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Biological Science

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Biological Time Vector Field

Boundary of the Permian and Triassic

Highlights

Protein Level in Human Female Saliva

Southern Guinea Savanna Agroecology

Discovering Thoughts, Inventing Future

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Contents of the Issue

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

1. New Representative of the Family Hydroscaphidae from Tunguska Basin, the Boundary of the Permian and Triassic (Coleoptera, Myxophaga) with Review on Myxophagan Fossil Records. *1-13*
2. Changes in Total Protein Level in Human Female Saliva during Ovulatory Functions and Metabolic Disorders. *15-18*
3. Closed Motion of a Biological Time Vector Field and the Cellular Theory of a Plant Organism. *19-32*
4. Nutraceutical Potentials of *Cissus Populnea* Linn Cultivated in the Southern Guinea Savanna Agroecology of Nigeria. *33-42*

v. Fellows
vi. Auxiliary Memberships
vii. Preferred Author Guidelines
viii. Index

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New Representative of the Family Hydroscaphidae from Tunguska Basin, the Boundary of the Permian and Triassic (Coleoptera, Myxophaga) with Review on Myxophagan Fossil Records

By Alexander G. Kirejtshuk, Alexander A. Prokin & Alexander G. Ponomarenko

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Abstract- *Palaeoscapha tunguskaensis* gen. et sp. nov., the oldest representative of the family Hydroscaphidae, is described from the Tunguska Basin in Siberia, dated from the boundary of the Permian and Triassic. It is assigned to the subfamily Triamyxinae, stat. nov. New diagnoses of the Hydroscaphidae and Triamyxinae are proposed. The new subfamily Leehermaniinae subfam. nov. (type genus *Leehermania*) is erected. The fossil record of the suborder Myxophaga is reviewed and revised with some taxonomic notes and synonymy of some generic and species names in the family Lepiceridae, known from Burmese amber. Different aspects of the evolutionary history of this suborder are discussed. A synonymy of *Haplochelus* and *Lepiceratus* is proposed.

Keywords: *Lepiceridae, Spheriusidae, Torridincolidae, Triamixinae, Leehermaniinae, evolutionary history, new subfamily, new genus, new species, new synonymy.*

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New Representative of the Family Hydroscaphidae from Tunguska Basin, the Boundary of the Permian and Triassic (Coleoptera, Myxophaga) with Review on Myxophagan Fossil Records

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Keywords: Lepiceridae, Spheriusidae, Torridincolidae, Triamixinae, Leehermaniinae, evolutionary history, new subfamily, new genus, new species, new synonymy.

I. Introduction

Like other myxophagan families, the Hydroscaphidae is a relatively small group in the Recent fauna, and it seems to be yet poorly known in fossils. The extant hydroscaphids were reviewed thanks to the efforts of many colleagues [4, 7, 9, 10, 23, 24, 25, 27, 28, 29, 35, 36, 37, 38, 39, 42, 45, 46, 47,etc.]. It currently comprises four genera and nearly 40 species [47], while the modern myxophagans include about 120 species in four families with apparently ancient origins. Recently one hydroscaphid specimen was found in materials in sediments from Tunguska Basin, Krasnoyarsky Kray (tentatively dated around the boundary between the Permian and Triassic). The suborder Myxophaga *sensu stricto* has only a few fossil records [3, 4, 8, 9, 12, 13, 15, 16, 18, 19, 26, 34], which need to be reviewed and discussed to clarify the composition of this group in the past. The paper is aimed to describe the oldest fossil myxophagan representative (*Paleoscapha tunguskaensis* gen. et sp. nov.), summarize the available data on the current fossil record of the myxophagans, and provide them with some essential comments, including the proposal of a new subfamily Leehermaniinae subfam. nov. (type genus *Leehermania* Chatzimanolis, Grimaldi, Engel et Fraser, 2012) [3], and a subfamily state for Triamyxidae Qvarnström, Fikáček, Wernström, Huld, Beutel, Arriaga-Varela, Ahlberg et Niedźwiedzki, 2021 (Triamyxinae stat. nov.) [26].

The suborder Myxophaga *sensu stricto* was proposed by Crowson [4] only for the groups from the Recent fauna with some characters similar to those in the suborder Archostemata (mostly apparently plesiomorphic), in contrast to others which are shared by both myxophagans and polyphagans and seem to be more derivative but probably homoplastic. The recent comprehensive studies of fossil beetles revealed close relations between the recent myxophagan families and extinct archostematan groups of the infraorder Schizophoriformia Kirejtshuk, 1991 [14] (=Schizophorimorpha, Ponomarenko, 2001 [30]) [32]. In some recent publications both these groups are sometimes regarded together in the suborder Myxophaga *sensu lato* (e.g.,[1, 47]; etc.). The authors of this paper consider that such a joining remains still not reasonable and prefer to interpret them in the traditional way, viz. separating an archostematan infraorder Schzophoriformia from a suborder Myxophaga *sensu stricto*).

II. Material and Methods

The specimen examined was buried in volcanic rock and partially replaced by silica. As a result, the beetle remains maintains a three-dimensional appearance. This circumstance explains its comparatively good preservation. This fossil remains is one of two insects, beetle and leaf-hopper [44] found in

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Untuun River outcrop. There is no findings of similar beetles among the oryctocenoses of other volcanogenic localities of the Siberian (including Tunguska) traps. The many kilometer thickness of traps could have been accumulated over a short time and consist of a sequence of explosive thermal tuffs and a sequence of erupted basaltic lavas. All crustaceans, insects, fishes, and tetrapods from these localities belong to the first sequence of the traps. The thermal effect of lavas on coals and carbonates of the Tunguska syncline probably led to the accumulation of atmospheric methane and carbon oxides, which led to the thermal maximum in the middle of the Induan time [50]. If these assumptions are correct, the temperature maximum has fallen at the end of the Induan. Therefore, the Permian-Triassic boundary on the Tunguska should be placed on the outpouring of basalts. In this case, all locations of fossil organisms on the Tunguska traps should be considered as early Triassic and Induan, as was usually treated before with reference to the character of their fauna and flora. and not Permian as suggested by Sadovnikov [40, 41].

The specimen under description was examined by A.G. Ponomarenko and A.A. Prokin using a Leica M16 light stereomicroscope with a Leica DFC420 digital camera and an attached Tescan Vega XMU scanning electron microscope. The line drawing was prepared using Adobe Photoshop CS3. Some additional fossil and recent specimens from the collections of the Zoological Institute of the Russian Academy of Sciences (St. Petersburg), Borissiak Paleontological Institute of the Russian Academy of Sciences (Moscow), and Muséum national d'Histoire naturelle (Paris) were studied for comparison of the specimen under description. Photographs of different focal depths were combined using Helicon Focus 5.1.28. Line drawing was prepared with the Autodesk AutoCAD 2015 software.

III. Systematic Paleontology

Order Coleoptera Linnaeus, 1758
Suborder Myxophaga Crowson, 1955 [4]
Family Hydroscaphidae Le Conte, 1874 [22]

Remarks: This family was under the attention of many researches (see above); however, the current review of available myxophagan fossils forces us to recompile an emended diagnosis for this family.

Diagnosis (*adults*): Body small and fusiform, more or less gently narrowing to abdominal apex; usually dark and pubescent. Head large, wide and short, prognathous to hypognathous; eyes usually large, not protruding; antennae 5-11-segmented, usually clubbed; labrum separated from frons (clypeus); ultimate maxillary palpomere small, subulate; ultimate labial palpomere small and narrowing apically; mentum very small to relatively large. Prothorax rather short to moderately long; propleura reaching anterior edge of prothorax; prosternum relatively short and with short and pointed process; procoxal cavities transversely oval to transverse; procoxae with exposed trochantin. Mesothorax short or moderately long, with depressions for receipt base of prolegs; mesocoxal cavities widely to narrowly separated, externally opened; mesocoxae oval. Metathorax usually moderately long; metepisterna moderately widened anteriorly and well exposed; metaventrite without trace of sutures or with expressed premetacoxal sutures; metacoxae widely separated to sub (contiguous), usually short and with more or less raised plates or without them. Abdomen usually with six ventrites either rarely with five (probably in females) or seven ventrites, ventrite 1 longest (when all following segments of similar structure); 1-2 terminal segments subconical and very long. Elytra shortened and transversely truncate at apex or slightly shortened and subacute at apices; hind wing without *oblongum*, with reduced anal field and fringed along edge. Legs moderately long; trochanters elongate; femora of usual shape; tibiae relatively narrow to moderately wide or rarely strongly dilate; three simple tarsomeres.

Subfamily Triamyxinae Qvarnström, Fikáček, Wernström, Huld, Beutel, Arriaga-Varela, Ahlberg et Niedźwiedzki, 2021 [26], stat nov.

Type genus. *Triamyxa* Qvarnström, Fikáček, Wernström, Huld, Beutel, Arriaga-Varela, Ahlberg et Niedźwiedzki, 2021 [26].

Remarks: The taxon 'Triamixidae' was proposed as a separate family among the suborder Myxophaga [26], based on characters from the list compiled by Lawrence et al. [20] and thanks to computer software for producing cladograms interpreted as follows: 'Bayesian analyses of Coleoptera and Myxophaga datasets with constrained molecular topology suggest a sister group relationship with the entire modern Myxophaga. Maximum parsimony analyses place it either in an unresolved basal polytomy (analyses with constrained molecular topology…) or as sister to Hydroscaphidae (unconstrained analyses)'. The character matrix adopted by Qvarnström et al. [26] for analyses and determination of the placement of the studied type specimens of the fossil species contain 77 characters extracted from the set of 359 characters elaborated and defined for modern species [21]. Qvarnström et al. [26] could not obtain adequate definitions of characters to compare fossil representatives with extant myxophagans. Besides, the latter researchers did not provide their description with a proper family diagnosis, and they published only some comparison ('differentiated diagnosis') after the formalization of character definition by Lawrence et al. [21] and 'table S1' with 'family' diagnosis and comparison with other groups of the suborder Myxophaga *sensu stricto*. The new

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comparison of myxophagan fossils allows us to join two fossil genera (*Triamyxa* and *Palaeoscapha* gen. nov.). The newly defined diagnostic group characters here revised gives a possibility to generate a new emended diagnosis for this suprageneric taxon with a new rank (subfamily).

Because many characters of this subfamily are poorly visible in the fossil compressions examined, the diagnostic group features of this taxon should be regarded as preliminary. They require further revision after future studies of new additional specimens. The large mentum is previously included in the triamyxid diagnosis, as it was observed in both *Triamyxa* and *Palaeoscapha* gen. nov. The precoxal part of prosternum is rather long in *Triamyxa*, but moderately long in *Palaeoscapha* gen. nov. *Triamyxa* has probably nine antennomeres with a three-segmented club, while the antennae in the holotype of *Palaeoscapha tunguskaensis* sp. nov. are missing. Therefore, the antennal characters in the subfamily diagnosis proposed below should be considered as putative for the new genus. The holotype of *Palaeoscapha tunguskaensis* sp. nov. has six exposed abdominal ventrites, while the specimens of the type series of *Triamyxa coprolithica* Qvarnström, Fikáček, Wernström, Huld, Beutel, Arriaga-Varela, Ahlberg et Niedźwiedzki, 2021 [26] have five or six exposed ventrites ([26]: 'females with 5 ventrites and males with 6 ventrites'). The reconstruction of the prothoracic structure made by Qvarnström et al. (2021) [26] needs some essential corrections. It was performed by forming a prothorax from separate pronotum and prosternum and then supposing that intervals between them could be interpreted as a space for propleura (Qvarnström et al., 2021 [26]: Fig. 3, R-T). However, it is visible that the left mesal outline of the pronotum in Fig. 3T is different from the right mesal outline of the same pronotum. Furthermore, if all three pronota (Fig. 3 R-T) are compared, it can be supposed that a narrow interval between the anterior mesal angle of the pronotum and the anterior lateral angle of the prosternum is quite acceptable. If so, the diagnostic character of Hydroscaphidae concerning the propleura reaching the anterior edge of the prothorax can certainly be defined as in the below diagnosis.

Diagnosis: Head apparently prognathous, with eyes located at base moderately arcuately protruding and rather short temples not projecting laterally. Antenna clubbed with apparently nine antennomeres. Mentum very large. Mesothorax comparable in length with prothorax. Metepisterna moderately and almost rectilinearly anteriorly widened, their mesal anterior angle very narrowly separated from outer edge of mesocoxal cavity. Metacoxae very narrowly separated to conjoining and without raised coxal plates. Elytra full to slightly shortened and narrowly transversely truncate or subacute at apex; epipleura extending behind level of metacoxae. Abdomen with 5-6 ventrites.

Composition: The type genus (*Triamyxa*) and *Palaeoscapha* gen. nov.

Genus ***Palaeoscapha*** Kirejtshuk, Prokin et Ponomarenko, gen. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:05BBDE9D-F07C-4659-BF0F-B4F83BDBF5DE

Type species: *Palaeoscapha tunguskaensis* sp. nov.

Etymology: The name of this new genus is formed from the Greek 'πᾰλαιός'= 'palaeo' (ancient, older) plus generic root 'scapha' (*Scapha*, *Hydroscapha*, *Scaphander*, *Scaphydra*). Gender feminine.

Remarks: The new genus is represented only by one species and, therefore, the diagnoses of both somehow coincide and can be used for both species and genus in accordance of the traditional principle '*descriptio generica specifica*'.

Diagnosis: Body somewhat elongate, rather convex ventrally. Head subtriangular, apparently prognathous and truncate at anterior edge of frons (clypeus); gular sutures arcuately divergent; eyes located at base and medium-sized, moderately protruding, from below looking like equally biconvex lens; mandibles apparently slightly raised and apparently arcuate along outer edge; mentum large, widest at base and arcuately narrowing anteriorly; antennal grooves distinct, arcuate and reaching level of posterior edge of mentum; temples arcuately narrowing posteriorly. Prothorax transverse, widest at widely rounded posterior angles, gently narrowing to apparently slightly projecting anterior angles; prosternum very short and subtriangular, prosternal process very narrow, short and nearly sharply acuminate at apex, reaching midlength of procoxae; propleura reaching anterior edge of prothorax, apparently with mesal process extending along anterior edge of procoxae; procoxal cavity large, transverse and seemingly open posteriorly. Procoxae narrowly separated rather than contiguous, subacutely angular laterally and with largely exposed trochantin. Mesepisternum and mesepimeron comparable in size; mesoventrite subequal in length with prothorax; mesocoxal cavity open externally. Mesocoxae large, suboval, comparatively narrowly separated. Metathorax somewhat longer than each of pro- or mesothoraces; metaventrite transverse and with paramedian nearly rectilinear lines from inner edge of mesocoxae to outer edge of metacoxae; premetacoxal sutures slightly expressed and subparallel to anterior edge of metacoxal cavities. Metacoxae relatively short, (sub)contiguous and rectilinearly located along entire posterior edge of metaventrite. Elytra covering abdomen, about 1.5 times as long as wide together and subangular at apex;

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epipleura reaching at least level of ventrite 1. Abdomen with six ventrites and comparatively short, only slightly longer than wide; ventrite 1 longest and about twice as long as each of ventrites 2-6; hypopygidium (ventrite 6) short, transverse and widely rounded at apex. Right anterior leg with elongate trochanter, femur of very usual shape and moderately wide; protibia dilated along inner side and relatively wide.

Note: Posterior edges of abdominal ventrites looking like even (without notches).

Comparison: The new genus differs from all hydroscaphids in the dilated protibiae and relatively short abdomen with all ventrites strongly transverse; and also from *Triamyxa* in the larger mentum, smaller eyes, distinct and curved antennal grooves, transverse procoxae, widely rounded posterior angles of the pronotum and subacute elytral apices.

Palaeoscapha tunguskaensis Kirejtshuk, Prokin et Ponomarenko**,** sp. nov.
Figs 1–3

http://zoobank.org/urn:lsid:zoobank.org:act: 37EAED97-A725-4CCA-8625-AE1F2303B33F

Type material: Holotype, sex unknown; specimen represented by three-dimensional remains mostly visible from underside, PIN no. 2757/1, demonstrating the most part of its body, although the right part of the pro- and pterothoraces, abdominal apex, and appendages are missing (except for the observable right anterior trochanter, femur and tibia). The pictures of the holotype were published by Kirejtshuk & Ponomarenko[20] and Ponomarenko & Prokin[34].

Type locality and horizon: Russia, Krasnoyarsky Kray, Evenkiysky National Okrug, Tunguska Basin, Untuun River; intertrappean deposits; probably early Triassic or terminal Permian, Induan or ?Tatarian.

Etymology: The epithet of the new species is formed from the name of its type locality (Tunguska).

Description (*holotype*): Body length 1.5, body width 0.6 mm. Integument mostly smoothed, moderately finely and sparsely punctured (puncture diameter about as great as that of eye facets); prosternum transversely striate, propleura rugose, ventrites partly shagreened. Head subtriangular, apparently prognathous; distance between gular sutures subequal with maximum width of mentum and smaller than that between eyes; mentum slightly transverse, trapezoid, subtruncate at base and slightly arcuately narrowing anteriorly. Precoxal part of prostenum noticeably shorter than mesal length of procoxae. Mesocoxae about twice as widely separately as procoxae. Metaventrite slightly transverse; metepisterna subtriangular; premetacoxal sutures slightly expressed closely subparallel to anterior edge of metacoxal cavities. Elytral integument seemingly smoothed.

IV. Discussion

The subfamily Hydroscaphinae of the family Hydroscaphidae of the superfamily Sphaeriusoidea was known only after the one compression fossil from the Cretaceous of the Yxian Formation (Huangbanjigou, Liaoning, China) [2]. However, most outlines of the sclerites of this fossil are rather unclear. Therefore, the suborder and family attributions of this taxon should be regarded as rather probable. The distinctness of separated meso- and metacoxae, and also three-segmented tarsi, which are used by the co-authors of this publication as the most essential arguments, can be only traced with some probability. This fossil has not enough characters to be compared with modern genera and to justify its attribution to *Hydroscapha* rather than to any other genus in Hydroscaphidae. Thus, the species name '*jeholensis'* should be attributed rather to a *genus incertus* or cf. *Hydroscapha* than strictly to the latter genus. Nevertheless, the assignment of this species to the subfamily Hydroscaphinae *sensu stricto* is clear enough after the key to the hydroscaphid subfamilies proposed below.

The second hydroscaphid subfamily Triamyxinae stat. nov. is extinct and known only after two findings from the Permian-Triassic boundary (*Palaeoscapha* gen. nov.*)* and late Triassic (*Triamyxa*). It is characterized in detail in the above diagnosis and remarks, and can be easily identified after the below key to hydroscaphid subfamilies.

The late Triassic *Leehermania prorova* Chatzimanolis, Grimaldi, Engel et Fraser, 2012 [3] from Cascade (near Martinsville, Virginia, USA; Cow Branch Formation, late Carnian – early Norian), originally described as the oldest Staphylinidae [3], was recently re-examined and re-interpreted as a probable 'ancestral' Hydroscaphidae [8]. The type species of this genus seems to have enough characters to put the genus *Leehermania* near the subfamily Triamyxinae stat. nov., but this genus, in contrast to *Triamyxa* and *Palaeoscapha* gen. nov. (see the above remarks to the subfamily Triamyxinae stat. nov.), has a markedly hypognathous head, pronotum widest at the midlength, relatively short meso- and metathoraces, very short elytra with truncate apices and seven abdominal ventrites with comparatively shorter ventrite 1. These mentioned differences make it possible to propose for this genus a new subfamily. Both descriptors [3] and re-examinators [8] of *Leehermania* indicated the presence of about six ventrites (derivatives of sternites III-VIII), while the pictures of lateral view in both publications clearly show seven segments on the underside of the abdomen, although Chatzimanolis et al. [3] pointed out that the 'lateral tergal sclerites of IX becoming tapered to

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narrow apex'. It is a rather important point that the specimens of *Leehermania prorova* have two basal ventrites vs. only one as indicated by Fikáček et al. [8] in the drawing-reconstruction of the Fig. 2H of their publication. This misinterpretation can be evident even comparing this reconstruction with the picture of the specimen used for this drawing-reconstruction (Fig. 2G). It is also clearly visible in Chatzimanolis at al. ([3]: Fig. 8). Besides, the abdominal ventrite 1 of *Leehermania prorova* has its length only slightly more significant than the length of the ventrites 2-6 (the proportion of the ventrite 1 is significantly greater in all other known hydroscaphids). The same also concerns the hypognathous head of *Leehermania prorova*, although some modern hydroscaphids could have their heads in hypognathous positions. The abovementioned arguments show a considerable distinctness of the considered species, which is treated as enough ground to propose a new extinct subfamily Leehermaniinae subfam. nov.

The second sphaeriusoid family is Sphaeriusidae Erichson, 1845 [6], also known in the fossil record as a single species from Burmese amber (*Burmasporum rossi*) described by Kirejtshuk [15] with 'generalized' structural features compared to the modern representatives of the family (members of the genus *Sphaerius* Erichson, 1845 [6]). It seems to have many possible plesiomorphic differences from the modern species, namely: the comparatively larger, longer and more projecting head with longer anterior part of frons and longer mouthparts, and distinctly elongate eyes; elongate scape and antennomere 2, small antennomere 3, loose antennal club with four antennomeres; movable and larger pronotum with shorter lateral parts and widely rounded anterior and posterior angles and comparatively long legs with simple and subparallel-sided femora and tibiae.

The super family Lepiceroidea is represented by the single family Lepiceridae in the Recent fauna. Some fossil representatives of this family are known from the Cretaceous Burmese amber. At first, this family had only three fossil specimens from the same source (Burmese amber), and all these specimens are rather poorly preserved for studies, so that some interpretations and conclusions appear rather ambiguous and partly inadequate because of the not quite clearly visible structures examined. On the other hand, the studies of these specimens were rather essential in understanding the myxophagan phylogeny. Therefore, the conclusions obtained after studies of the first two specimens by Kirejtshuk and Poinar [18, 19], and the conclusions obtained after comparison of the third specimen with previous information by Jałoszyński et al. [12] are different. The latter co-authors tried to find a compromise based on the available prior knowledge using the particular matrix of the characters for a cladogram or on an assumption that the facts of fossil representatives are essentially wrong [12]. As a result, the co-authors of the last publication regarded that all Mesozoic species belong to the same genus, which is also present in the modern fauna (*Lepicerus*). They treated the description of *Haplochelus* as obscure and unworthy. They decided that the diagnostic characters of both fossil genera *Haplochelus* Kirejtshuk et Poinar, 2006 [18] and *Lepichelus* Kirejtshuk et Poinar in Kirejtshuk, 2017 [16] (=*Lepiceroides* Kirejtshuk et Poinar, 2013 [19], non Schedl, 1957 [43]), and also modern *Lepicerus* could be treated as only specific. However, most characters of *Lepichelus pretiosus* (Kirejtshuk et Poinar 2013) [19], comb. nov. and *Lepichelus mumia* (Jałoszyński *et* Yamamoto in Jałoszyński et al., 2017) [12], comb. nov. are correspondent to each other. Differences between them could be depended upon preservation condition of examined specimens. The situation became more confusing because these co-authors some times declared that '*Lepicerus*' *mumia* has four antennomeres, as, in their opinion, should be in the modern members of *Lepicerus*, but the drawing in their paper (Fig. 2D) clearly shows five antennomeres as should be in *Lepichelus* (indeed according to Fikáček et al. [8] modern members of *Lepicerus* have four antennomeres in one species and five in others). Thus, the contradictions between the opinions of researchers can be proved or disproved only after the examination of additional specimens and further examination of the holotypes. The description of the poorly preserved material of *Lepichelus mumia* comb. nov. does not allow to clearly discriminate it from *L. pretiosus comb. nov.* [16]. The 'curved protarsomere 1' mentioned by Fikáček et al. [8] for *Lepichelus mumia* comb. nov. could be indeed a result of an optic aberration. These co-describers also indicated the following characters of *Lepichelus mumia* comb. nov. diagnostic from '*L. pretiosus*, in the epipleural concavity located behind the middle of the elytra (at the middle in *L. pretiosus*), and in the prosternal process narrowing, and not broadening posteriorly'. Perhaps, the problem of the conspecifity of specimens and synonymy of these species names has to be finally solved after a further re-examination of both type specimens and additional specimens. The extinct Cretaceous species differ from extant ones in the structure of elytral sides and less widely separated metacoxae (almost about comparably separated with mesocoxae). Anyway, it seems to be more correct to consider that the palaeoendemic genus *Lepichelus* comprising two species from Burmese amber are enough separated from the taxon with modern representatives, and it is not reasonable to join the modern and Cretaceous species in one generic taxon or tentatively consider them as two subgenera of the same genus.

The recent publication by Jałoszyński et al. [13], unfortunately, also contains some new wrong

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interpretations, but the good illustrations make it possible to solve some central problems in the contradictions and interpretations. In the last paper, the co-authors described the new genus *Lepiceratus* Jałoszyński, Luo, Yamamoto et Beutel, 2020 [13], which indeed completely fits with *Haplochelus* even in the number of antennomeres, the structure of elytra, comparatively narrowly separated all pairs of coxae of the type specimen of the genus type species (*L. ankylosaurus* Jałoszyński, Luo, Yamamoto et Beutel, 2020 [13]). The type species of both genera (*Haplochelus* and *Lepiceratus*) have some differences in the general body outline, different shapes of head and pronotum, eye sizes, presence of the visible discrimen only in *H. georyssoides* vs. absence in *L. ankylosaurus*, and some other features, which are better to consider as species differences than generic ones. Even the elytra of the latter species (*L. ankylosaurus*) are distinctly separated only in the artist's reconstruction of *L. ankylosaurus* ([13]: Fig. 1C) but not so distinctly in the photograph (Fig. 1A) and tomography reconstruction (Fig. 2A and 3A). It is thought that the sutural elytral edges can be relatively sharply elevated and firmly closed together to make an impression of the united fused ridge (as in the holotype of *Haplochelus georyssoides*). A median depression of the mentum with the sharp outline of this holotype could make an illusion of gular sutures [18], and also a more or less weak median depression is quite characteristic of other lepicerids. Thus, it is reasonable to considerthe name *Lepiceratus* as a junior synonym of *Haplochelus*.

Taking into consideration the small body size of all known extant and fossil myxophagans and a small probability of their coming into deposits (tanatocenoses), it can be supposed that the available fossil record of this group is rather scarce among coleopterans in general. Nevertheless, the suborder Myxophaga is rather ancient and originated not later in the Palaeozoic (as suborders Archostemata and Adephaga). D.E. Shcherbakov found one unique tiny disc-like beetle larva in the extensive collection from the Middle Permian (~265 Ma) of Kargala near Orenburg, European Russia (Borissiak Paleontological Institute, Russian Academy of Sciences), whose print demonstrates a considerable similarity to larvae of some modern members of Torridincolidae Steffan, 1964 [49] (superfamily Sphaeriusoidea). The current data on this suborder is not sufficient enough for grounded phylogenetic conclusions. The rather ancient origin of Myxophaga and the scarcity of its fossil record make it impossible to use the current methods to reconstruct its phylogeny. It is thought that the groups of four myxophagan families in the Recent fauna represent only rather separate splinters of the significant diversity of this suborder in the past, although hydroscaphids (and probably something like torridincolid-like larvae) could be present in the Palaeozoic faunas. Other two myxophagan families (Sphaeriusidae and Lepiceridae) and also true torridincolids with more specialized structural appearance could have the later (Mesozoic) origin (as traced in the current fossil record of the suborder). Nevertheless, many phylogenetic hypotheses after formal analyses of structural and molecular comparisons give a basal placement for the Hydroscaphidae ([8, 26]; etc.), i.e., showing some correspondence with the current fossil record. It is necessary to take into consideration that the result of structural analysis is somehow connected with the more generalized appearance of hydroscaphids (a greater proportion of plesiomorphies in general). At the moment, strict phylogenetic reconstructions are somewhat premature. However, it can be supposed that the phylogeny of this suborder could have a complexity analogous to that of cupedids from the suborder Archostemata. A grounded phylogenetic model will be more probable after getting more data on fossils of various ages and applying the principle of multiple parallelisms [17].

The very conspicuous peculiarity of this suborder was pointed out by Crowson [5] that the myxophagan families have structural and partly bionomically analogous groups among various small polyphagans. In particular, adults of modern Hydroscaphidae, according to the opinion of R.A. Crowson, have many similarities with 'staphylinoids', 'hydrophiloids', and basalmost eucinetids [5]. The lepicerids, in general appearance, resemble elmids [5] or georissids [18]. The species of Sphaeriusidae somehow are reminiscent of polyphagan clambids (Scirtoidea) and cybocephalines (Nitidulidae, Cucujoidea) (see notes on 'Calyptomeridae' in Crowson, 1967 [5]). Thus, in the phylogenetic reconstruction of myxophagans, it would be desirable to take into consideration many aspects of structural convergence, including adaptive and morphogenetic processes.

It can be supposed some kind of association between Palaeozoic and Mesozoic myxophagans and algae, which is somewhat similar to that between the modern representatives of this suborder and their food resource. As another Permian suborder Archostemata with little structural transformations reached nowadays mostly because of the maintenance of ancient lifestyle and habits, modern myxophagans could also have a considerable conservatism in their habitats and diet from the Palaeozoic together with their general appearance, keeping small body-size and simplification of some structures. Modern myxophagans with known bionomy, including hydroscaphids, usually inhabit aquatic to moist environments associated with algal growth [47]. Probably a certain similarity in throphic connections should also be expected in the case of the fossil *Palaeoscapha tunguskaensis* gen. et sp. nov. As to *Triamyxa coprolithica*, described from coprolite of

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dinosaur form *Silesaurus opolensis* from the lacustrine Krasiejów deposits [26], it is thought that this beetle inhabited aquatic and moist environments associated with algal mats (most likely growing at shores of water bodies or floating). Floating mats or their aggregates are known from different Mesozoic localities and proposed as probable habitats for many aquatic insects, including beetles, as well as food resources for vertebrates such as dinosaurs [31, 33, 34].

V. Taxonomic Conclusions

The available data of fossil records on Myxophaga, taking into consideration the above discussion, can be summarized as follows:

Superfamily *Sphaeriusoidea* Erichson, 1845 [6]

Family *Hydroscaphidae* LeConte, 1874 [22]

Subfamily *Hydroscaphinae* LeConte, 1874[22]

– (?) '*Hydroscapha*' *jeholensis* Cai, Short et Huang, 2012 [2] – China, Liaoning Province (Huangbanjigou), Yixian Formation; Lower Cretaceous, Lower Aptian

Subfamily *Triamyxinae* Qvarnström, Fikáček, Wernström, Huld, Beutel, Arriaga-Varela, Ahlberg et Niedźwiedzki, 2021 [26], stat. nov.

– *Triamyxa coprolithica* Qvarnström, Fikáček, Wernström, Huld, Beutel, Arriaga-Varela, Ahlberg et Niedźwiedzki, 2021 [26] – Krasiejów clay pit (near Ozimek), Upper Silesia, Poland, Upper Triassic
– *Palaeoscapha tunguskaensis* gen. et sp. nov. – Siberia, Tunguska Basin, Untuun; probably early Triassic or terminal Permian, Induan or ?Tatarian

Subfamily *Leehermaniinae* Kirejtshuk, Prokin et Ponomarenko, subfam. nov.

urn:lsid:zoobank.org:act:3B6B5BDD-1CD8-40AC-BFDB-461C76B23842

Type genus *Leehermania prorova* Chatzimanolis, Grimaldi, Engel et Fraser, 2012 [3]

Diagnosis. Head seemingly hypognathous with eyes located at base moderately arcuately protruding and very short temples. Antennae 11-segmented and clubbed. Mesothorax extremely short. Metathorax comparatively short. Metepisterna strongly and almost rectilinearly widened anteriorly, their mesal anterior angle very narrowly separated from outer edge of mesocoxal cavity. Metacoxae seemingly very narrowly separated to conjoining and apparently without raised coxal plates. Elytra somewhat shortened and widely transversely truncate at apex; epipleura seemingly not extending behind level of metacoxae. Abdomen with seven ventrites.

– *Leehermania prorova* Chatzimanolis, Grimaldi, Engel et Fraser, 2012[3] – USA, Virginia, Cascade (near Martinsville), Cow Branch Formation, Upper Triassic, Upper Carnian/Lower Norian

Family *Sphaeriusidae* Erichson, 1845 [6]

– *Burmasporum rossi* Kirejtshuk 2009 [15] – Myanmar, Burmese amber; Myanmar, Kachin, Hukawng Valley; 'mid'–Cretaceous, Albian/Ceno-manian

Superfamily *Lepiceroidea* Hinton, 1936[11]

Family *Lepiceridae* Hinton, 1936 [11]

– *Lepichelus pretiosus* (Kirejtshuk et Poinar, 2006), comb. nov. [19] [*Lepiceroides*] – Burmese amber; Myanmar, Kachin, Hukawng Valley; 'mid'-Cretaceous, Albian/Cenomanian
– Probable synonym *Lepichelus mumia* (Jaloszynski and Yamamoto, 2017), comb. nov. [12] [*Lepicerus*] – Burmese amber; Myanmar, Kachin, Hukawng Valley; 'mid'-Cretaceous, Albian/Cenomanian
– *Haplochelus georyssoides* Kirejtshuk et Poinar, 2006 [18] – Burmese amber; Myanmar,Kachin, Hukawng Valley; 'mid'-Cretaceous, Albian/ Cenomanian
– *Haplochelus ankylosaurus* (Jałoszyński, Luo, Yamamoto et Beutel, 2020), comb. nov. [13] [*Lepiceratus* Jałoszyński, Luo, Yamamoto et Beutel, 2020, syn. nov. [13]] – Burmese amber; Myanmar, Kachin, Hukawng Valley; 'mid'-Cretaceous, Albian/ Cenomanian.

VI. Key to Hydroscaphid Subfamilies

1. Elytra long, covering completely or most part of abdomen and subacute at apices; abdomen with five or six ventrites; –metacoxae (sub) contiguous; mentum large; antennae with three-segmented club . . . . . .Triamyxinae stat. nov.
– Elytra very short and truncate at apices, leaving 3-4 abdominal segments uncovered; abdomen with six or seven ventrites; – other characters different 2
2. Abdomen with six ventrites; antennae with five or nine antennomeres with ultimate sclub-shaped; metacoxae with coxal plate and clearly separated. . . . . Hydroscaphinae *sensu stricto*
– Abdomen with seven ventrites; antennae with three-segmented club; metacoxae without coxal plate and narrowly separated or (sub) contiguous . . . .Leehermaniinae subfam. nov.

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Appendix A

After acceptance of this paper (November 23, 2022) two new papers on the fossil members of Sphaeriusidae appeared (December 7, 2022 and January 11, 2023):

1. Fikáček M., Yamamoto S., Matsumoto S., Beutel R.G., and Maddison D.R. (2022). Phylogeny and systematics of Sphaeriusidae (Coleoptera: Myxophaga): minute living fossils with underestimated past and present-day diversity. Systematic Entomology 2022,1–17. https://doi.org/10.1111/syen.12571
2. li Y.D., Ślipiński A., Huang D.Y., Cai C.-Y. (2023) New fossils of Sphaeriusidae from mid-Cretaceous Burmese amber revealed by confocal microscopy (Coleoptera: Myxophaga). Frontier of Earth Science, 11 January 2023. https://doi.org/10.3389/feart.2022.901573

The first paper has a description of the sphaeriusid genus *Bezesporum* Fikáček, Yamamoto, Matsumoto, Beutel et Maddison, 2022 (type species: *Sphaerius minutus* Liang et Jia, 2018 – modern, China, Jiangxi Province, Xiping County) including as two modern species as one fossil Bezesporum burmiticum Fikáček, Yamamoto, Matsumoto, Beutel et Maddison, 2022 from Burmese amber; Myanmar, Kachin, Hukawng Valley; 'mid'-Cretaceous, Albian/Cenomanian. This fossil species is rather different from *Burmasporum rossi* after the diagnosis proposed by Fikáček et al. (2022), however it is quite problematic to compare modern and fossil species because of bad preservation of the latter. The modern and fossil species seem to differ at least by shape of main body sclerites, antennal club, maxillary palpi, and protibiae. Nevertheless, the taxonomic relation between them needs further consideration. In order to avoid confusion analogous with that in lepicerids (see above Discussion) it would be better to indicate fossil species as cf. *'Bezesporum' burmiticum*.

The second paper is devoted to descriptions of two sphaeriusid species from Cretaceous Burmese amber. One of them is very similar to modern species of the genus *Sphaerius* Waltl, 1838 and named as *S. martini* Li et Cai, 2023, while another species is different from all other congeners of the sphaeriusid genera and, therefore, it described with proposal a new genus *Crowsonaerius* Li & Cai, 2023 (*C. minutus* Li et Cai, 2023, designated as type species of the latter genus).

These new data increase the number of the known sphaeriusid genera in fossils till four ones originated from the same resource (Burmese amber). These materials and particularly finding of a species assigned by Li et. al. (2023) to the genus of *Sphaerius* known in the Recent fauna support the opinion on probable considerable diversity of this family in the Mesozoic and consevatism of the suborder Myxophaga in general (see the above discusion).

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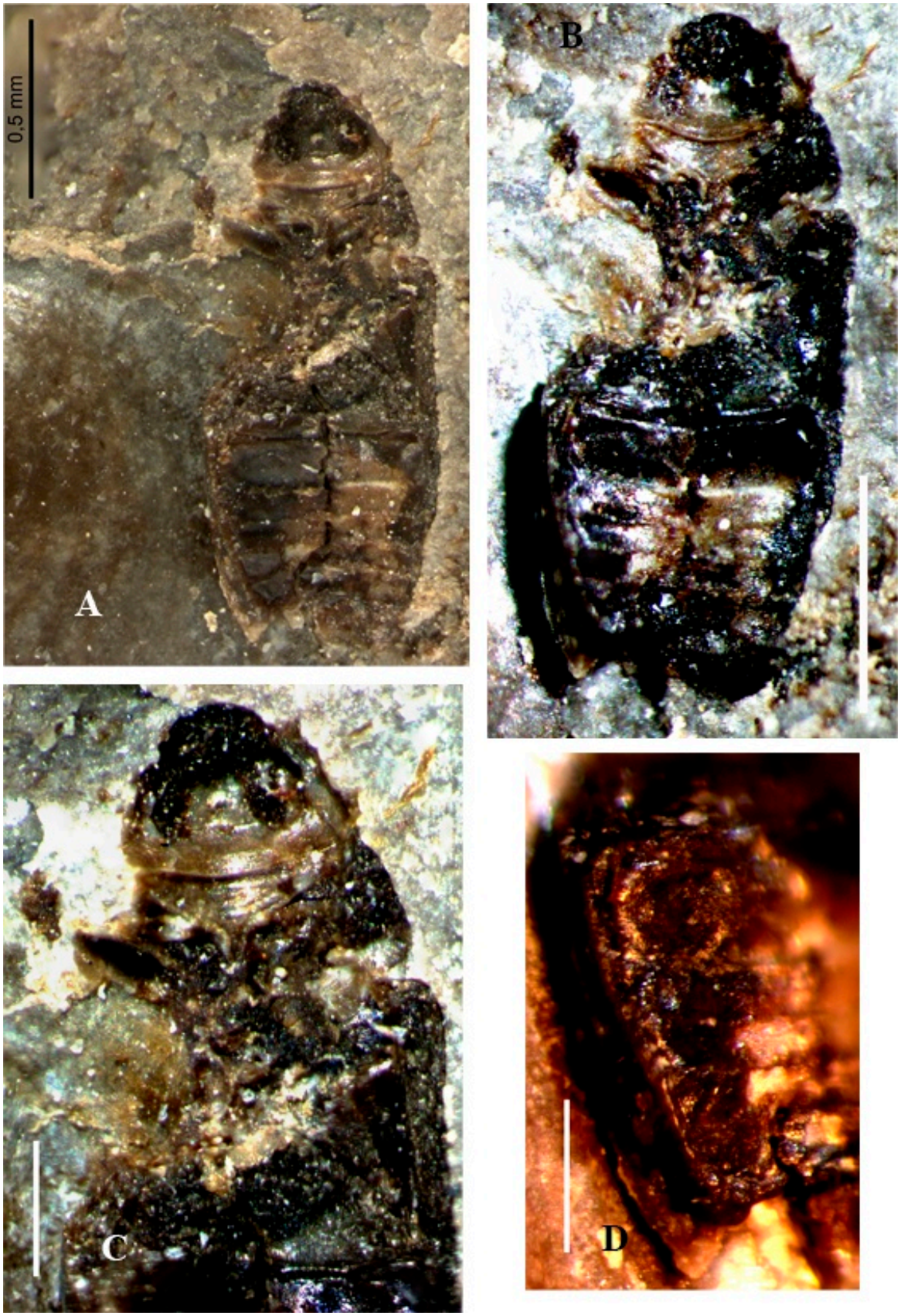

Figure 1: *Palaeoscapha tunguskaensis* gen. et sp. nov., holotype, photographs: A, B – habitus, C – head and thorax, D – abdomen. Scale bar 0.5 mm (A, B), 0.2 mm (C,D).

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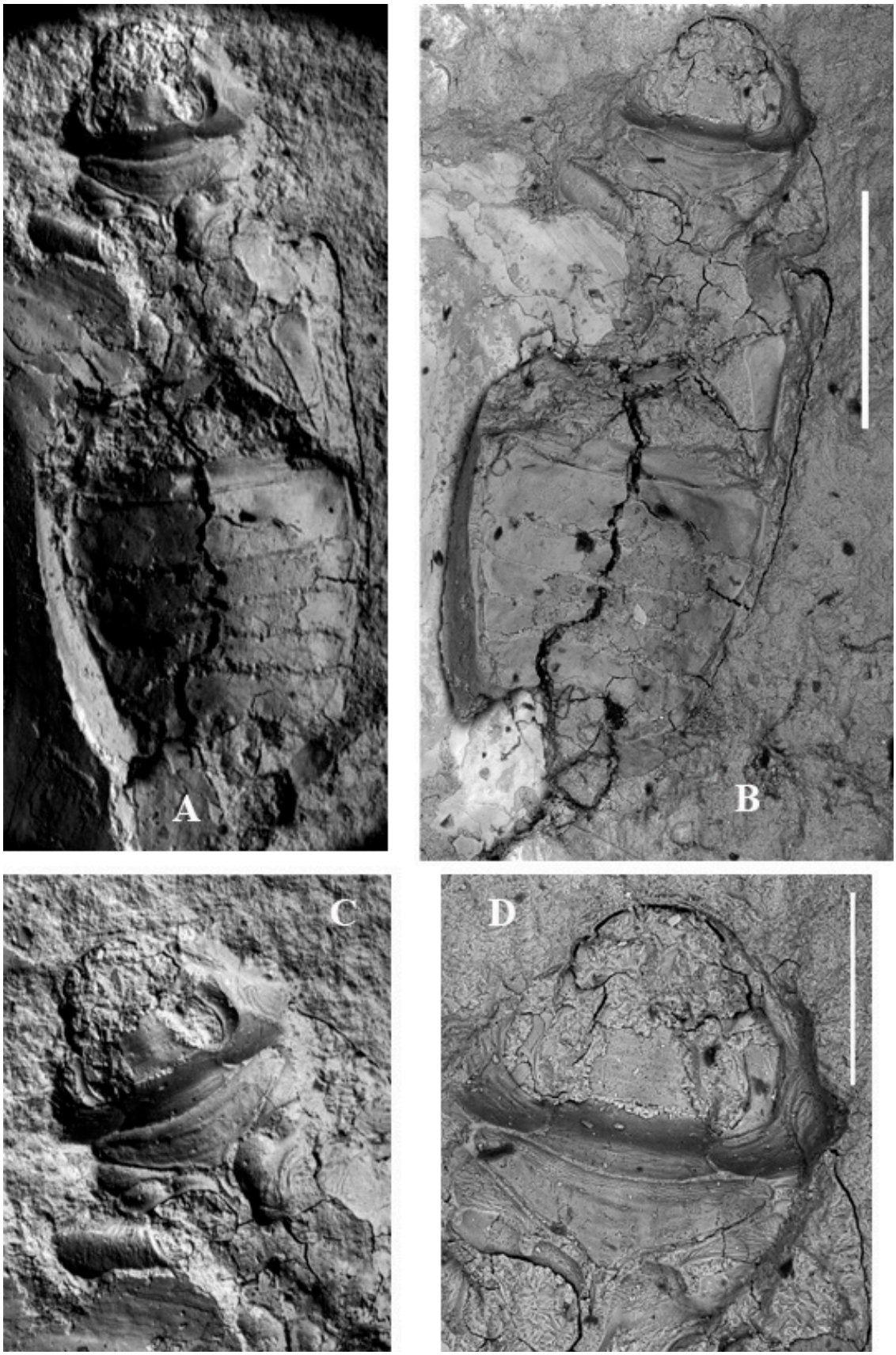

Figure 2: *Palaeoscapha tunguskaensis* gen. et sp. nov., holotype, SEM: A, B – habitus, C, D – head and prosternum. Scale bar 0.5 mm (B), 0.2 mm (D), not for scale (A,C).

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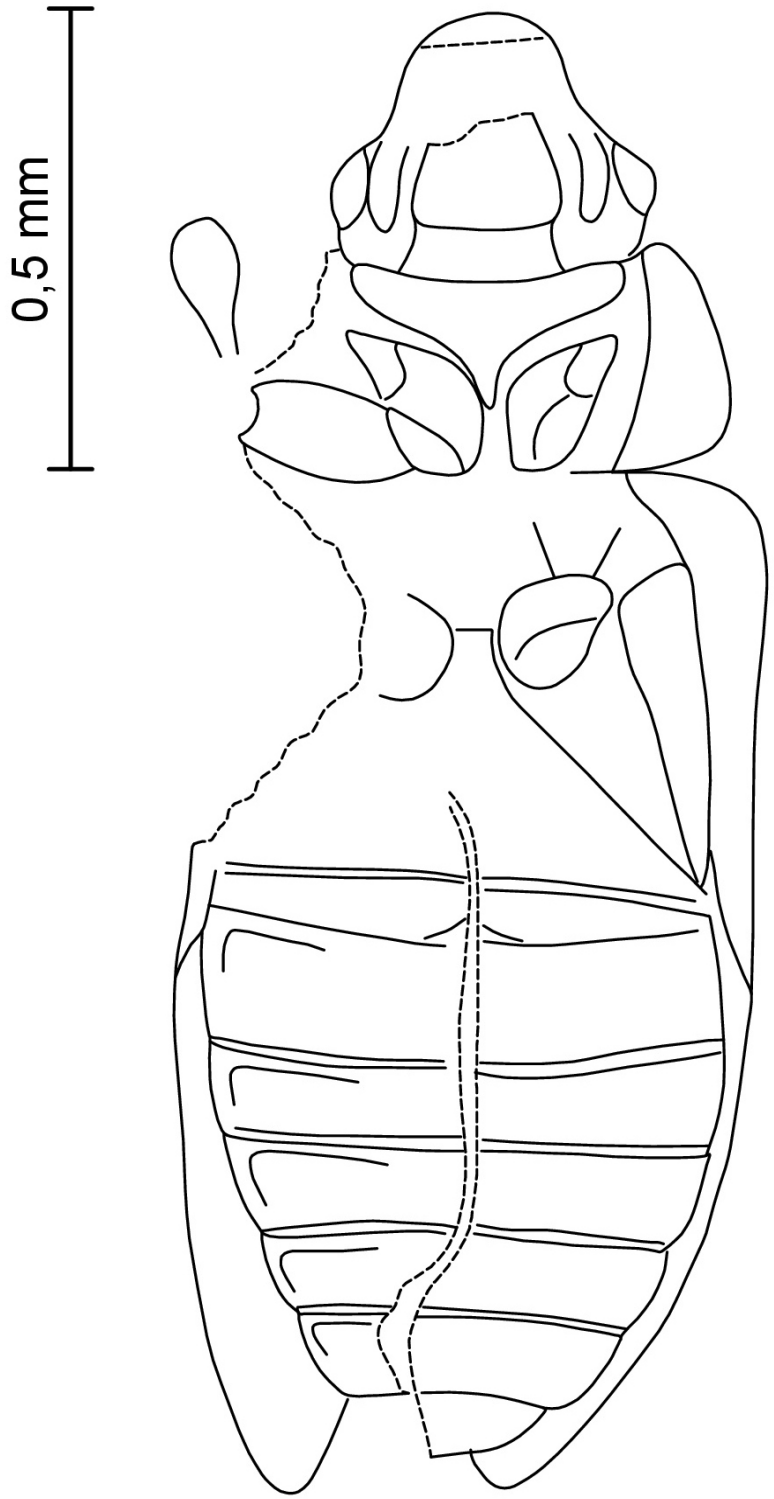

Figure 3: *Palaeoscapha tunguskaensis* gen. et sp. nov.: holotype, habitus, line drawing. Scale bar 0.5 mm.

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Changes in Total Protein Level in Human Female Saliva during Ovulatory Functions and Metabolic Disorders

By Shweta & Navi Ranjan
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Abstract- Saliva has been studied extensively as a potential diagnostic tool over the last decade due to its ease and non-invasive accessibility along with its abundance of biomarkers, such as genetic material and proteins. The activity of protein in saliva increased during ovulation. When we monitored salivary protein activity in 40 different women volunteers during various stages of reproduction like (prepubertal, parous, non-parous, menopausal and in the metabolic disorder state diabetic condition it has been observed that highly significant ($p<0.001$) increase in parous ovulatory & non parous ovulatory whereas, highly significant ($p<0.01$)increase was observed in non-parous postovulatory and a highly significant ($p<0.001$) decrease was observed in menopause and diabetic in comparison to prepubertal. A highly significant ($p<0.001$) decrease was observed in menopause in comparison to parous preovulatory, ovulatory, post ovulatory & non-parous ovulatory and postovulatory. A highly significant ($p<0.01$) decrease was observed in diabetic in comparison to menopausal human female subjects. The result revealed that the total protein was considered as testing the saliva instead of blood isa non-invasive loom and it can be used as a biomarker for ovulation detection.

Keywords: saliva, total protein, pre-pubertal, parous, non-parous, menopausal and diabetic.

GJSFR-C Classification: DDC Code: 574.192 LCC Code: QP514.2



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Changes in Total Protein Level in Human Female Saliva during Ovulatory Functions and Metabolic Disorders

Shweta [α] & Navi Ranjan [σ]

Abstract- Saliva has been studied extensively as a potential diagnostic tool over the last decade due to its ease and non-invasive accessibility along with its abundance of biomarkers, such as genetic material and proteins. The activity of protein in saliva increased during ovulation. When we monitored salivary protein activity in 40 different women volunteers during various stages of reproduction like (prepubertal, parous, non-parous, menopausal and in the metabolic disorder state diabetic condition it has been observed that highly significant ($p<0.001$) increase in parous ovulatory & non parous ovulatory whereas, highly significant ($p<0.01$)increase was observed in non-parous postovulatory and a highly significant ($p<0.001$) decrease was observed in menopause and diabetic in comparison to prepubertal. A highly significant ($p<0.001$) decrease was observed in menopause in comparison to parous preovulatory, ovulatory, post ovulatory & non-parous ovulatory and postovulatory. A highly significant ($p<0.01$) decrease was observed in diabetic in comparison to menopausal human female subjects. The result revealed that the total protein was considered as testing the saliva instead of blood isa non-invasive loom and it can be used as a biomarker for ovulation detection.

Keywords: saliva, total protein, pre-pubertal, parous, non-parous, menopausal and diabetic.

I. Introduction

As a diagnostic fluids Body fluids like blood, saliva, tears, sweat and urine are a source of pathological biochemical markers in which saliva is an excellent biological fluid that is useful for noninvasive exploration of the human diseases and physiological conditions (Villiger *et al*., 2018). Salivary protein concentration is dependent on gland production at time of day, diet, age, gender and presence of disease (Ferreiro *et al*., 2002). Saliva consists mainly of water, enzymes, ions and amino acids and performs several important functions in oral health. It contains various biomolecules such as proteins, enzymes and hormones. Saliva contains a large number of proteins that participate in the protection of the oral tissues, for instance lysozyme, lactoferrin, lactoperoxidase, immunoglobulins, ag- glutinin and mucins. Nieuw Amerongen and Veer- man 2002, Denny *et al.,* 2008 and Yan *et al.,* 2008 reported about two thousand proteins in human saliva using mass spectrometry. Sanjay *et al.,* 2008 reported significant high total salivary protein in the saliva of cancer patient. In the beginning of menstruation and during ovulation, the protein content of saliva increases considerably, which turns out to be a rich source of nutrient to bacteria, the count of which may increase during menstruation and ovulation. Protein in the serum is made up of albumin and globulin. Albumin is made mainly in the liver which helps tissue growth and healing. Globulins is made up of different proteins called alpha, beta and gamma. Some globulins are made by liver while others are made by immune systems. Salivary proteins, such as mucins, α-amylase, lysozyme, and peroxidase are synthesized and packed into secretory granules in acinar cells. Salivary protein secretion is strongly enhanced by the sympathetic nerve stimulation. Perinpanayagam *et al.,* 1995 reported that small peptide in saliva showed proteolytic activity. Chicharro *et al.,* 1998 indicated that the salivary level of total protein increases also through β –sympathetic activity in salivary glandssince saliva secretion is mainly evoked by the action of androgenic mediators. Hu *et al.,* 2004 and Huang, 2004 studied the structure and function of a large number of proteins in human saliva with traditional biochemical techniques including chromatography, gel electrophoresis, mass spectrometry, immunoassay. Tabak, 2001 and Simpson *et al.,* 2005 also detected various enzymes, enzyme inhibitors, hormones (growth factor) and cytokines (Interlukin-8) in the saliva. Van Nieum Amerongen & Veerman 2002 studied the functional aspect of important proteins in saliva which act as mineralization of the enamel, taste, perception, digestion, inhibition, cell proliferation, chemotaxis, and cell motility. Zachariasen, 1992 also indicated that changes in ovarian hormone levels during puberty, parous pregnancy menstrual cycle and after oral contraceptive use appear to co-relate with decrease in various proteins. Ben-Aryeh *et al.,* 1986 and Arranz *et al.,*1992 also reported increase in total protein and reduction in amylase activity with age in parotid saliva. Lakshmi *et al*., 2015 showed a higher level of salivary total proteins and α-amylase in patients with DM. Panchbhai *et al*., 2010 studied on salivary total proteins, and α-amylase of well-controlled and poorly-controlled DM patients

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compared with healthy individuals and showed a significantly lower level of salivary α-amylase in patients with well-controlled DM compared to healthy subjects.

II. Materials and Methods

The studies were performed in 63 different human female volunteers of age group (7 to 45yrs.) categorizedas prepubertal, parous, non-parous, menopausal and diabetogenic. The human female unstimulated whole saliva was collected during various periods of parousand non-parous volunteers, viz. pre-ovulatory, ovulatory and post ovulatory phases and also from pre-pubertal, menopausal and diabetogenic stages and were stored at -70°C for further use (Navazesh and Christensen, 1982). Subjects were asked not to swallow any saliva during the collection period. Saliva volume was measured with the tube sealed and then frozen in dry ice until taken back to the laboratory for processing. All the saliva samples of different category of female subjects were placed into salivate tubeusing a natural cotton swab insert and centrifuged at 400gfor 10 minutes at 4°C. The Total protein was measured as per method of Lowry *et al.,* 1951 by Folin-ciocalteau phenol reagent.

III. Results & Discussion

Table 1: Level of Total Protein in saliva of different conditions in human female subjects

Sl No.	Name of different conditions with symbols	Level of Total Protein(mg/dl), Mean and SE of 6 samples	P-Value
1	Pre- pubertal –(a)	9.51 ± 0.153	
2	Parous Pre-ovulatory –(b) Ovulatory – (c) Post ovulatory –(d)	 9.40 ± 0.184 16.50 ± 0.149 11.28 ± 0.181	 a to c – (p<0.001) HS a to e – (p<0.001) HS a to f – (p<0.001) HS a to g – (p<0.01) HS
3	Non- Parous Pre-ovulatory –(e) Ovulatory – (f) Post ovulatory –(g)	 7.47 ± 0.264 11.58 ± 0.147 10.38 ± 0.153	 b to e – (p<0.001) HS c to f – (p<0.001) HS d to g – (p<0.01) S
4	Menopausal –(h)	7.61 ± 0.150	a to h – (p<0.001) HS b to h – (p<0.001) HS c to h – (p<0.001) HS d to h – (p<0.001) HS f to h – (p<0.001) HS g to h – (p<0.001) HS
5	Diabetogenic – (i)	6.58± 0.164	a to i – (p<0.001) HS b to i – (p<0.001) HS c to i – (p<0.001) HS d to i – (p<0.001) HS e to i – (p<0.02) S f to i – (p<0.001) HS g to i – (p<0.001) HS h to i – (p<0.01) HS

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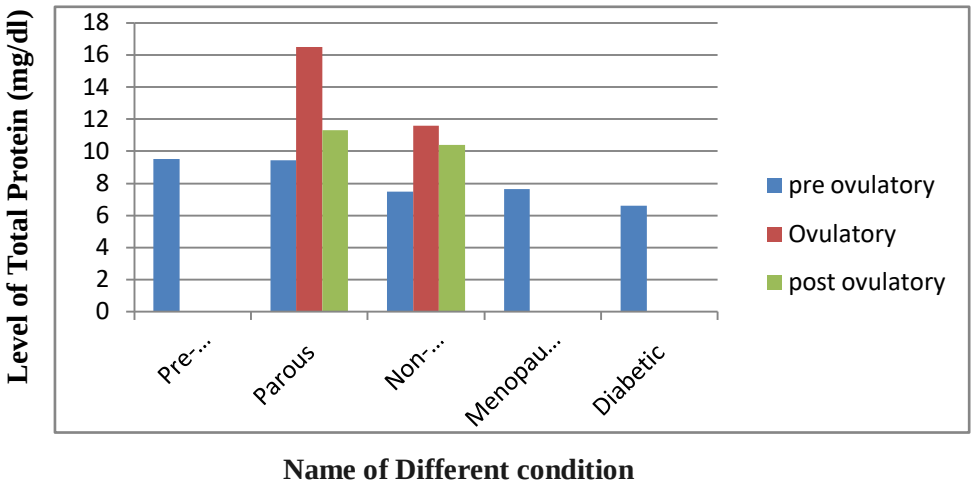

Figure

As per our findings a highly significant increased salivary total protein in parous and non-parous ovulatory and post ovulatory phase of menstrual cycle in comparison to prepubertal salivary total protein might be due to the diminished level of ovarian steroid estrogen & progesterone hormone in pubertal stage of female subject and active secretion and synthesis of steroid hormone in parous & non- parous human female subjects. A highly significant decreased level of salivary protein in non-parous preovulatory, ovulatory, post ovulatory phases of menstrual cycle than parous women phases might be an indication of disturbed hormone level in non- parous women. Earlier report of Alagendran *et al.,* 2013 indicated that protein range was highest at ovulatory phase than pre and post ovulatory phases due to estrogen peak. Thus, increased salivary protein level may be estrogen dependent. He further observed that total protein level in saliva were maximum during ovulation and minimum during post ovulatory phase. But in menopausal women's saliva the total protein showed as decreased level due to physiological endocrine-logical changes in menopausal women. As earlier report of Ambatipudi *et al.,* 2009 indicated that age related changes in protein abundance were observed. But in diabetogenic women a significant & highly significant increased level of salivary protein in comparison to prepubertal, parous and non-parous reproductive cycle phases salivary protein and from menopausal women might be due to disturbed metabolic activity in diabetic condition of women might be due to reduced salivary fluid secretion and various range of underlying pathogenic factors. Earlier report of Antonio *et al.,* 2004 support our findings that diabetic patients were attributed to reduced salivary fluid secretion. Our findings are consistent with the findings of Dodds and Dodds 1997 that increased salivary protein were observed in diabetic patients. Twetman *et al.,* 2002 also observed increased salivary total protein level in type -1 diabetic human female subjects than type-2.

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Closed Motion of a Biological Time Vector Field and the Cellular Theory of a Plant Organism

By Naumov Mikhail Makarovich

Odessa State Ecological University

Abstract- In this work, we continue to explore the time inside the plant organism. The beginning of the study of time inside the plant organism is given in [1]. It is shown that inside the plant organism biological time has the character of a vector time field. A dynamic system of two differential equations is constructed, corresponding to this field and corresponding to the whole plant organism (for sunflower). It is shown that such a vector time field divides the plant organism into cellular and subcellular structures. Such a time field has been studied and verified on the basis of data from agrometeorological yearbooks. The calculations of the time axis are made and the phase portrait of the field is obtained.

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Closed Motion of a Biological Time Vector Field and the Cellular Theory of a Plant Organism

Naumov Mikhail Makarovich

Annotation- In this work, we continue to explore the time inside the plant organism. The beginning of the study of time inside the plant organism is given in [1]. It is shown that inside the plant organism biological time has the character of a vector time field. A dynamic system of two differential equations is constructed, corresponding to this field and corresponding to the whole plant organism (for sunflower). It is shown that such a vector time field divides the plant organism into cellular and subcellular structures. Such a time field has been studied and verified on the basis of data from agrometeorological yearbooks. The calculations of the time axis are made and the phase portrait of the field is obtained.

I. Introduction

a) *Materials and Research Methods*

1. Mathematical apparatus
2. Construction of an autonomous system of two differential equations biological vector time field of the plant organism.
3. Study of the autonomous differential system of the vector time field of the plant organism in the Euclidean plane

clause. 3.1 Theoretical results.

clause 3.2. Practical results of calculations of the axis and field of biological time inside the plant organism according to agrometeorological data ?? yearbooks что это за слово?? оно нужно??

clause 3.3 Phase portrait of the autonomous dynamic system of the sunflower biological vector time field.

Literature

Discussion

II. Introduction

In this work, we will show that the closed trajectory of the movement of biological time (or simply time) in the plant organism during ontogenesis ensures its division into a cellular structure with subcellular organelles. In the work “On The Closed Trajectory of Movement of Biological Time Inside the Organism of a Plant in Ontogenesis and Everything Secret Becomes Apparent” [1], it was shown and proved that time in a plant organism moves along a closed trajectory. This closed trajectory of time, at its simplest, can be a circle or an ellipse.

Let us give an example: a closed trajectory of the movement of a biological e around the circle. The circle is written in parametric form:

$$T_F(j) = 1 - \cos(2\pi t(j)) \quad \text{(V.1)}$$

$$T_R(j) = \sin(2\pi t(j)) \quad \text{(V.2)}$$

Here *t(j)* - is a parameter - physical time, varies from small t_0 to *1*, *rel. units*, if *t(j)* is expressed in *days j*, then it is equal to $1/t_E$, t_E — the, where is of the entire ontogenesis, *days*; $T_F(j)$ - normalized value of the duration biological time corresponding to the process of photosynthesis, *rel. un.*; $T_R(j)$ - normalized value of biological time corresponding to the process of respiration, *rel.un.*; *j* - is the number of the day of the billing period. Day *j* changes from a small value, corresponding to the "plant shoots" phase, to a certain value of the day, corresponding to the "full maturity of the plant" phase. Number of days from year to year may vary depending on the prevailing agrometeorological conditions.

For the time ellipse of the plant organism, a slightly different parametric equation, see [1].

Thus, the main conclusion is that, as shown in [1], that in a living, growing, plant organism (one-year vegetation period), biological time, or simply time, has a closed nature of movement. This movement is ensured, respectively, by the unchanging movement of our physical time.

Biological time, or simply time, we can explore with the help of mathematical apparatus, and material processes observed in *Nature*, in the *plant organism*. Time is not material, like matter, but it can influence material processes.

III. Materials and Research Methods

a) *Mathematical apparatus*

In the beginning, it is necessary to acquaint you with the necessary mathematical apparatus. This mathematical apparatus is well described in the work of Ac. Pontryagina L.S. together with Ac. Andronov A.A. "Rough Systems" [2], in 1937. This is a system of two autonomous differential equations. In a later book by Bautin N.N. and Leontovich E.A. [3], 1990, methods are presented that allow solving a system of autonomous differential equations on a computer, see also the work of Reznichenko G.Yu. [4].

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In [2], a dynamical system of two equations of the first order:

$$dx/dt = P(x, y); dy/dt = Q(x, y), \qquad (A)$$

where x and y are Cartesian coordinates on the plane and where $P(x,y)$ and $Q(x,y)$ are analytic functions for all considered values of the variables x and y.

We consider such systems of two differential equations (A) for which there is a "*cycle without contact*". The system (A) is "*coarse*" in a given domain G if its conditions are satisfied, in contrast to systems that are not "*coarse*" [2].

Theorem I. If the system (A) is rough in the domain G, then in the domain G the system (A) can have only such equilibrium states for which the real parts of the roots of the corresponding characteristic equation are nonzero [2].

Theorem II. If the system (A) is rough in the domain G, then in the domain G the system (A) can have only such periodic motions for which the characteristic exponent is not equal to zero [2].

Theorem III. If system (A) is rough in G, then system (A) in G can have only such separatrices (saddle whiskers) that do not go from saddle to saddle [2].

Obviously, rough systems exist. In more detail signs and theorems of the existence of autonomous dynamical systems (A) are presented in the article [2].

The phase portrait of an autonomous system of two differential equations is considered in the Euclidean plane, which is called the phase plane, and depicts the set of system states (A). The point $M(x, y)$ is called the depicting or representing point. To construct a phase portrait of a dynamic system of two autonomous differential equations (A), the isocline method is used [4]. The main types of trajectories of a dynamic system (A) are shown in Fig. 1.

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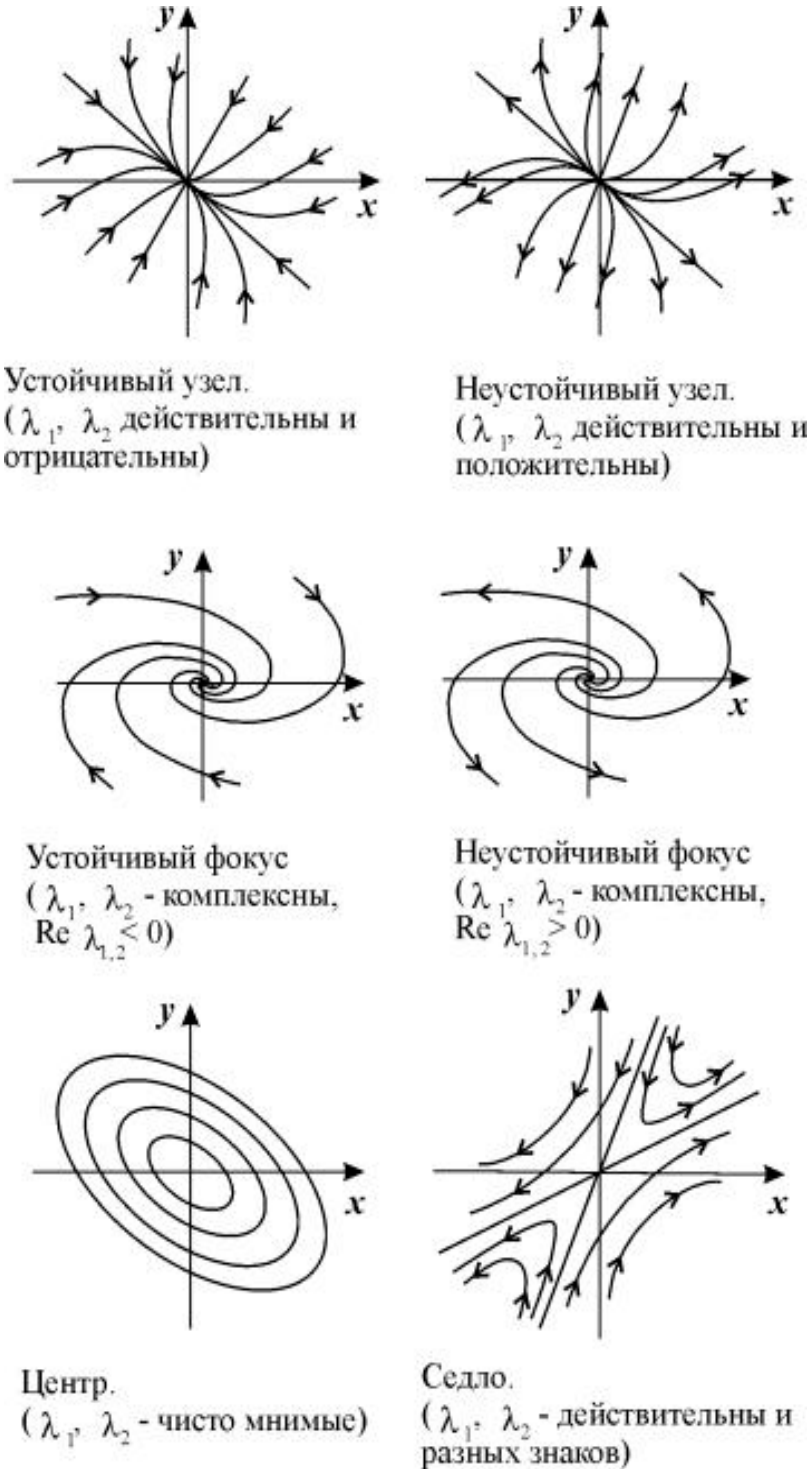

Fig. 1: The main types of phase trajectories of a dynamical system (A) in Cartesian coordinates, that is, in two-dimensional time space planes

1. Stable node — λ_1, λ_2 roots of the characteristic equation are real and negative;
2. Unstable node — λ_1, λ_2 the roots of the characteristic equation are real and positive;
3. Stable focus — λ_1, λ_2 the roots of the characteristic equation are complex $Re\ \lambda_{1,2} < 0$;
4. Unstable focus — λ_1, λ_2 the roots of the characteristic equation are complex $Re\ \lambda_{1,2} > 0$;
5. Center — λ_1, λ_2 the roots of the characteristic equation are purely imaginary;
6. Saddle — λ_1, λ_2 roots of the characteristic equation are real and have different signs.

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b) Construction of an autonomous system of two differential equations of the biological vector time field of the plant organism

We, in a natural way, will construct an autonomous system of differential equations of a biological vector time field. To do this, we first calculate the vector time axis of the plant organism:

$$dT(j)_{\text{environment}} = U(j)_{\text{environment}} \cdot dT_{opt} \qquad T_0 < T(j) < 1, \tag{1}$$

where dT_{opt} - is the biological time differential under optimal agrometeorological environmental conditions (Light; Heat; Moisture; Mineral Nutrition), constant, *rel. units*; *dT(j)* - environment is the biological time differential under the current agrometeorological environmental conditions, *rel. Units*; *U(j)* - environment - the level of tension of environmental factors, depends on the current agrometeorological conditions, *rel. units*, shows the ratio of CO_2 gas exchange of the whole plant organism under current agrometeorological environmental conditions to CO_2 gas exchange under optimal agrometeorological environmental conditions of the whole plant organism at the same moment of ontogenesis; T_0 - is the initial value of the biological time vector of the plant organism, its length, *rel. units*; *T(j)* - is the current value of the biological time vector of the plant organism, its length, *rel. Units.*

Equation (1) is considered in the time interval from "plant shoots" to "flowering", which corresponds to values from small T_0 to *1*.

Let us find the integral of equation (1):

$$T(j) = \int_{T_0}^{1=\text{flowering}} dT(j)_{environment} = \int_{T_0}^{1=\text{flowering}} [U(j)_{environment}]_{\text{average}}\, dT_{opt}, \qquad T_0 < T(j) < 1 \tag{2}$$

where $[U(j)_{\text{enironment}}]_{\text{average}}$ - is the average value of the level of tension of the external environment for the period of time from the "shoots" to the current moment of integration - *T(j)*, *rel.units*.

This integral (2), in a natural way, shows how the length of the biological time vector is summed up in the plant organism. At the same time, the original sum equation [5] was used in the integral:

$$T(j) = \sum_{j=1}^{n} \Delta T_{opt} \cdot U(j)_{environment} \tag{3}$$

where *j=1* - corresponds to the vegetative phase "shoots"; ΔT_{opt} - is the increase in biological time under optimal agrometeorological environmental conditions for one day of calculation, *rel. units*; *n* - respectively, the number of days before flowering.

In the integral (2), it is considered that the division of ontogenesis into parts is one day of calculation. The function of the biological time vector is continuous for the plant organism and changes from day to day.

In the integral (2), the level of intensity of agrometeorological environmental factors $[U(j)_{environment}]_{average}$ - is taken not for a given moment of ontogenesis, but as some average value from the beginning of the integration moment T_0 to the current integration moment *T(j)*, *rel.units*. Then the whole complex of agrometeorological factors of the external environment that influence the rate of biological time flow in a certain interval of integration is taken into account.

Thus, equation (2) expresses the vector axis of biological time in the first half of ontogeny, before the vegetative phase "flowering", in the time interval $T_0 \le T(j) \le T$ flowering.

Since the level of intensity of agromet eorological environmental factors $[U(j)_{environment}]_{average}$ does not depend on the biological time vector *T(j)* of the plant organism, this function can be taken out of the integration sign as a constant:

$$T(j) = [U(j)_{environment}]_{average} \int_{T_0}^{1=flowering} dT_{opt} \tag{4}$$

The average value of the intensity level of agrometeorological environmental factors $[U(j)_{environment}]_{average}$, for a certain interval of integration $[T_0; T(j)]$ is found as an integral:

$$[U(j)_{environment}]_{average} = \frac{1}{T(j)} \int_{T_0}^{T(j)} U(j)_{environment}\, dT_{opt} \tag{5}$$

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That is, the plant organism, in the process of its life, selects the average value of all environmental factors up to the current moment of ontogenesis.

We introduce the integral (5) into the equation of the integral (2). We will get a double integral, already in a two-dimensional time space. And this double integral is in the Euclidean time plane with its domain of definition G_{FR}:

$$T(j) = \frac{1}{T(j)} \iint_{G_{FR}} U(j)_{environment}\, dG_{FR} \tag{6}$$

The differential dG_{FR} consists of a simple product of two differentials: dT_F and dT_R:

$$dG_{FR} = dT_F dT_R \tag{7}$$

The domain of the G_{FR} of the double integral (6) is a square with its boundary. You can of course also consider a rectangle.

According to [1], in the organism of an annual plant, there is a closed region of integration of the vector biological time field. This is a closed circle or ellipse [1], obtained by Green's formula, the area of this contour is always equal to *1*:

$$T(j) = -2\oint_{V_\rho} -\beta\, T(j)_{R\rho}\, dT_{F\rho} + \alpha\, T(j)_{F\rho}\, dT_{R\rho} = -2\iint_{G_\rho} (\alpha+\beta)\, dT_{F\rho}\, dT_{R\rho} \quad , \tag{8}$$

where *T(j)* - is the work of the force field of the total biological time for the entire plant organism for the entire ontogeny, (*rel. units of biol. Time*)^2; V_ρ - is a closed contour, our time circle along which the integration of a closed curvilinear integral of the second kind takes place; α and β are dimensionless parameters, they should always equal *1* in their sum, and, they provide a change of a closed circle to a various closed ellipse, depending on their values; $T(j)_{F\rho}$ - parametric circle equation for time processes of photosynthesis, *rel. units* ; $T(j)_{R\rho}$ - is the parametric circle equation for temporary breathing processes, *rel. units*; $dT_{F\rho}$ and $dT_{R\rho}$ are the differentials, respectively, of the time processes of photosynthesis and the time processes of respiration of the plant organism; G_ρ - is a two-dimensional time domain of integration, located in the plant body, and which is closed, having its own area: the area of the time domain of the plant organism in relative units of time $G_\rho(E) = \pi \cdot \rho^2 = 1$ [1].

To go from time *T(j)* to physical time *t(j)*, the following equations are used for time differentials of photosynthesis and respiration processes:

$$dT_{F\rho} = \sin(2\pi \cdot t(j))dt \; ; \; dT_{R\rho} = \cos(2\pi \cdot t(j))dt \tag{9}$$

where *t(j)* - is the physical time, from the phenological phase “shoots” to the phenological phase “flowering”, days.

Taking into account equations (8) [1], where it is proved that the movement of biological time goes along a closed trajectory, for further consideration of equation (6), we need to switch to polar time coordinates, since the time domain G_ρ is a time circle of radius $\rho = \sqrt{\frac{1}{\pi}} = 0{,}5642$ [1]. Then the integral (6) can be written in the form:

$$T_\rho(j) = \frac{1}{T_\rho(j)} \iint_{G_\rho} U(j)_{environment}\, \rho(j) dG_\rho \tag{10}$$

The region of integration G_ρ will be a circle of radius ρ. In this case, the polar coordinates have a singular point $\rho = 0$. Such a value of ρ cannot exist, since in the seeds of plants there is always some non-zero, structural formation of biological time - a living seed. At the same time, the radius lying in negative values along the *x* axis is excluded from the region G_ρ. In our case, ρ can vary within:

$$\rho_0 \le \rho \le \frac{1}{\sqrt{\pi}} \tag{11}$$

for the interval of biological time [T_0; 1].

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Taking into account (11), the integral (10) takes the form, where we have passed to the iterated integral:

$$T_\rho(j)=\frac{1}{T_\rho(j)}\int_{-\varepsilon\pi}^{+\varepsilon\pi}d\theta\int_{\rho_0}^{1/\sqrt{\pi}}\mathrm{U(j)_{environment}}\frac{1}{\sqrt{\pi}}\rho(j)d\rho \quad , (12)$$

where θ - is the angle of rotation of the biological time vector, see also my works [5, 6];

To continue the reasoning, we need to consider the transition from the area of integration of the double integral (6) G_{FR} - which is a square, to the area of integration of the double integral (10) G_ρ - which is a circle.

Taking into account the fact that our physical time is a vector field, where the time field vector at any point of our three-dimensional space (approximately, not taking into account the influence of gravity) moves in a straight line, and the biological vector time field inside the plant organism is closed G_ρ [1], then we have a reflection that is carried out by the plant organism during ontogenesis, in the process of photosynthesis and respiration, and, in general, in the cells of the whole plant during their life:

$$T_F(j)=\rho(j)cos\Theta(j) \quad , \quad (13)$$

$$T_R(j)=\rho(j)sin\Theta(j) \quad (14)$$

Then we have another integral equation (10), where in the plant organism, during its life, there is a linear transformation of the vector Euclidean time plane (physical time) into a time vector plane with polar coordinates. Then, in the plant organism, we will have a closed trajectory of biological time. This fact can be represented by an equation, taking into account equations (13) and (14):

$$T_\rho(j)=\frac{1}{T(j)}\iint_{G_{FR}}U(j)_{environment}\frac{1}{\sqrt{\pi}}T(j)dT_F dT_R \quad (15)$$

where $T_\rho(j)$ - is the result of a linear transformation of the Euclidean temporal plane (physical time) into a temporal plane expressed in polar coordinates, thus, we get that in the plant organism, during its life, the vector biological time moves from the “seed” to "seed".

Consider the integrand of equation (15):

$$U(j)_{environment}\frac{1}{\sqrt{\pi}}T(j) \quad (16)$$

For such a consideration, we use Theorem I. From [2], which says: in the G_{FR} region, system (A) cannot have equilibrium states $x=x_0$, $y=y_0$:

a) for which

$$\Delta=\begin{vmatrix}P_x'(x_0,y_0) & P_y'(x_0,y_0)\\ Q_x'(x_0,y_0) & Q_y'(x_0,y_0)\end{vmatrix}=0 \quad (17)$$

b) for which

$$\Delta>0\ \sigma=-[P'_x(x_0,y_0)+Q'_y(x_0,y_0)]=0\ , \quad (18)$$

and Theorem II, which says: in the G_{FR} region, system (A) cannot have periodic motions $x=\varphi(t)$, $y=\psi(t)$ [$\varphi(t+\tau)=\varphi(t)$; $\psi(t+\tau)=\psi(t)$], for which:

$$h=\frac{1}{\tau}\int_0^\tau[P_x'(\varphi;\psi)+Q_y'(\varphi;\psi)]dt=0 \quad (19)$$

Our integrand (16) has a rotating matrix:

$$\Delta_\rho=\begin{Vmatrix}A\cos\theta & -B\sin\theta\\ C\sin\theta & D\cos\theta\end{Vmatrix} \quad (20)$$

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where:

$$A = \frac{U(t)_{environment}}{\sqrt{\pi}} \tag{21}$$

$$B = \frac{U(t)_{environment}}{\sqrt{\pi}} T(j) \tag{22}$$

$$C = 1 \tag{23}$$

$$D = T(j) \tag{24}$$

and the corresponding determinant.
In this case, we have, according to the condition of Theorem I (20):

$$P_x^{'}(x_0; y_0) = \frac{U(t)_{environment}}{\sqrt{\pi}} \cos\theta(j) \tag{25.1}$$

$$P_y^{'}(x_0; y_0) = -\frac{U(t)_{environment}}{\sqrt{\pi}} T(j) \sin\theta(j) \tag{25.2}$$

$$Q_x^{'}(x_0; y_0) = 1 \cdot \sin\theta(j) \tag{25.3}$$

$$Q_y^{'}(x_0; y_0) = T(j) \cdot \cos\theta(j) \tag{25.4}$$

In total there are 4!=24 substitutions of elements A, B, C and D. Depending on this, there will be different differential equations from the matrix (20). Let's provide a finding of one system of two differential equations of a vector temporary biological field of the first order. Then we have, according to equation (A):

$$\frac{dT(j)}{dt} = P(T(j); \theta(j)) = \frac{U(j)_{environment}}{\sqrt{\pi}} T(j) \cdot \cos\theta(j) + C_1 \tag{A.1}$$

$$\frac{d\theta(j)}{dt} = Q(j)(T(j); \theta(j)) = T(j) \cdot \sin\theta(j) + C_2 \tag{A.2}$$

where C_1 and C_2 are integration constants, represent, in our case, a bunch of time field, which can be located in chromosomes, and is a moving element, *rel. time ·day*$^{-1}$. Systems of equations (A.1 and A.2) where C_1 and C_2 are equal to zero are not considered.

Thus, an autonomous differential system of the vector time field was obtained, which is present in the seeds, and later, in the whole plant organism in the process of its growth and development.

c) *Study of the Autonomous Differential System of the Vector Time Field of the Plant Organism in the Euclidean Plane*

This representation of the time field (A.1 and A.2) immediately determines the structural organization of the field. Such a field is immediately divided into a finite number of connected cells, and each cell has its own sink and source. This representation of the time field corresponds to the cellular theory of the plant organism. Closed trajectories of the time field will determine the oscillatory process in the system of the plant organism. It is well known that light is the first necessary condition for the process of photosynthesis. Therefore, it can be assumed that such an oscillatory process corresponds to electromagnetic oscillations of Photosynthetic Active Radiation - FAR. The fact of mechanical vibrations of the material structure in the leaves of sunflower, corn and sugar beet [7], and for Plasmodium [8] was recorded.

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Clause 3.1 Theoretical results

In the work of Ak. Pontryagina L.S. and Ak. Andronova A.A. [2] gives 11 topological types of integral trajectories of a rough dynamical system of motions of differential equations (A). Let's just compare these trajectories with the organization of the plant organism, Table. 1. This table was obtained on the basis of a comparison of the results of [2] and the results of cytological studies presented in [11]. We will not present here the theorems and definitions given in [2]. We will only say that these theorems and definitions are important for the autonomous system of the vector time field (A.1 and A.2) of the plant organism. See also my works [9 and 10].

Table 1: A set of integral trajectories of a rough dynamic system (A) in comparison with the cellular structure of a plant organism

I. States of equilibrium:	The node (focus)	1	Ribosome
	Saddles	2	Chromatin
II. limit cycles		3	Cell nucleus (control structure)
III. Separatrixes:	Leaving the node (focus) or tending to the node (focus)	4	Mitochondria
	Collapsing from the limit cycle or tending to the limit cycle	5	Chloroplast
	Included in the field G_ρ	6	Cell wall
IV. Trajectories having as their limit trajectories only nodes (focus) and limit cycles located in the domain G_ρ	Leaving the node (focus) and tending to the node (focus)	7	Golgi apparatus
	Collapsing from the limit cycle and tending to the limit cycle	8	Plasmodesma
	Leaving the node (focus) and tending to the limit cycle (or vice versa)	9	Lysosome
V. Trajectories entering the domain G_ρ and not being separatrices:	Aspiring to the node (focus)	10	Vacuole
	Aspiring to the limit cycle	11	Endoplasmic reticulum

Clause 3.2. Practical results of calculations of the axis and field of biological time inside the plant organism according to the data of agrometeorological yearbooks

In order to carry out a practical verification of the provisions on biological time outlined in this work, it is first necessary to calculate the vector axis of biological time, in accordance with equation (3), on the integral sum of total increments. After that, it is necessary to determine the moment of violation of the roughness conditions of the autonomous system of the vector time field (A.1 and A.2) of the plant organism and the cardinal points on this time axis.

Let's carry out such a calculation for the sunflower culture, which grew in the Odessa region. Let's define the vegetative phases, "flowering", and then "budding". Calculations of the vegetative phase "full ripening" of sunflower are not given.

According to equation (3), it is considered that for the "seedlings" phase, the biological time vector is equal to $T_0(j)=0.01$ *relative time unit day*$^{-1}$; ΔT_{opt} - be as *1/65*, where *1* is the time interval from the "seedlings" phase to "flowering", *relative units*, 65 days is the average value of the number of days according to agrometeorological yearbooks for the period 1975-1989 from the phase "seedlings" to the "flowering" phase, and is taken equal to $\Delta T_{opt}=0.02$ *relative time unit day*$^{-1}$. The integral sum (3) was found with a step of 1 day. For the Odessa region, there are 8 agrometeorological stations. Then the average value was found in general for the Odessa region according to the data of these stations. The calculations were carried out on the basis of average ten-day values of environmental factors: "light - FAR", "heat - air temperature", "moisture - reserves of productive moisture in the soil of the 0-100 cm layer".

Also, calculations were made of the violation of the conditions for the roughness of the dynamic system of time in the body of a sunflower plant (A.1 and A.2) and compared with the date of the "budding" phase. Among the three theorems, necessary and sufficient conditions for the existence of a rough system, there is a characteristic exponent σ (18), [2]. The value of the characteristic index σ cannot be equal to zero $\sigma \neq 0$. In the case of $\sigma = 0$, the coarse system (A) and, therefore, (A.1 and A.2) ceases to be coarse and becomes a dynamic system of the first and other degrees of non-coarseness [2]. That is, the system (A) is rough if the separatrices (saddle whiskers) do not go from saddle to saddle, fig. 2.

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a b c

Fig. 2: Separatrices of saddles: a - separatrices go from saddle to saddle, the system (A.1 and A.2) becomes "not rough"; b, c – separatrices do not go from saddle to saddle, the system (A.1 and A.2) is “rough” (according to [3]).

For comparison, the "roughness" of the system of the biological vector time field of the plant organism (A.1 and A.2) is seen in the example of division in the cell of the aloe root - fig. 3.

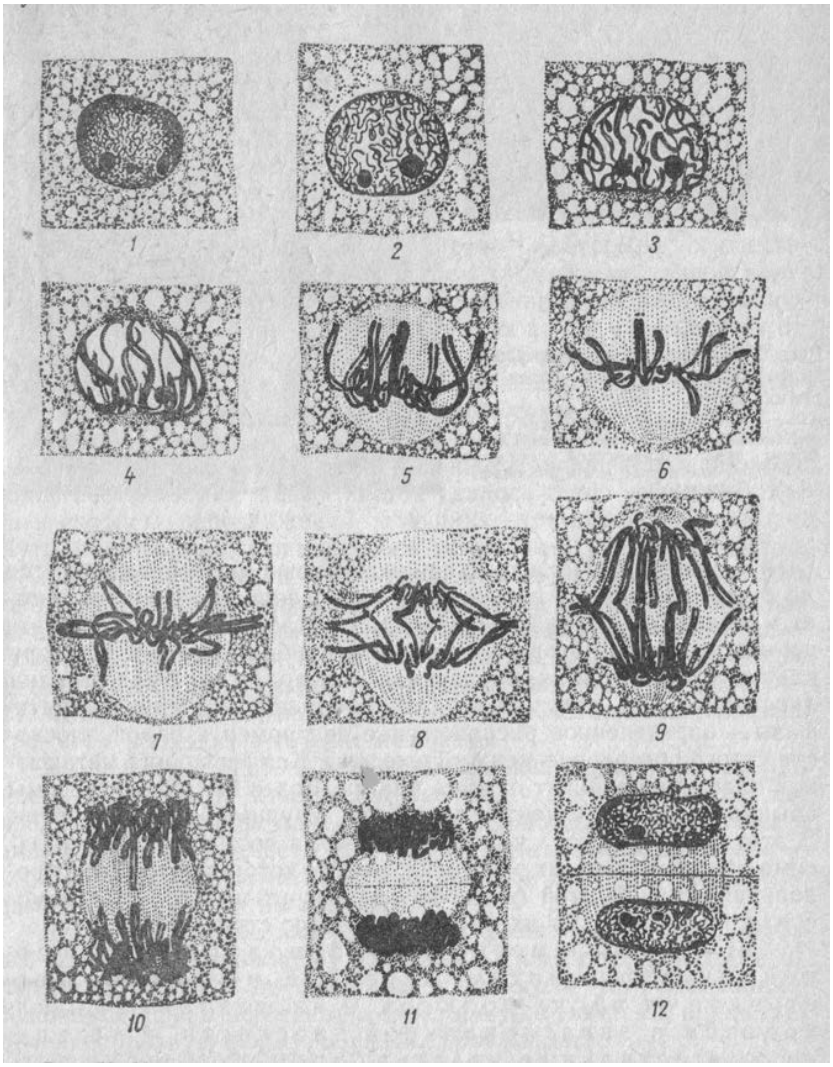

Fig. 3: Mitosis in aloe root cells (according to [11]).

Fig.3 clearly shows the chromatin of the cell. And this chromatin, as the division process proceeds, from the beginning of cell division, to the formation of two new cells, first “rough”, then “not rough” - separatrices of saddles go from saddle to saddle - and at the end of cell division, or mitosis, two cells with coarse chromatin. See also fig. 2 for comparison.

For practical calculations of the “non-roughness” date of the dynamic system of the vector time field (A.1 and A.2), in a growing sunflower organism, a special case of our characteristic index (18) has the form:

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$$\sigma = -\left[\frac{U(j)_{environment}}{\sqrt{\pi}}\cos\theta_o(j) + T(j)\cos\theta_o(j) + T(j)\cos\theta_0(j)\right] \quad (26)$$

or:

$$\sigma = -\left[\frac{U(j)_{environment}}{\sqrt{\pi}} + T(j)\right]\cos\theta_o(j) \quad (27)$$

With this characteristic exponent, there are two conditions when it is equal to zero:

$$-\left[\frac{U(j)_{environment}}{\sqrt{\pi}} + T(j)\right] = 0 \ , \quad (28)$$

$$\cos\theta(j) = 0 \quad . \quad (29)$$

Condition (29) is satisfied when the biological time vector is orthogonal to physical time. This means that the development of the plant organism is suspended. Suspension of sunflower development can be only if the level of environmental factors drops to biological zero. Which can happen very rarely. Usually the level of environmental factors is high enough to carry out growth and development in the climatic conditions of sunflower growth. At the same time, condition (28) is always fulfilled at the beginning of cell division in the stem meristem for the future basket. Taking into account the fact that the vector of the biological time field has the property of closure and moves in a circle [1], then our condition (28) turns into the following formula:

$$\frac{U(j)_{environment}}{\sqrt{\pi}} = \pm T(j) \quad (30)$$

On the interval of physical time from T_0 to *1*, which is equivalent to the phase "shoots" to the phase "flowering". Based on these conditions, calculations were carried out for the Odessa region, where the moment of fulfillment of condition (30) was noted. The calculation results are given in Table 2.

Table 2: Comparison of calculated and actual dates of sunflower flowering and violation of the conditions of "roughness" of the system (A.1 and A.2) with the "budding" phase. Odessa region

Year	Physiological event of sunflower plant			
	Phase "budding"		phase "flowering"	
	$\frac{U(j)_{environment}}{\sqrt{\pi}} = \pm T(j)$ Days from "shoots"	Fact Days from "shoots"	Calculation Days from "shoots"	Fact Days from "shoots"
1975	29	40	64	63
1976	29	43	69	68
1977	34	39	60	66
1978	29	41	58	66
1979	28	34	58	58
1980	31	42	55	66
1981	28	38	60	64
1982	25	40	60	64
1983	28	37	71	66
1984	---	---	---	----

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1985	26	32	58	66
1986	30	41	64	66
1987	30	37	69	62
1988	28	40	61	65
1989	27	44	65	65
Среднее	29	39	62	65

The average error of the estimated the phase "flowering" date deviates from the actual date by 6.7%.

As can be seen from the data presented in Table. 2, the moment of violation of the “roughness” of the dynamic system of the biological time field (A.1 and A.2) precedes the “budding” phase by an average of 10 days. If we take into account that the “budding” phase is visually fixed during observations of the culture, like a bud that has appeared, then 10 days are just necessary for such visual fixation.

Clause. 3.3 Phase portrait of the autonomous dynamic system of the sunflower biological vector time field

The phase portrait of the vector biological time field system was built using the finite increments method. By setting the increment $\Delta t>0$, we obtain the corresponding increments Δx and Δy from the expressions [4]:

$$\Delta x = P(x, y)\Delta t \,, \qquad \text{(B1)}$$

$$\Delta y = Q(x, y)\Delta t \,. \qquad \text{(B2)}$$

Then the direction of the vector at the point (*x, y*) will determine the structural organization of the field without an explicit, analytical solution of the system of equations (A) or, which is the same, our system of equations (A.1 and A.2). The construction of a phase portrait allows us to draw conclusions about the nature of the changes in the variables *x*, *y* without knowing the analytical solutions of the original system of equations (A.1 and A.2). Moreover, the system (A.1 and A.2) has no explicit solution.

The phase portrait of the system (A.1 and A.2) for two variables $T(j)$ and $\theta(j)$ is shown in fig. 4.

First of all, it should be noted that the dynamic system (A.1 and A.2) divides the domain of existence of the variables $T(j)$ and $\theta(j)$ into a finite number of connected cells – plant cells. At the same time, all topological types of integral trajectories of the vector biological time field of the plant organism are present in the results obtained.

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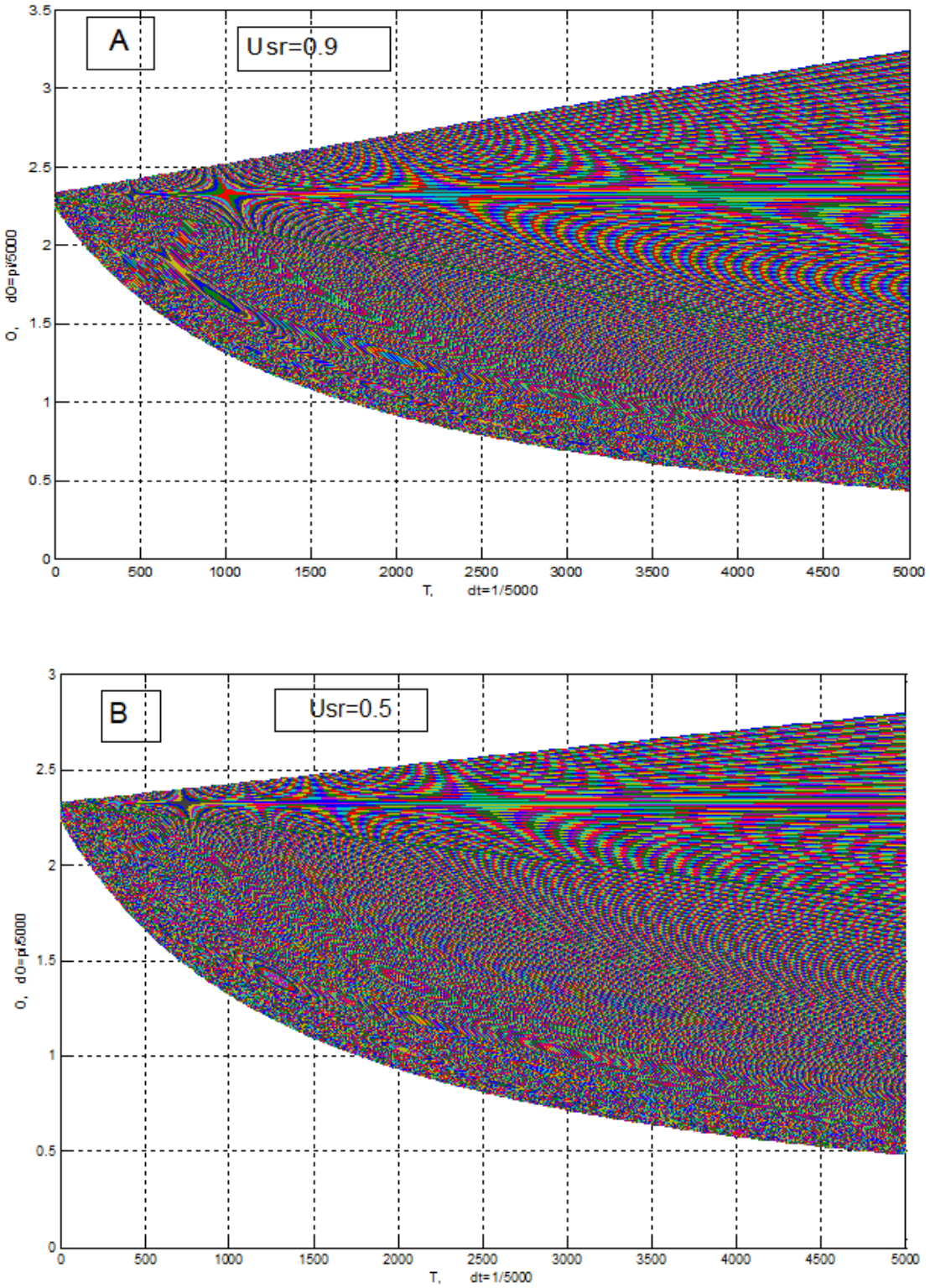

Fig. 4: Phase portrait of the dynamic system of the vector biological time field of the sunflower organism (A.1 and A.2) for two cases: A - the level of tension of environmental factors $U_{sr}=U(j)_{environment}$ *=0.9 rel.un.*, this value is accepted for the entire time interval from T_0 to *1*, or what is the same from the "shoots" phase to the "flowering" phase, or what is the same in the figure - from *0* to *5000*; B – tension level of environmental factors $U_{sr}= U(j)_{environment}$ *=0.5 rel.un.* ,from T_0 to *1*, or what is the same, you can see in the figure the variable *T(j)* changes from *0* to *5000*, and the variable *θ(j)* changes from *0* to *3.5* π, the resolution step is = *54 days·24 hours·60 minutes·60 seconds = 4,665,600 seconds*, or, divided by *5000 = 933.12* seconds or *15.552* minutes

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The integral trajectories fill the entire region of the space G_ρ. Integral trajectories of the vector biological temporal field have all possible directions and permeate the entire domain of definition, which corresponds to the integrity of the plant organism. The integration constants c_1 and c_2 correspond to the sunflower culture and make up the values: c_1=*0.65 rel. units biological time* and c_2=*0.28 rel. units biological time* and found as a result of a simple study by the substitution method. These constants can be written as: $c_1 = \sum_{i=1}^{N_1} c_i$ and $c_2 = \sum_{k=1}^{N_2} c_k$, where N_1 and N_2 are numbers that add up to N_1+N_2 to double the number of plant chromosomes, that is, time clots.

On fig. 4, for two figures A and B, the entire region G_ρ of the vector biological time field is divided by the integral straight line into two sub-regions: upper and lower. The upper sub-region corresponds to the above-ground part of the plant, and the lower sub-region corresponds to the roots. In the upper sub-region there is an integral trajectory (straight line) on which integral trajectories are sequentially arranged according to the saddle type. Moreover, the whiskers of these saddles go from saddle to saddle, which violates the conditions for the roughness of the dynamic system of the vector biological time field, intensive cell division occurs on these trajectories. Thus, these given trajectories correspond to the meristem of the stem, or tissue of the growth cone of the above-ground shoot. The zero points of successive saddles correspond to the moments of the beginning of the laying of leaves, and the last saddle corresponds to the laying of the reproductive organ. The lower subregion corresponding to the roots does not contain saddle trajectories, where the saddle whiskers go from saddle to saddle, which indicates the absence of a “limit cycle” control structure in the roots, which is the control structure of the growth cone of the above-ground shoot. Near the lower boundary of the subdomain corresponding to the roots, in Fig. 4 shows the intensive formation of small cells - root endings.

Depending on the state of environmental factors (Figure 4 - A and Figure 4 - B), the proportion of roots in the whole plant body changes. Thus, under more intense environmental conditions, $U(j)_{environment}$ =*0.5*, the proportion of roots in the plant organism increases. At the same time, the share of the aerial part of the plant organism decreases. Thus, the level of tension of environmental factors determines the growth and development of the plant organism.

IV. Discussion

We relied on the already proven work [1] on the closed trajectory of biological time in the plant organism. In this work, we have shown that the closed movement of the vector biological time field, inside the plant organism, provides this organism with division into cells and subcellular structures.

For this, various methods of studying time were involved. First of all, it should be noted that time is not material, like matter. Therefore, mathematical methods of research, general considerations, and data on the already well-known structure and organization of the plant organism were involved. In this work, we obtained: a). Autonomous system of two differential equations of the temporal vector field of the plant organism for the first half of ontogenesis.

This system of equations corresponds to the whole plant organism during its growth and development. b). An equation was obtained that depends on the agrometeorological factors of the external environment and describes the beginning of division in the growth cone of the sunflower stem, which in the future is observed visually as a bud. c). Numerical experiments were carried out on the basis of agrometeorological yearbooks to compare the violation of the conditions of "roughness" of the time system and the "budding" of sunflower to the definition. d). 24 types of autonomous dynamical systems of the organism's vector time field have been found, which can correspond to various plant objects. (I think not only plant objects. This issue should be carefully investigated). e). The study of the dynamic system of the vector biological time field has been made. The isocline method was adopted as the basis. e). two phase portraits of the dynamic time system were constructed for different environmental conditions. Conclusions are drawn. The work requires continued research time.

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Nutraceutical Potentials of *Cissus Populnea* Linn Cultivated in the Southern Guinea Savanna Agroecology of Nigeria

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Materials and Methods: The stems of *C. pulpunea* where sectioned and sent to the laboratory where Protocols for carbohydrates, crude fibre, crude protein, Crude Fat, mineral composition and oxalate were all determined. Means of the replicates were obtained and recorded.

Keywords: *cissus populnea, phytochemical screening, proximate, mineral, amino, acid vitamin analysis, medicinal plants.*

GJSFR-C Classification: *DDC Code: 577.550973 LCC Code: S441*



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Ochekwu, E. B [α] & Amadi, P [σ]

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Materials and Methods: The stems of *C. pulpunea* where sectioned and sent to the laboratory where Protocols for carbohydrates, crude fibre, crude protein, Crude Fat, mineral composition and oxalate were all determined. Means of the replicates were obtained and recorded.

Results: The percentage vitamins showed that Ascorbic acid had the highest value and the least was tocopherols. The essential and non-essential amino acid range from 0.10-14.48% for essential and 0.05-5.44% for non-essential in that order. The percentage total amino acid of *C. populnea* is 45.38%. The proximate composition of *C. populnea* are protein, ash, crude fibre, fat, nitrogen, moisture content and carbohydrate. The phytochemical results revealed the presence of some secondary metabolites found in *C populnea* to include flavonoids, phenolics, alkaloid, oxalate, glucosides, phytate, saponin, trypsin inhibitors and HCN. The mineral composition showed the presence of minerals as calcium, magnesium, potassium, phosphorus, sodium, manganese, iron, zinc and copper.

Conclusion: The study therefore supports the cultivation of *C. populnea* with the aim of improving the bioactive, components, directly providing medicinal and nutritional requirements of humans.

Keywords: cissus populnea, phytochemical screening, proximate, mineral, amino, acid vitamin analysis, medicinal plants.

I. Introduction

Man depend on plants for medicine, preservative sources of food, shelter and clothing because of the presence of secondary metabolites. Phytochemicals are biologically active plants compounds having disease hampering capabilities and preservative action. They are compounds present in plants that are used as food and medicine to protect against illness and to maintain human health[1]. Phytochemical have anti-oxidant or hormone-like effect which help in fighting against many disease including dermatological problems, simple body ailments, cancer heart disease, diabetes, high blood pressure[2]. The medicinal value of plants lies in their secondary metabolites which produce definite physiological action on the human body. Medicinal plants have been used for centuries as remedies for human disease because they contain components of therapeutic value[3]. Many of the plant materials used in herbal medicine are readily available in rural areas and this has made it relatively cheaper than orthodox medicine[4]. Medical plant has been found to be helpful in curing many common ailments such as ringworm, eczema, malaria, ashema, ulcer, fever etc and have always promoted the search for different extracts from plant[5]. Phytochemicals in *Ciccus populnea* are alkaloids: Alkaloids are basic nitrogenous compound with definite physiological and pharmacological activity. Alkaloids are natural plant compounds having basic characteristics and containing at least one nitrogen atom in a heterocyclic chain[5]; flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage. They have strong anti-cancer activity and protects against all stage of carcinogens. Flavonoids in the body are known to reduce the risk of heart disease[6], Tannis are a heterogenous group of complex compounds widely distributed in plants, phenols, saponins and oxalate[6]. Many physiological activities, host mediated tumor activity and wide range of anti-infective action have been trace to tannins. They are class of natural organic compound which are essential for growth and reproduction of plant and are produced as a response for defending injured plant against pathogen; Saponins are plant glycoides characterized by foaming in water when mixed and stirred. Oxalate is a common constituent of plants and several species including some crop plants, accumulate high level of this C_2 dicarboxylic acid anion. Depending on species, oxalate accumulates primarily as soluble oxalate, insoluble calcium oxalate, or a combination of these two

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Author's Contributions: This work was carried out in collaboration between both authors. Author OEB contributed to design of study, wrote protocol, performed preliminary data analysis, interpreted the data and produced initial draft and supervised the work. Author AP contributed to design of study, gathered the initial data, managed literature searches, made contributions and corrections to initial draft. Both authors read and approved the final manuscript.

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forms[6]. Some minerals in *C. populnea* like calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function; Potassium is net principal cation in intercellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na+/K+ - ATPase[6].

The plant *Cissus populnea* belongs to the family Amplidacea (Vitaceae) the plant is 2 to 3m high semi-circular which grows in the savannah and is widely distributed in Senegal, sudan, Uganda, Abyssinia and Nigeria[7]. It is commonly known as 'okoho' by the Idomas, Igbo and Igala tribes of Nigeria, 'Dafara' (Kano, Zaria); 'Latutuwa' (Katsina) by the Hausa language of the indicated towns of Northern Nigeria; 'Ajara' or 'Orogboro' by the Yoruba tribes of Northern Nigeria and Southern Nigeria[8]. The plant *Cissus populnea* is also called food gum. The gum is used for soup and as soup thickener. It is also widely used as medicine for the treatment of general disease and indigestion and as a drug binder. *Cissus populnea* has been used locally to treat many ailments such as veneral, stomach and skin infections, sore breast, interstinal parasites, oedema and eye problems resulting from attack from black cobra and also used as laxative or purgative[8], economically it has been used as binder in food and in lining dye pits. This study was aimed at evaluating the phytochemicals, screening, proximate composition, mineral and functional properties of *Cissus populnea* as a medicinal plant

II. Materials and Methods

a) Study Area and Sample Collections

The stem of *Cissus populnea* was collected from Anwule village of Oglewu (Longitude 7.1493 and Longitude 8.0276) district in Ohimini Local Government Area of Benue State. and was sent for analysis to International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The study was carried out in June 2018 – September 2018.

b) Preparation of Sample

The stem of *Cissus populnea* was sliced into strands of smaller size and thoroughly washed to remove the adhering and foreign material. The fresh stem was cut into file strips with a kitchen knife and was air dried. It was then reduced to a fine powder using mortar and sieve. The powdered sample was put inside an air tight polyethylene container, neatly labeled and kept in a refrigerator pending analysis.

c) Proximate Analysis

The recommended method of association of analytical chemist's Proximate analysis of *Cissus populnea* stem was to determine the moisture content, crude fat, Ash contents, crude fibre, crude protein, sugar and starch using various methods[9].

d) Moisture content determination

This is a measure of the percentage moisture[10, 11] loot due to drying at room temperature.

2.0g sample was weighed into aluminum dishes and dried in the oven at 103-105°C for 4-6hours. Dish was transfer into desiccator and allow to cool then weighed on cooling at room temperature[9].

$$\% \text{ moisture} = \frac{W1-W2}{weig\,ht\ of\ sample} x \frac{100}{1}$$

Where

W1 = Initial weight of crucible + sample

W2 = Final weight of crucible + sample

e) Determination of carbohydrates

Determination of carbohydrates (Clegg anthrone) plant samples weighing 1g were put into a 250ml volumetric flask. Distilled wale (10ml) and 13ml of 62% perchloric acid was added and the mixture was shaken in order for it to homogenize completely. The flask was made up to 250 ml with distilled water, the solution formed was filtered through a glass filter paper. Filtrate (10ml) was collected and transferred into a 100ml test tube; this was also diluted to volume with distilled water. The hydroxyl used solution was pipette into a clean fat tube and 5ml of anthrone reagent was added, they were then mixed together. The whole mixture was read at 630nm wave length using the 1m distilled water and the 5ml anthrone prepared as blank. Glucose solution of 0.1ml was prepared and this was treated with the Anthrone reagent. Absorbance of the standard glucose with calculated suing the formula below[12];

$$\% \text{ CHO} = \frac{25\ x\ Absorbance\ \ of\ sample}{(Absorbance\ \ of\ glucose\ )x\ 1g\ of\ smple\ \ used}$$

f) Ash content determination

2.0g of dried ground sample was weighed into a dish which has been previously ignited and weighed. The sample was ignited on a hot plate in a fume cup board to char the organic matter. The dishes were placed in the muffle furnace and maintained at 600°C for 6hours until completely ashed. The dish was directly transferred to the desiccators and allowed to cool then weighed[13].

$$\text{ASH content (\%)} = \frac{W2-W1}{weig\,ht\ of\ sample}\ x\ 100$$

Where

W2 = Weight of dish + ash

W1 = Weight of dish

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g) Crude fibre determination (Trichloro-acetic Acid (TCA) method)

1.0g of defatted sample was weighed into digestion beaker and 100ml of TCA digestion reagent was added. Place on the heating unit of digester open water supply to reflect condenser. Bring to boiling and reflux for exactly 40 minutes, counting from the time boiling commenced. Beaker was removed from the heater and allowed to cool a bottle bit, then filtered through Whatman No 4 (15.0 cm diameter), residue was washed with hot water and once with industrial spirit. The filter paper was opened out, the residue was removed with a spatula and was transferred to a previously ignited and pre-weighed dish then dried overnight in an oven at 150°C. desiccators were transferred and allowed to cool before weighing. Ash in a muffle furnace at 600°C for 6hours, then allowed to cool before reweighing[10].

$$\text{Crude fibre \%} = \frac{weig\,ht\ of\ crude\ \ fibre}{weig\,ht\ of\ sample}\ x\ 100$$

h) Crude Protein Determination

The crude protein was determined using micro-Kjeldah method. 1.0g of sample and catalyst mixture were weighed, 15ml of concentrated sulphuric acid was added from dispenser and mixed carefully by swirling the tube with a tube mixer. The digestion tube stand was placed with the prepared sample in front of the digester and the exhaust caps was fit.

The vacuum source was turned on to maximize air flow and ensure the fumes are contained. The tube was placed in the preheated digester inside the hood. As soon as the sample solution was cooled sufficiently, 75ml distilled water was diluted and mixed. 25ml boric acid was measured into receiver flask, then placed in the upper position on the form of the distilling unit. Prepared digestion tube was fixed to the corresponding tube bolder, 50ml of NaOH (55-40%) solution was dispensed automatically, steam supply was switch on to start distillation (usually 5 minutes). When the distillation was completed, digestion tube was removed with residue and placed back in the stand, receiver flask was removed and rest was distilled accordingly. Stirrer bar was added to the receiver flask solution and placed on the magnetic stirrer place of the filtrator[14].

This was filtrated 5 N standard HCl solution to a neutral grey colour. The result was expressed as

$$\%\ N = \frac{1.401\ x\ (ml\ HCL\ sample\ titre - ml\ HCL\ titre\ of\ blank) x\ NHCL}{sample\ weight}$$

i) Crude Fat (Soxhlet Extration method)

4.0g of sample was weighed accurately into a moisture dish and dried in an oven for 5-6hours at 100°C. Exactly 2-3g of the dried sample was weighed into a filter paper and wrapped properly then placed in thimble and transferred to extraction barrel-petroleum there was added through barrel into the extractor flask until it siphons over once into the extraction flask directly below it, more other was added until volume was enough to run through the duration of extraction. Water inlet tap was opened to make sure all joints are tight and flask was healed on the regulated heating mantle for 6hours at a condensation rate of at least 3-6 drop per seconds[9]. After extraction was completed, thimble was removed from the extractor barrel and dried in the oven, barrel was replaced and continued distilling until the extraction flask was almost dried then flask containing oil was then detached and dried in the oven overnight at 70°C to constant weight.

$$\text{Crude fat \%} = \frac{W2-W1}{W1}\ x\ \frac{100}{1}$$

Where *W1* = Weight of evaporating dish or extraction flask

W2 = Weight of evaporating dish and content after drying

S = Sample weight in g.

j) Determination of Amino acids using waters 616/626 LC (HPLC) Instrument

The sample preparation and determination were carried out in the following four Stages[9]:

1. *Hydrolysis*

The samples (0.5g) were weighed into a sterile furnaces hydrolysis tube 5 nmols Norleucine was added to the samples and then dried under a vacuum. The tube was again placed in a vial containing 10.05 N HCl with a small quantity of phenol, thereby hydrolyzing the protein by the HCl vapours under vacuum. This stage of hydrolysis of the sample lasted for between 20 – 23 hours at 108°C. After the hydrolysis, the samples were dissolved in ultra-pure water grade, containing ethylene diamine tetraacetic acid (EDTA). The EDTA chelates the metal was present in the samples. The hydrolysed samples now are stored in HPLC amino acid analyzer bottles for further analytical operations.

2. *Derivatisation*

The hydrolyzed samples were derivalised automatically on the water 616/626 HPLC by reacting the five amino acid, under basic situations with phenylisothiocyanate (PITC) to get phenylthiocarbamyl (PTC) amino acid derivatives. The duration for this is 45 minutes per sample, as calibrated on the instrument. A set of standard solutions of the amino acids were prepared from Pierce Reference standards H (1000

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μmol) into autosampler crops and they were also derivatised. These standards (0.0, 0.5, 1.0, 1.5, 2.0 μmol) were used to generate a calibration file that was used to determine the amino acids contents of the samples. After the derivatisation, a methanol solution (1.5 N) containing the PTC-amino acids was transferred to a narrow bore waters 616/626 HPLC system for separation.

k) HPLC separation and Quantization

The separation and quantization of the PTC-amino acids was done on a reverse phase (18 silica column) and the PTC chromophone was automatically and digitally detected at the wavelength of 254 nm. The elution of the whole amino acids in the samples took 30minutes. The buffer system used for separation was 140 mm sodium acetate pH 5.50 as buffer A and 80% acetonitrile as buffer B. The program was ran using a gradient of buffer A and buffer B concentration and ending with a 55% buffer B concentration at the end of the gradient[9].

l) Data processing/interpretation and calculation

The intensity of the chromatographic peaks areas was automatically and digitally identified and quantified using a Dionex chromeleon data analysis system which is attached to the waters 616/626 HPLC System. The calibration curve or file prepared from the average values of the retention times (in minutes) and areas (in Au) at the amino acids in 5 standards runs was used[9]. Since a known amount of each amino acid in the standard loaded into the HPLC, a response factor (Au/pmol) was calculated by the software that was interphased with the HPLC. This response factor was used to calculate the amount of each of the amino acid (in pmols) in the sample and displayed on the system digitally. The amount of each amino acid in the sample is finally calculated by the software by dividing the intensity of the peak area of each (corrected for the differing molar absorptivity's of the various amino acids) by the internal standard (Pierce) in the chromatogram and multiplying this by the total amount of internal standard added to the original sample. After the picomole by the intensity of the height of each amino acid has been ascertained by the software, the data, the digital chromatographic software extrapolate back to 5 nmoles of the internal standard (Norleucine), and displays for the total amount that was pipetted into the hydrolysis tube at the beginning of the analysis as below:

$$\frac{mg}{mL}(in\ extract) dilution\ factor\ x\ peak\ height\ intensity$$

$$\frac{mg}{mL}(in\ sample) = \frac{\mu \frac{g}{mL} in\ extract\ x\ sample\ volume}{weight\ of\ sample}$$

m) Phytochemical Screening

The extraction and analysis of *Cissus populnea* stem was screened for the presence of phytochemical constituents such as alkaloids, flavonoids, tannins, saponin, oxalate, phytate, glucosides, trypsin, phenol, using waters 616/626 HPLC.

n) Extraction and Analysis of alkaloid using waters 616/626 HPLC

10g of the sample was weighed into a 250ml beaker, 200ml of 20% acetic acid in ethanol (2:1) was added, covered and allowed to stand for 6hours to extract. This was shake for 25minutes and centrifuged at 3500 rpm (revolution per a minute). The supernatant, after filtration was concentrated on a water bath to half of the original volume, concentrated ammonium hydroxide was 4times drop wisely added for alkaloids extraction, until the precipitation was completed. The whole solution was allowed to set and the precipitate was collected and washed with dilute ammonium hydroxide, filtration was carried out and after that, the precipitate was dried[9].

o) Extraction and Analysis of Flavonoids using waters 616/626 HPLC

2.0mg of the sample was weighed in each of the extraction tubes. 2.0ml of methanol was mixed with the sample and shake for 25 minutes, it was allowed to stand for 1hour. 0.2ml of 40% aluminum trichloride in methanol, followed by a drop of acetic acid (CH_3COOH), and finally, diluted with methanol to 250ml. Shaked for 15minutes and allowed to stand for 45minutes and then transferred supernatants to a set of HPLC auto analyzer cups and stored for determination on waters 616/626 HPLC.

p) Extraction and Analysis of Glycosides using waters 616/626

0.5g of the sample was weighed into a set of plastic vails and 1.0ml of elimeth sulfoxide (DMSO) was added and vortexed for 2minutes. The vials containing the sample was transferred to the dark environment for 5minutes. 5% methanol containing hydrochlorie acid and 1.0mg was added. TBHQ was added. The vials were mixed using a vortex mixer for 5minutes. The solution was transferred to a set of centrifuge tubes and was allowed to stand for 2hours, then centrifuged for 10minutes at 3500 rpm. The supernatant was transferred to HPLC auto analyzer cups for analysis[9].

q) Extraction and Analysis of phenol using water 616/626

5.0g of the sample was weighed into a 250ml digestion tube. 40ml of 80% ethanol + 60ml of ultra-pure water was added and 10ml of the above solutions was transferred to a set of test tube; 0.8ml of 2N of the folinciocalter reagent and 2.5ml of 40% Na_2Co_3 solution was added and the final volume was made to 250ml with ultra-pure water and shacked vigorous for

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10minutes and allowed to stand for 4hours. The extract was sampled to a set of HPLC auto analyzer cups and then run on water 616/626 at the water length (1nm) of 765nm as follows[9].

r) Determinations of Saponins

A spectrophotometric method described by Brunner (1984) was used for saponin analysis. 1g of the finely ground sample was weighed into a 250ml beaker and 100ml of isobetyl alcohol was added. The mixture was shaken in a mechanical shaker for 5hour to ensure uniform mixturing. Subsequently, the mixture was filtered through a Whattman No. 1 filter paper into a 100ml beaker and 20ml of 40% saturated solution of magnesium carbonate was added[9].

The obtained mixture with saturated $mgCo_3$ was again filtered to obtain a clear colourless solution. 1ml of the colourless solution was pipetted into 50ml volumetric flask and 2ml of 5% $fecl_3$ solution was added.

s) Determination of Tannins

Tannins content was determined using the standard method[9]. 500mg of the sample was weighed into a 50ml plastic bottle and 50ml of distilled water was added and then shaken thoroughly for 1 hour in a mechanical shaker. The solution was filtered into a 50ml volumetric flask and made up to the mark. 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1m $fecl_3$ in 0.1N HCl and 0.008m potassium ferocyanide. The absorbance was measured at 120nm within 10mins.

t) Determination of Oxalate

5.0g of sample was weighed out. 100ml distilled water was added which was heated for 1 hour and allowed to cool, make up to 100ml with distilled water, then measured 25ml of sample into a flask, added 20ml of 2m H_2SO_4. Heat the sample on the heating mantle, put the thermometer as you are heating till 70_oC temperature, then bring down the flask. Titrate the sample with KMNO4. For oxalate standard, measure 25ml of oxalic acid into another flask and treat standard as sample[9].

u) Mineral composition

Mineral content of *C. populnea* were determine using atomic absorption and flame spectrometry according to standard method[9].

Wet digestion of sample was done by taking the powered sample in a digesting glass to which 12ml of HNO_3 was added and kept the mixture overnight at room temperature. 4ml of perchloric acid was then added to the mixture and digested in a fume block. The temperature was increased gradually, starting from 50°C to 300°C, where the appearance of white fumes indicated the completion of digestion. The mixture was left to cool down, the contents of the tube was transferred to volumetric flask and the volume of the content were increased to 100ml by adding distilled water. The wet digestion was transferred to labeled plastics. The digest stored and used for mineral determination. Determination of Iron (Fe), Zinc (Zn), Calcium (Ca), manganese (Mn) and magnesium (Mg) was analysed using atomic absorption spectrophotometer. Different electrode lamps were used for each mineral. The equipment was standard solution for each mineral before and during determination to check if it was working properly. The dilution factor for all mineral except p and mg was 100.

For determination of Mg, the original solution was further diluted to 0.5ml original solution and adding distilled water to obtain a volume of up to 100ml.

For the determination of calcium, 1ml of lithium oxide solution was added to the original solution to unmask Ca from Mg. The concentration of the minerals recorded are converted to Mg by multiplying the absorbency (in ppm) with a dilution factors and dividing by 100.

$$\text{MW} = \frac{Absorbency\ \ (ppm)}{Weight\ of\ sample} \text{x100}$$

Determination of sodium (Na) and potassium (k) was done by flame photometry. Standard solution of 20, 40, 60, 80, and 100mg/ml used both for Na and k. The calculation for total mineral intake involve the same procedure given involve the same procedure given in atomic absorption spectrophotometer above.

Phosphorus (P) in the sample was determined by spectrophotometry by mixing ammonium molybdate with 250ml of distilled water in a beaker (solution A). antimony potassium titrates 0.2g was taken and dissolved in 500ml H_2SO_4 solution in a volumetric flask, enough distilled water was added to make the solution up to 1000ml (solution B). The two solutions were mixed in a 200ml volumetric flask to get the mix reagent. The volume of the mixed reagent was increased to 200ml by a distilled water.

Ascorbic acid (0.7g) was mixed with 140ml of the mixed reagent in a beaker left until dissolved to make the colour reagent. Digested sample (1.0g) was taken into a plane bottle labeled property and 4ml of distilled water was added to make a diluted volume of 5ml colour reagent (5ml) was added to this volume and the total volume of this mixture (final solution) was increase to 25ml. The dilution factor of this solution was 2500. After sometime the colour of this final solution turned blue. Sample from the final blue solution was taken cuvette and read using a spectrophotometer. Reading of phosphorus was recorded in ppm. The calculation for the total mineral intake involve the same procedure as given in atomic absorption spectrotometry.

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III. Results

a) *Phytochemical screening of the stem of Cissus populnea*

The result of phytochemical screening from table 1 below, showed the presence of some secondary metabolites which are alkaloid, flavonoid, saponin, oxalate, Phytate, phenol, Glucosides, Trypsin, Tannins.

Table 1: Phytochemical Composition of *Cissus populnea*

Parameters	Stem of *C. populnea* (g/100g)
Pmm saponin	1.636
Pmm phytate	2.140
Pmm tannins	4.015
Ppm trypsin-inhibitor	1.216
Ppm total hemagykutinin	0.977
Ppm hydrogen cyanide (HCN)	0.024
g/100 total Phenolics	86.750
g/100 total Flavonoids	92.248
g/100 total Glucosides	5.893
g/100 total Alkaloids	11.131

Ppm (Part per million)

b) *Proximate Analysis Result of Plant Sample*

The result of proximate analysis showed the presence of Nitrogen, protein, moisture content, ash, crude fibre, fat and carbohydrate. Among all carbohydrate gave the highest value with 48.574% while Nitrogen gave the lowest value of 2.190% as shown in table 2.

Table 2: Proximate Composition of *Cissus populnea*

Parameter	Stem (%)
Nitrogen	2.190
Protein	13.690
Moisture Content	13.033
Ash	9.66
Crude fibre	11.331
Fat	3.705
Carbohydrate	48.574

c) *Mineral Analysis Results of Plant Sample*

Mineral analysis result showed the presence of calcium, potassium, magnesium, phosphorus, sodium, manganese, Iron, zinc, copper contents with Fe having the highest content in *Cissus populnea* with Ni with the least content (Table 3).

Table 3: Mineral Content of *Cissus populnea*

Elements/parameter	Stem (%)
Ca %	3.877
Mg %	0.598
K %	0.082
P %	0.48
Ppm Na	0.086
Ppm mn	85.781
Ppm Fe	94.798
Ppm Zn	41.957
Ppm Cu	5.655
Ppm Sn	0.024
Ppm Pb	0.024
Ppm Cd	0.017
Ppm Se	0.003
Ppm Cr	0.069
Ppm Co	0.016
Ppm Ni	0.002
Ppm As	0.020
Ppm Hg	0.019
Ppm Ag	0.004

Cissus populnea showed the presence of water soluble and fat-soluble vitamins. Vitamins were also present in *C. populnea,* Vitamin B2 (riboflavin) as the highest and vitamin E (tocopherols) having the smallest content (Table 4).

Table 4: Vitamins content of *Cissus populnea*

Types of vitamins	Vitamins(%)	
Water soluble	Vitamin B1 (thiamine)	2.347
	Vitamin B2 (riboflavin)	7.567
	Vitamin B3(niacine)	0.621
	Vitamin B6(pyridoxin)	1.829
	Vitamin B12 cobalamin)	3.128
	Vitamin C(ascorbic acid)	9.102
Fat soluble	VitaminA beta carotene)	5.641
	Vitamin E(tocopherols)	0.062

Twenty amino acid were present in *C. populnea* with asparagines having the least content and phenylamine the highest content of amino acid Table 5).

Table 5: Amino Acids content of *Cissus populnea*

Elements/parameter	Stem
Threonine %	3.26
Leucine %	3.19
Isoleucine %	0.90
Lysisne %	0.11
Methonine %	0.10
Phenylamine %	14.48
Tyrosine %	0.34
Valine %	3.76
Argine %	0.68
Histindine %	0.43
Alanine %	0.87
Aspartic acid %	0.11
Asparagines %	0.05
Glutamic acid %	0.76
Glutamine %	5.44
Glycine %	3.11
Proline %	2.48
Serine %	2.10
Tryptophane %	2.76
Crotine %	0.47
% total Amino acids	45.38

Glycoside analysis showed glucoside having the highest content and Metoprolol acid with the least (Table 6).

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Table 6: Glycosides contents of *Cissus populnea*

Elements/parameter	Stem
g/100 glycyrhizic acid	2.624
g/100 glycyrrhetinic acid	0.003
g/100 18-beta-glycyrrhetinic acid	0.013
g/100 E-strphanthin acid	0.067
g/100 Digoxin acid	0.005
g/100 Digitoxin acid	0.049
g/100 Oleandrin acid	0.004
g/100 varapamil acid	0.095
g/100 Infedipine acid	0.0255
g/100 Amedipine acid	1.326
g/100 lisinopril acid	0.748
g/100 Enalapril acid	0.030
g/100 captopril acid	0.023
g/100 Hydrochlorathiazide acid	0.008
g/100 furosemide acid	0.034
g/100 propranolol acid	0.0569
g/100 Atenonol acid	0.023
g/100 Metoprolol acid	0.017
g/100 total glucosides	5.893

Alkaloids of various kinds were also present in the stem of *C. populnea* with Ricinine as the highest quantity and Quinoline with the least (Table 7)

Table 7: Alkaloids constituent of *Cissus populnea*

Elements/parameter	Stem
g/100 caffeine	0.496
g/100 colchicine	0.069
g/100 cinchonine	0.017
g/100 Rauwolfia	0.062
g/100 morphine	0.045
g/100 Aptmorphine	0.045
g/100 Atropine	0.035
g/100 Apoatrpine	0.087
g/100 Quinine	0.235
g/100 Narcotine	1.222
g/100 codaine	0.689
g/100 papaverine	0.028
g/100 Nicotine	0.021
g/100 conine	0.007
g/100 Piperine	0.031
g/100 Ricinine	1.918
g/100 strychine	0.003
g/100 vincristine	0.015
g/100 eserine	0.080
g/100 Pilocarpine	0.006
g/100 Ehedrine	0.058
g/100 lobelline	0.004
g/100 Tubocurarine	0.112
g/100 Reserpine	0.301
g/100 Vinblastine	1.567
g/100 Piperidine	0.884
g/100 Heroin	0.035
g/100 Emetine	0.027
g/100 Guinidine	0.009
g/100 Peletrevine	0.040
g/100 Pyridine	0.736
g/100 Quinoline	0.001
g/100 Aeridine	0.006
g/100 Cocaine	0.031
g/100 Eryotamine	0.002
g/100 Norpseudoephedine	0.022
g/100 Nornicotine	0.002
g/100 Cinchoridine	0.043
g/100 Hyoscine	0.116
g/100 Berberine	0.601
g/100 Psychotrine	0.339
g/100 Theobromine	0.014
g/100 Theophylline	0.010
g/100 cephaline	0.003
g/100 B-carboline	0.015
g/100 Phenylethylamine	1.039
g/100 Total Alkaloids	11.131

All phenolic components were acidic with valnilic acid having the highest content and castarinol C1 acid with the least value (Table 8).

Table 8: Phenolics constituents of *Cissus populnea*

Elements/parameter	Stem
g/100 Cinnamic acid	4.054
g/100 Piperonic acid	2.317
g/100 veratoe acid	2.317
g/100 valnilic acid	18.243
g/100 genticitic acid	0.097
g/100 galic acid	0.494
g/100 salicylic acid	0.159
g/100 carreic acid	0.234
g/100 sinamic acid	16.806
g/100 ferulic acid	0.420
g/100 homogentisic acid	3.262
g/100 pyrogallic acid	3.424
g/100 syringic acid	0.290
g/100 Benzoic acid	0.424
g/100 Izoferulic acid	0.204
g/100 mendelic acid	2.466
g/100 salicitic acid	0.389
g/100 P-OH-Phenyloacetic acid	0.299
g/100 m-OH-benzoic acid	0.056
g/100 homovanillic acid	0.797
g/100 protocatelic acid	7.098
g/100 P-cumaric acid	0.508
g/100 Galic acid	0.793
g/100 cafein acid	3.680
g/100 sinagic acid	2.366
g/100 singlic acid	8.578
g/100 P-OH-benzoic acid	0.957
g/100 caffeic acid	0.090
g/100 cattaric acid	0.275
g/100 coumaric acid	0.005
g/100 contaric acid	1.100
g/100 cyanidin 30-glucoside	0.009
g/100 castarinol C1 acid	0.0025
g/100 castarinol C2 acid	0.058
g/100 castarinol C3 acid	0.027
g/100 castarinol C4 acid	0.055
g/100 cutissin acid	0.023
g/100 Astringin acid	2.432
g/100 catechin acid	0.878
g/100 Aesculetin acid	0.305
g/100 ethyl/caffeati acid	0.501
g/100 ethyl/gallon acid	0.005
g/100 ferteric acid	0.036
g/100 Total phenolics	86.750

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Flavonoid constituent was not left out in the analysis. Poncirin had the highest constituent and Thearubigin with the least constituent (Table 9)

Table 9: Flavonoids constituents of *Cissus populnea*

Parameters	Stem
g/100g Hesperidin	3.418
g/100g Nanitutin	1.953
g/100g Neoriocitin	1.953
g/100g Poncirin	15.380
g/100g didymin	0.082
g/100g Enocetrin	0.417
g/100g Rhoifolin	0.134
g/100g Diosmin	0.197
g/100g Nobiletin	14.169
g/100g Acacetin	0.354
g/100g raxifolin	2.750
g/100g sinerisetrin	0.134
g/100g tangeretin	2.887
g/100g neodiosmin	0.244
g/100g Naringin	0.358
g/100g Naringinenin	0.172
g/100g Quercetin	2.079
g/100g Erodictyol	0.328
g/100g myricetrin	0.252
g/100g Kaempterol	0.048
g/100g Apigenin	5.121
g/100g Isorharmnetic	5.984
g/100g Luteolin	8.302
g/100g Daidzein	5.844
g/100g Genistein	3.102
g/100g Glycitein	1.995
g/100g anthocyanin	7.232
g/100g Catechin	5.924
g/100g Epicatechin	0.076
g/100g Theoflarins	0.232
g/100g Thearubigins	0.003
g/100g Epigallocatechin	0.927
g/100g Epicatechin gallate	0.007
g/100g Epigallo catechin gallate	0.031
g/100g Proanthocyanidins	0.021
g/100g Hesperetin	0.049
g/100g Acacetin	0.022
g/100g Taxifolin	0.046
g/100g Tangeretin	0.020
g/100g Total Flavonoid	92.248

Plants, including most feeds and foods, produce a broad range of bioactive compounds via secondary metabolism. Venation in both nutrient and non-nutrient composition of plant materials have been reported to be influenced by age, culture practices, environment, seasons and the varieties. Thus the variation in the phytochemical properties of the species may present baseline information for their utilization in synthesis of pharmacological products for medications. Plants with potent bioactive compounds are often characterized as both poisonous and medicinal and may depend on the amount taken and the content.

IV. Discussion

The result of this study may be used in formulating antimicrobial products against pathogen which shows that of *C. populnea* is rich in plant compounds/secondary metabolites which are alkaloid, flavonoid, sapinin, oxalate, phytate, phenol, Glucosides, Trypsin and Tannins. The conducted phytochemical screening has shown that the plant part (stem) contain large amount of flavonoid, phenol, alkaloids (table 1). The flavonoid content in the stem of *C. populnea* seems to be very abundant as compared to other phytochemcials. The abundance of flavonoid in the stem is also indicative of its potent antioxidant effect, which suggest that the plant may be very useful as an antibacterial, anti-inflammatory, anti-allergic, anticlinal, antithrombotic, antimultagic and vasodicatory compound[15]. Plants containing alkaloid do not feature strongly in herbal medicine, yet the alkaloid have always been an important phytocompound used in allopathic systems[16].

Saponins, although non-toxic, can generate adverse physiological response in animals that consume them. Saponins have tumour-inhibiting effects in animals[17]. Their abundance in the stem and root of *C .populnea* support the use of the plant parts in many parts of Nigeria, particularly in the north, for traditional treatments of ailments[18]. Similarly, flavonoid and tannin compounds have also some anti-bacterial, antiviral[19] and antiparasitic effect. Their destruction or modification, in turn, plays an important role in the ripening of fruit[20]. The amount of tannins reported in this work suggested that *Cissus populnea* can also be useful in the production of drug for treatment of bacterial and viral infections. Its tannin content may also be useful in treating haemochromatosis a hereditary disease characterized by excessive absorption of dietary iron, resulting in pathological increase of total iron content stored in the body. The presence of other phytochemical also supported the fact that the plant is useful in many ways, as earlier reported by several authors.

The trend of the abundance of the mineral content in the stem of *C. populnea* in table 3 is as follows Fe > Mn > Zn > Cu > Ca > Mg > Na > K > P. The macro elements play a vital role in the body metabolism. From my results potassium content was (0.082), calcium (3.877), magnesium (0.598), phosphorus (0.48), sodium (0.86), manganese (85.781), iron (94.798), Zinc (41.957), copper (5.655). The stem *C, populnea* was high in iron, manganese, zinc, copper and calcium.

Calcium and magnesium play a significant role in photosynthesis, carbohydrate metabolism, nucleic acid and binding agent of eth cell wall. Calcium aids in teeth development.

Magnesium is an essential mineral for enzyme activity. Magnesium also play a role in regulating the

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acid-alkaline balance in the body. The value of sodium in the sample which is required in the body only in a small quantity[21] stated that sodium and potassium found in the Intracellular and extracellular fluid in human help to maintain electrolyte balance and membrane fluidity. The function of the cell of the immune system.

The proximate composition of the stem of *C. populnea* is presented in Table 2. Carbohydrate is the highest. However, moisture content obtained in this study for *C. populnea* stem was 13.033. The low moisture content helps to prevent the stem spoilage by microorganism. The fat content value of the stem of C. *populnea* was lower compare to other values. The ash-content is an indication of the level of inorganic mineral and organic matter present in the stem.

Crude fibre quantity in these stem is desirable because adequate consumption of dietary fibre may aid digestion. Fibre softens stool and therefore prevent constipation. Dietary fibre is also important in layering serum cholesterol level and reduce risk of disease such as coronary heart disease, hypertension, diabetes and breast cancer.

The amino acid profiles of *C. populnea* using HPLC are listed in table 5. This method identified and quantified twenty amino acids in *C. populnea.* The essential and non-essential amino acid range from 0.10 – 14.48% for essential and 0.05-5.44%, for non-essential. It is interesting to note that phenylalamine, Glutamine, tyrosine, valine, Glycine threonine, leucine, trypotophan, proline, serine, acids were the most concentrated amino acids in *C. populnea*. This observation has shown that the concentration and presence of amino acid vary from one plant to another. The medium of propagation of plant is also a key factor to the concentration of amino acid. The percentage total amino acids in *C. populnea* was 45.38%. This is an indication that *C. populnea* produced a higher concentration of amino acid.[22] reported that amino acid concentration increased in salt-stressed environment. Secondary metabolites such as alkaloid provide chemical defense for plant and amino acid are important in protein synthesis and precursor in the formation of secondary metabolism molecules[23]. Valine maintain the balance of branched chain amino acids, whereas alanine is involved on hepatic, autophagy, gluconeogenesis and transamination. Leucine regulats the protein turnover (mTOR signaling) and gene expression. Glycine, lysine, threnonine and glutamate help to maintain intestinal integrity and health.

The vitamin content of *C. populnea* as presented in Table 4. It shows that ascorbic acid content was found to be the highest followed by riboflavin then beta-carotene after which cobalamin followed and finally thiamine. The result revealed a higher percentage of vitamin in *C. populnea*. Vitamins are crucial in the functioning Vitamin C is a major antioxidant in the human body and possess the capacity to participate in enzymatic and hydroxylation reaction. It also prompts the absorption of microelements such as iron and copper, involves in trace element metabolism and process cells from damage caused by free radicals and environmental pollution.

Vitamin A is a micronutrient essential to most mammalian species. It is necessary in vision, growth and development, gene transcription, immunity dermatology[24,25] any bioactive components such as vitamin C and E, carotenoids, phenolic and thiol compound have been reported to have natural protective effect against many illnesses.

V. Conclusion

The phytochemical, proximate and mineral results present in the stem of *C. populnea* showed that certain chemical compounds in plants such as alkaloid, flavonoids, saponins, oxalates and tannins contains some bioactive component, which may have biological significance to living organisms and can be useful in pharmaceutical industry, ethnobotany and ethno-medicine. Traditionally, *C. populnea* is used for the treatment of different ailments.

VI. Significance Statement

This study discovers that *Cissus populnea* can be beneficial for not only as a food crop but also as a medicinal crop. This study will help the researcher to uncover the critical areas of medicinal properties of *C. populnea* that many researchers were not able to explore. Thus a new fact on the phytochemical screening, proximate composition, mineral content, amino acid and vitamin analyses of *Cissus populnea* linn as a medicinal plant may be arrived at.

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Acknowledgments

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Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
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- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
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- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
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- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
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The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

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c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

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i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

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16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

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Key points to remember:

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- Please note the criteria peer reviewers will use for grading the final paper.

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- Use present tense to report well-accepted matters.
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Reason for writing the article—theory, overall issue, purpose.

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- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

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The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
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- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
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Approach:

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- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

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Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

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- Skip all descriptive information and surroundings—save it for the argument.
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The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

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- Present a background, such as by describing the question that was addressed by creation of an exacting study.
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- Give details of all of your remarks as much as possible, focusing on mechanisms.
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Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

A

Aberration · 6
Antithrombotic, · 50
Apoptosis · 53, 56
Archostemata · 1, 6, 7, 9

C

Calyptomeridae · 7
Chaperone · 53, 54, 57, 58
Chatzimanolis · 1, 5, 8, 9
Coleoptera · 1, 2, 3, 9, 10, 11, 12
Cretaceous · 5, 7, 8, 9, 10, 12

E

Epipleura · 3, 4, 8

H

Haplochelus · 1, 6, 8, 10
Hydroscaphidae · 1, 2, 3, 4, 5, 7, 9, 10, 11
Hypognathous · 2, 5, 8

I

Isocline · 23, 38

L

Lacustrine · 7
Lepicerids · 6, 7, 12
Lepichelus · 6, 8

M

Metaventrite · 2, 4
Metepisterna · 2, 4

N

Norleucine · 42, 43

P

Permian · 1, 2, 4, 5, 6, 7, 8, 11
Plesiomorphic · 5
Poisonous · 49
Procoxae · 4
Prosternum · 2, 3, 4, 15

S

Sphaeriusoidea · 5, 7

T

Triassic · 1, 2, 4, 5, 8, 9, 10, 11
Trochanters · 2
Trochantin · 2, 4



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