi & Sadeghi, 2014; Borisov, 2015; Borisov & Haritonov, 2007; Dumont & Borisov, 1995; Dumont, Demirsoy, & Mertens, 1988; Ghahari, Thipaksorn, Naderian, Sakenin,

*Corresponding author. Email: geert.deknijf@inbo.be

© 2016 Worldwide Dragonfly Association

& Tajali, 2012; Heidari & Dumont, 2002; Kiany & Sadeghi, 2012; Salur & Kiyak, 2006; Schneider & Ikemeyer, 2016; Schneider & Krupp, 1996). Nevertheless, only very little information is available on its habitat characteristics, life cycle and conservation status. Recently, the species was assessed as "Near Threatened" in the Red List of the Eastern Mediterranean (Boudot & Kalkman, 2014).

Although reports of several early studies on the dragonflies of Cyprus have been published, for example Martin (1894), Valle (1952) and Kiauta (1963), it was only in the early 1990s that a systematic survey began. Lopau & Adena (2002) compiled all hitherto known records. Since 2002, several publications on the odonates of Cyprus have been published (Cottle, 2007; De Knijf & Demolder, 2013; Tamm, 2014), but none of them mention the presence of *I. intermedia* for Cyprus. Following the recent discovery of *I. intermedia* at several localities in the south-western part of Cyprus, we were able to collect much data on the life cycle and gained insight into the habitat preferences. This allows us to better understand the threats and pressures and to reassess the conservation status of this rare species. We further aim to determine the phylogenetic position of *I. intermedia* within the small *Ischnura pumilio* (Charpentier, 1825) clade (*sensu* Dumont, 2013), especially how closely related it is to *Ischnura forcipata*, believed to be its sister species (Dumont & Borisov, 1995). Finally, we disentangle and discuss the reported presence of all *Ischnura* species for Cyprus.

Material & methods

Data collection

Since 2012, over 50 sites throughout Cyprus have been selected for monthly or bimonthly monitoring of dragonflies by members of the Cyprus Dragonfly Study Group (CDSG), counting all specimens along fixed transects. The selection of sites was designed to ensure coverage of the different habitat types and to be island-wide. In the event that CDSG members encountered problems identifying specimens, individuals were netted, checked for hand characteristics and documented photographically. Furthermore, we inspected the small collection of dragonflies (Box 6 and 6a) currently housed in the entomological collection of the Agriculture Department in the Ministry of Agriculture, Rural Development and Environment building in Nicosia. The *Ischnura* specimens from Cyprus housed in the collection of the Natural History Museum (London) were also checked for identification. The flight period of *I. intermedia* was analysed using the bimonthly monitoring data collected during 2015, as most sites were regularly surveyed during this year. Monitoring data collected from the first two weeks (days 1–15) of each month are grouped in "Month I" and data from the second two weeks (days 16–30/31) are grouped under "Month II".

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from nine individuals, five from *I. intermedia* from Diarizos (five samples) and four from *Ischnura elegans* (Vander Linden, 1820) (one sample from Zakaki [−34.64361°N, 032.98777°E] and three from Agios Eirini [35.13888°N, 032.57944°E]) using the DNeasy Blood & Tissue Kit (Qiagen, Germany) and following the manufacturer's proposed protocol. Two partial DNA fragments, mtDNA cytochrome b (Cytb) and cytochrome oxidase subunit 1 (COI), were amplified using the polymerase chain reaction (PCR). The total length of the targeted fragments was 1446 bp, ~750 bp for Cytb and 700 bp for COI. Primer pairs CB-J-10933/TS1-N-11683 and COI-L/ COI-H (Sánchez-Guillén, Wellenreuther, Cordero-Rivera, &

Hansson, 2011) were used for the amplification of Cytb and COI respectively. Both selected loci were amplified under the same PCR conditions: pro-incubation at 94°C for 10 min, denaturation at 94°C for 1 min, annealing at 48°C for 1 min for both COI and Cytb, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. Denaturation, annealing and extension steps were repeated consecutively for 39 cycles. Amplified DNA fragments were purified using the Qiaquick purification kit (Qiagen). Purified PCR product was sent to Macrogen Europe in the Netherlands where cycle sequencing and sequencing was done. Both strands of DNA were sequenced with the same primers used for the amplification procedure and results were sent to us as chromatograms. All sequences were submitted to GenBank.

Phylogenetic analysis

Obtained sequences were aligned with ClustalX v.2.1 (Larkin et al. 2007) and genetic distances were estimated with MEGA v6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) using Tamura-Nei (TrN) model of evolution (Tamura & Nei, 1993). Sequence divergence was calculated separately for COI and Cytb. Complementary sequences of other congeneric species plus two sequences coming from non-congeneric species (*Enallagma civile* Hagen, 1861 and *Enallagma hageni* Walsh, 1863 (Coenagrionidae)) that served as outgroup, were obtained from GenBank and included in our analyses (Tables 1 and 2). DNA sequence data from the two genes were analysed separately, as for several species data from both genes for each taxon were not available. Two robust methods of phylogenetic analysis were used: Bayesian inference (BI) and maximum likelihood (ML). Bayesian inference was performed with MrBayes v3.2.3 (Huelsenbeck & Ronquist, 2001). ML analyses were performed with RAxML v.7.2.8 (Stamatakis, 2006) with 100 random addition replicates in RAxML Black box (Stamatakis, Hoover, & Rougemont, 2008). We used jModeltest v2.1.4 (Darriba, Taboada, Doallo, & Posada, 2012) for the selection of the nucleotide substitution model best fitting our data. Out of 88 candidate models for COI gene under Akaike information criterion (AIC) the model with the lowest likelihood score (–In = 2797.9765) was the TVM + G. For Cytb respectively, ignoring the models that include both gamma distribution and invariable sites (Yang, 2006) , the selected model was the HKY + G (–lnL = 3563.0168). Four independent runs were performed, with eight chains per run for 2×10^7 generations. A tree was sampled every 100 generations from each chain; hence, overall

Table 1. Specimens analysed in this study. For each we list representative haplotype and respective GenBank accession number for cytochrome c oxidase I (COI) sequence data.

Haplotype	Species	Accession no.
Hap 1	*Ischnura posita*	JN419854
Hap 2	*Ischnura verticalis*	JN41985
Hap 3	*Ischnura posita*	JN419852
Hap 4	*Ischnura aurora*	AB708503, KF369414, AB708500
Hap 5	*Ischnura elegans*	KY127432/ KY127433/KY127434/KY127437/KY127438/KY127439
Hap 5	*Ischnura graellsii*	HQ834804, KC912316, KC912319, KC912317
Hap 6	*Ischnura elegans*	KY127440
Hap 7	*Ischnura asiatica*	KC135957
Hap 8	*Ischnura asiatica*	KF966552
Hap 9	*Ischnura asiatica*	KF257113
Hap 10	*Ischnura senegalensis*	KF369416
Hap 11	*Ischnura senegalensis*	KC912320, KC91232, KC912323
Hap 12	*Ischnura pumilio*	KC878732
Hap 13	*Ischnura ezoin*	AB708471, AB708469, AB708470
Hap 14	*Ischnura intermedia*	KY127435/KY127436
Hap 15	*Ischnura forcipata*	India, Dehra Dun (Dumont, 2013)
Hap 16	*Enallagma hageni*	JN419714

Table 2. Specimens analysed in this study. For each we list representative haplotype and respective GenBank accession number for cytochrome b (Cytb) sequence data.

Haplotype	Species	Accession no.
Hap 1	*Ischnura ramburii*	AF067700
Hap 2	*Ischnura saharensis*	KC430170, KC430169
Hap 2	*Ischnura graellsii*	KC430167
Hap 2	*Ischnura genei*	KC430154, KC430153
Hap 3	*Ischnura saharensis*	KC430168
Hap 3	*Ischnura graellsii*	HQ834802
Hap 3	*Ischnura genei*	KC430152
Hap 4	*Ischnura fountaineae*	KC430172
Hap 5	*Ischnura fountaineae*	KC430174, KC430173
Hap 6	*Ischnura prognata*	AF067699
Hap 7	*Ischnura barberi*	AF067683
Hap 8	*Ischnura hastata*	AF067693
Hap 9	*Ischnura senegalensis*	KC430193
Hap 10	*Ischnura senegalensis*	KC430189
Hap 11	*Ischnura asiatica*	KC430196
Hap 12	*Ischnura asiatica*	KC430195
Hap 13	*Ischnura elegans*	KY127446
Hap 14	*Ischnura elegans*	KY127449
Hap 15	*Ischnura elegans*	KY127442/KY127448
Hap 16	*Ischnura elegans*	KY127447
Hap 17	*Ischnura elegans*	KY127443
Hap 18	*Ischnura elegans*	KY127441
Hap 19	*Ischnura pumilio*	KC430198
Hap 20	*Ischnura pumilio*	KC878732
Hap 21	*Ischnura intermedia*	KY127444/KY127445
Hap 22	*Ischnura posita*	AF067698
Hap 23	*Ischnura erratica*	AF067691
Hap 24	*Ischnura cervula*	AF067685
Hap 25	*Ischnura gemina*	AF067692
Hap 26	*Ischnura damula*	AF067686
Hap 27	*Ischnura verticalis*	AF06770
Hap 28	*Ischnura perparva*	AF067696
Hap 29	*Ischnura denticollis*	AF067688
Hap 30	*Ischnura demorsa*	AF067687
Hap 31	*Ischnura kellicotti*	AF067695
Hap 32	*Ischnura capreolus*	AF067684
Hap 33	*Enallagma civile*	AF067690

the summary of BI relied on 2×10^5 trees. Of the sampled trees 25% were discarded as burn-in phase. A majority rule consensus tree was constructed and the statistical support of the nodes was estimated from posterior probabilities of reconstructed clades.

Results

Altogether, 161 records comprising 1618 individuals (1470 ♂♂, 78 ♀♀, seven pairs in tandem, 24 pairs in copula and eight ovipositing females) of *I. intermedia* were assembled during the flight seasons of 2013, 2014 and 2015 by the CDSG. The species was found at seven localities in the Diarizos valley, at one locality in Ezousa and Mavrokolympos valleys and on three other small streams in western Cyprus (Figure 1). In the list below, we further mention the month of discovery of *I. intermedia* at each locality, followed by the highest number of imagines counted on a single day during the monitoring surveys; we also assess the local population size. The names of the localities correspond with those used in the CDSG monitoring scheme.

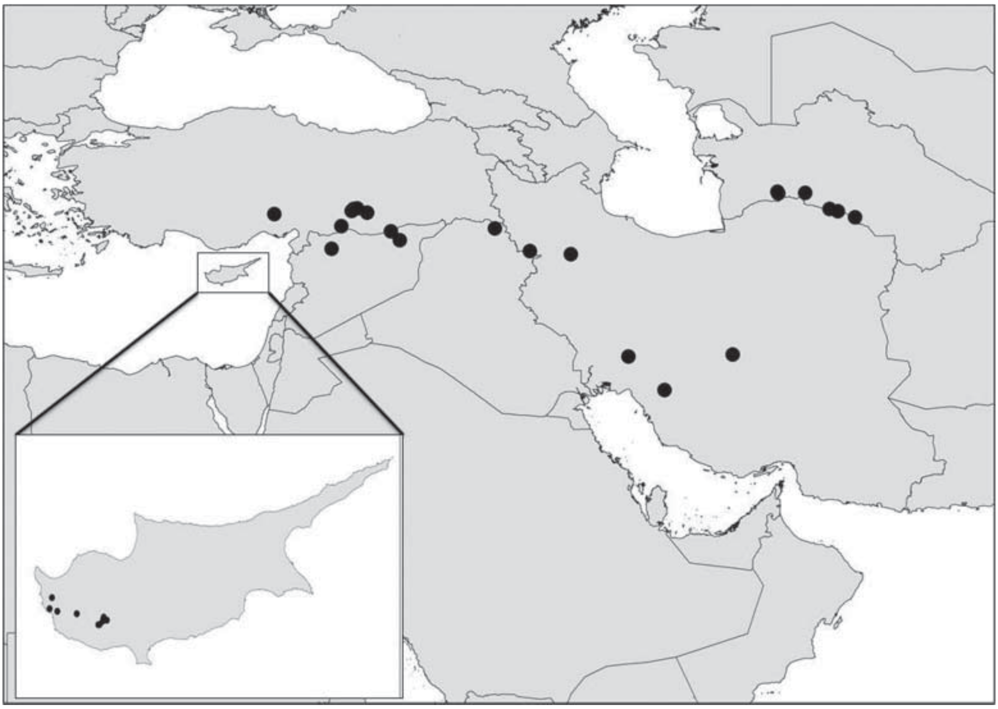

Figure 1. Compiled distribution of *Ischnura intermedia* (literature records plus this paper) with the recent findings in the west of Cyprus presented on the inset.

List of localities of I. intermedia in Cyprus

Diarizos river valley

- Loc. 1. Diarizos river 1, near village of Prastio; 34.785833°N, 32.685833°E; 220 m asl. Year round flow of water, first observation in April 2013 and medium-sized population present (maximum 21 individuals/day).
- Loc. 2. Diarizos river 2, near village of Prastio, *c*.300 m further upstream from Loc. 1.; 34.786111°N, 32.689444°E; 226 m asl. Year round flow of water, first observation in May 2013 and large population present (maximum 112 individuals/day).
- Loc. 3. Diarizos river 5, another 500 m upstream of Loc. 2 but separated by riverine forest, which is not suitable for the species; 34.788056°N, 32.695°E; 235 m asl. Partly dries up during summer. First observation in April 2013 and medium-sized population present (maximum 31 individuals/day).
- Loc. 4. Kidasi 2, Diarizos river, 3.5 km upstream of Loc. 3; 34.814167°N, 32.716389°E; 290 m asl. A marshy area on the riverbank fed by the overflow from Loc. 6. First observation in June 2013 and medium-sized population present (maximum 34 individuals/day). Site dried up in August 2013 and no further sightings since then. Population no longer present.
- Loc. 5. Kidasi 3, marshy area fed by spring water, near a track adjacent to the Diarizos river; 34.806667°N, 32.715833°E; 276 m asl. First observation in July 2013 and only very small numbers have been observed (maximum six individuals/day). Site dried up in September 2013 and no further sightings since then. Population no longer present.
- Loc. 6. Kidasi 4, marshy area fed from a spring below the bentonite quarry, located 1 km beyond the village of Kidasi; 34.815556°N, 32.717222°E; 290 m asl. Species was found only

in August 2013 (five individuals). Habitat was later destroyed and although visited several times in 2014, no further records. Population no longer present.
- Loc. 7. Kidasi 5, adjacent to a track on the eastern bank of the Diarizos river 2 km upstream of Loc. 4; 34.8275°N, 32.721111°E; 329 m asl. First observation in August 2014 and a medium-sized population present (maximum 21 individuals/day).

Ezousa valley

- Loc. 8. Letymvou, wide open stream with adjacent reed vegetation; 34.846944°N, 32.545°E; 211 m asl. First observation of two individuals in August 2013, followed by a male in September of the same year. No observations in 2014 and 2015, but in April 2016 again two individuals were seen. Probably no population present.

Mavrokolympos valley

- Loc. 9. Mavrokolympos feeder stream close to the entrance of the reservoir; 34.859722°N, 32.418056°E; 133 m asl. First observation in May 2013 and medium-sized population present (max 31 individuals/day).

Small stream courses in western Cyprus

- Loc. 10. Pegeia 3; 34.873889°N, 32.3675°E; 72 m asl. First observation in August 2015 and a small population present (maximum 10 individuals/day).
- Loc. 11. Lemba, permanent small stream fed from a spring; 34.811111°N, 32.408056°E; 73 m asl. First observation in April 2015 and a medium-sized population present (max 18 individuals/day).
- Loc. 12. Avakas Upper 2, temporary stream above and feeding the Avakas Gorge; 34.934722°N, 32.383056°E; 350 m asl. First observation in April 2016 and a small population present (max 12 individuals/day).

Diagnostic characters (see also Dumont, 1974; Kalkman, 2006) used to identify *I. intermedia* from the other species of the *Ischnura pumilio* group are the male's anal appendages and the shape and coloration of the pterostigma. The superior appendages of *I. intermedia* males are in lateral view only marginally longer than the inferior appendages and are shorter than S10, the former with a small apical hook. The upper anal appendages of *Ischnura forcipata* Morton, 1907 are much longer than the lower and the males *of I. pumilio* have much longer lower appendages than upper. Male pterostigma in forewing largely black with only a small fraction of the apical part whitish and hind wing pterostigma unicoloured and less than half the size of that in the forewing. Female pterostigma a unicoloured parallelogram in both pairs of wings and smaller in hind wing than in forewing. Males of *I. intermedia* and *I. forcipata* are easily distinguished from *I. pumilio* in the field by the black on segments 1 to 7 dorsally, the blue taillight on S8–9 and S10 being black. Furthermore *I. intermedia* and *I. forcipata* have non overlapping ranges. The side of the thorax and the side of the abdominal segments 1–3 are green, S4-half of S7 is yellow and basal half of S7–10 is blue. A seasonal change in the extent of the blue coloration on S8 and S9 in males has been observed (Figure 2). Males on the wing in the early season from the end of March to early June have a less extensive blue coverage than those from around early July to the end of the season. The seasonal variation appears to be consistent throughout all known locations.

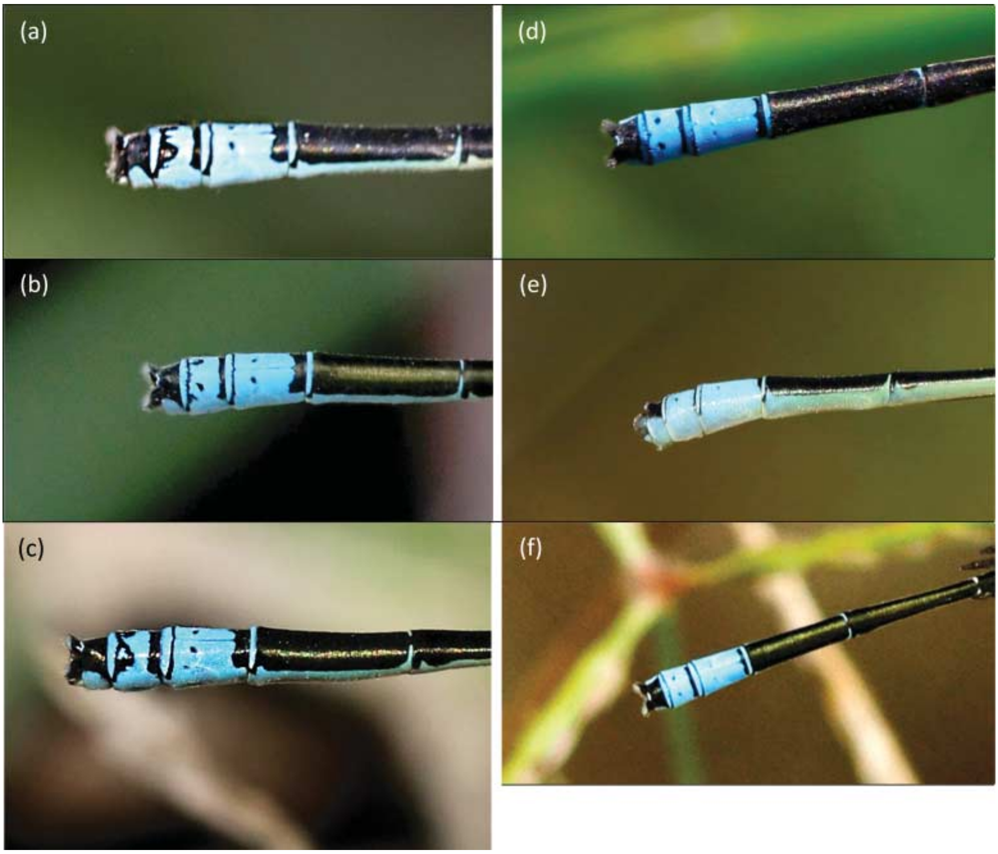

Figure 2. Seasonal change in the extent of the blue coloration on S8 and S9 in males. Individuals from the first generation (left) have reduced blue coverage compared with those from the second generation (right): (a) Letymvou 9 April 2016; (b) Diarizos 14 May 2016; (c) Avakas Upper 25 April 2016; (d) Diarizos 27 July 2013; (e) Diarizos 1 September 2013; (f) Diarizos 17 September 2013.

Flight period of I. intermedia

Based on the monitoring data of 2015 from Cyprus, the flight season of *I. intermedia* is presented for all known sites (Figure 3). The species has a long flight season and can be observed in good numbers from the first half of April until the second half of November, although yearly fluctuations occur. The figure shows a first peak from the second half of April until the first half of June. Numbers drop steeply in June and increase again from August onwards, suggesting a second generation. The earliest sighting was on 26 March 2014 at the Mavrokolympos feeder stream (Loc. 9) and the latest sighting was on 26 November 2015 at Diarizos 1 (Loc. 1).

Phylogenetic analysis

Of the 900 base pairs compared for Cytb and 707 bp for COI, 250 and 196 were variable, from which 177 and 164, respectively, were parsimony informative. Excluding outgroup sequences, there were 247 and 188 variable sites and 174 and 159 parsimony informative sites for Cytb and COI, respectively. Intraspecific sequence divergence for taxa for which we had more than one sequence available varied from 1.00% to 0.00% for COI, and from 3.60% to 0.00% for Cytb. Bayesian inference and maximum likelihood gave very similar topologies, for each gene dataset. In most of the cases, for both genes, each clade corresponds to a different morphological species.

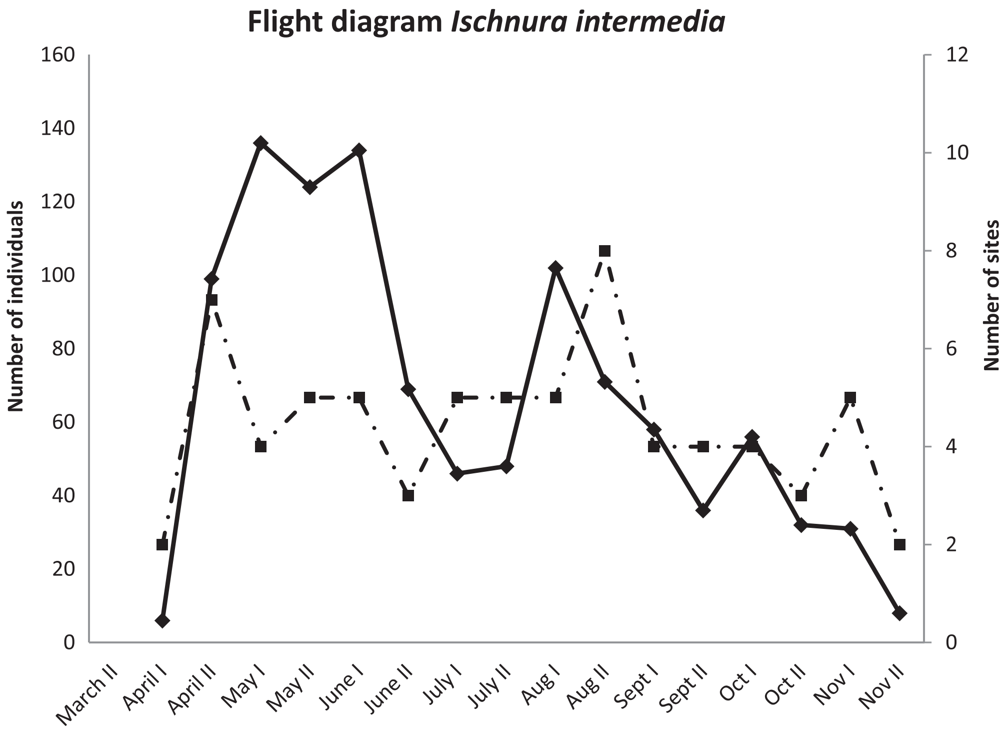

Figure 3. Flight diagram of *Ischnura intermedia* in Cyprus based on the bimonthly monitoring data from 2015 (line) with indication of number of surveyed localities (dotted line).

Table 3. Mean percentage (%) genetic distances for COI calculated under TN93 evolutionary model (Tamura & Nei, 1993) between clades as presented in Figure 4. Diagonal values are mean within-clade genetic distances.

Species name	1	2	3	4	5	6	7	8	9	10	11	12
1 *Ischnura asiatica*	0.76											
2 *Ischnura aurora*	13.35	0.15										
3 *Ischnura elegans*	13.21	14.05	0.52									
4 *Ischnura ezoin*	9.62	14.44	17.24	0.00								
5 *Ischnura forcipata*	13.72	17.87	16.78	12.79	–							
6 *Ischnura intermedia*	13.67	17.82	16.97	12.48	0.84	0.00						
7 *Ischnura graellsii*	14.76	15.14	1.43	18.08	16.99	17.00	0.13					
8 *Ischnura posita*	11.72	13.37	12.88	13.98	16.80	16.08	13.56	0.93				
9 *Ischnura pumilio*	11.66	15.73	14.45	12.66	14.07	14.27	15.59	14.10	–			
10 *Ischnura senegalensis*	14.20	13.24	7.46	16.79	14.71	15.16	8.02	12.87	17.69	1.33		
11 *Ischnura verticalis*	9.31	15.69	10.18	12.90	14.77	13.94	11.48	0.61	12.43	12.26	–	
12 *Enallagma hageni*	15.43	15.14	15.10	17.71	18.94	18.91	16.00	15.93	16.95	14.84	15.39	–

For COI gene data the *I. intermedia* specimens are grouped with *I. forcipata* specimens from which the genetic distance is 0.84% (Table 3, Figure 4), although the congeneric genetic distance between different taxa tends to be between 9.00–18.00% (Table 3). Low (0.61%), even lower than *I. intermedia*–*I. forcipata*, genetic divergence is also detected between *I. verticalis* Say, 1839 and *I. posita* (Hagen, 1861) (Table 3).

Concerning Cytb gene data (Table 4, Figure 5), the specimens of *I. intermedia* from Cyprus appear as a good monophyletic clade, with large distances (13.00–14.00%) from its sister clade (*I. pumilio*), supporting its status as a good separate species. The congeneric genetic distance varies between 0.14% and 18.00%.

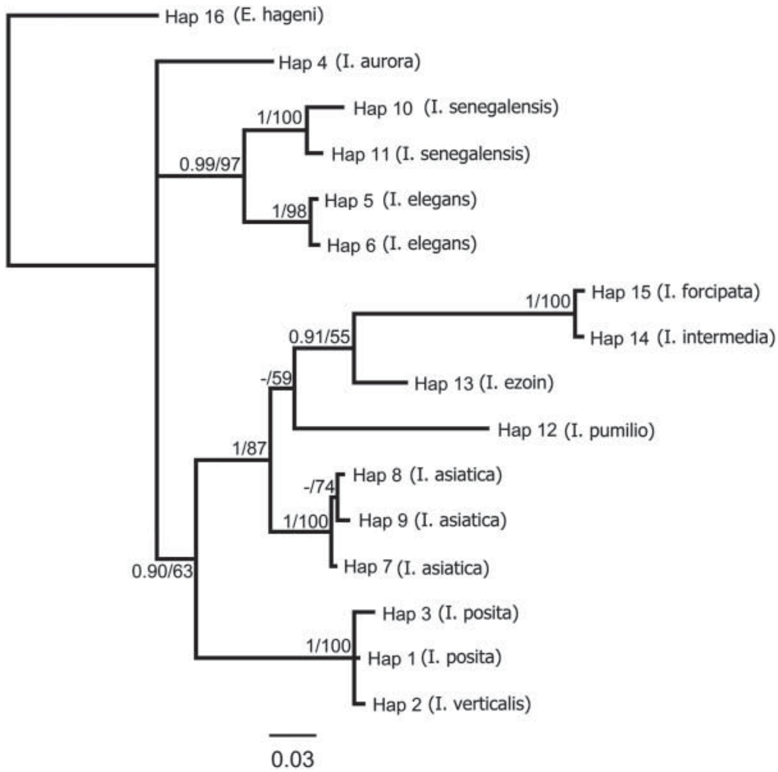

Figure 4. Phylogenetic tree obtained by Bayesian inference analysis of COI sequences. Bayesian posterior probabilities (> 0.95) and maximum likelihood bootstrap values (> 50) are indicated at nodes.

Museum collections

The inspection of the collection in the Ministry of Agriculture building in Nicosia yielded the unexpected finding of a specimen from 1947 labelled as *I. pumilio*, and as locality site, "Agios Pavlos Nicosia", det. C. Longfield. The specimen was in poor condition with abdominal segments 6–10 missing, as were the left wings. The right forewings and hind wings were, however, well preserved (Figure 6). Pterostigma in forewing and hind wing differs in shape, that of the forewing being larger, so excluding *Ischnura elegans*. The apical part of the pterostigma in the forewing is whitish and the basal part black and the hind wing pterostigma widens towards the top, both criteria given by Dumont (1974) and Kalkman (2006) to distinguish *I. pumilio* from *I. intermedia* and *I. forcipata*. Agios Pavlos is now a suburb of Nicosia, hence we consider this locality to be lost. Inspection of all *Ischnura* species from Cyprus present in the Natural History Museum of London yielded no records of *I. intermedia* nor of *I. pumilio*.

Discussion

Geographical distribution

Based on morphological characters *I. intermedia* is considered to belong to the *pumilio* group within the genus *Ischnura* (Dumont, 1974, 2013; Dumont & Borisov, 1995), where it has been regarded as taking an intermediate position (hence its name) between *I. forcipata*, a central Asian

Table 4. Mean percentage (%) genetic distances for Cytb calculated under TN93 evolutionary model (Tamura & Nei, 1993) between clades as presented in Figure 5. Diagonal values are mean within-clade genetic distances.

Species name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1. *Ischnura asiatica*	0.32																								
2. *Ischnura barberi*	14.92	–																							
3. *Ischnura capreola*	13.20	17.28	–																						
4. *Ischnura cervula*	9.81	16.10	14.34	–																					
5. *Ischnura damula*	9.85	13.93	11.80	4.31	–																				
6. *Ischnura demorsa*	10.24	13.93	12.02	4.52	1.20	–																			
7. *Ischnura dentricollis*	10.24	14.20	11.56	4.75	0.40	0.79	–																		
8. *Ischnura elegans*	10.03	13.31	14.25	12.76	12.44	13.12	12.66	0.65																	
9. *Ischnura erratica*	8.72	14.21	13.06	3.03	2.84	3.67	3.27	12.81	–																
10. *Ischnura fountaineae*	10.40	13.75	14.92	12.17	13.08	13.46	13.46	1.38	12.34	0.21															
11. *Ischnura gemina*	9.10	15.85	14.06	1.79	4.52	4.73	4.53	12.02	3.23	11.44	–														
12. *Ischnura genei*	11.96	13.99	14.47	12.68	12.26	13.40	12.63	2.11	12.58	1.29	11.95	0.21													
13. *Ischnura graellsii*	11.89	14.26	14.53	13.64	13.01	14.07	13.18	2.06	13.30	1.23	12.92	0.16	0.32												
14. *Ischnura hastata*	14.00	17.69	17.64	14.01	12.79	14.20	13.28	15.71	12.53	16.14	14.24	15.23	15.35	–											
15. *Ischnura intermedia*	12.55	15.98	17.27	13.34	12.64	13.69	13.37	15.84	12.38	16.75	13.27	17.24	16.99	16.27	0.00										

16. *Ischnura kellicotti*	11.97	14.49	14.38	9.12	8.48	8.72	8.48	14.38	8.02	15.12	8.89	14.69	15.10	16.50	16.89	–									
17. *Ischnura perparva*	10.21	13.90	12.03	5.15	0.79	2.00	1.19	12.79	3.26	13.45	5.36	12.63	13.35	13.03	12.97	9.38	–								
18. *Ischnura posita*	9.84	14.91	12.28	4.74	1.20	2.01	1.20	12.38	3.69	13.00	4.73	12.77	13.37	13.29	12.92	8.95	2.00	–							
19. *Ischnura prognata*	11.09	15.81	13.86	11.08	9.43	10.38	9.67	13.64	9.00	14.50	11.05	13.65	13.77	10.36	14.97	12.49	9.65	10.38	–						
20. *Ischnura pumilio*	12.37	16.94	17.59	15.76	13.64	14.68	14.01	17.09	13.58	17.40	14.98	17.24	16.96	16.03	14.45	15.90	14.01	14.45	14.20	1.62					
21. *Ischnura ramburii*	11.31	12.25	13.52	11.36	10.40	10.62	10.38	7.96	10.59	8.58	10.87	8.09	8.29	13.75	12.37	12.60	11.08	11.10	12.31	14.18	–				
22. *Ischnura saharensis*	11.96	13.99	14.47	12.68	12.26	13.40	12.63	2.11	12.58	1.29	11.95	0.14	0.16	15.23	17.24	14.69	12.63	12.77	13.65	17.24	8.09	0.21			
23. *Ischnura senegalensis*	11.07	13.49	14.85	11.52	10.94	11.53	11.31	7.75	10.52	7.74	10.80	7.62	7.68	11.82	14.76	13.52	11.30	11.72	13.52	15.81	8.48	7.62	3.61		
24. *Ischnura verticalis*	10.27	14.22	12.07	4.75	0.40	1.61	0.80	12.85	3.27	13.50	4.96	12.67	13.39	13.08	13.02	8.97	1.19	1.20	9.70	14.05	10.88	12.67	11.35	–	
25. *Enallagma civile*	12.51	15.09	15.44	11.16	11.70	12.19	11.50	11.96	10.97	12.74	10.69	12.88	12.80	14.54	14.56	14.11	11.93	11.68	13.00	17.77	11.48	12.88	12.13	11.98	–

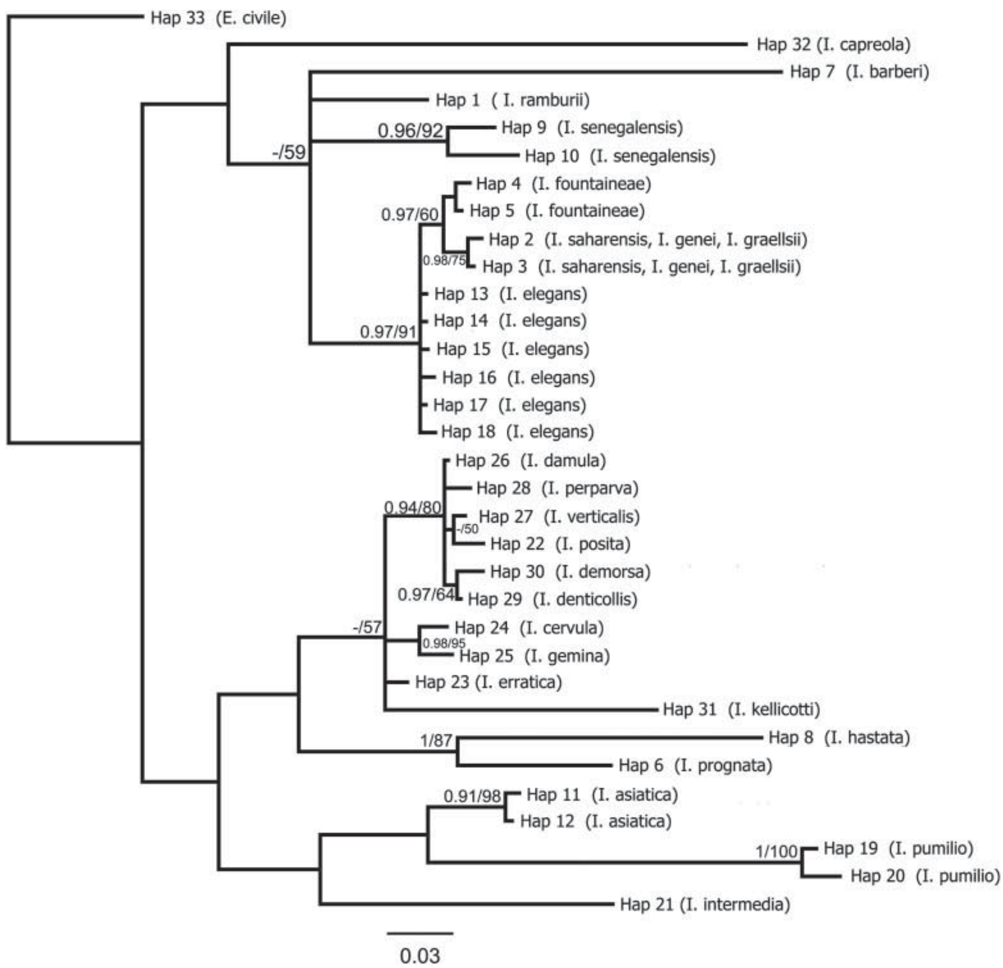

Figure 5. Phylogenetic tree obtained by Bayesian inference analysis of Cytb sequences. Bayesian posterior probabilities (> 0.95) and maximum likelihood bootstrap values (> 50) are indicated at nodes.

species (Borisov, 2014), and *I. pumilio*, widespread in Europe (Boudot et al., 2009) and extending eastwards as far as China. It is more closely related to *I. forcipata* than to *I. pumilio* (Dumont, 1977). Following the original description of *I. intermedia* from a tributary of the Euphrates River in eastern Turkey (Dumont, 1974), the species was rediscovered near the type locality (Dumont, Demirsoy, & Mertens, 1988). The type locality in Turkey became flooded due to the creation of the Ataturk dam and no longer exists. For nearly 20 years, the species was only known from south-eastern Turkey. In 1996, it was found by Schneider & Krupp (1996) at Ras-al-Ayn in north-eastern Syria. A second unpublished observation from Syria dates from June 2010 and was made at an irrigation channel north of Lake Jaboul, south-east of Aleppo (pers. comm. Johan van 't Bosch). In the meantime, several populations were also discovered in the region of the Kopet Dagh in southern Turkmenistan and Iran (Bakhshi & Sadeghi, 2014; Borisov, 2015; Borisov & Haritonov, 2007; Dumont & Borisov, 1995; Ghahari et al., 2012; Heidari & Dumont, 2002; Kiany & Sadeghi, 2012; Salur & Kiyak, 2006) (Figure 1). To the east, *I. intermedia* is separated by a series of deserts (Karakum, Dash-e-Kavir, Dash-e-Lut) from *I. forcipata* (Borisov & Haritonov, 2007; Dumont & Borisov, 1995). To the south, *I. intermedia* seems to be limited by the Syrian Desert, although it is likely that *I. intermedia* will be found further south along the Euphrates and

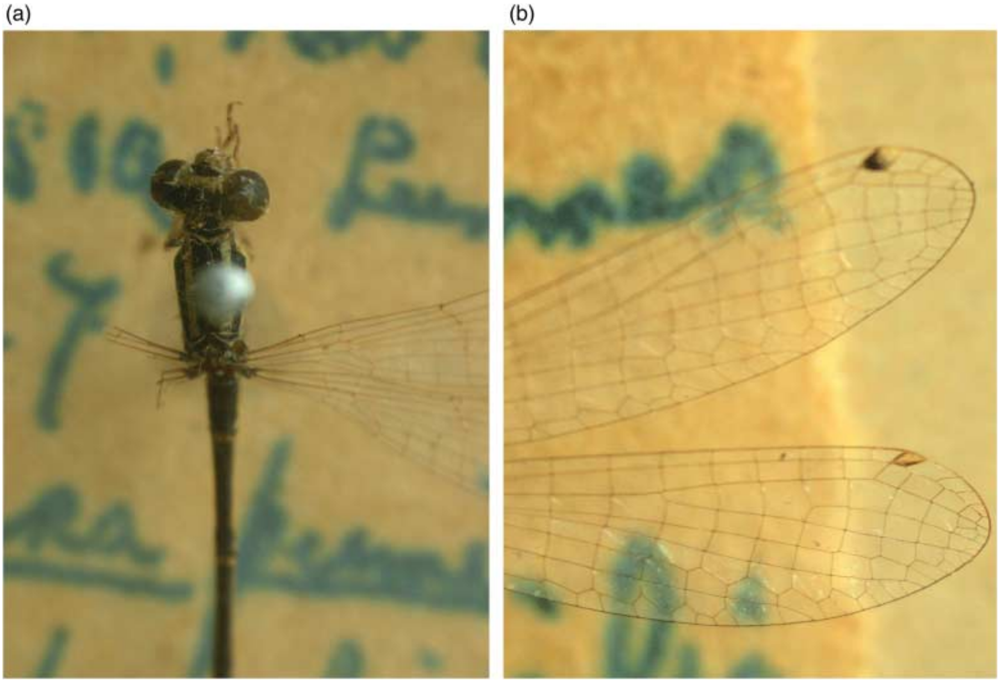

Figure 6. *Ischnura pumilio* (a) and detail of right-hand wings (b) preserved in the collection of the Ministry of Agriculture Building in Nicosia (photo Despina Filippou).

Tigris rivers in Syria and Iraq. Salur & Kiyak (2006) found one male at Aladağ (Büyüksofulu) near Adana, being the first record for the Mediterranean region of Turkey. No information is given about the existence of a local population, but this seems likely. Until recently, this was the most westerly record of *I. intermedia*. The finding of several populations of *I. intermedia* in the south-western part of Cyprus extends the range of the species by 350 km to the west (Figure 1).

The occurrence of *I. intermedia* on Cyprus appears to be restricted to the south-west, where it has been discovered in a cluster of seven sites in the Diarizos valley, at a single site on the feeder stream of the Mavrokolympos dam, at three sites on small streams in western Cyprus and at one locality in the Ezousa valley, where probably no population is present. At all these localities *Ischnura elegans* (Vander Linden, 1820) seems to occur sympatrically with *I. intermedia.* Coexistence of both species was also found in Syria (Schneider & Krupp, 1996), where even the much more closely related *I. pumilio* was present. Following the first sighting in April 2013, members of the CDSG have been meticulously checking populations of *I. elegans* around the island for the presence of *I. intermedia*, but without further success. We surmise that *I. intermedia* has been present on Cyprus for a long time, but had remained undetected owing to its similarity with the very widespread and common *I. elegans*.

Flight period of Ischnura intermedia

Our data demonstrate that *I. intermedia* has a flight period from the end of March until the end of November, with good numbers already on the wing from April onward. In Turkey (Kalkman & van Pelt, 2006), *I. intermedia* shows a flight season from May until August, but as this was based on only three records, one from mid-May and two from the first half of August, this can be no more than a crude indication of the flight period for the species, reflecting the paucity of available data. Although our data are from the most western part of the species' range, which is possibly not representative of the whole range, we can certainly conclude that *I. intermedia* has a very long flight season. Our results show a first peak in spring, from the end of April until mid-June. Numbers drop steeply in June and increase again during August, suggesting a second generation from August onwards. This correlates well with the seasonal variation in the extent of the blue coloration on S8 and S9 in males, whereby the first generation has a less extensive blue coverage than individuals of the second generation (Figure 2). This phenomenon of seasonal dimorphism was already noticed in *Ischnura graellsii* in Spain, notable in the amount of black in the postocular spots and in the extension of the male antehumeral lines (Cordero-Rivera, 1988; Jurzitza, 1964). Spring specimens were darker than individuals of the second, summer generation. This suggests a general pattern of lighter individuals of the second generation in summer, when temperatures are higher, and darker individuals of the first generation in spring. Further research is needed to demonstrate whether the increase in numbers during the first part of October corresponds with a third generation. These results, along with observations of freshly emerged individuals over much of the summer, are typical for species with two (bivoltine) or more (multivoltine) generations a year. Our results seem to be in accordance with the life-cycle given for *I. forcipata* in Central Asia by Borisov (2014), who mention a bivoltine life-cycle and in warm years even three generations.

Characterization of the habitat and conservation status

From the results of the CDSG monitoring scheme, *I. intermedia* appears to be very habitat-specific, much more so than the generalist *I. elegans.* All localities in Cyprus can be described as small secondary channels adjacent to streams and rivulets, where the current slows and water is retained. Marshy areas and small swamps occur locally in or near the streambed and taller grassy margins (often reed-vegetation, up to 4 m) grow adjacent to the stream (Figure 7). This seems to be in accordance with the limited available information on its habitat in south-western Asia (Dumont, 1974; Kiany & Sadeghi, 2012) and with the recent findings of *I. intermedia* in Syria

Figure 7. Habitat of *Ischnura intermedia*, Diarizos valley, Cyprus, 17 April 2015.

and Turkey (pers. comm. Johan van 't Bosch). Although less exclusively, *I. pumilio* also shows clear preferences for such biotopes. *I. intermedia* is not an active flier and imagines, both males and females, are rather inconspicuous and can be found sitting and resting in low open spaced grassy vegetation along the stream. Settling was noted only some 10–30 cm above the water. Several of the sites in Cyprus (Locs 3, 4 and 5) dried up during the summer months in recent years, followed by the complete disappearance locally of *I. intermedia*. We therefore presume that reasonably sized populations of the species are found only at sites that have permanent water, or at least where some marshy pools remain behind in summer. Because of river damming and rainfall pattern, rivers dry up during summer months, but there are sections of streams in the higher Troodos and some spring-fed sections lower down that flow throughout the year. The habitat of *I. intermedia* is threatened, not only in Cyprus but probably more so in the Middle East, where drought and hot summers are even more extreme, resulting in unfavourable and unstable ecological conditions, putting the species under severe pressure.

Proposal and rationale for a European and global Red List assessment

Our study is the first to demonstrate the presence of this species in Europe and provides enough data to assess its status for the regional European Red List. At present, *Ischnura intermedia* is known only from south-western Cyprus, being one of the species with the smallest distribution range in Europe (Boudot & Kalkman, 2015). For the European regional Red List, the species qualifies for IUCN threatened category "Endangered" based on its restricted geographic range and the inferred continuing decline of the quality of the habitat: B1, B2 + abiii (IUCN, 2012). The extent of occurrence (EOO) is less than 500 km^2 and the area of occupancy (AOO) is less than 10 km^2. It is only known from five river valleys (accounting for 12 localities) and the quality of the habitat is likely to be declining and is even expected to deteriorate further in the future due to precipitation deficit caused by climate change, and the construction of dams in Cyprus. Since its discovery in 2013, by 2016 the species was no longer present at four of the 12 original localities due to habitat destruction or desiccation. We urge the need to develop a species action plan for Cyprus. Additionally, the species might also qualify for "Endangered" under criterion D (which considers very small or restricted populations), as very low numbers of individuals were seen at each visit (< 250). Our results together with recent field surveys in West Asia, especially in Iran (Bakhshi & Sadeghi, 2014; Ghahari et al., 2012; Kiany & Sadeghi, 2012; Schneider & Ikemeyer, 2016) results in a better knowledge about its distribution. On a global scale, the species qualifies for category "Vulnerable" based on its low number of locations scattered across a relative large range, resulting in a fragmented distribution and the inferred continuing decline of the area and quality of the habitat: B2 + abiii (IUCN, 2012). Although the extent of occurrence (EOO) is greater than 20,000 km^2, the area of occupancy (AOO) is less than 100 km^2 and the number of locations is fragmented and low (23). At many locations only single individuals have been observed, and besides one locality where the species is considered as "very common" (Borisov, 2015), all other populations are small and relatively isolated, resulting in a reduced probability of recolonization. Some localities are already destroyed due to the construction of dams, like the type locality in Turkey (Dumont & Borisov, 1995). The area and the quality of the habitat, small streams, is likely to be declining and is even expected to deteriorate further in the future due to precipitation deficit caused by climate change and increased water extraction for local agriculture in most of its range.

Phylogenetic analysis

The genus *Ischnura* is recovered as monophyletic and is believed to consist of two main clades, called the *I. elegans* clade and the *I. pumilio* clade (Dumont, 2013). Our study did not have the aim of providing the phylogeny of the genus *Ischnura*, but we wanted to clarify the phylogenetic position of *I. intermedia* within the *I. pumilio* clade. Based on morphological characters, *I. intermedia* is considered to occupy an intermediate position between *I. pumilio* and *I. forcipata* (Dumont, 1974; Dumont & Borisov, 1995). For the first time, the genetic divergence among the three species of the *I. pumilio* clade has been quantified. Our study demonstrates that based on the phylogenetic reconstruction *I. intermedia*, *I. forcipata* and *I. pumilio* form part of the same cluster, the "*I. pumilio*" cluster. However, *I. intermedia* appears to be more closely related to *I. forcipata* than to *I. pumilio* (Figure 4). Although the calculated TN93 genetic distances among *I. intermedia* and *I. forcipata* are relatively low (0.84%), it is similar to the genetic distance detected among other closely related Palaearctic species in the genus, such as between *I. elegans*, *I. graellsii*, *I. genei* and *I. saharensis* (Sánchez-Guillén, Córdoba-Aguilar, Cordero-Rivera, & Wellenreuther, 2014). A low genetic distance has also been found between several species of *Cordulegaster* Leach, 1815 in Europe (Froufe, Ferreira, Boudot, Alves, & Harris, 2014). Our results confirm that *I. intermedia* is indeed the sister-species *of I. forcipata* and that the distinction between them is very small, but it has retained some characters of their sister-species *I. pumilio*. It is believed that *I. intermedia* and *I. forcipata* share a common ancestor, whose distribution became fragmented after the formation of the Karakum desert in Turkmenistan and the Dash-e-Kavir and Dash-e-Lut deserts in eastern Iran. These processes facilitated allopatric speciation into two well-defined species, *I. forcipata* to the east of these deserts (Borisov & Haritonov, 2007; Dumont & Borisov, 1995) and *I. intermedia* to the west. This speciation is of recent origin and probably only dates to the late Pleistocene, as is the case with other Palaearctic dragonflies (Dumont, 2013; Dumont, Vanfleteren, De Jonckheere, & Weekers, 2005; Froufe et al., 2014). The phylogenetic conclusions presented here, confirm the existence of *I. intermedia* as a *bona fide* species and of its close relationship to *I. forcipata*.

Comments on the species of the genus Ischnura in Cyprus

The most widely distributed species of Zygoptera on the island is *Ischnura elegans*, which has already been mentioned by several authors (De Knijf & Demolder, 2013; Lopau & Adena, 2002; Martin, 1894; Tamm, 2014; Valle, 1952). The species seems to prefer standing water as found at dams and ponds but also occurs along the margins of flowing water. It is also tolerant of some salinity, and has been observed in brackish water around 50 m from the shoreline (Sparrow, Sparrow, & De Knijf, 2016). Besides this very common *Ischnura* species, the presence of a second species of this genus for Cyprus has been reported by several authors. Martin (1894) was the first to discuss the odonate fauna of Cyprus and mentioned *Ischnura genei* (Rambur, 1842) but without giving the locality. It is now accepted that this should be considered a misidentification, as *I. genei* is endemic for the islands in the western part of the Mediterranean (Boudot et al., 2009). Without having examined Martin's material, Valle (1952) reasoned that this specimen could not be *I. genei* but belonged to *I. pumilio* or to *I. fountaineae*. As already stated by Kiauta (1963), this note should be regarded as merely speculative. Since then, it has been accepted that the specimen of Martin belonged to *I. pumilio* (Boudot et al., 2009; Dumont, 1977; Lopau & Adena, 2002). As no observations were known for more than 100 years, Lopau & Adena (2002) considered *I. pumilio* extinct on Cyprus. With our finding of several populations of *I. intermedia* on Cyprus, the question of the identity of the specimen mentioned by Martin is again raised. To complicate the matter further, we tracked down a previously unpublished pinned male specimen from 1947, labelled as *Ischnura pumilio*, in the collection housed in the Ministry of Agriculture in Nicosia. Although the specimen was in very poor condition, the right wings were in good condition (Figure 6) and were sufficient to confirm that *I. pumilio* has occurred on Cyprus at least once. However, there have been no reports of its presence since 1947 and during the last three years we have been unable to find this species, despite diligent searches in its preferred habitats, and therefore *I. pumilio* should be considered extinct on Cyprus.

Acknowledgements

Many thanks go to other members of the Cyprus Dragonfly Study Group who provided records for sightings of *Ischnura intermedia*: Dave Bowler, Pete Evans, Danne Johnston, Rosalyn Sparrow and Bill Stokes. Despina Filippou of the Agriculture Department, Ministry of Agriculture, Rural Development and Environment made available and photographed the *Ischnura pumilio* specimen in the Ministry's collection. Ben Price (Natural History Museum, London) kindly photographed all the *Ischnura* specimens from Cyprus, present in the museum collection. Henri Dumont was kind enough to provide us with the sequences of *Ischnura forcipata*. Rosa Ana Sánchez-Guillén was of great help in discussing the phylogenetic relationships within the genus *Ischnura*. We wish to thank Fons Peels and Jean-Pierre Boudot for exchange of ideas about variation in the blue "taillight". We are grateful to Eddie John for his proofreading of the text and to Martin Schorr for providing additional references. The authors wish to thank the anonymous reviewers for their insightful comments.

References

Bakhshi, Y., & Sadeghi, S. (2014). Odonata fauna of Khuzestan province (Iran) with a note on an Oriental species *Trithemis aurora* (Burmeister, 1839) as a new record for the south-west of Iran. *Zoology in the Middle East*, *60*, 372–374. doi:10.1080/09397140.2014.966522

Borisov, S. N. (2014). Distribution and ecology of *Ischnura forcipata* Morton, 1907 (Odonata, Coenagrionidae) in Tien-Shan and Pamir-Alai. *Euroasian Entomological Journal*, *13*, 323–328. Retrieved from http://kmkjournals.com/journals/EEJ

Borisov, S. N. (2015). Dragonflies (Odonata) of thermal springs in Central Asia. *Entomological Review*, *95*, 1203–1211. doi:10.1134/S0013873815090080

Borisov, S. N., & Haritonov, A. Y. (2007). The dragonflies (Odonata) of Middle Asia. Part 1. Caloptera, Zygoptera. *Euroasian Entomological Journal*, *6*, 343–360. Retrieved from http://kmkjournals.com/journals/EEJ

Boudot, J.-P., & Kalkman, V. J. (2014). Chapter 5. Odonata (dragonflies and damselflies). In K. G. Smith, V. Barrios, W. R. T. Darwall, & C. Numa. (Eds.), *The status and distribution of freshwater biodiversity in the Eastern Mediterranean* (pp. 57–69). Cambridge, UK, Malaga, Spain and Gland, Switzerland: IUCN.

Boudot, J.-P., & Kalkman, V. J. (Eds). (2015). *Atlas of the European dragonflies and damselflies*. Zeist, The Netherlands: KNNV publishing.

Boudot, J.-P., Kalkman, V. J., Azpilicueta Amorín, M., Bogdanovic, T., Cordero-Rivera, A., Degabriele, G., . . . Schneider, W. (2009). Atlas of the Odonata of the Mediterranean and North Africa. *Libellula, Supplement*, *9*, 1–256. Retrieved from http://www.libellula.org/wp-content/uploads/2015/12/Libellula_Supplement_9_Boudot_et_al_2009_Atlas_Odonata_Medit-1.pdf

Cordero-Rivera, A. (1988). Ciclomorfosis y fenologia en *Ischnura graellsi* Rambur, 1842 (Odonata: Coenagrionidae). *Actas III Congresso Ibérico de Entomologia*, 419–430.

Cottle, N. (2007). *Brachythemis leucosticta* (Burmeister, 1839) (Anisoptera: Libellulidae): A new dragonfly species for Cyprus and *Trithemis arteriosa* (Burmeister, 1839) (Anisoptera: Libellulidae): A rarely recorded dragonfly species in Cyprus. *The Bulletin of the Amateur Entomologists' Society*, *66*, 59–63. Retrieved from https://www.amentsoc.org/publications/bulletin/archive/2007/april/

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772. doi:10.1038/nmeth.2109

De Knijf, G., & Demolder, H. (2013). Early spring observations of Odonata from Cyprus. *Libellula*, *32*, 59–74. Retrieved from http://www.libellula.org/wp-content/uploads/2016/10/32_1-2_DeKnijf_Demolder.pdf

Dijkstra, K.-D. B., & Kalkman, V. J. (2012). Phylogeny, classification and taxonomy of European dragonflies and damselflies (Odonata): A review. *Organisms, Diversity & Evolution*, *12*, 209–227. doi:10.1007/s13127-012-0080-8

Dumont, H. J. (1974). *Ischnura intermedia* spec. nov. from Turkey, and its relations to *I. forcipata* Morton, 1907 and *I. pumilio* (Charpentier, 1825). *Odonatologica*, *3*, 153–165. Retrieved from http://natuurtijdschriften.nl/search?smode = advanced;portal = natuur;text = Odonatologica;f1-journal = Odonatologica;expand = year;f2-year = 1974;f3-author = Dumont,%20H.J

Dumont, H. J. (1977). A review of the dragonfly fauna of Turkey and adjacent Mediterranean Islands (Insecta Odonata). *Bulletin et Annales de la Société royale belge d'Entomologie*, *113*, 119–171. Retrieved from http://www.srbe-kbve.be/cm/sites/default/files/publications/Tables%20et%20Index/table%20g%E9n%E9rales%201981%20%E0%202008.PDF

Dumont, H. J. (2013). Phylogeny of the genus *Ischnura*, with emphasis on the old world taxa (Zygoptera: Coenagrionidae). *Odonatologica*, *42*, 301–308. Retrieved from http://www.odonatologica.com/volumes-2013/

Dumont, H. J., & Borisov, S. N. (1995). Status and range of the species-pair *Ischnura forcipata* Morton, 1907 and *Ischnura intermedia* Dumont, 1974 (Insecta: Odonata: Coenagrionidae). *Biologisch Jaarboek Dodonaea*, *62*, 157–163.

Dumont, H. J., Demirsoy, A., & Mertens, J. (1988). Odonata from south-east Anatolia (Turkey) collected in spring 1988. *Notulae Odonatologicae*, *3*, 22–26. Retrieved from http://natuurtijdschriften.nl/search?identifier = 593419; keyword = notulae

Dumont, H. J., Vanfleteren, J. R., De Jonckheere, J. F., & Weekers, P. H. H. (2005). Phylogenetic relationships, divergence time estimation, and global biogeographic patterns of Calopterygoid damselflies (Odonata, Zygoptera) inferred from Ribosomal DNA sequences. *Systematic Biology*, *54*, 347–362. doi:10.1080/10635150590949869

Froufe, E., Ferreira, S., Boudot, J.-P., Alves, P. C., & Harris, D. J. (2014). Molecular phylogeny of the Western Palaearctic *Cordulegaster* taxa (Odonata: Anisoptera: Cordulegastridae). *Biological Journal of the Linnean Society*, *111*, 49–57. doi:10.1111/bij.12190

Ghahari, H., Thipaksorn, A., Naderian, H., Sakenin, H., & Tajali, A. A. (2012). A faunistic study on the Odonata (Insecta) from Kurdistan province and vicinity, western Iran. *Linzer Biologische Beiträge*, *44*, 1079–1085.

Heidari, H., & Dumont, H. J. (2002). An annotated check-list of the Odonata of Iran. *Zoology in the Middle East*, *26*, 133–150. doi:10.1080/09397140.2002.10637929

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*, 754–755. doi:10.1093/bioinformatics/17.8.754

IUCN (2012). *IUCN Red List Categories and Criteria: Version 3.1*. *Second edition*. Gland, Switzerland and Cambridge, UK: IUCN.

Jurzitza, G. (1964). Saisondimorphismus bei *Ischnura graellsi*. *Nachrichtenblatt der Bayerischen Entomologen*, *13*, 84–87.

Kalkman, V. J. (2006). Key to the dragonflies of Turkey, including species known from Greece, Bulgaria, Lebanon, Syria, the Trans-Caucasus and Iran. *Brachytron*, *10*, 3–82. Retrieved from http://www.brachytron.nl/Brachytron/Brachytron 101inhoud.html

Kalkman, V. J., & van Pelt, G. J. (2006). The distribution and flight period of the dragonflies of Turkey. *Brachytron*, *10*, 83–153. Retrieved from http://www.brachytron.nl/Brachytron/Brachytron101inhoud.html

Kiany, M., & Sadeghi, S. (2012, September). A preliminary study on Odonata fauna of Yazd province. *Abstracts of the 17th National & 5th International Iranian Biology Conference*, Shahid Bahonar University, Kerman, Iran.

Kiauta, B. (1963). Additions to the Cyprus list of Odonata, with some notes on the annotated list given by K. J. Valle. Societas Scientiarum Fennica. *Commentationes Biologicae*, *26*, 1–6.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., . . . Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, *23*, 2947–2948. Retrieved from http://bioinformatics.oxfordjournals.org/content/23/21/2947.full.pdf + html

Lopau, W., & Adena, J. (2002). Die Libellenfauna von Cypern. *Naturkundliche Reiseberichte*, *19*, 1–71.

Martin, R. (1894). Odonates de Chypre. *Bulletin de la Société zoologique de France*, *19*, 135–138.

Salur, A., & Kiyak, S. (2006). Additional records for the Odonata fauna of East Mediterranean region of Turkey. *Munis Entomology & Zoology*, *1*, 239–252. Retrieved from http://www.munisentzool.org/yayin/vol1/issue2/239-252.pdf

Sánchez-Guillén, R. A., Wellenreuther, M., Cordero-Rivera, A., & Hansson, B. (2011). Introgression and rapid species turnover in sympatric damselflies. *BMC Evolutionary Biology*, *11*, 210. doi:10.1186/1471-2148-11-210

Sánchez-Guillén, R. A., Córdoba-Aguilar, A., Cordero-Rivera, A., & Wellenreuther, M. (2014). Genetic divergence predicts reproductive isolation in damselflies. *Journal of Evolutionary Biology*, *27*, 76–87. doi:10.1111/jeb.12274

Schneider, W., & Krupp, F. (1996). A possible natural hybrid between *Ischnura elegans ebneri* Schmidt, 1939 and *Ischnura fountainei* Morton, 1905 (Odonata: Coenagrionidae). *Zoology in the Middle East*, *12*, 75–81. doi:10.1080/09397140.1996.10637692

Schneider, T., & Ikemeyer, D. (2016). Notes on Odonata species in South-West Iran including *Platycnemis kervillei* (Martin, 1909) as a new species for Iran. *Entomologische Zeitschrift*, *126*, 3–8. Retrieved from http://www.wissenschaftlicherverlag.com/product_info.php?products_id = 138

Sparrow, D. J., Sparrow, R. L., & De Knijf, G. (2016). Odonata. In D. J. Sparrow & E. John (Eds), *An Introduction to the Wildlife of Cyprus*. (pp. 64–131). Lemesos: Terra Cypria.

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690. doi:10.1093/bioinformatics/btl446

Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, *57*, 758–771. doi:10.1080/10635150802429642

Tamm, J. (2014). Libellenfunde im späten Frühjahr auf Zypern. *Libellula*, *33*, 177–188. Retrieved from http://www.libellula.org/wp-content/uploads/2016/10/33_3-4_Tamm.pdf

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*, 512–526. Retrieved from http://mbe.oxfordjournals.org/content/10/3/512.full.pdf + html

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, *30*, 2725–2729. doi:10.1093/molbev/mst197

Valle, K. J. (1952). Die Odonatenfauna von Zypern. Societas Scientiarum Fennica. *Commentationes Biologicae*, *13*, 1–8.

Westfall, M. J., & May, M. L. (1996). *Damselflies of North America*. Gainesville, FL: Scientific Publishers.

Yang, Z. (2006). *Computational molecular evolution*. Oxford: Oxford University Press.