Freshwater conservation assessments in (semi-)arid regions: Testing river intermittence and buffer strategies using freshwater mussels (Bivalvia, Unionida) in Morocco

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ABSTRACT

The IUCN Red List assessments are essentially based on population trends and range, namely Area of Occupancy (AOO) and Extent of Occupancy (EOO). Range estimations are based on fixed grids, but this is likely inappropriate for species living in river networks. Furthermore, AOO and EOO are measured using the whole hydrographic network, therefore disregarding temporary sections, which is particularly problematic in arid and semi-arid regions. Here we mapped the permanent hydrographic network of Morocco using satellite imagery, complemented with field surveys to collect samples for molecular analyses of the five freshwater mussel species present and assess their distribution. The phylogeographic patterns are described for each species and used to identify priority areas and evolutionary significant units for conservation. Permanent hydrographic river sections represent only 18.3% of the whole hydrographic network. A north-to-south gradient of genetic diversity, species richness and distribution range was found, being coherent with water availability and river intermittence. Isolated evolutionary units were detected in southern basins that should also receive particular attention in conservation planning. We propose the mean river width multiplied by the extent of the river network as the best and the most adequate way to estimate both EOO and AOO. Given the worldwide degradation of freshwater systems and biodiversity, an accurate (re)assessment of species conservation status supported with maps of intermittent water bodies will be essential for prioritizing and guiding conservation actions and management plans, especially in arid and semi-arid regions.

1. Introduction

Freshwater ecosystems support disproportionally high biodiversity when considering the respective occupied earth surface (around 0.8%) (Balian et al., 2008). This is due to their physical and spatial structure, which favours isolation and impairs long distance or cross-basin dispersal, resulting in high levels of endemism and speciation (Strayer and Dudgeon, 2010). Freshwater ecosystems provide a wide range of

services (Carpenter et al., 2011; Vaughn, 2018); however, the growing human population and agriculture and industrial development have enhanced the need for water resources and increased the pressure upon freshwater systems (Dudgeon et al., 2006; Reid et al., 2019).

Studies on freshwater biodiversity have gathered increasing interest in the last decades, but most tend to favour vertebrates over invertebrates (Lydeard et al., 2004; Cardoso et al., 2011a), even though the latter usually dominate in abundance and biomass (Cardoso et al., 2011a). Freshwater bivalves of the order Unionida, Grey, 1854 are globally spread organisms, playing fundamental roles in ecosystem functions and services such as cycling of nutrients, matter and energy, water purification, bioturbation, among others (Vaughn and Hakenkamp, 2001; Spooner et al., 2013; Lopes-Lima et al., 2017; Vaughn, 2018). Despite all of this, freshwater mussels are globally threatened and many populations are in pronounced decline (Strayer et al., 2004; Graf and Cummings, 2007; Lopes-Lima et al., 2014b, 2018). Recent efforts have been implemented to better understand and conserve this group (Lopes-Lima et al., 2014a, 2014b, 2018), but there is still much to be done (see Ferreira-Rodríguez et al., 2019). Freshwater mussels are considered "permanent refugial organisms" (Sheldon et al., 2010), meaning they have limited dispersal abilities even under flow conditions, and are restricted to areas where water availability is permanent across many years. This makes freshwater mussels highly vulnerable to alterations in flow regime.

The Mediterranean region is one of the world's major hotspots of biodiversity and conservation priorities (Myers et al., 2000). The region's complex geo-climatic history has been proposed as the main factor shaping the phylogeographic patterns of many taxa (Myers et al., 2000; Husemann et al., 2012, 2014), including freshwater mussels (e.g. Araujo et al., 2009, 2010, 2016; Khalloufi et al., 2011; Reis et al., 2013; Froufe et al., 2014, 2016a, 2016b). In the more arid areas, the varying permanence of water likely assumed a preponderant role on population persistence and dynamics. North-African countries, such as Morocco, are particularly vulnerable to climate change and prone to extreme climate events (Schilling et al., 2012; Schyns and Hoekstra, 2014). Droughts are becoming longer and more frequent (Schilling et al., 2012); therefore, intermittent rivers will play an even larger role in ecosystem management. This creates an urgent need for proper data and suitable conservation strategies, but also an opportunity to study and develop a framework for a globally, highly relevant and expanding type of landscape.

Even though urgent conservation planning is needed, basic data like the diversity and distribution of freshwater mussel species is often outdated or even absent, particularly in North Africa (Graf and Cummings, 2011). Moroccan freshwater mussels have been the focus of some studies during the 20th century (Pallary, 1918, 1928; Van Damme, 1984; Mandhal-Barth, 1988; Daget, 1998), but only in the last decade was it possible to start tackling the uncertainties regarding current distributions and taxonomy, mostly due to molecular approaches (Araujo et al., 2009, 2010; Khalloufi et al., 2011; Froufe et al., 2016a, 2016b; Sousa et al., 2016, 2018). Presently, there are five recognised native freshwater mussel species in Morocco: four from family Unionidae Rafinesque 1820: *Anodonta* sp. (Lamarck, 1799), *Potomida littoralis* (Cuvier, 1798), *Unio gibbus* (Spengler, 1793) and *Unio foucauldianus* (Pallary, 1936); and one from family Margaritiferidae Henderson 1929 (1910): *Pseudunio marocanus* (Pallary, 1918). With the exception of the *Anodonta* sp., the taxonomic status of all Moroccan species has been recently tested and validated (Araujo et al., 2009, 2016; Froufe et al., 2016a, 2016b). The current distribution of all five species is poorly known and/or outdated, since almost all species have apparently suffered great declines over their range in the last decades (Araujo et al., 2009, 2010; Froufe et al., 2016a, 2016b; Sousa et al., 2016, 2018).

Intermittent rivers are among the most endangered of freshwater ecosystems. They are characterized by natural periodical interruption of flow and drastic fluctuations in the extent of freshwater habitat

(Stanley et al., 1997; Sheldon et al., 2010). These rivers constitute more than half of the length of the global river network, and are increasing in number and length in response to climate change, land-use alteration, and water abstraction (Datry et al., 2014; Datry et al., 2017). Despite significant biodiversity (e.g. Kingsford et al., 1998) and ecosystems services generated (e.g. nutrient cycling and groundwater renewal) (Sophocleous, 2002; Bernhardt and Palmer, 2011), the conservation significance of intermittent rivers is generally ignored (Leigh et al., 2016). They usually lack suitable management practices, protective policies and legislation, which are generally designed for perennial systems (Datry et al., 2014; Leigh et al., 2016). Consequently, efficient management will require a better understanding of the dynamics of shifting between lotic, lentic and dry conditions, and the improvement of river mapping (Datry et al., 2014, 2016; Leigh et al., 2016, 2019).

The increasing pressure on freshwater systems has a direct impact on already declining freshwater diversity (Strayer and Dudgeon, 2010; Carpenter et al., 2011; Reid et al., 2019). Correctly assigning conservation status of species is essential to effectively prioritize and guide management plans. The Red List proposed by International Union for Conservation of Nature (IUCN) is the most widely used framework for this important purpose (IUCN, 2001). For IUCN red-listing, categories are attributed according to five standard criteria, one of which (B) evaluates species extinction risk using its distribution area in two parameters: the area of occupancy (AOO, area occupied by a species) and extent of occurrence (EOO, region encompassing all possible presence sites, usually a minimum-convex polygon or a α-hull). These parameters are calculated using a 2×2 km square grid following IUCN guidelines (IUCN, 2001). This strategy may be suitable to fit terrestrial organisms, but is likely misleading for freshwater taxa, particularly when their distributions are limited to water bodies.

Given the urgency of having realistic river network maps, of optimizing conservation status assessments of freshwater organisms, and of updating the distribution, diversity and conservation status of freshwater mussel species, the aims of this study were to: i) map permanent river segments of the Moroccan hydrographic network; ii) update the current distribution and phylogeographic knowledge of the five species of Unionida present in Morocco, iii) clarify the taxonomic status of the Moroccan *Anodonta* sp.; iv) develop a more realistic approach for calculating the AOO and EOO to efficiently assign freshwater species conservation status; and v) use all this information to identify key areas of conservation for freshwater mussels in Morocco.

2. Material and methods

2.1. Sample and data collection

A total of 189 sites were surveyed across the country, encompassing all major river basins in Morocco. The surveys were carried between May 2013 and August 2018. Many different habitat types were assessed including permanent and temporary rivers and streams, and even artificial irrigation channels and dam reservoirs. The assessment considered previous information on the distribution of freshwater mussels in Morocco (Pallary, 1918, 1923, 1928; Van Damme, 1984; Mandhal-Barth, 1988; Daget, 1998). At each site, the search was conducted by wading, diving and hand-sampling following the rapid bioassessment methods for freshwater bivalves described by Cummings et al. (2016). The coordinates of each sampling site were recorded using GPS. Distribution data collected during the present study was supplemented by bibliographical data (Araujo et al., 2009, 2010, 2016; Froufe et al., 2016a, 2016b; Sousa et al., 2016, 2018). All collected individuals were morphologically identified on site and a small tissue snip was collected from each specimen following a non-lethal protocol (Naimo et al., 1998) for genetic analyses (Table 1). The freshwater mussels were then returned to their original positions.

In total, 153 individuals were collected for molecular analyses across Morocco (7 *P. marocanus*, 12 *Anodonta* sp., 19 *P. littoralis*, 75 *U.*

Table 1
List of all sampled individual: species; codes; population; river basin and respective GenBank accession codes.

Code	Species	Population	River Basin	Country	GenBank
BIV 2000	*Anodonta anatina*	Oum Er-Rbia	Oum Er-Rbia	Morocco	MK733420
BIV 2001	*Anodonta anatina*	Oum Er-Rbia	Oum Er-Rbia	Morocco	MK733421
BIV 2002	*Anodonta anatina*	Oum Er-Rbia	Oum Er-Rbia	Morocco	MK733422
BIV 2003	*Anodonta anatina*	Oum Er-Rbia	Oum Er-Rbia	Morocco	MK733423
BIV 2004	*Anodonta anatina*	Oum Er-Rbia	Oum Er-Rbia	Morocco	MK733424
BIV 2006	*Anodonta anatina*	Oum Er-Rbia	Oum Er-Rbia	Morocco	MK733425
BIV 2580	*Anodonta anatina*	Loukos	Loukos	Morocco	MK733426
BIV 2581	*Anodonta anatina*	Loukos	Loukos	Morocco	MK733427
BIV 2582	*Anodonta anatina*	Loukos	Loukos	Morocco	MK733428
BIV 2583	*Anodonta anatina*	Loukos	Loukos	Morocco	MK733429
BIV 2584	*Anodonta anatina*	Loukos	Loukos	Morocco	MK733430
BIV 2585	*Anodonta anatina*	Loukos	Loukos	Morocco	MK733431
BIV2635	*Pseudunio marocanus*	Bouhlou	Bouhlou, Sebou	Morocco	MK733432
BIV2640	*Pseudunio marocanus*	Bouhlou	Bouhlou, Sebou	Morocco	MK733433
BIV2641	*Pseudunio marocanus*	Bouhlou	Bouhlou, Sebou	Morocco	MK733434
BIV 2642	*Pseudunio marocanus*	Bouhlou	Bouhlou, Sebou	Morocco	MK733435
BIV2643	*Pseudunio marocanus*	Bouhlou	Bouhlou, Sebou	Morocco	MK733436
BIV2644	*Pseudunio marocanus*	Bouhlou	Bouhlou, Sebou	Morocco	MK733437
BIV2645	*Pseudunio marocanus*	Bouhlou	Bouhlou, Sebou	Morocco	MK733438
BIV2035	*Potomida littoralis*	Douar Laalaoua Bridge	Oum-Er-Rbia	Morocco	MK733439
BIV2037	*Potomida littoralis*	Douar Laalaoua Bridge	Oum-Er-Rbia	Morocco	MK733440
BIV2038	*Potomida littoralis*	Douar Laalaoua Bridge	Oum-Er-Rbia	Morocco	MK733441
BIV2039	*Potomida littoralis*	Douar Laalaoua Bridge	Oum-Er-Rbia	Morocco	MK733442
BIV2040	*Potomida littoralis*	Douar Laalaoua Bridge	Oum-Er-Rbia	Morocco	MK733443
BIV2041	*Potomida littoralis*	Douar Laalaoua Bridge	Oum-Er-Rbia	Morocco	MK733444
BIV0626	*Potomida littoralis*	Tilougass	Amaghouss, Massa	Morocco	MK733445
BIV2536	*Potomida littoralis*	Dar Bel Amri	Beth, Sebou	Morocco	MK733446
BIV 2541	*Potomida littoralis*	Dar Bel Amri	Beth, Sebou	Morocco	MK733447
BIV 2538	*Potomida littoralis*	Dar Bel Amri	Beth, Sebou	Morocco	MK733448
BIV3223	*Potomida littoralis*	Zewiherate Taoughilt	Ouergha, Sebou	Morocco	MK733449
BIV3224	*Potomida littoralis*	Zewiherate Taoughilt	Ouergha, Sebou	Morocco	MK733450
BIV3225	*Potomida littoralis*	Zewiherate Taoughilt	Ouergha, Sebou	Morocco	MK733451
BIV3194	*Potomida littoralis*	Tetouan	Hajera, Martil	Morocco	MK733452
BIV3195	*Potomida littoralis*	Tetouan	Hajera, Martil	Morocco	MK733453
BIV3196	*Potomida littoralis*	Tetouan	Hajera, Martil	Morocco	MK733454
BIV 3197	*Potomida littoralis*	Tetouan	Hajera, Martil	Morocco	MK733455
BIV3198	*Potomida littoralis*	Tetouan	Hajera, Martil	Morocco	MK733456
BIV3199	*Potomida littoralis*	Tetouan	Hajera, Martil	Morocco	MK733457
UD636	*Unio foucauldianus*	Kenitra	Mda	Morocco	MK733458
UD639	*Unio foucauldianus*	Kenitra	Mda	Morocco	MK733459
UD638	*Unio foucauldianus*	Kenitra	Mda	Morocco	MK733460
BIV663	*Unio foucauldianus*	Douar Chebabate	Abiod, Sebou	Morocco	MK733461
BIV664	*Unio foucauldianus*	Douar Chebabate	Abiod, Sebou	Morocco	MK733462
BIV665	*Unio foucauldianus*	Douar Chebabate	Abiod, Sebou	Morocco	MK733463
BIV3229	*Unio foucauldianus*	Bouhlou	Bouhlou, Sebou	Morocco	MK733464
BIV3230	*Unio foucauldianus*	Bouhlou	Bouhlou, Sebou	Morocco	MK733465
BIV805	*Unio foucauldianus*	Sidi Said Maachou	Oum Er-Rbia	Morocco	MK733466
BIV806	*Unio foucauldianus*	Sidi Said Maachou	Oum Er-Rbia	Morocco	MK733467
BIV602	*Unio foucauldianus*	Guelmim	Noun	Morocco	MK733468
BIV603	*Unio foucauldianus*	Guelmim	Noun	Morocco	MK733469
BIV614	*Unio foucauldianus*	Tilougass	Amaghouss, Massa	Morocco	MK733470
BIV615	*Unio foucauldianus*	Tilougass	Amaghouss, Massa	Morocco	MK733471
BIV2509	*Unio foucauldianus*	Oumnass	Nfiss, Tensift	Morocco	MK733472
BIV2510	*Unio foucauldianus*	Oumnass	Nfiss, Tensift	Morocco	MK733473
BIV2511	*Unio foucauldianus*	Oumnass	Nfiss, Tensift	Morocco	MK733474
BIV2512	*Unio foucauldianus*	Oumnass	Nfiss, Tensift	Morocco	MK733475
BIV2513	*Unio foucauldianus*	Oumnass	Nfiss, Tensift	Morocco	MK733476
BIV2514	*Unio foucauldianus*	Oumnass	Nfiss, Tensift	Morocco	MK733477
BIV2614	*Unio foucauldianus*	Douar Ellil	Moulouya	Morocco	MK733478
BIV2615	*Unio foucauldianus*	Douar Ellil	Moulouya	Morocco	MK733479
BIV2616	*Unio foucauldianus*	Douar Ellil	Moulouya	Morocco	MK733480
BIV2617	*Unio foucauldianus*	Douar Ellil	Moulouya	Morocco	MK733481
BIV2618	*Unio foucauldianus*	Douar Ellil	Moulouya	Morocco	MK733482
BIV2619	*Unio foucauldianus*	Douar Ellil	Moulouya	Morocco	MK733483
BIV3174	*Unio foucauldianus*	Tetouan	Hajera, Martil	Morocco	MK733484
BIV3175	*Unio foucauldianus*	Tetouan	Hajera, Martil	Morocco	MK733485
BIV3176	*Unio foucauldianus*	Tetouan	Hajera, Martil	Morocco	MK733486
BIV 3177	*Unio foucauldianus*	Tetouan	Hajera, Martil	Morocco	MK733487
BIV3178	*Unio foucauldianus*	Tetouan	Hajera, Martil	Morocco	MK733488
BIV3179	*Unio foucauldianus*	Tetouan	Hajera, Martil	Morocco	MK733489
BIV3214	*Unio foucauldianus*	Zewiherate Taoughilt	Ouergha, Sebou	Morocco	MK733490
BIV3215	*Unio foucauldianus*	Zewiherate Taoughilt	Ouergha, Sebou	Morocco	MK733491
BIV3216	*Unio foucauldianus*	Zewiherate Taoughilt	Ouergha, Sebou	Morocco	MK733492
BIV3217	*Unio foucauldianus*	Zewiherate Taoughilt	Ouergha, Sebou	Morocco	MK733493

Table 1 (*continued*)

Code	Species	Population	River Basin	Country	GenBank
BIV3218	*Unio foucauldianus*	Zewiherate Taoughilt	Ouergha, Sebou	Morocco	MK733494
BIV3219	*Unio foucauldianus*	Zewiherate Taoughilt	Ouergha, Sebou	Morocco	MK733495
BIV2557	*Unio foucauldianus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733496
BIV2558	*Unio foucauldianus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733497
BIV2565	*Unio foucauldianus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733498
BIV2566	*Unio foucauldianus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733499
BIV2562	*Unio foucauldianus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733500
BIV 2577	*Unio foucauldianus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733501
BIV 2578	*Unio foucauldianus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733502
BIV 2579	*Unio foucauldianus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733503
BIV 4864	*Unio foucauldianus*	Amghasse	Oued Tigrigra, Beth, Sebou	Morocco	MK733504
BIV 4865	*Unio foucauldianus*	Amghasse	Oued Tigrigra, Beth, Sebou	Morocco	MK733505
BIV 4866	*Unio foucauldianus*	Amghasse	Oued Tigrigra, Beth, Sebou	Morocco	MK733506
BIV 4867	*Unio foucauldianus*	Amghasse	Oued Tigrigra, Beth, Sebou	Morocco	MK733507
BIV 4868	*Unio foucauldianus*	Amghasse	Oued Tigrigra, Beth, Sebou	Morocco	MK733508
BIV 4869	*Unio foucauldianus*	Amghasse	Oued Tigrigra, Beth, Sebou	Morocco	MK733509
BIV 2599	*Unio foucauldianus*	Larache	Unnamed tributary, Loukos	Morocco	MK733510
BIV 2598	*Unio foucauldianus*	Larache	Unnamed tributary, Loukos	Morocco	MK733511
BIV2600	*Unio foucauldianus*	Larache	Unnamed tributary, Loukos	Morocco	MK733512
BIV2605	*Unio foucauldianus*	Larache	Unnamed tributary, Loukos	Morocco	MK733513
BIV2606	*Unio foucauldianus*	Larache	Unnamed tributary, Loukos	Morocco	MK733514
BIV2603	*Unio foucauldianus*	Larache	Unnamed tributary, Loukos	Morocco	MK733515
BIV3236	*Unio foucauldianus*	Aghbal	Bouregreg	Morocco	MK733516
BIV3238	*Unio foucauldianus*	Aghbal	Bouregreg	Morocco	MK733517
BIV3239	*Unio foucauldianus*	Aghbal	Bouregreg	Morocco	MK733518
BIV3240	*Unio foucauldianus*	Aghbal	Bouregreg	Morocco	MK733519
BIV3241	*Unio foucauldianus*	Aghbal	Bouregreg	Morocco	MK733520
BIV3257	*Unio foucauldianus*	Aghbal	Grou, Bouregreg	Morocco	MK733521
BIV3258	*Unio foucauldianus*	Aghbal	Grou, Bouregreg	Morocco	MK733522
BIV3259	*Unio foucauldianus*	Aghbal	Grou, Bouregreg	Morocco	MK733523
BIV3260	*Unio foucauldianus*	Aghbal	Grou, Bouregreg	Morocco	MK733524
BIV3261	*Unio foucauldianus*	Aghbal	Grou, Bouregreg	Morocco	MK733525
BIV 3262	*Unio foucauldianus*	Aghbal	Grou, Bouregreg	Morocco	MK733526
BIV 5106	*Unio foucauldianus*	Rabat-Salé-Kénitra	Grou, Bouregreg	Morocco	MK733527
BIV 5107	*Unio foucauldianus*	Rabat-Salé-Kénitra	Grou, Bouregreg	Morocco	MK733528
BIV5076	*Unio foucauldianus*	Mohammedia	Nefifikh	Morocco	MK733529
BIV5077	*Unio foucauldianus*	Mohammedia	Nefifikh	Morocco	MK733530
BIV5078	*Unio foucauldianus*	Mohammedia	Nefifikh	Morocco	MK733531
BIV5081	*Unio foucauldianus*	Mohammedia	Nefifikh	Morocco	MK733532
BIV5082	*Unio foucauldianus*	Mohammedia	Nefifikh	Morocco	MK733533
BIV5083	*Unio foucauldianus*	Mohammedia	Nefifikh	Morocco	MK733534
BIV3237	*Unio gibbus*	Aghbal	Bouregreg	Morocco	MK733535
BIV3250	*Unio gibbus*	Aghbal	Bouregreg	Morocco	MK733536
BIV3252	*Unio gibbus*	Aghbal	Bouregreg	Morocco	MK733537
BIV3253	*Unio gibbus*	Aghbal	Bouregreg	Morocco	MK733538
BIV725	*Unio gibbus*	Guelmim	Noun	Morocco	MK733539
BIV727	*Unio gibbus*	Guelmim	Noun	Morocco	MK733540
BIV728	*Unio gibbus*	Guelmim	Noun	Morocco	MK733541
BIV729	*Unio gibbus*	Guelmim	Noun	Morocco	MK733542
BIV730	*Unio gibbus*	Guelmim	Noun	Morocco	MK733543
BIV811	*Unio gibbus*	Sidi Said Maachou	Oum Er-Rbia	Morocco	MK733544
BIV821	*Unio gibbus*	Sidi Said Maachou	Oum Er-Rbia	Morocco	MK733545
UG4	*Unio gibbus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733546
UG5	*Unio gibbus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733547
UG6	*Unio gibbus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733548
BIV2537	*Unio gibbus*	Dar Bel Amri	Beth, Sebou	Morocco	MK733549
UG11	*Unio gibbus*	Kenitra	Mda	Morocco	MK733550
UG16	*Unio gibbus*	Kenitra	Mda	Morocco	MK733551
UG13	*Unio gibbus*	Kenitra	Mda	Morocco	MK733552
UG14	*Unio gibbus*	Kenitra	Mda	Morocco	MK733553
UG15	*Unio gibbus*	Kenitra	Mda	Morocco	MK733554
PL392	*Unio gibbus*	Unkonwn	Barbate	Spain	MK733555
BIV5079	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733556
BIV5080	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733557
BIV5085	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733558
BIV5086	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733559
BIV5087	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733560
BIV5088	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733561
BIV5089	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733562
BIV5090	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733563
BIV5092	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733564
BIV5095	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733565
BIV5096	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733566
BIV5097	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733567
BIV5098	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733568

Table 1 (*continued*)

Code	Species	Population	River Basin	Country	GenBank
BIV5099	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733569
BIV5100	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733570
BIV5101	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733571
BIV5102	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733572
BIV5104	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733573
BIV5105	*Unio gibbus*	Mohammedia	Nefifikh	Morocco	MK733574

foucauldianus and 40 *U. gibbus*).

2.2. Permanent freshwater sections and distribution maps

In order to map intermittent river segments, Hydrosheds (Lehner et al., 2008) river network was used as a base, removing the sections subjected to any period of desiccation during the last 15 years. Temporary dry sections were identified using Google Earth satellite imagery from 2003 to 2017. The whole Moroccan hydrographic network was visually checked for water absence in each image for all years available. When at least one image of river section revealed a clear absence of water, that section was identified as intermittent and removed from the Hydrosheds layer. Images where there was no clear view of the water level, e.g., due to tree or rock cover, shadow or low resolution were not removed and therefore may lead to an over-estimation of permanent water surface. However, error should be low given the generally high image quality, and the fact that Google Earth satellite imagery from different years and/or months was available throughout Morocco, which allowed for a standardized identification of intermittent sections.

Potential areas of occurrence, for each basin and each species included river sections between recent confirmed presence sites. Since the presence of empty shells does not provide any indication on how far upstream a population extends, shells (i.e. dead animals) were considered as presences when upstream of sites with living individuals; or in rivers where only shells were found. Maps of potential distribution for each species were obtained by overlapping presence and putative presence points with the Hydrobasin level 8 polygons (Lehner and Grill, 2013). A species richness map was then produced by overlapping species maps.

2.3. DNA extraction, amplification and sequencing

Total DNA was extracted using a high-salt protocol (Sambrook et al., 1989). The F-type COI mtDNA (Ca. 700 bp fragment) was amplified with LCO_22me and HCO_700dy primers (Walker et al., 2007), using PCR conditions described in Froufe et al. (2014) with the annealing temperatures varying from 48 °C to 55 °C. Sequences were obtained using the BigDye sequencing protocol (Applied Biosystems 3730xl) by Macrogen Inc., Korea, using the same primers.

2.4. Phylogenetic and Phylogeographic analyses

Forward and reverse Chromatograms were analysed and edited using ChromasPro 2.6.2 (Technelysium, Tewantin, Australia). The new sequences were deposited in GenBank (Table 1). For each species, an alignment was constructed using ClustalW, in Bioedit v7.2.5 (Hall, 1999), including the previously published sequences from Morocco, and/or from other relevant geographical areas outside Morocco, e.g. sequenced *U. gibbus* from Spain (Araujo et al., 2009, 2010, 2016; Khalloufi et al., 2011; Froufe et al., 2014, 2016b, 2017; Froufe et al., 2016a; Klishko et al., 2018; Sousa et al., 2018 and references within).

For the *Anodonta* sp., given the many uncertainties that still exist about which species is present in Morocco, a phylogenetic analysis was conducted, including all the *Anodonta* sp. from the present study, the four main mtDNA clades of *Anodonta anatina* previously described

(Froufe et al., 2014, 2017; Klishko et al., 2018), and all available sequences for *Anodonta cygnea* and *Anodonta exulcerata* (Porro, 1838). *Cristaria plicata* (Leach, 1814) (GenBank: EU698940), *Anemina arcaeformis* (Heude, 1877) (GenBank: NC_026674), *Sinanodonta woodiana* (Lea, 1834) (GenBank: HQ283346) were used as outgroups. The most appropriate model of molecular substitution for each codon position was selected using jModeltest 2 (Darriba et al., 2012). The best scoring models using Akaike Information Criterion were GTR + I for the first COI codon position, F81 for the second, and GTR for the third. Phylogenetic relationships were estimated by Bayesian inference using MrBayes v. 3.2.6 (Ronquist et al., 2012) in the HPC Black Box of the San Diego Supercomputer Center through the CIPRES S1cience Gateway (https://www.phylo.org). Two independent runs of 2.5×10^7 generations were sampled at intervals of 1000 generations producing a total of 25,000 trees. The burn-in value was determined upon convergence of log likelihood and parameter values, using Tracer v1.7 (Rambaut et al., 2018).

The relationships among closely related haplotypes within each species were evaluated by the construction of a maximum parsimony network under a 95% criterion using TCS 1.2.1 (Clement et al., 2000) and plotted using tcsBU (Múrias Dos Santos et al., 2015). The uncorrected *p*-distances were calculated using MEGA 7.0 (Kumar et al., 2016) and the nucleotide diversity measures were calculated using DnaSP v5.10.01 (Rozas and Rozas, 1995). In order to test for the molecular demographic signature of a hypothesized Pleistocene range expansion, Fu's Fs (1997) and Tajima's D (1989) statistics were calculated using ARLEQUIN v3.5.2.2 (Excoffier and Lischer, 2010).

2.5. Conservation status assessment

AOO and EOO were calculated using two river networks (total and permanent-only), and five area strategies: a) 2 km grid overlay, b) 1 km grid overlay, c) 2 km and d) 1 km buffer along river length, and e) mean river width × length. Mean river width was obtained per basin as the average of six equidistant points within a species' potential range. AOO comprised only confirmed presence points with a 300 m buffer along the river length, while EOO included also the potential segments. EOO and AOO values were then used to assess the Red List species conservation status of each species in Morocco (see Appendix A for the full accounts).

3. Results

3.1. Permanent freshwater sections and distribution maps

Intermittent rivers are more prevalent in the southern regions of Morocco, with most permanent streams being located north of the Atlas Mountains (Fig. 1; Shapefile as Appendix B). Notably, permanent segments can still be found in the relict endorheic basins of Ziz and Ghir. Total permanent river length was estimated at 14,892 km, 18.3% of the total river network.

Sites with extant populations are more numerous in the North of Morocco for all species, with *P. littoralis*, *U. foucauldianus* and *U. gibbus* being distributed throughout the country, while *A. anatina* and *P. marocanus* seem to be restricted to only a few localities (Figs. 2–6).
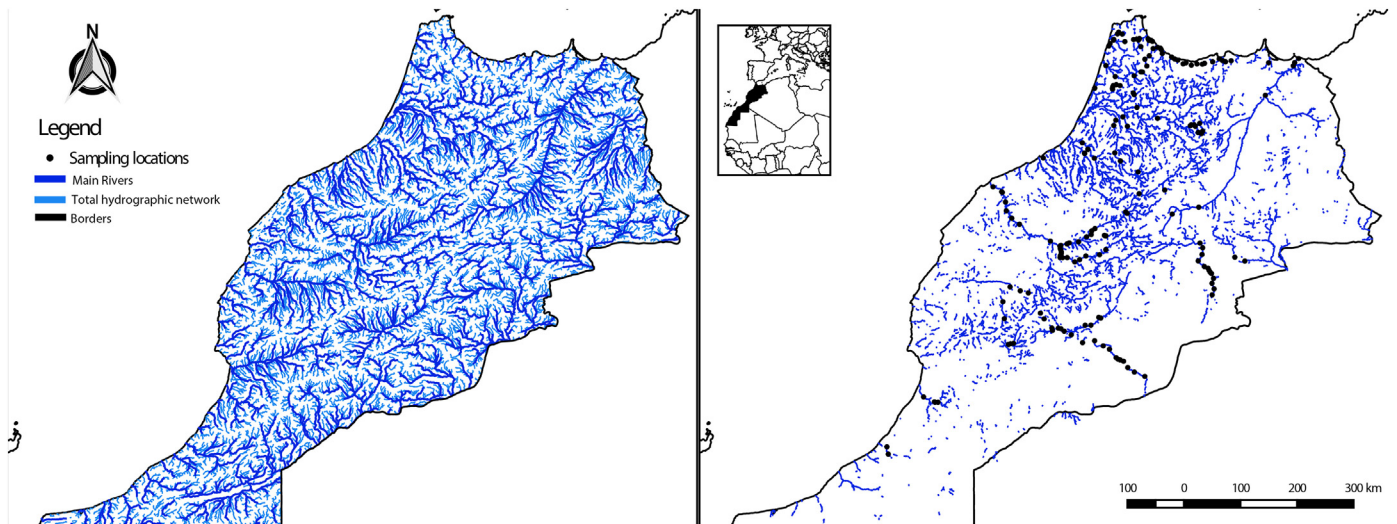
Fig. 1. Complete hydrographic network of Morocco (Hydrosheds). b) Permanent river network obtained by removing the river's sections subject to any period of desiccation during the last 15 years, with sampling locations superimposed.

Based on shell occurrence, unsampled populations may be present for: *A. anatina* in Oum Er Rbia and Mda basins (Fig. 2); *P. littoralis* in Mda, Loukos, Sebou and Oum Er Rbia basins (Fig. 3); *U. foucauldianus* in Mda, Loukos and Oum Er Rbia basins (Fig. 4) and *P. marocanus* in the lower Moulouya basin (Fig. 6).

The northern regions of the country support the highest species richness (Fig. 7). Oum Er Rbia is the unique basin containing all five freshwater mussel species, although there is no level-8 basin with more than four species. The highest species count is located in Mda (Fig. 7). Species richness per basin is lower in southern regions, with only Noun and Massa supporting more than one species.

3.2. Phylogeny of Anodonta and phylogeographic structure of freshwater mussels in Morocco

All mtDNA sequences obtained were considered F-Type given that no sequence similar to M-Type was detected. No insertions, deletions or stop codons were observed after translating all sequences to amino acids.

From the preliminary analyses on the *Anodonta* specimens, the present results show that the Moroccan sequences cluster within the current concept of *A. anatina*. The three previously identified mtDNA clades within *A. anatina* (Froufe et al., 2014) were replicated in this new phylogeny: a first clade including all the individuals from Iberia (except those from the Ebro basin) and the single Moroccan haplotype; a second clade including all the European non-Iberian and non-Italian individuals; and a third clade including all the individuals from Italy and from the Ebro basin. All except the third clade show high support (Fig. 8). Therefore, based on the low genetic divergence (see below), we hereafter consider all *Anodonta* Moroccan specimens as *A. anatina*.

The *A. anatina* dataset included 191 sequences, and the alignment was 555 nucleotides (nt) long. All the 12 Moroccan individuals sampled (6 from each basin), shared the same haplotype corresponding to a newly described haplogroup, closely related to the Iberian haplogroups (Fig. 2). The uncorrected *p*-distances between all haplogroups ranged between 0.96% and 3.28% (Table 2).

The *P. littoralis* dataset included 114 sequences and the alignment was 607 nt long. The most frequent haplotype was present in 31
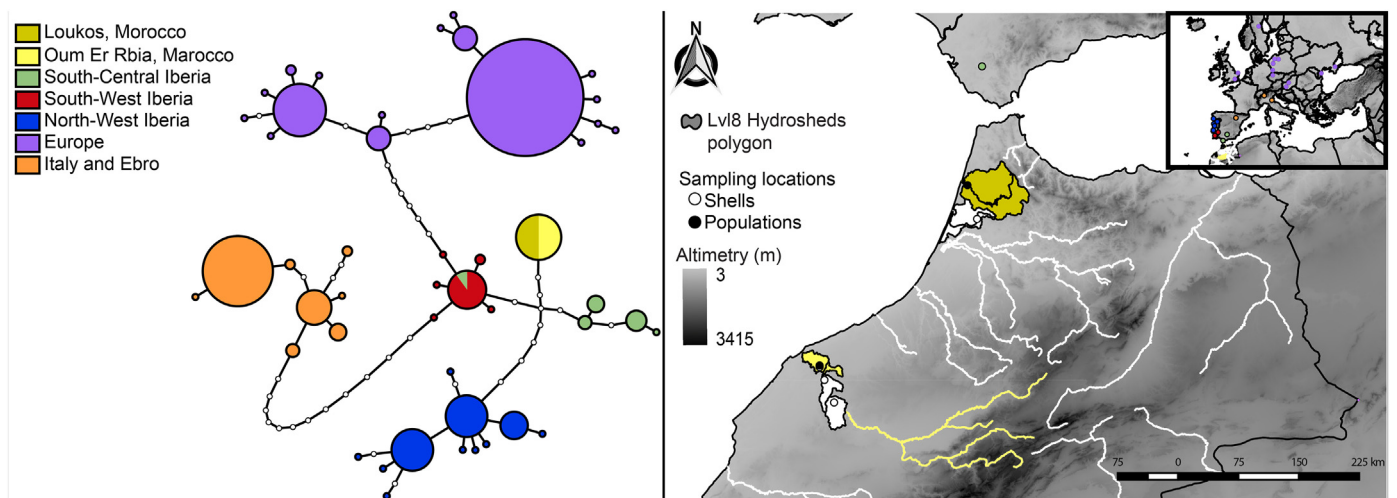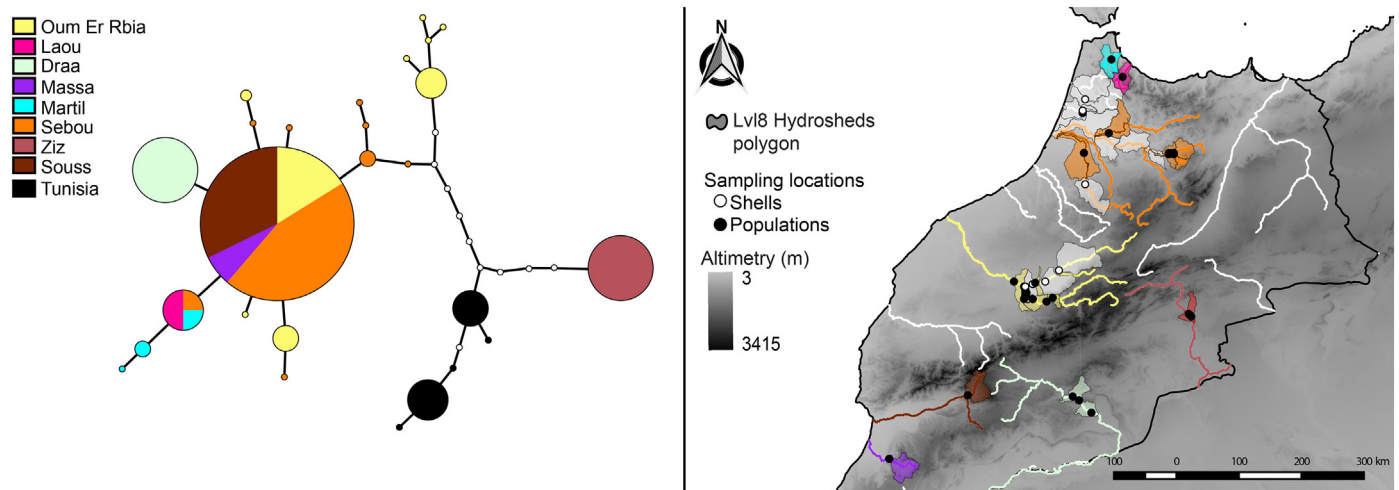

Fig. 2. a) COI haplotype (TCS) network showing the relationships of *A. anatina* haplotypes. Circle size is proportional to the observed haplotype frequencies and white points represent unobserved haplotypes and potential intermediates. Colours correspond to the geographic distribution as seen in b). b) Map showing the distribution of *A. anatina*, black dots represent the recently sampled individuals, white dots represent sampled shells. Lvl8 hydrosheds polygon layer shows the estimated distribution of the species, where white colour represents the potential distribution and other colours represent effective distribution sites. These colours correspond to the haplotypes represented in the network. The colours of the rivers are according the colours represented in the haplotype network as well.

Fig. 3. a) COI haplotype (TCS) network showing the relationships of *P. littoralis* haplotypes. Circle size is proportional to the observed haplotype frequencies and white points represent unobserved haplotypes and potential intermediates. Colours correspond to the geographic distribution as seen in b). b) Map showing the distribution of *P. littoralis*, black dots represent the recently sampled individuals, white dots represent sampled shells. Lvl8 hydrosheds polygon layer shows the estimated distribution of the species, where white colour represents the potential distribution and other colours represent effective distribution sites. These colours correspond to the haplotypes represented in the network. The colours of the rivers are according the colours represented in the haplotype network as well.

individuals and widely distributed. Oum Er Rbia and Sebou basins contained 11 out of 15 total singletons (Fig. 3). The highest values of haplotype diversity were observed in *P. littoralis*, Hd = 0.887 (Table 3). Three haplogroups were retrieved: the first including all individuals from Ziz River (Fig. 3); the second containing all the remaining Moroccan individuals; and the third including only Tunisian individuals. The uncorrected *p*-distances varied from 1.11% to 1.54% (Table 2) and signs of recent population expansion were found in Sebou basin (D = −1.65639, $p < 0.05$; Table 3).

The *U. foucauldianus* dataset included 121 sequences and the alignment was 624 nt long. Most individuals (N = 78) shared the same haplotype (Fig. 4), and the tests of recent population expansion were significant for the species (D = −1.56240, $p < 0.05$; Fs = −9.13433, $p < 0.02$) (Table 3).

The *U. gibbus* dataset included 65 sequences and the alignment was 559 nt long. Out of the 12 haplotypes, nine were from Morocco, four from Tunisia and one from Spain (Fig. 5). One side of the network is

formed by one haplotype present in four Moroccan populations, only one mutation from the Spanish haplotype; the other side is formed by the four Tunisian haplotypes (Fig. 5). The uncorrected *p*-distances varied from 0% to 2.25% for *U. gibbus* (Table 2).

The *P. marocanus* dataset included 22 sequences and the alignment was 657 nt long. The haplotype diversity within Oum Er Rbia basin (Hd = 0.711) was higher than in Sebou basin (Hd = 0.303) (Table 3) and the haplotype network is shown in Fig. 6. Demographic history tests were not significant, showing no evidence for recent expansion (Table 3).

3.3. Conservation status

EOO and AOO varied considerably across measurement strategies, with the 2 × 2 km grid having 32 to 583 times larger values than the mean river width strategy (Table 4, Fig. 8). The latter strategy resulted in all species being considered as CR according to criterion B, whereas
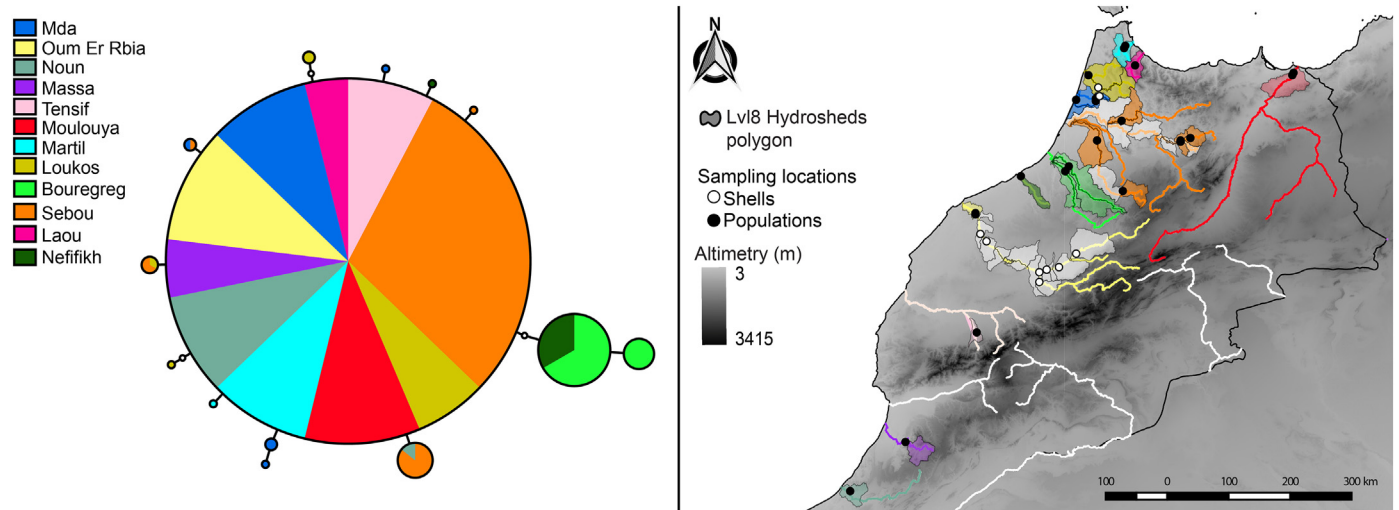
Fig. 4. a) COI haplotype (TCS) network showing the relationships of *U. foucauldianus* haplotypes. Circle size is proportional to the observed haplotype frequencies and white points represent unobserved haplotypes and potential intermediates. Colours correspond to the geographic distribution as seen in b). b) Map showing the distribution of *U. foucauldianus*, black dots represent the recently sampled individuals, white dots represent sampled shells. Lvl8 Hydrosheds polygon layer shows the estimated distribution of the species, where white colour represents the potential distribution and other colours represent effective distribution sites. These colours correspond to the haplotypes represented in the network. The colours of the rivers are according the colours represented in the haplotype network as well.

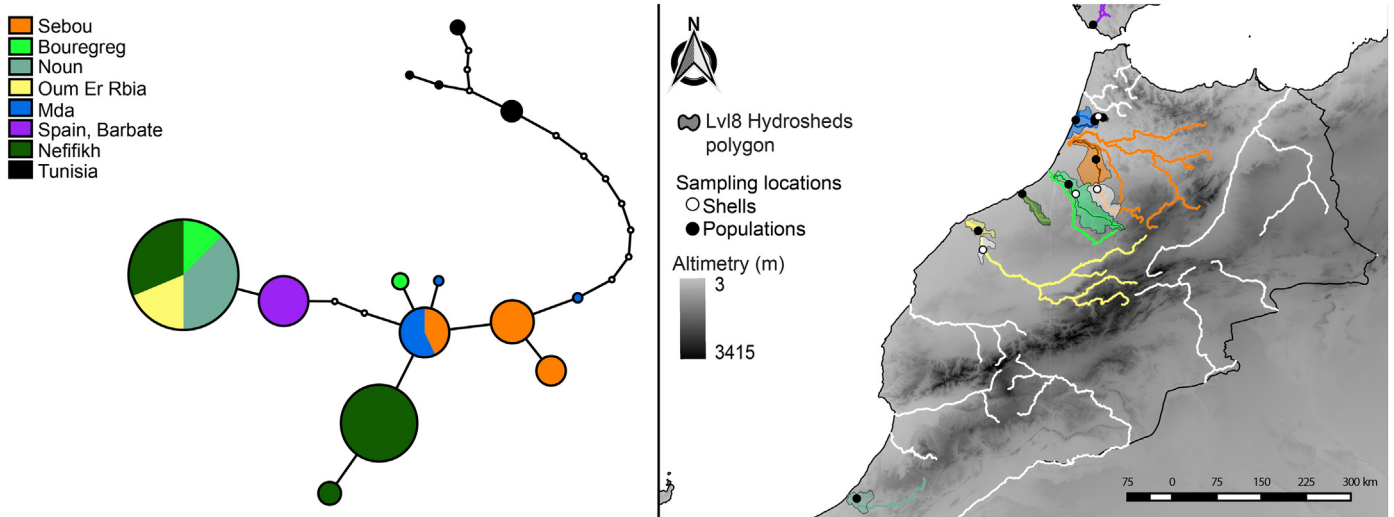**Fig. 5.** a) COI haplotype (TCS) network showing the relationships of *U. gibbus* haplotypes. Circle size is proportional to the observed haplotype frequencies and white points represent unobserved haplotypes and potential intermediates. Colours correspond to the geographic distribution as seen in b). b) Map showing the distribution of *U. gibbus*, black dots represent the recently sampled individuals, white dots represent sampled shells. Lvl8 hydrosheds polygon layer shows the estimated distribution of the species, where white colour represents the potential distribution and other colours represent effective distribution sites. These colours correspond to the haplotypes represented in the network. The colours of the rivers are according the colours represented in the haplotype network as well.

the 2 km grid/buffer returns a mix of EN or CR. Considering just the permanent river network resulted in estimated EOO decreases of up to 13%, but without influence in conservation statuses. Following these results, all complete conservation status accounts are presented in Appendix A and were assessed using the mean river width strategy for the estimation of AOO and EOO.

4. Discussion

This study represents the first all-inclusive genetic diversity and distribution assessment of all species of freshwater mussels in Morocco, providing the basis for future conservation management actions and further research. The comparison of AOO and EOO calculation methods contributes towards a more accurate way of determining the

conservation status of sessile and permanent refugial freshwater taxa. Additionally, the mapping of river intermittency here performed will be highly valuable for future studies and allow better management policies for freshwater taxa conservation in this semi-arid region. This study has shed light on the long lasting taxonomic uncertainties concerning *Anodonta* sp., and increased the phylogeographic knowledge on the five native species, *A. anatina*, *P. marocanus*, *P. littoralis*, *U. foucauldianus* and *U. gibbus* present in Morocco.

4.1. Phylogeographic structure of the freshwater mussels of Morocco

The north-south gradient of genetic diversity, species richness and distribution range size is strongly coherent with water availability and river intermittence. Whereas north of High and Middle Atlas Mountains
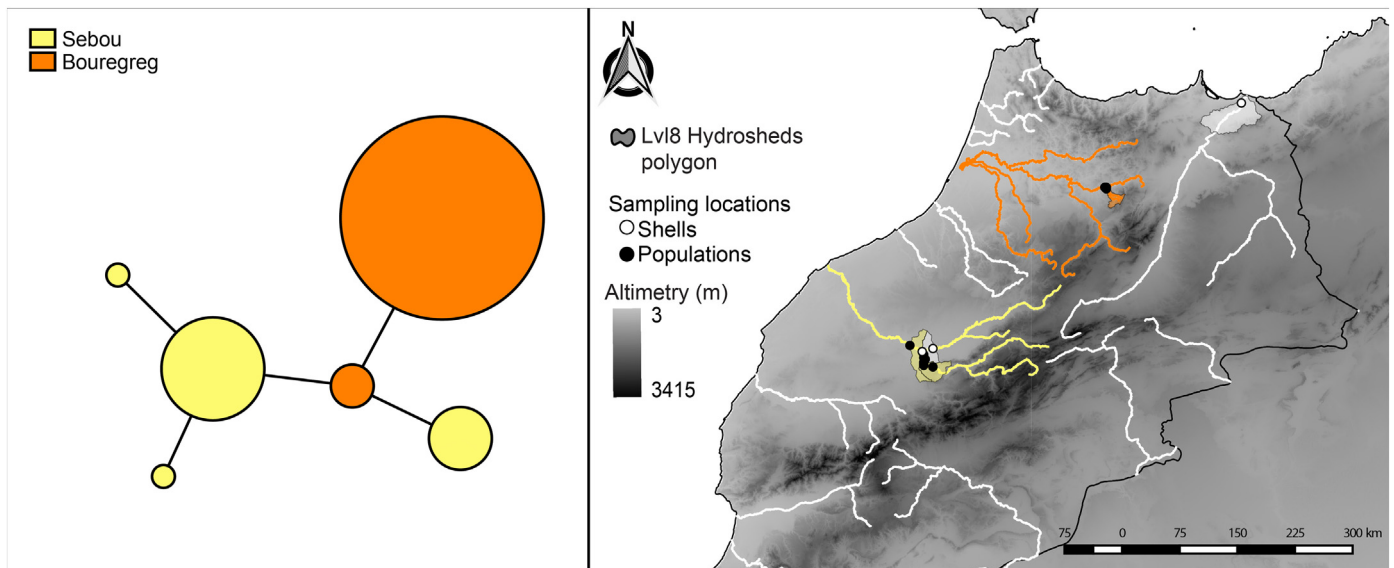

Fig. 6. a) COI haplotype (TCS) network showing the relationships of *P. marocanus* haplotypes. Circle size is proportional to the observed haplotype frequencies and white points represent unobserved haplotypes and potential intermediates. Colours correspond to the geographic distribution as seen in b). b) Map showing the distribution of *P. marocanus*, black dots represent the recently sampled individuals, white dots represent sampled shells. Lvl8 Hydrosheds polygon layer shows the estimated distribution of the species, where white colour represents the potential distribution and other colours represent effective distribution sites. These colours correspond to the haplotypes represented in the network. The colours of the rivers are according the colours represented in the haplotype network as well.
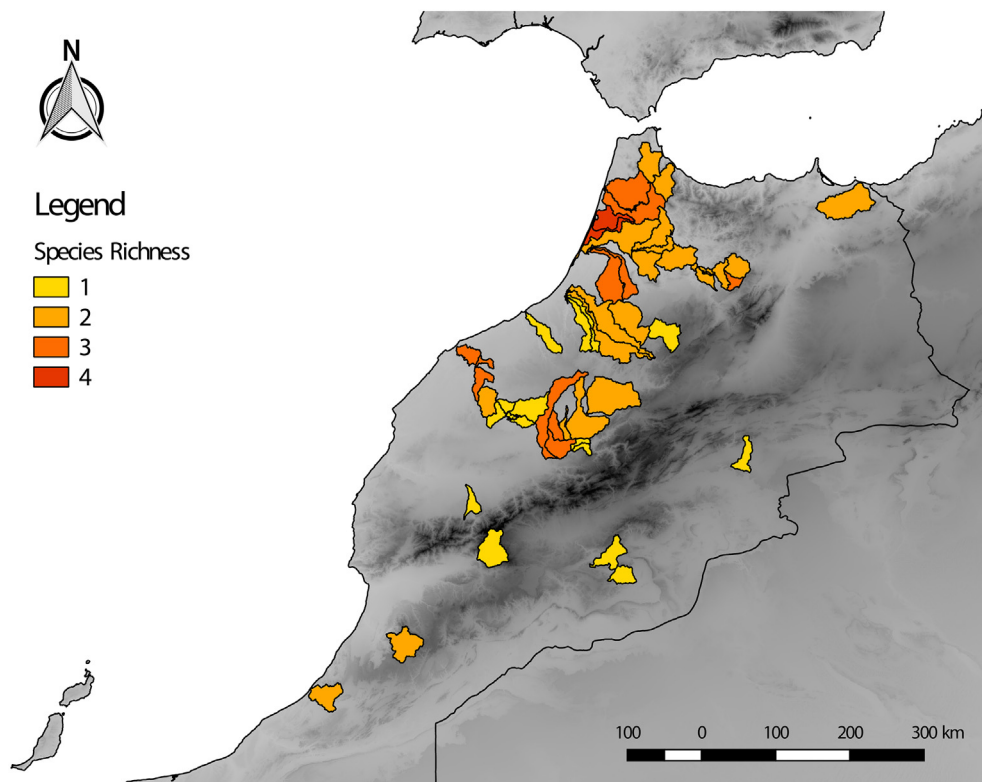
Fig. 7. Map of species richness representing the overlapping potential areas of distribution. Colours differ according to the number of species present in each area.

many rivers are permanent, in southern regions mussel populations are usually restricted to small pools subjected to very harsh abiotic conditions (e.g. oxygen, conductivity, pH and temperature; Sheldon and Fellows, 2010; Sheldon et al., 2010). During extreme drought conditions, most of the southern rivers in Morocco probably dry out, causing local extirpations. The consequently more pronounced genetic drift results in lower intraspecific genetic diversity. Similar patterns are found in other taxa, and have been attributed to the onset of the Sahara Desert and climatic shifts between humid and arid phases during the Plio-Pleistocene (Le Houérou, 1992). Although humid periods allow demographic expansions and recolonization of more arid (southern) regions, only the more resilient and adaptable species can do so and persist there (Brito et al., 2014). Even after the last Glacial Maximum (~18,000 years) the fluctuation continued, sometimes changing every 100 years (Sarnthein, 1978; Schuster et al., 2006).

Another prevalent pattern was the phylogeographic separation between Tunisian and Moroccan haplotypes (observed in *P. littoralis* and *U. gibbus*). This has been found not only in freshwater mussels (Khalloufi et al., 2011; Araujo et al., 2016; Froufe et al., 2016b) but also in other animals (e.g. fishes, Machordom and Doadrio, 2001; amphibians and reptiles, Recuero et al., 2007; Beddek et al., 2018), but like in many other taxa, sampling gaps in Algeria preclude further conclusions.
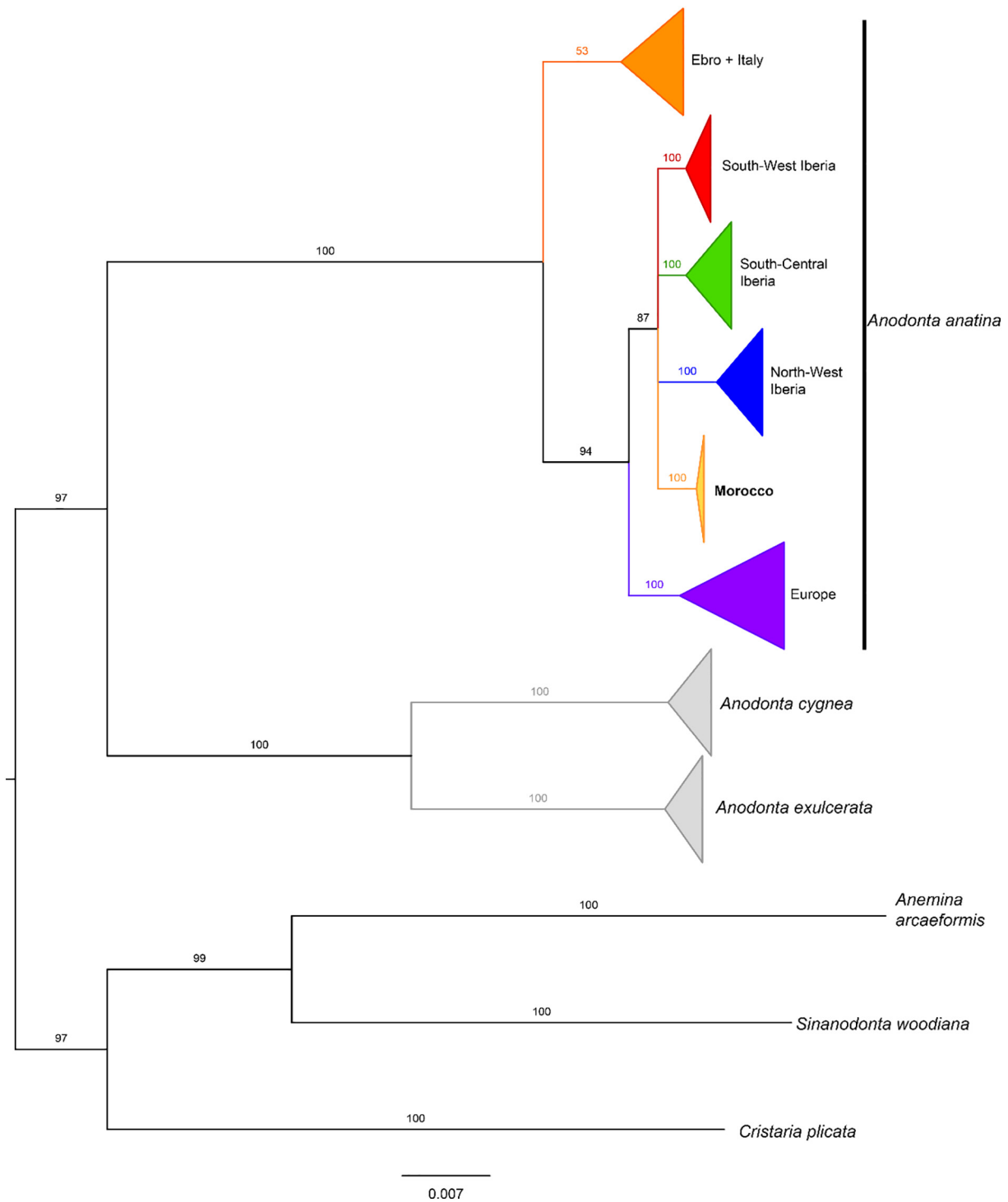
4.1.1. Anodonta anatina

Regarding *Anodonta* sp., many uncertainties persisted about which species exist in Morocco and the only recent records of its presence are from the Oum Er Rbia basin (Sousa et al., 2016). It has been speculated that Morocco either holds an endemic species named *Anodonta pallaryi* (Bédé, 1932) (Haas, 1969; Van Damme and Ghamizi, 2010a), or a wide-ranged European species, *Anodonta anatina* (Linnaeus, 1758) (Graf and Cummings, 2015). To date, no studies using molecular markers have been performed in order to support either classification. The present molecular data clusters the single Moroccan *Anodonta* haplotype within *A. anatina*, with a low genetic distance from the Southern Iberian haplogroups (Figs. 2 and 8 and Table 2). Additional nuclear data from a

small number of individuals show that Iberian and Moroccan populations share the same haplotype for the Histone 3 (H3) and 28S ribosomal genes (data not shown). Therefore, we consider the previously nominal taxa *Anodonta* (*Liouvillea*) *pallaryi* Bédé, 1932, *Anodonta* (*Liouvillea*) *theryi* Bédé, 1932 and *Anodonta* (*Liouvillea*) *gruveli* Perès, 1938 as junior synonyms of *Anodonta anatina* (Linnaeus, 1758). Still, a thorough taxonomic revision should be performed in the future using nuclear molecular markers together with morphological and ecological traits. The grouping of the Moroccan haplotype with the Iberian haplogroups (Fig. 2), whose genetic structure resulted from a likely refugia within refugia process (i.e. several distinct refugia within Iberian Peninsula; Froufe et al., 2014, 2017), further highlighting Morocco as an additional refugium in this species' phylogeographic history (Husemann et al., 2014; Prendergast et al., 2016). The wide European distribution and the close genetic proximity of the Moroccan and Southern Iberian populations (Figs. 2 and 8 and Table 2), suggest an Iberian origin for the colonization of the Maghreb. Given that the Mediterranean Sea represents an effective barrier, colonization may have taken place over the land bridge that was formed around 5.6 Mya between Europe and Africa. Post-Messinian vicariance has already been associated with divergences between other species of freshwater mussels (Araujo et al., 2016; Bolotov et al., 2016; Froufe et al., 2016a, 2016b). However, in those cases, divergence between Maghrebian and Iberian populations was much higher (3.2% for *Unio* spp. and 9.2% for Margaritiferidae, compared to 1%), which may imply faunal exchanges over previous land bridges (Bolotov et al., 2016), or even, in the case of *A. anatina*, a posterior event of dispersal.

4.1.2. Potomida littoralis

This species' high haplotypic diversity and wide distribution in Morocco (Fig. 3 and Table 3) indicates a higher adaptability to the environmental conditions found throughout Moroccan freshwater ecosystems. Further support comes from *P. littoralis* being the only freshwater mussel species found in the Draa and Ziz basins, both located in arid regions and being mostly intermittent along their extent (Clavero

Fig. 8. *Anodonta* Bayesian phylogenetic tree of the COI fragment. *Cristaria plicata*, *Anemina arcaeformis* and *Sinanodonta woodiana* were used as outgroups. The values above the branches indicate posterior probabilities. Within *A. anatina*, colours highlight the major mtDNA groups corresponding to the geographic distribution as seen in Fig. 2.

et al., 2015, 2017). The haplotype from the endorheic Ziz basin is closer to Tunisian haplotypes than to Moroccan ones, suggesting gene-flow during past wet periods in the Sahara like during the mid-Holocene (Drake et al., 2011; Clavero et al., 2015).

Even though the demographic history tests were inconclusive, when excluding Ziz haplotype the Fu's Fs results are significant for a recent expansion. Although genetic distinctiveness among basins suggests the species persisted in several basins, Sebou and Oum Er Rbia basins possess the highest genetic diversity. This is probably due to a more

stable environment during Plio-Pleistocene climatic fluctuations and more constant water flow from the mountains.

4.1.3. Unio foucauldianus

This study represents a great increase in the data available for *U. foucauldianus* (see Froufe et al., 2016a), expanding the known range to 12 basins. However, unlike *P. littoralis*, *U. foucauldianus* must have suffered a region-wide bottleneck, resulting in much lower genetic diversity (Hd = 0.566 – Table 3; 65% of the individuals sharing the same

Table 2
Mean genetic divergences for the COI dataset, among the populations and/or haplogroups of *Anodonta anatina*, *Potomida littoralis* and *Unio gibbus*.

Species	Populations/Haplogroups	1	2	3	4	5	6	7	8	9
Anodonta anatina	1 - European									
Anodonta anatina	2 - Italy/Ebro	0.03282								
Anodonta anatina	3- South West Iberia	0.02148	0.02852							
Anodonta anatina	4 -South Central Iberia	0.02253	0.03041	0.0096						
Anodonta anatina	5 - North West Iberia	0.02623	0.03505	0.01491	0.01641					
Anodonta anatina	6 - Morocco	0.02282	0.02988	0.00973	0.0112	0.01602				
Potomida littoralis	1 - Martil									
Potomida littoralis	2 - Massa	0.003								
Potomida littoralis	3 - Oum Er Rbia	0.008	0.005							
Potomida littoralis	4 - Sebou	0.004	0.001	0.005						
Potomida littoralis	5 - Laou	0.001	0.002	0.006	0.002					
Potomida littoralis	6 - Draa	0.005	0.002	0.006	0.003	0.003				
Potomida littoralis	7 - Ziz	0.018	0.015	0.017	0.015	0.016	0.013			
Potomida littoralis	8 - Souss	0.003	0	0.005	0.001	0.002	0.002	0.015		
Potomida littoralis	9 - Tunisia	0.017	0.014	0.016	0.015	0.016	0.013	0.011	0.014	
Potomida littoralis	1 - Moroccan Haplogroup									
Potomida littoralis	2 - Ziz Haplogroup	0.0154								
Potomida littoralis	3 - Tunisian Haplogroup	0.0149	0.0111							
Unio gibbus	1 - Bouregreg									
Unio gibbus	2 - Noun	0.0045								
Unio gibbus	3 - Oum Er Rbia	0.0045	0							
Unio gibbus	4 - Beth, Sebou	0.0064	0.0091	0.0091						
Unio gibbus	5 - Mda	0.0054	0.0081	0.0081	0.0024					
Unio gibbus	6 - Barbate, Spain	0.0045	0.0018	0.0018	0.0073	0.0063				
Unio gibbus	7 - Nefifikh	0.0059	0.0066	0.0066	0.0054	0.0044	0.0057			
Unio gibbus	8 - Tunisia	0.0221	0.0212	0.0212	0.0204	0.0209	0.0194	0.0225		

haplotypes - Fig. 4).

4.1.4. Unio gibbus

Unio gibbus seems to have a higher resilience to wide-ranging climatic shifts when compared with *U. foucauldianus*, given the higher genetic diversity in spite of a narrower distribution (Figs. 4 and 5 and Table 3). The nesting of the Spanish haplotype within Moroccan ones, and the very restricted current and historical distribution in Southern Spain (Araujo et al., 2010; Lopes-Lima et al., 2017), suggest that Maghreb was the origin of Iberia's colonization (Fig. 5). The close genetic proximity between Spanish and Moroccan haplotypes (uncorrected *p*-distance 0.18%–0.73% - Table 2) suggests a colonization much more recent than the last land bridge between North Africa and Iberian Peninsula (i.e. 5.6 Mya). This raises the possibility of a human-mediated introduction through fish host or mussel translocations. Anthropogenic mediated introductions of other taxa in South West Europe from Morocco have already been reported (e.g. mammals Dobson, 1998; and amphibians Recuero et al., 2007). This hypothesis should be further investigated as it may have implications in the application of conservation actions for this species in Spain.

4.2. Conservation

Occupancy parameters (EOO and AOO) are expected to be heavily influenced by river intermittence. The low differences here reported (below 13%) are probably an effect of sparse sampling and our conservative estimation of species distribution ranges. Given that we considered as potential presence only the river sections between two confirmed presence points, the base range was already biased towards large rivers, which are more likely to be permanent. Studies with finer sampling, or using taxa that occur in smaller rivers, are still expected to benefit greatly from river intermittence maps. Constructing maps of river intermittence will have important conservation and management implications at a worldwide scale. Intermittent rivers are among the freshwater ecosystems that receive less conservation attention and are generally poorly managed (Leigh et al., 2016, 2019). These systems tend to be overlooked by countries' authorities and, in some cases, not even legally recognised as part of the river network (Acuña et al., 2014;

Marshall et al., 2018). The generalized disregard of these systems partially results from inefficient characterization of water flow regimes associated with being ungauged, unmapped, or inaccurately depicted in topographic maps (Datry et al., 2014, 2017). Generating typologies that represent the natural heterogeneity of intermittent rivers is crucial to guide adequate management planning in both time and space and ensure restoration of the natural flow regimes (Datry et al., 2017). Identifying permanent water section refuges and ensure the undisrupted connectivity during flow seasons is essential for the long term survival and recovery of aquatic communities (Karaouzas et al., 2018). The results here presented clearly show the potential of combining topographically-derived hydrographic networks with the open source satellite imageries to create a realistic depiction of the intermittent river networks. Moreover, this strategy can be easily replicated in any river network and, given the high quality and quantity of satellite imagery available, it can even be applied at different spatial and temporal scales.

Regarding the EOO and AOO calculations, the higher sensitivity of the buffer strategies to differentiate between total and permanent-only river networks indicate an advantage. Furthermore, the 1 and 2 km buffers are a gross over-estimation of most mean river widths and therefore of the distribution range of aquatic species, and even some riparian species. The use of the 1 or 2 km standard buffer can greatly underestimate the threat status of many species, as observed in Table 4 and Fig. 9. Therefore, we propose here that for riverine species, AOO and EOO should be estimated with the mean length of the considered river network and not by the fixed grid or buffer area.

Transposing our approach of AOO and EOO estimation from a regional demonstration to a global implementation can have a fundamental impact on freshwater species preservation. Being the most globally relevant list of species at risk of extinction (Cardoso et al., 2011b), the IUCN Red List assessments have a fundamental role in guiding conservation efforts and funding, prioritizing protection acts, evaluating site irreplaceability and vulnerability, mobilizing environmental policies and legislation and monitoring biodiversity (Cardoso et al., 2011b; Carrizo et al., 2017 and references within). Thus, realistically assigning species conservation status must be a primary concern.

Considering the high species richness found in Northern rivers (Fig. 7), where all five native species are present, it is clear that these

Table 3

Summary of indices of genetic diversity estimated from the COI sequencing data for all populations all species: sample size (N), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π). Values of test of growth within each specie, i.e. the results of Fu's Fs and Tajima's D neutrality test. Statistically significant values are followed by an asterisk ($p < 0.05$ for Tajima's D and $p < 0.02$ for Fu' Fs).

Species	Haplogroup/populations	N	h	Hd	π	Fu's FS	Tajima's D
Anodonta anatina	European	72	17	0.758	0.00641	−4.98025	−0.28149
Anodonta anatina	Italy/Ebro	41	8	0.734	0.00403	−1.16817	−0.17893
Anodonta anatina	South West Iberia	15	6	0.648	0.00141	−3.61846*	−1.66013*
Anodonta anatina	South Central Iberia	14	5	0.791	0.00398	0.32272	−0.45993
Anodonta anatina	North West Iberia	37	11	0.803	0.00256	−5.4955*	−1.57827*
Anodonta anatina	Morocco	12	1	0	0	–	–
Pseudunio marocanus	Boulhou	12	2	0.303	0.00046	0.297	−0.19492
Pseudunio marocanus	Oum Er Rbia basin	10	4	0.711	0.00203	−0.277	−0.21888
Pseudunio marocanus	All Moroccan distribution	22	6	0.745	0.00213	−1.107	0.06716
Potomida littoralis	Martil	6	3	0.733	0.00139	−0.42679	−0.05002
Potomida littoralis	Massa	2	2	0	0	–	–
Potomida littoralis	Oum Er Rbia	23	9	0.858	0.00633	−0.50165	0.318
Potomida littoralis	Sebou	25	9	0.683	0.00192	−5.1001*	−1.65639*
Potomida littoralis	Laou	4	1	0	0	–	–
Potomida littoralis	Draa	13	1	0	0	–	–
Potomida littoralis	Ziz	13	1	0	0	–	–
Potomida littoralis	Souss	10	1	0	0	–	–
Potomida littoralis	Tunisia	18	5	0.68	0.00288	0.23226	0.74839
Potomida littoralis	All Moroccan distribution	114	26	0.887	0.00857	−4.45264	−0.54629
Unio foucauldianus	Mda	12	5	0.667	0.00146	−2.14438*	−1.10317
Unio foucauldianus	Sebou	33	5	0.491	0.00087	−2.15389*	−1.11821
Unio foucauldianus	Oum Er Rbia	8	1	0	0	–	–
Unio foucauldianus	Noun	6	2	0.333	0.00053	−0.00275	−0.93302
Unio foucauldianus	Massa	6	1	0	0	–	–
Unio foucauldianus	Tensift	6	1	0	0	–	–
Unio foucauldianus	Moulouya	8	1	0	0	–	–
Unio foucauldianus	Martil	8	2	0.25	0.0004	−0.18197	−1.05482
Unio foucauldianus	Loukos	9	4	0.694	0.00232	−1.26031	−0.91004
Unio foucauldianus	Bouregreg	16	2	0.500	0.00080	1.24657	1.30896
Unio foucauldianus	Lou	3	1	0	0	–	–
Unio foucauldianus	Nefifikh	6	2	0.333	0.00160	1.60944	−1.23311
Unio foucauldianus	All Moroccan distribution	121	14	0.566	0.00173	−9.13433*	−1.56240*
Unio gibbus	Bouregreg	4	2	0.667	0.00596	3.15252	2.12492
Unio gibbus	Noun	6	1	0	0	–	–
Unio gibbus	Oum Er Rbia	3	1	0	0	–	–
Unio gibbus	Beth, Sebou	13	3	0.692	0.00151	0.43557	0.87897
Unio gibbus	Mda	6	3	0.6	0.00179	−0.18945	−1.23311
Unio gibbus	Barbate, Spain	7	1	0	0	–	–
Unio gibbus	Nefifikh	19	3	0.602	0.00401	3.53364	1.75913
Unio gibbus	Tunisia	7	4	0.81	0.00494	0.49468	0.64916
Unio gibbus	All Moroccan distribution	51	9	0.827	0.00516	0.24080	0.85075

Table 4

Areas of occupancy (AOO) and Extent of occurrence (EOO) values for the five freshwater mussel species of Morocco. Five strategies using either a buffer or square grids (UTM29) were applied to two river networks: total hydrosheds network, and permanent-rivers only. Cells are shaded according to the resulting conservation status according to IUCN Red List criterion B: yellow – Endangered; red – Critically endangered.

	Calculated values of EOO and AOO (km²)									
	Anadonta anatina		*Potomida littoralis*		*Unio foucauldianus*		*Unio gibbus*		*Pseudunio marocanus*	
	EOO	AOO	EOO	AOO	EOO	AOO	EOO	AOO	EOO	AOO
2 × 2 km grid – Permanent	168	8	1748	120	2564	104	516	44	232	44
2 × 2 km grid – Total	168	8	1772	120	2600	104	532	44	232	44
2 km buffer – Permanent	169	8	1569	124	2246	108	398	52	185	52
2 km buffer – Total	169	8	1616	124	2323	108	449	52	184	52
1 × 1 km grid – Permanent	91	2	887	30	1302	27	237	11	106	12
1 × 1 km grid – Total	91	2	908	30	1329	27	253	11	106	12
1 km buffer – Permanent	83	2	780	31	1118	27	197	13	91	13
1 km buffer – Total	83	2	803	31	1156	27	222	13	91	13
River length × width – Permanent	4.16	0.02	54.17	0.48	63.91	0.28	5.61	0.08	3.52	0.08
River length × width – Total	4.17	0.02	55.87	0.48	65.62	0.28	6.01	0.08	3.52	0.08
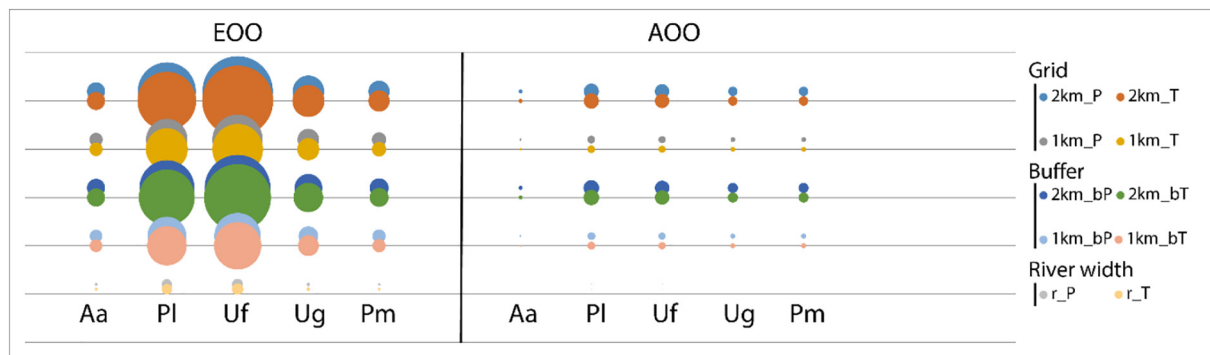
Fig. 9. Areas of occupancy (AOO) and extent of occurrence (EOO) values for the five freshwater mussel species of Morocco: *A. anatina* (Aa), *P. littoralis* (Pl), *U. foucauldianus* (Uf), *U gibbus* (Ug) and *P. marocanus* (Pm). Five strategies were applied to two river networks - total Hydrosheds network (T), and permanent-rivers (P); and using either a buffer or square grids (UTM29) - 2 km grid (2 km), 1 km grid (1 km), 2 km buffer (b 2 km) and 1 km buffer (b 1 km) along river length, and river width ∗ length (r).

areas represent broad priority areas for conservation of freshwater mussels. However, protecting some of the southern basins is paramount to safeguard phylogenetically unique populations.

Anodonta anatina in Morocco is likely restricted to Oum Er Rbia and Loukos basins, since the old records from Bouregreg/Grou River were not confirmed, and only shells were found in Mda. Based on the estimated EOO and AOO the species is here listed as Critically Endangered in Morocco B1ab(i,ii,iii,iv), B2ab(i,ii,iii,iv) (Table 4; see Appendix A for complete assessment). The species is very common in Europe, colonizing a wide variety of habitats (Hinzmann et al., 2013; Lopes-Lima et al., 2017). So it is unclear if the phylogeographic distinctiveness of the Moroccan populations warrants a CR status. The latter could possess unique adaptations to aridity (invaluable when considering future climate scenarios), but that is still speculative.

Potomida littoralis, widely distributed in South-west Europe and in Maghreb region, was recently listed as Endangered, globally (Lopes-Lima et al., 2014a). This species is widely distributed in Morocco, but most of the populations are highly fragmented, in some cases occurring in one location within each basin. The populations from Draa and Ziz basins (Fig. 3), relicts from the Green Sahara, present high genetic and phylogeographic uniqueness and should receive particular conservation attention. Based on the estimated EOO and AOO the species is here listed as Critically Endangered in Morocco B1ab(i,ii,iii,iv), B2ab (i,ii,iii,iv) (Table 4; see Appendix A for complete assessment).

Unio foucauldianus is still listed as Critically Endangered by the IUCN (Van Damme and Ghamizi, 2010b) but has been in need of conservation status reassessment since the results of a recent taxonomic revision revealing the species distribution to be wider than previously believed (Froufe et al., 2016a). In fact, the present study confirms that *U. foucauldianus* is present in several basins, although the distribution seems highly fragmented (often one population per basin). In spite of the recently proposed IUCN conservation status down-listing of *U. foucauldianus* (Froufe et al., 2016a), the reassessment of the species still resulted in Critically Endangered B1ab(i,ii,iii,iv), B2ab(i,ii,iii,iv) status due to the very low estimated EOO and AOO (Table 4; see Appendix A for complete assessment). Populations from Bouregreg and Nefifikh basins are of particular interest given their genetical distinctiveness.

Unio gibbus is present from Morocco to Tunisia and in Southern Spain, where only a small population persists. For this reason, the species is listed as Critical Endangered in Europe (Araujo, 2011). In Morocco, *U. gibbus* is present in several basins, although the distribution is highly fragmented (mostly one population per major basin). The species abundance is generally low across all sites (e.g. 3 individuals across the many sampling sites in Oum Er Rbia), and mainly occurs in downstream areas where it is highly exposed to human disturbance. The species is here assessed as Critically Endangered in Morocco B1ab (i,ii,iii,iv), B2ab(i,ii,iii,iv) due to the very low estimated AOO and EOO

(Table 4; see Appendix A for complete assessment). Bouregreg and Nefifikh basins should receive particular attention; the first given the apparent poor status of the population (four individuals found) and their high diversity; and the second due to genetic distinctiveness.

Pseudunio marocanus is currently listed as Critically Endangered based on its dramatic decline over the last one hundred years, being currently restricted to two basins (Oum Er Rbia and Sebou; Sousa et al., 2016, 2018). *Pseudunio marocanus* represents an obvious conservation priority. Shells found in Moulouya River could indicate the presence of an undocumented population in this river (Fig. 6), so additional surveys are urgently needed. Meanwhile, considering EOO and AOO values (Table 4), is here listed as Critically Endangered B1ab(i,ii,iii,iv), B2ab (i,ii,iii,iv) globally and in Morocco (see Appendix A for complete assessment).

4.3. Conclusions

This work substantially increases the present knowledge about the current distribution and phylogeographic patterns of freshwater mussels in a poorly studied area, where freshwater ecosystems are particularly threatened by water shortage and increasing aridity. Here we show that the permanent sections correspond to only 18.3% of the whole hydrographic network of Morocco. Scientific studies and conservation plans need to consider the water-body intermittence and not rely solely in topographically-derived hydrographic networks, particularly in arid and semi-arid regions. Using standard (1–2 km) square grids to evaluate freshwater taxa may highly underestimate the threat status, and we propose the mean river width as a more sensible approach that requires a minimal increase in effort. Using tailored approaches to each ecosystem is technically feasible and urgently needed in order to anticipate the effects of climate and land- and water-use changes and effectively protect the highly threatened freshwater taxa.

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