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Analysis of the microtheriofauna of the Alta Murgia National Park (Apulia)

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Abstract

The study presents the first data on the community of micromammals present in the Alta Murgia National Park (Puglia, Italy) by analyzing pellets from several species of raptors. A total of 300 pellets were considered: 131 of Barn Owl, *Tyto alba,* 5 of Long Eared Owl, *Asio otus*, 94 of Kestrel, *Falco tinnunculus* and 70 of Little Owl, *Athene noctua*. The analysis of the pellets allowed to determine 1039 individuals of micromammals belonging to 8 species. Savi's pine vole, *Microtus savii* resulted as the most frequent species. Of particular interest is the discovery of a specimen of Italian water vole, *Arvicola italicus*. A correlation between the frequency of presence of *M. savii* and *Suncus etruscus* and the landscape diversity index (Hill number) was found.

Keywords: micromammals, raptors, pellets, Alta Murgia

Riassunto

Questo studio presenta i primi dati sulla comunità di micromammiferi presenti nel Parco Nazionale dell'Alta Murgia (Puglia, Italia) analizzando borre di diverse specie di rapaci. Sono state analizzate in totale 300 borre: 131 di Barbagianni, *Tyto alba*, 5 di Gufo comune, *Asio otus*, 94 di Gheppio, *Falco* *tinnunculus* e 70 di Civetta, *Athene noctua*. L'analisi delle borre ha consentito di determinare 1039 individui di micromammiferi appartenenti a 8 specie. L'arvicola di Savi, *Microtus savii* è risultata la specie più frequente. Di particolare interesse è stato il ritrovamento di un esemplare di arvicola d'acqua italiana, *Arvicola italicus*. È stata riscontrata una correlazione tra la frequenza di presenza di *M. savii* e *Suncus etruscus* e l'indice di diversità del paesaggio (numero di Hill).

Parole chiave: micromammiferi, rapaci, borre, Alta Murgia

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Introduction

Distribution data about micromammals in Italy is abundant (e.g. Amori et al. 2008, Piccari and Szpunar 2012, Amori et al. 2014, Gaggi et al. 2014), anyway its geographic coverage is discontinuous, and many areas are still poorly investigated. This is the case of Apulia, despite its remarkable position that makes it a natural bridge between Europe and the East Mediterranean. An exhaustive distributional analysis of Apulian microtheriofauna is still lacking and some areas, such as the Alta Murgia district, are almost unknown.

Micromammals occupy an intermediate level of the trophic pyramid and are a key element to support the diversity and abundance of predators at higher levels (Amori et al. 2014). Their burrowing activities contribute to the bioturbation of the soil and increase water retention (Golley et al., 1975). Besides, their tunnels can host complex communities of arthropods (Carpaneto et al., 2011). Therefore, an optimal management of micromammal populations is a core element in the conservation of the target species and of the communities that co-evolved with them (Carpaneto et al. 2011). It is well known that pellet analysis is a valid tool in gathering data on micromammals (Contoli 1981, Nappi 2001) and is one of the most used methods for the study of microteriocenosis (Cheylan 1976, Morton and Martin 1979, Contoli 1980, 1981, Campbell et al. 1987, Contoli et al. 1991).

In the present contribution we present the first data on the community of micromammals present in the Alta Murgia National Park by comparing pellets from several species of raptors.

The Park was established in 2004 (DPR March 10 2004) and extends for about 68.077 ha in the western Province of Bari, including the highest elevations of the Murge Plateau. It falls within Natura 2000 SIC/ZPS IT9120007 "Murgia Alta" which covers about 128.880 ha.

Most of the Alta Murgia territory is characterized by mosaic areas in which semi-natural grasslands are found (Fig. 1). Semi-natural grasslands of the Western Palaearctic region are considered among the most species-rich habitats in the world (Dengler et al. 2012) and their biodiversity increased during millennia of extensive land-use and management practices, including grazing and deliberate burning regimes (Willems 1990, Turbé et al. 2010, Dengler et al. 2014). Due to the presence of habitats of Community interest and species of high conservation value, the Park is included in the Habitats Directive 92/43 CEE and the Birds Directive 79/409 CEE.

Materials and Methods

The study was carried out in 17 sites located in different sectors of the Alta Murgia National Park from October 2018 to October 2019 (Fig. 2). A total of 300 pellets were analyzed: 131 of Barn Owl, *Tyto alba,* 5 of Long Eared Owl, *Asio otus*, 94 of Kestrel, *Falco tinnunculus* and 70 of Little Owl, *Athene noctua*. Species were determined by referring to the identification keys available in literature, by an analysis of cranial parts and postcranial bones (Toschi and Lanza 1959, Toschi 1965, Nappi 2001, Amori et al. 2008). The determination of the sex of each single specimen was performed on the pelvis following Nappi (2001). Sex ratio was computed when possible. A correlation analysis was performed by computing Pearson coefficients between the frequency of presence of each species for each sampling site and the landscape diversity index (Hill number) calculated on a circular buffer with a radius of 2.5 km radius, i.e. considering the mean radius of predation of the different raptors of study (Wijnandts 1984, Gilli 1998, Salvati et al. 2002, Kross et al. 2016). The Hill number (Hill, 1973) expresses the effective number of land uses that contribute to the diversity of a given landscape. As the number of Hill increases,

Figure 1: A semi-natural grassland with an abandoned farm hosting raptor roosts sampled in the present work (Minervino Murge).

there is an increase in the dominance of some landscape patches over the others, with a consequent decrease in the complexity of the mosaic analyzed and its simplification, as the landscape is dominated by a lower number of types of land uses.

Results and Discussion

The analysis of the pellets allowed to determine 1039 individuals of micromammals belonging to 8 species: *Microtus savii* 871 (83,83% of total), *Apodemus sylvaticus* 91 (8,76%), *Apodemus* sp. 18 (1,73%), *Rattus rattus* 3 (0,29 %), *Arvicola italicus* 1 (0,1 %) *Crocidura suaveolens* 12 (1,15 %), *Crocidura leucodon* 25 (2,4%), *Crocidura* sp. 6 (0,58 %), *Suncus etruscus* 7 (0,68 %) and *Talpa* sp. 5 (0,48%). Out of the 1039 individuals analyzed, Savi's pine vole, *M. savii* resulted as the most frequent species with a value higher than 83%. This finding agrees with the studies conducted in central Italy by Pennesi and Battisti (2003), in Salento by Battisti et al. (1997) and in several areas of Apulia by Bux et al. (2000, 2009). *M. savii* is a very plastic species, typical of areas with herbaceous cover or cultivated, where the tree cover has been greatly reduced.

Of particular interest is the discovery of a specimen of Italian water vole, *A. italicus* in the site of "Masserie Nuove", in the Corato area. Costa (1871) indicates that this species is "rare" in Apulia and is recorded only in Brindisi (BR) and Capo di Leuca (LE). Records from other areas Apulia date to 1916 (seven finds from Cerignola, FG at the Museum of Genoa "G. Doria") and to 1951 (a tooth found at Lake Lesina by Pasa) (Amori et al. 1986). Recent investigations on the microteriofauna of Apulia carried out

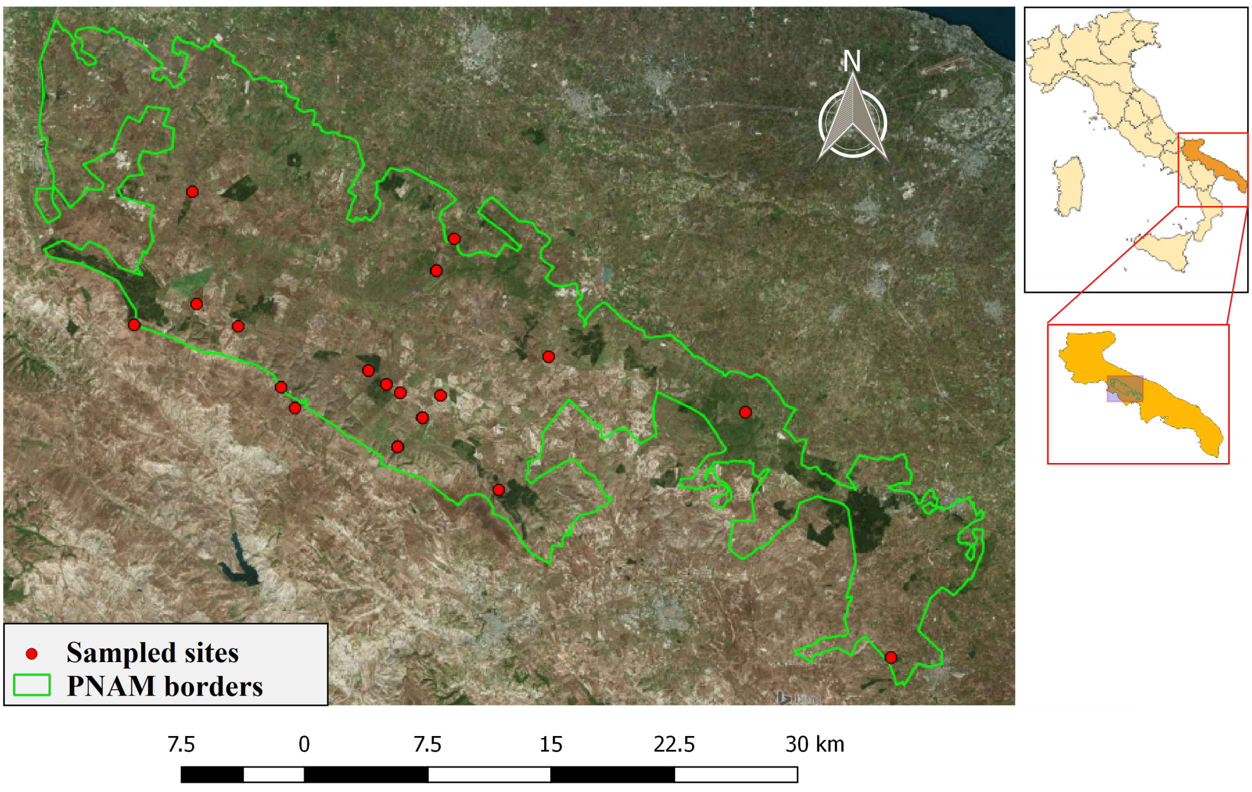

Figure 2: Geographical location of the sampled sites in the Alta Murgia National Park (PNAM).

through the analysis of a large number of raptors pellets (Battisti et al. 1997, Bux et al. 1999, 2000, 2001, 2009) have not confirmed the presence of this species. In a recent survey of micromammals from barn owl pellets in one out of five sites in the Maremma of Latium, Ferri et al. (2021) found some remnants of A. italicus. This site has the highest coverage of wet habitats among those sampled, confirming the semiaquatic habits of this declining species in peninsular Italy and thus the possible reason for the scarceness of findings in Apulia. Regarding murids, the presence of the wood mouse, *Apodemus sylvaticus* is recorded with a frequency of 8.76%. For the *Apodemus* genus, many findings were bone fragments so it was impossible to identify them at the species level by applying the morphological-morphometric index of Filippucci et al. (1984), so they were ascribed to *Apodemus* sp. Anyway, they probably belong to the widespread *A. sylvaticus*, since its congener *Apodemus flavicollis* in Apulia is found only in the area of the Gargano promontory (Umbra Forest; Amori et al. 1986) in which wooden coenoses with an elevated tree cover are found (Lovari et al. 1976).

As far as the synanthropic species, only a few specimens of black rat, *R. rattus* have been found. The absence of *Rattus norvegicus* could be due to the lack of waterways or in any case wetland and sewerage areas near the sites studied (Amori et al. 2008). The absence of *Mus domesticus* could be explained by the lack of urban centers near the analyzed sites (Amori et al. 2008).

Eulipotyphla have a low percentage (5.5%) compared to rodents, with *C. leucodon* as the most widespread species. *C. suaveolens* and *S. etruscus* individuals are rare. Their presence confirms the high naturalistic value of these areas that host suitable sites despite the intense agricultural exploitation. Crocidurini, in particular the two last cited species are considered excellent bioclimatic indicators (Contoli 1980, Bonvicino et al. 2002), since they are typical and exclusive species of thermo-xerophilous environments of macro- and mesoclimate (Spagnesi and De Marinis 2002): in fact, they select mostly arid and dry environments, similar to those sampled in this study.

Shrews of Genra *Sorex* and *Neomys* are completely absent; this could depend on the fact that they are more shade tolerant than the Crocidurini since they prefer colder, more humid environments with a minimum vegetation cover (Ricci and De Ascentiis 2002).

Sex determination was possible only for some *M. savii* and *A. sylvaticus* specimens, since the pelvic bones were often absent or broken. In *M. savii* sexes are almost equally distributed, with a slight prevalence in favor of males. In fact, the value of sex ratio, calculated on a sample of 224 individuals, was 1.33, in line with the values calculated for the species in other geographical areas (Caroli et al. 2000, Ettorre et al. 2013, Zaccaroni et al. 2015). On the opposite, a slight deviation towards females is observed in *A. sylvaticus* (n = 43; sex ratio = 0.87). This result is similar to capture-marking-recapture data from Sicily (Sarà and Casamento 1992). A positive correlation ($r = 0.044$, $n=11$, $p < 0.05$) was found for *S. etruscus* suggesting preference of this species for diversified and heterogeneous areas from a structural point of view. The negative correlation ($r = -0.041$, $n=11$, $p < 0.05$) found for *M. savii* confirms the plasticity and generalist attitudes of this species to frequent the unselected and homogeneous habitat from the structural point of view. This result is in accordance with previous data (Amori et al 2008), in fact, this species is regarded as euryecious, being able to adapt to diversified environmental conditions, and is more or less ubiquitous, versatile, and sometimes even invasive.

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The effect of hydrogeomorphological disturbances on the macroinvertebrate communities in two Mediterranean watersheds with different land management criteria

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Abstract

A macroinvertebrates sampling campaign was performed between May 2009 and June 2010 in two Calabrian (South Italy) watersheds, Trionto river (Ionian coast) and Savuto river (Tyrrhenian coast). Results showed that the development of exceptional rainfalls, interacting with sides degradation due to timber harvesting and diffuse fires, promoted in Trionto watershed a very strong hydrogeomorphological perturbation resulting in long lasting effects on macroinvertebrate communities. On the other hand, Savuto river was mostly affected, upstream by permanent water regimentation while a more sustainable land management preserved the watercourse from severe impacts due to winter rains.

Keywords: macroinvertebrates, rainfall, fine sediment transport, physical-chemical water column data.

Riassunto

Un campionamento di macroinvertebrati è stato effettuato tra maggio 2009 e giugno 2010 in due bacini idrografici della Calabria (Sud Italia), il fiume Trionto (costa ionica) e il fiume Savuto (costa tirrenica). I risultati hanno dimostrato che lo sviluppo di piogge eccezionali, insieme alla compromissione dei versanti a causa del disboscamento e degli incendi diffusi, hanno promosso nel bacino del Trionto una forte alterazione idrogeomorfologica che ha avuto effetti duraturi sulle comunità di macroinvertebrati. Il fiume Savuto, invece, è risultato maggiormente interessato, a monte, da una regimentazione permanente delle acque, mentre una gestione più sostenibile del territorio ha preservato il corso d'acqua da gravi impatti dovuti alle piogge invernali.

Parole chiave: macroinvertebrati, precipitazioni piovose, trasporto di sedimenti fini, dati fisico-chimici della colonna d'acqua

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Introduction

In Mediterranean regions, temperature and rainfall show clear seasonal trends, with dry and warm late spring-summer periods and rainy and relatively cold autumn-winter periods. The macroinvertebrate communities exhibit biotic control of their structure in low-flow months (Bonada et al., 2007), mainly due to the number and intensity of intraspecific and interspecific interactions, whereas its re-establishment in high-flow months depends on the intensity of discharge increases as well as the time elapsing between two such events (Dudgeon et al., 2006; Jaiswal and Pandey, 2021). However, when the levels of anthropization are high, human pressures can overlap with natural hydrological disturbances, making it difficult to separate their relative contribution and resulting in fragile, more or less simplified communities prone to quick degradation following a natural perturbation. In this context geomorphological alterations are the most long-lasting anthropogenic impacts (Goudie, 2020; Oglecki et al., 2021) because of permanent habitat destruction, hydrological modification and change or loss of autochthonous or allochthonous productivity sources (Allan, 2004; Elosegi et al., 2010; Kaplan and Cory, 2016; Shields et al., 2021). As regard Calabria region (South Italy), although the Ionian side is warmer and drier than the Tyrrhenian side, it experiences the effect of North African atmospheric disturbances, resulting in tropical cyclones with extremely intense rainfall (Greco et al., 2020). On the other hand, tectonic activity and the lithology (granites, gneiss, mica-schists and phyllites that are deeply altered and broken) of the middle and southern parts result in the formation of sandy dishomogeneous masses that are easily eroded and highly crumbling (Melidoro, 1966; Cortese, 1983). Intense reforestation efforts devoted to reducing the side instability were thwarted by massive forest exploitation (Bombino, 2009) and very high fire frequency and intensity during summer season (Regione Calabria, 2018). Agriculture is diffuse, involving about 50% of regional surface. However, when arboriculture (vineyards and olive trees) prevails on sowable soils it reduces the risks of sides degradation. In the view of the above mentioned scenarios, and the increased frequency in flash floods events promoted by developing climatic changes, a monitoring campaign involving macroinvertebrate assemblages was carried out, between 2009 and 2010 in two watersheds, one Ionian (Trionto river) and one Tyrrhenian (Savuto river) showing different land management criteria and fire hazard of sides and climate characteristics. The aims of the work were: 1) to evaluate if macroinvertebrate communities show a variation mainly associated to temporal (seasonal) or spatial (from upstream to downstream) trends, 2) to detect the factors mainly driving general community parameters changes, 3) to analyze the communities structure and related most characterizing taxa as well as the representativeness of functional feeding groups.

Materials and Methods

Study area

Table 1 reports the main characteristics of the two watersheds, whereas Figure 1 shows the location of the sampling stations along the watercourses ranging, in case of Trionto, from 1000 m a.s.l. to 136 m a.s.l. and in case of Savuto from 1235 m a.s.l. to 167 m a.s.l.
Sides in the Trionto watershed are affected by severe soil erosion (DiMase and Iovino, 1988) especially in the middle upper part where very high slopes combined to timber harvesting and an extremely high fire hazard (Coschignano et al., 2019; PSR Calabria, 2014-2020) results in both an erosion rate of 0.92 mm/yr in the 40% of the total area (Olivetti et al., 2012) and triggering landslide events ranging between 21 and 100 per year, from 1921 to 2010 (Gariano et al. 2018). In the middle-lower part of the

Table 1: Natural and anthropogenic characteristics of the Trionto and Savuto watersheds.

Length of river (km)	Watershed surface (km^2)	Total inhabitants of main towns	Geology	Land use
			Trionto River	
40	288	14564	Conglomerates and Sandstones, Granites, Granodiorites, Gneiss, Clays	**Middle-upper part**: permanent meadows, mixed forests, broadleaf and coniferous forests, intensive timber harvesting. **Middle-lower part**: sowable fields, olive groves, mixed annual and permanent crops, mixed forests, permanent meadows.
			Savuto River	
55	411	10221	Gneiss, Phyllites, Clays, Quartzite-Clays	**Middle-upper part**: broadleaf forests, vineyards, orchards, sowable fields, permanent meadows. **Middle-lower part**: mixed annual and permanent crops, mixed forests, olive groves, sowable fields, permanent meadows.

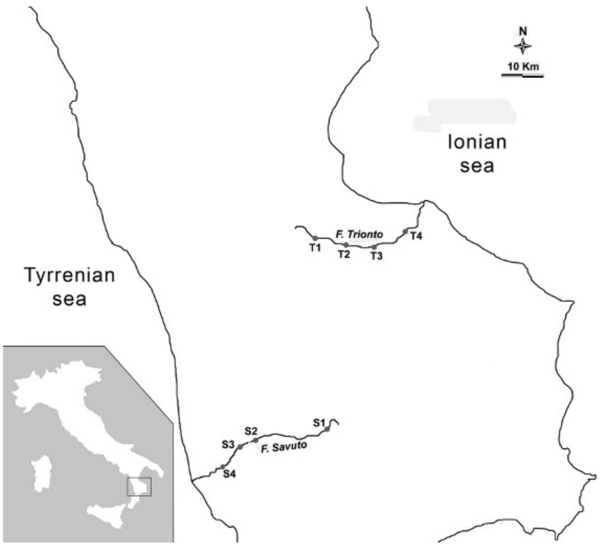

Figure 1: Placement of sampling sites along watercourses.

watershed, prevailing land components are extended olive groves and sowable fields.

Permanent meadows and crops and olive and vineyards cover 65% of Savuto watershed surface, to whom should be added more than 5000 hectares of woods included in farms (ARSAC, 2021). Fire hazard shows a patchy distribution ranging from very high to very low level. The increase in slope together with predominance of phyllitc schists, in the middle-lower part of watershed, result in virtual high erosion rates (0.60-0.80 mm/yr) (Le Pera and Sorriso-Salvo, 2000), however compensated by less soil exploitative agriculture. Indeed, the landslide events frequency ranged from 11 to 50 per year, again between 1920 and 2010 (Gariano et al., 2018). In the upper reaches, damming of the river at Poverella location resulted in the formation of a relatively small multipurpose reservoir (1 billion cubic metres) (Viceconte, 2004). The rainfall trends are consistent with the Mediterranean climate of Calabria, although Trionto watershed shows a higher frequency and intensity of very strong precipitation events i.e. days with more than 100 mm of rain per day (Protezione Civile, 2010) (Table 2).

Sampling and analytical procedures

Macroinvertebrates, water-column chemistry and substrate percentage composition were monitored from May 2009 to June 2010 on

Table 2: Rainfall data from the ARPACAL (Environmental Protection Agency of Calabria Region) database. The Longobucco and Rogliano pluviometric stations are located in the upper-middle parts of the two watersheds while the Cropalati and Savuto stations are in the lower-middle parts (available chronology: Longobucco 89 years, Cropalati 87 years, Savuto 77 years, Rogliano 90 years, all data up to 2010 except Savuto up to 2001).

Rainfall	Trionto		Savuto	
	Longobucco	Cropalati	Rogliano	Savuto
Total % of rainy days	24.7	19.9	26.44	31.65
Mean amount of rain (mm) y^{-1}	1277	1016	1164	1355
Total number of days with >100 mm of rainfall	59	82	5	10
Total % of days with >100 mm of rainfall	0.734	1.29	0.057	0.112
Total % of rain associated with rainfalls >100 mm	7.24	13.48	0.613	1.12
Mean Spacing Time (years) between two >100 mm rainfalls	1.44	1.04	24.52	4.5
Minimum and maximum (days) Spacing Times between two >100 mm rainfalls	1-3330	1-1813	1713-16780	1-6935

the following dates: May 25th, October 10th, March 25th and June 8th. Daily and monthly rainfall measurements (included three months before May 2009) came from the ARPACAL database (2022). The monthly amounts of rain were used in place of discharge variation due to the lack of stream gauges and because there is a well-known correlation between them (Ankan and Ekmekci, 1985; Gregory and Walling, 1973; Kelly et al., 2019; Pandzic and Trninic, 1999; Rebora et al., 2016; Rhoads, 2020). Macroinvertebrates samplings were conducted following a time-standardized multi-habitat proportional semi-quantitative sampling procedure (Lucadamo et al., 2008). The samples were separated from sediment and stored in 70% alcohol. Once in the laboratory, all sampled organisms were counted and identified at the family level under a Leica stereomicroscope (except for a few taxa identified at the genus level for calculation of the Extended Biotic Index) (Campaioli et al., 1994; Sansoni, 1988; Tachet, 2000). This allowed estimation of the following community parameters: Abundance, Taxonomic Richness (TR), No. EPT (Ephemeroptera, Plecoptera and Trichoptera) Taxa, Dominating Taxon (DT) (i.e. the taxon with the highest percentage within the sampled community whose value suggests the development of a perturbation when approaches 50%, Lucadamo et al., 2008, Extended Biotic Index (EBI) (Ghetti, 2001), Biological Monitoring Working Party (BMWP) (Alba-Tercedor and Sanchez-Ortega, 1988), Shannon index (calculated at family level, see Karr and Chu 1999, Zu et al. 2020), Functional Feeding Group (FFG) classification (Merrit and Cummins, 2007) and PC-RO (Precocious Colonizers-Remaining Organisms) ratio (Gallo et al. 2010). The last is the ratio between the abundance of taxa most frequently indicated in the literature as typical of the first stages of biological colonization (including the families Chironomidae, Simuliidae (Diptera) and Hydropsychidae (Trichoptera), and the genus *Baetis* (Ephemeroptera)) (Hemphill and Cooper, 1983; Downes and Lake, 1991; Ulfstrand et al., 1974; Peckarsky, 1986; Hershey and Lamberti, 2001), defined here as Precocious Colonizers, and the organism abundance of all other taxa (RO). pH, temperature and conductivity were determined in the field with a multiparameter probe (Hanna Instruments, model HI991300), whereas dissolved O_2 and relative saturation percentage were measured with an oximeter (Hanna Instruments, model HI9143). NH_4^+, NO_3^-, PO_4^{3-}, suspended solids and Chemical Oxygen Demand (COD) were evaluated in a 2-litres water sample according to APAT-CNR/IRSA methods (2003). Percentage substrate composition was estimated based on a visual criterion (EPA, 2010) and, at the same time, the Coarse Substrates - Sand ratio (CS/S) was calculated as a measure of the prevalence in erosive or depositional processes (Lucadamo et al., 2010). Spatial and temporal changes of macroinvertebrates communities structure and general community parameters were analysed using respectively Multi-Response Permutation Procedure (MRPP) and Indicator Species Analysis (ISA) and 1 Way Analysis of Variance with post hoc comparison (Tukey test), whereas association between abiotic factors and community parameters was tested by calculation of parametric correlation coefficient (Person). Multivariate Analyses were performed by PC-ORD (version 4) software and uni e bivariate analyses by Minitab (version 16) software.

Results

Analysis of the monthly rainfall clearly revealed (Fig. 2) a lower total amount of rain collected by each of the two rain gauge stations in the Trionto watershed compared to the Savuto station but a strong rainfall peaked at both Trionto watershed stations in September 2009. Indeed, 84.38% (499.4 mm) and 48.97% (192.6 mm) of the September rainfalls were concentrated in three days at Longobucco station (24th, 25th and 26th September) and in one day (25th September) at Cropalati station, respectively. As regard the physical-chemical data of water-column the mean pH values of both watersheds were largely consistent with the nature of the geological substrates (Table 3). When the average values of conductivity measured at the lowest station are compared between Trionto and Savuto watersheds, the load in ions/charged molecules was much higher in the former (413 μS cm^{-1}) vs the latter (274 μS cm^{-1}). The outcome of Trionto river probably is ascribable to the significant levels in suspended solids and maybe in COD but

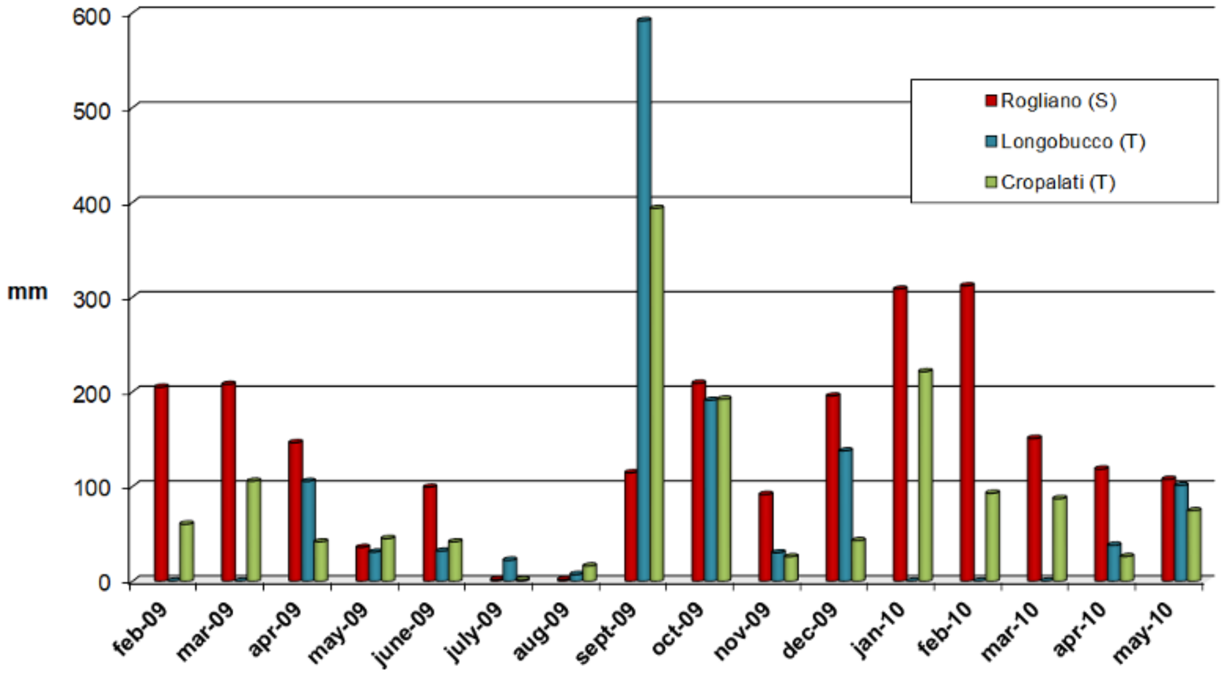

Figure 2: Pluviometric levels detected at rain gauge stations in Trionto (Longobucco and Cropalati) and Savuto (Rogliano) watersheds. S = Savuto, T = Trionto.

Table 3: Mean values of the water-column parameters related to each of the studied watersheds.

Parameters	Trionto river	Savuto river
pH	7.94	7.74
Temperature (°C)	18.27	17.48
Conductivity (μS cm^{-1})	245.50	235.63
COD (mg O_2 L^{-1})	28.21	15.47
O_2 (mg L^{-1})	8.86	9.43
% O_2	91.13	96.09
Solidi sospesi (mg L^{-1})	65.66	21.57
NH_4^+(μgg L^{-1})	2.76	43.26
NO_2^-μgg L^{-1})	0.20	0.22
NO_3^-(μgg L^{-1})	2.32	3.51
PO_4^{2-}(μgg L^{-1})	8.52	14.69
CS/FS	9.93	4.73
% Sand	26	18.75

Table 4: Macroinvertebrates community parameters. PC/RO = Precocious Colonizers/Remaining Organisms of community; EBI = Extended Biotic Index; BMWP = Biological Monitoring Working Party; WQC = Water Quality Class; EWQ = Excellent Water Quality; NC = Not Calculated; A = Absent; (*) Dominating taxon was considered absent when no organisms were collected or the residual number was so small that the condition of taxa dominance was considered meaningless (Lucadamo et al., 2008). Environmental impairment detected by water quality indexes if EBI<10 and BMWP<101.

Station	Date	No. Taxa	No. Taxa EPT	Abundance	Dominating taxon (%)	PC/RO	EBI	WQC-EBI	BMWP	WQC-BMWP	Shannon index
T1	**05/09**	35	15	632	38.92	0.614	11	I	200	EWQ	2.143
	10/09	17	9	233	A*	0.913	6	III	116	I	1.379
	03/10	9	5	72	A*	0.425	8	II	50	III	2.784
	06/10	18	9	1475	39.1	0.535	9	II	104	I	1.488
T2	**05/09**	35	18	883	35.02	0.634	10	I	223	EWQ	2.247
	10/09	-	-	-	A*	0.000	-	V	-	NC	0
	03/10	15	10	104	A*	0.372	7	III	108	I	1.826
	06/10	19	11	2533	61.65	0.904	8	II	123	I	1.205
T3	**05/09**	20	10	1350	46	0.887	8	II	120	I	1.520
	10/09	-	-	-	A*	0.000	-	V	-	NC	0
	03/10	10	6	47	A*	0.818	-	V	67	II	1.458
	06/10	18	7	1768	52.26	0.939	7	III	101	I	1.197
T4	**05/09**	14	5	820	38.29	0.894	7	III	63	II	1.687
	10/09	-	-	-	A*	0.000	-	V	-	NC	0
	03/10	1	-	2	A*	0.000	-	V	4	NC	0
	06/10	13	4	1223	52.08	0.975	6	III	56	III	1.032
S1	**05/09**	19	8	209	A*	0.519	8	II	88	II	1.974
	10/09	7	4	217	A*	0.977	7	II	91	II	0.599
	03/10	7	4	36	A*	0.583	4	IV	45	III	1.579
	06/10	14	8	836	70.33	0.748	9	II	90	II	1.036
S2	**05/09**	17	11	1764	56.85	0.864	9	II	123	I	1.383
	10/09	28	11	1096	22.11	0.354	10	I	162	EWQ	2.383
	03/10	6	4	9	A*	0.556	-	V	39	III	1.676
	06/10	23	11	988	39.67	0.789	9	II	140	I	1.783
S3	**05/09**	14	7	607	37.6	0.831	7	III	84	II	1.582
	10/09	19	10	975	26.76	0.691	9	II	126	I	1.841
	03/10	4	4	5	A*	0.600	0	V	29	V	1.054
	06/10	18	8	878	44.43	0.871	8	II	98	II	1.511
S4	**05/09**	13	8	604	33.44	0.815	8	II	94	II	2.005
	10/09	17	8	530	26.45	0.666	8	II	117	I	1.932
	03/10	-	-	-	A*	0.000	-	V	-	NC	0
	06/10	13	6	448	59.24	0.882	7	III	68	II	1.438

not to the nutrient concentrations that result to be respectively 15 (NH_4^+), 1.5 (NO_3^-) and 1.7 (PO_4^{2-}) times higher in Savuto water-column. Such a result is consistent with the high potential soil erosion of Trionto watershed sides, and the need to support the trophic requests of permanent crops in Savuto watershed with mineral nitrogen and phosphorous (Lawniczak et al., 2016).

The mean values of O_2 saturation percentage were substantially good (Ghetti, 2001), although some sub-optimal levels were detected at the Trionto stations, perhaps due to prolonged fine sediment deposition. The worst level of O_2 saturation percentage was recorded at station S1 in October 2009, owing to a prolonged period of regimentation coupled with the previous high number of degree-days (Vannote and Sweeney, 1980).

Table 4 shows the values of the general community parameters calculated in occasion of the 4 sampling dates. When their variation is matched to that of abiotic drivers,

Table 5: Statistically significant Pearson correlation coefficient between abiotic drivers and community parameters calculated at the level of both watersheds (CS/S = Coarse Sediments/Sand).

Correlations	r	r²	p
Rainfalls-Shannon index	-0.836	0.698	0.01
Rainfalls-N. of Taxa	-0.714	0.509	0.047
Rainfalls-N. of EPT	-0.773	0.597	0.024
Rainfalls-PC/RO	0.561	0.314	0.033
Rainfalls-EBI	-0.735	0.540	0.038
Rainfalls-CS/S	0.720	0.518	0.044
Suspended solids-Abundance	-0.475	0.225	0.006
Suspended solids-PC/RO	-0.546	0.001	0.298
CS/S-Shannon index	-0.716	0.512	0.046

as cumulative effect at the levels of both watersheds, the only significant association were found with rainfalls, suspended solids and ratio between Coarse Substrates and Sand (CS/S) with values of Pearson coefficient mostly indicating negative correlations, ranging from -0.475 to -0.836, except in case of covariance between amount of precipitations and PC/RO and CS/S (Table 5).

The comparison of mean community parameters made between both watersheds and stations selected within each watersheds do not show any significant difference suggesting the missing of relevant spatial gradients developing along the rivers. This is the consequence of the very high average slopes of sides together with the short length of the watercourses making the rhytral morphology dominant for about ¾ of the length.

On the contrary, Anova performed on the sampling dates (Table 6) clearly indicates that most of the variation affecting communities is associated to the seasonality. Indeed, in case of Trionto river four parameters (Shannon index, N. of Taxa, Abundance and Dominating taxon) showed significant values of F, whereas Savuto river displayed such an outcome for N. of Taxa, N. of EPT Taxa and Dominating Taxon.

As regard the water quality indexes applied to the present study EBI detected as well more cases of environmental alterations than BMWP (Gallo et al., 2003) as the presence of a higher amount of stenoecious taxa in the upper tract of Trionto compared to Savuto river with both of them clearly showing strong value changes associated to sampling dates. In addition, the indexes suggested that, in absence of hydro-geomorphological pressures, most of the stations are

Table 6: Statistically significant results of 1-way ANOVA and post hoc multiple comparison (Tukey test) performed on the 4 different sampling dates, separately per each watershed.

Watershed	Month	Taxon	Indicator Value	p
Trionto	March 2010	Taenyopterigidae	71.5	0.035
	June 2010	Baetidae	67.9	0.003
		Ephemerellidae	78.4	0.037
		Chironomidae	91.1	0.009
		Simuliidae	67.8	0.033
	May 2009	Glossosomatidae	100	0.003
		Dixidae	75	0.021
		Hydropsychidae	54.5	0.026
		Tabanidae	100	0.003
Savuto	October 2009	Leuctridae	70.7	0.016
	June 2010	Ephemerellidae	73.9	0.007
		Ceratopogonidae	80	0.01
		Tipulidae	75	0.03

characterized by a sub-optimal suitability to host well-structured communities. When the same analysis is transferred at the level of community structure (family composition) the result matches that obtained for the general communities parameters. Performing a Multiresponse Permutation Procedure (MRPP) on the whole database (stations-sampling dates x collected taxa), a significant value of test statistic T resulted only when using as factor the sampling dates (Trionto: T=-4622, Chance-corrected within group agreement A=0.3110, Probability of a smaller or equal delta p=0.00092, Savuto: T=-4.9903, Chance-corrected within group agreement A=0.2047, Probability of a smaller or equal delta p=0.00028).

Indicator Species Analysis devoted to the identification of families responsible of this temporal segregation revealed that within the range of statistically significant Indicator Values, 61% were associated to the Trionto sampling dates (May, March and June) while only 23% gave the same result for Savuto

Table 7: Statistically significant results of Indicator Species Analysis performed on the database.

Watershed	Parameters	F	p	SSD
Trionto	Shannon index	3.56	0.048	May 09> October 09
	N. of Taxa	6.47	0.007	May 09 > October 09, March 10
	Abundance	24.48	<0.00005	May 09 > October 09, March 10 June 10 > May 09, October 09, March 10
	Dominating Taxon	106.03	<0.00005	May 09 > October 09, March 10 June 10 > May 09, October 09, March 10
Savuto	N. of Taxa	5.75	0.011	May 09, October 09, June 10 > March 10
	N. of EPT Taxa	5.47	0.013	May 09, October 09, June 10 > March 10
	Dominating Taxon	9.04	0.002	June 10 > October 09, March 10 May 09 > March 10

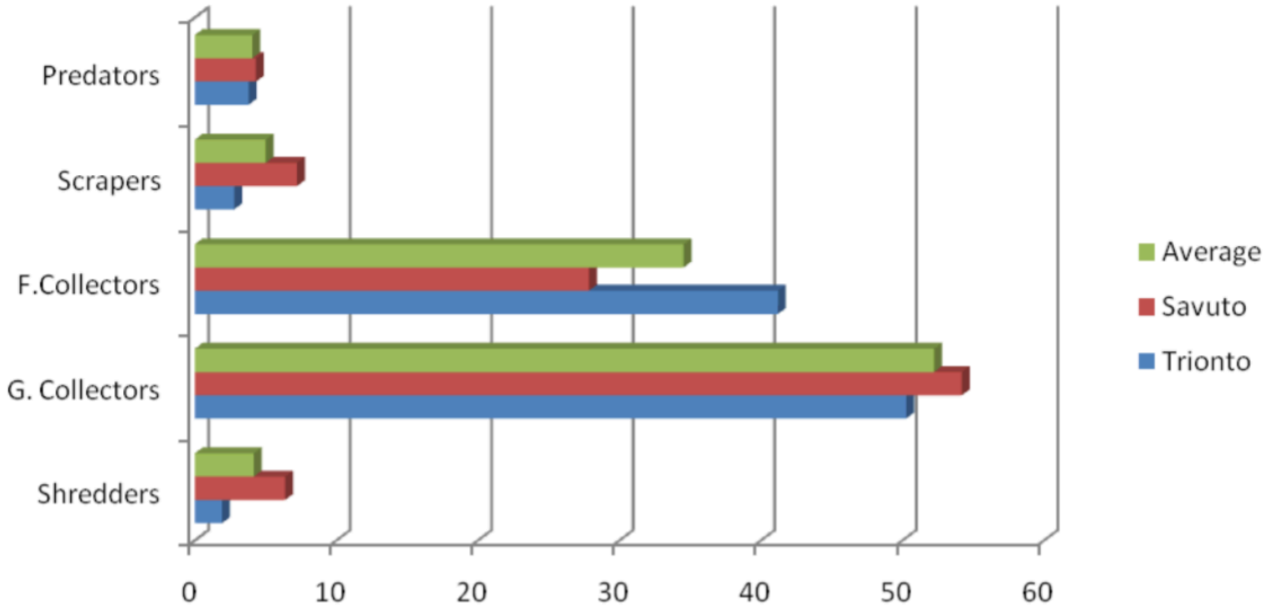

Figure 3: Representativeness of Functionl Feeding Groups in the monitored watersheds (means across stations and sampling dates). F = Filtering, G = Gathering.

watershed (October, June) (Table 7) suggesting a less frequent occurrence of family-specific selective conditions in case of the latter.

Analysis of representativeness of functional feeding groups revealed that the collectors contributed 86.8% of total sampled organisms (Figure 3). Savuto percentage in shredders and scrapers were respectively 3.31 and 2.58 times higher than those of Trionto river indicating better levels of allochthonous and autochthonous primary productivity in the case of the former watercourse. On the other hand, Trionto watershed showed a comparable percentage of gathering collectors but an amount of filtering collectors around 50% higher than that of Savuto. The average value of Filtering Collectors - Gathering Collectors ratio equals to 0.819, suggesting a remarkable load of suspended FPOM (Merrit and Cummins. 2007), that resulted to be 60% higher than that detected in Savuto river (0.514).

Discussion

The three months preceding the beginning of the monitoring period were substantially lacking of significant precipitation events and this suggests that the structure of the macroinvertebrates communities recorded in May 2009 would be passing under the control of biotic factors (Monk et al., 2006; Poff and Ward, 1989) except in the presence of human pressures that favour the settlement of taxa tolerating environmental alterations, a result that seem to be detected by values registered by Shannon index (Ravera, 2001), EBI and BMWP (Table 4).

The remarkable precipitation that took place at the end of September 2009 in the Trionto watershed can be really considered exceptional. In fact, such a high amount falling in a few successive days has never been recorded at the Longobucco station (minimum: 232 mm, maximum: 404 mm) and only once (516 mm in three days) in 1930 at the Cropalati station suggesting a recurrence time of 80 years. The average value of CS/S of Trionto watershed was about the double of that of Savuto watershed (Table 3). This ratio equalled to 50 at station T2 in October sampling, while reduced to 0.5-0.7 at downstream stations in October and March, suggesting a remarkable flushing of fine sediments in upper tract resulting in substrate armouring a phenomenon that produces strong habitat simplification and depression of biological colonization (Svendsen et al., 2009; Addy et al. 2016). On the other side, a comparable deleterious effect on macroinvertebrates community may arise from excess in fine sediment deposition, especially when sand approaches 40-50% in substrate composition (Spindler, 2004; Lucadamo et al., 2010) a process that took place in the lower-middle part of watershed (stations T3 and T4) following upstream flushing. Our data suggests that particularly high value in flow (following extremely infrequent rainfalls) and riverbed disturbance temporary depressed taxa richness and biodiversity, i.e. promoting disappearing of families as indicated by 1-way ANOVA (Table 6). On the other hand, change in abundance, included significant niche dilatation, persisted until June 2010 (Table 6). They were related to both increase of suspended solids (Table 4), probably as a consequence of removal of organisms inhabiting exposed surfaces, and dispersion strategies as indicated by ISA (Table 7) showing as characterizing families, in the last sampling date, Chironomidae, Baetidae and Simuliidae, typical r-strategy taxa, that were less represented in the May 2009 sampling (- 20%). EBI and BMWP displayed a similar result as a consequence of the weight, in their calculation, of these euriecious organisms that contributed to lowering the respective final scores.

Post-flood recovery times are usually shorter than that recorded in our study for fauna pre-adapted to frequent floods (Collins et al., 1981; Fisher et al., 1982; Mundahl and Hunt, 2011; Rader et al., 2007; Scrimgeour, 1988), as should be the case for the Trionto watershed given the historical pluviometric data. Several factors can have contributed to such an outcome.

First of all, the interaction between sides soil erosion, caused by poor land management criteria, and a very strong rainfall (and discharge) event. Indeed, scientific literature suggests that relevant factors promoting increase in as well superficial and sub-superficial runoff as load in fine sediments to watercourses are timber harvesting and forest fires associated to strong precipitations (Bruijnzeel, 2004; Cook et al., 2020; Richardson and Béraud, 2014; Soulis et al., 2021; Schawrtz et al., 2021), the former constantly affecting Trionto watershed and the latter occurring just during the present sampling campaign. Secondly, the timing of the exceptional rainfalls; in fact, most of the strongest daily rains in the Trionto watershed occur, based on historical data, between October and February and only 7-8% in September, suggesting that the effect of the hydrogeomorphological disturbance on benthic communities could have been exacerbated by the unusual timing of such extremely intense short-term precipitation, compromising sensitive life stages of macroinvertebrates organisms such as egg lying or emergence (Ghetti, 2001; Cattaneo et al., 2006; Allan and Castillo, 2007). Thirdly, a potential involvement of hyporheos, suggested by the macroscopic morphological change experienced by riverbed, destroying most of refuge zones (Goudie, 2020), may have contributed to delay the appearance, in the communities, of stenoecious taxa, with a slower colonization capacity.

The hydrogeomorphological temporal changes occurred in Savuto river did not involve the organisms abundance but rather the short lasting variations of on local-scale spatial heterogeneity and associated number of taxa (Table 6). The timing of watercourse disturbance was different from that of Trionto, because the strongest rainfalls occurred in winter, with manifestation of severe communities impairment associated to the March 2010 sampling (Table 6). On the contrary, water quality and Shannon indexes pointed to an improvement of environmental conditions in half of the stations in October sampling and in the view of crops grown in the Savuto watershed (especially vine-yards and olive-yards) (Eynard and Dalmasso, 1990; Istruzione Agraria Online, 2010; Rossi, 2010) it can be associated to a reduction of chemicals (fertilizers and pesticides) fluxes to hydrological network. A different trend was detectable at station S1 due to the regimentation promoted by river damming. Indeed, in three out of four sampling dates the segment showed clear effect of the reservoir activities i.e. drop in oxygen, sand accumulation and large woody debris heaped on riparian zones, suggesting a remarkable interruption of river connectivity with related consequences on macroinvertebrates communities (Allan and Castillo, 2007; Pringle, 1996). In fact, same rheophilous families, adapted to relatively high current values (Ghetti, 2001), like Hydropsychidae, Lepidostomatidae, Athericidae and Blephariceridae, well represented in the other tracts, disappeared. As regard the other stations, only that closest to the mouth resulted to be still affected by a drop in EBI and biodiversity levels in June 2010 compared to the values of May 2009 probably due to the cumulative effect of hydrogeomorphological and chemicals impact (maximum load in sediments, fertilizers and pesticides). The analysis of feeding functional structure is consistent with the previous environmental picture (Table 8). Indeed, FPOM results to be the most abundant energy resource in both watersheds, a condition typically detected in unstable watercourses (Johnson et al., 2018; Kelso and Baker, 2020; Jenneau et al., 2018; Jacinthe et al., 2004). However, the filtering collectors are much more represented in Trionto probably also due to the higher potential erodibility of sides. This result shows a satisfying match with that of average concentration of COD: it is 28.2 in Trionto and 15.5 in Savuto with a ratio of 1.82, again supporting the dilation of niches of suspended FPOM feeders detected in the former. Accordingly to the more conservative agriculture practiced in Savuto watershed, the condition of riparian woods seem to be better than that of Trionto as indicated by the higher average amount of shredders collected and the peaks in October and June respectively of Leuctridae and Tipulidae, well known consumers of Coarse Particulate Organic Matter (CPOM). On the other side, the much lower levels of suspended solids in Savuto, probably results in a lower water-column turbidity promoting a better colonization of benthic primary productivity utilizers.

Conclusions

The very intense tectonic activity and peculiar lithology of Calabria coupled with strong rainfalls make the watercourse network susceptible to severe fluctuations in flow and sediment load. The results of the present work suggested that when anthropogenic pressures, like those causing further changes in discharge variation and sediment fluxes from the valley sides interact with the aforesaid environmental background, the natural short-to-medium-term physiognomic evolution of river channels is strongly impaired, resulting in severe disturbances to the freshwater macroinvertebrates. In such a condition the main trophic resource was the FPOM resulting from land run off and sediment resuspension because of the absolute reduction in benthic primary productivity and riparian CPOM. In the case of the Trionto River a very strong rainfall pulse perturbation was coupled with diffuse timber harvesting and fires resulting in an extreme hydrogeomorphological alteration. This consisted, upstream in riverbed armouring and consequently, downstream in substantial burial of substrate both severely delaying the recovery of macroinvertebrate communities. Damming in the Savuto River caused permanent flow abatement and hydrological connectivity interruption which changed rhithral physiognomy to one looking like a potamal morphology, eliminating more rheophilous taxa. The communities of lower reaches, because of a better sides soil management and a less severe rainfall perturbation, being pre-adapted to climatic alteration, quickly recovered. Due to the lack of any other relevant pressure systematically affecting the watercourses, toxic fluxes of agriculture chemicals driven by irrigation run-off may be considered the main factor, in the low-flow period, causing a general depression of biodiversity in both watersheds although the magnitude of its effect was not investigated.

Author contributions

All the authors equally contributed to this work

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Winter roosts of great cormorant *Phalacrocorax carbo sinensis* in Campania region: distribution and multi-year analysis of the population, from 1998 to 2022

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Abstract

The great cormorants' (*Phalacrocorax carbo sinensis*) habit of gathering in roosts in winter makes it easier to monitor the wintering populations. In Campania region (Southern Italy) winter roosts have been counted since the 1980s. However, since January 1998 the monitoring has been standardized and made constant over the years. Therefore, there is a 25-year historical series on which analyzes were carried out on the trend, periodicity, nature of the various roosts monitored in the considered period, and the geographical origins of the wintering specimens. A moderate increasing trend emerged, also confirmed by the comparison of the median number of animals present in the decade 2003-2012 with those of the decade 2013-2022. No periodicity phenomenon was found.

Keywords: Great cormorant, wintering roosts, trend, periodicity

Riassunto

L'abitudine dei cormorani (*Phalacrocorax carbo sinensis*) di radunarsi in inverno in dormitori rende più semplice il monitoraggio delle popolazioni svernanti. In Campania (Italia meridionale) si effettuano conteggi ai dormitori invernali dagli anni '80 del XX secolo. A partire dal gennaio 1998 però il monitoraggio è stato standardizzato e reso costante negli anni. Si dispone oggi quindi di una serie storica di 25 anni sulla quale si sono effettuate analisi sull'andamento, la periodicità, la natura dei vari dormitori monitorati nel periodo preso in esame, e le origini geografiche degli esemplari svernanti. Ne è emersa una tendenza moderata all'incremento, confermata anche dal confronto del numero mediano di animali presenti nel decennio 2003-2012 con quelli del decennio 2013-2022. Non si è riscontrato un fenomeno di periodicità.

Parole chiave: Cormorano, dormitori invernali, andamento, periodicità

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Introduction

During winter, several species of birds use to gather at sunset in roosts to spend the night. This attitude makes it possible to carry out easily the censuses of the wintering populations in a specific territory. The great cormorant, *Phalacrocorax carbo* is among them.

In Campania region (Southern Italy) the subspecies *P. c. sinensis* appears to be migratory, wintering, breeding, and summer breeding (Fraissinet 2015; Fraissinet & Usai 2021). Nesting began in 2007 in the WWF Oasis Lake Campolattaro (Giannotti et al., 2011).

The first news of the regular wintering of the great cormorant in Campania dates to the first half of the 1980s (Mancuso et al., 2001). At the same time, the first counts of animals in roosts began in the mid-1980s. In the period between winter 1984/1985 and winter 1986/1987, as part of the monitoring of the wintering aquatic avifauna in 14 inland or coastal wetlands in Campania region, the species was found in 7 localities, approximately sited by the coast except for WWF Oasis of Serre - Persano, located in the middle course of the Fiume Sele (Fraissinet et al., 1988). Starting from the winter of 1997/1998, regular and standardized monitoring was carried out throughout the whole region. In the decade from winter 1997/98 to winter 2006/2007, the species was monitored in the months of December, January, and February, following the protocol of the IUCN/Wetlands International Cormorant Research Group (Fraissinet et al., 2003). A first analysis of the trends related to the first decade of standardized monitoring of dormitories was published in 2008 (Fraissinet et al., 2008).

The continuation of the monitoring allowed us to acquire data for 25 consecutive years. Therefore, we considered it appropriate to carry out an updated analysis on the distribution, the environmental characteristics of the roosts, and the trend of the overall population present in the dormitories.

Materials and Methods

The study area is the entire Campania Region, whose extension is 13,595 square kilometres (Fig.1). The count in the roosts was carried out from one hour before sunset to nightfall.

The counts were carried out through the

Figure 1: Location of the Campania region on the Italian peninsula.

visual census technique and were performed each January from 1998 to 2022. The dates of the counts are placed within the time window set by the IWC - International Waterbird Census - for monitoring wintering waterbirds. Since the counts were carried out during IWC monitoring, the protocol for such monitoring was followed, which provides for a very limited time interval in which to carry out the counts, therefore each dormitory was visited only once in January of each year.

The substrates on which the cormorants roosted were also noted and classified into 4 categories: trees near lake or riverbanks, sea crags, dead trunks emerging from the water in basins where the flooding killed the pre-existing trees, and anthropogenic structures (cranes, buoys, etc.).

The research was carried out to bring together all the reports of marked specimens found in Campania region, to know the origin of the wintering populations.

The study of the overall trend was verified through the linear correlation between the counts of the sites surveyed each year. Since this turned out to be high every time, to avoid cases of incorrect estimation due to the variation in the number of sites over the years, it was decided to divide the total number of animals investigated each year by the total number of sites investigated in the same year, to have an average value for each year in every sites independent the number of sites investigated.

To apply the exponential trend estimation method of the TRIM calculation program (Pannekoek & van Strien 2013) of the averages calculated each year, only the whole number was considered, excluding the decimals, to prevent the program from giving errors. Once the data were obtained, it was possible to estimate the exponential growth model through the TRIM program. This model was then used to detrend the historical series and verify if components of this series were present with the turning points test (ps), and the test of the sign of differences (SDI) (Di Fonzo & Lisi 2013). For the comparison between the median number of animals present in the decade 2003-2012 with those of the decade 2013-2022, the non-parametric Wilcoxon-Mann-Whitney test was used (Siegel & Castellan 1988).

Results

In the period under consideration, 25 dormitories were identified (Fig. 2), with an annual average of 9 dormitories, distributed throughout the region with a prevalence for the coastal areas, in particular for the Campi Flegrei area. Five dormitories have registered continuous attendance for 20 years or more and are still occupied (Tab.1).
Figure 3 shows the trend in the number of dormitories and specimens counted overall for each year. The average in the period under review was 1155.16. A first increase can be observed from 2003, reaching its maximum in January 2007 with 2788 specimens. The following year, 2008, the lowest value was recorded, but this could be explained by the lower number of dormitories monitored in that year due to logistical difficulties. A new increase has been recorded starting from January 2013. The overall trend shows an exponential growth rate of 4.61% ± 1.85% ($p < 0.05$) equivalent to TRIM to moderate increase (Fig.4).
The search for any periodicity of the time series employing the test of turning points (ps) and the sign of differences (SDI), did not reveal significant results, resulting respectively: ps = 0.84 p = 0.401 and SDI = 0.35 p = 0.729.

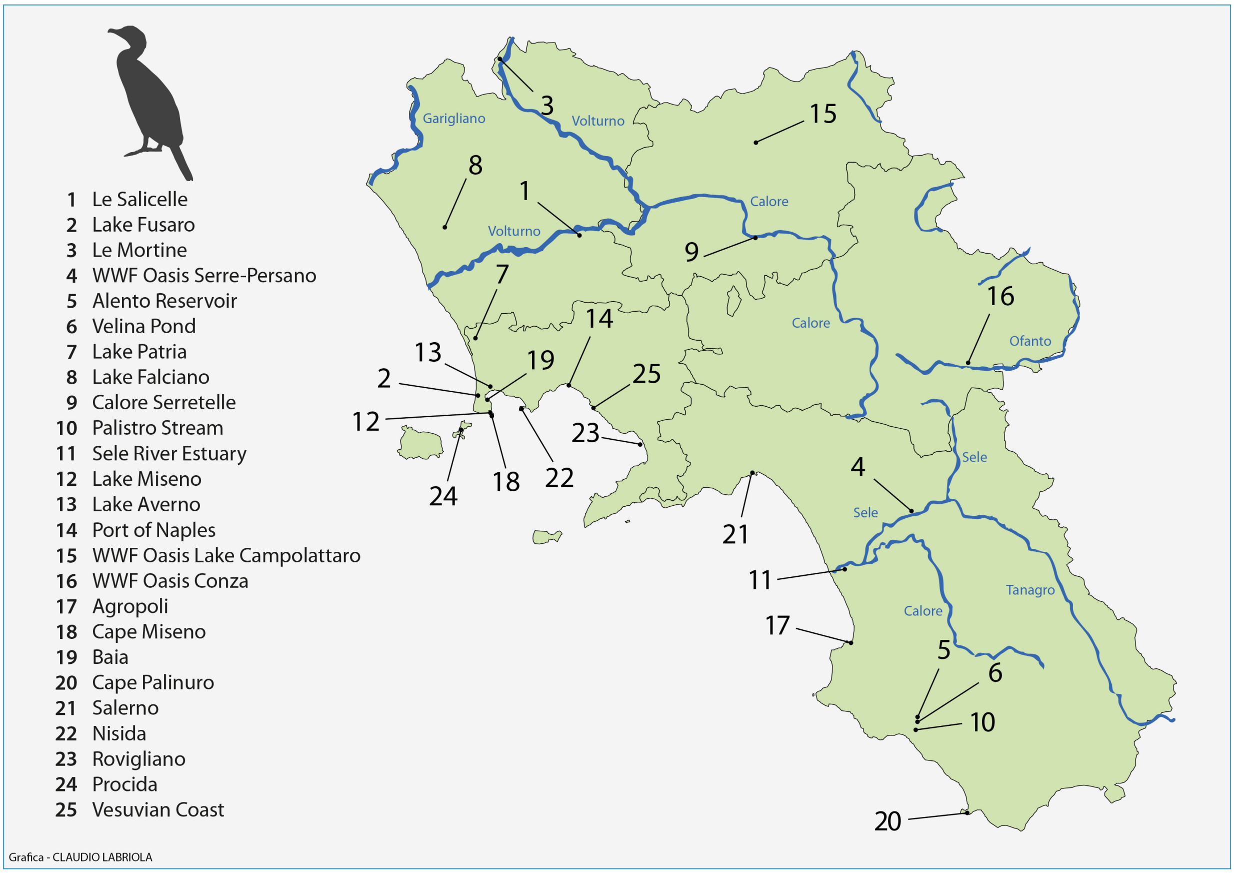

Figure 2: Location of the roosts on the regional territory.

Table 1: Number of years of actual use of the roost.

roost	number of years of use
Le Salicelle	11
Lake Fusaro	24
Le Mortine	20
WWF Oasis Serre - Persano	25
Alento Reservoir	9
Velina Pond	6
Lake Patria	10
Lake Falciano	4
Calore Serretelle	10
Palistro Stream	2
Sele River Estuary	1
Lake Miseno	7
Lake Averno	4
Port of Naples	3
WWF Oasis Lake Campolattaro	22
WWF Oasis Conza	25
Agropoli	1
Cape Miseno	2
Baia	2
Cape Palinuro	2
Salerno	10
Nisida	9
Rovigliano	11
Procida	1
Vesuvian Coast	2

Besides, in the decade 2003-2012, the median number was 99 specimens, while for the decade 2013-2022 it was 169 specimens. The ratio between the median of average number of animals in the roosts in the decade 2013-2022 with that of the decade 2003-2012 is equal to 1.71, thus showing an almost doubling of the population (Fig. 5).

Most dormitories are positioned on trees located on the banks of rivers or lakes, with a prevalence of *Populus nigra*, and on sea cliffs. There are dormitories located on man-made structures such as artificial breakwater cliffs, port cranes, and floating buoys in mussel farming facilities. In a couple of cases, in the WWF Oases Conza and Campolattaro the cormorants exploit trunks of dead trees emerging from the waters of the artificial basin (Fig.6).

There are only nine reports of ringed specimens, all from abroad (Fig.7). Four specimens have been ringed in Germany, two in Denmark and Estonia, and one in Hungary. All the reports were from the wetlands of the Caserta coast and refer to

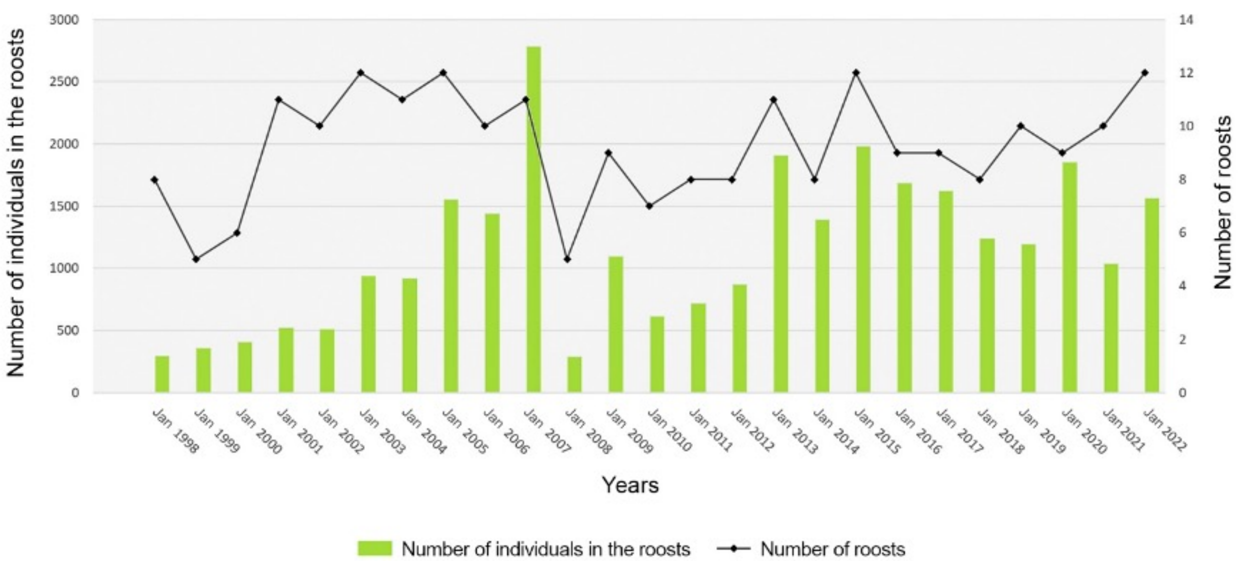

Figure 3: Total number of individuals in dormitories and number of dormitories for each monitoring year.

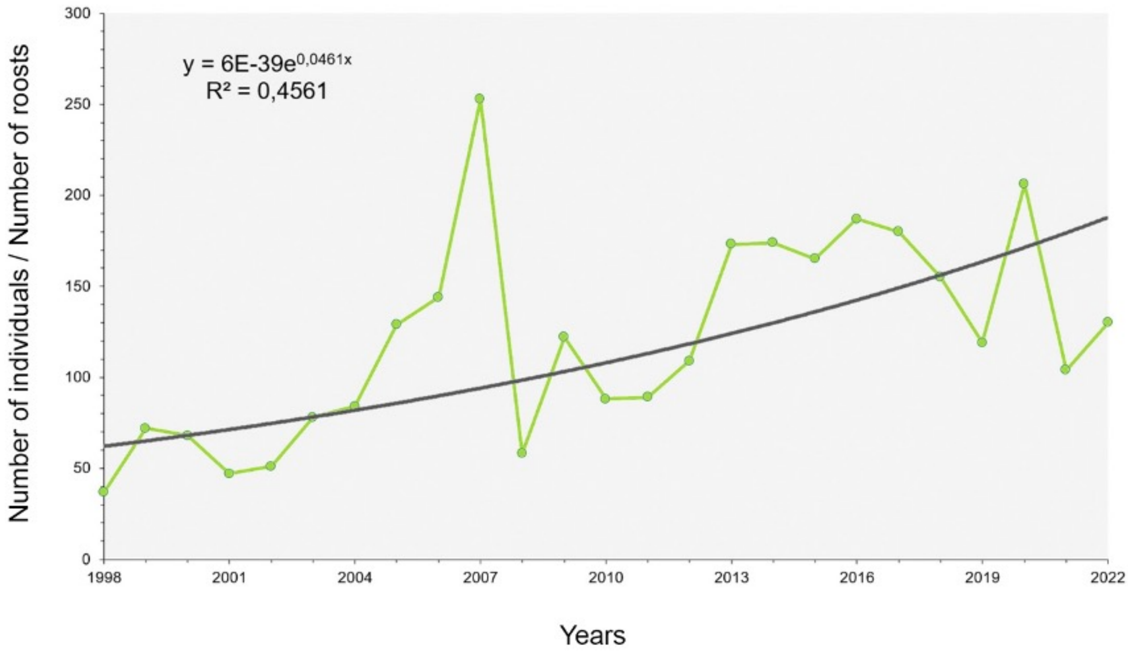

Figure 4: Historical series of the average number of individuals in the roost per year.

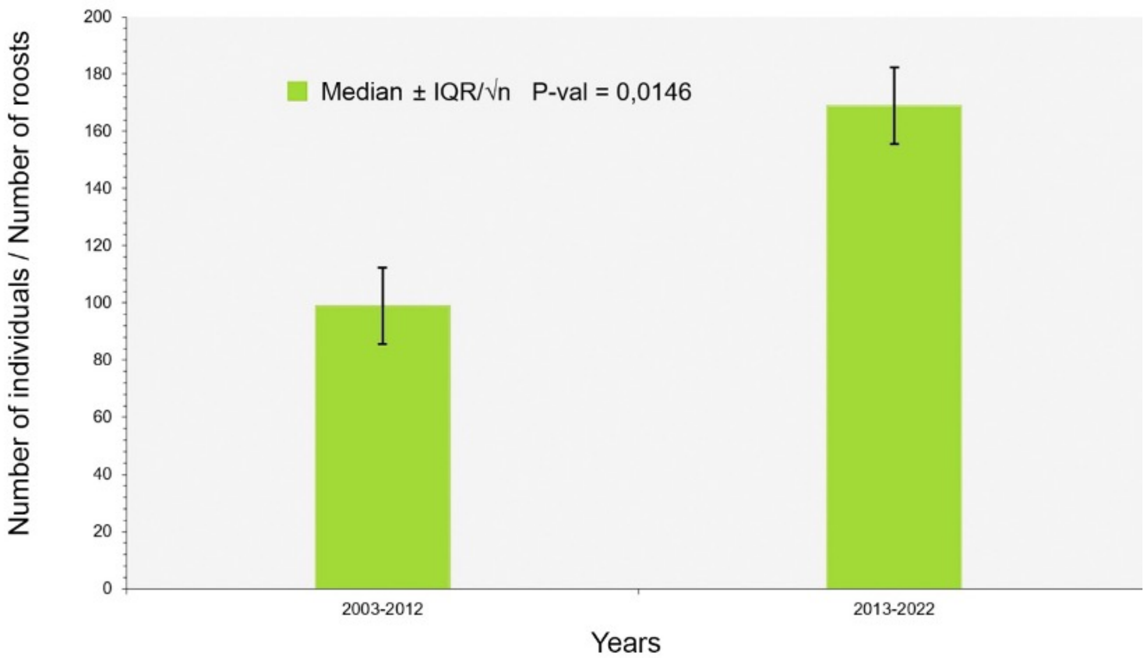

Figure 5: Median ± IQR / √n of the population surveyed at roosts in the two decades 2003/2012 and 2013/2022. IQR = Interquartile Range, √n = square root of the sample size.

killed specimens or, more recently, to the reading of the colored rings from living individuals. One report dates back to 1965 (from Germany), two to the 1980s (from Germany and Denmark), one to 1990 (from Germany), and five in the first two decades of the 21st century (from Denmark, Germany, Estonia, and Hungary). No reading took

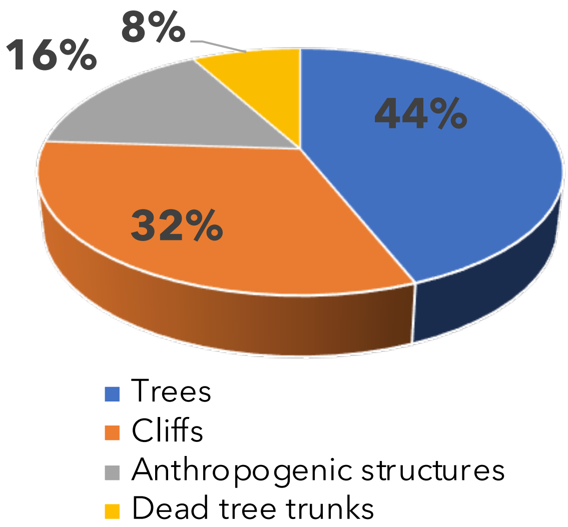

Figure 6: Substrates of the different Campanian dormitories.

place in the roosts. This can be due to the difficulties of finding and reading the rings for the twilight time and the position that the animals assume in the roosts, especially if laid on trees and then shielded by branches or other specimens.

Discussion

The roosts are in different places in the region, in areas characterized by high fishing, irrespective of their coastal or inland position (Fig.2). Of the total of 25 dormitories found during the 25 years of monitoring, only 5, 20%, were frequented continuously for more than 20 years (Tab.1). Most perches consist of riparian tree formations and sea cliffs (Fig. 6).

As for the trend of the monitored population in the roosts over time, there is a moderate increase with a growth rate of 4.6% per year. Deepening the analysis with the comparison between the last two decades confirms the growth, with almost a doubling of the median annual value per dormitory in the second decade. The increase in the wintering population is in line with BirdLife International (2017) in Europe, although the growth rate registered by us is less than 5%, a value that TRIM classifies as moderate.

Therefore, the graph in Fig. 4 allows to hypothesize a first consistent growth as a possible consequence of the conservation measures at the European level (see Marzano et al., 2013). Then, a slowdown follows probably imputable to saturation of resources. In any case, the wintering population in Campania region is in line with the moderate growth reported by recent literature (Frederisken et al., 2018).

The small number of marked individuals does not allow to make many assessments on the origins of wintering stocks in Campania region (Fig.7).

We note the absence of ringed individuals in Italy and that the origins of these specimens are from central-northern and central and north-eastern Europe. Frederisken et al. (2018), and more recently Bregnballe et al.

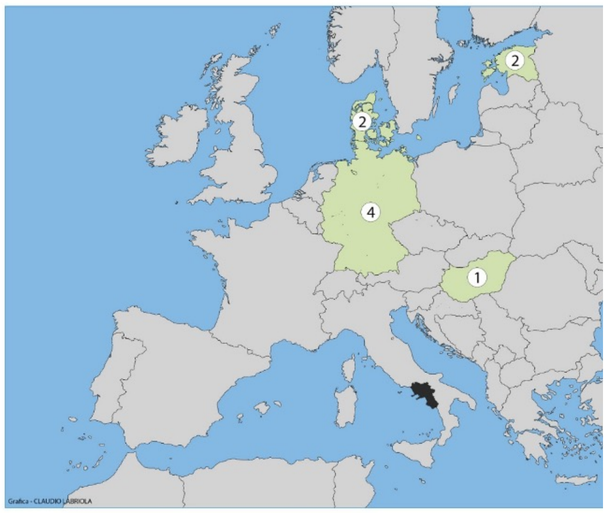

Figure 7: Putative origin of the cormorant specimens in winter. Green areas indicates the countries in which the specimens have been ringed. The number indicates how many recaptures come from that country.

(2021), report a change over the years, as a result of both climate change and the increase of great cormorant breeding populations on the Baltic Sea coasts. It can be hypothesized that in Italy the populations from Holland, Denmark and Norway are shifting their wintering sites towards France and the British Isles, whereas the Baltic population is increasing.
The three specimens from Central and North-Eastern Europe found since 2006 are still too few to confirm the data of literature, therefore it is desirable that future readings of colored rings will help to better understand the current origin of the wintering populations in Campania region.

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Author contributions

Maurizio Fraissinet, Leandro Buongiovanni, Beatrice Bigu, Marcello Bruschini, Camillo Campolongo, Anna Digilio, Lucio Dinacci, Bruno Dovere, Diego Errico, Elio Esse, Giuseppe Farace, Claudio Labriola, Remigio Lenza, Patrizia Loffredo, Vincenzo Mancini, Sabrina Maria Marsala, Danila Mastronardi, Alessandro Motta, Luca Nelisio, Stefano Piciocchi, Alfonso Maria Piromallo, Filippo Tatino, Costantino Tedeschi, Marilena Terminio and Alessio Usai collected data in field work. Maurizio Fraissinet coordinated the monitoring and catalogued data. Stefano Giustino provided the statistical analysis. Sabrina Maria Marsala wrote the English text.

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Elio Abatino, a naturalist devoted to education

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Abstract

We report biographical notes on Elio Abatino, Neapolitan naturalist and geologist, expert in environmental popular science, with particular reference to the Campania region. Former CNR (National Research Council) researcher in the field of electron microscopy, author of multiple publications, he dedicated himself particularly to the training and scientific updating of middle and high school teaching staff, first as a member of ANISN (Associazione Nazionale Insegnanti di Scienze Naturali), and then as founder of IREDA (Istituto di Ricerca e Didattica Ambientale).

Keywords: Abatino Elio, electron microscopist, geologist, naturalist, popular science

Riassunto

Si riportano i cenni biografici di Elio Abatino, naturalista e geologo napoletano, esperto di divulgazione ambientale, con particolare riferimento al territorio campano. Già ricercatore del CNR (Consiglio Nazionale delle Ricerche) nel settore della microscopia elettronica, autore di molteplici pubblicazioni, si è dedicato particolarmente alla formazione e aggiornamento scientifico del personale docente delle scuole medie e superiori, prima come socio dell'ANISN (Associazione Nazionale Insegnanti di Scienze Naturali), e poi come fondatore dell'IREDA (Istituto di Ricerca e Didattica Ambientale).

Parole chiave: Abatino Elio, microscopista elettronico, geologo, naturalista, divulgazione scientifica

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Elio Abatino was born in Naples on July 20, 1933, and passed away there on June 21, 2022.

He devoted himself to the collection and study of shells, minerals and fossils since a very young age. After graduating cum laude in Geological Sciences from the University of Naples, with an experimental thesis on the volcanic island of Ponza (Tutor: Prof. Angiola Maria Maccagno) he won the competition for researcher at CNR (National Research Council) and later he became facilities Manager at the Centre for Electron Microscopy and the Chemistry Department of the National Research Council. He was also responsible for public relations, and consultant for the Library and the Historical Museum of the CNR's Engine Institute, where for many decades (1973-2000) he

Elio Abatino (1933-2022).

successfully carried out research on several projects using electron microscope. In this regard, we would like to mention the '*Scipionix samniticus*' International Palaeontology Prize in Benevento received by Abatino on 24 April 2009 for his work "History of microscopy applied to optical and electronic micropalaeontology". From 1971 to 1975, he was the national coordinator of the Earth Sciences Group of the SIME-Italian Society for Electron Microscopy. Unfortunately, the intense research activity under electron microscope caused him a severe visual impairment, which afflicted him for the rest of his life.

For five years he was the coordinator-secretary of the Biology Group in the Didactic Seminar of the Faculty of Science at the University of Naples (Director Prof Giulio Cortini), a department responsible for refresher courses for teachers of natural sciences.

Trained in the school of Professor Arturo Palombi, he intensively collaborated with the Campana Section of ANISN (Associazione Nazionale Insegnanti di Scienze Naturali/ National Association of Natural Science Teachers) (VV AA, 2019) as editor and organizer of educational excursions for schoolteachers. After a long series of prestigious assignments (Puntillo, 2021; Vitiello, 2021), in 1986 he founded the IREDA (Istituto di Ricerca e Didattica Ambientale/ Institute of Environmental Science and

Education) awarded both at regional and European level with numerous merits.
Since 1984 he taught in the free Faculty of Tourism Sciences founded by Professor Umberto Fragola, and in 1986 he was awarded an Honorary Degree in Tourism Sciences. For three years he was director of the caves of Castelcivita (Salerno, Italy) to which he devoted in-depth studies. He has made study and research trips to almost every country in the world.
He participated in the reconstruction of the New 'Giovan Battista Alfano' Mineralogical Museum in Pompeii by drawing up a detailed guide to the museum collections and presenting an extension and restoration project on behalf of the Tourist Promotion Agency. For the Municipality of Castelcivita he also realised a project for the implementation of a Museum of Prehistory and Natural History in the area, to be located in a deconsecrated church in the medieval village.
Passionate about Giovanbattista Della Porta, he did painstaking research on the life and works of this Neapolitan scholar who lived in Naples between the late Renaissance and the Scientific Revolution.
During his life, he was awarded numerous titles, prizes and honors from the scientific world, such as diplomas, plaques from: CNR, Sorrento City Council, Naples City Council, Arcipesca FISA Italian Federation of Sport, Environment, Education and Image; in 1998 he was awarded the 'Silver Primrose' honour by the Scientific Committee of the magazine published by Electa 'Uomo & Natura' for merits acquired in the protection of the Environment and for his many years of teaching naturalistic and environmental disciplines. In 1986, he was awarded an honorary degree in Tourism Sciences, a faculty where he held various positions in Natural Sciences, Geography and Surveying and Planning of Tourist Routes. In April 2006 he received a Diploma and a Gold Medal for Scientific and Didactic Merit from the Councillor for Education and Training and Culture Policies of the Veneto Region, Dr. Elena Donezzan, for the active collaboration carried out in favor of the Civic Museum of Natural History of Jesolo (VE).
He was a member of the Order of Journalists of Campania since 1973 as director of the "Bulletin of the Activities of Ireda" and he was a member of the Advisory Commission for the establishment of Regional Parks and of the Commission on Bathing of the Campania Region.
He participated in the implementation of the Scientific Project of the Marine Park of Santa Maria di Castellabate on behalf of the Zoological Station "Anton Dohrn" (Director Dr. Pietro Dohrn) and the local Diving Center.
He was a member of the Società Italiana di Malacologia (Italian Society of Malacology) since 1988 and of the Società dei Naturalisti in Napoli (Society of Naturalists in Naples) since 1971. With the latter society in 1981 he was a member of the organizing committee and the "Promoting Committee" for the celebration of the 1st centenary of the Society which took place in November 1981. Specifically, in the Minutes of the General Meeting of February 27, 1981 published in Volume 90 of 1981, it is reported that member Abatino was chosen because he was "particularly appreciated for his organizational skills."
He was a member of numerous other Italian and foreign Scientific Academies and Cultural Associations such as: Società Geologica Italiana, Società di Mineralogia e

Petrologia, Società Italiana di Paleontologia, Associazione Nazionale Musei Scientifici (Orti Botanici, Giardini Zoologici e Acquari), Società Speleologica Italiana, Naturalisti Bolognesi, Naturalisti Ferraresi, Gruppo Mineralogico Geologico Napoletano, Unione Esperti Europei del Turismo, Associazione Geoturismo di Genova, Associazione Italiana di Geologia e Turismo - Bologna, Società Napoletana di Storia Patria, Società Italiana di Microscopia Elettronica, Società Italiana di Cinematografia Scientifica, Touring Club Italiano, Archeoclub, Arca.

After his retirement on 1998 his activities as a scientific popularizer and trainer of natural sciences teacher multiplied.

His volcanic activity is evidenced by more than 90 papers on environmental issues, scientific dissemination (popular science) and biodiversity, many of which are intended to enhance hidden treasures of the territory (Crovato & Maio, 2021).

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Author contributions

Nicola Maio and Paolo Crovato cured equally all steps for the composition of the manuscript.

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Chromosome analysis on Central and Southern Italy population of the common toad, *Bufo bufo* (Amphibia, Anura)

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Abstract

Amphibians constitute a very good model to explore the historical aspects of species distributions due to their low dispersal capacity and low individual vagility. Bufonidae are one of the most speciose family of Anura, including taxa, such as *Bufo bufo*, widespread in Eurasian regions.

We performed a karyological study with standard and sequential C-banding + fluorochromes (Chromomycin A_3 (CMA) and Diamidinophenylindole (DAPI) on several tadpoles from different populations of Central and Southern Italy. All the examined tadpoles exhibited the standard *Bufo* karyotype of 2n = 22 biarmed chromosomes, with the first six pairs larger than the other five (7 - 11) pairs and NOR associated heterochromatin distal on the long arms of the 6^{th} chromosome pair, that was also the only chromosome CMA-positive region. C-banding evidenced centromeric heterochromatin, DAPI positive, on all the chromosomes in all the studied populations from Central Italy. The Southern Italy populations differed in additional paracentromeric C-bands on the short arms of chromosomes 1, 3 and 5. These results support the partition of Central populations of *B. bufo* from the Southern ones, as evidenced also from molecular phylogenetic studies.

Keywords: *Bufo bufo*, C-banding, heterochromatin, karyotype, NORs

Riassunto

Gli anfibi costituiscono un ottimo modello per esplorare gli aspetti storici della distribuzione delle specie a causa della loro bassa capacità di dispersione e della bassa vagilità individuale. I bufonidi sono una delle famiglie di Anuri più ricche di specie, tra cui il rospo comune, *Bufo bufo*, diffuso nelle regioni eurasiatiche.
Abbiamo eseguito uno studio cariologico con tecniche standard di bandeggio C e sequenziali con fluorocromi (Cromomicina A_3 (CMA) e Diamidinofenilindolo (DAPI) su diversi girini di diverse popolazioni del Centro e Sud Italia. Tutti i girini esaminati presentavano il cariotipo *Bufo* standard, di 2n = 22 cromosomi a due braccia, con le prime sei coppie più grandi delle altre cinque (7 - 11), e l'eterocromatina NOR-associata distale sui bracci lunghi della sesta coppia di cromosomi, che era anche l'unica regione cromosomica CMA-positiva. Il bandeggio C ha evidenziato eterocromatina centromerica, DAPI positiva, su tutti i cromosomi in tutte le popolazioni studiate dell'Italia centrale. Le popolazioni dell'Italia meridionale differivano per bande C paracentromeriche aggiuntive sui bracci corti dei cromosomi 1, 3 e 5. Questi risultati supportano la suddivisione delle popolazioni centrali di *B. bufo* da quelle meridionali, come evidenziato anche da studi filogenetici molecolari

Parole chiave: *Bufo bufo,* pattern di Bandeggio-C, eterocromatina, cariotipo, NORs

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Introduction

DNA sequencing has provided great advancement in biological studies, such as phylogenetics, systematics and taxonomy, nevertheless conventional and molecular cytogenetic analyses are still relevant to study the genomic/chromosomal changes during evolution. Comparative chromosome analyses can be useful to identify plesiomorphic and apomorphic characters and the occurrence of different evolutionary lineages (Mezzasalma et al. 2015, 2021). Chromosome rearrangements may either precede or follow molecular evolution and directly promote the speciation, or as by product after phylogenetic diversification (King 1993; Mezzasalma et al. 2017). In either case, they represent discrete evolutionary markers able to detect different evolutionary trends in the taxa studied (Olmo, 2008; Mezzasalma et al. 2022a, 2002b).

Amphibians constitute a very good model to explore the historical aspects of species distributions due to their low dispersal capacity and exhibit low individual vagility, often accompanied by high philopatry to natal sites retention (Beebee, 1996). Moreover, they are very sensitive to climatic changes, which make them optimal organisms for discriminating the effects of glacial cycles and other environmental changes upon their genetic structure and biogeographic patterns (Zeisset and Beebee, 2008).

Bufonidae is the third largest Anura family with 640 species so far recognised (AmphibiaWeb 2022; Frost 2022), among which *Bufo bufo*, widespread in almost all Eurasian regions.

Bufo bufo is part of a species complex, formerly considered as its subspecies, namely: the Caucasian toad (*B. verrucosissimus*); the Japanese common toad (*B. japonicus*), the European common toad (*B.bufo*) and a new species, *B. eichwaldi*, described by Litvinchuk et al. (2008), morphologically and genetically distinct from *B. bufo*, living in south Azerbaijan and Iran. (Recuero et al. 2012; Arntzen et al. 2013, 2016; 2017). Garcia-Porta et al. (2012) analysed the phylogenetic relationships between the Eurasian and North African species of the *B. bufo* group and specified a first split of *B. eichwaldi* from the main lineage occurred around to thirteen - nine million years ago. Next split of *B. spinosus* dated about five million years ago. Finally, the splitting between *B. bufo* and *B. verrucosissimus* occurred about three million years ago during the Pleistocene.

Several karyological studies, using different chromosome banding, have been performed on *B. bufo* species group (Schmid 1978; Birstein and Mazin 1982; Matsui et al., 1985; Spasić-Bošković, et al. 2000; Skorinov et al. 2018; Guzmán-Markevich et al. 2022). Concerning the Italian population, the only one study was conducted by Morescalchi (1964), who used standard chromosome staining, on a population from Southern Italy.

In this paper we carried out a chromosomal study using standard and C-banding staining methods on tadpoles of *B. bufo* from several Central and Southern Italy populations aiming to detect eventual differences on the chromosomes and heterochromatin distribution among the considered populations.

Material and Methods

The number and origin of the examined tadpoles of the common toad, *B. bufo* are given in table 1.

Specimen identity of all studied tadpoles (Table 1) was determined by the molecular barcoding method, using a segment of the mitochondrial 16S rDNA gene, which is widely used in phylogenetic analyses on this toad taxon (e.g. Kutrup et al. 2006; Garcia-Porta et al. 2012; Recuero et al. 2012; Arntzen et al, 2017; Chiocchio et al., 2021). DNA was extracted from cell suspensions following Sambrook et al. (1989). Primer pairs were: 16Sa (CGCCTGTTTATCAAAAACAT) and 16Sb (CCGGTCTGAAACTCAGATCAGT) (Palumbi et al. 1991), allowing to amplify a segment of about 550 bp. The PCR running parameters were: 5 min. at 95°C (denaturation step); 35 cycles at 94°C for 30 s; 55°C for 30 s and 72°C for 1 min. (amplification cycles); 7 min. at 72°C (termination step). After gel electrophoresis on 1.5% agarose gel, the bands of the amplified products were excised, purified from the gel with GenElute kit (Sigma), and sequenced in both orientations using the BigDye Terminator kit v1.1 and the automatic sequencer ABI Prism 310 (Applied Biosystems, Foster City). Chromatograms were checked and edited using Chromas Lite 2.6.6 and BioEdit 7.2.6.1

Table 1: Number and origin of studied tadpoles of *B. bufo*; cl.e6 and cl.e7 refer to the clades of the tree designed by Garcia-Porta et al. (2012).

Origin	Nr.	% Id, vs Seq- GenBank
Conero, Ancona (Marche)	2	100% vs JQ348788 (Monteleone d'Orvieto, PG) (cl. e6)
Minturno, Latina (Lazio)	2	100% vs JQ348786 (Campa di Segni, Roma) (cl. e6)
Rio Santa Croce, Formia, Latina (Lazio)	3	100% vs JQ348786 (Campa di Segni, Roma) (cl. e6)
Lago Penitro, Formia, Latina (Lazio)	2	100% vs JQ348786 (Campa di Segni, Roma) (cl. e6)
Cesinali, Serino, Avellino (Campania)	3	100% vs AY555020 (Matera) (cl. e7)
Altamura, Bari (Puglia)	2	100% vs JQ348795 (Bari) (cl. e7)
Gallo Matese, Caserta (Campania)	2	99.6% vs AY555020 (Matera) (cl. e7)
Lago Letino, Letino, Caserta (Campania)	2	99.6% vs AY555020 (Matera) (cl. e7)
Agnone Cilento, Salerno (Campania)	2	99,8% vs JQ348794 (Piaggine, Salerno) (cl. e7)
Montecorice, Salerno (Campania)	3	99,8% vs JQ348794 (Piaggine, Salerno) (cl. e7)
San Nicola Arcella, Cosenza (Calabria)	2	100% vs JQ348763 (Cetraro, CS) (cl. e7)

(Hall 1999). Sequences were deposited in GenBank: OQ301661 - OQ301672.

Chromosome analysis

The chromosome analysis was performed using the scraping method (Sharma and Sharma 1980), as modified by Petraccioli et al. (2015). Chromosomes were derived from intestine (cleaned form debris) as described by Petraccioli et al. (2012). In brief, after immersion of the specimen in tricaine methanesulfonate (0,1%), the intestine of each tadpole was removed and incubated for two hours in one ml of calf serum and inactivated at 56°C for 30 min in a solution containing 50 μl of colcemid at 10 μg/ml. Subsequently, the intestine was incubated for 30 min. in hypotonic solution (KCl 0.075 M + sodium citrate 0.5%, 1:1) and fixed for 15 min. in methanol: acetic acid, 3:1. Cell dissociation of the intestine was made on a 100-mesh sieve and chromosomes were prepared dropping 25 μl of the obtained cell suspensions on the slides. The chromosome staining was performed with traditional staining (5% Giemsa solution at pH 7 for 10 min) and sequential C-banding + CMA_3 + DAPI according to Mezzasalma et al. (2022a). Karyotype reconstruction was performed after scoring at least five plates per sample and chromosomes were classified following Levan et al. (1964).

Results

The results of 16S analysis confirmed the taxonomic attribution of the studied tadpoles to the taxon *Bufo bufo* (see Table 1 for the results of queries in GenBank deposited). Within each population the tadpoles show an unique haplotype, but it should be stressed that the inter-populational diversification of the segment of 16S sequences here considered is very scarce, e.g. the identity is 99.81%. between the two more distant population studied, San Nicola Arcella (Cosenza) and Conero (Marche).

Chromosomes were obtained from tadpoles of all populations, except for those from Cesinali (Avellino). All the other tadpoles, regardless of the origin, showed a karyotype of 2n = 22 biarmed chromosomes, with the first six pairs (1-6) distinctively bigger than the other five pairs (7-11) (Fig. 1). Sequential C-banding + CMA + DAPI evidenced that all chromosomes were uniformly stained with the first fluorochrome, except for the peritelomeric regions of long arms of chromosomes of the sixth pair that were strongly CMA-positive (Fig. 1). C-banding + DAPI showed centromeric C-band positive to this fluorochrome on all chromosomes of tadpoles from Conero (Ancona), Minturno (Latina), Rio Santa Croce (Latina), Lago Penitro (Latina), and Lago Letino (Caserta) and Gallo Matese (Caserta) (Fig. 1). The tadpoles from Altamura (Bari); Agnone Cilento (Salerno), Montecorice (Salerno), Corleto (Salerno) and San Nicola Arcella (Cosenza) in addition to centromeric C-bands on all chromosomes showed para-centromeric C-bands DAPI positive on the short arms of the chromosomes of pairs 1, 3 and 5 (Fig. 1 D'-F').

Discussion

The first chromosomal study on common toad was conducted on French specimens and date back to the first years of the last century (Lebrun, 1902). This study reported an inexact number of elements, that successive analyses on European and Asian specimens established to be 2n = 22 (Stohler, 1927-1928; Minouchi and Iriki, 1931; Tchou-Su, 1931; Galgano, 1933; Witschi, 1933; Wickbom, 1945). Next studies on specimens from Monte Cerreto (Salerno, Italy) by Morescalchi (1964) and from Germany by Ullerich (1966) confirmed the karyotype of 2n = 22 chromosomes, all metacentric except for the submetacentric 4th and 8th pairs. These investigations described a secondary constriction terminal to the long arms of chromosomes of the 6th pair and denied the presence of heteromorphic sex chromosomes. This chromosome formula was confirmed in European pet trade toads of unknown origin by Schmid (1978), in the first of his long, very interesting, series of papers on chromosome banding in Amphibia. This author confirmed the presence of NORs peritelomeric on the long arms of the 6th pair and provided the pattern of C-banding positive heterochromatin (see Fig. 2). Similar C-banded karyotypes were displayed by Serbian (Spasić-Bosković et al., 2000) and Russian (Matsui et al. 2013) specimens (see Fig. 2 for the corresponding ideograms). Furthermore, chromosome analysis conducted on taxa formerly considered as subspecies of *B. bufo*, namely *B. spinosus*, *B. verrucosissinus*, *B. japonicus* and *B. eichwaldi*, showed that they conserved the localization of NORs peritelomeric on the long arms of the chromosomes of the 6th pair and each exhibited a distinctive pattern of C-banding positive heterochromatin (Birstein and Mazin 1982; Matsui et al. 1985; Skorinov et al. 2014; Guzmán-Markevich et al. 2022). All exhibited centromeric C-bands on all chromosomes but differed on the pattern of paracentromeric, interstitial and/or telomeric heterochromatin. In Urodeles evidence from studies on satellite DNAs (Hutchinson and Pardue 1975; Macgregor and Sessions 1986; Macgregor 1991), which are a major component of heterochromatin (John 1988), proposed the following evolutionary steps on the chromosome

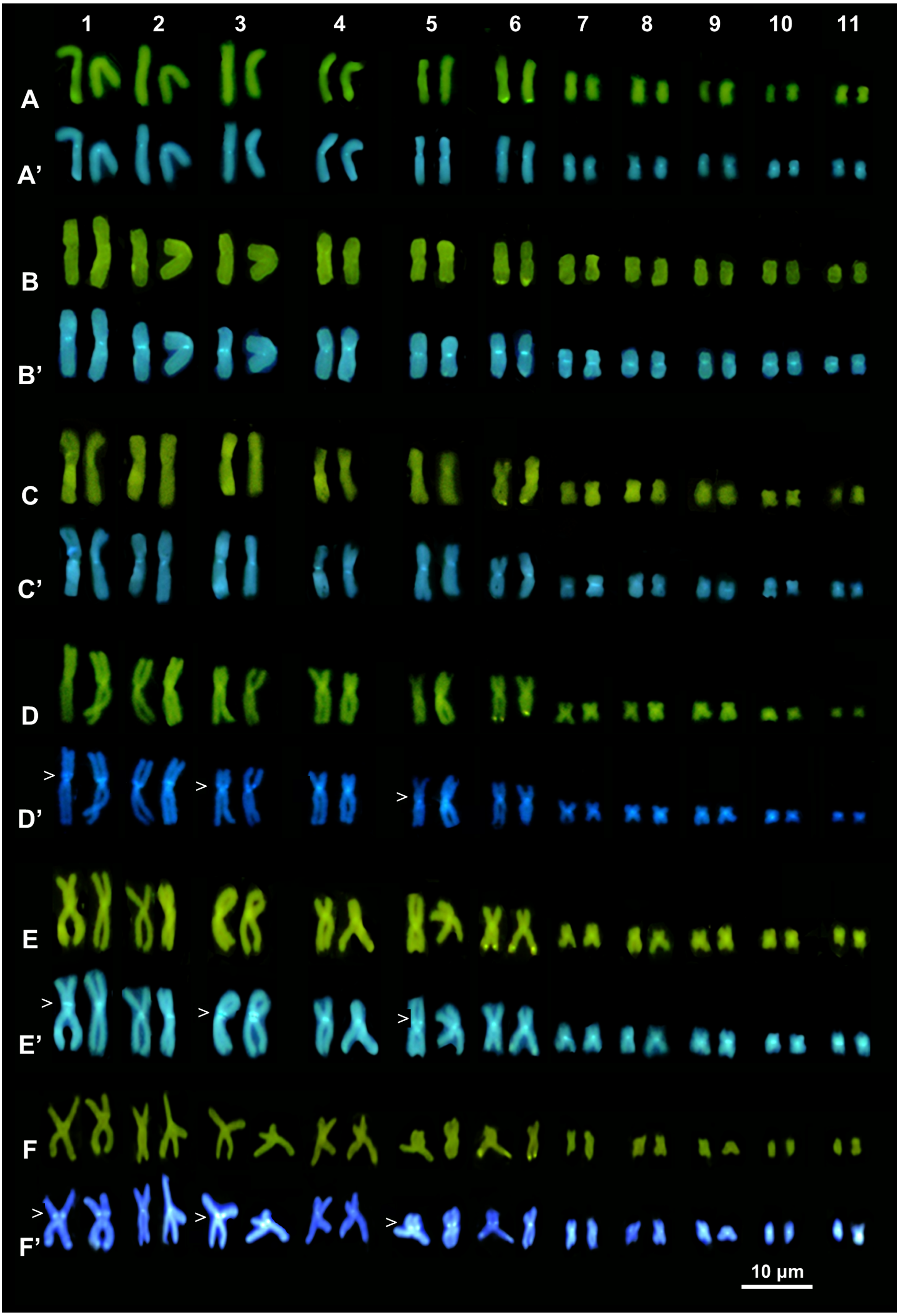

Figure 1: Karyotypes stained with sequential C-banding + CMA (**A**-**F**) + DAPI (**A'**-**F'**) of specimens of *B. bufo* from: Conero (Marche) (**A**, **A'**); Minturno, Rio Santa Croce, Lago Penitro (Lazio) (**B**, **B'**); Lago Letino, Gallo (Campania) (**C**, **C'**); Altamura (Puglia) (**D**, **D'**); Agnone Cilento, Montecorice, Corleto (Campania) (**E**, **E'**); San Nicola Arcella (Calabria) (**F**, **F'**). Bar refers to all karyotypes. > point to paracentromeric, DAPI positive C-bands.

heterochromatin distribution: i) an initial amplification of satellite DNA arrays occurring at centromeric/ pericentromeric regions; ii) intrachromosomal rearrangements satellite sequences on interstitial and/or telomeric regions, with some arrays as remnants of the original amplified satellite sequence still evident on centromeric C-bands. The results of C-banding staining here obtained show a different pattern of heterochromatin between population of common toad from central (Ancona, Latina + Caserta) and southern (Salerno and Cosenza) populations. In turn, the C-banding patterns of above population also differ from those available in literature, which concerned populations from Serbia, near Moscow and an unknown European population (Schmid 1978; Birstein 1982; Spasić-Bošković et al. 2000), that we have superimposed on the image (opportunely modified) of the distribution areas of clades of *B. bufo* by Garcia-Porta et al. 2012 (Fig. 2).

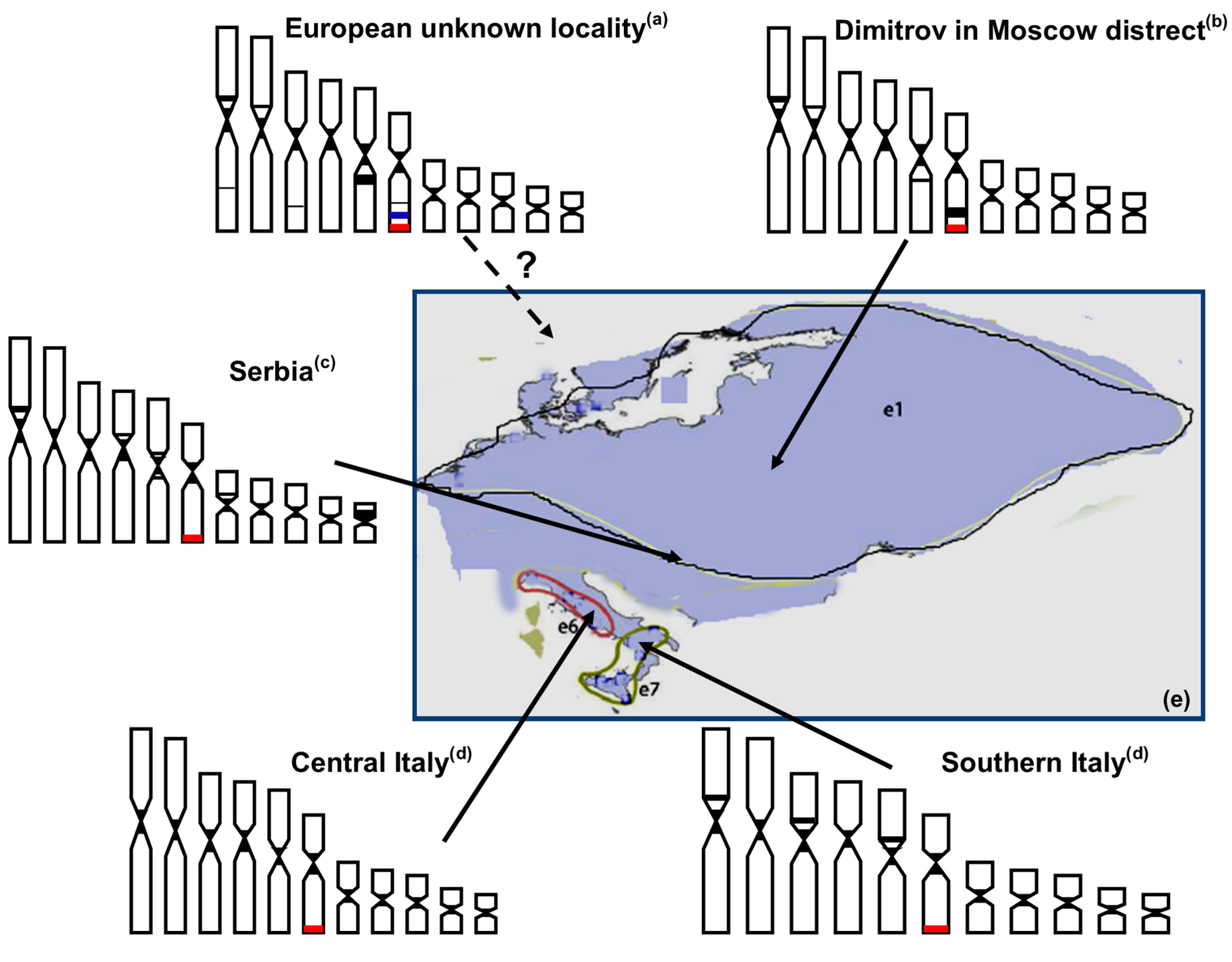

Figure 2: Schematic haploid karyotype ideograms of populations of *B. b. bufo* superimposed on the geographic distribution of its clades e1, e6 and e7 according to: Garcia-Porta et al. (2012); Schmid 1978 (a); Birstein 1982 (b); Spasić-Bošković et al. 2000 (c); present paper (d). The Figure was modified from Garcia-Porta et al. (2012). Dark, blue and red bands refer, respectively, to C-banding positive heterochromatin, NOR-associated heterochromatin and Loci of NORs.

Interestingly, the central and southern Italian populations are in the clades e6 and e7, respectively, while the Russian and Serbian population are in the clade e1. Note, that the Serbian population are in southern margin of distribution of the e1 clade, leaving uncertain its attribution at this clade. So, C-band patterns reveal a population variability regarding heterochromatin distribution, which in turn appear to support/discriminate the distribution areas of clades e6 and e7 of *B. bufo* (Garcia-Porta et al. 2012). However, some cautions should have to consider C-bands, specifically the subtle interstitial ones. These bands may be not reproducible or not visible, due to methodological differences among laboratories to prepare, which are in more condensed chromosomes, as by preparation from cell cultures or from testis. In any case solid paracentromeric and or interstitial C-bands are independent from the employed methodology and tissues to obtain chromosomes. So, they can be used as landmarks to discriminate the different populations of *B. bufo*. Furthermore, the chromosome evidence here obtained supports the hypothesis advanced by Chiocchio et al. (2021) that the Central and Southern Italy lineages of *B. bufo* expanded form their ancestral glacial refugia along Tyrrhenian coastal refugia, with the Volturno-Calore rivers lower basin as suture zone, establishing a secondary contact zone before the last interglacial.

In conclusion, the chromosome C-banding patterns seem useful in distinguishing taxonomically different forms of *B. bufo*, but their use in relation to speciation events or systematic relationships need further analysis.

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