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Phytoplankton biomass and main functional groups of the Sele river mouth (Southern Italian Tyrrhenian coast) in a severe drought period

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Abstract

The phytoplankton community structure of the terminal stretches of the Sele river, one of the most important rivers of the southern Tyrrhenian coast of Italy, was investigated. Main functional groups and total biomass were determined from samples collected in proximity of drainage channels and under a period of severe drought. The overall sampling area was characterized by a strong vertical stratification driven by intrusion of seawater, with large hypoxic or anoxic areas near the bottom. High concentrations of Chl-a up to 95.73 μg/l were observed in the surface water layer (0.1-1.0 m depth) characterized by oxygen oversaturated waters. Cryptophytes and diatoms were the dominant groups, but high variability of minor groups was observed among stations. Results highlighted critical ecological conditions suggesting the need for further studies to cope with the high variability of such a complex system, particularly the utilization of more appropriate sampling scales.

Keyword: Eutrophication, phytoplankton, chemotaxonomy, biodiversity, river ecosystem

Riassunto

In questo studio è stata analizzata la struttura della comunità fitoplanctonica, in termini di gruppi funzionali principali e di biomassa totale, nel tratto terminale del Sele, uno dei fiumi più importanti della costa meridionale italiana, durante un periodo di grave siccità. I punti di campionamento sono stati posizionati nei pressi dei canali di drenaggio che affluiscono nel fiume. Complessivamente, l'area di campionamento è stata caratterizzata da una forte stratificazione verticale indotta dall'intrusione di acqua di mare sul fondo, dove sono state riscontrate ampie zone ipossiche o anossiche. Sono state riportate concentrazioni di Chl-a elevate, fino a 95,73 µg/l, e lo strato di acqua superficiale compreso tra 0,1 ed 1,0 m di profondità era caratterizzato da acque soprasature di ossigeno. Criptofite e diatomee erano i gruppi dominanti, ma è stata osservata un'elevata variabilità dei gruppi funzionali minori tra le stazioni. I risultati suggeriscono la presenza di punti critici nel tratto finale del fiume Sele, ed è quindi importante approfondire con ulteriori studi le dinamiche ecologiche di questo sistema complesso, sulla base di scale di campionamento adeguate.

Parole chiave: Eutrofizzazione, fitoplancton, chemotassonomia, biodiversità, ecosistema fluviale

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Introduction

Sele river is the second largest river in southern Italy in terms of average water volume, second to Volturno. Its lower part is located within a wide alluvial coastal plain, with a drainage basin of 3235 km^2 and a solid flow of 500,000 m^3/yr (Cocco et al., 1989). This territory has a relevant interest from the point of view of the interaction between natural and anthropic dynamics, to such an extent that allows to this relationship as a paradigmatic example of the interaction between natural dynamics and a cultural niche. In particular, it must be highlighted a territorial feature which is not unique to the Piana del Sele, but it is present in several other Mediterranean coastal context, that is to show dynamics that range from the regional scale to the entire Mediterranean basin in terms of anthropic history. The history of the human presence in the Piana del Sele is well documented since the Eneolithic and went on with more or less relevant events to the present day. The literature regarding the anthropic settlement in the Piana del Sele and the more relevant aspects of the evolution of its natural features are reported in a rich series of paper leaving both with the settlement history (Ferrara & Greco, 2017) and the morphological evolution of its physical features (Alberico et al., 2012). As for more recent times, starting from the mid-nineteenth century, it has undergone intense agricultural exploitation up to the current levels of over-exploitation. Along with the agriculture, a zootechnical activity was carried out based on the water buffalo, since middle age. In this general frame, it has to be mentioned the relevance of both natural and artificial drainage structures, all leading to the river Sele. The point in which these channels reach the river were the object of the present paper. The construction of a dense net of artificial drainage channels and the presence of the Dam of Persano strongly influenced both the coastal erosion and the river environment (Alberico et al., 2012; Arienzo et al., 2020). The Dam of Persano is located 16.2 linear km from the river mouth, and was built between 1929 and 1932, creating a basin of 1.5 million m^3 which affects the downstream sediment deposition causing the consequent retreat of the coast (https://www.bonificadestrasele.it/site/wp-content/uploads/2017/12/Relazione-Diga.pdf).

The significant geomorphological alteration of Sele mouth and the presence of pollutants has been documented recently by Arienzo et al. (2020) with conspicuous loads of organic matter deriving from anthropogenic activities over the entire system. One of the most important effects of pollutants on rivers ecosystems is a net increases in primary production processes, including an excessive growth of phytoplankton biomass, as a result of nutrient supply (mainly phosphorus and nitrogen). The impact of nutrient enrichment on rivers is often season specific and complicated by their dynamic nature (Newman et al., 2005). Rivers are highly selective environments, where flow rate and turbidity are the two critical factors limiting the development of phytoplankton communities so that only a relatively small number of genera can achieve dominance (Rojo et al., 1994; Reynolds 1994a). One of the reasons why phytoplankton represents a significant component of primary production only in slow-moving rivers of lowland regions, is that the retention time of water is longer than the generation time of the plankton (Lampert & Sommer, 1997). The dominance of centric diatoms and coccal green algae in lowland regions has been explained by Reynolds et al., (1994a), as a consequence of the dynamics of vertical mixing of a turbid and turbulent water column, that selects species with the appropriate traits (Reynolds & Descy, 1996; Gray 1989).

Due to the lack of information on the phytoplankton community of the river waters, a study was carried out along its terminal stretch (the final 3 km) in order to contribute to fill these gaps. The relationship between phytoplankton and biomass with water chemical and physical properties was evaluated in proximity of the confluence of several drainage channels during a period of severe drought when the river mouth appeared completely occluded by sediment deposition impeding any water exchange with the open sea.

Material and Methods

A one day sampling was carried out on July 31, 2017 in the Sele river, in proximity of drainage channels in the first 3 km from the mouth. A total of 9 stations were sampled, from the mouth to the inner part of the river (Fig. 1; Tab. 1), for the determination of phytoplankton biomass, main functional groups and physical and chemical properties of the water. Salinity, temperature (°C), and saturation percentage of dissolved oxygen (%) profiles were acquired by an Idromar XMAR212 probe interfaced with a GPS (Garmin Map 78S). In order to assess the phytoplankton biomass and diversity in terms of chemotaxonomical groups, at each station (except stations 1, 3 and 6) 2 l of surface water were collected with a Niskin bottle for pigment spectra determination. One litre of water was then drawn from the Niskin and filtered on GF/F Whatman filters (25-mm diameters and 47-mm) for spectrofluorimetric and HPLC analyses (Vidussi et al.,1996; Mangoni et al., 2016). Filters were stored in -20°C until the analyses in laboratory. The amount of chlorophyll-a (Chl-a) was used to indicate the total phytoplankton biomass, while the contribution of the main phytoplankton groups to the total Chl-a was estimated on the basis of the concentrations of biomarker pigments, using the CHEMTAX software as indicated by Mangoni et al., (2008).

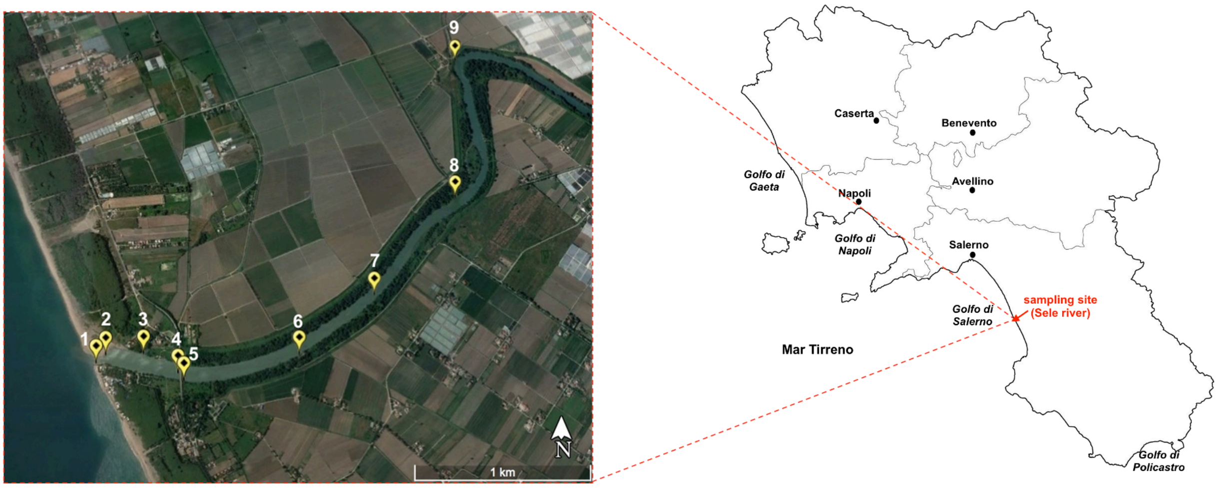

Figure 1: The terminal stretch (final 3 km) of the Sele river, with sampled stations (yellow mark).

Table 1: Sampled stations and related coordinates.

Station	Latitude N	Longitude E
1	14,943003	40,481503
2	14,943663	40,481942
3	14,946342	40,482025
4	14,948773	40,481002
5	14,949187	40,480607
6	14,957369	40,482022
7	14,963108	40,485324
8	14,970075	40,491178
9	14,971769	40,500494

Results

Fig. 2 displays the vertical and longitudinal distributions of salinity, temperature, and saturation percentage of dissolved oxygen. The distribution of salinity shows the presence of a strong water stratification, with a net halocline between 1.5 and 2 m depth, except at station 8, and at the mouth of the river where tidal movements exerted a strong influence (Fig. 2a). The first 1.5 m

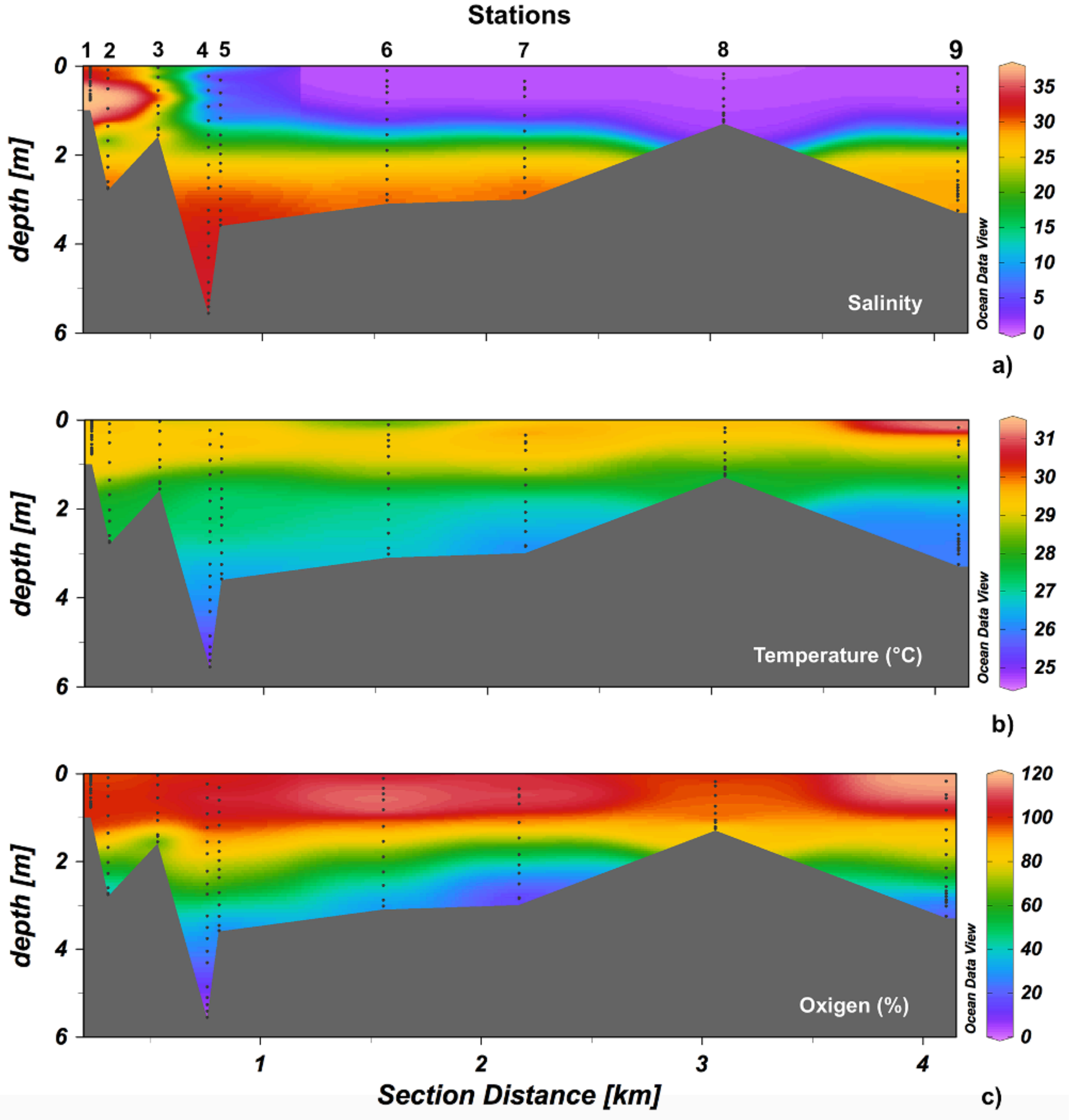

Figure 2: Vertical and longitudinal distributions of: a) salinity, b) temperature, and c) percentage of dissolved oxygen. The colour on the Z axis indicates the range of variability of each variable.

water layer between stations 3 and 9 was characterized by freshwater with values ranging from 1.5 to 8; below this water depth, salinity strongly increased forming a sharp halocline (~0.3 m thickness) with 15-20 units higher than the mean level of the surface layers. Except for station 8, the bottom layer was characterized by the presence of salty water. Within this stratified structure, the observations reveal the presence of a clear increasing gradient of salinity from the inner part toward the mouth and from the surface to the river bed, with higher values between stations 1 and 2 (about 100 m apart) as a consequence of tidal inflow. The temperature trend was similar at all stations with values slightly decreasing from the surface to the bottom and ranging from a minimum of 24.59 at station 4 (5.5 m) to a maximum of 31.34°C at station 9 (0 m) (Fig. 2b). The saturation percentage of dissolved oxygen showed the presence of three distinct layers: a 0- 1.0 m layer characterized by oversaturated oxygen conditions and up to 117% at station 9, located in the inner part of the sampling area (Fig. 2c); a 1.0-2.5 m layer characterized by a strong gradient, with values from 100% at 1.0 m to 65% at 2.0 m; a layer below 2.5 m depth characterized by severe oxygen depletion, especially at the deeper stations, with values reaching the minimum of 1.5% at station 4.

The mean value of Chl-a was 62.72 µg/l, with values ranging from 15.05 µg/l at station 2 to 95.73 µg/l at station 8, showing higher values in the inner part of the river (Fig. 3). Chemotaxonomic composition revealed that diatoms and cryptophytes were the dominant functional groups, with the former dominating at stations 4, 7, 8 and 9, and the latter at stations 2, 5, and 8 (Fig. 4). The presence and abundance of other groups strongly differing among stations.

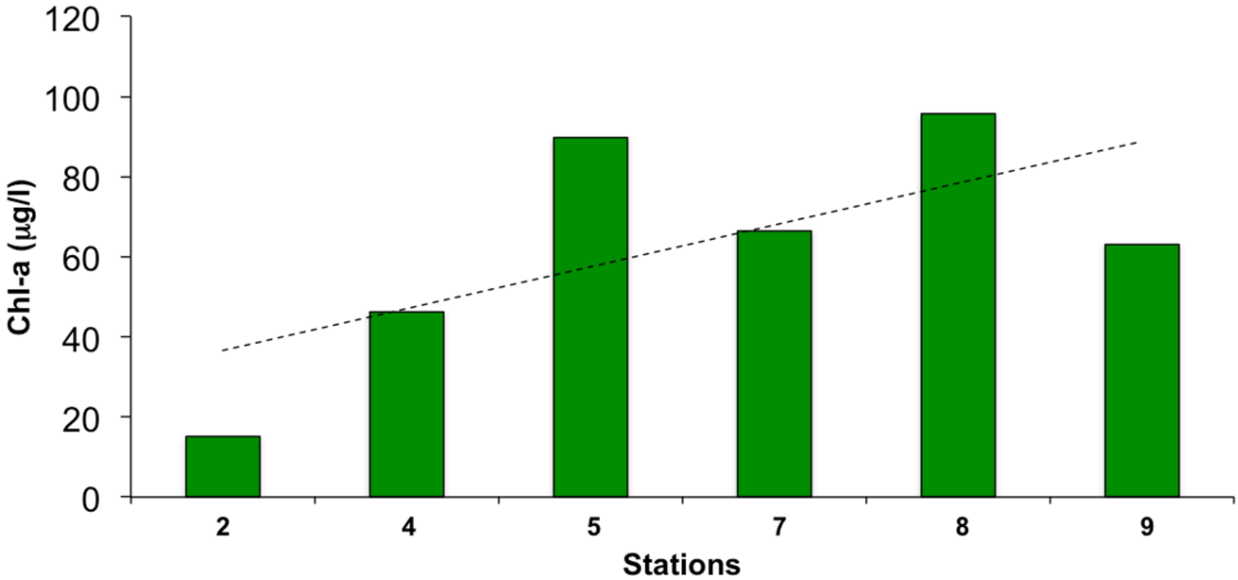

Figure 3: Phytoplankton total biomass (Chl-a, µg/l) at each sampled station, with related regression line (black points). For technical problems, some data stations were not considered for the elaborations.

Rapidophytes were completely absent at station 4, cyanophytes were only present at station 2 and xantophytes were only present at station 8 and 2. Dinoflagellates were absent at stations 8 and 2, while prasinophytes were only present at stations 4, 5 and 7.

Discussion

Phytoplankton is commonly used as an ecological indicator in the assessment of anthropic impact on freshwater environments since phytoplankton blooms are usually the result of excessive nutrient loading and extended water residence time induced by the artificial flow control (Paerl et al., 2007; Reynolds, 2003; Waylett et al., 2013). Not surprisingly, the study of phytoplankton in rivers is a required quality element in the WFD (Directive 2000/60/EC of the European Parliament and of the Council) establishing a framework for the Community action in the field of water policy.

Although the presence of human settlements on the Sele river date back to the second millennium B.C. (Bronze Age), a significant anthropic influence on the evolution of the area started around the middle of 19th century with the construction of a first set of artificial drainage and irrigation channels, that have allowed the development of intensive agricultural and livestock sectors. The presence of a stratification characterized by a salty and

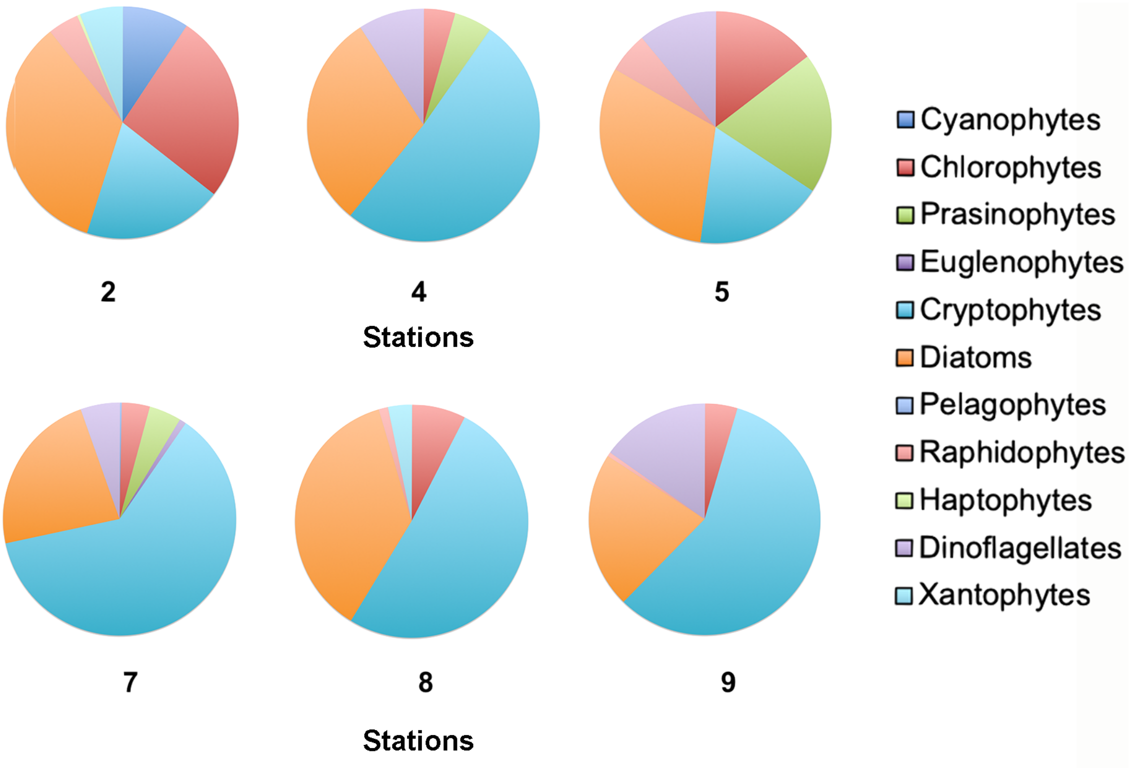

Figure 4: Phytoplankton total biomass (Chl-a, µg/l) at each sampled station, with related regression line (black points). For technical problems, some data stations were not considered for the elaborations.

dense layer of sea waters at the bottom represents an important physical and chemical barrier affecting the functioning of this system. Our sampling was carried out when the mouth of the river was completely occluded by sediments (Fig. 5) preventing an exchange with the sea and strongly contributing to the development of the eutrophic status of the river, as an effect of the ensuing the water retention time and preventing the enlivening of the river deeper waters. This feature is absent at stations 1 and 2 due to the interstitial inflow through the sandbar. The mean value of phytoplankton biomass was high with a mean of 62.72 (±27) µg/l over the entire sampled stretch. This, together with the presence of anoxic conditions in deeper layer represent critical conditions for the benthic assemblages, that otherwise, could be potentially involved in algal removal by filtering or grazing activities (Newman et al., 2005). The observed large variability of the main functional groups over a limited space indicated that drainage channels could significantly contribute to shape the phytoplankton community. The dominance of diatoms agrees with data reported in literature (Reynolds & Descy, 1996; Genkal 1997), but the high percentage of cryptophytes represents a novel observation. The lack of information on the Sele river ecosystem, and the limited number of stations analyzed, does not allow an exhaustive interpretation of phytoplankton community structure and dynamics in such a

Figure 5: July 31, 2017. The mouth of the Sele river occluded by sediments.

complex ecosystem. However, our data clearly suggest the presence of a high heterogeneity in terms of physical-chemical and biological properties of waters, mainly due to strong anthropogenic pressures (Arienzo et al., 2020). Although it is not possible at the moment to interpret the data in a coherent overall picture, they nevertheless constitute a significant starting point to improve our knowledge on the environmental status this area. Further research has been planned in order to define the temporal and spatial dynamics of phytoplankton community. To this end, it is necessary to use appropriate temporal and spatial scales of investigation in order to frame the system in relation to human pressures (Reynolds 1994b) and, considering that the management of large rivers requires a balance between human needs and ecological integrity. As the Sele River plays a central role not only in biodiversity but also in the economy and culture of its catchment area, the deepening of the knowledge of its environmental conditions, of the origins of the criticalities observed in view of a recovery plan for this transitional environment is now an avant-garde objective on which the authors intend to pursue.

In conclusion the relevant aspects of this preliminary study can be represented by 1) the confirm diatoms as the main component of river phytoplankton; 2) the significant presence, in our case, in the composition of the community of other groups, such as cryptophytes; 3) the temporal limitation of our sampling period; 4) the physiognomy of the river phytoplankton community represents a firs reference point to the evolution of the community following regimes of higher discharge by the river in the annual hydrographic cycle.

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Author contributions

Conceptualization: F.B, O.M., L.F., M.A. Data curation: F.B, O.M. Formal analysis: F.B, O.M. Investigation: F.B., L.F. Methodology: F.B, O.M. Resources: O.M. Writing – original draft: F.B., L.F., M.A., O.M. Writing – final draft preparation: F.B., L.F., M.A., O.M.

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Review Article

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The origins of life: novel perspectives over an old problem

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Abstract

Life probably appeared on Earth around 4 billion years ago and was globally diffused within the next 500 million years. It is debated whether life emerged in a superficial terrestrial environment, as generally maintained by "primordial-soup" models, or in association with submarine hydrothermal vents. Simulation experiments show that abiotic formation of simple organic molecules from CO_2 and H_2, and of peptides from free amino acids is thermodynamically favoured under hydrothermal-vent conditions. In contrast, proposed pathways of abiotic synthesis of nucleotides and RNA fit better with superficial scenarios subject to wet/dry cycles. The "RNA World" hypothesis posits that a critical step towards life was the appearance of RNA enzymes (ribozymes) that catalysed RNA replication and random α-amino acid polymerization. The narrative presented here suggests that ribozyme interaction with peptides underpinned the emergence of populations of "protoribosomes" and virus-like RNA "protochromosomes" depending on each other for replication and subject to Darwinian evolution. The establishment of a genetic code coupled RNA and peptide evolution. RNA chaperoning of peptides positively selected self-folding peptide sequences, thus paving the way to the evolution of biologically active protein architectures. Association of informationally interlinked protoribosomes and protochromosomes with liquid-crystal bilayers produced the first protocells, self-replicating structures that evolved an increasingly complex metabolism by replacing ancestral ribozymes with more efficient protein enzymes. The addition of the Sec translocon machinery and of integral lipid-synthesizing enzymes converted self-assembled protomembranes into hereditary encoded membranes. The transition to DNA as the repository of genetic information established the genotype-ribotype-phenotype tripartite organization of modern cells. Cell evolution was the first and most conspicuous expression of ecological inheritance in life history. Primordial-soup models favour a heterotrophic ancestral metabolism, whereas the alkaline-vent scenario points to a chemioautotrophic origin. In line with the latter, phylogenomic analysis suggests that the last universal common ancestor (LUCA) was a CO_2-fixing, H_2-dependent, N_2-fixing, thermophilic organism. Life utilized only a tiny part of the virtually limitless space potentially available in carbon chemistry, probably due to the need to control harmful spontaneous reactions in the overcrowded intracellular environment.

Keyword: Cell evolution, Genetic membranes, Genotype-Ribotype-Phenotype, Last Universal Common Ancestor, RNA World, Translation machinery

Riassunto

La vita è probabilmente apparsa sulla Terra intorno a 4 miliardi di anni fa e si è globalmente diffusa nei successivi 500 milioni di anni. Non è chiaro se essa sia nata in un “brodo primordiale” sulla superficie del pianeta, o in associazione con fumarole alcaline nelle profondità marine. Simulazioni *in vitro* mostrano che la formazione abiotica di piccole molecole organiche da CO_2 e H_2, e di peptidi da aminoacidi liberi è termodinamicamente favorita nelle condizioni esistenti nelle fumarole alcaline. In contrasto, i meccanismi proposti di sintesi abiotica di nucleotidi e RNA sono meglio compatibili con ambienti di superficie soggetti a cicli di idratazione e disidratazione. L’ipotesi del “Mondo dell’RNA” assume che un evento cruciale nell’evoluzione della vita sia stata l’apparizione di ribozimi, molecole di RNA capaci di catalizzare la polimerizzazione casuale di aminoacidi in peptidi e la replicazione dell’RNA. La narrativa qui presentata suggerisce che l’interazione tra ribozimi e peptidi abbia prodotto popolazioni di “protoribosomi” e “protocromosomi” simili a virus a RNA, reciprocamente dipendenti per la replicazione e soggetti a evoluzione darwiniana. L’affermazione del codice genetico accoppiò poi l’evoluzione dell’RNA a quella dei peptidi. L’interazione peptidi-RNA favorì l’emergenza di sequenze peptidiche capaci di ripiegarsi in strutture regolari, dando così avvio all’evoluzione di architetture proteiche complesse. RNA e peptidi interconnessi dal codice genetico si associarono a protomembrane di origine abiotica, producendo protocellule, strutture autoreplicanti che svilupparono un metabolismo di crescente complessità rimpiazzando i ribozimi ancestrali con più efficienti enzimi proteici.

L'incorporazione di un sistema Sec di traslocazione e di complessi enzimatici per la sintesi di lipidi convertì le protomembrane in "membrane genetiche" trasmesse di generazione in generazione. L'adozione del DNA come veicolo dell'informazione genetica al posto dell'RNA introdusse l'organizzazione tripartita in genotipo, ribotipo e fenotipo che caratterizza le cellule moderne. L'evoluzione della cellula fu il primo e più cospicuo caso di eredità ecologica nella storia della vita. I modelli basati sul concetto di "brodo primordiale" favoriscono un metabolismo ancestrale di tipo eterotrofo, mentre lo scenario delle fumarole alcaline postula un'origine chemioautotrofa. In linea con l'ipotesi chemioautotrofa, l'analisi filogenomica suggerisce che l'ultimo progenitore universale comune (LUCA) fosse un organismo termofilo che fissava N_2 e CO_2 utilizzando H_2 di origine geochimica. La vita ha esplorato solo una minuscola parte dello spazio virtualmente illimitato della chimica del carbonio, probabilmente per la necessità di controllare dannose reazioni spontanee nel sovraffollato ambiente intracellulare.

Parole chiave: Evoluzione cellulare, Genotipo-Ribotipo-Fenotipo, Membrane genetiche, Mondo dell'RNA, Apparato di traduzione, Ultimo Progenitore Universale Comune

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1. Introduction

Unraveling the origins of life is an appealing problem per se; nobody really cares, but everybody would like to know (Nicolas Galtier)

The origins of life has been the central problem of biology since when Louis Pasteur and John Tyndall definitely disproved spontaneous generation in the mid-19th century. Panspermy hypotheses postulating an origin of life from extra-terrestrial spores do nothing more than transferring the problem to another place in the Universe (Chandra 2011). Panspermy cannot be rejected by principle but, in the almost complete absence of scientific evidence, it is generally assumed that terrestrial life originated on Earth.

Extant living beings, although as diverse as to warrant two or three Domains of life to categorize them (da Cunha et al. 2018; Williams et al. 2020), share a set of fundamental traits, most notably the translation apparatus, the genetic code and the use of DNA for storing genetic information. No less revelatory is the universality of many if not all existing protein folding domains and of fundamental protein architectures. The simplest explanation for this shared biology is that all living organisms inherited it from a Last Universal Common Ancestor (LUCA), implying that essential hallmarks of extant life were most probably established long before the appearance of LUCA.

Direct and indirect evidence suggests that life was already present on Earth around 4 billion years ago (Ga), only about 600 million years after the formation of the Earth-Moon system (Dodd et al. 2017; Bell et al. 2015). The Earth surface was at the time almost entirely covered with an ocean because continental landmasses were scarcely developed and mostly submerged, with only volcanic edifices exposed to the atmosphere (Kamber 2015). It is estimated that the total volume of the ocean was initially about twice as much as today, the missing water having been transferred to the mantle by subduction of hydrated oceanic crust and in minor part lost by photodissociation (Genda 2016). The atmosphere consisted mainly of nitrogen, carbon dioxide and water, with minor amounts of hydrogen, methane and sulphur dioxide of geochemical origin, and traces of nitrogen oxides produced from nitrogen and water under the action of lightning. Because carbon dioxide was much more abundant than today, the ocean was acidic, with a pH in the range 5-6. Sodium chloride was directly added as vapour to the Earth atmosphere after the formation of the Moon and then, in cooler condition, dissolved in the ocean, which therefore was saline from the beginning (Camprubì et al. 2019).

The origins of life coincided with the origins of the cell, the essential unit of life. The cell is traditionally viewed as a duality of phenotype and genotype, the first providing material and energy for reproduction, the latter providing information. Ribosomes and RNA mediate the interaction between genotype and phenotype under the rules of the genetic code. The translation apparatus is generally considered as a part of the phenotype, yet in 1981 Barbieri described it as a third fundamental component of the cell, which he called the *ribotype*, and proposed that life ultimately derived from a self-replicating ancestral ribotype. Major steps in Barbieri's scenario of cell evolution were the emergence of self-replicating ribosome-like structures, or "ribosoids", their aggregation into nucleolus-like "nucleosoids", the acquisition of a bounding membrane, and the incorporation of DNA, initially as a parasite and then as the repository of genetic information. Based on his model, Barbieri described the cell as *a colony of ribonucleoproteins engaged in producing other colonies of ribonucleoproteins*. We will see in this review that recent work revives in part Barbieri's model.

Ribosomes are essential to protein synthesis and, indirectly, to RNA synthesis. Yet, although a pre-formed complement of ribosomes is transmitted to daughter cells at each cell division, ribosomes cannot be considered as hereditary structures because ribosomal RNA and proteins self-assemble into new ribosomes without requiring pre-extant ribosomes as templates. This is instead much the case for biological membranes, supramolecular architectures that carry essential information for making new membrane. Cavalier-Smith (2001, 2004) distinguished *hereditary* and *non-hereditary membranes*. The first are membranes vertically transmitted from cell to cell and amplified upon request by addition of new components (lipids and proteins) in their pre-existing molecular framework. The process involves integral protein complexes including lipid-synthesizing enzymes and Sec translocons (du Plessis et al. 2011; Nyathi et al. 2013). A hereditary membrane can originate only from a membrane of the same type. The hypothetical loss of a hereditary membrane would be lethal because the cell could no longer recover it. Major instances of hereditary membranes are the cell membrane in all organisms, the outer membrane in gram-negative bacteria (negibacteria), the thylakoid membrane in cyanobacteria and chloroplasts, the ER, and probably the Golgi apparatus in eukaryotes. Non-hereditary membranes derive from a hereditary membrane and, if lost, can be recovered. Examples in eukaryotes are the nuclear envelope, which re-forms after mitosis from endoplasmic reticulum, and the bounding membrane of lysosomes, endosomes and vacuoles, deriving from the Golgi complex and continuously recycled. Akin to Cavalier-Smith's concept of hereditary membrane is the notion of "encoded membrane" introduced by Lane and Martin (2012) to denote a key step in pre-cellular evolution in which key components of membranes started being encoded by genes.

Membrane compartmentation is so central to the functioning of the cell that membrane-bound protocells and a rudimentary form of membrane heredity have been suggested to predate genetic heredity (West et al. 2017). Compartmentation is, however, only one of the conditions required for the emergence of life. Living systems exist in a physical state that is extremely far from thermodynamic equilibrium or, equivalently, has extremely low entropy and thus low probability. To maintain this condition, living systems need coupling with an external disequilibrium, namely a supply of "negentropy" (Branscomb and Russell 2018a). The loss of thermodynamic coupling is what we call "death", dead organisms rapidly and irreversibly degrading to equilibrium. A third condition is a source of organic compounds providing the starting molecular framework for the construction of biological architectures. Last, living systems need a sink to get rid of waste without affecting chemical disequilibria.

Glossary

Annotation (of genes). The process of identifying the locations of coding regions (genes) in a genome (structural annotation) and determining what those sequences do (functional annotation). Once a genome is sequenced, it needs to be annotated to make sense of it.

Asgards. A recently proposed superphylum of archaea, resolved as the closest prokaryotic relative of eukaryotes in some phylogenetic trees (Williams et al. 2017, 2019)

Autotrophy. From Greek *autòs* "self" and *tròphein* "to nourish"). Organisms that obtain more than 50% of their carbon from CO_2 (or bicarbonate) are autotrophs. Organisms that obtain less than 50% of their cellular carbon from CO_2 are heterotrophs (Schönheit et al. 2016). This conventional definition takes considers that carboxylation reactions are universally present in all extant organisms, including humans.

Chemioautotrophy. A type of autotrophic metabolism that uses energy from the oxidation of inorganic compounds. Photoautotrophs instead use light energy.

Darwinian evolution. A population of self-reproducing systems evolves in a Darwinian way when the relative frequency of alternative hereditary traits arising from random mutation changes across generations, either in response to competition for resources or by chance.

Hadean. The geological eon preceding the appearance of the first known rocks (4.6-4.0 billion years ago).

Heterotrophy. By convention, heterotrophy is a form of metabolism in which less than 50% of total carbon is obtained from CO_2 or bicarbonate (cf. autotrophy).

Homologous genes. Genes derived by mutation from the same ancestral gene. Homologous genes are recognizable from sequence similarities.

Horizontal gene transfer (HGT). Transfer of genetic material from organism to organism independent of kinship relationships. HGT is distinct from vertical inheritance, viz. parent-to-offspring gene transfer. Genes acquired by HGT do not reflect common ancestry and need to be excluded from phylogenetic analysis. HGT frequency is relatively high in prokaryotes, much lower in eukaryotes.

Orthologous genes (orthologs). Genes derived from a common ancestral gene by a speciation event. Orthologous genes control the same function in related species.

Paralogous genes (paralogs). Genes derived by duplication of an ancestral gene in the same organism and diverged for novel, sometimes subtly different functions. Genes coding for different forms of tubulin in eukaryotes, or of collagen in animals are examples of paralogous genes. Molecular phylogeny compares the sequences of orthologous genes in separate lineages whilst excluding paralogous versions.

Protein domain. A segment of a polypeptide chain that folds autonomously from the rest of the filament. A domain may have a length of 40 to 350 amino acid residues. Single polypeptide chains may be comprised of one to several domains. Domains in multidomain proteins may affect each other's folding. Domain shuffling, viz. random recombination of gene sequences coding for domains belonging to different proteins, is an important mechanism of biological innovation. Most existing proteins are mosaics of domains from other proteins.

Secondary (protein) structures. Three-dimensional arrays of local peptide segments deriving from the formation of hydrogen bonds between the carbonyl oxygen and amide nitrogen of the peptide backbone. The most common secondary structures found in native peptides are alfa-helices and beta-sheets. Amino acids vary in their ability to form secondary structure elements. Proline and glycine are sometimes known as "helix breakers" because they disrupt the regularity of the alfa helical backbone.

2. The environmental context

The debate around the origin of life has long revolved around "primordial-soup" scenarios originally proposed by Alexander Oparin and J.B.S. Haldane and supported by experimental work of Stanley Miller (Miller 1953). Traditional models postulate abiotic formation of simple organic molecules from carbon dioxide and reducing compounds under the action of energy sources such as UV light, lightning, volcanism, or meteoritic impacts. Although differing in details, most primordial-soup models share the view that abiotic organic compounds accumulated in shallow superficial basins and, interacting with catalytic minerals, polymerized into molecules of increasing complexity. Under appropriate conditions, abiotic polymers in turn aggregated into self-replicating complexes that primed the evolution of living cells. This kind of narrative has been subject to extensive criticism from thermodynamic, biochemical and geochemical perspectives. Branscomb and Russel (2018a) view it as a "Frankenstein" scenario that fails to provide a steady source of disequilibrium to support pre-cellular systems.

Life is an emerging property of systems of linear polymers (nucleic acids and proteins) with specific monomer sequences. The spontaneous polymerization of monomers in solution proceeds up to an equilibrium characterized by small amounts of polymers with random sequences, and a majority of free monomers. The polymerization of monomeric units is generally a thermodynamically disfavoured reaction because it involves an increase of free energy ($\Delta G>0$); for the equilibrium to favour polymer formation, polymerization must be coupled with a reaction that has a negative G, as occurs in living systems.

The submarine hydrothermal scenario has been proposed relatively recently as an alternative to primordial-soup models (Martin and Russell 2007; Russell et al. 2010, 2013; Sleep et al. 2011; Sojo et al. 2016; Branscomb and Russell 2018b). Discovered at the onset of the current millennium (Kelley 2001), submarine hydrothermal vents are solid chimneys deriving from the interaction of marine water with peridotite rock along mid-ocean ridges. These edifices consist of silicates, clays, carbonates, and sulphides, and have a porous structure in which mineral-enriched water flows slowly across myriads of apertures, with prolonged interaction with ocean water (Cardoso and Cartwright 2017; Fig. 1).

Alkaline vents have several properties considered favourable to the emergence of life:

- A finely compartmented scaffold rich in catalytic minerals such as mackinawite (iron and nickel sulphide), greigite (Fe_3S_4), silica (SiO_2) and fougerite (a complex of ferrous-ferric iron, hydroxide ions and carbonate).
- A continuous supply of reactive compounds, in particular hydrogen, methane, hydrogen sulphide, ammonia, formate ($HCOO^-$), cianide (CN^-).
- A constant source of disequilibrium in the form of temperature, pH and redox potential gradients.
- Temperatures high enough to accelerate chemical reactions but compatible with the stability of organic molecules involved in biology.
- The possibility to discharge waste in the ocean, thus preventing the establishment of unfavourable equilibria. This is an

important aspect, usually neglected in "primordial-soup" models.

CO_2 and H_2, and the polymerization of free amino acids into peptides under

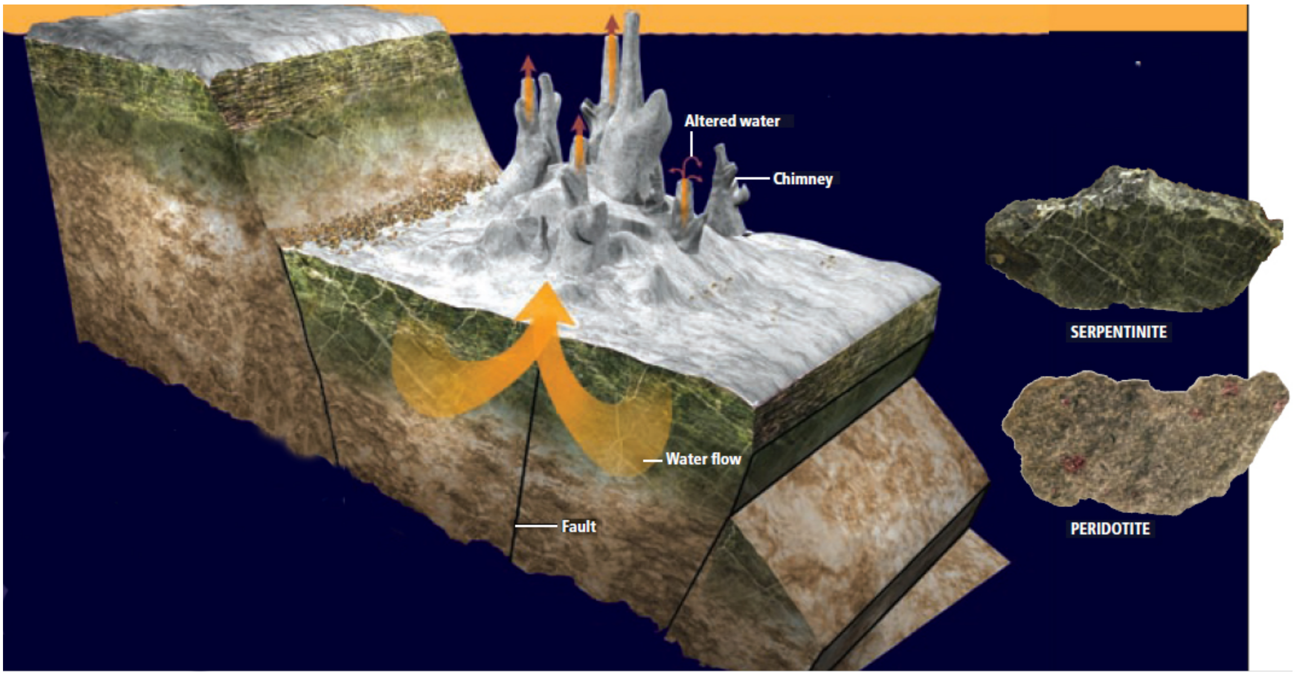

Figure 1: Alkaline hydrothermal vents develop along mid-ocean ridges from the interaction of marine water with newly formed oceanic crust rich in reactive peridotite rock. The process, known as serpentinization, converts peridotite into serpentinite; the reactions involved are exothermic and impart water an alkaline pH. Marine water penetrates the crust across faults. Circulating across the rock, the water charges with a diversity of minerals some of which contain biologically important inorganic catalysts such as iron, nickel, manganese, molybdenum, tungsten, and emerges at emission points at a temperature in the range 40-90 °C. In contact with cold oceanic water, dissolved minerals precipitate into solid chimneys that can attain heights of tens of meters. Alkaline vents depend on newly formed oceanic crust for activity; when the reactive minerals are exhausted, the vents die out, but others develop on new oceanic crust. The average duration in activity of alkaline hydrothermal vents is in the order of 10^4 years.

hydrothermal vent conditions are exergonic, thermodynamically favoured reactions (Lemke et al. 2009; Amend et al. 2013).

Field observations (Proskurowski et al. 2008; Lang et al. 2010) and simulation experiments (reviewed in Colín-García et al. 2016) have shown that the conditions existing in alkaline vents are conducive to spontaneous formation of organic molecules from carbon dioxide and hydrogen or other inorganic reductants. Abiotic organic synthesis may involve not only simple molecules such as formate and acetate, but also aminoacids and a diversity of sugars including ribose and deoxyribose. Most remarkably, the formation of simple organic molecules from

Less straightforward are the results of research on abiotic synthesis of nucleic acids. Geochemically plausible pathways have been described for pyrimidine but not purine nucleotides (Sutherland 2010). Formamide generated from ammonia and formate was found to be a precursor for abiotic synthesis of nucleic acid bases and purine acyclonucleosides in conditions simulating alkaline vents (Saladino et al.

2012a,b). A major problem with the alkaline vent scenario is the tendency of RNA to hydrolyse spontaneously into free nucleotides in alkaline solution, which would prevent accumulation of abiotic RNA (Bernhardt 2012). In fact, experiments simulating conditions in alkaline vents using circular ribonucleotides, imidazole-activated ribonucleotides with montmorillonite catalyst, or ribonucleotides in the presence of lipids, only managed to produce RNA oligomers up to four units in length (Burcar et al. 2015). In contrast, Da Silva et al. (2015) reported mononucleoside polymerization into RNA chains of 20-100 units, upon exposition to multiple cycles of hydration-dehydration at elevated temperatures and in presence of monovalent salts, suggesting the involvement of molecular alignment at water/air interfaces. Higgs (2016) developed a theoretical model accounting for the effect of repeated wet/dry cycles on spontaneous monomer polymerization. In thermodynamics terms, the entropy increase associated with water evaporation compensates the loss of entropy due to monomer polymerization. Nam et al. (2018) reported spontaneous synthesis of purine and pyrimidine ribonucleosides within aqueous microdroplets containing phosphoric acid, ribose, nucleobases and magnesium ions as a catalyser. The authors suggested that alignment at the air–water interface of microdroplet surfaces permitted the reactants to overcome the thermodynamic barrier for condensation reactions. The demonstration that wet/dry cycles may provide conditions favourable to spontaneous polymerization favours terrestrial models in opposition to the submarine scenario. Taking on board the novel information, Pearce et al. (2017) suggest that the bulk of nucleotide precursors of pre-biotic RNAs arrived from space with meteorites and interplanetary dust and polymerized under the action of wet/dry fluctuations in superficial ponds.

Based on similar premises as those underlying the alkaline vent hypothesis, Mulkidjanian et al. (2012) have proposed terrestrial anaerobic geothermal fields as the possible set for early life evolution (Fig. 2). These environments may provide essential conditions for the emergence of life including a supply of organic compounds, catalytic minerals, compartmentation, and steady chemical disequilibria. Moreover, unlike submarine vents, terrestrial geothermal fields might have benefited from exposition to wet/dry cycles. Geothermal systems like those proposed by Mulkidjanian et al (2012) still exist today and may have been in existence on Hadean Earth, despite the likely low extension of exposed landmass at that time (Camprubì et al. 2019). The hypothesis suggests that the high K/Na ratio that characterizes the living cytoplasm is a legacy of the special conditions that characterize terrestrial geothermal fields. Based on the assumption that ancestral cells, or protocells, probably had leaky membranes, Mulkidjanian et al. (2012) infer that the universal preference of extant life for potassium is incompatible with an origin in a marine set, where sodium largely predominates over potassium. Moreover, the frequency of Zn-dependent enzymes in modern life does not fit well with extremely low estimates of Zn concentrations (10^{-12}-10^{-16} M) in the Hadean ocean. The geothermal terrestrial scenario incorporates most features of submarine hydrothermal vents considered favourable to the emergence of life and adds more, notably exposition to wet/dry cycles. The model proposes that CO_2 reduction in light-mediated reactions provided a steady source of organic compounds whilst zinc and manganese sulfide afforded early biological systems protection from UV. Becker et al. (2018) propose a plausible abiotic pathway from simple inorganic precursors to nucleotides driven by wet/dry cycles and fluctuations of physicochemical

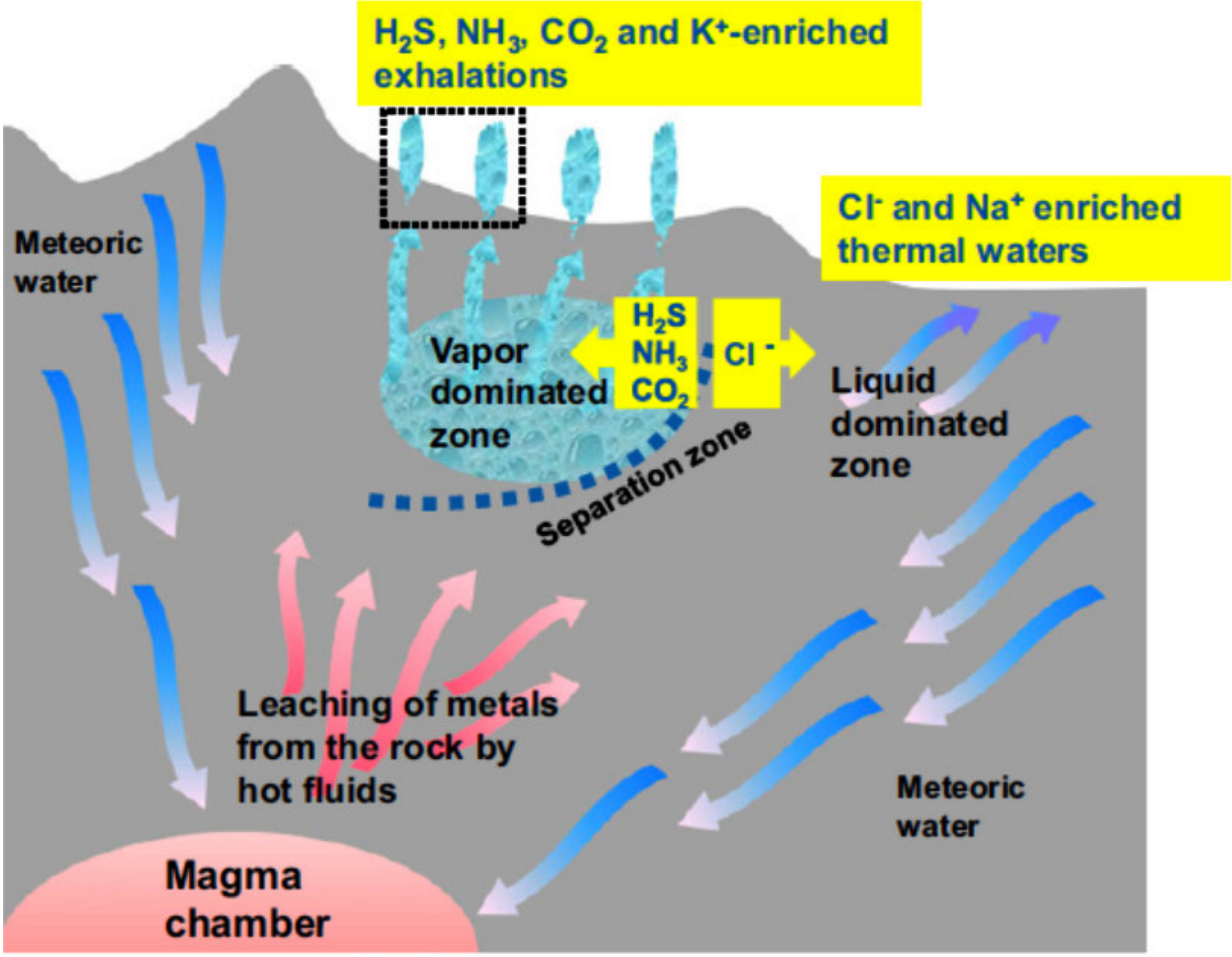

Figure 2: A terrestrial geothermal system proposed as the possible set for the birth of life on Hadean Earth. The system is essentially a lake or a system of lakes lying above a volcanic magmatic chamber and fed mostly by water from rain and snow (meteoric water). In the deep underground, water mixes with cation- and anion-enriched magmatic fluids and is heated to 300-500 °C. Ascending toward the surface, hot water interacts with the rock and is enriched in metal cations and anions such as Cl^-, HS^-, and CO_3^{-2}. At shallower depths, the rising hot water starts to boil because of lower pressure. Separation of a vapor phase from the liquid phase causes chemical separation of dissolved compounds, with some (e.g. chloride ions) mostly remaining in the liquid phase, and others such as the gaseous compounds CO_2, NH_3, and H_2S redistributing into vapor. The liquid phase emerges to the surface following the crevices within the rock. The vapor rises upward and spreads within the rock; the subsurface area that is filled by steam and gas is called the vapor-dominated zone. Part of the steam condenses near the surface and is ejected at thermal springs, and the rest of the steam reaches the surface through fissures of the rock to form fumaroles. Metal cations are carried by both the liquid and vapor phases, although the K^+/Na^+ ratio is higher in the vapor phase. From Mulkidjanian et al. (2012) under conditions granted by PNAS licence to publish.

parameters in a terrestrial geochemical scenario. Crucially, terrestrial geothermal fields like those considered by Mulkidjanian and co-workers might remain active for several million years, which is a more plausible time frame for the transition from molecules to life than the relatively short lifetime of submarine alkaline vents.

3. The RNA World hypothesis

In living organisms, proteins, RNA and DNA mutually interact in a closed circuit in which each type of molecule depends on the others for replication. Proteins catalyse the synthesis of RNA and DNA from nucleotides; RNA provides the information required for amino acid polymerization in cognate sequences; DNA provides the information for the synthesis of preordained sequences of RNA and for its own replication.

It is extremely unlikely that the three types of polymers and their complex interactions emerged together from scrap. Unlike DNA, RNA is a flexible polymer that can produce a diversity of molecular architectures made of alternating single- and double-filament regions and interconnecting loops (Hiller and Strober 2011). The discovery that RNA is not only able to carry genetic information (as in RNA viruses) but can also perform “non-canonical” functions, led to the hypothesis that RNA preceded proteins and DNA in a pre-biotic evolutionary phase dubbed the “RNA World ” (Robertson and Joyce 2012).

Research has revealed several instances of RNA sequences that might be heritages of the putative “RNA World”. Among these are riboswitches, non-coding traits of mRNA that bind specific metabolites and may affect gene expression at the transcription or translation level (Breaker 2010). Even more interesting in the present context are ribozymes, RNA sequences that perform enzymatic functions. The best-known example of a ribozyme is the peptidyl-transferase activity responsible for the formation of the peptide bond between the amino acid and elongating peptide during protein synthesis. This catalytic activity lies in the large ribosome subunit and depends on a region of rRNA known as the peptidyl transferase complex (PTC), with no protein directly participating in the process. With a turnover rate of 10-20 peptide bonds per second and an error frequency of 10^{-4}, the PTC has a catalytic efficiency comparable to that of many protein enzymes (Fox 2010; Moore and Steitz 2010).

A second example of ribozymes is from “self-splicing” sequences that catalyse their own excision from longer RNA filaments. These include type-I and type-II introns present in mRNA, tRNA and rRNA precursors in all three domains, as well as hairpin and hammerhead ribozymes common in viruses but also found in plants and animals (Woodson 2005; Ferré D’Amaré and Scott 2010; Lambowitz and Zimmerly 2011). A further type of ribozyme is RNA in spliceosomes, which directly participates in the excision of spliceosomal introns in precursors of messenger RNAs, a type of introns unique to eukaryotes (Will and Lührmann 2011). Mono and di-nucleotides such as ATP, GTP, coenzyme-A, NAD and FAD, which perform essential functions in all extant organisms, might be vestigial remnants of a “RNA World” (Yarus 2011).

Although RNA may have enzymatic properties, no RNA is known that can self-replicate autonomously, namely without the assistance of a protein enzyme (e.g. an RNA-dependent RNA polymerase). Several research groups have tried to produce self-replicating RNA molecules by natural selection. Random RNA sequences were copied through numerous replication cycles mediated by a protein RNA-dependent RNA polymerase; with time, many variant copies were produced due to the accumulation of copying errors. The products were tested at intervals and molecules with catalytic properties were selected for further cycles of replication and selection. This process led to the identification of RNA sequences that were able to function as an RNA ligase, i.e. to bind together short RNA sequences by phosphodiester bonds. A self-replicating system was assembled using two complementary ribozymes, each able to produce copies of the other by linking together two shorter sequences (Lincoln and Yoice 2009; Yoice 2009). More recently, an experimental protocol devised by Wochner and co-workers permitted the selection of a novel ribozyme family capable of accurately replicating templates with a length up to 206 nucleotides (Wochner et al. 2011; Attwater et al. 2013). These ribozymes, however, were extremely slow and depended on primers with a specific sequence; no instance of self-replicating RNA sequence has been reported to date.

Besides phosphoryl transfer (the cleavage or ligation of the RNA phosphodiester backbone) and peptide bond formation, the catalytic repertoire of known ribozymes includes RNA aminoacylation, a reaction analogous to amino acid activation by aminoacyl~tRNA synthetases (Chumachenko et al. 2009; Yarus 2011).

The RNA World hypothesis assumes that life started from RNA polymers. The possibility that primordial nucleic acids were not identical to RNA (“XNA”), and that RNA took over at a later stage has been considered (Schrum et al. 2010; Robertson and Joyce 2012). Interestingly, ribose and its 2’-deoxyribose analogue in DNA perform better than almost any other scaffolding molecules for supporting Watson-Crick pairing (Benner 2010). Moreover, ribose and deoxyribose appear to be particularly easy carbohydrates to synthesize prebiotically (Herschy et al. 2014).

A polymer required to perform catalysis as well as to carry biological information faces contrasting demands. In fact, the two functions are not easily coupled, because catalysis requires three-dimensional folding, whereas genetics requires the polymer not to fold to function as a template for its complement. In addition, an ancestral ribozyme replicase should have been able to replicate itself into a complementary filament and then copy this into the original, functional polymer. The co-existence of complementary filaments might have resulted into the formation of reverse double-stranded filaments scarcely prone to replication. In modern cells, this does not cause any difficulty, as the nucleic acid is either double-stranded or protected from hybridization by single-strand-binding proteins. Taylor (2006) suggests that the high temperatures postulated in hydrothermal/geothermal pre-biotic scenarios favoured the formation of parallel-stranded duplexes (made of filaments with the same 3’ to 5’ orientation) that were more prone to replication than antiparallel strands. RNA appears to be quite special among alternative polymeric systems in its ability to strike a balance between the contradicting needs of catalysis and genetics. Attempts at replacing the ribose-phosphate backbone in RNA with alternative backbones more plausible in a "prebiotic" scenario have shown that RNA performs much better than alternative polymers, thus supporting the RNA-first hypothesis (Neveu et al. 2013).

Why did life choose polymers with repeated negative charges on their backbone for storing genetic information? In general, even modest changes in the structure of an organic molecule significantly affect its physical properties. For transmitting and expressing genetic information, however, living systems need molecules that tolerate minor structural changes without significantly modifying their physical properties. The negative charges on the backbone of nucleic acids impart these polymers a strong polyanionic character that is barely affected by changes in the base sequence on which the biological properties closely depend. A repeating charge might be a general rule for any genetic molecule acting in water (Bennet 2010).

4. The Genetic Code

There could be no cell, however rudimentary, without a genetic code, so a code must have evolved at a pre-biotic stage. The origin of the genetic code is one of the most perplexing problems in evolutionary biology, so much so that little novel insight has emerged in the last decades, despite dramatic progress in molecular biology (Wolf and Koonin 2007; Koonin and Novozhilov 2009, 2017).

In living organisms, the amino acid sequence of proteins assembled by ribosomes mirrors the nucleotide sequence of messenger RNAs (mRNAs) under the rules of the 64-triplet code. The link is established by "transfer" RNAs (tRNAs) that function as adaptors. One of the most critical steps in this process is the binding of free amino acids at the CCA end of cognate tRNAs, namely tRNAs bearing a nucleotide triplet (anticodon) complementary to a triplet (codon) for the same amino acid. Aminoacylation of tRNA is catalysed by aminoacyl~tRNA synthetases, a family of enzymes with medium specificity (the rate of mis-aminoacylation is one in 10^4 cases, implying an average frequency of error of 0.03 amino acids for a peptide of 300 amino acids). The code is virtually universal among extant life forms and is therefore known as the *Standard Genetic Code* (SGC); although many deviations from the SGC exist, particularly in organelles and prokaryotes with small genomes, these are limited in scope and obviously secondary in origin (Maynard Smith and Szathmáry 1995; Koonin and Novozhilov 2017).

The design of the translation system in even the simplest modern cells is extremely complex. At the heart of the system is the ribosome, a large assemblage of at least three RNA molecules and 60-80 proteins arranged in a precise spatial architecture and interacting with other components of the translation system in a most finely choreographed fashion (Section 5). Other essential components include the complete set of tRNAs for the 20 amino acids (only about 40 tRNA species, due to the general occurrence of isoacceptor tRNAs), a set of aminoacyl~tRNA synthetases, and a complement of at least seven-eight translation factors (Smith et al. 2008; Fox 2010; Moore and Steitz 2010; Opron and Burton 2019).

Simpler, albeit less efficient solutions must have preceded such a hugely complex biosynthetic system. The observation that aminoacyl~CCA complexes (CCA is the three-nucleotide tail shared by all tRNAs) can participate in peptide bond formation in ribosomes suggests that protein synthesis began as a non-coded process and that the tRNA adaptors were a late addition introduced when the system was able to produce coupling enzymes (aminoacyl~tRNA synthetases) with the necessary specificity (Fox 2010). Maynard Smith and Szathmáry (1995) suggested that the ancestral aminoacyl~tRNA synthetases were ribozymes, later replaced by protein enzymes; this hypothesis received support from the observation that *in-vitro* evolved short RNA molecules were able to catalyse RNA aminoacylation (Chumachenko et al. 2009; Yarus 2011) and is consistent with recently proposed scenarios of ribosome and protein evolution (Sections 5 and 6).

The structure of the code is non-random and ensures high robustness to mutational and translational errors. For example, for most codons, the third base may be one of the two purines (adenine and guanine) or one of the two pyrimidines (uracil and cytosine) without changing the meaning. This suggests that the primordial genetic code was based on couples of nucleotides, which allowed for 16 possible codons (the number of combinations with repetition of four elements in groups of two is 4^2). Thus, the amino acids initially used in peptide synthesis might have been less than twenty, the rest having been co-opted later, when a more complex metabolic network had developed and the translation system had been refined. Ten amino acids are consistently produced in prebiotic chemistry experiments, in the following order of relative abundance: glycine, alanine, aspartic acid, glutamic acid, valine, serine, isoleucine, leucine, proline, tyrosine. This order reflects the free energies of their synthesis, the first being those thermodynamically more stable. The same amino acids, with the same relative abundances, also occur in meteorites. Several lines of evidence suggest that these ten amino acids are older than the others, in the sense that they were probably present in the first proteins whereas the others were not (Koonin and Novozhilov 2017).

With the addition of a third nucleotide, stereospecificity and the accuracy of synthesis increased, but the possible combinations became 64 (4^3). The sharp increase in the number of combinations available allowed the recruitment of novel amino acids. Because the number of amino acids deployed in protein synthesis was ancestrally set to twenty, the genetic code underwent “degeneracy”, with most amino acids being assigned two or more codons. Code degeneracy had the positive effect of alleviating the consequences of point mutations (mutations that substitute a single nucleotide for another). Increasing the number of encoded amino acids above twenty would have enhanced the diversity and structural versatility of peptides chains but probably would also have reduced the accuracy of translation. Setting the number to twenty was probably the optimal solution between the two opposite effects (Maynard Smith and Szathmáry 1995). Codes based on codons of four or more nucleotides require a greater level of degeneracy, therefore affording enhanced resilience to point mutations and greater translation accuracy, but arguably they would be slower and more expensive than shorter codification systems. A code based on 64 triplets succeeded as the best solution among a suite of alternatives tested by evolution, thus becoming universal.

The debate on the origin and evolution of the genetic code currently revolves around four competing perspectives. (i) The *stereochemical hypothesis* suggests that direct affinity between single amino acids and codons (or anticodons) played a pivotal role in primordial translation before being replaced by the extant indirect mechanism. (ii) The *coevolution model* proposes that the code structure coevolved with amino acid biosynthesis pathways. (iii) The *error minimization* scenario assumes that the code emerged from selection to minimize adverse effects of point mutations and translation errors. (iv) The *frozen accident* idea holds that the standard code has no special properties but was fixed simply because all extant life forms share a common ancestor, with subsequent re-assignment of codons generally precluded by deleterious effects on protein structure. These four perspectives are not mutually exclusive, thus adding further complexity to the problem. For example, mathematical analysis has shown that the SGC is more robust than approximately every million randomly chosen codes. Nevertheless, the SGC is far from being the best possible code: given the astronomical number of codes that are theoretically possible with three-base codons and four bases ($>10^{84}$), there are billions of variants more robust than the actual universal code. We can infer that, once attained a sufficient level of reliability, the SGC was irreversibly fixed in evolution, becoming in part a "frozen accident" (Koonin and Novozhilov 2009, 2017; Facchiano and Di Giulio 2018).

Modern rRNAs and tRNAs are chiral molecules containing D ribose, and during translation they work together to make chiral proteins exclusively with α-L-amino acids. This is highly advantageous to modern organisms because mixed chirality would interfere with self-organization of structural motifs such as α-helices and β-sheets, which are fundamental in modern proteins. Structural analysis of the ribosomal peptidyl transferase complex indicates that the chirality of the sugar ring in RNA is well paired with the choice of α-L-amino acids, this possibly being another instance of "frozen accident" (Fox 2010). As already observed, homochirality is not only a property of proteins but virtually applies to any biological compound that may exist in isomeric forms. In modern living systems, biological molecules are the products of enzyme-catalysed reactions, and homochirality is maintained by stereospecific interaction with the active site of enzymes. There is no reason to exclude that enzymes working with different stereoisomers of the same compound may appear by mutation. Mutant enzymes of this sort, however, are quickly eliminated by selection because their products cannot interact with other enzymes in the metabolic network. Was a similar constrain present in pre-biotic systems? The answer is "*probably yes*" because selection favoured the emergence of well-integrated metabolic networks. Initially, the choice between enzyme/substrate variants was probably random; yet selection had to choose one working isoform and eliminate the other(s) to avoid competition and futile cycles, thus making homochirality a general property of the living world. It is important to notice that this did not involve a sort of hindsight: alternative competing solutions disappeared because of lower efficiency or simply by chance.

5. Ribosome evolution and the roots of life

Modern ribosomes consist of small (SSU) and large (LSU) subunits that associate together during protein synthesis and separate again in conjunction with the release of the finished protein. Each subunit is an RNA/protein complex. In bacteria and archaea, the LSU typically contains a 23S rRNA and a 5S rRNA, whereas the SSU contains a 16S rRNA. In eukaryotes, the LSU contains three rRNA molecules (28, 5.8 and 5 S), the SSU contains a 23S rRNA. Associated with rRNAs are a number of ribosomal proteins (r-proteins). There are 31 and 21 r-proteins in the LSU and SSU of *E. coli*, and 49 and 33 in the LSU and SSU of mammals, respectively. Prokaryotic ribosomes have a molecular mass of about 2.5 MDa; eukaryotic ribosomes vary around 4.5 MDa, most of the variability being due to LSU sizes. The secondary structure of the LSU rRNA reveals the presence of six domains named with the roman numbers *I* to *VI*, with the PTC being located in Domain V. The SSU rRNA is comprised of four domains named with roman numbers *I* to *IV* or, based on the position, *3'm, 3'M, C* and *5'*. The SSU domains can be assembled independently, whereas the LSU is monolythic. Cells contain from thousands to millions of ribosomes, depending on their sizes and metabolic activity, ribosomal mass typically accounting for about one third of cellular dry mass. Because of this, rRNAs and r-proteins are the most abundant macromolecules in the biological world (Bowman et al. 2020).

The functional core of the SSU is the decoding centre (DCC) and the functional core of the LSU is the peptidyl transferase centre (PTC). The DCC recognizes codons on mRNA by coupling them with the cognate tRNA. The PTC catalyses peptide bond synthesis and forms a nanopore that permits the elongating peptide to enter an exit tunnel extending through the LSU and ultimately be released from the ribosome. Every coded protein ever produced by life on Earth has passed through the exit tunnel, which could therefore be viewed as the "birth canal of biology" (Bowman et al. 2020). The LSU is able to catalyse peptide bond formation in vitro, in the absence of the SSU. For further details on ribosome functioning, see Opron and Burton (2019).

Prokaryotic and eukaryotic ribosomes share a common structural core made of parts belonging to both the LSU and SSU. The common core comprises 34 conserved proteins and about 4,400 RNA bases, and harbours the major functional sites, i.e. the PTC, the tRNA-binding sites and the decoding site. Superimposed to the common core is a set of moieties specific to each domain: expansion segments of rRNAs, domain-specific r-proteins, and insertions and extensions of shared r-proteins. Interaction between ribosomal subunits depends on several contact points at the interface, called bridges (Melnikov et al. 2012).

Ribosomal RNA and r-proteins have been sequenced in an expanding range of organisms. Concurrently, x-ray crystallography and cryo-electron microscopy have provided atomic-resolution structures of ribosomes in representatives of all three domains of life. Integrated analysis of the data has produced a wealth of novel insight (Fox 2010; Melnikov 2012; Petrov et al 2014, 2015; Bernier et al. 2018; Bowman et al. 2020).

Of special interest in the present context are the following points.

- The common core, encompassing 2800 nucleotides, 19 LSU r-proteins and 15 SSU r-proteins to a total mass of nearly 2 million Daltons, is conserved over the entire tree of life in sequence and especially in three-dimensional structure.
- The common core was finalized around 3.8-4.2 GYA, preceding the emergence of LUCA.
- Bacterial ribosomes are almost entirely composed of the common core (around 90% of total rRNA), with only minor deviations between different lineages, suggesting that the rRNA of bacteria has remained essentially static in size from divergence from LUCA
- Archaeal ribosomes are slightly larger and more variable than bacterial ribosomes. For example, the LSU rRNA of *P. furiosus* is 248 nucleotides larger than the common core rRNA. The largest archaeal rRNA expansions are found in some Asgards.
- Eukaryotic ribosomes have expanded much beyond the common core by addition of novel rRNA segments and novel r-proteins, most of which lie at the periphery of the ribosome.
- The common core rRNAs of bacterial and archaeal ribosomes are most similar, followed by those of archaeal and eukaryotic ribosomes. The common cores of bacterial and eukaryotic ribosomes are the most divergent.

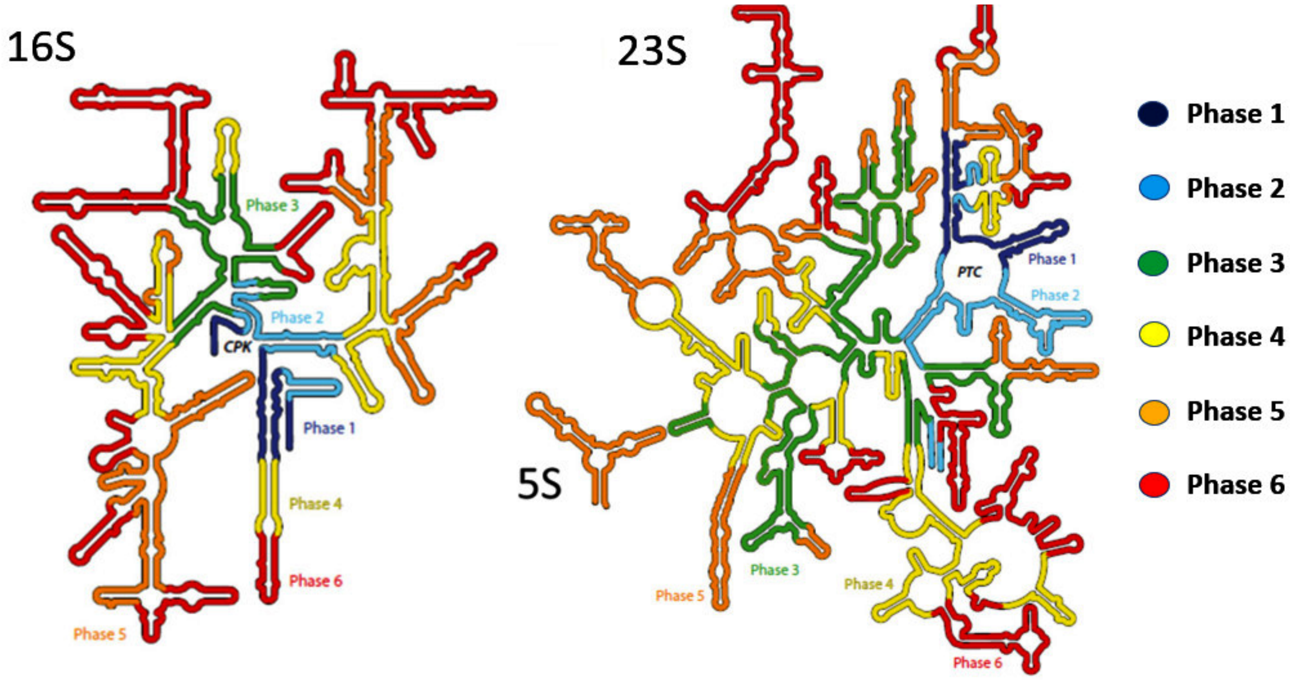

Figure 3: Six-phase accretion model mapped on small- and large-subunit rRNAs of the bacterium Escherichia coli. 16S and 23S rRNAs evolved by accretion of "expansion segments" to originally much smaller molecules (dark-blue segments). The small and large ribosome subunit evolved independently from phase 1 to phase 3 and started working cooperatively in phase 4. The 5S rRNA was recruited in the large subunit in phase 5. The ribosomal core attained the final size before the appearance of LUCA. Modified from Petrov et al. (2015) under conditions granted by PNAS licence to publish.

- rRNA expansion segments have been added without perturbing the molecular architecture of the pre-existing core.
- rRNA size in eukaryotes generally increases with organismal complexity.

Comparative analysis of two- and three-dimensional reconstructions of LSU and SSU rRNAs from prokaryotes and eukaryotes has enabled researchers to infer the order of addition of individual RNA segments and create a hierarchical map of rRNA structure. The evidence thus obtained suggests that ribosome evolution has proceeded by superimposing new layers over pre-existing parts, building up an onion-like molecular edifice made of an ancient core and progressively more recent parts outwardly. LUCA's ribosomes were most likely not radically different from ribosomes in present-living prokaryotes. Early in eukaryote evolution, ribosomes acquired an extra shell of eukaryote-specific rRNA extensions and r-proteins. Further ribosome elaboration occurred in complex animals, with the addition of tentacle-like rRNA projections (Bokov and Steinberg 2009; Petrov et al. 2014, 2015; Bowman et al. 2020).

Petrov et al. (2015) identified six major phases in ribosome evolution from the beginning to the emergence of the first cells (Fig. 3).

- **Phase 1:** *appearance of self-folding RNA oligomers* forming a double-helix stem and a single-strand loop. Folding made RNA molecules less prone to chemical degradation, thus elongating their average life, and favoured the emergence of catalytic properties.
- **Phase 2:** *emergence of RNA replicase ribozymes and of ancestral forms of LSU and SSU rRNA, tRNA* and *mRNA*. The emergence of ribozymes with RNA replicase activity not only ensured the maintenance of a steady RNA population, replacing RNA lost by degradation or leakage, but also gave the start to Darwinian selection for more efficient self-replicating ribozymes, The primordial LSU rRNA was able to catalyse peptide bond formation, producing uncoded oligopeptides. Primordial mRNA was a random population of single-stranded oligomers. Primordial tRNAs consisted of a simple CCA tail and subsequently acquired the double-helix amino acid acceptor stem.
- **Phase 3:** *SSU and peptide recruitment into pre-proto-ribosomes*. The LSU expanded to form a short exit tunnel and an embryonic subunit interface, by which it bound to SSU, which therefore became a component of the emerging peptide-synthesizing complex. LSU–SSU association was mediated by interaction with proto-mRNAs and tRNAs. The proto-tRNA minihelix was extended by an insertion to form prototypes of modern L-shaped tRNAs. Catalytic efficiency of the PTC and product length increased. Uncoded peptides started being incorporated into pre-proto-ribosomes. Darwinian selection started operating on proto-rRNAs, proto-tRNAs, and proto-mRNAs in a coordinated way.
- **Phase 4**: *birth of proto-ribosomes*. A primitive genetic code started coupling RNA and peptide evolution (Sections 3 and 4). The evolutionary trajectories of LSU, SSU, proto-mRNAs, and proto-tRNAs were more strongly integrated. The addition of novel RNA segments expanded the subunit interface, elongated the exit tunnel, and formed well-defined pockets for tRNA binding; proto-tRNAs

were optimized to form base-pair triplets with proto-mRNAs, which remained a population of single-stranded oligomers.

- **Phase 5**: *addition of a GTP-dependent ratcheting system* responsible for the coordinated movement of the ribosome and mRNA. This innovation marked the transition from a catalytic system driving spontaneous reactions to a decoding machine driving coupled reactions. The genetic code improved.
- **Phase 6**: *the rRNA common core was finalized and the genetic code froze in the modern form*. The addition of novel protein-binding RNA segments expanded the set of ribosomal proteins.

The SSU rRNA might have derived from a pre-existing RNA replicase ribozyme that was secondarily co-opted in peptide synthesis (Smith et al. 2008; Fox 2010). This hypothesis gains support from evidence suggesting that large and small subunit rRNAs initially evolved independently (Petrov et al. 2014, 2015). An ancestral RNA replicase activity of the small subunit rRNA is consistent with the fact that here lies the decoding site responsible for codon-anticodon interaction in modern ribosomes. A hybrid dimer, half RNA replicase and half peptide polymerase, could perform mRNA translocation before the advent of protein elongation factors (Taylor 2006). Putative ribozymes extinguished, or were reduced to simple cofactors such as NAD, FAD and CoA, when more efficient protein enzymes replaced them at a later stage of evolution. With the emergence of a genetic code, mRNAs that had passed initial selection aggregated into longer molecules, thus reducing the rate of dispersion of functional sequences. Association of these enlarged RNAs with peptides might have produced virus-like "protochromosomes" that on phase 5 took control of peptide synthesis and of their own replication, thus establishing a separation between catalytic and informational functions.

Analysis of the six reading frames in the rRNA of the bacterium *Escherichia coli* (three in the 5′ to 3′ direction, and three in the opposite direction) has demonstrated the occurrence of segments with high sequence homology with tRNAs for all twenty amino acids (Root-Bernstein and Root-Bernstein 2015). The entire set of tRNAs is represented in the 16S rRNA, whereas 23S rRNA directly encodes only six tRNAs. Its complementary sequence, however, contain segments with high levels of homology with all tRNAs. This result points to a strong evolutionary link between rRNA and tRNA, although there is no clue to establish whether tRNAs derived from rRNA or vice versa. The same research also showed that rRNA contains sequences encoding for segments of ribosomal proteins and of proteins involved in ribosomal function such as RNA polymerase and amino acyl-tRNA synthetases, thus suggesting the existence of an ancestral link also between rRNA and mRNA.

The transition from short oligonucleotides to functional ribosomes working under the rules of a fully developed genetic code (phase 1 to 6) most likely covered a long time interval. This does not easily conciliate with "primordial-soup" scenarios linked to transient sets such as volcanic eruptions, lightning, meteoritic impacts. Indeed, the same also applies to the alkaline vent hypothesis, the average lifetime of single vents in the order of 10^4 years being probably insufficient to foster such a complex succession of events. The problem might be easier to handle under the assumption that RNA-peptide complexes were able to migrate and "colonize" new favourable locations, thus substantially elongating their evolutionary trajectory. In the alkaline vent scenario, for example, populations of RNA-peptide complexes bound to mineral fragments might have moved from old to young vents following ocean currents. On the other hand, terrestrial geothermal fields like those considered as the possible cradle of life by Mulkidjanian et al. (2012) may remain active for several million years independently of local climatic conditions. In addition, geothermal pools in the same field may exchange materials through rains or overflowing, thus increasing the chances for evolution. It should also be considered that, in a lifeless world, organic molecules probably had longer lifespans than today, at least in protected environments, being not subject to biological decomposition.

6. How did life discover folding peptides in a universe of unstructured sequences?

Nucleic acids and proteins must assume defined three-dimensional structures for biological activity, yet their ability to do so is starkly different. Nucleic acids fold spontaneously, based primarily on simple base-pairing rules, and can in general be denatured and renatured reversibly by chemical agents or warming/cooling cycles. Protein folding into complex architectures is much more a delicate process subject to severe constraints. For example, secondary folding into structures such as α-helices and β-sheets requires the regular formation of hydrogen bonds between the amino hydrogen and carboxyl oxygen atoms in the peptide backbone, which is possible only under certain conditions. Likewise, peptide folding into tertiary structures depends on the formation of a hydrophobic core isolated within a shell of secondary structures. Proteins require stable folds, robust to environmental fluctuations, but they must also find solutions that are flexible enough for allostery and complex interactions. This is not an easy task. The number of sequence possibilities for a random polypeptide chain exceeds the number of atoms in the known universe already at a chain length of around 60 residues. Yet only an extremely small fraction of these sequences exhibit folding competence, thus precluding the possibility for proteins to evolve by random substitution of single amino acids. Biological proteins, despite their apparent limitless diversity, are in fact combinations of "only" about 10,000 basic domains, indicating that the main mechanism underpinning protein evolution is domain shuffling rather than elaboration of novel sequences. Although relatively numerous, however, these domains still account for only an infinitesimal fraction of possible peptide sequences. How did evolution manage to find folding motifs in an almost limitless universe of unstructured sequences?

Combined sequence and structural analysis of a diversity of proteins across the whole spectrum of life has permitted the discovery of a much more circumscribed number of structural motifs (possibly around 100) with an average length of 24 amino acids, which are universally spread in nature and particularly abundant in iron-sulfur- and nucleic acid-binding proteins. It has been suggested that these motifs emerged in a pre-biotic RNA-Peptide World and were fixed in evolution as structural subunits of protein domains (Alva et al. 2014).

Because of its onion-like structure, the ribosome provides a window that looks back to the very beginning of peptide evolution. Molecular dissection of prokaryotic ribosomes has revealed that the inner sphere with a radius of 20 Å from the geometrical centre contains no protein structure. Because this area coincides with the peptidyl transferase ribozyme, the absence of protein is viewed as a legacy from the putative RNA World. The area immediately outside, 20 to 50 Å from the centre, contains few r-protein segments that form random coils with little or no secondary structures. The ribosomal sphere between 50 and 70 Å from the centre is more protein-rich; moreover, the protein chains located here have an increased content of secondary structures. Ribosomal proteins located between 70 and 90 Å from the centre are organized into secondary structures that may also form super secondary arrangements (https://en.wikipedia.org/wiki/Supersecondary_structure). These proteins, however, still lack a tertiary structure. Globular proteins resembling cytoplasmic proteins are only found in the more peripheral shell of the ribosome. When these data are mapped against the relative ages of rRNA, they provide a reconstruction of natural protein evolution (Lupas and Alva 2017; Kovacs et al. 2017). The first abiotic peptides, either formed by spontaneous polymerization or produced by RNA ribozymes without a genetic code, had random sequences and were most likely unstructured. Peptide association with RNA probably increased the chemical stability of both polymers. Besides using non-specific acid base interactions, peptides may stereospecifically bind to RNA by hydrogen bonds. Specific binding to folded RNA may have guided, and even forced unstructured peptides into more complex conformations with novel properties. This might have favoured the emergence of a primordial code and most likely exerted a strong selection pressure for better-structured peptides after the code was established. The broad diversity of protein domains in nature might have evolved by accretion of a limited number of motifs that emerged in ribosomal proteins in response to interaction with rRNA. At some stage, this led to the appearance of proteins no longer dependent on RNA for folding, which therefore could work outside of the ribosome. In this perspective, cytoplasmic proteins might be viewed as the outermost shell built by ribosomes under selection pressure for more effective self-replication (Alva et al. 2015; Lupas and Alva 2017).

The appearance of complex three-dimensional protein architectures permitted the evolution of a diversity of enzymes with superior catalytic properties, which replaced pre-existing analogous ribozymes. The emergence of protein enzymes paved the way to two further innovations needed for making a cell: a self-replicating bounding membrane and an autonomous metabolism.

7. From self-assembling bilayers to encoded membranes

Far from being a simple bag for living matter, the cell membrane is a highly selective barrier that controls the inflow and outflow of a diversity of molecules. Most importantly, the cell membrane can build electrochemical gradients and use them for making ATP or for transporting solutes against their electrochemical gradient. Because of this, any attempt at reconstructing the evolution of life should address not only the emergence of a genetic system and a metabolism, but also the origin of cell membranes.

The basic components of membranes are amphipathic lipids, molecules consisting of a hydrophilic (polar) and hydrophobic (non-polar) part. Interacting with water, these molecules spontaneously arrange into bilayers that isolate the hydrophobic parts inside, exposing the hydrophilic parts on either side (Chen and Walde 2010). Cells do not make new membrane by assembling together single molecules from naught, as a bricklayer does in building a wall. The cells can only expand pre-existing membranes by adding new molecules one by one into the pre-existing framework, and then pinch off them to form separate compartments. This is the way membranes are transmitted to daughter cells during cell division. Thus, much like chromosomes, biological membranes are hereditary structures (Cavalier-Smith 2001, 2004). If membranes are essential to life and cells can make them only from pre-existing membranes, how did membranes evolve?

Supporters of the alkaline vent scenario suggest that at an early stage of pre-cellular evolution, peptides of abiotic origin bound to mineral microcompartment walls and modified their properties. Under the repulsive force of water, peptide-mineral pellicles might have incorporated amphipathic lipids, isolating their hydrophobic tails from water circulating on either side. This model receives support from the observation that the addition of peptides and RNA to the solution circulating in a simulated hydrothermal vent modified the elemental stoichiometry of the chimneys, perhaps epitomizing the putative takeover of abiotic metabolism by organic polymers (McGlynn et al. 2012). It has been suggested that organic-mineral protomembranes were able to harness chemiosmotic energy of geochemical origin (Lane and Martin. 2012; Russell et al. 2013; Sousa et al. 2013). Ancestral membranes did not require lipids as complex as those forming modern membranes, simple long-chain organic acids or monoglycerides being more likely precursors in a prebiotic world. Peptides with a hydrophobic tail of glycine residues and a hydrophilic head of aspartic acid are also able to form stable bilayers in an aqueous environment subject to wet/dry cycles (Smith et al. 2008; Mulkidjanian et al. 2009; Mansy 2010; Schrum et al. 2010; West et al. 2017).

To generate self-replicating protocells, ancestral membranes needed three further innovations: (i) a stable association with cognate (viz. informationally interlinked) protoribosomes and protochromosomes; (ii) mechanisms mediating the incorporation of amphiphilic peptides and lipids for membrane growth; (iii) a chemiosmotic machinery (Section 8). The evolution of the Sec protein insertion machinery (du Plessis et al. 2011; Nyathi et al. 2013) and integral lipid-synthesising complexes converted protomembranes into "encoded membranes" (Lane and Martin 2012), hereditary structures vertically transmitted during replication.

Phylogenomic analysis indicates that LUCA had a bounding membrane containing all fundamental components of modern membranes (Jekely 2006), thus the innovations outlined above probably predated LUCA. Reiterating Virchow's classic aphorism "omnis cellula e cellula" (every cell from a cell), cell biologist Günter Blobel (Blobel 1980) wrote "omnis membrana e membrana", every membrane from a membrane. Because life is almost certainly monophyletic, this implies that all membranes existing today, however modified and specialized, arise from one ancestral membrane. Yet, because of major divergences in membrane architecture between the archaea and all the other extant organisms, some researchers have proposed a dual origin (Section 10).

8. Early metabolic pathways

Metabolism (from Greek metabolè, change) is the set of life-sustaining chemical transformations that enable living organisms to produce building materials and to obtain energy. It is generally agreed that a primitive form of metabolism preceded the emergence of life. Among the scenarios proposed, however, only the submarine hydrothermal model analyses in some extent the likely mechanisms involved. Active oceanic vents present today on the Earth are rich with life, thus it is extremely difficult to verify the existence of abiotic sources of organic compounds. Radiometric investigation in the field, data from artificial systems and theoretical models suggests that reductants of geochemical origin such as hydrogen, hydrogen sulphide and ferrous iron, could spontaneously react with carbon dioxide, producing a diversity of organic molecules (Proskurowski et al. 2008; Lang et al. 2010). Moreover, experimental evidence suggests that, in the conditions presumably existing in alkaline vents in the Hadean Earth, the pH gradient across mineral pellicles supported the synthesis of energy-rich molecules such as pyrophosphate ($HP_2O_7^{-3}$) and trimetaphosfate ($H_3P_3O_{10}^{-3}$). These compounds could accumulate in pre-biotic microcompartments by thermophoresis (Baaske et al. 2007) and/or by binding to mineral surfaces (Martin and Russell 2007; Russell et al. 2013; Sojo et al. 2016). Proponents of the terrestrial geothermal model assume similar properties for their geochemical scenario, with wet/dry cycles driving thermodynamically unfavoured reactions (Mulkidjanian et al. 2012).

In a context characterized by the presence of reactive chemical species and stable sources of disequilibrium, ribozymes could work co-operatively with peptides and inorganic catalysers, generating metabolic networks of increasing complexity. With time, proteins took on enzymatic functions, whilst RNA role as a catalyser progressively lessened. The replacement of ancestral ribozymes with limited catalytic performances by more versatile protein enzymes vastly increased the diversity, abundance and complexity of the proto-biotic organic pool. Catalytic metals possibly played a major role in this fundamental transition. The suite of metal-enzymes in extant organisms, such as ferredoxin (iron-sulphur), cytochromes (iron), plastocyanin (copper), carbonic anhydrase (zinc), nitrogenase (molybdenum), urease and hydrogenase (nickel), are probably modern descendants of pre-biotic inorganic catalysers (Nitschke et al. 2013).

A fundamental step towards the emergence of an autonomous metabolism was the appearance of a form of bioenergetics, namely a mechanism that that utilized environmental disequilibria to make ATP or its likely precursor acetyl-phosphate (Martin and Russell 2007). Modern cells have two ways of producing ATP. The first is substrate-level phosphorylation (SLP), the second is chemiosmosis. SLP directly couples exergonic redox reactions to ATP synthesis. As an example, the oxidation of 3-phosphoglyceraldehyde to 3-phosphoglycerate and subsequent conversion of the latter to pyruvate are sequentially coupled to the synthesis of two ATP molecules. Chemiosmosis instead couples redox reactions to the building of an electrochemical gradient across a membrane, generally redistributing protons. Proton flow down their electrochemical gradient is coupled to ATP synthesis by ATP synthase, an enzymatic complex inserted within the membrane (Schoepp-Cothenet et al. 2013). SLP is a biochemically simple and extremely fast way of making ATP; in contrast, chemiosmosis is a relatively complex and slow process, yet it is much more efficient that SLP in extracting energy from reactions including those with extremely low energy yields.

It is debated whether early life was heterotrophic or autotrophic. Primordial-soup models favour a heterotrophic early life, whereas the submarine vent hypothesis points to a chemioautotrophic ancestral metabolism (Martin and Sousa 2016). If heterotrophic, early life most likely employed SLP to produce ATP; if autotrophic, instead, it probably employed chemiosmosis. Having detected no enzymes involved in autotrophic pathways in a set of universal proteins, Mulkidjanian et al. (2012) assumed that early life was heterotrophic, probably obtaining energy from anaerobic oxidation of organic compounds of abiotic origin. By analogy with metabolic pathways in extant acetogenic bacteria and methanogenic archaea, supporters of the submarine scenario suggest that early life used hydrogen of geochemical origin to reduce carbon dioxide to acetate (eq. 1) or methane (eq. 2) through the acetyl-CoA (or Wood-Ljungdahl) pathway (Lane and Martin 2012; Sousa et al. 2013; Schönheit et al. 2016).

$$\mathbf{8H_2 + 4HCO_3^- + 2H^+ \rightarrow 2CH_3COO^- + 8H_2O}$$

(eq. 1)

$$\mathbf{4H_2 + CO_2 \rightarrow CH_4 + 2H_2O}$$

(eq. 2)

A deep ancestry of the acetyl-CoA pathway is supported by phylogenetic analysis of the enzymes involved, such as the bifunctional enzyme carbon monoxide dehydrogenase/acetyl-CoA synthase reducing CO_2 to CO, the molybdo/tungstopterin proteins reducing CO_2 to a formyl moiety, and [NiFe] hydrogenases extracting electrons from hydrogen (Sousa et al. 2013; Schoepp-Cothenet et al. 2013; Weiss et al. 2016, 2018).

Alternatively, Russell and Nitsche (2017) proposed that early life could make a live from the oxidation of abiotic methane from serpentinization (Section 2), using nitrogen oxides (eq. 3) or ferric ions (eq. 4) as electron acceptors. Putative methanotrophic life could use metabolic intermediates of methanotrophy as a source of organic carbon.

$$\mathbf{3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 6H_2O + 4N_2}$$

(eq. 3)

$$\mathbf{CH_4 + 8Fe^{+3} + 2H_2O \rightarrow CO_2 + 8Fe^{+2} + 8H^+}$$

(eq. 4)

Paleogeochemical inference suggests that the reaction of atmospheric molecular nitrogen with carbon dioxide under the effect of lightning or meteorite impacts could generate substantial amounts of nitrogen oxides despite the lack of free oxygen on the Hadean Earth (Ducluzeau et al. 2009; Wong et al. 2017). Likewise, photo-oxidation of ferrous iron (Fe^{+2}) by solar ultraviolet could produce ferric iron (Fe^{+3}) that precipitated to the ocean bottom, becoming accessible to early life for redox processes (Nitschke and Russell 2013; Russell et al. 2013).

9. DNA recruitment gave the start to modern biology

A network of entangled metabolic pathways and the genetic system evolved together. Evolutionary pressure for novel and more efficient protein catalysts promoted the diversification of RNA templates, or "replicators". The persistence of numerous independent replicators enhanced recombination, as occurs still today in some viruses, but also increased the risk that cooperative sequences were separated and potential synergies dispersed (Maynard Smith and Szathmáry 1995). Aggregation of smaller replicators into larger molecules was positively selected because it favoured (a) the synthesis of novel, larger and more versatile peptides, and (b) coordinate expression, replication, and transmission of functionally related sequences (Koonin and Martin 2005). These expanded replicators might have been similar to negative-strand RNA viruses, consisting of a long peptide-bound RNA filament functioning as a template for more transient mRNAs.

At a point, the replicators attained sizes that raised problems of instability: being the single-strand RNA filament highly flexible, it tended to spontaneously break and/or to fold into structures that interfered with transcription, translation or replication. Thanks to its double-helix structure and the absence of the oxygen atom in the position 2 of the sugar (deoxyribose, instead of ribose), DNA is much more stable that RNA. A further advantage of the double helix structure is that the two strands carry the same information, yet only one is decoded, the other being used for correcting errors during replication, thus affording the genetic system enhanced stability. DNA is only one of several possible forms of double-strand nucleic acid that could have evolved in a RNA-based biological world. The fact that all extant life uses DNA implies that the transition from a single-strand to double-strand nucleic acid occurred only once or, more likely, that only the contemporary DNA form survived early selection (Benner 2010).

As for other major innovations, the transition from RNA to DNA occurred gradually, for example first utilizing double-filament RNAs for information storage and replication, and single filament RNAs for expression, and then separating the two functions completely with the use of different nucleotide precursors: deoxyribonucleotides for double-filament replicators and ribonucleotides for single filament "messengers". The separation also involved replacement of uracil with thymine in the replicator, thus reducing the risk of mutation due to the tendency of cytosine to convert into uracil by deamination; a misplaced uracil cannot be recognized in RNA, whereas it can be pinpointed as an alien base in DNA and removed by repair systems (Forterre et al. 2004).

RNA replacement by DNA as the repository of biological information established the genotype-ribotype-phenotype tripartite organization typical of modern cells. This transition increased the efficiency of storage, replication and transmission of genetic information and activated a strong evolutionary pressure towards functional aggregation of genes. Less "co-operative" genes were unavoidably suppressed, favouring the emergence of highly integrated gene communities, or genomes. Gene aggregation into large genomes enhanced combinatorial sequence shuffling, fostering the emergence of an expanding diversity of multidomain proteins with novel biological functions (Alva and Lupas 2018). Surprisingly, the DNA polymerases involved in DNA replication in bacteria, archaea, and eukaryotes display no appreciable homology. The apparent diversity of the replication machineries among the three Domains sharply contrasts with the conservation of proteins involved in transcription and translation. The lack of homology among DNA polymerases in the three lineages precludes the reconstruction of the ancestral state, suggesting multiple origins for DNA replication and even the possibility that LUCA was an RNA-based cell. The discovery of sequence homology between the catalytic core of the archaeal DNA polymerase PolD and that of the large subunit of the RNA polymerases responsible for DNA transcription in all three Domains fostered a more parsimonious scenario. Koonin et al. (2020) suggested that RNA polymerases and replicative DNA polymerases in the three Domains evolved from a common ancestor that functioned as an RNA-dependent RNA polymerase in the RNA-protein world before the advent of DNA.

10. The membrane gap: ancestral or derived?

Barring the prokaryote/eukaryote divide, the largest gap in the biological world is in the molecular architecture of membranes. The two gaps do not coincide, the latter setting the bacteria and eukaryotes into an assemblage separate from the archaea. In all three domains of life, the main membrane lipids are glycerophospholipids, amphipathic molecules made of glycerol bound to two long hydrophobic "tails" and to a phosphate group. Apart from this commonality, glycerophospholipids could not be more different in the two assemblages (Fig. 4). In bacteria and eukaryotes, glycerol is in the L form, the hydrophobic tails are linear aliphatic chains bound to C1 and C2 of glycerol by ester bonds, and phosphate is bound at the C3 position. In the archaea, glycerol is in the D form, the hydrophobic molecules are isoprenoid chains bound at C2 and C3 positions by ether bonds, and phosphate is bound at the C1 position (Shimada and Yamagishi 2011).

Isoprenoid chains are produced by the mevalonate (MVA) pathway in eukaryotes and a modified form of the same in archaea (Boucher et al. 2004), whereas the bacteria mostly employ the non-homologous methylerythritol phosphate (MEP) pathway. Photosynthetic eukaryotes acquired the MEP pathway through the chloroplast endosymbiosis. The occurrence of the MVA pathway in some bacteria has traditionally been interpreted as a trait acquired by horizontal gene transfer from an eukaryotic or archaeal donor. At odd with this claim, more recent phylogenomic evidence suggests that the MVA pathway ancestrally occurs in all three domains and was probably present in the last common ancestor (Lombard and Moreira 2011).

The evolutionary significance of the membrane divide between G3P lipid stereochemistry in bacteria and eukaryotes

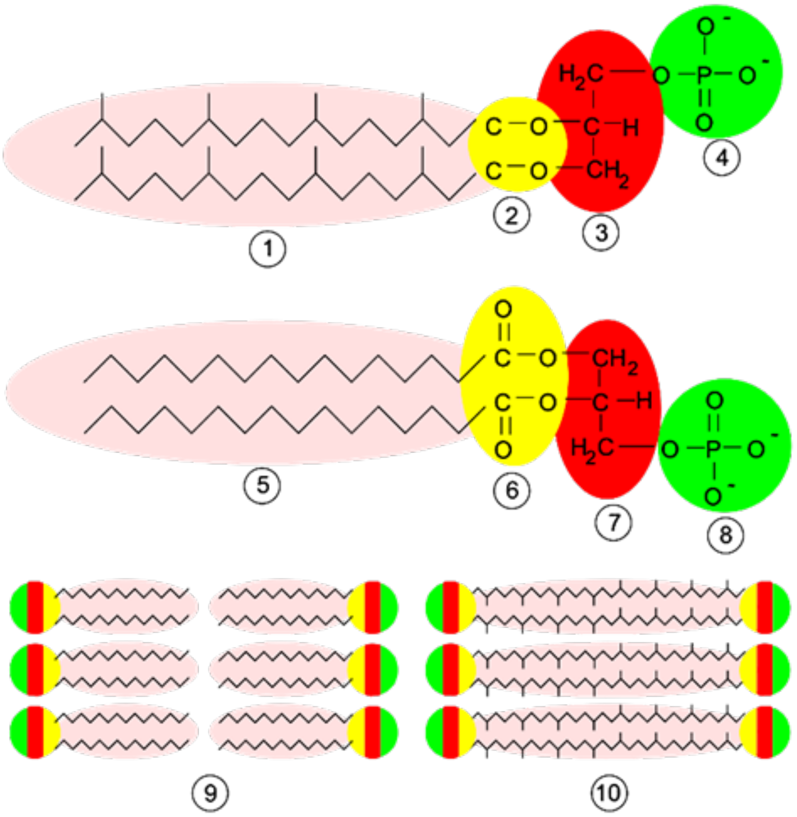

Figure 4: (1-5) Glycerolipid structure in the archaea: (1) isoprenoid chains, (2) ether bonds, (3) D-glycerol, (4) phosphate group. The isoprenoid chains are bound to glycerol at the C2 and C3 positions by ether bonds, phosphate is bound at the C1 position. (5-8) Glycerolipid structure in bacteria and eukaryotes. (5) Linear fatty acid chains, (6) ester bonds, (7) L-glycerol, (8) phosphate. The fatty acid molecules are bound to glycerol at C1 and C2 positions by ester bonds, phosphate is bound at the C3 position. (9) Bilayered membrane organization as typical of bacteria and eukaryotes. (10) Unilayered membrane of tetraether lipids in hyperthermophilic archaea. Non-hyperthermophylic archaea have bilayered membranes as in bacteria and eukaryotes, but their glycerolipids have archaeal stereochemistry. From: https://commons.wikimedia.org/wiki/File:Archaea_membrane.svg

and G1P in archaea is controversial. The current mainstream debate is focused on two competing scenarios. The first holds that the bacteria and archaea diverged from a common ancestor that either lacked true membranes (Martin and Russell 2003, 2007) or possessed heterochiral membranes made of both G3P and G1P lipids (Wächtershäuser 2003; Peretó et al. 2004; Lombard 2012; Koga 2014). After splitting, the bacteria evolved or retained a G3P membrane stereochemistry, whereas the archaea evolved or retained a G1P stereochemistry, the choice depending on adaption to different environmental conditions. Under this scenario, the eukaryotes derived by the symbiosis of an archaeon and a bacterium, the latter having been converted into the mitochondrion (Lòpez-Garcìa and Moreira 2019; Spahn et al. 2019). It is proposed that cellular integration of the two symbionts involved the loss of the archaeal G1P lipid biosynthetic machinery and its replacement with the symbiont's G3P machinery, thus explaining why eukaryotes have bacterial-type G3P membranes. Importantly, the Sec protein translocon is universal in the three domains, indicating that the last common ancestor did have a true membrane (Jekely 2006). The hypothesis of a heterochiral common ancestor for archaea and bacteria receives support from the demonstration that vesicles (liposomes) made of heterochiral membranes not only are stable, but are even more resistant to high temperature (in terms of proton permeability) than homochiral G3P vesicles (Shimada and Yamagishi 2011). Caforio et al (2018) have demonstrated that the same also applies to living cells. They engineered a strain of the bacterium *Escherichia coli* to produce archaeal lipids in addition to its normal lipids; the transformed cells produced hybrid membranes made of lipids of ether-linked isoprenoids and ester-linked fatty acids bound to a G1P or a G3P backbone, respectively. The cells were perfectly viable, with growth rates comparable to the wild type, and showed a higher tolerance to heat treatment compared with control strains. Overexpression of archaeal lipids above 30% of the total lipid content, however, was associated with disturbances including the release of membranous vesicles in the growth medium and asymmetric cell division.

The alternative scenario, sponsored by Forterre (2013) and Cavalier-Smith (2014), posits that the three Domains derive from a common ancestor with G3P stereochemistry. In this perspective, the bacteria and eukaryotes retained ancestral G3P membranes, whereas the archaea secondarily evolved G1P membranes by adapting to hyperthermal acidic habitats. Like the competing hypothesis, this scenario implies an intermediate phase in which G3P and G1P lipids co-existed but provides a strong selection pressure to explain the transition.

The archaeal domain typically encompasses hyperthermophilic and acidophilic forms with optimum temperature above 80 °C and optimum pH below 3, yet mesophilic (optimum temperature in the 20-45 °C range) and even psychrophilic archaea (-20 to +10 °C) are also known. The paradox is only apparent, as archaeal membranes are stable at high temperature but remain fluid and functional also at low temperatures. A unique trait of hyperthermophilic archaea is that their membranes consist of only one layer of bipolar lipids with a tetraether structure (Fig. 4.10). These special membranes are more stable and less permeable to protons that two-layered membranes in conditions of high temperature and low pH. Molecular evidence points to hyperthermophyly and underpinning tetraether G1P membranes as the ancestral condition in archaea (Gribaldo et al. 2006). This suggests that two-layered membranes secondarily evolved in derived archaeal lineages that lost tetraether lipids by adapting to less extreme habitats but retained the ancestral isoprenoid G1P stereochemistry (Cavalier-Smith 2014). This scenario has received support from recent phylogenomic work (Williams et al. 2017).

Hyperthermophyly also occurs among bacteria, and at least two bacterial lineages (the Thermotogales and Aquificales) possess tetraether lipids; these lipids, however, have G3P stereochemistry and are made with non-isoprenoid fatty chains, thus they most likely evolved independently of archaeal tetraether lipids (Schouten et al. 2007; Glansdorff et al. 2008). Hyperthermophyly has been suggested to be an ancestral trait of the Bacteria domain, possibly inherited from LUCA (Di Giulio 2003). Phylogenetic analysis by Lake et al. (2009) does not support a hyperthermophilic root of the tree of life. A phylogenetic analysis based on ribosomal proteins resolves the Thermotogales and Aquificales as sister groups and places them in a derived position in the bacterial tree (Yutin et al 2012). In contrast, a more recent analysis based on a large dataset from metagenomic sequencing places the Thermotogales and Aquificales in a basal position, thus supporting the view that hyperthermophyly is an ancient trait in bacteria (Schulz et al. 2017). Some archaea possess a G3P dehydrogenase besides a G1P dehydrogenase, whereas some bacteria have a G1P besides G3P dehydrogenase, both occurrences being interpreted as the likely outcome of horizontal gene transfer (Peretó et al. 2004). The Firmicutes, a gram-positive group of bacteria, possess a geranylgeranylglyceryl phosphatase (the enzyme responsible for the formation of the ether bond in archaeal lipids), probably acquired from an archaeon by horizontal gene transfer (Valas and Bourne 2011).

Spontaneous racemization (*viz.* the conversion of the L isoform into the D isoform) of aspartic acid residues in proteins increases exponentially with temperature, thus obliging microorganisms living at high temperatures to continuously replace their proteins to maintain enzymatic activity (Onstott et al. 2014). Such a major constraint over life at high temperature provides robust evidence against hyperthermophyly being an ancestral trait of life.

From the analysis of a large dataset of prokaryotic sequences, Weiss et al. (2016, 2018) traced back to LUCA 355 protein families. The properties of these proteins depict LUCA as an anaerobic, CO_2-fixing, H_2-dependent with the acetyl-CoA (or Wood-Ljungdahl) pathway (Section 8), nitrogen-fixing and thermophilic (not hyperthermophilic) organism. The hypothesis of ancestral chemioautotrophy receives support from evidence for ^{13}C-depleted organic carbon in extremely ancient sedimentary rocks (Ueno et al. 2006; Dodd et al. 2017; Tashiro et al. 2017). It is important, however, to emphasize that LUCA might have been the last microbial population preceding the divergence of bacteria and archaea, yet it was not the first organism or the first cell and certainly does not represent the origin of life.

11. Concluding remarks

Life is a chemical system that exists in a condition of disequilibrium and evolves in a Darwinian way. Access to an external source of disequilibrium is an essential condition for the emergence of life from "inanimate" matter. Among a diversity of sources potentially available on the Hadean Earth, modern models almost unanimously point to geochemical disequilibria inherent to volcanic activity, a conclusion in line with evidence of a chemioautotrophic ancestral metabolism. The ultimate driver of volcanic activity on Earth is gravitational separation of an iron-nickel core and a silicate-rich mantle exposed to the surface (viz. not segregated under massive amounts of hydrogen/helium as in gaseous giant planets). Because this is the natural fate of "terrestrial" planets in the Solar system and most likely also elsewhere in the Universe, it is tempting to infer that (i) terrestrial planets have an intrinsic propensity to generate life, (ii) life is probably widespread in the Universe, and (iii) the ultimate source of the chemical disequilibria that started life on Earth and possibly on other planets is gravitational energy stored during Universe expansion. This position shares the view that life as defined above is *a likely, almost necessary occurrence whenever and wherever the right conditions happen to develop* (Russell et al. 2013; Hazen 2017), rather than *a most rare occurrence if not a unique trait of our planet* (Monod 1971).

Terrestrial life as we see it today is a highly refined product of billions of years of evolution, yet it retains traits that probably appeared at the very beginning of its history.

The peptidyl transferase ribozyme, unstructured protein chains in the ribosome core, universal use of proton gradients in energy transduction and of transition metals in catalysis are likely legacies of a pre-cellular stage intermediate between the living and non-living world. The narrative presented in this review is compatible in essential points with both deep-ocean and terrestrial geothermal scenarios. Partnership between RNA and protein has probably dominated biology since the very beginning. Inherited from LUCA and retained virtually unchanged for several billion years, the translation machinery is the fundamental hallmark that defines the universal tree of life (Bowmann et al. 2018).

The appearance of the first cells - self-reproducing systems with at least one hereditary membrane, a genetic system, and a metabolism - was a crucial, irreversible step that put an end to pre-biotic evolution, because cells were immensely more efficient than any pre-cellular system in exploiting environmental resources. From then on, the chance for independent emergence of alternative life forms on Earth was virtually nil, as pre-existing life would immediately out-compete them. The same probably applies for hypothetical extra-terrestrial life reaching the Earth in the form of resting cells.

It is generally assumed that self-replicating molecules and a rudimentary metabolism appeared independently, and that life emerged when a translation system based on a genetic code linked them together. The notion of living organisms as mortal vehicles for immortal genes as popularized by Richard Dawkins (Dawkins 1976) is a useful metaphor that helps catching the way natural selection works, yet biological inheritance implies not only the transmission of genes but also of the biochemical machinery necessary for their functioning. To replicate, genes must fit in a cell, a structured and predictable environment that depends on genes for maintenance and reproduction but cannot be built by genes from scratch. A long phase of pre-biotic evolution was necessary not only to produce self-replicating molecules but also to accommodate them in a reproducible house - the cell - made of inter-linked genotype, ribotype and phenotype. The transgenerational transmission of a cellular organization is thus the most ancient and conspicuous expression of *ecological inheritance*, or *niche construction*, namely the ability of life to modify the environment and transmit the changes across generations (Laland et al. 2016).

A further point worth of attention is the relatively low chemical diversity of living systems. Estimates of the total number of carbon-based compounds with molecular masses in the same range as those of living systems, *i.e.* below 500 Da, give numbers in excess of 10^{60}. Life uses only a tiny fraction of this potential “chemical space” (Dobson 2004). The first explanation that comes to mind is that life chemistry is still focused on ancestral molecular diversity, namely the few types of compounds of abiotic origin wherefrom life started plus their close derivatives. This is unlikely. Under positive selection, billions of years of evolution would have certainly produced much a greater chemical diversity. The most likely explanation stems from the consideration that, for thermodynamic reasons, cells are crowded systems containing high concentrations of molecules, either free or in the form of polymers. This condition is conducive to spontaneous, uncontrolled reactions that may interfere with metabolic pathways and/or damage key cellular components. Thus, life has evolved under strong chemical constraints, being obliged to exclude potentially noxious molecular architectures. Even keeping chemical diversity under tight control, life had to accept compromises, a most prominent example being that of glucose. This is a six-carbon sugar with an aldehyde functional group, universally employed as a substrate in respiration and as a source of carbon chains in most biosynthetic pathways. The highly reactive aldehyde group can easily be blocked by glucose polymerization into glycogen or another polysaccharide, but the free form tends to react with amino groups in proteins, causing structural damage. Presumably, glucose emerged as the most abundant and widespread natural monosaccharide because it is less reactive with proteins than other monosaccharides. Damage to proteins exposed on cellular surfaces from excessive glucose concentration in the blood is at the origin of diabetes in humans.

Life restriction to a limited range of carbon compounds does in no way imply that forms of life arisen independently in the Universe are obliged to explore the same molecular space. Even without considering chirality, the overall chemical space available to life is most likely much wider than that exploited on Earth. This means that, in contrast with commonplace science-fiction, no alien creature could feed on terrestrial organisms, nor could humans feed on non-terrestrial life. If ever possible, therefore, colonization of other planets will require that humans take with them a large complement of organisms, both prokaryotic and eukaryotic, to establish self-supporting "terrestrial" ecosystems. Great job opportunities for "terraforming" ecologists in a possible remote future.

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The unsustainability of sustainability: a new perception of applied ecology against the granfalloon's mission

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Abstract

The unsustainability of sustainability wants to underline the improper and widespread use of a complex concept that cannot be trivialized through the proposal of technological solutions. Although technological solutions represent "comfortable" tools for social reassurance, the risk is a social drift generated by the phenomenon of the "granfalloon". Behind the technological solutions there are new market proposals, which have nothing to do with the real resolution of the sustainability of the global socio-economic system. The perception of the whole socio-economic system as a single metabolic socio-ecological system, makes us interpret the role of technological solutions differently. The knowledge we have gained over the last 50 years on the functioning of natural systems represent consolidated bases for interpreting and providing solutions on the sustainability of mankind. This perception, while providing uncomfortable solutions, should change the perception of applied ecology within the academy community. Therefore, applied ecology no longer as a study of human effects on the ecosphere, but as a discipline that teaches mankind how to structure its socio-economic metabolism compatibly with the constraints imposed by the ecosphere.

Keyword: Sustainability; MuSIASEM; Applied Ecology; Jevons' paradox; Uncomfortable Knowledge

Riassunto

L'insostenibilità della sostenibilità vuole sottolineare l'uso improprio e diffuso di un concetto complesso che non può essere banalizzato attraverso la proposta di soluzioni tecnologiche. Sebbene le soluzioni tecnologiche rappresentino strumenti "comodi" di rassicurazione sociale, il rischio è una deriva sociale generata dal fenomeno del "granfalloon". Dietro le soluzioni tecnologiche ci sono nuove proposte di mercato, che nulla hanno a che fare con la reale risoluzione della sostenibilità del sistema socio-economico globale. La percezione dell'intero sistema socio-economico come un unico sistema metabolico socio-ecologico, ci fa interpretare diversamente il ruolo delle soluzioni tecnologiche. Le conoscenze che abbiamo maturato negli ultimi 50 anni sul funzionamento dei sistemi naturali rappresentano basi consolidate per interpretare e fornire soluzioni sulla sostenibilità delle attività umane. Questa percezione, pur fornendo soluzioni scomode, dovrebbe cambiare la percezione dell'ecologia applicata all'interno della comunità accademica. L'ecologia applicata, quindi, non più come studio degli effetti dell'uomo sull'ecosfera, ma come disciplina che insegna all'umanità come strutturare il proprio metabolismo socio-economico compatibilmente con i vincoli imposti dall'ecosfera.

Parole chiave: Sostenibilità; MuSIASEM; Ecologia Applicata; Paradosso di Jevons; Conoscenza Scomoda

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Introduction

The concept of sustainability in the last decades has become part of the social lexicon: is it used in an appropriate way? This question is pertinent, since in many contexts the word, and the concept it carries with it, appears misused as a fashionable word. Such a concept and its application to the development of the social fabric can correspond to what Walter Bryce Gallie (1956) has defined as an "essentially contested concept" (ECC). According to Gallie, a concept is essentially contested if there is agreement on the means and objectives of a concept but disagreements on how to define it, on which units of analysis to use to capture the adaptive capacity, which are the conceptual cornerstones and which methodology of investigation is appropriate. This is what is actually being observed today on the tangled issues of sustainability about which, in my opinion, intense semantic fantasy activity is in full force. In fact, we all agree on what we want (a sustainable society) but not everyone agrees on how, because there is no univocal definition, there is no analytical system able to grasp the complexity and adaptive capacity of the system we observe, in the final analysis not everyone observes the same thing, since we are looking at a complex system. Can we assign the sustainability label to products, for example, bioplastics, biofuels, energy carrier, or to services, for example, tourism, transport sector? The answer to this question is not simple but, above all, it is not currently answered by the dominant narrative that makes strong reference to technology as a solution tool and to a reductionist science as an evaluation approach.

Trying to synthetize the dominant storyline developed in the recent decades, we can notice it is based around the link between innovation, efficiency and sustainability and it's conducted by a techno-scientistic thinking driven by the wind of the neoclassical economy which can be summarized by the following statement: "we have developed alternative and innovative technologies to modify production mechanisms, compatible with the limits and needs of the ecosphere, to build new forms of income and guarantee the social fabric to maintain the *status quo*". The *status quo* is represented by the unchanged need to ensure economic growth, decoupling it from the limits imposed by the ecosphere (Giampietro, 2019; Renner and Giampietro, 2020). In fact, the most widely accepted and therefore cited definition of sustainability is probably the one produced by the Brundtland report (World Commission for the Environment and Development, 1987). It states that: *sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs*. It is no coincidence that this definition is recognized and cited by the United Nations in the great global project represented by the "Sustainable Development Goals". The UN Department of Public Information cites the Bruntland definition and stresses that, to achieve the objectives, three elements must be harmonized: economic growth, social inclusion and environmental protection. These objectives, in tension with each other, recall the English idiomatic proverb saying: you can't have your cake and eat it too. What is being observed is a blind belief in human technological skills, satisfying gluttony saving the cake. Unanimous chorus was generated among various international bodies (International Monetary Fund, World Bank, United Nations, European Union, Organization for Economic Co-operation and Development, Food and Agriculture Organization) and national governments that amplify strategies as "green growth" and "blue growth" - whether smart, inclusive or responsible - through strategies of circular economy, bioeconomy and digitalization (Giampietro and Funtowicz, 2020). The storytellers of this vision are many, starting with scientists (do they have developed appropriate models of investigations?), passing through politicians and entrepreneurs (do they have been well informed?) to arrive at public figures of the show and culture (do they have the skills to do so?).

Elrich and Holden (1974) defined the impact of human activity on the environment with a simple equation, known as IPAT; it defines the impact as: $I = P \times A \times T$. The equation links the environmental impact (I) to the population (P), to the levels of consumption (affluence, A) and to the impact per unit of resource used, which depends on the use of technology (T). Changing processes toward sustainability, that are developing globally, are exclusively based by developing new technologies, since in the immediate future it is difficult to stem the reduction of the world population and since we continue to rely on

GDP (therefore high levels of consumption) as an indicator of the well-being of the social fabric. The stability of the *status quo* increasingly depends on rosy visions portraying painless solutions to sustainability problems thus avoiding uncomfortable discussions about our current life style and standard of living (Giampietro and Funtowicz, 2020; Funtowicz and Ravetz, 1994).

In the light of those who have developed a different view of the facts, based on the awareness of biophysical limits, these strategies appear to be based on shaky scientific foundations. The belief in the decoupling of economic growth from the use of natural resources through the unlimited power of the invisible hand of the market and human ingenuity - defying thermodynamic laws - should be considered a legend (Giampietro and Funtowicz, 2020). This blind belief in new technologies was defined by Jasanoff and Kim (2015) "the economy of technological promises". The same authors have defined socio-technical imaginaries as the production of collective visions of good and attainable futures through the advancement of science and technology. As discussed by Funtowicz and Ravetz (1994) this narrative about the sustainability of the economy, grounded over the technological promise, has led to a situation akin to the ancien régime syndrome: "a state of affairs in which the ruling elites become unable to cope with stressors and adopt instead a strategy of denial, refusing to process either internal or external signals, including those of danger".

Obviously, this background of facts affects social behavior and generate new governance conflicts, mainly the creation of inflated and unfulfilled expectations about sustainability, precisely the "*granfalloon*" (Giampietro and Funtowicz, 2020). Granfalloon is a term coined from Vonnegut's narrative fantasy, in his book "Cat's Cradle" (1963), within which many people loyal to an invented religion that takes the name of "Bokononism" are stirring. The foundation of this belief is that all existing religions - and therefore also Bokononism - are made up solely of lies. Those who believe in them can have a happy life thus achieving the purpose of Bokononism which, coincidentally, is happiness (the banner raised by the logic of the neoclassical economics). This term entered the lexicon of social psychology as the "paradigm of the minimum group" on which a powerful persuasion technique is based which, as always, addresses emotions and not reason; in other words it describes "a proud and meaningless association of human beings" who imagine (or are manipulated to believe) that they are involved in an important mission. The Granfalloon neatly characterizes this situation were inflated expectations are unfulfilled (Giampietro and Funtowicz, 2020). This social behavior is the reaction of a society that is unable to accept the awareness of the crisis, especially in an era that has made technology the main tool of development. The "global social experiment" emerged from the experience of the Covid, in spite of us, is a demonstration of how much today's society is unable to accept changes, at least in the short time and when the disturbance is unexpected.

On the discourse of sustainability, it seems rather bizarre to record public statements by ecologists of the academy who trust in technology to ensure economic growth, while safeguarding the ecosphere. If I think of the famous assertion of economist Kenneth Boulding - *"Anyone who believes that exponential growth can go on forever in a finite world is either a madman or an economist"* - raises a question about the position expressed by ecologists: have they been phagocytized by economists?

Why do I think these positions taken by some ecologists of the academy are bizarre? Because what they teach from university professorships, through established basic ecology programs, should lead them to think otherwise.

This paper has two main objectives: (i) it intends to direct readers to a correct interpretation of the complex world of sustainability, to avoid the improper use of the concept of sustainability (the unsustainability of sustainability), highlighting some of the critical issues that cannot make technology a saving strategy; (ii) in doing this, I will refer to the consolidated concepts of basic ecology that have been present for years in the study programs of university courses in order to trigger a reflection on the definition of applied ecology: wouldn't it be appropriate to enhance applied ecology as a discipline that allows you to translate the behavior of natural systems towards socio-economic ones, since both are complex adaptive systems that tend to move away from equilibrium like in the thermodynamic systems? The narratives developed in a conspicuous literature, for whose references I refer to Giampietro (2019), suggest a strong analogy between the processes of self-organization of ecological systems and social systems: both require the existence of favorable boundary conditions and the capacity to exploit them.

This paper is organized as follows. Session 1 is an attempt to synthetize the complexity of information to provide a definition of sustainability that is compatible with the need to define the relationship between socio-economic systems and the ecosphere. Session 2 deals with the concept of development from a socio-ecological perspective to demonstrate, only in broad terms, the structural difficulties that the global society of the future will have to face in becoming "sustainable". Session 3 addresses the concept of efficiency to demonstrate that relative efficiency does not affect absolute efficiency, in the case of system aiming for growth. Finally, session 4 draws the conclusions with the aim of providing points for reflection for the readers.

Sustainability rationale deals with the metabolism of socio-ecological system

We can all agree on a fundamental principle of sustainability: the concept of sustainability refers to the human socio-economic system and therefore the science of sustainability deal with this system as its "object of observation".

The etymology of sustainability is sufficiently explanatory (it derives from the Latin verb *sustĭnēre - sus* "under" and *tĭnēre* "to keep") to give a concise but effective definition of it: sustainability is the survival ability of the socio-economic system.

The socio-economic system is: (i) a complex adaptive system (Kampis, 1991; Gell-Mann, 1994; Holland, 1995, 2006); (ii) with its own metabolism (Giampietro and Mayumi, 2000a, b; Giampietro et al., 2012); (iii) in its structures and functions it is organized by holons at various hierarchical levels (holarchy) contributing to the so-called emergent properties (Koestler 1967; Giampietro et al., 2006) and therefore observable over different scales (Giampietro and Mayumi, 2018); (iv) it works thanks to cybernetic principles (autocatalytic loop) (Ashby, 1958; Odum, 1971; Giampietro and Mayumi, 2018) and autopoietic properties (systems capable of producing themselves) (Maturana and Varela, 1980; Maturana and Varela, 1992); (v) like all metabolic systems it is a self-organized and open system escaping from thermodynamic equilibrium gathering resources from their environment and dispose wastes into it (Schrödinger, 1967; Nicolis and Prigogine, 1977); (vi) for all these reasons it cannot be observed and treated with reductionistic and deterministic approaches (Ashby, 1958; Giampietro and Mayumi, 2018).

All these characteristics can certainly appear complex, the figure 1 helps to summarize and explain in order to define the socio-economic system. If we observe superficially the socio-economic system, as a "metabolic black box" (Fig. 1a), we can identify two main objectives of the system: (i) move away from thermodynamic equilibrium (purpose 1); (ii) increase its size (purpose 2). In order to achieve these two purposes, the system must be organized with structural elements with specific functions: the holons. Typical examples of holons are the different components of the social fabric (social sectors), each of which is organized in appropriate productive and consumption sectors making possible to stabilize a given metabolic pattern by means of energy and matter. Georgescu-Roegen, after the concept proposed by Lotka (1956), distinguished two distinct forms of metabolism of modern societies: (i) endosomatic metabolism, it refers to the food energy converted inside the human body to preserve and sustain the physiological activity of humans, that in turn are used to preserve and express structural and functional elements of the society, (ii) exosomatic metabolism, it refers to the energy converted outside the human body, but under human control, with the goal of amplifying the output of useful work associated with human activity (e.g., animal power, machineries, stuffs, buildings). The exosomatic metabolism became tremendously important in shaping the identity of modern societies after the industrial revolution (Cottrel, 1955, Hall et al., 1986). In fact, the accumulation of technical capital implied a dramatic increase in the productivity per hour of human activity. This allows the social fabric to use the flows of energy and matter to increase the number of individuals in a population (purpose 2 of figure 1a, equivalent to the reproductive fitness of natural populations) and to use the surplus to generate a parallel flow expressed by monetary added value of goods for economic growth (purpose 2 of figure 1a, represented by economic fitness expressed as GDP). To give an idea of the relative importance of the two types of metabolism of energy, in a developed society the metabolism of endosomatic energy (food) lies in the range of 10-12MJ/day per capita (approximately 2,400-3,000 kcal/day) whereas the metabolism of exosomatic energy (measured in primary energy sources) can be estimated at 500-900MJ/day per capita (or 200-320 GJ/year). Thus, the exo/endo energy ratio typical of developed societies falls within the range 50/1-75/1, while that of pre-industrial societies is

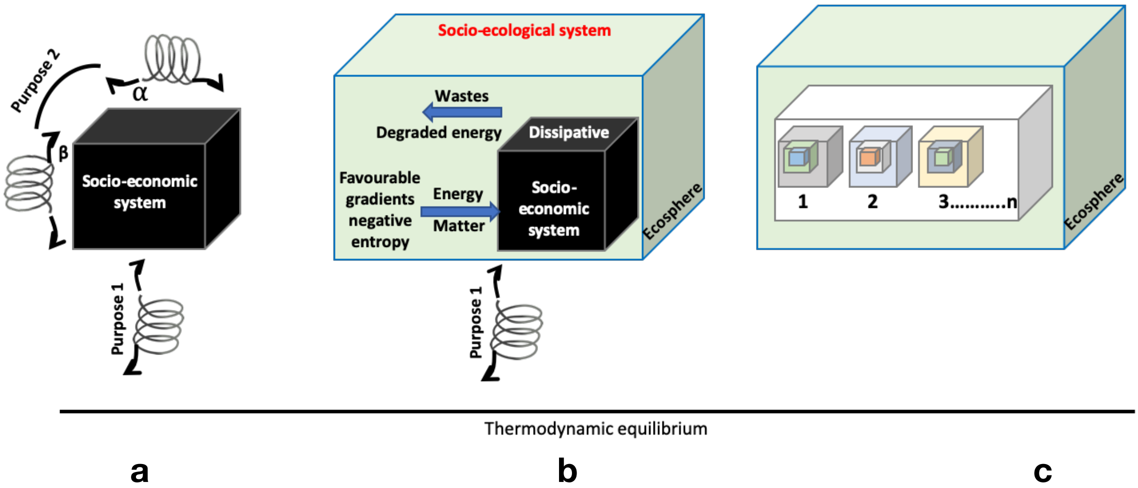

Figure 1: Metabolic perception of the socio-ecological system.

typically only about 5/1 including energy used for cooking, heating and illumination as well as animal power and local sources of mechanical power such as waterfalls or wind (Giampietro and Mayumi, 2009; Giampietro et al. 2012). A suggestive example is from the food systems. Due to the structure and spatial arrangement of the population in developed economy countries, where about 75% of the population lives in cities, large amount of energy is invested, besides food production in agriculture, in other activities such as food processing in the food industry, packaging, transportation, final distribution, home storage and preparation. In modern food systems, the post-harvest sector uses four times more energy than the agricultural sector (Heller and Keoleian, 2000).

The socio-economic system is an open system and as such it is unable to express its metabolic functions without interacting with the external environment, that is the ecosphere (Fig. 1b). Just like the socio-economic system, the ecosphere also aims to escape thermodynamic equilibrium. It succeeds in this task above all through the photosynthetic process of transforming solar energy into chemical energy, as regards the energy supply, and of matter gradients due to bio-geological events. Metabolic processes occur on a local and global scale for the different ecosystems and will be bound by the environmental conditions to ensure the transformation of solar energy and the recycling of the catabolites of their own metabolism, this translates into a steady state condition of the system. The socio-economic system is a dissipative system (Fig. 1b) whose conditions for the survival are determined by an expected pattern of interaction between the dissipative structure, generating a positive entropy flux needed to express its structures and functions and the environment, providing a flux of negative entropy compensating the continuous destruction of favorable gradients by the dissipative structure (Prigogine, 1980). This ineluctable thermodynamic constraint explains the impossibility of guaranteeing economic growth to a system that aspires to be circular and therefore decoupled from the ecosphere (Giampietro, 2019).

Georgescu-Roegen (1971), in his bioeconomic view of the socio-economic system, made a distinction between flows, stocks and funds for describing the process of interaction between the socio-economic system (technosphere) and the ecosphere. (i) Flows are quantities disappearing or appearing over the duration of analysis. They can be further divided into primary flows, requiring primary sources and primary sinks beyond human control and crossing the border between technosphere and ecosphere; and secondary flows that are produced and consumed inside the technosphere and transformed under human control. (ii) Stocks are quantities of accumulated flows that change their identity through the duration of the analysis because of outflows (stock depletion) and/or inflows (sink filling). Hence, in contrast to its use in economic jargon, in Georgescu-Roegen's analytical framework a stock is not a constituent component of the system, but an accumulated flow that changes its size in time. (iii) Funds are agents capable of both producing and consuming flows inside the metabolic pattern of the socio-economic system. Funds do preserve their original identity throughout the duration of the analysis (e.g., the human population, the work force, technological capital, land use). Fund elements define what the system is made of.

For all these reasons, it is no longer correct to speak of a socio-economic system but rather of socio-ecological system. A socio-ecological system can be defined as the complex of functional and structural components operating within a prescribed boundary that is controlled in an integrated way by the activities expressed by a given set of ecosystems (in the biosphere) and a given set of social actors and institutions (in the technosphere) (Giampietro,2018). Socio-ecological systems are open systems depend on their context for maintaining their current level of activity and size of production factor and must be adaptive and anticipatory in order to survive in time because of their option space being constrained by processes beyond control.

If we open the black box, we can appreciate its holarchy (Fig. 1c), that is the different structural and functional components (holons) organized in a multi-level space that guarantee the metabolic identity of the system (technosphere), compatibly with the constraints imposed by the ecosphere. These elements interact according to impredicative relationships and for this reason study approaches capable of considering relation analysis are necessary. The performance of the socio-economic system is tied to the "emergent property" determined by the interaction of lower-level functional components (e.g., economic sectors) made up of structural elements (i.e., expressing the physical processes) (Fig. 1c). The emergent property is represented by the ability of the economy to reproduce and adapt according to its internal values and aspirations, while interacting with its context (Giampietro et al., 2012).

In this framing, it should be quite evident that observing, describing and analyzing a complex system is a very difficult task, especially when different perceptions of the facts and the mobilization of huge monetary resources raises different interests, mainly in form of public subsides. An inclusive observation and, above all, not conditioned by interests must be able to move transversally through the different domains of analysis according to a multi-criteria perception (Fig. 2). The complex socio-ecological system is structured around three wide descriptive domains, environmental, social and economic, each of which can be described and evaluated by multiple

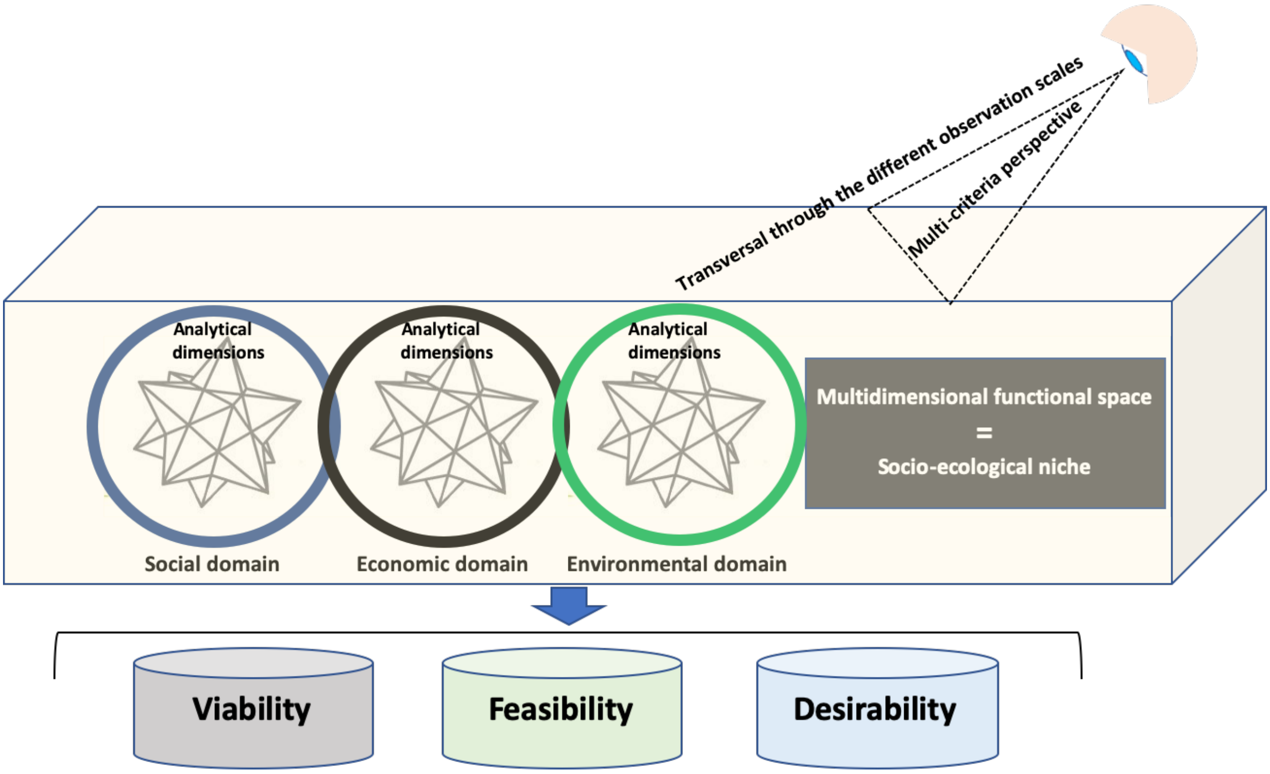

Figure 2: Multi-criteria perspective that allows to observe the multi-domain and multi-dimensional characteristics of the socio-ecological system. The system interpretation outputs are expressed according to the MuSIASEM jargon.

dimensions of analysis. Ultimately, it is necessary to represent in the best way a multi-dimensional functional space which is none other than the socio-ecological niche of the metabolic system. We need to find the proper balance between avoiding the excessive simplification of information, which is not able to adequately represent the complex system, and generating an excessive number of information that can prevent a consistent and fast interpretation of the system. In the jargon of MuSIASEM (Multi-Scale Integrated Analysis of Societal and Ecosystem Metabolism), an accounting method used to analyze the metabolic pattern of social-ecological systems (Giampietro and Mayumi, 2000a; Giampietro and Mayumi, 2000b; Giampietro et al., 2009), the information space is enclosed inside three performance container: (i) *feasibility* (compatibility with external constraints determined by processes outside human control); (ii) *viability* (compatibility with internal constraints determined by processes under human control); (iii) *desirability* (compatibility with institutions and normative values) (Fig. 2). It is based on maintaining coherence of the quantitative representations generated using different metrics across different scales and dimensions (e.g. economic, social, demographic, ecological, technical).

Development to ensure "the needs of the present"

The current structural and functional organization of the socio-ecological system was developed to ensure "the needs of the present" (Bruntland quote). Will a change in the socio-ecological development model still guarantee this societal need? There are many critical issues in the currently proposed "sustainability" models based on technological solutions. Let's try to understand why.

The concept of development is transversal, along the scale of organization of metabolic systems, from single cell up to socio-ecological system. Development is a progressive quantitative and qualitative change of the structural elements of a system which must guarantee specific functions. The temporal sequence of changes is organized over the short, medium and long term, according to the factors that act on the system to induce it to change and according to the system's ability to respond. It is known that in economics the concept of development refers to a society that passes from an economy consisting of primary activities (agriculture and exploitation of natural resources) to an economy focused on industrial production activities and in the tertiary sector with the aim of generating more value added. According to a metabolic perception, the constituent components of a society (i.e. its functional parts guaranteeing its metabolism) can be divided into: (i) the primary sectors (such as agriculture and energy and mining) that represent the catabolic part, taking advantage of favorable gradients provided by nature to supply the required inputs to the rest of society; and (ii) the "other sectors", representing the anabolic part, using secondary inputs supplied by the primary sectors to maintain and reproduce the society. The "other sectors" include: manufacturing and construction, service and government and the household (residential) sector. These constituent components depend on each other in terms of essential inputs. The household sector uses inputs from all the others to reproduce and supply hours of human activity (labor) to the rest; the primary sectors use human activity, primary sources and secondary inputs to provide secondary inputs of food, energy and raw materials to the others; the manufacturing and construction sector uses human activity and secondary inputs to supply technology and infrastructures to the entire society whereas the service and government sector uses human activity and secondary inputs to reproduce institutions and maintain people. According to the MuSIASEM jargon, a distinction between dissipative activities and hypercyclic activities is performed. The concept of hypercycle *vs* dissipative is taken from theoretical ecology of Ulanowicz, where it is used to describe the factors that stabilize complex metabolic networks. An hypercycle is a loop in which the output is larger than the input. Dissipative activities are those that consume biophysical flows and use exosomatic devices, without producing either of them. They are household sector (HH) and service and government (SG). This implies that in the same society we must find other activities that generate a net supply of flows and exosomatic funds, in alternative the flows and exosomatic funds consumed have to be imported (the activities generating a net supply of flows and funds are externalized to other societies). The demand generated by dissipative activities defines the required supply of flows and exosomatic funds. The hypercyclic compartment is composed by agriculture and fishering sector (AF), energy and mining sector (EM) and manufacturing and construction (MC). These compartments have to provide this supply or it has to be integrated by imports. Examples of hypercycle are the agricultural sector, which produces more vegetal and animal products than it consumes, and the energy sector, which produces more electricity and fuels than it consumes and manufacturing and construction producing more exosomatic funds that they consume. For this reason, the primary and secondary sectors can provide net flows of food, energy and exosomatic funds to the dissipative compartments of the society.

Figure 3 shows the societal structure of the Campania Region (ISTAT data for the year 2015) and therefore the respective functional roles of the individual sectors which, with a good approximation, is representative of a developed country. We can observe the limited contribution of the hypercyclic activities, less than 9% in a population of almost six million inhabitants, with an annual investment of working hours of just 1 Giga. Even more limited is the contribution of the catabolic sectors (AF and EM) that take advantage of favorable gradients provided by nature to supply the required inputs to the rest of society. The remaining 91.2% of the population is dissipative, with as much as 72.5% in the household sector. This "picture" of the developed society also helps us to better understand the economic impact caused by the sudden "perturbation of

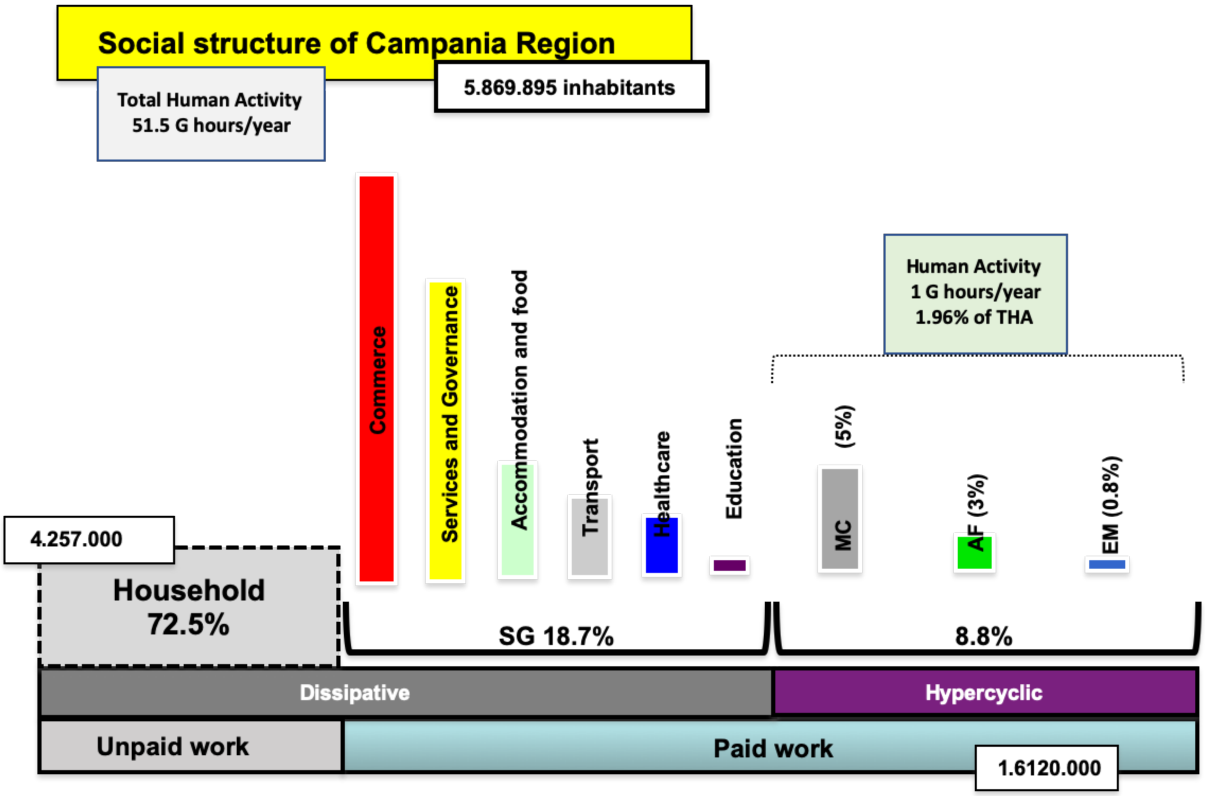

Figure 3: Structural and functional organization of the Campania Region as a representative example of a developed economy society (ISTAT, 2015).

Covid". The economic crisis was not triggered by the lack of production and the main factors that support it (i.e. the availability of energy) but rather by the lack of consumption.

Why has the social structure of the Region been able to take this form? The answer is because society has been able and still can have a high input of energy and matter from the fossil stock (stock flow economy). The current level of productivity of production factors (labor, capital, land) is obtained by altering the pace and density of the flows naturally occurring in the biosphere in managed ecosystems (human land-uses). In doing so, society can express structures and functions (associated with a given rate of positive entropy generation) that would otherwise not be possible (if relying on the negative flux generated by natural processes) (Smil, 2015). The level of power supply is the pivotal feature. Information about power levels, in fact, is fundamental to understand the viability of a societal metabolism and its interdependence with the social structure. The high metabolism of developed societies requires a high power level, which in turn requires a concentrated flow of energy as input. The power density of the energy source, that is to say the rate of energy flux per unit of area (W/m^2), is a key indicator (Smil, 1983, 2003, 2010, 2015). As described in the figure 4, the current developed economy societies (power density in end uses) have been structured on the high power density offered by fossil energy (power density in supply), this is a stock-flow economy mode. The energy supply of modern society predominantly consists of a linear exploitation of non-renewable stocks of fossil energy allowing a density and pace of flows that are orders of magnitude higher than those of circular renewable fund flows, such as biomass (Smil, 2003; Smil, 2015; Giampietro and Mayumi, 2009). The move to circular fund-flow mode, inevitably has to pay the price of low power density. For example, fossil fuels perform power density from 300 to 3000 times better than the biofuel. Fierro et al (2019) assessed a power density value of second generation bioethanol of 0.11 W/m^2. Smil (2015) report values ranging from 4 to 10 and from 0.5 to 1.5 for photovoltaic and wind energy, respectively. Giampietro and Mayumi (2009), argue that developed societies, in order to sustain their level of metabolism, require an energy throughput in the energy sector ranging from 10,000 to 20,000 MJ per hour of labor. Developed societies, thanks to a developed technosphere, gathers and concentrates material and energy forms required for its maintenance and reproduction, to achieve this result they heavily rely on non-renewable energy sources. Inside the technosphere both the densities and paces of flows per unit of societal funds (flow/fund ratios) are much larger than those of the natural flows per unit of ecological funds (flow/fund ratios) in the biosphere (Giampietro et al., 2012). The two graphs in figure 4 explain the progressive increase of urban populations on our planet: the massive use of fossil energy guarantees a high spatial density in the supply of energy inputs that enables a high spatial density in the supply and consumption of food, goods and services.

The combined effect of the changes that took place during the past two centuries in the agricultural and the energy sector of modern economies is well represented in figure 5. This figure clearly illustrates the essence of the industrial revolution that

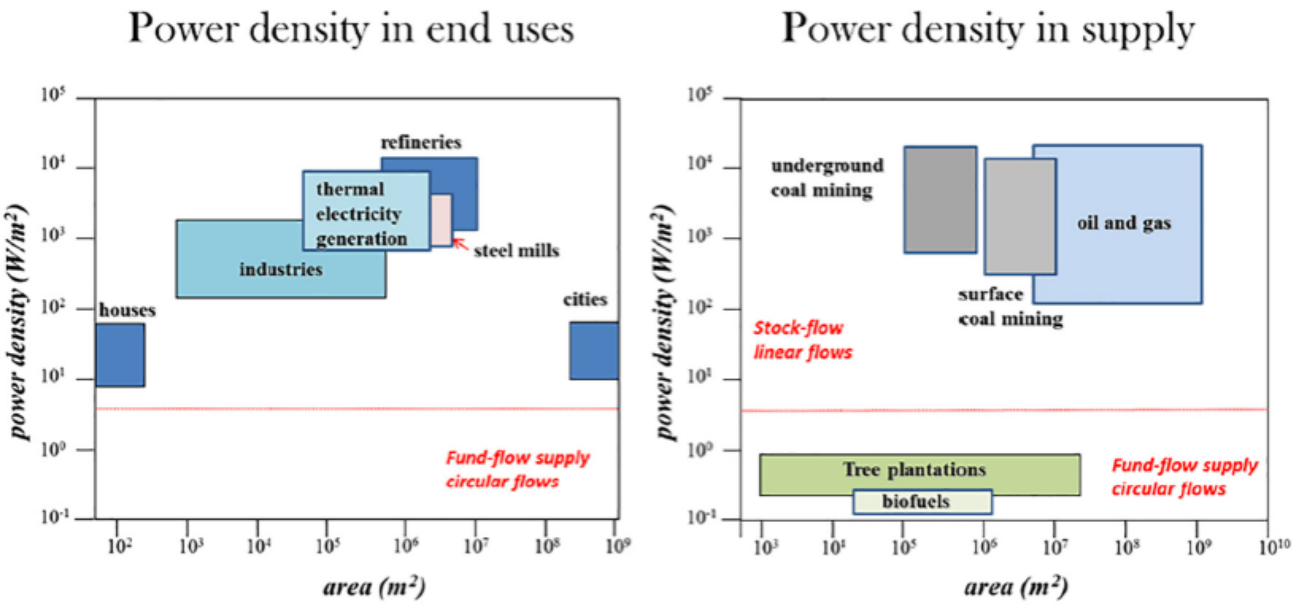

Figure 4: The power density, an indicator of how the current social fabric of developed economy countries depends on energy density (after Smil, 2015).

shaped contemporary society. The mode of energy and food production changed dramatically from being almost entirely based on circular fund-flows (inputs produced and wastes absorbed by ecological funds) to almost complete dependence on linear stock flows (inputs extracted from stocks and wastes overwhelming environmental sink capacity). The current condition of the socio-economic systems of countries with developed economies is comparable with the condition of eutrophic systems. Eutrophication is a known dysfunction for many aquatic systems, it occurs when the system changes its pattern of receiving and using the main nutrients, passing from a fund-flow mode to a stock-flow mode, causing an explosion of the algal biomass and the consequent accumulation of this biomass in the detritus chain, with a collapse of the oxygen concentration and substantial consequences on fish populations. The observation time scale is important for understanding the normal situation of a system. Who observes a water body in the time scale of one year, may have the ability to recognize the condition of normality (system that works according to a fund-flow mode) compared to a temporary condition of abnormality represented by the system in a phase of eutrophication (system which works in a stock-flow mode). No ecologist would have the presumption to define the eutrophicated system as the condition of normal functioning of the system. The only exception may be in the event that the flow of nutrients becomes chronic, in this case the system will change its structural and functional condition to represent "normality". If in a similar way an observer were limited to observing the socio-ecological system in a short time scale, for example a decade, he would notice the society as described in figure 3, equivalent to the eutrophic society in stock-flow mode which could represent the normal condition. If, on the other hand, the observer would expand the time scale by jumping back two centuries (circular flow mode in Figure 5), he would observe a different social structure,

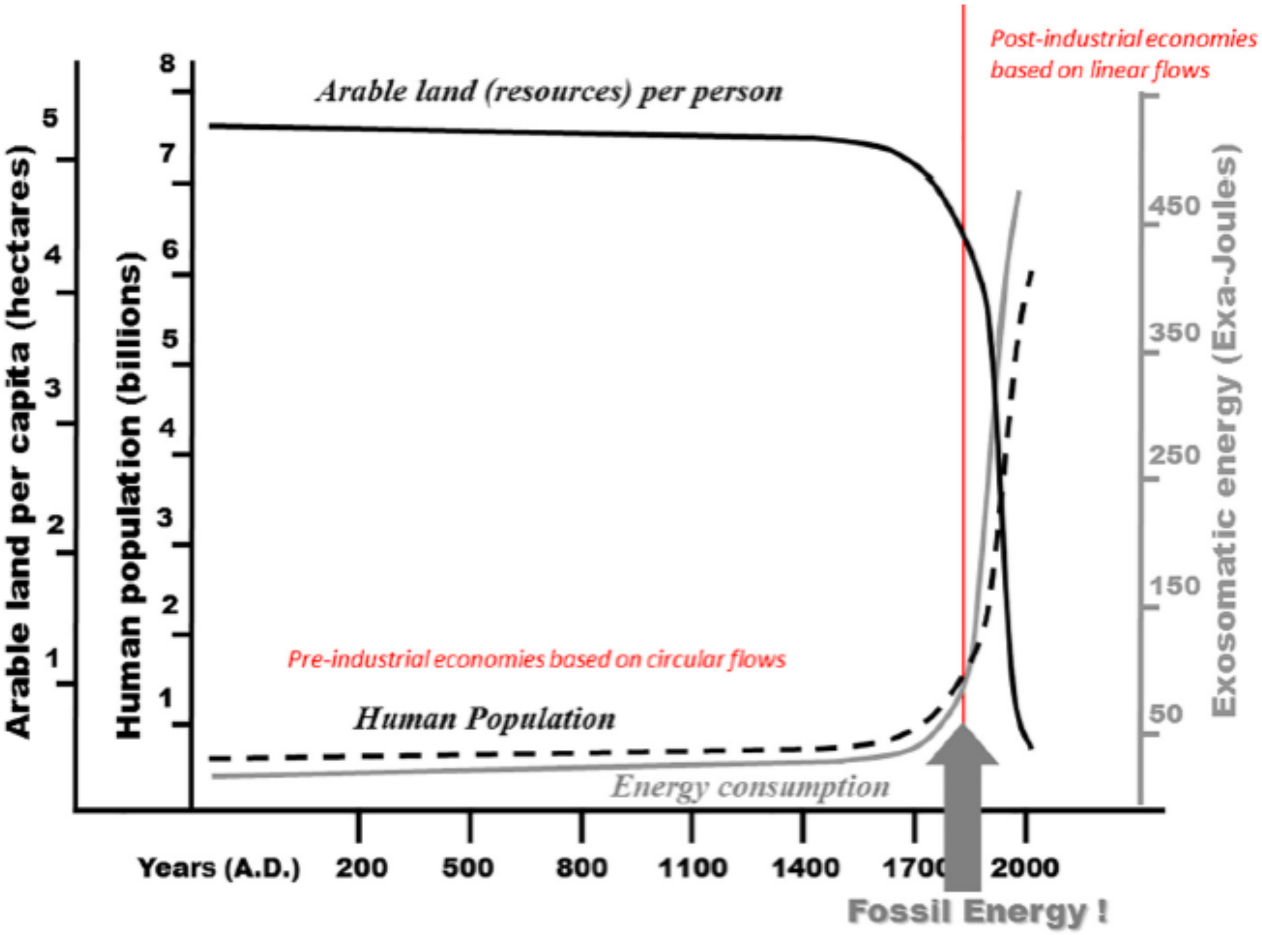

Figure 5: The industrial revolution changed radically the world economy, moving from exploitation of circular flows to dependence on linear stock flows (after Giampietro and Mayumi 2009).

that is to say the society structured on the basis of the constraints imposed by the ecosphere without having the auxiliary input of fossil energy. Which of the two represents the normal condition? It would therefore be difficult for anyone to affirm that a society that decides to become sustainable, thus moving from a stock-flow to a fund-flow mode, can continue to guarantee "the needs of the present".

In the end just listen to Jevons wisdom

Jevons paradox is an implacable sentence against human efforts to aspire technological innovation in ensuring the "sustainable" permanence of current socio-economic system inside the limits imposed by the ecosphere. For those interested in a more in-depth analysis of the issue, I refer to the work of Giampietro and Mayumi (2018). The Jevons Paradox states that, in the long term, an increase in efficiency in resource use will generate an increase in resource consumption rather than a decrease. Jevons, in his books, reported these words:

"Now, if the quantity of coal used in a blast-furnace, for instance, be diminished in comparison with the yield, the profits of the trade will increase, new capital will be attracted, the price of pig-iron will fall, but the demand for it increase; and eventually the

greater number of furnaces will more than make up for the diminished consumption of each. And if such is not always the result within a single branch, it must be remembered that the progress of any branch of manufacture excite a new activity in most other branches" (Jevons, 1865, p. 141).

A classic example, to translate Jevons's claim to modern times, is that of automobile sector. It is known that in recent decades the efficiency of cars has progressively increased such as to reduce the pipe emissions. This improvement has not translated into an overall reduction in emissions due to the tremendous increase in cars production.

I will give another simple example to make it clear how much the choice of "apparently sustainable" solution fail in its intent when applied to the current socio-economic system. If an individual decides to renounce the car for daily transport needs and to opt for the use of the bicycle, this choice will certainly have some advantages: (i) an "environmentally friendly" alternative since there will be no environmental impacts associated with the construction, use and disposal of the car; (ii) substantial economic savings. In a socio-economic system whose monetary value is the main element of performance (economic growth) the individual, who has made this choice, will have the need to allocate the money saved; they will be invested in the purchase of other consumer goods and as such they will always be associated with a dissipative system to produce them. If, absurdly, all the inhabitants of the planet were to renounce the car, this will cause the collapse of the automobile industry. In a system that aspires to economic growth, this will result in the reallocation of flows and funds to other productive sectors. During a phase of economic expansion (upward causation) the insurgence of the Jevons Paradox is practically inevitable. Whether it is because of an uneven distribution of wealth or a strong aspiration for a higher material standard of living, it is unlikely that an energy surplus generated by an increase in efficiency will not be consumed by a society to fix a problem or improve living conditions (Giampietro and Mayumi, 2018).

Briefly, we can state that complex adaptive systems work thanks to two principles: (i) the principle of minimum entropy production and (ii) the principle of maximum energy flux (Giampietro and Mayumi, 2018). They contemplate the functioning of complex adaptive systems, operating away from thermodynamic equilibrium, in an internal and external sphere of observation. By means of the minimum entropy production principle we can understand the efforts developed by each metabolic holon (inside the black box of figure 1c) to improve the efficiency (output/input efficiency). Therefore, we deal with lower hierarchical levels of the system operating under a strict set of constraints within stable boundary conditions. Under these conditions, system performance is well-defined and it is reasonable to assume a steady trend of learning new ways of reducing the required energy and matter input for sustaining a given function.

By means of maximum energy flux principle we can understand the phenomenon by observing the metabolic black box from the outside (outside the black box of figure 1c) and therefore its growth pattern (2 of figure 1a). In brief, if a certain unit of energy enters a system, if some internal metabolic systems use it efficiently, the saved energy will be reallocated to other metabolic elements to ensure the growth of the entire metabolic system. This happens in today's societies because expanding the ability to produce more in order to consume more, maximizing the energy flux, is a common attractor for socioeconomic systems. The principle of maximum energy flux in economics has been formalized in terms of the maximization of profit and welfare (Giampietro and Mayumi, 2018). A quick economic growth implies a continuous expansion of the activities of the metabolic pattern associated with a continuous enlargement of the economic process both in terms of the size of the metabolic system (population, technologies and infrastructures) and in terms of the pace of activity per unit of size (the pace and density of flows of resources consumed per capita). Therefore, we deal with the level of the whole complex adaptive system it is reasonable to expect that it will express as many functions as possible in order to enhance its chances of survival and well-being in its interaction with the context.

The functioning of natural ecosystems on the basis of the two principles set out above, is a well-known topic in the university curricula of basic ecology and appears with the concept of ecological efficiencies. This topic is part of systemic ecology, it explains that the capacity of energy flow along the food chain is conditioned by the metabolic characteristics of each trophic level along the chain, through different forms of transformation efficiencies. Environmental conditions are equally important in ensuring these forms of efficiency. In any case, the distribution of the amount of energy along the two energy and matter bifurcations of ecosystems (the trophic chain and the detritus chain) is, in any case, conditioned by the maximum power energy available to the system, i.e. direct and indirect solar energy and the ability of the different transformers to convert the energy.

Final reflections

The "unsustainability of sustainability" is about the simplified, improper and opportunistic use of this term. In the last two decades, also thanks to the mobilization of huge public funds, the discussion on sustainability has taken on a purely technical shape. The complexity of the facts has been reduced to a simple equation: technological innovation + new economic models = sustainability. According to the narrative, the algebraic binomial can generate new economic wealth by decoupling human being from the constraints imposed by nature. The main actors in this narrative are the old business lobbies that have recycled themselves (ancien régime). Thanks to the sounding board of the media, which they often own, they have spread the mission of the granfalloon.

What has been explained in the previous sessions should clarify that sustainability is about understanding a complex system, both to evaluate it in existence and in development. To avoid that it remains an "essentially contested concept" a systemic view is necessary, that cannot be based on scientific reductionism that allows solving single problems. The Bruntland definition is not robust enough to define sustainability. It prefigures a "virtuous" path of the globalized society but, in fact, does not define sustainability. It indicates a path to follow in time, but it does not tell us whether A in time t0 will remain A in time t1 or will become B in time t1. Even if the statement "*needs of the present*" leaves no doubt, in time t1 A will remain A. Continuing to guarantee the "*needs of the present*" means that it is unlikely that a socio-economic system, that is structured on a stock-flow economic model, continues to be the same when transformed into a fund-flow model. Defining sustainability in a solid and unanimous way is an epistemological necessity to structure the appropriate analytical models capable of providing robust information for the "science of governance". We must not only define the virtuous path about *what we want*; above all we must understand and indicate *how to do it* and *if it can be done* with reference to what we are observing. When we adjective the term "development", we refer to a process of forming a system. In our case the system is represented by the human socio-economic system. "Sustainable" quality refers to what? To who? What are the quality characteristics? This semantic problem arises because in fact, through the concept of sustainable development, the "substance" of sustainability is not defined: that is, how to appropriately define the aspects that characterize that system so that it is sustainable.

A metabolic definition allows to define the relationships between human capabilities to transform energy and matter (technosphere) and the relationships that associate these capabilities with the constraints imposed by the ecosphere. A metabolic perception therefore allows us to define the functional boundaries of the socio-ecological system (socio-ecological niche) in order to evaluate its "*ability to survive*", changing certain characteristics and conditions. When adopting the metabolic view of the whole socio-economic or, through a progressive breakdown, of the different production systems that compose it (multi-scale perception of the different structural and functional holons), inevitably we must understand how each of them interact with the external environment. In this view, the issue of sustainability boils down to the compatibility between: (i) the size and the metabolic pace of the fund elements operating in the technosphere and determining the flux of positive entropy, and (ii) the size and the metabolic pace of the fund elements operating in the biosphere and determining the flux of negative entropy. Put in another way, the identity of the fund elements entails a constraint on the pace and density of the flow throughput both in biosphere and technosphere.

A sustainable economy based on "renewable" flows coming from fund-flow relations that respect and maintain the identity of the funds. Biophysical representation based on the rationale of metabolic systems thus describes the "production factors" as fund elements, contrary to the economic representation in which they are considered stocks. In the view of Georgescu-Roegen, the sustainability of the economic process is not about stabilizing the flows of goods and services produced and consumed in the economy, but about reproducing the fund elements that are associated with the stabilization of the metabolized flows.

Therefore, the question is not just finding a technological solution that reduces environmental pressures (both in the withdrawal of resources and in the emission of metabolic waste), it is a question of understanding whether the new production system satisfies this new metabolic structure to stabilize new flows. Based on the MuSIASEM jargon, it is a question of understanding whether the new production system can survive compatibly with technical and economic constraints (viability), compatibly with environmental constraints (feasibility) and compatibly with the social structure of Figure 3 to ensure that there are no tragic changes for society (desirability). To ensure the current lifestyle, represented above all by the exosomatic metabolism, the socio-ecological system has taken on a particular conformation in its structural parts. A change in the economic modality, leaving the paradigm of economic growth unchanged, could compromise the possibility of guaranteeing the same material lifestyle but would also involve a structural change that could not be desirable for society.

The perception of sustainability not only requires to study how a new technology solves one or two problems, above all, it must understand the functioning of the whole metabolic system: we cannot assign the "sustainable label" to a product or service. Jevons' paradox is an inexorable sentence for all human efforts to produce new innovative technologies. We can also find infinite technological solutions to improve the "relative" sustainability of a single production process, what determines real sustainability is the behavior of the entire socio-economic system (absolute sustainability). Since at the present stage governments continue to trust GDP as a valid indicator for the objectives of a society, the need to rely on the maximum power principle must necessarily exist. It would therefore be difficult for a society that is structured on the availability of high gradients of energy and matter (stock flow mode) to maintain a high GDP by switching to a fund-flow mode with a slowdown in the flows of energy and matter. This change of functional modality will also involve the shift of working hours from the secondary sectors to the primary sectors, a solution that certainly does not satisfy the social desirability of producing high added values. In other words, sustainability is about understanding and anticipating the new identity of the society after changing. Since, the transition from a stock-flow to a fund-flow economy will radically change our social structure and expected functions.

Words are extremely important in understanding what a nation want do. In reference to the EU the words, which reflect the policies for the coming decades, clearly express a change in the management of resources to ensure economic growth. Therefore, the claims that with the green deal, circular economy, bio-based economy, in the next 30 years, the EU will be able to substitute fossil fuel, decarbonize the electricity sector, reduce the environmental pressures, make its agriculture competitive and capable of guaranteeing food security no longer depending on imports, show a remarkable lack of scientific and political understanding of these issues. It is becoming ostensibly clear that the current pattern of economic growth is incapable to solve growing concerns about inequity, environmental protection, dangerous dependence on disappearing resources and on the exploitation of less powerful social ecological systems. To avoid the risk of a collapse in the credibility of the EU system, it is the right time to move from the present class of "yes we can" narratives to the class of narratives "Houston we have a problem" (Giampietro and Funtowitcz, 2020). Proposing solutions for a sustainable socio-economic model today appears to be affected by many critical issues, thus evidence based policy may result in a dramatic simplification of the available perceptions, in flawed policy prescriptions and in the neglect of other relevant world views of legitimate stakeholders. In accepting the "yes we can" narrative, the flip side of evidence based policy prevails, namely policy based evidence. (Saltelli and Giampietro, 2017). A much more comfortable situation for those who want to aspire to funding by following the policy requests.

In a historical phase of large public economic investments many social actors, not least the class of scientists, insinuate their "yes we can" comfortable narrative, both in relation to the policies of the EU on the Green Deal and Blu Growth and in relation to Recovery Fund for post-Covid. It therefore makes us reflect the positions taken by many scholars, including ecologists, in full agreement with the "yes we can" narrative. Ecologists should have the cognitive tools to highlight the criticalities of these political strategies. For this reason, I highlight the need to evaluate the concept of applied ecology differently, no longer as a discipline that evaluates the effects of human pressure on the environment but as a discipline capable of transferring the knowledge of the functioning of natural systems to the functioning of socio-economic systems, which are nothing more than socio-ecological systems. I remember that this path was already paved over half a century ago by the speculative work of Georgescu-Roegen and by theoretical ecology.

On this playground of sustainability, made up of many interests, we are witnessing a dangerous cultural drift. A tangible risk of the "minimum group paradigm" is manifesting with a process of "cancel culture": the exclusion from debates and from funding opportunities for those who profess "politically incorrect" positions because they are not in line with the comfortable narrative of "yes we can". Steve Rayner (2012) describes this phenomenon: "To make sense of the complexity of the world so that they can act, individuals and institutions need to develop simplified, self-consistent versions of that world. The process of doing so means that much of what is known about the world needs to be excluded from those versions, and in particular that knowledge which is in tension or outright contradiction with those versions must be expunged. This is uncomfortable knowledge".

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The Checklist of Birds from Campania Region (updated to 31th January 2021)

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Abstract

In the Campania region 361 bird species reside, 214 Non-Passeriformes and 147 Passeriformes. To these species, we must add 31 species in AERC list E and 7 species that were rejected as regional avifauna by the Italian Rarity Commitee (CISO-COI). We recorded 154 breeding birds (147 regular): 70 Non-Passeriformes and 84 Passeriformes; two are non-native species to the region and have self-sustaining breeding populations: *Psittacula krameri* and *Acridotheres tristis*. Outside these, six species have now an uncertain breeding status and two others are regionally extinct. 90 species have a status of resident birds. 51 species are transient migrants and 70 (19%) have been classified as vagrant with eight that have a "historical" presence (AERC code: B).

The present study updates the last published checklist (Fraissinet 2015) making a critical review of the previous list in order to assess the status categorization of each taxon. Moreover the list adapts the systematic classification of the Italian Checklist (Brichetti & Fracasso 2015) and the recent version of IOC World Bird List (Gill et al., 2021). We also publish the first list of birds escaped from captivity observed in the region (AERC list E). Ornithological Value Index calculated on breeding birds is 126,66 and the chorological spectrum has a prevalence of Palearctic species. The purpose of this study is to realize a new base for ornithological research in the next years and to identify priority lines of monitoring and conservation of bird-areas in the region.

Riassunto

La check-list della Campania annovera 361 specie di uccelli, 214 non-passeriformi e 147 passeriformi. A queste specie, vanno aggiunte 31 specie in lista AERC E e 7 specie in lista AERC X, che non sono state accettate dal Comitato di Omologazione Italiano (CISO-COI). Abbiamo registrato 154 specie nidificanti (147 regolari): 70 Non Passeriformi e 84 Passeriformi; due sono specie non autoctone della regione ed hanno popolazioni riproduttive autosufficienti: *Psittacula krameri* e *Acridotheres tristis*. Al di fuori di queste, sei specie hanno attualmente uno stato riproduttivo incerto e due sono dichiarate estinte a livello regionale. 90 specie hanno lo *status* di uccelli residenti. 51 specie sono esclusivamente migratrici e 70 (19%) sono state classificate accidentali, con otto che hanno una presenza "storica" (codice AERC: B).

Il presente studio aggiorna l'ultima checklist pubblicata (Fraissinet, 2015) effettuando una revisione critica della precedente lista al fine di valutare la classificazione dello stato di ogni taxon. La lista segue la classificazione sistematica della Check-list italiana (Brichetti & Fracasso, 2015) e la recente versione della IOC World Bird List (Gill et al., 2021). Viene pubblicato anche il primo elenco degli uccelli di origine aufuga osservati in ambito regionale (lista AERC E). L'Indice del Valore Ornitologico calcolato sugli uccelli nidificanti è pari a 126,66 e lo spettro corologico presenta una prevalenza di specie paleartiche. Lo scopo di questo studio è realizzare una nuova base per la ricerca ornitologica dei prossimi anni.

Parole chiave: Checklist, Uccelli, Campania, Italia meridionale

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Introduction

Localization and correct classification of a taxonomic group represented, and still represents, a need by scientific operators in the wild field, in nature conservation, in laws and administrations (Mayr & Bock 1994; Bruce 2003; Brichetti & Fracasso 2015). The species list of a single *taxon*, with related status on a certain territory, is the knowledge base to carry out researche and analysis, habitat management, conservation actions, law proposals and all the other interventions related to nature protection (Fig. 1).

The raising ornithological research on the field due to the presence of bird-ringing station in key sites, and their operability in constant effort, as well as birdwatchers and nature photographers observed in last decade in Campania (Fig. 2), have given a considerable boost to knowledge of regional avifauna. All that has provided relevant data on the distribution, population size, presence and identification of birds concerning the Mediterranean basin.

These reasons moved authors to draw up a new checklist, five years after the last one (Fraissinet 2015), with interesting changes and new species, collecting published works

Figure 1: Some typical environments of the Campania region. A, marine coast (Licosa); B, volcanic lake (d'Averno); C, river (Sele); D, stream (Lete); E, Forest and mountain pastures (Montola); F, Mountain forest (Cervati); G, agricultural (Eboli); H, small town (Controne); urban city (Naples). Photos by Domenico Fulgione.

on ornithological journals and unpublished data by our or by many enthusiastic persons interested, for various reasons, in wild birds of Campania

Another purpose of this updates checklist is the need to carry out a critical review of previously assigned status in some species, because new data occurred, specific survey are made and a prudential criteria was established on the status attribution. In a few cases it might seem as a "comeback" (e.g. species with a previous migratory status are reclassified as a vagrant) but other data analysis showed an effective rarefaction (e.g. species with regular migratory or wintering status became irregular). For these, and for all species where we retained necessary, a specific note was made.

Finally, yet importantly, there is the aim to connect and the need to update the Campania checklist with the taxonomic innovations occurred in recent years and, therefore, to produce a new list on which to base future comparisons.

Materials and Methods

Our checklist is also based on a careful and critical review of the previous regional checklists published in the last thirty years (Milone et al., 1989; Scebba 1993; Fraissinet et al., 2001; Fraissinet 2003; Fraissinet et al., 2007; Fraissinet, 2015) combined with new data collected in the last five years, in terms of acquisition of species and status change in Campania Region.

Figure 2: The Campania region in the Mediterranean region.

Collection of new data is based on the standard techniques used in ornithology, bird-ringing station activities, observation and song interpretation.

When possible, uncertain status reported in previous checklists is clarified, providing specific annotation for each species. Data, for determinations on the status of each species, are collected into the databases of *Associazione Studi Ornitologici Italia Meridionale - ASOIM* and *Istituto di Gestione della Fauna - IGF*. Furthermore, online databases (as: eBird, Ornitho, UBird), specialized mailing-lists (ASOIM-list, EBN Italia-list), and social pages of birdwatchers and nature photographers are consulted by authors. The checklist is updated to January 31th 2021.

Taxonomy follows the *IOC World Bird List*, using the latest version 11.1 (Gill et al., 2021). Authors decided to accept this taxonomy in contrast with the National Checklist (Fracasso et al., 2009; Baccetti et al., 2019) that follows the taxonomy recommended by *HBW-BirdLife Checklist* (HBW & BirdLife International 2017). Although the differences in taxonomies are minimal, among the species present in the Campania region, our decision principally moved on these reasons: the *IOC World Bird List* splits "Gulls "in genus *Larus*, *Chroicocephalus*, *Leucophaeus*, *Ichthyaetus* while *HBW* taxonomy only includes the *Larus* genus; *Corvus cornix* is admitted as a species and not as a subspecies. In addition to these reasons, *IOC World Bird list* is constantly updated to latest changes accepted in taxonomy and also that list has been used for the last published Italian Checklist (Brichetti & Fracasso 2015) and for the CISO-COI list of Italian birds of naturalized species and the accessory categories (Baccetti et al., 2014). The choice of the authors to follow this taxonomy is determined by to use same taxonomy present in previous checklists in order to carry out assessments and comparisons, primarily with the last version of the regional checklist (Fraissinet 2015).

English names of birds reported in the list follow the recommended birds name present in *IOC World Bird List* (Gill et al., 2021). In all lists, the Italian name of species primarily refers to the national checklist (Brichetti & Fracasso 2015), excluding *Columba livia* var. *domestica* that we named "Colombo domestico" in according to Boano et al. (2018). Italian names for released, escaped or non-naturalized species are taken from the specific national checklist (Baccetti et al., 2014); where no match was found, we report the name indicated by the web resource AVIBASE - The World Bird Database (2021).

The algorithm of Ornithological Value Index (IVO), elaborated by Massa et al. (2004) is applied on the breeding species present in checklist. Algorithm is the following:

$$\begin{aligned} \mathrm{IVO} = S_{Tot}\Big[\big(S_{Spec1} * 1\big) + \big(S_{Spec2} * 0{,}75\big) + \big(S_{Spec3} * 0{,}5\big) \\ + \big(S_{Non\ SpecE} * 0{,}25\big) + (S_{CR} * 1) + (S_{EN} * 0{,}75) \\ + (S_{VU} * 0{,}5) + (S_{LR} * 0{,}25) + S_{147}\Big] * 100^{-1} \end{aligned}$$

Where: S_{Tot} is the number of total breeding species; S_{Spec1}, S_{Spec2}, S_{Spec3}, $S_{Non\ SpecE}$ is the number of breeding species categorized as SPEC (BirdLIfe International, 2004; BirdLife International 2017); S_{CR}, S_{EN}, S_{VU}, S_{LR} is the number of breeding species categorized in the Italian Red List of Birds (Gustin et al., 2019); S_{147} is the number of breeding species reported in Annex I in Directive on the conservation of wild birds (2009/147/EC).

Categories used for the analysis of chorological spectrum refer to those indicated by Brichetti & Gariboldi (1997).

Status assessment

The attribution of status categories for each species is done applying the abbreviated traditional codes:

B - Breeder (*nidificante*);
S - Resident (*sedentaria*);
M - Migrant (*migratrice*);
W – Wintering (*svernante*);
E - Summer visitor (*estivante*), continuous presence of no resident birds outside the breeding territories;
N - Naturalized (*naturalizzata*);
reg - regular (*regolare*);
irr - irregular (*irregolare*);
? – uncertain status (*status incerto*);
A - Vagrant (*accidentale*), species with less than 10 records or with an uncertain number.

The code may have the number of known records and for those up to three years of observation are indicated.

The status codes sequence is determine by the importance that each category assumes for the species on the regional territory.

In order to provide a complete information on the status, in addition to the traditional classification, we categorize each species applying methodology indicated in the CISO-COI Italian Birds Checklist (Fracasso et al., 2009), reporting an alphanumeric code composed by three parts.

First code (letter/s) indicate the categories following the recommendations of the *Association of European Rarities and Records Committees* (AERC 1999; AERC TAC 2003):

A - species which has been recorded in an apparently wild state at least once since 1.1.1950;

B - species which has been recorded in an apparently wild state only between 1800 and 1949;

C - released or escaped species which has established a self-supporting breeding population in the own country; also birds coming from a category C population of another country (with the species not breeding in the own country);

D - every species unless it is almost certainly a genuine vagrant (in wich case it enters Cat. A), or almost certainly an escape from captivity (Cat. E);

E - escapees from captivity.

Authors used category "**AC**" to indicate species o the regional territory that present populations in category "A" and in "C".

An extra category, in according to Baccetti et al. (2014), is applied:

X - in this category we include all species excluded from the categories A | B | C, because their record is not submitted (or not accepted) to the *COI Italian Ornithological Committee*, deleted for insufficient data information or suspended by authors waiting for other data.

Second and third codes (numbers) indicate, respectively, the general (presence) and breeding status (Volet et al., 2000; Volet 2006; Fracasso et al., 2009).

Status:

1 -regular, species recorded in at least 9 years out of 10;

2 - irregular, species recorded more than 10 times and in less than 6 years after 1950 but in fewer than 9 years out of 10.

3 - accidental, species recorded 1-10 times or in 1-5 years after 1950;

4 - species recorded at least once but not since 1950.

Breeding status:

1 - regular breeder, recorded breeding in at least 9 years out of 10;

2 - irregular breeder, recorded breeding in at least 4-8 years out of 10;

3 - accidental breeder, recorded breeding only in at least 1-3 years out of 10 or more;

4 - former breeder, species which regularly bred during an earlier period but which has not been recorded breeding in last 10 years.

0 - species never recorded breeding.

Results and Discussion

The number of species recorded in the Campania region is 361, divided as follows: 342 reported in AERC category A; 8 in B; 8 in C; 3 in AC. This total is composed by 214 species from the non taxonomic group of Non-Passeriformes and 147 species to the Passeriformes Order.

The checklist of birds of Campania Region, updated on 31th January 2021, is as reported in Table 1.

Table 1: Checklist of birds of Campania Region (AERC A|B|C Lists) updated to 31th January 2021.

	Scientific name	English common name	Italian common name	Fenology	Code AERC	note
	ANSERIFORMES					
	Anatidae					
1	*Branta ruficollis*	Red-breasted Goose	Oca collorosso	A 1 (1940)	B40	
2	*Anser anser*	Greylag Goose	Oca selvatica	M irr, W irr	A20	
3	*Anser fabalis*	Taiga Bean Goose	Oca granaiola	M irr	A20	
4	*Anser albifrons*	Greater White-fronted Goose	Oca lombardella	M irr, W irr	A20	
5	*Cygnus olor*	Mute Swan	Cigno reale	M irr, W irr	AC11	
6	*Cygnus columbianus*	Tundra Swan	Cigno minore	A 1 (2015)	A30	
7	*Tadorna tadorna*	Common Shelduck	Volpoca	M, W, B?	A10	1
8	*Tadorna ferruginea*	Ruddy Shelduck	Casarca	M reg?	A10	
9	*Spatula querquedula*	Garganey	Marzaiola	M, W irr	A10	
10	*Spatula clypeata*	Northern Shoveler	Mestolone	M, W	A10	
11	*Mareca strepera*	Gadwall	Canapiglia	M, W	A10	
12	*Mareca penelope*	Eurasian Wigeon	Fischione	M, W	A10	
13	*Anas platyrhynchos*	Mallard	Germano reale	M, SB, W	A11	
14	*Anas acuta*	Northern Pintail	Codone	M, W	A10	
15	*Anas crecca*	Eurasian Teal	Alzavola	M,W, B?	A10	2
16	*Marmaronetta angustirostris*	Marbled Duck	Anatra marmorizzata	A 1 (1858)	B40	
17	*Netta rufina*	Red-crested Pochard	Fistione turco	M, W, B irr	A13	3
18	*Aythya ferina*	Common Pochard	Moriglione	M, W, B	A11	
19	*Aythya nyroca*	Ferruginous Duck	Moretta tabaccata	M, W, SB	A11	
20	*Aythya fuligula*	Tufted Duck	Moretta	M, W	A10	

21	*Aythya marila*	Greater Scaup	Moretta grigia	M irr, W irr	A20
22	*Somateria mollissima*	Common Eider	Edredone	M irr, W irr	A20
23	*Melanitta fusca*	Velvet Scoter	Orco marino	M, W	A10
24	*Melanitta nigra*	Common Scoter	Orchetto marino	M, W	A10
25	*Clangula hyemalis*	Long-tailed Duck	Moretta codona	A 1 (2016)	A30
26	*Bucephala clangula*	Common Goldeneye	Quattrocchi	A 9	A30
27	*Mergellus albellus*	Smew	Pesciaiola	A 1 (1991)	A30
28	*Mergus serrator*	Red-breasted Merganser	Smergo minore	M, W	A10
29	*Oxyura jamaicensis*	Ruddy Duck	Gobbo della Giamaica	A 3 ('90, 2007, 2020)	C30
30	*Oxyura leucocephala*	White-headed Duck	Gobbo rugginoso	A 7	B40
	GALLIFORMES				
	Phasianidae				
31	*Alectoris graeca*	Rock Partridge	Coturnice	SB	AC11
32	*Perdix perdix*	Grey Partridge	Starna	SB?	C13
33	*Coturnix coturnix*	Common Quail	Quaglia	M, B, W irr	A11
34	*Phasianus colchicus*	Common Pheasant	Fagiano comune	SB	C11
	CAPRIMULGIFORMES				
	Caprimulgidae				
35	*Caprimulgus europaeus*	European Nightjar	Succiacapre	M, B	A11
	APODIFORMES				
	Apodidae				
36	*Tachymarptis melba*	Alpine Swift	Rondone maggiore	M, B	A11
37	*Apus apus*	Common Swift	Rondone comune	M, B	A11
38	*Apus pallidus*	Pallid Swift	Rondone pallido	M, B	A11
	OTIDIFORMES				
	Otididae				

39	*Otis tarda*	Great Bustard	Otarda	A 2 (1969, 1989/90)	A30	
40	*Tetrax tetrax*	Little Bustard	Gallina prataiola	A 2 (1965, 1967)	A30	
	CUCULIFORMES					
	Cuculidae					
41	*Clamator glandarius*	Great Spotted Cuckoo	Cuculo dal ciuffo	M, B	A11	
42	*Cuculus canorus*	Common Cuckoo	Cuculo	M, B	A11	
	COLUMBIFORMES					
	Columbidae					
43	*Columba livia* var. *domestica*	Rock Dove	Colombo domestico	SB	AC11	4
44	*Columba oenas*	Stock Dove	Colombella	M, W, B irr	A12	
45	*Columba palumbus*	Common Wood Pigeon	Colombaccio	SB, M, W	A11	
46	*Streptopelia turtur*	European Turtle Dove	Tortora selvatica	M, B	A11	
47	*Streptopelia decaocto*	Eurasian Collared Dove	Tortora dal collare	SB	A11	
	GRUIFORMES					
	Rallidae					
48	*Rallus aquaticus*	Water Rail	Porciglione	SB, M, W	A11	
49	*Crex crex*	Corn Crake	Re di quaglie	M	A10	
50	*Porzana porzana*	Spotted Crake	Voltolino	M, W irr	A10	
51	*Gallinula chloropus*	Common Moorhen	Gallinella d'acqua	SB, M, W	A11	
52	*Fulica atra*	Eurasian Coot	Folaga	W, M, SB	A11	
53	*Porphyrio porphyrio*	Western Swamphen	Pollo sultano	A 2 (1884, 1897)	B40	
54	*Zapornia pusilla*	Baillon's Crake	Schiribilla grigiata	A	A30	5
55	*Zapornia parva*	Little Crake	Schiribilla	M, W irr?	A10	
	Gruidae					
56	*Grus grus*	Common Crane	Gru	M, W irr	A10	
	PODICIPEDIFORMES					

	Podicipedidae					
57	*Tachybaptus ruficollis*	Little Grebe	Tuffetto	M, W, SB	A11	
58	*Podiceps grisegena*	Red-necked Grebe	Svasso collorosso	A 3 (1854, 1910, 2009)	A30	
59	*Podiceps cristatus*	Great Crested Grebe	Svasso maggiore	M, W, SB	A11	
60	*Podiceps auritus*	Horned Grebe	Svasso cornuto	A 4	A30	
61	*Podiceps nigricollis*	Black-necked Grebe	Svasso piccolo	M, W	A10	
	PHOENICOPTERIFORMES					
	Phoenicopteridae					
62	*Phoenicopterus roseus*	Greater Flamingo	Fenicottero	M, W	A10	
	CHARADRIIFORMES					
	Burhinidae					
63	*Burhinus oedicnemus*	Eurasian Stone-curlew	Occhione	M	A10	
	Haematopodidae					
64	*Haematopus ostralegus*	Eurasian Oystercatcher	Beccaccia di mare	M	A10	
	Recurvirostridae					
65	*Himantopus himantopus*	Black-winged Stilt	Cavaliere d'Italia	M, B, W irr	A11	6
66	*Recurvirostra avosetta*	Pied Avocet	Avocetta	M, W irr, B irr	A13	7
	Charadriidae					
67	*Vanellus vanellus*	Northern Lapwing	Pavoncella	M, W	A10	
68	*Pluvialis apricaria*	European Golden Plover	Piviere dorato	M, W	A10	
69	*Pluvialis fulva*	Pacific Golden Plover	Piviere orientale	A 2 (2017, 2020)	A30	
70	*Pluvialis squatarola*	Grey Plover	Pivieressa	M, W irr	A10	
71	*Charadrius hiaticula*	Common Ringed Plover	Corriere grosso	M	A10	
72	*Charadrius dubius*	Little Ringed Plover	Corriere piccolo	M, B, W irr	A11	
73	*Charadrius alexandrinus*	Kentish Plover	Fratino	M, B, W	A11	
74	*Charadrius morinellus*	Eurasian Dotterel	Piviere tortolino	M	A10	

	Scolopacidae					
75	*Numenius phaeopus*	Eurasian Whimbrel	Chiurlo piccolo	M	A10	
76	*Numenius tenuirostris*	Slender-billed Curlew	Chiurlottello	A 5	A30	8
77	*Numenius arquata*	Eurasian Curlew	Chiurlo maggiore	M, W	A10	
78	*Limosa lapponica*	Bar-tailed Godwit	Pittima minore	M	A10	
79	*Limosa limosa*	Black-tailed Godwit	Pittima reale	M	A10	
80	*Arenaria interpres*	Ruddy Turnstone	Voltapietre	M	A10	
81	*Calidris canutus*	Red Knot	Piovanello maggiore	M	A10	
82	*Calidris pugnax*	Ruff	Combattente	M, W irr	A10	
83	*Calidris falcinellus*	Broad-billed Sandpiper	Gambecchio frullino	M	A10	
84	*Calidris ferruginea*	Curlew Sandpiper	Piovanello comune	M	A10	
85	*Calidris temminckii*	Temminck's Stint	Gambecchio nano	M, W	A10	
86	*Calidris alba*	Sanderling	Piovanello tridattilo	M, W	A10	
87	*Calidris alpina*	Dunlin	Piovanello pancianera	M, W	A10	
88	*Calidris minuta*	Little Stint	Gambecchio comune	M, W	A10	
89	*Calidris subruficollis*	Buff-breasted Sandpiper	Piro piro fulvo	A 1 (2007)	A30	
90	*Calidris melanotos*	Pectoral Sandpiper	Piovanello pettorale	A 2 (2010, 2020)	A30	
91	*Scolopax rusticola*	Eurasian Woodcock	Beccaccia	M, W	A10	
92	*Lymnocryptes minimus*	Jack Snipe	Frullino	M, W	A10	
93	*Gallinago media*	Great Snipe	Croccolone	M	A10	
94	*Gallinago gallinago*	Common Snipe	Beccaccino	M, W	A10	
95	*Xenus cinereus*	Terek Sandpiper	Piro piro del Terek	M reg?	A10	
96	*Phalaropus lobatus*	Red-necked Phalarope	Falaropo beccosottile	A 4	A30	
97	*Phalaropus fulicarius*	Red Phalarope	Falaropo beccolargo	A 3 (1869, 1870, 1956)	A30	
98	*Actitis hypoleucos*	Common Sandpiper	Piro piro piccolo	M, W	A10	
99	*Tringa ochropus*	Green Sandpiper	Piro piro culbianco	M, W	A10	

100	*Tringa totanus*	Common Redshank	Pettegola	M, W	A10
101	*Tringa stagnatilis*	Marsh Sandpiper	Albastrello	M	A10
102	*Tringa glareola*	Wood Sandpiper	Piro piro boschereccio	M, W irr	A10
103	*Tringa erythropus*	Spotted Redshank	Totano moro	M, W	A10
104	*Tringa nebularia*	Common Greenshank	Pantana	M, W	A10
	Glareolidae				
105	*Cursorius cursor*	Cream-colored Courser	Corrione biondo	A 1 (1975)	A30
106	*Glareola pratincola*	Collared Pratincole	Pernice di mare	M, B irr	A12
	Laridae				
107	*Rissa tridactyla*	Black-legged Kittiwake	Gabbiano tridattilo	M, W irr	A20
108	*Chroicocephalus genei*	Slender-billed Gull	Gabbiano roseo	M, W	A10
109	*Chroicocephalus ridibundus*	Black-headed Gull	Gabbiano comune	M, W, E, B irr	A13
110	*Hydrocoloeus minutus*	Little Gull	Gabbianello	M, W	A10
111	*Rhodostethia rosea*	Ross's Gull	Gabbiano di Ross	A 1 (1997)	A30
112	*Leucophaeus pipixcan*	Franklin's Gull	Gabbiano di Franklin	A 1 (2014)	A30
113	*Ichthyaetus audouinii*	Audouin's Gull	Gabbiano corso	SB, M, W	A11
114	*Ichthyaetus melanocephalus*	Mediterranean Gull	Gabbiano corallino	M, W, E	A10
115	*Ichthyaetus ichthyaetus*	Pallas's Gull	Gabbiano di Pallas	A 1 (2004)	A30
116	*Larus canus*	Mew Gull	Gavina	M, W	A10
117	*Larus marinus*	Great Black-backed Gull	Mugnaiaccio	M irr, W irr	A20
118	*Larus argentatus*	European Herring Gull	Gabbiano reale nordico	M irr, W irr	A20
119	*Larus cachinnans*	Caspian Gull	Gabbiano reale pontico	M irr, W irr	A20
120	*Larus michahellis*	Yellow-legged Gull	Gabbiano reale	SB, M, W	A11
121	*Larus fuscus*	Lesser Black-backed Gull	Zafferano	M, W	A10
122	*Gelochelidon nilotica*	Gull-billed Tern	Sterna zampenere	M	A10
123	*Hydroprogne caspia*	Caspian Tern	Sterna maggiore	M	A10

124	*Thalasseus bengalensis*	Lesser Crested Tern	Sterna di Rueppell	A 5	A30	9
125	*Thalasseus sandvicensis*	Sandwich Tern	Beccapesci	M, E, W	A10	
126	*Sternula albifrons*	Little Tern	Fraticello	M	A10	10
127	*Sterna hirundo*	Common Tern	Sterna comune	M	A10	
128	*Sterna paradisaea*	Arctic Tern	Sterna codalunga	A 2 (2004, 2006)	A30	
129	*Chlidonias hybrida*	Whiskered Tern	Mignattino piombato	M	A10	
130	*Chlidonias leucopterus*	White-winged Tern	Mignattino alibianche	M	A10	
131	*Chlidonias niger*	Black Tern	Mignattino comune	M	A10	
	Stercorariidae					
132	*Stercorarius skua*	Great Skua	Stercorario maggiore	A 4	A30	
133	*Stercorarius pomarinus*	Pomarine Jaeger	Stercorario mezzano	M, W	A10	
134	*Stercorarius parasiticus*	Parasitic Jaeger	Labbo	M, W	A10	
135	*Stercorarius longicaudus*	Long-tailed Jaeger	Labbo codalunga	A 1 (1974)	A30	
	Alcidae					
136	*Alle alle*	Little Auk	Gazza marina minore	A 1 (1978?)	A30	
137	*Alca torda*	Razorbill	Gazza marina	A 8	A30	
138	*Fratercula arctica*	Atlantic Puffin	Pulcinella di mare	A 5	A30	
	GAVIIFORMES					
	Gaviidae					
139	*Gavia stellata*	Red-throated Loon	Strolaga minore	M, W	A10	
140	*Gavia arctica*	Black-throated Loon	Strolaga mezzana	M irr, W irr	A20	
	PROCELLARIIFORMES					
	Hydrobatidae					
141	*Hydrobates pelagicus*	European Storm Petrel	Uccello delle tempeste	M reg?	A20	
	Procellariidae					
142	*Calonectris diomedea*	Scopoli's Shearwater	Berta maggiore	M	A10	

143	*Puffinus yelkouan*	Yelkouan Shearwater	Berta minore	M, W	A10	
144	*Puffinus mauretanicus*	Balearic Shearwater	Berta balearica	A 2 (2011, 2012)	A30	11
	CICONIIFORMES					
	Ciconiidae					
145	*Ciconia nigra*	Black Stork	Cicogna nera	M, B, E	A11	
146	*Ciconia ciconia*	White Stork	Cicogna bianca	M, B	A11	
	SULIFORMES					
	Sulidae					
147	*Morus bassanus*	Northern Gannet	Sula	M, W	A10	
	Phalacrocoracidae					
148	*Microcarbo pygmaeus*	Pygmy Cormorant	Marangone minore	M, SB	A11	
150	*Phalacrocorax carbo*	Great Cormorant	Cormorano	M, W, SB	A11	
149	*Phalacrocorax aristotelis*	European Shag	Marangone dal ciuffo	M	A10	12
	PELECANIFORMES					
	Threskiornithidae					
151	*Threskiornis aethiopicus*	African Sacred Ibis	Ibis sacro	A	C30	13
152	*Plegadis falcinellus*	Glossy Ibis	Mignattaio	M, B, W	A11	
153	*Platalea leucorodia*	Eurasian Spoonbill	Spatola	M, W, E	A10	
	Ardeidae					
154	*Botaurus stellaris*	Eurasian Bittern	Tarabuso	M, W	A10	
155	*Ixobrychus minutus*	Little Bittern	Tarabusino	M, B	A11	
156	*Nycticorax nycticorax*	Black-crowned Night Heron	Nitticora	M, B, W	A11	
157	*Ardeola ralloides*	Squacco Heron	Sgarza ciuffetto	M, B	A11	14
158	*Bubulcus ibis*	Western Cattle Egret	Airone guardabuoi	SB, M, W	A11	
159	*Ardea cinerea*	Grey Heron	Airone cenerino	M, W, SB	A11	
160	*Ardea purpurea*	Purple Heron	Airone rosso	M, B	A11	

161	*Ardea alba*	Great Egret	Airone bianco maggiore	M, W, E	A10	
162	*Egretta garzetta*	Little Egret	Garzetta	M, SB, W	A11	
163	*Egretta gularis*	Western Reef Heron	Airone schistaceo	A 2 (1976, 2014)	A30	
	Pelecanidae					
164	*Pelecanus onocrotalus*	Great White Pelican	Pellicano comune	A 3 (2009, 2011, 2013)	A30	
	ACCIPITRIFORMES					
	Pandionidae					
165	*Pandion haliaetus*	Western Osprey	Falco pescatore	M, W irr	A10	
	Accipitridae					
166	*Neophron percnopterus*	Egyptian Vulture	Capovaccaio	M irr, B estinto	A24	
167	*Pernis apivorus*	European Honey Buzzard	Falco pecchiaiolo	M, B	A11	
168	*Gyps fulvus*	Griffon Vulture	Grifone	M reg?	C10	
169	*Aegypius monachus*	Cinereous Vulture	Avvoltoio monaco	A 1 (2015)	C30	
170	*Circaetus gallicus*	Short-toed Snake Eagle	Biancone	M, B, W irr	A11	
171	*Clanga pomarina*	Lesser Spotted Eagle	Aquila anatraia minore	A 2 (2017, 2020)	A30	
172	*Clanga clanga*	Greater Spotted Eagle	Aquila anatraia maggiore	A 3 (1-'900, 2-'50/'60)	A30	
173	*Hieraaetus pennatus*	Booted Eagle	Aquila minore	M, W	A10	
174	*Aquila heliaca*	Eastern Imperial Eagle	Aquila imperiale	A 1 (2013)	A30	
175	*Aquila chrysaetos*	Golden Eagle	Aquila reale	SB	A11	
176	*Aquila fasciata*	Bonelli's Eagle	Aquila di Bonelli	A 2 (1889, 1957)	A30	15
177	*Accipiter nisus*	Eurasian Sparrowhawk	Sparviere	SB, M, W	A11	
178	*Accipiter gentilis*	Northern Goshawk	Astore	SB, M irr	A11	
179	*Circus aeruginosus*	Western Marsh Harrier	Falco di palude	M, W, E	A10	
180	*Circus cyaneus*	Hen Harrier	Albanella reale	M, W	A10	
181	*Circus macrourus*	Pallid Harrier	Albanella pallida	M, W irr	A10	
182	*Circus pygargus*	Montagu's Harrier	Albanella minore	M	A10	

183	*Milvus milvus*	Red Kite	Nibbio reale	SB, M, W	A11	
184	*Milvus migrans*	Black Kite	Nibbio bruno	B, M, W	A11	
185	*Haliaeetus albicilla*	White-tailed Eagle	Aquila di mare	A 3 (1977, 1982, 1992)	A30	
186	*Buteo rufinus*	Long-legged Buzzard	Poiana codabianca	M irr	A20	
187	*Buteo buteo*	Common Buzzard	Poiana	SB, M, W	A11	
	STRIGIFORMES					
	Tytonidae					
188	*Tyto alba*	Western Barn Owl	Barbagianni	SB	A11	
	Strigidae					
189	*Athene noctua*	Little Owl	Civetta	SB	A11	
190	*Otus scops*	Eurasian Scops Owl	Assiolo	M, B, W	A11	
191	*Asio otus*	Long-eared Owl	Gufo comune	M, W, B	A11	
192	*Asio flammeus*	Short-eared Owl	Gufo di palude	M, W irr	A10	
193	*Bubo bubo*	Eurasian Eagle-Owl	Gufo reale	SB estinto	A24	16
194	*Strix aluco*	Tawny Owl	Allocco	SB	A11	
	BUCEROTIFORMES					
	Upupidae					
195	*Upupa epops*	Eurasian Hoopoe	Upupa	M, B	A11	
	CORACIIFORMES					
	Coraciidae					
196	*Coracias garrulus*	European Roller	Ghiandaia marina	M, B	A11	
	Alcedinidae					
197	*Alcedo atthis*	Common Kingfisher	Martin pescatore	M, W, SB	A11	
	Meropidae					
198	*Merops apiaster*	European Bee-eater	Gruccione	M, B	A11	
	PICIFORMES					

	Picidae					
199	*Jynx torquilla*	Eurasian Wryneck	Torcicollo	M, B, W	A11	
200	*Dendrocoptes medius*	Middle Spotted Woodpecker	Picchio rosso mezzano	SB	A11	
201	*Dryobates minor*	Lesser Spotted Woodpecker	Picchio rosso minore	SB	A11	
202	*Dendrocopos major*	Great Spotted Woodpecker	Picchio rosso maggiore	SB	A11	
203	*Dryocopus martius*	Black Woodpecker	Picchio nero	SB	A11	
204	*Picus viridis*	European Green Woodpecker	Picchio verde	SB	A11	
	FALCONIFORMES					
	Falconidae					
205	*Falco naumanni*	Lesser Kestrel	Grillaio	M	A10	17
206	*Falco tinnunculus*	Common Kestrel	Gheppio	SB, M, W	A11	
207	*Falco vespertinus*	Red-footed Falcon	Falco cuculo	M	A10	
208	*Falco eleonorae*	Eleonora's Falcon	Falco della regina	M reg?	A10	
209	*Falco columbarius*	Merlin	Smeriglio	M, W reg?	A10	
210	*Falco subbuteo*	Eurasian Hobby	Lodolaio	M, B	A11	
211	*Falco biarmicus*	Lanner Falcon	Lanario	SB?	A12	18
212	*Falco cherrug*	Saker Falcon	Sacro	A 2 (1993, 2003)	A30	
213	*Falco peregrinus*	Peregrine Falcon	Falco pellegrino	SB, M, W	A11	
	PSITTACIFORMES					
	Psittaculidae					
214	*Psittacula krameri*	Rose-ringed Parakeet	Parrocchetto dal collare	SBN	C11	
	PASSERIFORMES					
	Laniidae					
215	*Lanius collurio*	Red-backed Shrike	Averla piccola	M, B	A11	
216	*Lanius minor*	Lesser Grey Shrike	Averla cenerina	B, M	A11	
217	*Lanius excubitor*	Great Grey Shrike	Averla maggiore	M irr, W irr	A20	19

218	*Lanius senator*	Woodchat Shrike	Averla capirossa	M, B	A11
	Oriolidae				
219	*Oriolus oriolus*	Eurasian Golden Oriole	Rigogolo	M, B	A11
	Corvidae				
220	*Garrulus glandarius*	Eurasian Jay	Ghiandaia	SB	A11
221	*Pica pica*	Eurasian Magpie	Gazza	SB	A11
222	*Pyrrhocorax pyrrhocorax*	Red-billed Chough	Gracchio corallino	SB	A11
223	*Coloeus monedula*	Western Jackdaw	Taccola	SB, M, W	A11
224	*Corvus frugilegus*	Rook	Corvo comune	A 5	A30
225	*Corvus cornix*	Hooded Crow	Cornacchia grigia	SB	A11
226	*Corvus corax*	Northern Raven	Corvo imperiale	SB	A11
	Bombycillidae				
227	*Bombycilla garrulus*	Bohemian Waxwing	Beccofrusone	A 2 (1965, 1966)	A30
	Paridae				
228	*Periparus ater*	Coal Tit	Cincia mora	SB, M, W	A11
229	*Lophophanes cristatus*	European Crested Tit	Cincia dal ciuffo	A 2 (XIX sec, 1891)	B40
230	*Poecile palustris*	Marsh Tit	Cincia bigia	SB	A11
231	*Cyanistes caeruleus*	Eurasian Blue Tit	Cinciarella	SB, M, W	A11
232	*Parus major*	Great Tit	Cinciallegra	SB	A11
	Remizidae				
233	*Remiz pendulinus*	Eurasian Penduline Tit	Pendolino	M, W, SB	A11
	Panuridae				
234	*Panurus biarmicus*	Bearded Reedling	Basettino	M irr, W irr	A20
	Alaudidae				
235	*Lullula arborea*	Woodlark	Tottavilla	SB, M, W	A11
236	*Alauda arvensis*	Eurasian Skylark	Allodola	M, W, SB	A11

237	*Galerida cristata*	Crested Lark	Cappellaccia	SB	A11	
238	*Eremophila alpestris*	Horned Lark	Allodola golagialla	A 2 (1868, 1874)	B40	
239	*Calandrella brachydactyla*	Greater Short-toed Lark	Calandrella	M, B	A11	
240	*Melanocorypha calandra*	Calandra Lark	Calandra	SB, M, W	A11	
	Hirundinidae					
241	*Riparia riparia*	Sand Martin	Topino	M, B?	A14	20
242	*Hirundo rustica*	Barn Swallow	Rondine	M, B, W irr	A11	
243	*Ptyonoprogne rupestris*	Eurasian Crag Martin	Rondine montana	SB	A11	
244	*Delichon urbicum*	Common House Martin	Balestruccio	M, B	A11	
245	*Cecropis daurica*	Red-rumped Swallow	Rondine rossiccia	M, B irr	A12	
	Cettiidae					
246	*Cettia cetti*	Cetti's Warbler	Usignolo di fiume	SB	A11	
	Aegithalidae					
247	*Aegithalos caudatus*	Long-tailed Tit	Codibugnolo	SB	A11	
	Phylloscopidae					
248	*Phylloscopus sibilatrix*	Wood Warbler	Luì verde	M, B	A11	
249	*Phylloscopus bonelli*	Western Bonelli's Warbler	Luì bianco	M, B	A11	
250	*Phylloscopus humei*	Hume's Leaf Warbler	Luì di Hume	A 1 (1989)	A30	
251	*Phylloscopus proregulus*	Pallas's Leaf Warbler	Luì di Pallas	A 1 (2003)	A30	
252	*Phylloscopus trochilus*	Willow Warbler	Luì grosso	M	A10	
253	*Phylloscopus collybita*	Common Chiffchaff	Luì piccolo	M, W, SB	A11	
	Acrocephalidae					
254	*Acrocephalus arundinaceus*	Great Reed Warbler	Cannareccione	M, B	A11	
255	*Acrocephalus melanopogon*	Moustached Warbler	Forapaglie castagnolo	M, W	A10	21
256	*Acrocephalus schoenobaenus*	Sedge Warbler	Forapaglie comune	M	A10	
257	*Acrocephalus scirpaceus*	Eurasian Reed Warbler	Cannaiola comune	M, B	A11	

258	*Acrocephalus palustris*	Marsh Warbler	Cannaiola verdognola	M reg?	A20	
259	*Iduna pallida*	Eastern Olivaceous Warbler	Canapino pallido orientale	A 1 (1998)	A30	
260	*Hippolais polyglotta*	Melodious Warbler	Canapino comune	M, B	A11	
261	*Hippolais icterina*	Icterine Warbler	Canapino maggiore	M	A10	
	Locustellidae					
262	*Locustella luscinioides*	Savi's Warbler	Salciaiola	M	A10	
263	*Locustella naevia*	Common Grasshopper Warbler	Forapaglie macchiettato	M irr	A30	22
	Cisticolidae					
264	*Cisticola juncidis*	Zitting Cisticola	Beccamoschino	SB	A11	
	Sylviidae					
265	*Sylvia atricapilla*	Eurasian Blackcap	Capinera	SB, M, W	A11	
266	*Sylvia borin*	Garden Warbler	Beccafico	M	A10	
267	*Curruca curruca*	Lesser Whitethroat	Bigiarella	M, W irr	A10	
268	*Curruca hortensis*	Western Orphean Warbler	Bigia grossa occidentale	M irr	A20	
269	*Curruca ruppeli*	Rüppell's Warbler	Bigia di Rueppell	M irr	A20	
270	*Curruca melanocephala*	Sardinian Warbler	Occhiocotto	SB, M, W	A11	
271	*Curruca subalpina*	Moltoni's Warbler	Sterpazzolina di Moltoni	M	A10	
272	*Curruca cantillans*	Eastern Subalpine Warbler	Sterpazzolina comune	M, B	A11	23
273	*Curruca communis*	Common Whitethroat	Sterpazzola	M, B	A11	
274	*Curruca conspicillata*	Spectacled Warbler	Sterpazzola della Sardegna	M, B	A11	
275	*Curruca sarda*	Marmora's Warbler	Magnanina sarda	M irr	A20	
276	*Curruca undata*	Dartford Warbler	Magnanina comune	SB, M, W	A11	
	Regulidae					
277	*Regulus ignicapilla*	Common Firecrest	Fiorrancino	SB, M, W	A11	
278	*Regulus regulus*	Goldcrest	Regolo	M, W	A10	
	Troglodytidae					

279	*Troglodytes troglodytes*	Eurasian Wren	Scricciolo	SB, M, W	A11
	Sittidae				
280	*Sitta europaea*	Eurasian Nuthatch	Picchio muratore	SB	A11
	Tichodromidae				
281	*Tichodroma muraria*	Wallcreeper	Picchio muraiolo	M, W	A10
	Certhiidae				
282	*Certhia familiaris*	Eurasian Treecreeper	Rampichino alpestre	SB	A11
283	*Certhia brachydactyla*	Short-toed Treecreeper	Rampichino comune	SB	A11
	Sturnidae				
284	*Acridotheres tristis*	Common Myna	Maina comune	SBN	C11
285	*Pastor roseus*	Rosy Starling	Storno roseo	A 2 (post 1950, 1962)	A30
286	*Sturnus vulgaris*	Common Starling	Storno	M, W, SB	A11
287	*Sturnus unicolor*	Spotless Starling	Storno nero	A 1 (1992)	A30
	Turdidae				
288	*Turdus torquatus*	Ring Ouzel	Merlo dal collare	M reg?	A10
289	*Turdus merula*	Common Blackbird	Merlo	SB, M, W	A11
290	*Turdus obscurus*	Eyebrowed Thrush	Tordo oscuro	A 1 (1891)	B40
291	*Turdus pilaris*	Fieldfare	Cesena	M, W	A10
292	*Turdus iliacus*	Redwing	Tordo sassello	M, W	A10
293	*Turdus philomelos*	Song Thrush	Tordo bottaccio	M, W, B	A11
294	*Turdus viscivorus*	Mistle Thrush	Tordela	SB, M, W	A11
	Muscicapidae				
295	*Cercotrichas galactotes*	Rufous-tailed Scrub Robin	Usignolo d’Africa	A 2 (1978, 1979)	A30
296	*Muscicapa striata*	Spotted Flycatcher	Pigliamosche	M, B	A11
297	*Erithacus rubecula*	European Robin	Pettirosso	M, W, SB	A11
298	*Luscinia svecica*	Bluethroat	Pettazzurro	M, W	A10

299	*Luscinia luscinia*	Thrush Nightingale	Usignolo maggiore	A 1 (1986)	A30	
300	*Luscinia megarhynchos*	Common Nightingale	Usignolo	M, B	A11	
301	*Tarsiger cyanurus*	Red-flanked Bluetail	Codazzurro	A 2 (2008, 2014)	A30	
302	*Ficedula parva*	Red-breasted Flycatcher	Pigliamosche pettirosso	A 1 (2014)	A30	24
303	*Ficedula semitorquata*	Semicollared Flycatcher	Balia caucasica	M irr	A20	
304	*Ficedula hypoleuca*	European Pied Flycatcher	Balia nera	M	A10	
305	*Ficedula albicollis*	Collared Flycatcher	Balia dal collare	M, B	A11	
306	*Phoenicurus ochruros*	Black Redstart	Codirosso spazzacamino	M, W, B	A11	
307	*Phoenicurus phoenicurus*	Common Redstart	Codirosso comune	M, B	A11	
308	*Monticola saxatilis*	Common Rock Thrush	Codirossone	M, B	A11	
309	*Monticola solitarius*	Blue Rock Thrush	Passero solitario	SB, M, W	A11	
310	*Saxicola rubetra*	Whinchat	Stiaccino	M	A10	25
311	*Saxicola rubicola*	European Stonechat	Saltimpalo	M, W, B	A11	
312	*Saxicola maurus*	Siberian Stonechat	Saltimpalo siberiano	A 1 (2018)	A30	26
313	*Oenanthe oenanthe*	Northern Wheatear	Culbianco	M, B	A11	
314	*Oenanthe isabellina*	Isabelline Wheatear	Culbianco isabellino	A 1 (1971)	A30	
315	*Oenanthe deserti*	Desert Wheatear	Monachella del deserto	A 4	A30	
316	*Oenanthe hispanica*	Western Black-eared Wheatear	Monachella	M, B	A11	
317	*Oenanthe pleschanka*	Pied Wheatear	Monachella dorsonero	A 1 (1961)	A30	
	Cinclidae					
318	*Cinclus cinclus*	White-throated Dipper	Merlo acquaiolo	SB	A11	
	Passeridae					
319	*Passer domesticus*	House Sparrow	Passera oltremontana	A 2 (1991, 2020)	A30	
320	*Passer italiae*	Italian Sparrow	Passera d'Italia	SB	A11	
321	*Passer hispaniolensis*	Spanish Sparrow	Passera sarda	M, W, SB	A11	
322	*Passer montanus*	Eurasian Tree Sparrow	Passera mattugia	SB	A11	

323	*Petronia petronia*	Rock Sparrow	Passera lagia	SB	A11	
324	*Montifringilla nivalis*	White-winged Snowfinch	Fringuello alpino	M, W, B irr	A13	27
	Prunellidae					
325	*Prunella collaris*	Alpine Accentor	Sordone	M, W	A10	
326	*Prunella modularis*	Dunnock	Passera scopaiola	M, W	A10	28
	Motacillidae					
327	*Motacilla flava*	Western Yellow Wagtail	Cutrettola	M, B	A11	
328	*Motacilla citreola*	Citrine Wagtail	Cutrettola testagialla orientale	A 2 (2015, 2017)	A30	
329	*Motacilla cinerea*	Grey Wagtail	Ballerina gialla	SB, M, W	A11	
330	*Motacilla alba*	White Wagtail	Ballerina bianca	M, W, SB	A11	
331	*Anthus richardi*	Richard's Pipit	Calandro maggiore	M irr, W irr	A30	29
332	*Anthus campestris*	Tawny Pipit	Calandro	M, B	A11	
333	*Anthus pratensis*	Meadow Pipit	Pispola	M, W	A10	
334	*Anthus trivialis*	Tree Pipit	Prispolone	M, B	A11	
335	*Anthus cervinus*	Red-throated Pipit	Pispola golarossa	M	A10	
336	*Anthus spinoletta*	Water Pipit	Spioncello	M, W, B	A11	
337	*Anthus petrosus*	European Rock Pipit	Spioncello marino	A 1 (2004)	A30	
	Fringillidae					
338	*Fringilla coelebs*	Common Chaffinch	Fringuello	M, W, SB	A11	
339	*Fringilla montifringilla*	Brambling	Peppola	M, W	A10	
340	*Coccothraustes coccothraustes*	Hawfinch	Frosone	M, W, B	A11	
341	*Pyrrhula pyrrhula*	Eurasian Bullfinch	Ciuffolotto	SB	A11	
342	*Bucanetes githagineus*	Trumpeter Finch	Trombettiere	A 3 (1994, 2004, 2010)	A30	
343	*Carpodacus erythrinus*	Common Rosefinch	Ciuffolotto scarlatto	A 2 (1981, 2020)	A30	
344	*Chloris chloris*	European Greenfinch	Verdone	SB, M, W	A11	
345	*Linaria cannabina*	Common Linnet	Fanello	M, W, SB	A11	

346	*Acanthis flammea*	Common Redpoll	Organetto	A 1 (1912)	B40	
347	*Loxia curvirostra*	Red Crossbill	Crociere	M irr, W irr, B irr?	A13	30
348	*Carduelis carduelis*	European Goldfinch	Cardellino	SB, M, W	A11	
349	*Serinus serinus*	European Serin	Verzellino	SB, M, W	A11	
350	*Spinus spinus*	Eurasian Siskin	Lucherino	M, W	A10	
	Calcariidae					
351	*Calcarius lapponicus*	Lapland Longspur	Zigolo della Lapponia	A 2 (1964, 2013)	A30	
352	*Plectrophenax nivalis*	Snow Bunting	Zigolo delle nevi	A 5	A30	
	Emberizidae					
353	*Emberiza calandra*	Corn Bunting	Strillozzo	SB, M, W	A11	
354	*Emberiza citrinella*	Yellowhammer	Zigolo giallo	SB, M, W	A11	
355	*Emberiza cia*	Rock Bunting	Zigolo muciatto	SB, M irr	A11	
356	*Emberiza hortulana*	Ortolan Bunting	Ortolano	M irr	A20	31
357	*Emberiza caesia*	Cretzschmar's Bunting	Ortolano grigio	A 1 (1989)	A30	
358	*Emberiza cirlus*	Cirl Bunting	Zigolo nero	SB, M, W	A11	
359	*Emberiza pusilla*	Little Bunting	Zigolo minore	A 1 (2007)	A30	
360	*Emberiza melanocephala*	Black-headed Bunting	Zigolo capinero	M, B	A11	
361	*Emberiza schoeniclus*	Common Reed Bunting	Migliarino di palude	M, W	A10	

43% of the total species (N = 154) is a breeder, regular (N = 147) or irregular (N = 7). Of these, 70 are Non-Passerines (NP) and 84 are Passerines (P), with an NP/P ratio of 0.83 and NP/Total ratio of 0.45. Two naturalized species (*Psittacula krameri* and *Acridotheres tristis*) with self-sustaining breeding populations (Fraissinet 2015; Mori et al., 2020; Fraissinet & Capasso 2020). Six species (*Perdix perdix, Tadorna tadorna, Anas crecca, Falco biarmicus, Riparia riparia, Loxia curvirostra*) are uncertain breeder, while two species (*Neophron percnopterus* and *Bubo bubo*) are declared extinct breeder in the region. In Fig. 3 the number of breeder species reported in the published Campania checklists from 1989 to 2020 is shown, excluding the uncertain breeders.

Comparing the previous checklists, it is possible to observe an increase in breeder species and relative to the most recent (Fraissinet 2015) there are 4 additional species. This is due to the arrival of *Netta rufina* and *Montifringilla nivalis* on the regional territory as certain breeder in recent years and to the confirmation of *Microcarbo pygmaeus* and *Recurvirostra avosetta*, previously considered uncertain breeders. Families with the highest number of breeding species are: *Muscicapidae* with 11 species; *Accipitridae* and *Fringillidae* with 8; *Ardeidae* with 7.

The conservation status of breeding species is evaluated by SPEC categories (BirdLife International, 2017), as follows: 7 are classified SPEC1; 12 are SPEC2; 27 are SPEC3; 24 are NON SPEC^E. Threatened species, based on IUCN criteria, are defined on the Italian Red List of Birds (Gustin et al., 2019): no species classified CR (Critically Endangered); 9 species EN (Endangered); 13 species VU (Vulnerable); 10 species NT (Near Threatened). At least, 34 species mentioned in Annex I of the Directive on the

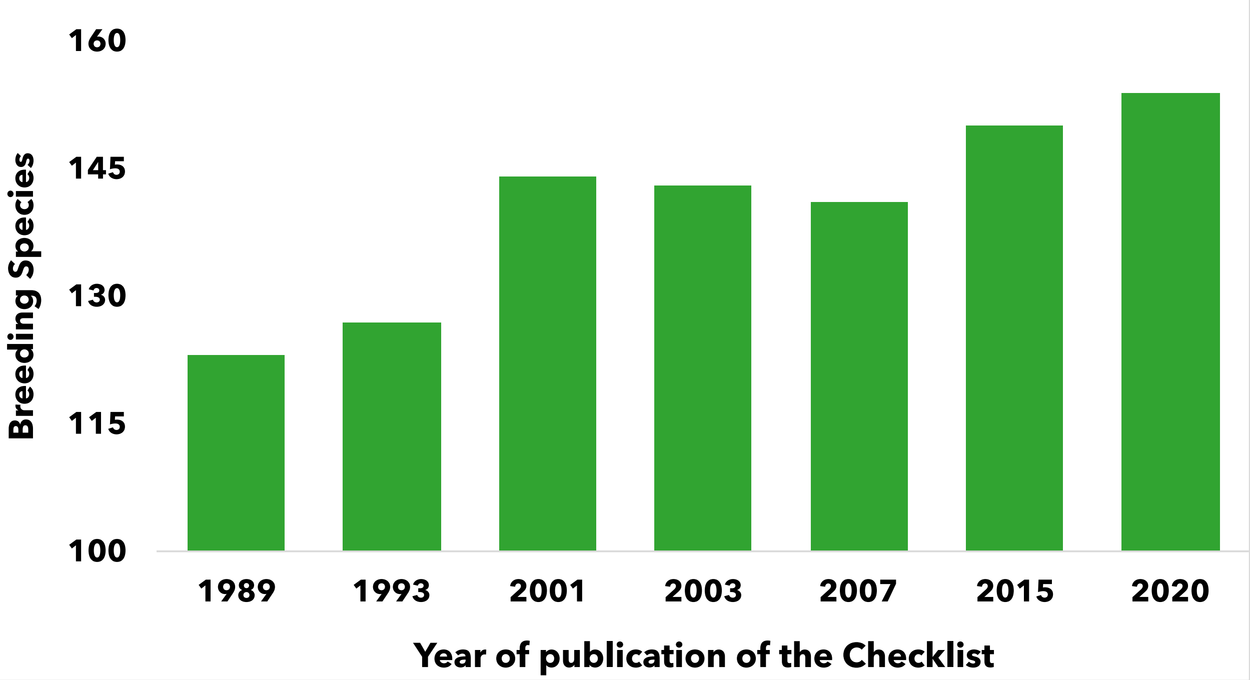

Figure 3: Number of breeding species reported in the Campania checklists that have occurred over time.

conservation of wild birds (2009/147/EC). The Ornithological Value Index (IVO) is equal to 126.66.

Chorological spectrum of breeding species in Campania (Fig. 4) shows an expected prevalence of Palearctic species, while the Eastern Palearctic, European and Mediterranean categories have about a 10% of the species each one.

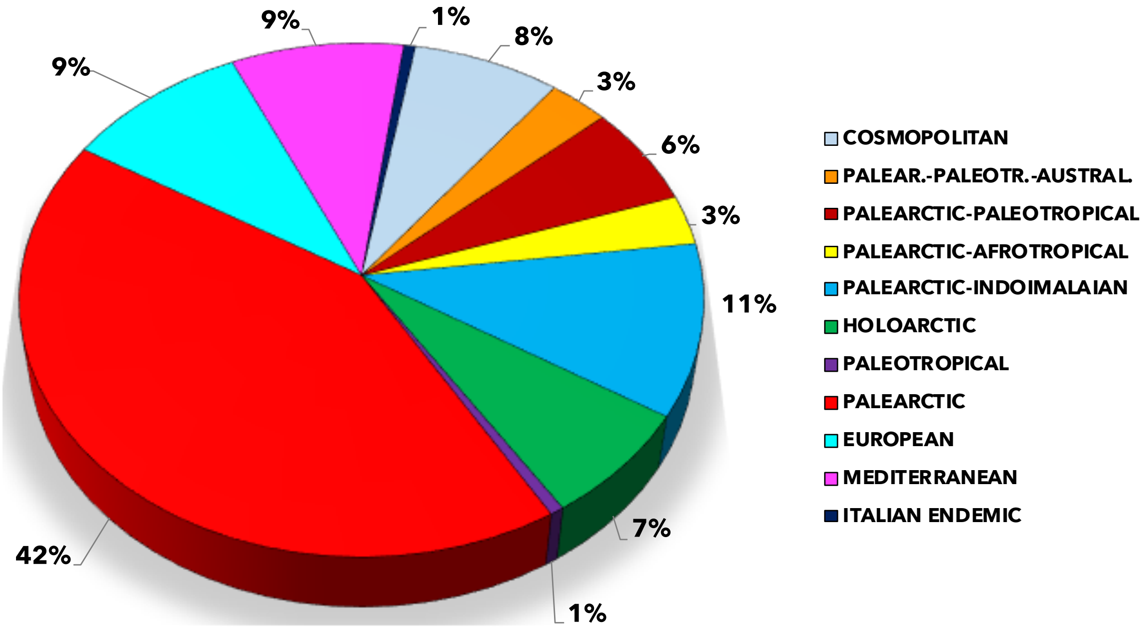

Figure 4: Chorological categories of breeding species in Campania Region.

The 70% (N = 253) of the total of species are migratory birds. Of these: 124 are Migrant Breeder (M, B); 153 are Winter Migrant (M, W); 75 are Breeder and Winter Migrant (M, B, W); 51 are Passage Migrant (M). Species with status of Resident Breeder (SB) are 90 (25%) but, anyway, some of these have migrant populations. A significant weight is given by Vagrant species (A) which represent the 19% (N = 70) of the total of species and, of these, 57 have ≤ 3 records.

For the first time, we have published the checklist of "escaped birds", AERC category E, that include a total of 31 species (Tab. 2) and the checklist of "rejected birds", the accessory category X, made up of 7 species (Tab. 3).

Authors report that the populations in list E of *Gallus gallus* and *Pavo cristatus*, both located in the Gussone Park annexed to the Bourbon Palace of Portici (NA), breeding without human support but, for now, we do not consider them self-sustaining breeding populations with characteristics of species in AERC category C. Anyway, we believe that the species in list E present in the Campania region are in greater numbers than those reported and, in the future, it will improve.

Table 2: Released, escaped or non-naturalized species recorded in Campania Region - Checklist (AERC E List) updated to 31th January 2021.

	Scientific name	English common name	Italian common name
	ANSERIFORMES		
	Anatidae		
1	*Anser cygnoides*	Swan Goose	Oca cigno
2	*Cygnus atratus*	Black Swan	Cigno nero
3	*Alopochen aegyptiaca*	Egyptian Goose	Oca egiziana
4	*Cairina moschata*	Muscovy Duck	Anatra muta
5	*Aix galericulata*	Mandarin Duck	Anatra mandarina
6	*Callonetta leucophrys*	Ringed Teal	Alzavola spallerosse
	GALLIFORMES		
	Odontophoridae		
7	*Colinus virginianus*	Northern Bobwhite	Colino della Virginia
	Phasianidae		
8	*Alectoris chuckar*	Chukar Partridge	Coturnice orientale
9	*Coturnix japonica*	Japanese Quail	Quaglia giapponese
10	*Gallus gallus*	Red Junglefowl	Gallo bankiva
11	*Pavo cristatus*	Indian Peafowl	Pavone
	COLUMBIFORMES		
	Columbidae		
12	*Spilopelia senegalensis*	Laughing Dove	Tortora delle palme
	GRUIFORMES		
	Gruidae		
13	*Grus virgo*	Demoiselle Crane	Damigella della Numidia
	CHARADRIIFORMES		
	Charadriidae		
14	*Vanellus miles*	Masked Lapwing	Pavoncella mascherata
	CICONIIFORMES		
	Ciconiidae		
15	*Mycteria ibis*	Yellow-billed Stork	Tantalo africano
16	*Leptoptilos crumenifer*	Marabou Stork	Marabù
	SULIFORMES		
	Sulidae		
17	*Sula nebouxii*	Blue-footed Booby	Sula zampeblù
	PELECANIFORMES		
	Threskiornithidae		
18	*Geronticus eremita*	Northern Bald Ibis	Ibis eremita
	ACCIPITRIFORMES		

	Accipitridae		
19	*Parabuteo unicinctus*	Harris's Hawk	Poiana di Harris
	PSITTACIFORMES		
	Cacatuidae		
20	*Nymphicus hollandicus*	Cockatiel	Calopsitta
	Psittacidae		
21	*Amazona aestiva*	Turquoise-fronted Amazon	Amazzone fronteblù
22	*Melopsittacus undulatus*	Budgerigar	Pappagallino ondulato
23	*Agapornis roseicollis*	Rosy-faced Lovebird	Inseparabile facciarosa
	PASSERIFORMES		
	Pycnonotidae		
24	*Pycnonotus leucogenys*	Himalayan Bulbul	Bulbul guancebianche
	Sturnidae		
25	*Gracula religiosa*	Common Hill Myna	Gracula religiosa
26	*Lamprotornis nitens*	Cape Starling	Storno splendente alirosse
	Ploceidae		
27	*Ploceus cucullatus*	Village Weaver	Gendarme
28	*Quelea erythrops*	Red-headed Quelea	Quelea testarossa
29	*Quelea quelea*	Red-billed Quelea	Quelea beccorosso
	Estrildidae		
30	*Amandava amandava*	Red Avadavat	Bengalino comune
	Fringillidae		
31	*Serinus canaria*	Atlantic Canary	Canarino

Table 3: Species rejected or suspended in Campania Region - Checklist (AERC X List) updated to 31th January 2021.

	Scientific name	English common name	Italian common name	Notes
	CHARADRIIFORMES			
	Laridae			
1	*Ichthyaetus leucophthalmus*	White-eyed Gull	Gabbiano occhibianchi	32
2	*Sterna dougallii*	Roseate Tern	Sterna di Dougall	33
	PROCELLARIIFORMES			
	Diomedeidae			
3	*Thalassarche melanophris*	Black-browed Albatross	Albatro sopracciglineri	34
	Procellariidae			
4	*Daption capense*	Cape Petrel	Procellaria del Capo	35
	PASSERIFORMES			
	Phylloscopidae			
5	*Phylloscopus orientalis*	Eastern Bonelli's Warbler	Luì bianco orientale	36
	Turdidae			
6	*Turdus atrogularis*	Black-throated Thrush	Tordo golanera	37
	Motacillidae			
7	*Anthus hodgsoni*	Olive-backed Pipit	Prispolone indiano	38

Notes to the Checklist

1	*Tadorna tadorna* - presence of adults in breeding period and observations of juveniles, with uncertain flight, in suitable habitat. Specific research carried out but no nests or chicks found.
2	*Anas crecca* - A female with 6 chicks observed and photographed in August 2005 inside Campolattaro WWF Oasis (BN). The photographic documentation is no longer recoverable and verifiable. In some years, couples in reproductive period observed in suitable habitats without further breeding evidence.
3	*Netta rufina* - first breeding record ascertained in 2018 (Usai et al., 2019) and reconfirmed in subsequent years.
4	*Columba livia* var. *domestica* - last records of the wild form dating back to the 80s of the twentieth century. Wild form believed by the authors to be extinct in Campania.
5	*Porzana pusilla* - a revision of historical reports and the absence of recent records lead the authors to consider it as Accidental, without numerical indications.
6	*Himantopus himantopus* - wintering ascertained for the first time in winter season 2019/2020 and reconfirmed in 2020/2021 by one of the authors (AU).
7	*Recurvirostra avosetta* - first breeding record ascertained in 2019 (Usai et al., 2019) and reconfirmed in 2020.
8	*Numenius tenuirostris* - 5 records for the region, of which three before 1950 (1877, 1884, 1904) and two post (1972, 1986).
9	*Thalasseus bengalensis* - previously considered M irr. Authors attribute it the status of Accidental pending further records.
10	*Sternula albifrons* - also reported in previous checklists with the status "B est", deleted in last revision (Fraissinet 2015) due to the absence of indisputable documentation.
11	*Puffinus mauretanicus* - previously considered M reg?. Authors attribute it the status of Accidental pending further records.
12	*Phalacrocorax aristotelis* - in June 2019 and 2020, 1 adult with 4 juveniles and 1 adult, 1 immature and 4 juveniles, respectively, observed on the islet of Vetara - Amalfi coast (SA). There is a lack of breeding evidence as prescribed by the protocols used for ornithological atlases (Hagemeijer & Blair 1997).
13	*Threskiornis aethiopicus* - less than 10 records are known. Erratism started from 2015 with increasing frequency and permanence in sites. Authors attribute it the status of Accidental, without numerical indication, pending further observations.
14	*Ardeola ralloides* - two winter records are kwon but without documentation or subsequent contacts. Authors suspend wintering status pending other data.

15	*Aquila fasciata* - in previous checklists indicated with A 3 (4) phenology; a review of the reports lead us to assign it the status of A 2, beacuse the third record refears to a dubious specific attribution (Scebba 1993) while there is not certain evidence on the fourth (Fraissinet 2015).
16	*Bubo bubo* - specific research missions, including recent ones, in historical sites and potential habitats, have given negative results.
17	*Falco naumanni* - observations in breeding period and in suitable habitat occurred in Alta Irpinia (AV) in the year 2020. There is a lack of breeding evidence as prescribed by the protocols used for ornithological atlases (Hagemeijer & Blair 1997).
18	*Falco biarmicus* - last nesting ascertained in 2008. Subsequently there were other observations in breeding period but without successful evidence. A new specific survey will be necessary to verify if the species is still a regular breeder.
19	*Lanius excubitor* - previously considered M and W reg. Decreasing of the observations during the last decade and absence of records for continuous years. Currently, authors consider it as an irregular migratory and wintering for the region.
20	*Riparia riparia* - specific research missions, including recent ones, in historical sites and potential habitats, have given negative results. A new specific survey will necessary to verify if the species is still a regular breeder.
21	*Acrocephalus melanopogon* - a review of the reports lead us to the exclusion of the species as a breeder in the Campania region. The historical records refer to birds in singing during migratory period.
22	*Locustella naevia* - less than 10 records are known; 6 from ringing with last in 2020. Due to its elusiveness, authors retain to assign it the phenological status of irregular migratory but with application of AERC code A30.
23	*Sylvia cantillans* - during the drafting of this Checklist, the split *S. cantillans* and *S. iberiae*, according to Zuccon et al. (2020), was accepted by the IOC. The nominal subspecies is a regular breeder in Campania while *S. iberiae* should be migratory. Waiting for other data to the regional phenology, authors consider appropriate to apply any subdivision in a future update.
24	*Ficedula parva* - other observations known but not supported by adequate documentation.
25	*Saxicola rubetra* - a review of the reports led us to the exclusion of the species as a breeder in the region. The historical records refer to birds in migratory period or a wrong identification.
26	*Saxicola maurus* - a record of one bird documented at Vasche di Ischitella (or "Le Soglitelle) (CE) in January 2018 by Janni & Cavaliere. Report approved by Italian Ornithological Commission as "Saltimpalo di Hemprich" - *Saxicola torquatus hemprichii* follow sequence and nomenclature of the latest CISO-COI List of Italian Birds (Fulco & Liuzzi 2019).

27	*Montifringilla nivalis* - first breeding record ascertained in 2016 (Capobianco et al., 2019).
28	*Prunella modularis* - a review of the reports led us to the exclusion of the species as a breeder in the region. No evidence of certain breeding birds.
29	*Anthus richardi* - less than 10 records are known; 3 from ringing. Due to its elusiveness, authors retain to assign it the phenological status of irregular migratory but with application of AERC code A30. First wintering record documented in January 2021 by one of the authors (AU).
30	*Loxia curvirostra* - the last observation in breeding period, without any further evidence of reproduction, occurred in 2010 (Fraissinet 2015). Fledged birds observed in 1994 (Moschetti et al., 1995) and that report induced the authors of previous checklists to apply B irr phenology to the species. In absence of recent observations, authors consider it appropriate to insert the uncertainty (?) on breeding.
31	*Emberiza hortulana* - previously considered M reg. Observations and ringed birds in rarefication during last decade. Currently, authors consider it as an irregular migratory for the Campania region.
32	*Ichthyaetus leucophthalmus* - a record of one bird observed at islet of Licosa (SA) in winter 1973 (Milone & Grotta 1988). Report not submitted to the Italian Ornithological Commission (Milone 1999).
33	*Sterna dougallii* - a record of one bird observed at Punta Licosa (SA) in winter 1970 (Milone & Grotta 1988). Report not submitted to the Italian Ornithological Commission (Milone 1999).
34	*Thalassarche melanophris* - a record of one bird observed along the coast between Battipaglia and Eboli (SA) in January 2005 (Gatto S. in EBN Italia 2005). Report not submitted to the Italian Ornithological Commission.
35	*Daption capense* - a record of one bird captured by a fishing boat off the coast of Cetara (SA) in December 1977 (Milone 1999). Report not submitted to the Italian Ornithological Commission.
36	*Phylloscopus orientalis* - previously considered M reg?. Before the split, ringed birds of *P. bonelli* identifcated as ssp. *P. b. orientalis* during spring migration. Authors attribute it in this list pending further records.
37	*Turdus atrogularis* - a record of one bird hunted along the course of the Ofanto, near Sant'Andrea di Conza (AV) in November 1961 (Scebba 1993). Species deleted from the previous checklist (Fraissinet et al., 2007).
38	*Anthus hodgsoni* - a record of one bird ringed in the isle of Vivara (NA) in April 1985 (Fraissinet 2015). Report not approved by the Italian Ornithological Commission.

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Author contributions

Maurizio Fraissinet and Alessio Usai cured all steps for the composition of the manuscript.

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De pulvere pro lupis occidendis: wolf poisoning in Southern Italy during the XIII century

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Abstract

In this study, we present some documents showing the official acknowledgement of the role of *luparius* in Southern Italy during the XIII century. *Luparii* were professional wolf-killers, prevalently coming from three regions of the Kingdom of Naples: Abruzzo, Terra di Lavoro, Apulia, but active throughout the kingdom. Different techniques were adopted by *luparii*, but one of the most widespread was the so called *pulvis*, a powder probably obtained from an unknown poisonous plant. Dioscorides and Galen reported that a plant named *akoniton* was used in the Mediterranean world to poison wild animals, and according to the XVI century physician Pietro Andrea Mattioli, *luparii* used the *Aconitum* to kill wolves. Historical sources show that the problems related to the identification of *Aconitum* was harshly debated among the botanists contemporary to Mattioli, and that *Doronicum pardalianches* L. and *Aconitum napellus* L. were the species most frequently proposed. However, in Southern Italy both plants are scarcely present, whereas cognate species are diffused, as *Doronicum columnae* Ten. and two subspecies of *Aconitum lycoctonum, A. lycoctonum* L. subsp. *neapolitanum* (Ten.) Nyman or *A. lycoctonum* L. subsp. *vulparia* (Rchb. ex Spreng.) Nyman.

Keywords: wolf hunting; luparius; XIII century; *pulvis*; *Aconitum*

Riassunto

In questo studio, presentiamo alcuni documenti che attestano il ruolo ufficiale dei lupari nell'Italia meridionale durante il XIII secolo. I lupari erano cacciatori di lupi, provenienti prevalentemente da tre regioni del Regno di Napoli: Abruzzo, Terra di Lavoro, Puglia, ma attivi in tutto il regno. Diverse erano le tecniche adottate dai lupari per uccidere i lupi, ma una delle più diffuse prevedeva l'uso della cosiddetta *pulvis*, una polvere ottenuta probabilmente da una pianta velenosa sconosciuta. Dioscoride e Galeno riportano che una pianta chiamata *akoniton* era usata nel mondo mediterraneo per avvelenare gli animali selvatici, e secondo il medico del XVI secolo Pietro Andrea Mattioli, i luparii usavano l'*aconitum* per uccidere i lupi. Le fonti storiche mostrano che i problemi relativi all'identificazione dell'*aconitum* furono aspramente dibattuti tra i botanici contemporanei a Mattioli, e che *Doronicum pardalianches* L. e *Aconitum napellus* L. furono le specie più frequentemente proposte. Tuttavia, nel Sud Italia entrambe le piante sono scarsamente presenti, mentre sono diffuse specie affini, come *Doronicum columnae* Ten e due sottospecie di *Aconitum lycoctonum*, *A. lycoctonum* L. subsp. *neapolitanum* (Ten.) Nyman e *A. lycoctonum* L. subsp. *vulparia* (Rchb. ex Spreng.) Nyman.

Parole chiave: cacciatori di lupi; lupari; XIII secolo; *pulvis*; *Aconitum*

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Introduction

The negative image of the wolf in many human cultures is undeniable, although reverence and admiration are also frequently expressed. From totemic animal and guide in the *Ver Sacrum* to unclean beast, from symbol of wilderness to personification of evil, the wolf has been the protagonist in bestiaries, fairy tales, as well as in manuals about farm conduction (de Clamorgan 1588). Early men and wolves had strict relationships, not only in terms of competition but also because they shared many aspects of their societies, from leadership hierarchies to common care and training of juveniles (Fritts et al. 2003). In the Mediterranean region human attitude toward the wolf had been relatively positive for very long times: Greek and Italic populations shared religious practices related to wolves as totemic animals and generally respected them, but, on the other hand, in VI century BCE Solon's legislation instituted in Athens a five drachmae reward for each wolf killed. Romans did not use wolves for *venationes* nor hunted them, due to the religious importance that they assigned to this animal (Rissanen 2014). In IV century CE, Servius Marius Honoratus, in commenting Vergil's Georgics (Emmenessius 1680) stated: «constat enim luparios carnibus tinctis veneno lupos necare». This is one of the first citation of *luparii*, and their activity seems to be linked with the uses of poisonous substances since the beginning. During the following centuries, wolves were viewed as evil by the Catholic Church, becoming a negative symbol for thousands of years, and a serious problem for human activities (Boitani 1995, Rao 2018). An early written document is reported in the *Capitularia Regum Francorum* and refers to the year 813: it is allowed to hunt wolves, particularly the young ones, «cum pulvere et hamis, quam cum fossis et canibus» (Baluze 1772). To this purpose, in the Capitularium it is also stated that each Vicar (officials at the services of the Earl) had two *luparii* with him, who had to deliver the wolves' skins to the court, receiving a bushel of wheat (von Buri 1788).

Starting from the so-called Medieval Climate Anomaly (approximately 1100-1400 PE), many forests were converted to agricultural farms and livestock pastures, keeping territories also at elevations above 700 m (Mensing et al. 2016). The reduced amount of forest and uncultivated land led wolves to prey animals bred by man, causing conflictual relationships between farmers and wolves. Moreover, the reestablished central authority over rural territories allowed investments of large economical resources on wolf hunting (Rao 2018). Wolves were just considered as a pest to be destroyed, and rewards for their killing were offered in numerous European countries, such as Spain, France, Switzerland and Italy (Moriceau 2014). This new attitude is incisively shown by the importance acquired by the *lupariu*s, that became a professional killer of wolves paid by central Governments (Fig.1).

Based on a survey of historical sources, this study aims to put in evidence the professional status of *luparii* in Southern Italy during the XIII century, focusing on the role of the so-called *pulvis* in their hunting techniques, and suggesting a possible identification of the plant used for this preparation.

Materials and Methods

The most important sources for investigating the role of *luparii* during the 13th century were the *Historia diplomatica Friderici Secundi* edited by Huillard-Bréholles (1852), [https://books.google.it/books?id=Gh8r5pyhdz4C&hl=it], and the *Archivi della Cancelleria Angioina* (www.accademiapontaniana.it/pubblicazioni/).

This second bibliographic source, an immensely important and valuable tool for the study of European history, survived for about seven centuries until September 30, 1943, when, having been transferred the registers at San Paolo Belsito (Napoli) to

Figure 1: Wolf hunt with traps and arquebus, drawing by Jan van der Straet (Philips Galle, Venationes Ferarum, Avium, Piscium. Pugnae bestiariorum et mutuae bestiarum depictae, [Antwerp?], 1580.

preserve them from bombing during the Second World War, they were set on fire by the retreating German army (Jamison 1949). Today, we owe the recovery of their contents to the patient and incessant work of some scholars who, starting from 1950, have carried out a momentous work of reconstruction.

Results and Discussion

An early document mentioning wolf killers, *luparii*, from Southern Italy dates back to 1021 (Federici 1925), while numerous documents from the XIII century from the official Archives of the Kingdom attest the payment of *luparii*. Here we have chronologically reported the most detailed documents dealing with this topic.

1239 (October 14) – *It has come to our attention that in our park of Milazzo there are in great quantity foxes and wolves and they kill small animals, so that in a short time few of them will survive there, if you do not provide for them with some remedy. Therefore, we entrust to your devotion in advising immediately after reading this letter about a wolf powder that you undertake to find and so you order, as it is usual and as it must happen, the one [powder] that can kill only wolves and foxes and can plentifully increase the small animals and cannot* *destroy them. Nevertheless, let you endeavor and see to it that all game in your place of jurisdiction be protected, and about the powder against wolves let you that it be placed where it may be useful* (Huillard-Bréholles 1859).

1239 (November 6) – *Ruggero di Petrasturmina has written about the imperial mandate to the Giustiziere of Abruzzo, so that he may search and find in the territories of his jurisdiction two men who know how to kill wolves with powder and send them to Riccardo di Montenegro, Giustiziere of the Terra di Lavoro, to place them in the wood of Patria [area of Lake Patria] to kill wolves and foxes* (Huillard-Bréholles 1859).

1240 (March 31) – *Concerning the finding of the powder for the elimination of the wolves following our mandate, we have understood what you have done, and we have instructed Thomas of Aquinas, our faithful Count of Acerra, to send you immediately the same powder in abundance; I also send you two men instructed in the preparation of the powder and in the killing of wolves, who, when they come to you, will show you the necessary expenses* (Huillard-Bréholles 1859).

1270 – *Charles I of Anjou with his constitution of 1270 formed the* «Luparii pro occidendis lupis in Aratriis Regiis», *most of which were in Puglia and not far from Lucera* (Lombardi 1748).

1278 – *Immunity for some luparii, who kill wolves with powder to defend us. Immunity for Sora Valley luparii, who kill wolves with powder in the royal forests* (Orefice De Angelis 1967).

1278 (March 14) – *We wrote to the* Secretum *of the Principato, of Terra di Lavoro and Abruzzo. Having to kill the wolves with powder through our* defensa *of Terra di Lavoro and other places outside the same* defensa *in which the luparii Iacobo di Cassano and Guglielmo di Nusco had been useful for a greater convenience of the wild animals that exist in the same* defensa *and also of our animals and the animals of private citizens. Our Highness has given an estimate of the expenses for our curia... we send ... expenses at the rate of fifteen gold tarì per month for each of them (luparii) from the moment they began to perform the same service and from before until they continuously performed the same service for the entire month of April* (Mazzoleni 1964).

1279 – *Granting immunity to 20 men of the Sora Valley who are named luparii because they went hunting for wolves and foxes* (Mazzoleni 1969)

1291 (November 20) – *In November 20, two ounces of gold for two months were given to Rainaldo de Dominicis, Pietro de Blasio, Domenico di Giovanni and Basidio da Balsorana who had to go to the forests of S. Gervasio and Lagopesole to kill wolves* «cum pulvere». *On the 27th the pay of 15 tarì per month is paid to Tommaso di Giovanni and Onofrio di Nicola sent to the forest of Orta in Capitanata. The luparii for all the time of service had exemption from any collection or* *bounty that could be imposed on the inhabitants of their countries* (Cubellis 1996).

1292 (August 24) – *order to the Giustizieri of Principato and Abruzzo, because they search, everyone in the own province, four luparii* «pro occidendis lupis cum pulvere in forestis ubi animalia araciarum curie in pascuis morantur» (Schipa 1890, Palmieri 1987).

-*...the mentioned Charles Martel, Vicar General of the Kingdom in the absence of his parent, ordered to the Giustiziere of Principato ultra, to find four luparii and send them in the royal forests to kill wolves, with a certain powder* (Camera 1860).

1292 (November 20) – *Having reported Ughetto de Palafredis, master of the royal marescallia and of the races in Puglia, that a certain number of mares, foals and other animals had been devoured by wolves, which appeared in great multitude in those places, it is ordered from Naples on November 20, to call in Puglia Rainaldo de Dominicis, Pietro de Blasio, Domenico de Iohanne and Casidio de Valletorana, destined to kill the wolves* «cum pulvere» *in the forests of San Gervasio and Lagopesole, to destroy them, and to the Giustiziere of Basilicata to pay to the luparii for two months two ounces of gold* (Palmieri 1999).

1292 (November 27) – *it is approved the payment of fifteen tarì for a month to Tommaso di Giovanni and Onofrio de Nicola, both of Balsorana, sent to kill with powder other wolves in the forest of Orte in Capitanata. To the luparii, for the duration of their service, was granted exemption from any grant or collection or bounty that could be imposed on the inhabitants of their countries* (Schipa 1890).

- *The king approves the payments made by the treasurer to Colino de Berro for the expenses of Anastasia, daughter and heir of the late Guido de Montfort, for six ounces of gold, to Tommaso di Giovanni and Onofrio de Nicola, both of Balsorana, who were sent to Capitanata to kill wolves* «cum pulvere» *for a month* (Palmieri 1999).

As can be seen, during the XIII century the activity of *luparii* was diffused from Campania to Sicily and was strictly connected with the use of *pulvis,* a powder containing highly toxic substances, but, to the best of our knowledge, no official document gives any detail about the composition of this powder. Poison hunting is reported at every latitude and seems to be an early acquisition of human communities, probably dating back to more than 20.000 years ago (Osborn 2016). Early hunters applied animal- or plant-based poisons on spears and arrows, but toxic plants were also managed to prepare traps to kill predatory mammals like wolves and foxes throughout Europe. Plants like *Anamirta coccolus* in South-East Asia, and lichens like *Letharia vulpina* have been used in Scandinavia for this purpose (Svanberg and Ståhlberg 2018), but in the Mediterranean Region wolfsbane (*Aconitum* spp.) was one of the plants of choice species since the Classic era (Cilliers and Retief 2000). Indeed, *Aconitum* spp. are worldwide known as a source of a potent venom (Borgia et al. 2017), due to the presence of a suite of alkaloids responsible for cardiac and neurotoxic action (Nyirimigabo et al. 2014). The genus *Aconitum* (Ranunculaceae) comprises more than 300 hundred species, distributed throughout arctic regions and mountain environments of all continents, except Australia. According to recent research, the genus *Aconitum* originated 24.7 Mya (Park et al. 2020), and consists of different subgenera, among which *Aconitum* L. subgen. *Aconitum*, is the largest one, including 22 species native to Europe (Boroń et al. 2020). The name ἀκόνιτον (akoniton) occurs in medical literature of classical antiquity, particularly in the works dealing with toxicological matters. It would derive from *Akone*, a toponym assigned to more than one locality of Pontus, or ἀκονᾶν (akonàn), a term that defines whetstone, or also from the Greek verb ἐγκονέω (enkonèo) 'I accelerate', because aconitum accelerates death. Finally, other scholars link *akoniton* to ἄκον (àkon) and ἀκή (akè), the Greek words denoting an arrow or a point, because these plants were applied to arrowheads, as previously described (Reichenbach 1820).

The chapter on *akoniton* in the Medical Matter of Dioscorides (Gunther 1959), focuses on its toxicity to animals, and two types of *akoniton* are described, whose botanical identification has been a matter of controversy for centuries. The first *akoniton* is named by Dioscorides as παρδαλιαγχὲς or leopard's bane, but also κυνοκτόνον (kynoktonon), dog killer, and is briefly described as a small plant, with three or four leaves with scarce hairs, similar to those of cyclamen, and a root with a shape resembling the tail of a scorpion. The second one, ἀκόνιτον ἕτερον (akoniton eteron), is morphologically completely different from the former, being taller and with leaves like those of *Platanus*, and, according to Dioscorides, it was particularly diffused on Vestini Mounts (Abruzzo, Southern Italy). Galen in *De simplicium medicamentorum temperamentis ac facultatibus* (Kühn 1821-1833) reports that the *akoniton* is also called λυκοκτόνον (lykoktonon), wolf-killer, whilst Nicander describes a complex symptomatology due to *akoniton* intoxication, centered on abdominal effects (stomach and abdominal pains) coupled with head heaviness, blurred visions, and stupefaction (Skaltsa et al. 1997).

Although more than 257 plants are mentioned in the *Corpus Hippocraticum* (Riddle 2013) and more than 500 species are named in the works of Theophrastus (Irwin and Irby-Massie 2016) and Dioscorides (Staub et al. 2016), botanical descriptions in these texts are either entirely absent or lacking, if we consider them from a "modern" point of view. In Greece, the collection of plants in the wild, and thus their identification, was a professional activity carried out by expert herbalists, called ῥιζοτόμοι (rhizotomoi) (Roze 1898), whose key role in the trade of plant species had been progressively consolidated in Attica over the centuries, to the point of giving rise to a guild recognized as such in 5th Century BPE in Athens (Samama 2006). We know that Sophocles wrote a tragedy, which has not come down to us, entitled Οἵ ῥιζοτόμοι (oi rhizotomoi), and that Theophrastus considered both ῥιζοτόμοι and φαρμακοπῶλαι (farmacopolai, sellers of medicinal plants) mostly as charlatans, although they were able to provide useful information (Scarborough 2006). In most cases, the ability to recognize and name plants, as well as the knowledge of the territories and habitats in which they were found, was a skill not fully possessed by most of those who practiced the medical arts, although they made constant use of plant products for the treatment of diseases. This ability to identify plants in the wild was also a prerogative of specific populations to whom was credited deep knowledge regarding poisons and drugs of animal and vegetable origin: with regard to the Italic Peninsula, the Marsi, coming from Abruzzo, were also consulted by Galen, for their knowledge about medicines and antidotes (Nutton 1985), whereas in the rest of the Mediterranean Region Psylli, Nasamoni and Palaeotebani played the same role of contact point between the civilized world and the secrets of Nature (Cilliers and Retief 2000).

The identification of the two *akoniton* species described by Dioscorides was attempted by the most prominent XVI-XVII Century botanists; the first species was attributed to *Paris* by Fuchs, *Tora by* Gessner, *Doronicum* by Dodonaeus (Palmer 1985). In the same years, Giacomo Antonio Cortusio, a Paduan botanist, administered *Doronicum* roots to different animals, demonstrating it had lethal effects (Palmer, 1985).

Nowadays, the prevailing attributions of the two Dioscoride's *akoniton* respectively point toward *Doronicum pardalianches* L., and *Aconitum napellus* L. or *A. lycoctonum* L. (Been 1992). The genus *Doronicum* L. is a member of the tribe Senecioneae, Asteraceae, which comprises 26 species growing from Asia to Europe and North Africa, growing in forests and on rocky places from the sea level up to 5000 m (Fernández 2003). In Southern Italy, the presence of *D. pardalianches* is presently not attested, whereas *D. columnae* Ten. is found, a species prevalently growing on rocky and shady places from 500 to 2000 m. *A. napellus* has a distribution confined to Northern Italy, whilst *A. lycoctonum* L. subsp. *neapolitanum* (Ten.) Nyman (Fig. 2) and *A. lycoctonum* L. subsp. *vulparia* (Rchb. ex Spreng.) Nyman are recorded over the entire Italian territory, except Apulia, Sicily and Sardinia (Pignatti 2019).

Figure 2: Specimen of *Aconitum lycoctonum* L. subsp. *neapolitanum* (Ten.) Nyman collected by Michele Tenore at Piano di Verteglia, Terminio, Montella (Campania region, Italy). Courtesy of the Herbarium Neapolitanum, Orto Botanico di Napoli.

According to Mattioli (1548) «Quello [*aconitum*] della prima spetie, (Fig. 3) che ammazza i leopardi, et le panthere, ho più volte ricolte io in su'l Trentino, ove nasce copiosamente: et di questa spetie è quello, che usano alcuni in terra di Roma, et nel Reame di Napoli, che non fanno altra arte, che ammazzare i lupi, et vendonne spesse volte le radici in su'l ponte di Santo Agnolo à chi ne vuole: imperoche queste radici ammazzano subito i lupi». It is well known that Mattioli was doubtful about the

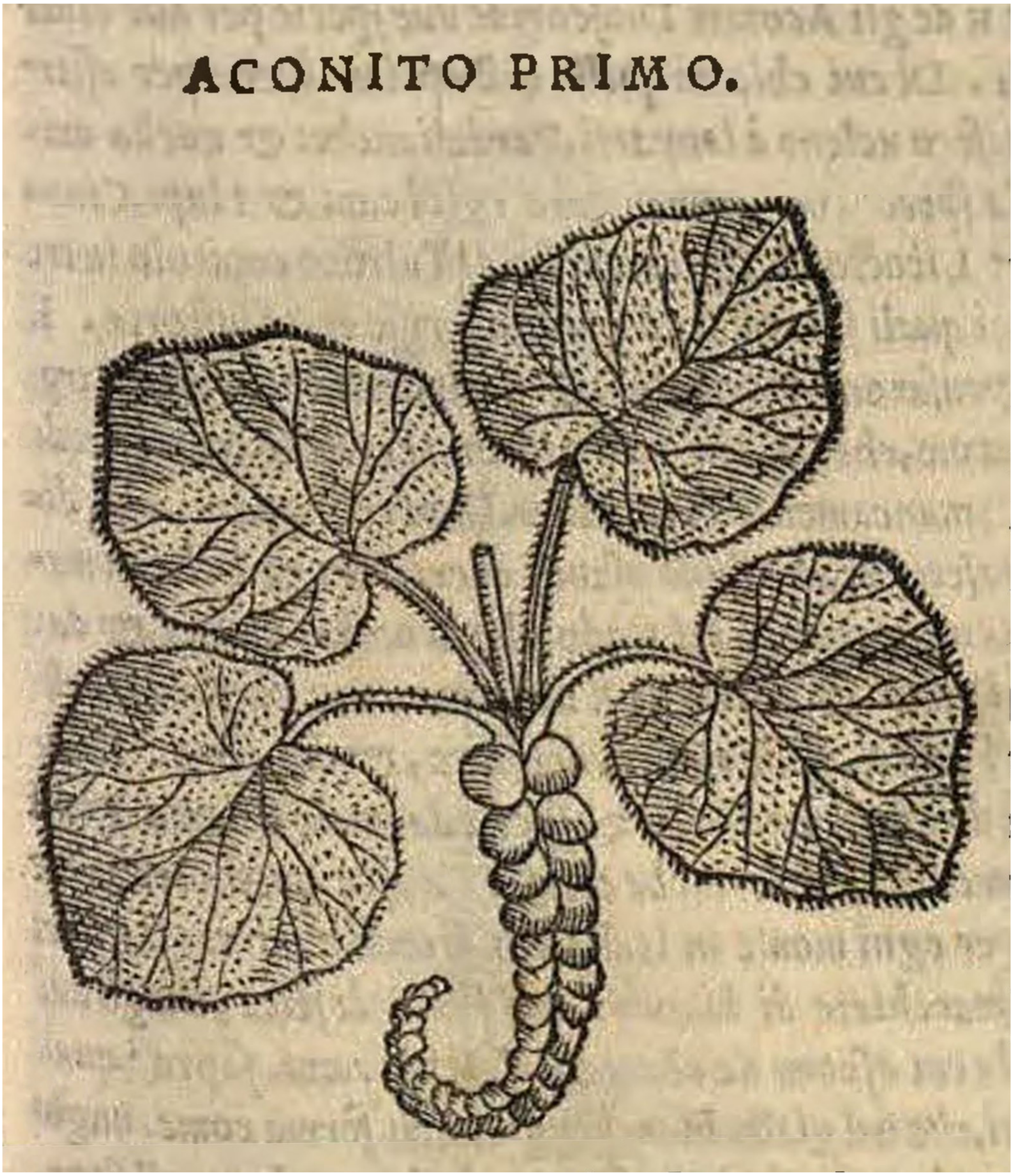

Figure 3: Drawings of "aconito primo". In: Mattioli, M.P., *I discorsi di M. Pietro Matthioli sanese, […]*, Appresso Vincenzo Valgrisi, Venetia, 1563.

recognition of the plant and only the experimental evidence of the poisoning action of *Doronicum* presented by Cortusio convinced him that this was the correct identification (Palmer 1985). However, the comment of Mattioli represents an explicit link between the activity of the *luparii* and a plant species. In recent times, Cuozzo (2005) hypothesized that the powder prepared to kill wolves by Thomas I d'Aquino Count of Acerra under request of Frederick II, could be identified with gunpowder. Conversely, the evidence presented in this research shows that the so called *pulvis* was obtained from a plant, independently of its identification. Moreover, the statement of Mattioli suggests that the high reputation of *luparii* from Southern Italy survived at least until his time. Indeed, in 1585 the Duke of Ferrara called to court many *luparii* from the Kingdom of Naples and, to pay them for several years, a 1500 scudi tax was imposed on grazing livestock (Frizzi 1796). The *luparii* survived in some mountain regions of Southern Italy, such as the territory of Matese, between Campania and Molise, up to the second half of XX century (Guacci 2007). Over the centuries, they adopted new, very effective strategies against wolves, that led the species to near extinction. The new hunting techniques were the result of a combined utilization of traps and firearms, but no mention of a powder based on a poisonous plant has been reported, although lethal poisons like strychnine and arsenic were occasionally used (Guacci 2007). Probably, the gradual loss of traditional knowledge about wild plants and their activity, affecting many Italian rural communities, led to the progressive disappearance of the practice.

Author contributions

Both authors equally contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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