



Research Article

Phylogenetic study of Indian Collembolan: an evaluation in Uttar Pradesh

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Abstract: Springtails (Collembola) from the largest of the three lineages of modern hexapods that are no longer considered insects (the other two are the Protura and Diplura). Although the three orders are sometimes grouped together in a class called Entognatha because they have internal mouthparts, they do not appear to be any more closely related to one another than they all are to insects, which have external mouthparts. Collembolans are omnivorous, free-living organisms that prefer moist conditions. They do not directly engage in the decomposition of organic matter but contribute to it indirectly through the fragmentation of organic matter and the control of soil microbial communities. The word "Collembola" is from the ancient Greek "Glue" and "Peg"; this name was given due to the existence of the colophore, which was previously thought to stick to surfaces in order to stabilize the insect. It is necessary to study the phylogeny of collembolans to explore evolutionary status.

Keywords: Springtails, Collembola, Phylogeny, Ventral tube, Retinaculum and Furcula.

1. Introduction

These insects were known in ancient India as "utpatikya" on account of their springing habit. The systematic position of collembolan is disputed, Handlirsch (1895) regarded them as an independent class of Arthropoda because they differ from other insects having only six abdominal segments in which the primitive abdominal appendages other than tall cerci have been preserved and modified into special organ. Tillyard (1908) regarded collembolan as remnants of the primitive group on account of primitive abdominal appendages and few postcephalic segments and from which other insects evolved. Mouth appendages which are based on Insecta plan and appendages being opisthogoneate hexapods, they are regarded as insects.

They generally live in organic debris, leaf mold, and dung or wherever there are sufficient moisture and soil to support their existence. Collembolan are mostly confined to wet or damp surroundings and inhabit moss, humus, caves nests or termites and ants, soles, underleaves, litter and other organic debris as well as among plant roots or bark, in fungi, on the water and in the intertidal areas among the seashores where they may be completely submerged for a considerable time in the crevices of rock and sometimes on the damp soil by for a part of one family. In which there is poorly developed

tracheal system. The respiration in this order is cutaneous and the skin. Must be kept moist in order to the respiration process may function. The integument is so delicate i.e., low humidity the body dries out rapidly. The members of some genera are covered with scales of various shapes and size, and within this protective covering, they may withstand the dry atmosphere more than their scaleless relatives.

Sexes are similar in collembolan, there being no external genitalia. The genital aperture in both sexes is placed near the hind margin of V sternum while the anus is located on VI sternum and in some Sminthuridae the genital duct opens united on the genital papillae on V sternite. Some collembolan of genus *Rhodonella salmon* shows sexual dimorphism. The male bears long curved horns on the top of the head and in *Spinisotoma* Stach of the same family the male is with terminal abdominal spines.

In some Symphypleona the male is provided with a clasping organ of hooks and spines, dorsally on VI abdominal segments and sometimes the antennae of male are also modified for clasping.

The collembolan represents an early possible primitive phase in the evolution of insects and, so far as the head is concerned, exhibit to quite distinct groupings. The first group which approximately close to the hypothetical ancestral form with a prognathous head

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and an elongated segmented trunk region includes the commonly recognized Collembola-Arthopleona. In the second group Symphyleona, the axis of the head become reoriented so that the head assumes a completely hypognathous or obliquely hypognathous position. This accompanied by a tendency for the fusion and telescoping of the trunk segments which advances progressively as an evolution of the group proceeds. Therefore, collembolan occupies a unique position among Apterygota.

2. Materials and Methods

Large number of collembolan have been collected from different parts of Agra and from various localities from Uttar Pradesh in the month of August, September, and October. The specimen was mostly procured from under heaps of fallen leaves among mosses edges of streams and rivers from the hilly part of India. Large number of specimens were collected from sugarcane, wheat and rice crop and cultivated fields during monsoon season near about Agra (U.P.). Some specimens were also collected in the vicinity of stagnant water. The specimens were collected with the help of camel hair brush moistened with 90% alcohol. Canada balsam is unsuitable as mounting for collembolan.

For the microscopical study of the structure of the abdominal appendages, specimens were first put into dilute KOH and the mounted as a whole on a slide under a binocular microscope and mounted in Salmon's - Polyvinyl alcohol-lactophenol medium prepared as follows:

1. Polyvinyl alcohol solution - 10ml
2. Lactic acid - 10ml
3. Glycerine - 1ml

The medium is a colorless oily liquid slightly viscous. It lacks the phenolic smell and brown color in bulk when exposed to daylight. Mounts prepared in this medium kept in over at 40°C for three months did not show any sign of shrinkage or distortion. Specimens can be mounted directly into PL₃ from water, alcohol and various preserving and fixing solutions without the necessity of passing through the tedious dehydration procedure. The collembolans collected are sorted out and their locality and habitat label are put inside the tube. The collembolans are preserved in 70% alcohol plus formaldehyde plus the little amount of glycerine and they are properly identified and their detailed study is made under high power microscope and the different diagrams with the help of the camera Lucida have been made.

3. Results and Discussion

3.1 Phylogeny of the group

Keeping the life in mind we must consider in what direction the taxonomic characters were specialized. Here we present such specialization in a tentative table as follows in (Table 1). Natural classification is based on phylogeny, although they represent phylogeny imperfectly. Ever since the invention of the phylogenetic tree by Haeckel, it has been customary among taxonomists to express phylogenetic conclusion in the form of diagrams. In spite of their numerous shortcomings, such diagrams are useful summarizations of the author's concept of the evolutionary history of a group (Mayer, Linsley and Usinger, 1953). Concerning collembolan, there is a lot of evidence which shows the most basic or unmodified primitive forms are sought in the hemiedaphon-normal category of animals such as the family Hypogastruridae. From this, divergence of the characters might be considered to occur in several different ways.

Table 1. Specialization of structural characters of Collembola.

S. No.	Characters	Primitive of basic state	Specialized state
1.	Segmentation of body	Prothorax not reduces, Setaceous dorsally, Abdominal Segments not ankylosed.	Prothorax more or less reduced, covered by mesonota, without setae, Some of the abdominal segments ankylosed (Mostly Abds. IV-VI)
2.	Head	Head prognathous	Head hypognathous
3.	Antennae	Normally 4-segmented, moderately long, no sub-division occur	Excessive elongation of shortening; subdivisions of the segments.
4.	Mouthparts	Masticatory with normally constructed mandible and maxilla.	Sectorial, more or less silent-shaped or absence or mandible.
5.	Ant.-org.III and PAO	Rather simple	Absence of the organs (Epigeon), or excessive complication (Euedaphon)
6.	Ocelli	Eight or five on each side	Reduction in number, in extreme case, absent.
7.	Claw	Unguis and Unguiculus simple without superficial accessories.	They are provided with superficial accessories or reduction of Unguiculus.
8.	Ventral tubes	Ventral tube small or prominent with minute or very long and much elongated vesicles.	Excessive reduction or development of this organ.
9.	Tenaculum	With normally constructed corpus and rami	Excessive reduction or development of this organ.
10.	Furcula	With normally constructed manubrium, Dens, and Mucro	Excessive reduction or development of the elements.
11.	Body wall	Normally granular	Strongly tuberculate or smooth.
12.	Hairing & scaling	Setae simple, and without Scales or sensuale	Setae complex, and in son forms, scales and sensual developed.

Note: In the present investigation only Ventral tube, Retinaculum and Furcula have been studied.

By far several attempts of phylogenetic diagram are made without fossil evidence (Börner, 1909; Gisin, 1966). This involves the basic but unproved assumption that the degree of resemblance of recent collembolan is a direct function of their respective ages, the most divergent forms having branched off earlier than more closely related groups. The classification of collembolan is essentially due to Börner's "Das system der Collembolan" in 1913, although great advances have been made in the taxonomy at the specific level during the past decade. Their classification as it now stands is based primarily upon morphological similarities without regard to fundamental phylogenetic relationships. But they are very important and useful in the classification of the main group in the higher taxa of collembolan.

On the embryology of an insect, segmentation of the body is one of the earliest major phases of development of the embryo from the egg. In this processor segmentation, the segment of the head region is the first to be differentiated in the protopod phase of embryology. When fully segmented at the oligopod phase of its development the embryo displays a prognathous head region and completely trunk region. It would seem reasonable to accept that in the hypothetical ancestral insect the long axis of the head projects forward in line with the long axis of the body i.e. prognathous. The hypothetical ancestor would then, like the Symphyla, have a prognathous head and segmented trunk. And then any reorientation of the long axis of the head must be later developed and hence of secondary origin. On this basis, a hypognathous head would be a later specialization in evolution.

There is virtually no palaeontological record, the only known fossil species are *Rhyniella praecursor* Hirst and Maulik, 1926, described from four specimens in the middle Devonian. Massoud (1967) reported that the composition of the mandible with rudimentary molar plate and the stylet-like maxilla, allows one to place *Rhyniella* among the genera of a highly specialized group of actual collembolan. Sharov (1966) pointed out that the imaginary ancestral stock ought to be sought in the protosymphyla from which Symphyla, Diplura insects were originated, besides collembolan.

The collembolan represents an early, possibly primitive phase in the evolution of insects and, so far as the head concerned, exhibit two quite distinct groupings. The first group which approximates closely to the hypothetical ancestral with a prognathous head and an elongated segmented trunk region includes the Collembola-Arthropleona. In the second group, the axis of the head become reoriented so that the head assumes a completely hypognathous or obliquely hypognathous position. This accompanied by a tendency for the fusion and telescoping of the trunk segments which advances progressively as an evolution of the group proceeds. Intercalated by the genus *Podura*, this tendency reaches full expression in the suborder Symphypleona. This development is seen in its early stages not only in the

genus *Podura* but also in the genus *Actaletes*. The genus *Podura* has always been included at the beginning of the suborder Arthropleona in all recent schemes of classification, but, on the basis of its hypognathous head, the genus must now be removed from the Arthropleona and associated with *Actaletes* and the Symphypleona in a few grouping. For such group which comprises *Podura* and *Actaletes* Salmon (1964) has erected the suborder Metaxypleona. But suborder Metaxypleona in this investigation is not used because the genus *Actaletes* has not been studied in this work.

Referring back once more to the hypothetical ancestral insect; this would be equipped with simple mouthparts adapted for biting, chewing and tearing which would be fashioned on a basic plan that utilised mandibles and maxillae, both bearing teeth, ridges, or other tearing or grinding a structure as laterally moving jaws. The mandibles and maxillae are appendages of the head region and are among the first appendages to appear in the developing insect embryo. They are therefore fundamental structures of great importance as indicators of natural groupings in taxonomy. Any deviation from the basic pattern of the structure and function of the mouthparts which appear in any group of insects can then be regarded as of later a secondary origin and used as an indicator of basic patterns of evolution. Comparing the homogeneous symphypleonids with the Collembola-Arthropleona the latter group is revealed as a very heterogeneous assemblage which has grown up over the years. Based on a small nucleus of old established genera, entirely through morphological conceptions, especially the abdominal appendages like ventral tune, retinaculum, and furcula. When the principles outlined in the fundamental characters such as micro are applied to this assemblage it breaks down into two groups. One group is based upon the fundamental pattern of furcula especially micro which shows great variation. Looked at in this way the collembolan. Arthropleona must be subdivided on the basis of the furcula into two taxonomic units. Salmon (1964) proposed to create the suborder Neoarthropleona for those forms with Arthropleona to designate the suborder containing all those forms with biting and chewing mouthparts. This reorientation of the classification involved considerable revision of existing taxonomic arrangements, especially in the groups at present recognized as Brachystomellidae, Anuridae, Neanuridae, and Pseudachorutidae.

If we pursue the development of the primitive embryonic insect further it follows that integumentary characters such as scales, hairs, setae, spines. Integumentary swelling and outgrowths bosses and granules though of considerable taxonomic value are not nearly so fundamental or origin as the body segmentation from and the structures of the abdominal appendages. Even though these characters as the attributes to these forms acquired by the adaptation to

the environment. Bosses and other tuberculate cuticular outgrowths are probabilistic in such a nature which could have evolved merely as an analogy on numerous occasions in different, possibly even unrelated groups likewise the setae: spines, scales, and many other sensory organs. As Salmon pointed out, these structures must be used with considerable caution if used as the basic characters in any classificatory scheme for collembolan. Generally to say the characters of body covering might suitably be used for the classification of the group in the specific level or at most in the generic rank.

Gisin (1966) stated in his "La systématique idéale" as follows – these criteria are based essentially on the synthetic theory of evolution and on the quantum theory of taxonomy. An analysis of an example of modern classifications shows that they tend to be an unclear compromise of incompatible principles each of which is sound in its self but has quite distinct function in taxonomy. These functions have to be sharply in taxonomy. As such, important step methods and goals of obtaining information about genealogies discontinuities and evolutionary quanta are explained and exemplified. Thus, the legs, ventral tube, retinaculum and furcula which have no doubt evolved from the primitive appendage of the body segments must be more fundamentals and basic to the collembolan and hence of greater fundamental importance in taxonomy. However, knowledge of collembolan is by no means sufficiently advanced to be dogmatic on this point and even though circumstantial evidence may suggest such a possibility. Therefore, in the classification presented in this investigation the size, shape of the different part of furcula and the presence of spines. The furcula has been used in the normally excepted way has good family characters.

This present deal with the studies on collembolan from Uttar Pradesh, India. Collembolan is small soft-bodied insects and in Uttar Pradesh, they are largely neglected, due to their small size and inconspicuous retiring habits. The aim of the present work would serve a good purpose for future workers in India. A considerable amount of attention has been paid to the present information and this thesis as up to date as possible. However, the present work cannot be claimed to be complete as some of the confirmatory experiments are eliminated. Moreover, such details are not essential in the study of this nature.

In the course of my work, several new and little-known species, some of which are already described through the kindness of Head of Zoology Department, school of Entomology, St. Johns College, Agra. It was also possible for me to study the collection of collembolan in the School of Entomology, St. Johns College, Agra. Several species inadequately described by earlier workers are fully described and rightly placed in the view of recent taxonomic work. The work appended brief diagnostic keys to the superfamilies, subfamilies, families Genera and species studied.

References

- [1]. Baum, D.A. & Larson, A. (1991). Adaptation reviewed: A phylogenetic methodology for studying character macroevolution. *Systematic Zoology*, 40(1): 1–18, <https://doi.org/10.1093/sysbio/40.1.1>.
- [2]. Brooks, D.R. & McLennan, D.A. (1991). Phylogeny, ecology, and behavior: A research program in comparative biology. University of Chicago Press.
- [3]. Brown, R.W. (1956). Composition of scientific words. Smithsonian Institution Press. Washington, D.C. 882 pp.
- [4]. Carpenter, J.M. (1989). Testing scenarios: wasp social behavior. *Cladistics*, 5: 131-144.
- [5]. Chang, B.S. & Donoghue, M.J. (2000). Recreating ancestral proteins. *Trends in Ecology and Evolution*, 15: 109-114.
- [6]. Coddington, J.A. (1988), Cladistic Tests of Adaptational Hypotheses. *Cladistics*, 4: 3-22. doi:10.1111/j.1096-0031.1988.tb00465.x.
- [7]. Cunningham, C.W., Omland, K.E. & Oakley, T.H. (1998). Reconstructing ancestral character states: a critical reappraisal. *Trends in Ecology and Evolution*, 13: 361-366.
- [8]. Donoghue, M.J. (1989). Phylogenies and the analysis of evolutionary sequences, with examples from seed plants. *Evolution*, 43(6): 1137-1156. doi: 10.1111/j.1558-5646.1989.tb02565.x.
- [9]. Donoghue, M.J. & Sanderson, M.J. (1992). The Suitability of Molecular and Morphological Evidence in Reconstructing Plant Phylogeny. In: Soltis, P.S., Soltis, D.E. & Doyle J.J. (eds). *Molecular Systematics of Plants*. Chapman and Hall, N.Y. pp. 340-368.
- [10]. Doolittle, W.F. (1999). Phylogenetic classification and the universal tree. *Science*, 284: 2124-2128.
- [11]. Edwards, A.W.F. & Cavalli-Sforza, L.L. (1964). Reconstruction of evolutionary trees. In: Heywood, V.H. and McNeill, J. (eds.), *Phenetic and Phylogenetic Classification*, Systematics Association Pub. No. 6, London. pp. 67–76.
- [12]. Eldredge, N. and Cracraft, J. (1980). *Phylogenetic Patterns and the Evolutionary Process: Method and Theory in Comparative Biology*. Columbia University Press, New York.
- [13]. Farrell, B.D., Mitter, C. & Futuyma, D.J. (1992). Diversification at the Insect-Plant Interface: Insights from phylogenetics. *BioScience*, 42(1): 34–42. doi: <https://doi.org/10.2307/1311626>.
- [14]. Farris, J.S. (1970). Methods for computing Wagner Trees. *Systematic Zoology*, 19: 83-92.
- [15]. Farris, J.S. (1983). The logical basis of phylogenetic analysis. In: Platnick, N.I. and Funk, V.A. (eds.), *Advances in Cladistics*, Vol. 2. Proceedings of the Second Meeting of the Willi Hennig Society. Columbia University Press, N.Y. pp. 1-36.

- [16]. Felsenstein, J. (1979). Alternative Methods of Phylogenetic Inference and their Interrelationship. *Systematic Zoology*, 28(1): 49–62. <https://doi.org/10.1093/sysbio/28.1.49>.
- [17]. Felsenstein, J. (1985). Phylogenies and the Comparative Method. *The American Naturalist*, 125(1): 1-15.
- [18]. Felsenstein, J. (1988a). Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics*, 22: 521-565.
- [19]. Felsenstein, J. (1988b). Phylogenies and quantitative characters. *Annual Review of Ecology and Systematics*, 19: 445-471.
- [20]. Felsenstein, J. (2004). *Inferring Phylogenies*. Sinauer Associates, Inc., Sunderland, Massachusetts.
- [21]. Fink, W.L. (1982). The Conceptual Relationship between Ontogeny and Phylogeny. *Paleobiology*, 8(3): 254-264.
- [22]. Fitch, W.M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology*, 20: 406-416.
- [23]. Futuyma, D.J. (1988). Sturm und Drang and the evolutionary synthesis. *Evolution*, 42(2): 217-226. doi: 10.1111/j.1558-5646.1988.tb04126.x.
- [24]. Futuyma, D.J. & McCafferty, S.S. (1990). Phylogeny and the evolution of host plant associations in the leaf beetle genus *Ophraella* (Coleoptera, Chrysomelidae). *Evolution*, 44: 1885-1913. doi:10.1111/j.1558-5646.1990.tb04298.x.
- [25]. Gittleman, J.L. (1988). The comparative approach in ethology: Aims and limitations. In: Bateson, P.P.G. and Klopfer, P.H. (eds.). *Perspectives in Ethology*, 8: 55–83. New York: Plenum.
- [26]. Goodman, M., Czelusniak, J., Moore, G.W., Romero-Herrera, A.E. & Matsuda, G. (1979). Fitting the Gene Lineage into its Species Lineage, a Parsimony Strategy Illustrated by Cladograms Constructed from Globin Sequences. *Systematic Zoology*, 28(2): 132–163. <https://doi.org/10.1093/sysbio/28.2.132>.
- [27]. Graur, D. and Li, W.-H. (1999) *Fundamentals of Molecular Evolution*. 2nd Edition. Sinauer Associates, Sunderland, Massachusetts.
- [28]. Harvey, P.H., Leigh Brown, A.J. and Maynard Smith, J. (1995). New uses for new phylogenies: editors' introduction. *Philosophical Transactions of the Royal Society London Series B*, 349: 3-4.
- [29]. Harvey, P.H. and Pagel, M.D. (1991). *The Comparative Method in Evolutionary Biology*. Oxford: Oxford University Press.
- [30]. Hennig, W. (1966). *Phylogenetic Systematics*. University of Illinois Press, Urbana.
- [31]. Hillis, D.M. (1987). Molecular Versus Morphological approaches to systematics. *Annual Review of Ecology and Systematics*, 18: 23-42.
- [32]. Hillis, D.M., Bull, J.J., White, M.E., Badgett, M.R. & Molineux, I.J. (1992). Experimental phylogenetics: generation of a known phylogeny. *Science*, 255: 589-592.
- [33]. Hillis, D.M., Huelsenbeck, J.P. & Cunningham, C.W. (1994). Application and accuracy of molecular phylogenies. *Science*, 264: 671-677.
- [34]. Hopkin, S.P. (1997). *Biology of the Springtails—Insecta: Collembola*. Oxford University Press. 330 pp.
- [35]. Huelsenbeck, J.P. & Rannala, B. (1997). Phylogenetic Methods Come of Age: Testing Hypotheses in an Evolutionary Context. *Science*, 276: 227-232. DOI: 10.1126/science.276.5310.227.
- [36]. Huelsenbeck, J.P. & K.A. Crandall (1997). Phylogeny Estimation and Hypothesis Testing Using Maximum Likelihood. *Annual Review of Ecology and Systematics*, 28: 437-466.
- [37]. Huey, R.B. & A.F. Bennett (1987). Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution*, 41: 1098-1115.
- [38]. Koshi, J.M. & Goldstein, R.A. (1996). Probabilistic reconstruction of ancestral protein sequences. *Journal of Molecular Evolution*, 42: 313-320.
- [39]. Lauder, G.V. (1982). Historical biology and the problem of design. *Journal of Theoretical Biology*, 97: 57-67.
- [40]. Maddison, D.R. (1994). Phylogenetic Methods for Inferring the Evolutionary History and Processes of Change in Discretely Valued Characters. *Annual Review of Entomology*, 39: 267-292.
- [41]. Maddison, D.R. & W.P. Maddison (2000). *MacClade 4: Analysis of phylogeny and character evolution*. Sinauer Associates, Sunderland, Massachusetts.
- [42]. Maddison, D.R., Ruvolo, M. & Swofford, D.L. (1992). Geographic origins of human mitochondrial DNA: Phylogenetic evidence from control region sequences. *Systematic Biology*, 41: 111-124.
- [43]. Maddison, W.P. (1990). A method for testing the correlated evolution of two binary characters: Are gains or losses concentrated on certain branches of a phylogenetic tree? *Evolution*, 44: 539-557. doi: 10.1111/j.1558-5646.1990.tb05937.x.
- [44]. Maddison, W.P. (1995). Calculating the probability distributions of ancestral states reconstructed by parsimony on phylogenetic trees. *Systematic Biology*, 44: 474-481.
- [45]. Maddison, W.P. (1996). Molecular approaches and the growth of phylogenetic biology. Pages 47-63 in J.D. Ferraris and S.R. Palumbi (eds.). *Molecular Zoology: Advances, strategies, and protocols*. Wiley-Liss, New York.
- [46]. Maddison, W.P. (1997). Gene trees in species trees. *Systematic Biology*, 46: 523-536.
- [47]. Maddison, W.P. & Maddison, D.R. (1992). *MacClade 3: Analysis of Phylogeny and*

- Character Evolution. Sinauer Associates, Sunderland, Massachusetts.
- [48]. Merritt, R.W., Cummins, K.W. & Berg, M.B., Eds. (2008). Aquatic Insects of North America. 4th Edition, Kendall/Hunt, Dubuque.
- [49]. Mitter, C., Farrell, B. & Wiegmann, B. (1988). The Phylogenetic Study of Adaptive Zones: Has Phytophagy Promoted Insect Diversification? *The American Naturalist*, 132(1): 107-128.
- [50]. Morrison, D.A. (1996). Phylogenetic tree-building. *International Journal for Parasitology* 26: 589-617.
- [51]. Nelson, G. & N.I. Platnick (1981). Systematics and Biogeography: Cladistics and Vicariance. New York: Columbia University Press.
- [52]. O'Hara, R.J. (1988). Homage to Clio, or, Toward an historical philosophy for evolutionary biology. *Systematic Zoology*, 37: 142-155.
- [53]. Page, R.D.M. & E.C. Holmes (1998). Molecular Evolution: A Phylogenetic Approach. Blackwell Science, Oxford.
- [54]. Pagel, M. (1994). Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London Series B* 255: 37-45.
- [55]. Pagel, M. (1997). Inferring evolutionary processes from phylogenies. *Zoologica Scripta*, 26: 331-348. doi:10.1111/j.1463-6409.1997.tb00423.x.
- [56]. Pagel, M. (1999a). Inferring the historical patterns of biological evolution. *Nature*, 401: 877-884.
- [57]. Pagel, M. (1999b). The Maximum Likelihood Approach to Reconstructing Ancestral Character States of Discrete Characters on Phylogenies. *Systematic Biology*, 48: 612-622.
- [58]. Penny, D., Hendy, M.D. and Steel, M.A. (1992). Progress with methods for constructing evolutionary trees. *Trends in Ecology and Evolution*, 7: 73-79.
- [59]. Richard, E. White, Donald J. Borror, Roger Tory Peterson (1998). A Field Guide to Insects: America North of Mexico. Houghton Mifflin Co.
- [60]. Ridley, M. (1983). The Explanation of Organic Diversity: The Comparative Method and Adaptations for Mating. Oxford: Clarendon Press.
- [61]. Sessions, S.K. & Larson, A. (1987). Developmental correlates of genome size in plethodontid salamanders and their implications for genome evolution. *Evolution*, 41:1239-1251. doi: 10.1111/j.1558-5646.1987.tb02463.x.
- [62]. Sillén-Tullberg, B. (1988). Evolution of gregariousness in aposematic butterfly larvae: A phylogenetic analysis. *Evolution*, 42:293-305. doi: 10.1111/j.1558-5646.1988.tb04133.x.
- [63]. Steel, M. & D. Penny (2000). Parsimony, likelihood, and the role of models in molecular phylogenetics. *Molecular Biology and Evolution*, 17: 839-850.
- [64]. Swofford, D.L. & Maddison, W.P. (1992) Parsimony, character-state reconstructions, and evolutionary inferences. In: Mayden, R.L. (ed.), Systematics, Historical Ecology, and North American Freshwater Fishes, Stanford: Stanford University Press, pp. 187-223.
- [65]. Swofford, D.L. & Olsen, G.J. (1990). Phylogeny reconstruction. In: Hillis, D.M. and Moritz, C. (eds.), Molecular Systematics, Sinauer Associates, Sunderland, Massachusetts. pp 411-501.
- [66]. Swofford, D.L., Olsen, G.J., Waddell, P.J. & Hillis, D.M. (1996). Phylogenetic Inference. In: Hillis, D.M., Moritz, C. and Mable, B.K., Eds., Molecular Systematics, 2nd Edition, Sinauer Associates, Sunderland (MA), 407-514.
- [67]. Tuffley, C. & Steel, M. (1997). Links between maximum likelihood and maximum parsimony under a simple model of site substitution. *Bulletin of Mathematical Biology*, 59: 581-607.
- [68]. Wanntorp, H., Brooks, D., Nilsson, T., Nylin, S., Ronquist, F., Stearns, S. & Wedell, N. (1990). Phylogenetic Approaches in Ecology. *Oikos*, 57(1), 119-132. doi: 10.2307/3565745.
- [69]. Wiley, E.O. (1981). Phylogenetics: The Theory and Practice of Phylogenetic Systematics. Wiley.
- [70]. Yang, Z.H. (1994b). Estimating the pattern of nucleotide substitution. *Journal of Molecular Evolution*, 39: 105-111.
- [71]. Yang, Z.H. (1993). Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution*, 10: 1396-1401.
- [72]. Yang, Z.H. (1994a). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution*, 39: 306-314.
- [73]. Yang, Z., Kumar, S. & Nei, M. (1995). A new method of inference of ancestral nucleotide and amino acid sequences. *Genetics*, 141: 1641-1650.