Bajopas Volume 2 Number 2 December, 2009

Bayero Journal of Pure and Applied Sciences, 2(2): 159 - 164
Received: August, 2009
Accepted: November, 2009

THE COMPLEXITIES IN THE CLASSIFICATION OF PROTOZOA: A CHALLENGE TO PARASITOLOGISTS

Imam, T.S.
Biological Sciences Department, Bayero University, Kano, PMB 3011, Kano, Nigeria.
E-mail Address: tsimam2001@yahoo.com

ABSTRACT
This paper revisits the controversial issues bedevilling classification of the parasitic protozoa as a result of varying interest by different scientists belonging to protozoology and parasitology axes. In recent years, the availability of a number of molecular markers has made it possible to analyse relationships between protozoa that would not have been possible using morphological characters alone. Three terms are currently widely used: Protozoa, Protoctista, and Protista. However, parasitologists tend to be very conservative and the term Protozoa is now almost universally used by scientist working with those parasitic unicellular organisms that infect humans and domesticated animals. With the creation of 5 kingdoms, status of Protozoa was raised to that of a kingdom, which formerly was a Phylum. Thus, the subordinate groups automatically became Phyla. The increase in the number of parasitic protozoa recorded from humans and the accumulation of knowledge about their biology led to the creation of taxonomic and other groupings at the subgenus and subspecie levels. Corliss (1994) in his scheme has used both traditional and contemporary approaches and has attempted to retain familiar names as far as possible. It is important that any classification should reflect modern thinking about the classification as a whole, while retaining sufficient traditional material so as to permit every reference to information retrieval systems

Keywords: parasitic protozoa, parasitologists, classification, molecular markers, morphological characters

INTRODUCTION

The protozoa are defined as single celled eukaryotic organisms, but this definition is very simplistic and it has been an enigmatic one, largely because of the small size of their cells, the relative lack of morphological features and absence of any meaningful evolutionary history. The fact that protozoa are organisms that lie somewhere between prokaryotic and higher eukaryotic organisms, sharing of each characteristics make their classification a little bit complex (Collier *et al.*,1998).

The word Protozoa meaning 'first animals' was coined by Goldfuss in 1818 since then has been in use in a modified form ever since. Siebold,(1848) like Goldfuss regarded the protozoa as primitive invertebrates and divided the protozoa between two classes, the Infusoria and Rhizopoda (roughly equivalent to ciliates and amoebae). Hogg (1860) introduced the term 'Protoctista' to embrace those forms that had plant, animal or no clear affinities. Haekel (1876) used the term 'Protista'. Haekel's ideas were translated into a system of classification that divided the Animal kingdom into single celled organisms, Protozoa and multicellular organisms, Metazoa. This has remained almost unchanged in zoological books until the present time and its basic concepts are clearly and unambiguously set out by Craig (1926).

There are > 200, 000 named species of protozoa of which nearly 10, 000 are parasitic in invertebrates and in almost every species of vertebrate (Collier *et al.*, 1998). This range from forms that are never pathogenic to those that cause some major diseases of tropical countries: such as malaria, sleeping sickness, Chagas disease, and Leishmaniasis, which together threaten over one-quarter of the world's population (Collier *et al.*, 1998). Craig opined that the protozoa are animals composed of a single cell. Copeland (1938) grouped together all those single celled organisms that did not have obvious animal or plant affinities and used Haekel's term Protista, which was later changed to Hogg's Protoctista on grounds of priority. Thus, three terms are currently widely used: Protozoa, Protoctista, and Protista. However, parasitologists tend to be very conservative and the term Protozoa is now almost universally used by scientists working with those parasitic unicellular organisms that infect humans and domesticated animals (Collier *et al.*, 1998).

This paper aimed at revisiting the controversial issues bedevilling classification of the parasitic protozoans as a result of varying interest by different scientists in the field.

Kingdoms in Disarray

Carl Linnaeus in his 1758 classification of all living things, recognised two Kingdoms, Animalia and Plantae, but by the middle of the 19^{th} century scientists had begun looking for a third category in which to classify organisms like the single celled ones, which were then being discovered, with increasing frequency.

Several scientists including Hogg (1860) and Haekel (1876), argued for the creation of an additional kingdom to accommodate these forms. Haekel applied evolutionary trends to the classification of single celled organisms, and he is often credited with the concept of 3 Kingdoms of living things: Animalia, Plantae and Protista which was expanded to 4 Kingdoms: Animalia, Plantae, Protoctista and Mychota by Copeland(1938). With the removal of the fungi from the plant kingdom the creation of a fifth kingdom, Fungi was necessary and sixth was added by Jahn and Jahn (1949), who modified the kingdoms as thus, Archetista(viruses), Monera(bacteria and blue-green algae), Metazoa(multi cellular organisms), Fungi and Protista. Jahn and Jahn's classification became widely adopted and forms the basis of what is now known as Whittaker's 5 kingdoms Classification: Monera(prokaryote), Animalia, Plantae, Fungi and Protista (Whittaker, 1969).

Essentially what this did was to remove the viruses (regarded as non living) and to make readjustments between the fungi and plants. However, studies at the biochemical and molecular levels have thrown up a number of problems making it necessary to adopt a rather more radical approach such as that set out by Cavalier-Smith (1993; 2002) and adopted by Corliss (1994). Cavalier noted that eukaryotes and archaebacteria form the Clade Neomura, and are 'sisters' as shown by genes fragmented only in archaebacteria and by many sequence trees (Clade refers to a group of organisms e.g. a species that are considered to share a common ancestor, a word coined from a Greek word *Klados* meaning branch). These radical innovations occurred in a derivative of the neomuran common ancestor, which evolved immediately prior to the divergence of eukaryotes and archaebacteria (figure 1). As Corliss noted '...there is pressing need now for a useful/usable interim system treating the protists overall in a manner understandable to the general protozoologist/phycologist/mycologist and the myriads of cell and evolutionary biologists, biochemists and general biologist (Corliss, 1994). As has already been pointed out, there is some confusion at the highest taxonomic level because three terms are in existence that can be applied to the unicellular eukaryotic organisms: Protozoa, Protoctista and Protista (Rothschild,1989). Each has its own supporters; parasitologists tend to favour Protozoa(Cox, 1992), while protozoologists prefer to use Protista (Corliss,1994) or sometimes Protoctista (Margulis and Corliss, 1990). Corliss recognises 6 kingdoms in what he calls the empire 'Eukaryota'. The kingdoms are Archezoa, Protozoa, Chromista, Plantae, Fungi and Animalia. The first 3 contain only single celled organisms, although a number of single celled organisms occur in both Fungi and few in Animalia (Corliss, 1994).

Phyla and Classes: A Melting Pot of Instability

With the additional creation of 3 kingdoms, status of Protozoa (formerly a phylum) was raised to that of a Kingdom. Thus, the subordinate groups automatically became phyla. (Collier *et* al.,1998). Goldfuss (1818) established the concept of 3 great groups of Protozoa (amoebae, flagellates and ciliates) on the basis of their mode of locomotion and this number was increased to 4 by the addition of the sporozoans by Butschli(1883).

By the beginning of the 1960s, with the availability of increasingly sophisticated ways of studying protozoa, the Society of Protozoologists in 1964 published a revised classification (Honingberg and Balamuth,1964), which included grouping the sarcodina(amoebae) and mastigophora(flagellates) together and removed the myxosporidians and microsporidians from the sporozoa. One particularly controversial effort was to remove the piroplasms from the sporozoa and to place them among the sarcomastigophora, which was rejected by protozoologists and almost all parasitologists, although this classification was widely accepted and appeared in standard textbooks (Levine,1973).

In 1980, the Society of Protozoologists published its second classification(Levine and Corliss,1980), which recognises 7 phyla: Sarcomastigophora, Apicomplexa(sporozoa), Ciliophora, Microspora, Myxozoa, Ascetospora and Labyrinthomorpha, which was ably outlined by Orihel and Ash(1995) in their Traditional Classification.

Genera and Specie: The Most Stable Portion of Protozoa Classification

The binomial system of nomenclature of Protozoa that infect humans have been remarkably consistent throughout the upheavals that characterized the classification of the protozoa over the past few years(Collier *et al.*,1998).

The main changes have been the omission of the enigmatic species *Blastocystis hominis* and *Pneumocystis carinii*, now classified among the fungi (Collier *et al.*,1998), and the addition of species of the coccidians *Cryptosporidium* and *Cyclospora* and the microsporidians *Encephalizoon, Enterocytozoon* and *Septata*, that cause opportunistic infections in HIV infected individuals, *Balamuthia* too is added into the protozoa which causes accidental infection. Also, *Sarcocystis* spp which were previously unidentified are now found to be a protozoa (Collier *et al.*,1998). The other main changes observed is the growth in the number of species in the genus *Leishmania* (Collier *et al.*, 1998). So also, *Rhinosporidium* which was placed under the fungi and are now classified as protozoa (Fredericks,2000).

Readjustments in the Protozoa Classification Below The Genera And Below The Specie Levels

The increase in the number of parasitic protozoa recorded from humans and the accumulation of knowledge about their biology led to the the creation of taxonomic and other groupings at the subgenus and subspecie levels (Collier *et al.*,1998). The genus *Leishmania* has received the greatest attention. Formerly 2 species of the Old World were observed *L.tropica* (cutaneous form) and *L.donovani*(visceral form).

But *L.tropica* subsequently was differentiated as 2 varieties: *L.tropica var. minor* and *L.tropica var. major* and later subspecies *L.t.minor* and *L.t.major* which have moved to specie level as *L.tropica* and *L.major.* Two subspecies of *L.donovani,* i.e*. L.d.donovani* and *L.d. infantum* are now raised to species level, where *L.d.donovani* has now been raised to *L.donovani* while *L.d. infantum* has been raised to *L.infantum*. In the New World, the cutaneous form *L.tropica var. mexicana* is changed to *L.mexicana*. The New World visceral form *L.donovani* subsequently became *L.d. chagasi* and then *L.chagasi* (Table 1).

Initially each new discovery was assigned either to *L.mexicana* or *L.braziliensis* as a subspecie for example *L.b.braziliensis*. Later 2 subgenera evolved which are *Leishmania* embracing all Old World species and *Viannia* which include the New World species (Lainson and Shaw,1987).

Among the amoebae, *Entamoeba histolytica* has drawn a lot of controversy because it is the most important parasite in the group. For long it has been known that 2 types exist, which are the pathogenic and non-pathogenic forms. This led to the scientists naming the pathogenic form as *E.histolytica,* while non-pathogenic as *E.dispar* (Collier *et al.,*1998). Subgenera and subspecies have been also used for the classification of the trypanosomes,*Trypanosoma* spp. Large numbers of trypanosomes parasitise all groups of vertebrate, thus, Hoare(1972) proposed the creation of subgenera within which to classify the trypanosomes of mammals. These subgenera were classified under 2 groups: Stercoraria containing *Megatrypanum,Herpetosoma* and *Schizotrypanum*, and Salivaria containing *Duttonella, Nannomonas, Pycnomonas* and *Trypanozoon* (table 1). The New World Trypanosome of humans *T.cruzi* is classified in the subgenus *Schizotrypanum* and *T.rangeli* is placed in the subgenus *Herpetosoma,* whereas both the Old World forms, *T.brucei gambiense* and *T.b.rhodesiense* are placed in the subgenus *Trypanozoon.* However the use of *Trypanosoma(Schizotrypanum) cruzi* and *Trypanosoma (Trypanozoon) brucei* was clumsy and the practice has now almost been abandoned (Collier *et al.,*1998).

The malaria parasites belonging to the genus *Plasmodium* have been classified into subgenera on the same grounds as the trypanosomes. Nine subgenera were proposed: *Plasmodium,Vinckeia, Laverania, Haemamoeba, Giovannolaia, Novyella, Huffia, Sauramoeba* and *Carinia*, of which 3 occur in mammals, 4 in birds and 2 in lizards respectively. The human species *Plasmodium falciparum,* was placed in the subgenus *Laverania* whereas, others *P.malariae, P.ovale* and *P.vivax* were placed in the subgenus *Plasmodium* (Garnham,1966).

Giardia duodenalis is another intestinal parasite that causes a range of symptoms and what constitutes this species remains to be resolved, mainly because of the genetic variability that exists even within cloned lines (Thompson, *et al*., 1993). *G.intestinalis* and *G.duodenalis* tend to be used interchangeably in western Europe and Australia. *G.lamblia* is used in the USA and *Lamblia intestinalis* is used in eastern Europe. Electrophoretic analysis suggests that the species in human is morphologically *G.duodenalis*, which parasitizes a number of mammals but that it could be afforded specific status as *G.intestinalis* on grounds of host specificity (Mayrhofer and Andrews,1995). The peculiar morphological features of *G.intestinalis* trophozoite is pear shaped, about 10-20µm, motile with two nuclei and eight flagella which is spoon-shaped laterally. The cyst assumes an oval, ellipsoidal or round shape, 8-19µm, non motile with four nuclei without flagella and having a longitudinal fibres on its surface (Collier *et al*.,1998).

Application of Molecular and Biochemical Analysis to the Classification of the Parasitic Protozoa

1. Isoenzyme Profiles: The first most widely used technique is isoenzyme profiles which have been extremely useful tools for distinguishing between apparently identical parasites. The technique involves using a number of characteristic enzymes to type different populations of parasite isolates in parallel and then with previously characterised control. Isoenzymes have been used to distinguish the various species of *Leishmania,* subspecies of African trypanosomes, pathogenic and non-pathogenic forms of *Entamoeba histolytica*, and pathogenic and non-pathogenic forms of *Toxoplasma gondii*. It is widely used in the typing of *G.duodenalis*(Tibayrenc,1993), and also, is useful for phylogenetic classification of *Cryptosporidium hominis,* and *P.falciparum* (Robert *et al.,*2003).

2. DNA and RNA Technology: is increasingly being used for diagnosis of parasitic infections as well as for resolving taxonomic and phylogenetic problems. Johnson and Baverstock (1989) were the first to attempt to produce a comprehensive phylogenetic tree of the protozoa with special reference to the parasitic forms, using data derived from a small subunit of ribosomal RNA(srRNA).

Both DNA and RNA can be used to determine evolutionary distance as nucleotide sequences tend to diverge over time and do evolve at a more regular rate than do morphological characters. Different kinds of RNA particularly small nuclear RNA (16S and 18S snRNA) and srRNA have been extensively used for taxonomic and phylogenetic investigations. DNA probes have been extensively used for studies on *Leishmania* spp both for diagnosis and determining relationships.

The development of the Polymerase Chain Reaction (PCR) has revolutionised the use of DNA techniques in parasitology and has been used to confirm the existence of non-virulent strains of *T.gondii* (Guo and Johnson,1995).

3. Molecular Karyotyping: another technique being used is molecular karyotyping, which involves measuring size differences between chromosomes, which has now been applied to New World *Leishmania* species and has confirmed conventional geographical groupings (Dujardin and Dujardin,1995).

Traditional Classification of Parasitic Protozoa

This classification (Table 3) is simply outlined, focusing on the central problem of protozoa classification, the need to meet up with the protozoologists requirement as well as providing a useful reference for parasitologist (especially medical parasitologists). It is based on 1980 Classification published by The Society of Protozoologists and that of Lee, *et al*. (1985) as outlined by Orihel and Ash (1995).

Utilitarian Classification of the Parasitic Protozoa of Humans

In recent years, the availability of a number of molecular markers has made it possible to analyse relationships between protozoans that would not have been possible using morphological characters alone. Corliss (1994) in his scheme has used both traditional and contemporary approaches and has attempted to retain familiar names as far as possible. It is important that any classification should reflect modern thinking about the classification as a whole, while retaining sufficient traditional material so as to permit every reference to information retrieval systems (Table 4).

This classification by Corliss (1994) is widely used by the majority of protozoologists and almost all parasitologists that are interested in the parasitic protozoa of humans. Two kingdoms under the Empire Eukaryota were created: Archezoa (Haekel, 1894) and Protozoa (Goldfuss,1818). Most parasitologists, largely basing their evidence on rRNA, now regard *Giardia* as well as other genera placed in the kingdom Archezoa as very primitive and consider them as representing early stage of eukaryote evolution (Collier *et al*., 1998).

Table1: Outline of the Readjusted Classification of the Kinetoplastids (Collier *et al*., 1998).

Order	Family	Genus	Section	Subgenus	Specie
Kinetoplastida	Trypanosomatidae	*Trypanosoma*	Salivaria	*Nannomonas*	*T.(N.)congolense*
				Trypanozoon	*T.(T.)brucei*
					T.(T.)b.brucei
					T.(T.)b.gambiense
					T.(T.)b.rhodesiense
					T.(T.)equiperdum
					T.(T.)evansi
				Pycnomonas	*T.(P.)suis*
			Stercoraria	*Schizotrypanum*	*T.(S.)cruzi*
				Duttonella	*T.(D.)vivax*
		Leishmania		*Leishmania*	*L.(L.)donovani*
				(Saf'yanova 1982)	*L.(L.)chagasi*
					L.(L.)mexicana
					L.(L.)amazonensis
					L.(L.)garnhami
				Viannia	*L.(V.)braziliensis*
				(Lainson and Shaw 1987)	*L.(V.)guyanensis*
					L.(V.)equatorensis
					L.(V.)peruviana
					L.(V.)colombiensis
					L.(V.)naifi

Table 2: Outline of the Classification of *Plasmodium* spp (Garnham, 1966).

Order	Family	Genus	Section	Subgenus	Specie
Haemosporida	Plasmodidae	*Plasmodium*	Reptiles	*Carinia*	*P. (C.) minasense*
				Sauramoaeba	*P. (S.) agamae*
				Ophidiella	*P. (O.) wenyomi*
			Mammals	*Vinckeia*	*P. (V.) berghei*
					P. (V.) yoelii
					P. (V.) vinckei
					P. (V.) chabaudi
				Plasmodium	*P. (P.) fragile*
					P. (P.) rhodaini
					P. (P.) brasilianum
					P. (P.) simium
					P. (P.) cynomolgi
					P. (P.)coatneyi
					P. (P.) knowlesi
					P. (P.) simiovale
					*P. (P.) ovale**
					*P. (P.) vivax**
					*P. (P.) malariae**
				Laverania	*P. (L.) reichenowi*
					*P. (L.) falciparum**
			Birds	*Novyella*	*P. (N.) juxtanuclrare*
				Haemameba	*P. (H.) gallinaceum*
					P. (H.) relictum
					P. (H.) cathemerium
				Giovannolaia	*P. (G.) fallax*
					P. (G.) durae
					P. (G.) circumflexum
					P. (G.) lophurae
				Huffia	*P. (H.) elongatum*

**Plasmodium* spp causing human malaria.

Table 3: Outline of Traditional Classification (Orihel and Ash, 1995).

- **Kingdom** Protista
  - **Subkingdom** Protozoa
    - **Phylum** Sarcomastigophora
      - **Subphylum** Mastigophora.e.g. *Giardia, Chilomastix,Trichomonas, Trypanosoma, Leishmania,Dientamoeba*
      - **Subphylum** Sarcodina: e.g. *Entamoeba,Iodamoeba, Endolimax, Acanthamoeba, Balamuthia, Naegleria*
    - **Phylum** Apicomplexa (the sporozoans)
      - **Class** Sporozoea
        - **Subclass** Coccidia
          - **Order** Eucoccidiida
            - **Suborder** Eimeriina e.g. *Isospora, Sarcocystis, Toxoplasma, Cryptosporidium, Cyclospora*
            - **Suborder** Haemosporina e.g. *Plasmodium*
        - **Subclass** Piroplasmea e.g. *Babesia*
    - **Phylum** Microspora e.g. *Enterocytozoon, Encephalitozoon, Septata*
    - **Phylum** Ciliophora(the ciliates) e.g. *Balantidium*

Table 4: Outline of Utilitarian Classification of Parasitic Protozoa (Corliss, 1994).

Empire	Kingdom	Phylum	Class	Order	Representative Member(s)
Eukaryota	Archezoa Haekel 1894	Metamonada	Trepomonada	Diplomonadida	*Giardia*
				Enteromonadida	*Enteromonas*
			Retortamonadea	Retortamonadida	*Chilomastix*
					Retortamonas
		Microspora	Microsporea	Microsporida	*Encephalitozoon*
					Enterocytozoon
					Nosema
					Septata
					Trachipleistophora
	Protozoa Goldfuss 1818	Percolozoa	Heterolobosea	Schizopyrenida	*Naegleria*
		Parabasala	Trichomonadea	Trichomonadida	*Dientamoeba*
					Trichomonas
		Euglenozoa	Kinetoplastidea	Trypanosomatida	*Leishmania*
					Trypanosoma
		Ciliophora	Litostomatea	Vestibuliferida	*Balantidium*
		Apicomplexa	Coccidea	Eimeriida	*Cryptosporidium*
					Cyclospora
					Isospora
					Sarcocystis
					Toxoplasma
			Haematozoea	Haemosporida	*Plasmodium*
				Piroplasmida	*Babesia*

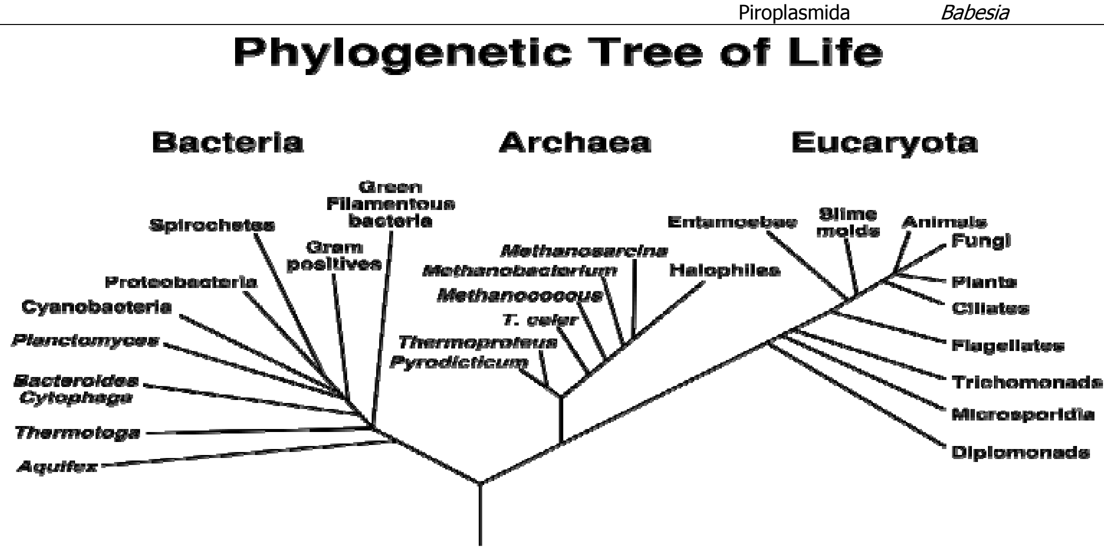

Figure 1: Modified Phylogenetic Tree of Living Organisms (adapted from Cavalier-smith, 2002).

Conclusion

The protozoa are defined as single celled eukaryotic organisms, but this definition is very simplistic and it has been an enigmatic one, largely because of the small size of their cells, the relative lack of morphological features and absence of any meaningful evolutionary history. The fact that protozoa are organisms that lie somewhere between prokaryotic and higher eukaryotic organisms, sharing any of the characteristics of each make their classification a little bit complex. In recent years, the availability of a number of molecular markers has made it possible to analyse relationships between protozoans that would not have been possible using morphological characters alone. Three terms are currently widely used: Protozoa, Protoctista, and Protista. However, parasitologists tend to be very conservative and the term Protozoa is now almost universally used by scientist working with those parasitic unicellular organisms that infect humans and domesticated animals (Collier *et al.*, 1998).

The increase in the number of parasitic protozoa recorded from humans and the accumulation of knowledge about their biology led to the creation of taxonomic and other groupings at the subgenus and subspecie levels (Collier *et al.*, 1998). Corliss (1994) in his scheme has used both traditional and contemporary approaches and has attempted to retain familiar names as far as possible. It is important that any classification should reflect modern thinking about the classification as a whole, while retaining sufficient traditional material so as to permit every reference to information retrieval systems. This classification by Corliss (1994) is widely used by the majority of protozoologists and almost all parasitologists that are interested in the parasitic protozoa of humans.

REFERENCES

Butschli,O.(1883).*Protozoa, Klassen und Ordnugen des Thier-ReichsBronn,* vol.1,ed Bronn,H.G., Winter,C.F. Leipzig.

Cavalier-Smith,T.(1993).Kingdom Protozoa and its 18 Phyla, *Microbiol Rev,* **57:**953-94.

Cavalier-Smith,T.(2002).The phagotrophic origin of eukaryotes and phylogenetic classification of protozoa. *Int. J. Syst. And Evol. Microbiology,***52**(2).

Collier,L.,Balows,A. and Sussman,M.(1998).*Parasotology in:Topley and Wilson's Microbiology and Microbial Infectio*ns,9th edtn,Arnold,UK.

Copeland,H.F.(1938). The Kingdoms of Organisms,*Quart Rev Biol,***13:**383-420.

Corliss,J.O.(1994).An interim Utilitarian(user-friendly) Hierarchical Classification and Characterization of the Protists,*Acta Protozoologica,***33:**1-51.

Cox,F.E.G.(1992).A New Classification of Parasitic Protozoa,*Protozoological Abs,***5:**9-14.

Craig,C.F.(1926).*A Manual of the Parasitic Protozoa of Man*, J.P.Lippincott,London,1-2

Dujardin,J.C. and Dujardin,J.P.(1995).Karyotype Plasticity in Neotropical *Leishmania*: an index for measuring genomic distance among *L.(V.)peruviana* and *L.(V.)braziliensis* populations,*Parasitology,**110:***521-8.

Fredericks,D.N.(2000). *Rhinosporidium seeberi*: a human pathogen from novel group of aquatic protistan parasites, *Emerg. Infect. Dis.**6***273-282.

Garnham,P.C.C.(1966).*Malaria Parasites and other Haemosporidia,*Blackwell Scientific Publications,Oxford.

Goldfuss,G.A.(1818).*Uber die Entwicklungsstufen des Thieres,*Nurenberg,**18:**21.

Guo,Z-G, and Johnson,A.M.(1995).Genetic Characterizatio of *T.gondii* strains by random amplified Polymerase Chain Reaction,*Parasitology,**111:***127-32.

Haeckel,E.(1876).*The History of Creation:or the development of the earth and its inhabitants by the action of natural causes,*Appleton,NY.

Hoare,C.A.(1972).*The Trypanosomes of Mammals,*Blackwell Scientific Publications.

Hogg,J.(1860).On the distinctions of a plant and an animal and on a fourth kingdom of nature,*Edinburgh New Phil J.**12:***216-2.

Honingberg,B.M. and Balamuth,W.(1964).A revised Classification of the phylum Protozoa,*J.Protozool,***11:**7-20.

Jahn,T.L. and Jahn,F.F.(1949).*How to know the Protozoa,*William Brown,Dubuque,Iowa.

Johnson,A.M. and Baverstock,P.R.(1989).Rapid ribosomal RNA sequencing and phylogenetic analysis of Protists,*Parasitol Today,***5:**102-5.

Lainson,R. and Shaw,J.J.(1987).*Evolution,Classification and Geographical distribution,the Leishmaniases in Biology and Medicine,*vol.1 eds Peters,W.,Killick-Kendrick,R.,Academic Press,London,1-120.

Lee,J.J,Huntner,S.H. and Bovee,E.C.(1985).An Illustrated Guide to the *Protozoa* ,Society of Protozoologists,Lawrence,Kansas.

Levine,N.D.(1973).*Protozoan Parasites of domestic animals and of man,*2nd edtn,Burgess,Minneapolis.

Levine,N.D. and Corliss. J.O.(1980).A newly revised classification of the Protozoa,*J.Protozoo,***27:**37-58.

Margulis,L. and Corliss,J.O. eds.(1990).*Handbook of Protoctista,*Jones and Bartlett,Boston.

Mayrhoffer,G. And Andrews,R.H.(1995).Division of *Giardia* isolates from humans into two genetically distinct assemblages by electrophoretic analysis of enzymes encoded at 27 loci and comparison with *G.muris,Parasitology,***111:**11-17.

Orihel,T.C. and Ash,L.R.(1995).*Parasites in human tissues,*American Society of Clinical Pathologists, Chicago.

Robert,H.O.,Ryab,H.L.,Bruce,R.D.,Robert,M.S. and Anderson,A.C.(2003). Phylogenetic classification of protozoa based on the structure of the linker domain in the bifunctional enzyme, Dihydrofolate reductase-thymidilate synthase.*J.Biol.Chem.**278**(52).*

Rothschild,L.J.(1989).Protozoa,Protista,Protoctista:What is in a name?*J.History Biology*, **22**(2).

Saf'yanova,V.M.(1982).The problems of *Leishmania* taxonomy(in Russian),*Protozoologiya(Leishmanii)*Leningrad,**7:**3-109.

Siebold,C.T.E von.(1848).*Lehrbuch der Vergleicherden Anatomie der wirbellosen Thiere,*Thiel,Berlin.

Tibarync, M.(1993).*Entamoeba,Giardia,* and *Toxoplasma*: clones or cryptic species?*Parasitol Today,**9:**102-5.*

Thomas,R.C.A., Reynoldson,J.A. and Lymbery,A.J.(1993). *Giardia:* from molecule to disease and beyond, *Parasitol Today*, **9**:313-5.

Whittaker,R.H.(1969).New Concepts of Kingdoms of Organisms,*Science,* **163:**150-60.